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Annals of Agrarian Science

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Aims and Scope

The aim of “Annals of Agrarian Science” is to overview problems of the following main disciplines and subjects: Agricultural and Biological Sciences, Biochemistry, Genetics and Molecular Biology, Engineering, Environmental Science. The Journal will publish research papers, review articles, book reviews and conference reports for the above mentioned subjects.

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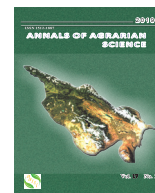
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Laser diffraction spectra's deconvolution in the study of soil texture

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ABSTRACT

The deconvolution procedure for separating the original particle size distribution spectrum by laser diffraction (LD) gives a different share of the fractions compared to the common method, when the number of particles is determined in the vertical walls at the group boundaries according to the FAO classification or other classification. In addition, a new indicator provides information on the properties of individual fractions by particle size: the LD fractions dispersion (D). Vertisols have a harder texture after deconvolution, especially in the Stavropol Vertisols, and the less extent in the Texas Vertisols. Vertisols have relatively low dispersion $D < 0.7$, which means relatively homogeneity of LD-fractions. Andosols dispersion (D) is very high; as a result of small number LD fractions, less than the number of FAO fractions. Very high D values can be attributed to preservation aggregates due to incomplete dispergation of soil. A strong disturbance in the distribution of LD-fractions and a high degree of their superposition makes it possible to assume that micro aggregates will remain in them after not adequate chemical preparation for LD analysis.

Keywords: Deconvolution procedure, Splitting of LD-spectrum, Soil texture, Vertisols, Andosols. Dispergation of soil.

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Introduction

At present, there are two common methods for analyzing the grain size composition of soils and sediments. The older method, a sieve analysis combined with a thin-particle pipette analysis, is based on determining the mass fraction of individual fractions [1]. Later, a method of laser diffraction analysis (LDA) was developed, based on the determination of the volume part of fractions [2]. Laser diffractometer provides fast analysis and does not require a greater sample mass. Another important advantage of LDA is to obtain an almost continuous spectrum of particle content.

The main task of any analysis of grain size, including LDA, is to determine the texture of the soil / sediment, that is, the proportion of each fraction, expressed as a percentage. Is this problem solved now, in relation to the LDA?

Now the concept of an “impenetrable vertical” boundary between fractions is conditional. So, we see that the usual schemes for decrypting LD-spec-

tra entail serious errors due to the assumption of vertical barriers on the border between fractions [3–5]. But in reality, the particles are distributed statistically, forming a series of independent sets, as a rule, in accordance with the law of normal distribution [2, 6]. Therefore, between the boundaries of the factions taken in any classification, there are no rigid vertical boundaries.

To circumvent the error in determining the soil / sediment texture, a different approach to deciphering continuous LD-spectra is needed.

Objective: to propose a universal methodology for decrypting LD-spectra.

Characteristics of LD-fractions

Nine classic size fractions of the dominant particles with different mineralogy are allocated in the range 0–2000 μm according to FAO Classification. Thus, each of the groups: clay (C), silt (Si) and sand (S) are subdivided into 3 fractions: fine (f), medium (m) and coarse (c), total 9 classic fractions. Each

fraction is marked by the index (Table 1). Usually, the each FAO-fraction content is determined by area under the LD-curve limited by vertical walls at the borders between the factions. In other words, content of FAO-fraction (S_{FAO}) is determined by the area (S), limited by top LD-spectra, and the side verticals in the points with the low and high limits fraction in a classification. For example, the contents cSi fraction is determined by the vertical walls at 20 and 63 μm .

But in fact, using the vertical boundaries between the FAO fractions is a very blatant admission. The normal distribution of the particles does not take into account the specified vertical borders between the fractions. As this problem is solved when analyzing soil LD-spectra?

The simplest form of statistical particles distribution is described by gauss form [7]. The meaning of deconvolution (with respect to decoding the energy dispersive X-ray fluorescence spectrum) is described by Savichev and Stepanov [7] and in relation to texture composition, defined by the LDA, is described earlier [8].

The main indicator of particle size distribution is the content of some fraction in the soil. It is possible to solve the problem of grain-size composition characteristics using the maximum position of LD-fraction on the axis diameter: $d_{\text{max}} = d_{\text{average}}$, placing it between the fraction bounds. In other words, we will name the LD-fraction, on the basis of the conditions: $d_1 < d_{\text{average}} < d_2$, where d_1 and d_2 are the lower and upper grain diameter of this fraction. For a precise definition of the centre fraction d_{average} is need to “deconvolute” or to split LD-spectrum.

Since laser diffraction is not able to detect fine clay of 0–0.2 μm , we use the eight fractions in the calculations. Thus, the average diameter (d_{average}) of the LD-fractions can be used to identify LD-fractions by placing it in the classical borders of the 8 groups (clay, silt and sand) of FAO Classification [1].

It is obvious that the vertical borders between the fractions are an abstraction, not taking into account the statistical grain probability distribution within the fraction. This assumption would be true if the

minima were reduced to zero. But, as can be seen from Figures 1-3, particle content is never reduced to zero.

The main indicator of LD-fraction is the position of its center on the axis of diameters: d_{average} . The second indicator is the area under the Gauss-line, which defines the share fraction S_{LD} in %. The third indicator is the fraction dispersion – D.

The deconvolution procedure allows solving two problems. First, it is possible to clarify the usual indicators, which are determined from the initial dependence of the particles on their diameter. In addition, the share of neighboring fractions can be distorted due to their superposition on the initial curve. The overlap effect is revealed as a result of the deconvolution of the original spectra. At the same time, it is possible to correct the ratio between the LD-fractions.

Secondly, perhaps the most important advantage of deconvolution is the ability to study the fraction dispersion D. If each fraction were completely separated from the neighboring fractions, then on the initial curve the minimum concentration would decrease to zero. Usually this does not happen, which means the superposition of fractions. It is clear that the greater the dispersion D, the lower its homogeneity and the higher the likelihood of the presence of microaggregates.

Dispersion D is lower in fully isolated fractions without superposition (a partial contribution of particles of a given fraction to the content of particles of neighboring fractions) and increases with an increase in the effect of superposition. We calculated the dispersion of completely isolated fractions on the basis of the ideal distribution of the grain size fractions of rocks [6]. Their dispersion is low $D = 0.26-0.38$, on average 0.32. This value is taken as the reference dispersion of completely isolated LD-fractions.

High values $D > 1$ can be associated with two factors. Stable particles are considered the first cause of the weak weathering fraction. The second reason is to save some micro-aggregates before LD-analysis.

Table 1. Index and borders (μm) between the soil size fractions [1]

Index	fC	mC	cC	fSi	mSi	cSi	fS	mS	cC
d, μm	0-0.2	02-0.63	0.63-2.0	2.0-6.3	6.3-20	20-63	63-200	200-630	630-2000

Objects and Methods

1. *Vertisols from different regions.* We studied two soil complexes with gilgai microrelief in the temperate and subtropical climates. The various elements of the microrelief differ significantly in the amount of precipitation flow.

Vertisol soil complex of temperate climate was described and sampled in the southeastern European part of Russia (44°38'17"N, 42°15'04" E) in the Stavropol region, North Caucasus. Soils are formed on marine clay under native steppe vegetation. According to Soil Taxonomy [9], this soil complex consists of Sodic Haplusterts (microhigh) and Typic Epiaquet (microlow).

The second Vertisol soil complex is located in the southern part of the United States, in Texas. 12-m-long trench has been formed on the second terrace of the Brazos River, Burleson County, near city of College Station (30°29'21"N, 96°28'44"W). The soils are developed from clayey alluvium, consisting of derivatives of the red-earth Permian and Triassic clays. The soils have been classified as Typic Haplusterts (microhigh) and Udic Haplusterts (microlow).

Grain size soil composition was determined on the laser diffraction particle size Analyzer "Sizer Analysette 22 comfort" (FRITSCH, Germany). Before analysis soil samples in the form of suspension were treated by ultrasound without chemical processing. The original soil samples are mixed with a rubber pestle and sieved through a 0.25 mm sieve. The hitch (0.10-0.13 g) was added 30 ml of distilled water and soil was treated by ultrasonic dispenser Digital Sonifir 250 (Branson Ultrasonics) with a dispersive element probe type, operating on a frequency of 20 kHz, in non-impulse mode, the energy of ultrasound – 450 J/ml.

2. *Andosols. (Southern Italy).* We studied recent soils and paleosols in quaternary fluvio-lacustrine sediments in the basin of Bozhano in Southern Italy, described by Colombo [4]. Recent soils and paleosols were formed as a result of pedogenesis on the products of interaction of pyroclastic material with alluvial clay sediments, some of which are enriched in carbonates.

Recent soils are called andosols, their thickness is 1.5 m. The parent rocks C-1 and C-2 at depths of 80-150 cm are strongly enriched in carbonates: the calcite content reaches 70-77%. Below, up to a depth of 11 m, paleosols are opened, which according to morphology are divided into four solums: Solum I-IV. Their parent rocks are lake sediments.

Before analyzing the grain size of andosols, to disperse microaggregates, samples < 2 mm were treated with H₂O₂ to oxidize organic matter, and then with dithionite-citrate-bicarbonate (DCB) to dissolve ferrous cement. In samples of carbonate rocks underlying recent soil (samples RS-C1 and RS-C2), carbonates were removed by treatment with Na acetate, buffered at pH = 5 [4].

Particle size distribution was determined using an "Analysette 22 comfort" laser diffraction particle size analyzer (FRITSCH, Germany). The device uses the method of "reverse Fourier optics", this is a system of a converging laser beam (helium is a neon laser with a wavelength of 632.8 nm). Soil samples were sonicated before analysis in the form of a suspension without additional chemical treatment [4].

After this preparation, the soil composition was determined by laser diffraction on a Malvern Mastersizer 2000 analyzer. The analysis parameters are the following: the pump speed is 2500-3000 rpm, the number of measurements is 6-10, the refractive index is 1.52, and the absorption index is 0.1 [4]. The particle size distribution was obtained using the full Mie scattering function for spheres, which provides a more accurate estimate of the particle size in the clay fraction than the Fraunhofer function [10].

The obtained particle distribution spectra were interpreted by deconvolution. Then a LD-fraction share as area of elementary contour obtained after deconvolution (S_{LD}) was compared with a share of FAO-fractions (S_{FAO}). The most important fractions of soil/sediment difference was estimated: $\Delta S = S_{LD} - S_{FAO}$ for clay (<2 μ m).

Results and discussion

Characteristics of the of Stavropol Vertisols's LD-fractions

The content of the LD fractions differs from the content of the FAO fractions and it was calculated according to the old method, assuming that the vertical walls at the fractions boundaries (Fig. 1). Deconvolution procedure showed the Vertisol heavier texture. Clay content of LD-fractions with average diameter < 2 microns is increased to 10-11% in comparison with the calculation based on vertical borders between particles FAO-fractions.

The homogeneity of the particle size fractions was evaluated by the values of their dispersions. The dispersion data of the Gaussians of each of the fractions are shown in Table 2.

In Vertisols of temperate climate common regularities were observed: the homogeneity of particles of fine fractions is higher than that of large fractions. Thus, the dispersion D is 0.50 for the three thinnest fractions, and D is 0.60–0.63 for the largest fractions. From this it follows that the coarse fractions are less homogeneous than the fine ones. Overall uniformity of LD-fractions of the Vertisols is high, because $D < 0.7$.

Characteristics of the Texas Vertisols LD-fractions

The LD-fractions contents are different from contents of FAO-fractions, calculated under the old methodology, assuming vertical walls at the frac-

tions borders (Fig. 2). Deconvolution procedure is showed the Vertisol heavier texture. Clay content of LD-fractions with an average diameter < 2 microns is increased to 7-8% in comparison with the FAO-fractions (Table 2).

In the Texas Vertisols the homogeneity of clay and silt fractions is lower than in Vertisols of Stavropol region. The relatively high dispersion D of fine fractions of Texas Vertisols reflects their lower weathering compared to Vertisols of Stavropol region. Probably, in Texas Vertisols, the absence of coarse silt is explained by the lithological peculiarities of the parent material, but not by the preservation of aggregates after preparing the soil for laser diffraction.

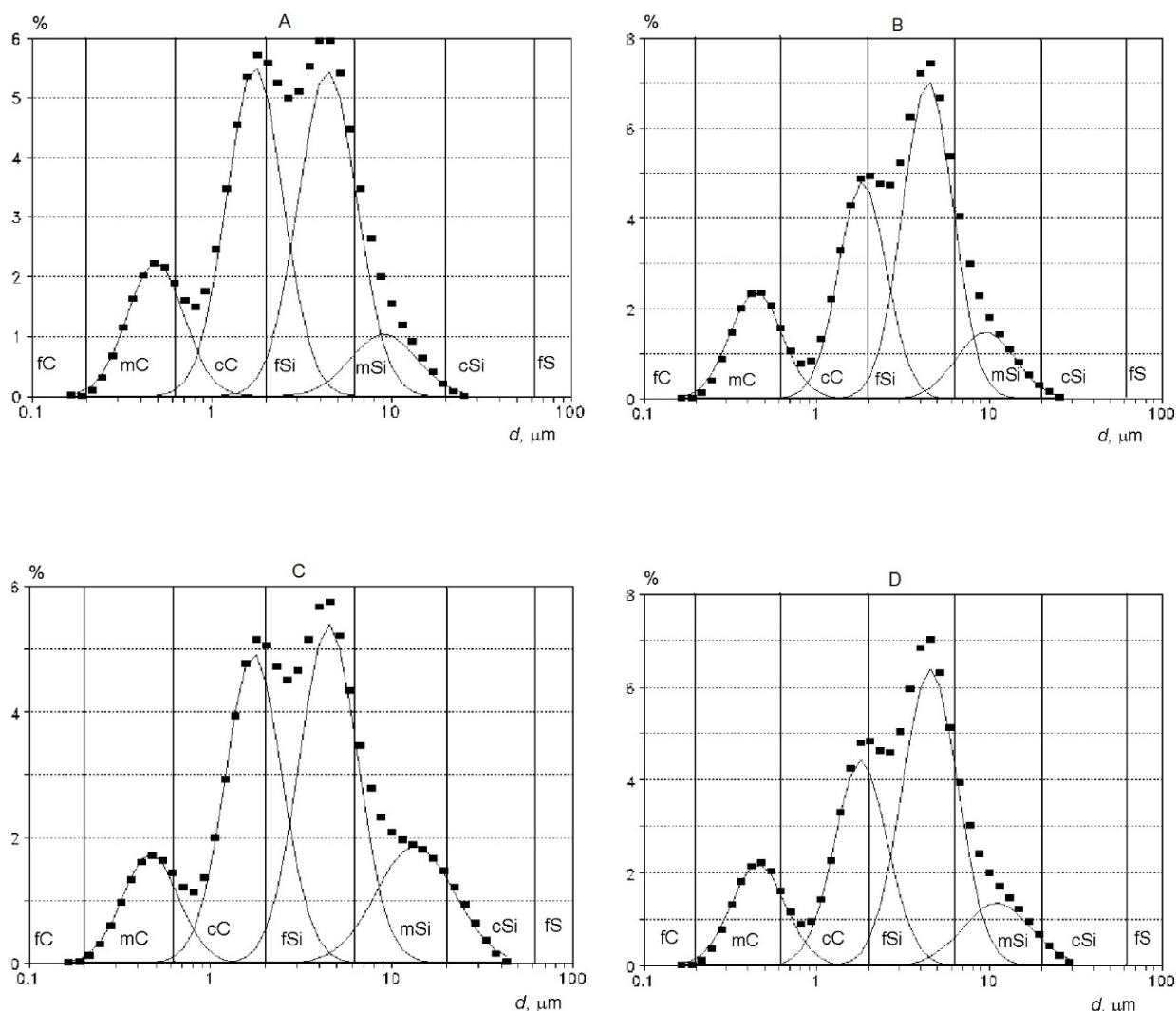


Fig. 1. The distribution of particle diameters (Stavropol, Vertisols). The points are the initial differential distribution; thin lines are elementary contours. A - microhigh, AU-horizon; B - microhigh, Cv-horizon; C - microlow, AU-horizon; D - microlow, Cv-horizon.

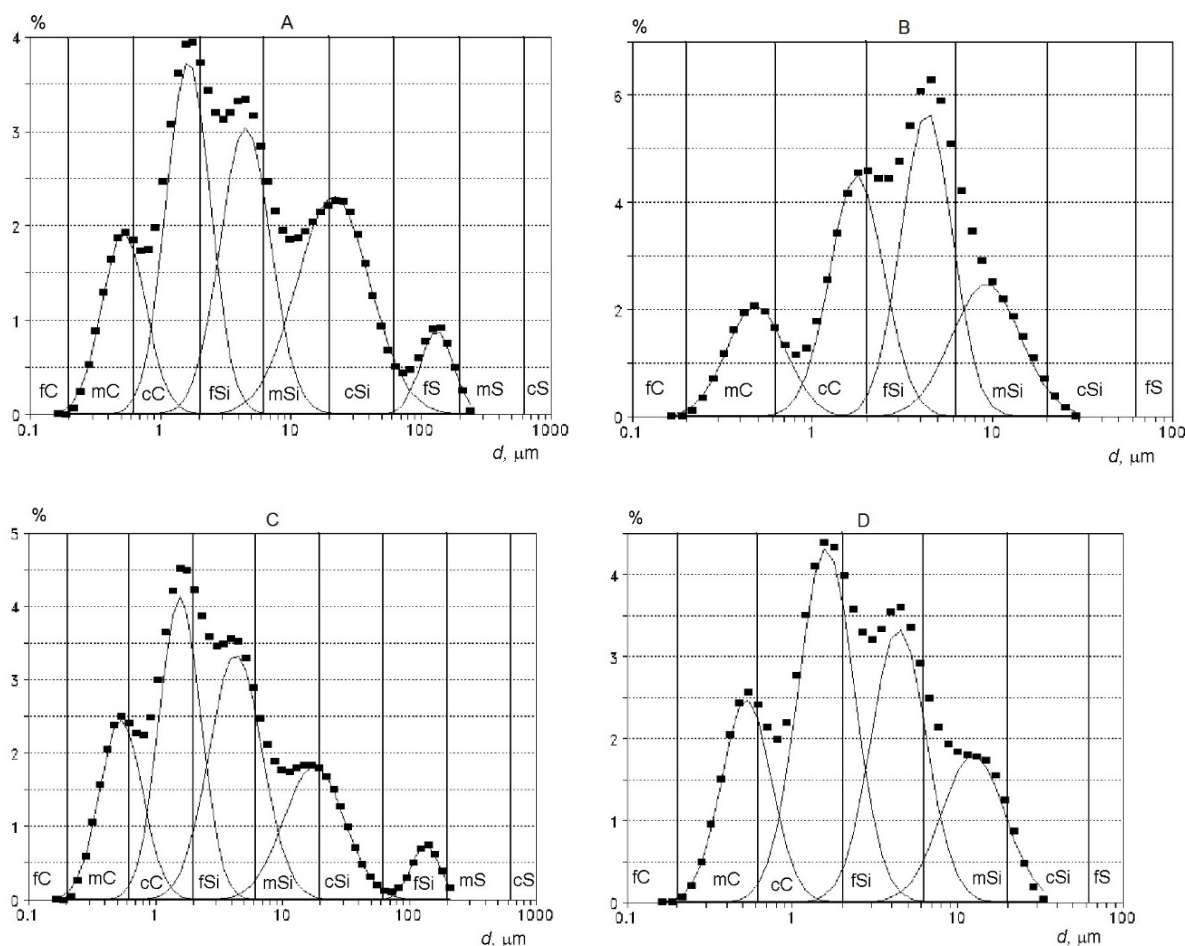


Fig. 2. The distribution of particle diameters (Texas, Vertisols). The points are the initial differential distribution; thin lines are elementary contours. A - microhigh, AUca-horizon; B - microhigh, Cv-horizon; C - microlow, AUca-horizon; D - microlow, Bca-horizon.

Table 1. Index and borders (μm) between the soil size fractions [1]

Group (μm)	Share S_{FAO} , %	After deconvolution			Share S_{FAO} , %	After deconvolution		
		Share S_{LD} , %	d , μm	D		Share S_{LD} , %	d , μm	D
Stavropol, Vertisols								
Microhigh, AU-horizon					Microhigh, Cv-horizon			
mC (0.2-0.63)	12.9	15.3	0.49	0.51	13.7	15.6	0.44	0.49
cC (0.63-2.0)	28.4	37.7	1.73	0.51	20.6	29.1	1.84	0.45
fSi (2.0-6.3)	47.2	38.7	4.39	0.53	52.1	44.4	4.39	0.47
mSi (6.3-20)	11.3	8.3	9.02	0.60	13.4	10.9	13.6	0.64
ΣC , %	41.3	53.0	$\Delta=11.7$		34.3	44.7	$\Delta=10.4$	
Microlow, AU-horizon					Microlow, Cv-horizon			
mC (0.2-0.63)	5.5	6.1	0.46	0.49	13.0	14.9	0.43	1.38
cC (0.63-2.0)	17.2	25.4	1.80	0.50	20.9	29.6		

fSi (2.0-6.3)	39.1	32.6	4.53	0.48	50.0	44.5		
mSi (6.3-20)	27.0	14.4	10.7	0.58	15.6	11.6	8.5	1.44
cSi (20-63)	11.0	21.8	22.2	0.65				
$\Sigma C, \%$	22.7	31.5	$\Delta=8.8$		33.9	44.5	$\Delta=10.6$	
Texas, Vertisols								
Microhigh, AUca-horizon					Microhigh, Cv-horizon			
mC (0.2-0.63)	11.2	14.1	0.53	0.55	12.3	14.7	0.49	0.53
cC (0.63-2.0)	23.5	27.6	1.65	0.55	21.9	29.6	1.77	0.49
fSi (2.0-6.3)	28.7	25.6	4.53	0.63	46.9	35.0	4.31	0.46
mSi (6.3-20)	18.5				18.6	20.6	9.21	0.62
cSi (20-63)	12.2	28.1	21.3	0.91				
fS (63-200)	5.7	4.9	133.0	0.41				
$\Sigma C, \%$	34.7	41.7	$\Delta=7.7$		34.2	44.3	$\Delta=10.1$	
Microlow, AUca-horizon					Microlow, Bca-horizon			
mC (0.2-0.63)	14.0	18.0	0.54	0.55	15.8	18.7	0.53	0.49
cC (0.63-2.0)	27.9	29.3	1.57	0.53	30.3	36.5	1.60	0.55
fSi (2.0-6.3)	31.0	30.4	4.31	0.68	34.5	27.7	4.39	0.54
mSi (6.3-20)	16.9	18.7	17.8	0.77	18.0	17.2	12.4	0.62
cSi (20-63)	6.4				1.3			
fS (63-200)	3.4	3.7	134.0	0.37				
$\Sigma C, \%$	41.9	47.3	$\Delta=5.4$		46.1	55.2	$\Delta=9.1$	
Italy, Andosols								
Recently soil, RS-Bw1-horizon					Recently soil, RS-C-1			
mC (0.2-0.63)	2.2				2.1			
cC (0.63-2.0)	10.4				10.0			
fSi (2.0-6.3)	17.0	35.2	2.1	1.52	16.8	33.4	3.0	1.52
mSi (6.3-20)	25.1				19.8			
cSi (20-63)	29.1	59.6	27	1.24	17.4	31.4	21	1.24
fS (63-200)	9.4				19.9	29.5	129	0.96
mS (200-630)	6.1	7.0	372	0.68	13.1	7.3	412	0.62
$\Sigma C, \%$	12.6	0.0	$\Delta=-12.6$		12.1	0.0	$\Delta=-12.1$	
Paleosols, SI-1-horizon					Paleosols, SIV-3			
mC (0.2-0.63)	3.5				2.7			
cC (0.63-2.0)	19.9				15.3			
fSi (2.0-6.3)	34.4	80.3	3.6	1.46	23.9	53	3.1	1.52
mSi (6.3-20)	24.0				23.1			
cSi (20-63)	11.9	16.6	29.3	1.18	21.6	44	30.0	1.40
fS (63-200)	2.9				9.1			
mS (200-630)	2.9	6.1	428	0.70	3.7	3	388	0.52
$\Sigma C, \%$	13.4	0.0	$\Delta=-13.4$		18.0	0.0	$\Delta=-18.0$	

Characteristic of the South of Italy Andosols LD-fractions

The main features of Andosols LD-fractions. The LD-fractions contents are different from contents of FAO-fractions. In addition, it is not individual LD-clay fractions, with an average diameter of $<2 \mu\text{m}$ (Fig. 3).

Clay particles are allocated traditionally on vertical limits of only 13%. But in fact clay particles are belonging to “tail” of fine silt LD-fraction. The “tail” of this fraction is very long, as evidenced by the unusually high the fine silt fraction dispersion $D = 1.5$ (Table 2).

Secondly, there is no independent fine sand LD-fraction 63-200 microns. A small fine sand content by traditional analysis ($<1\%$), is the result of superposition “tails” of neighbor large silt and medium sand LD-fractions.

Thus, grain size deconvolution is reduced drastically the number of LD-fractions by excluding as

independent fractions, consisting long LD-fractions tails. The reason is the very high LD-fractions dispersion $D = 1.5$. Smooth (absent-mindedness) of grain size fractions can be linked to two factors: 1) weak weathering of minerals; 2) preservation aggregates due to incomplete dispersion of soil. The first reason is possible if there are many resistant to weathering particles of feldspar and quartz. Meanwhile, Andosols are dominated by X-ray amorphous minerals (allofan and imogolite), distinguished by extremely low resistance. Therefore, the first assumption about the reason of weak weathering minerals for andosols is not suitable.

The second assumption remains an abnormally high dispersion, that indicates the preservation of micro-aggregates. One possible reason is preservation of aggregates due to incomplete dispersion of soil. This suggests the probable conservation of microaggregates after preparing the soil for laser diffraction.

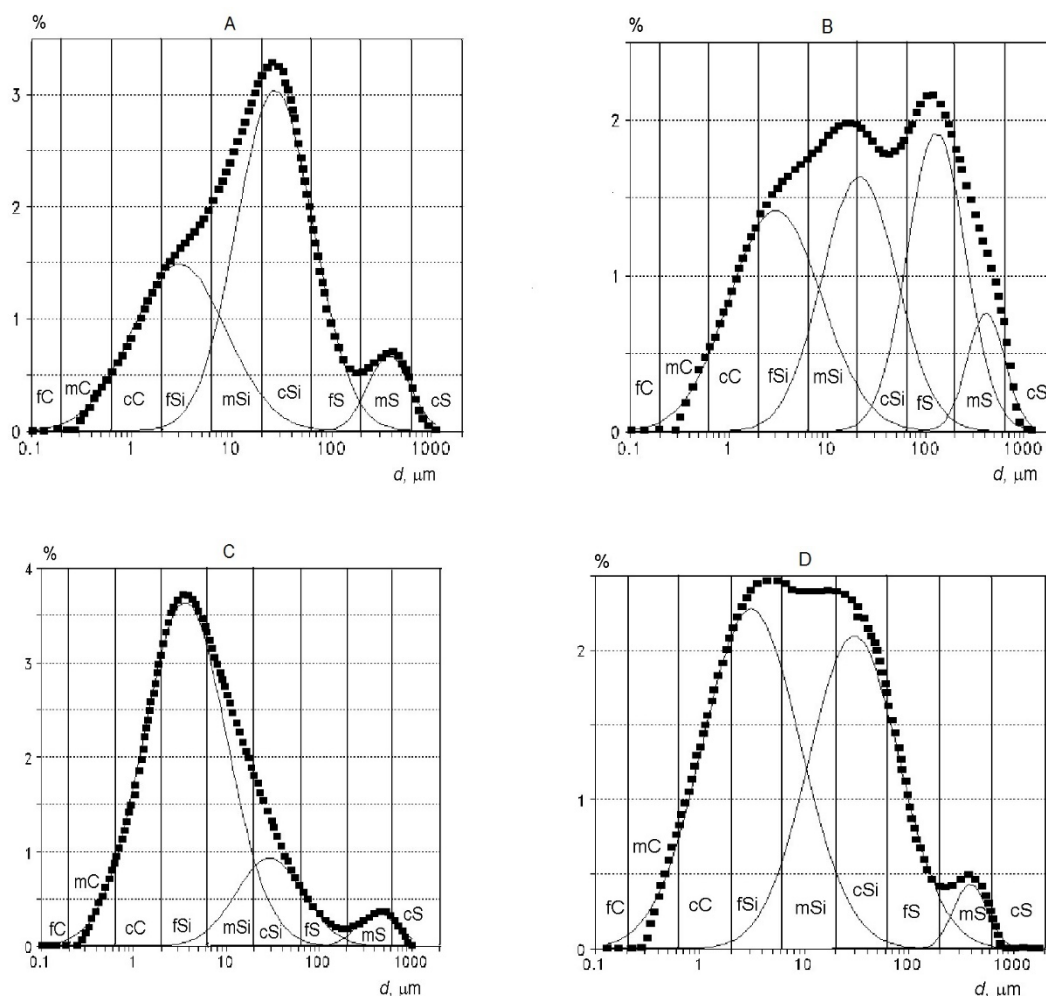


Fig. 3. The distribution of particle diameters (Italy, Andosols). The points are the initial differential distribution; thin lines are elementary contours. A - recently soil, RS-Bw1; B - Recently soil, RS-C-1; C - paleosols, SI-1; D - paleosols, SIV-3.

The mineralogical composition of Andosols in a neutral medium is determined by the hydrolysis of volcanic glass [11]. This leads to the formation of typical minerals of Andosols: clay minerals (allophane and imogolite) and Fe-hydroxide: ferrihydrate $2\text{Fe}_2\text{O}_3 \cdot \text{FeOOH} \cdot 4\text{H}_2\text{O}$ [11]. The role of mineral cement in Andosols is performed by active particles of allophane, imogolite and ferrihydrate, connected with organic matter [12].

Allophane and imogolite are nano-sized aluminosilicate with hollow spherule and tube structures with the diameter 3-5.5 nm; the minerals have high cation/anion exchange capacity as well as extensive, variable-charged surfaces [12, 13]. Together with Fe-containing minerals of short-range order, which can easily dissolve and precipitate during oxidation-reduction changes, these nano-sized minerals of short-range order and the organic matter associated with them can act as a strong binding agent for the formation of aggregates [14]. In addition, Al-organic (and, to less extent, Fe-organic) complexes formed via covalent bonding between monomeric Al and Fe ions with organic functional groups are also relatively abundant in Andosols [12, 13].

DCB effectively dissolves Fe-cement in strongly ferruginized soils, for example, in ferrallitic soils [15]. Feature of these soils is high content of gross iron, much more above the Clarke of the lithosphere - 6.2% [16]. But the total iron content in the Italian Andosols and paleosols is very low: 0.7-2.8%. This already indicates a weak ferrigation of Andosols. The most of thin microaggregates of Andosol have very high strength. It is obvious that DCB is not able to completely destroy solid micro-aggregates of Andosols.

A thorough study using the fine absorption structure of near X-rays showed that organic matter forms a weak bond with the smooth surface of large crystals [13]. This explains the reason not to attend such minerals like kaolinite, hematite, goethite in the formation of micro-aggregates in Andosol. But organic matter is formed a strong connection with unordered minerals such as allophane, imogolite and ferrihydrite [13].

Colombo [4] randomly used a reagent (DCB) that is not very selective reagent to Andosols. But this allowed us to obtain new information on the composition of soil microaggregates. In the future, it is possible to apply deliberately soft processing to obtain information about firmly connected soil microaggregates, not only in Andosol.

Conclusion

The deconvolution procedure for splitting the original spectrum of particle size distribution gives another fractions share compared with usual method, when the number of particles is determined within the vertical walls at the borders of groups according to the FAO classification or other classification. In addition, a new indicator provides information on the properties of individual particle size fractions: dispersion of LD-fractions (D). Dispersion value ranges from 0.32 in the complete absence of LD-fractions superposition of up to 1.8 from Andosols with very long “tails” of the distribution.

After deconvolution vertisols have the harder texture, especially in the vertisols of Stavropol and, to a lesser extent at Texas vertisols. Vertisols have relatively low dispersion $D < 0.7$, which means relative homogeneity of LD-fractions.

In Northern Italy Andosols dispersion D of LD-fraction is very high; resulting in the real fraction number is less than the number of FAO-fraction. Very high values of the dispersion can be attributed to preservation aggregates due to incomplete dispersion of soil. A strong disturbance in the distribution of LD-fractions and a high degree of their superposition makes it possible to assume that microaggregates will remain in them after not adequate chemical preparation for laser diffraction, in particular, this applies to paleosols.

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Cerrena unicolor BCC306 - new potent producer of lignin-modifying enzymes

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ABSTRACT

In this study, the nutritional requirements of *Cerrena unicolor* BCC306 providing enhanced production of lignin-modifying enzymes have been elucidated in the submerged fermentation of plant raw materials. Mandarin peels followed by the wheat bran and pomegranate residues manifold enhanced laccase production. All tested lignocellulosic materials 2-6-fold stimulated secretion of phenol red oxidizing manganese peroxidase (MnP_{610}) as compared with the control medium, only mandarin peels more than 3-fold increased the Mn oxidizing manganese peroxidase (MnP_{270}) activity whereas addition of pomegranate or wheat straw 5- and 11-fold decreased this enzyme activity, respectively. Mandarin peels and pomegranate ensured accumulation of appreciated levels of extracellular lignin peroxidase (LiP). Variation of mandarin peels concentration from 0 to 6% revealed that the higher was the growth substrate concentration the higher was enzyme activity. Supplementation of mandarin peels-based medium with an additional nitrogen source (20 mM N) favored of laccase secretion, none of them increased MnP_{610} activity and only peptone and casein hydrolysate enhanced the MnP_{270} activity. The highest LiP activity was revealed during the fungus cultivation in the medium supplemented with KNO_3 as an additional nitrogen source. Cultivation of *C. unicolor* 306 in 7 L laboratory fermenter resulted in accumulation of 302.4 U/mL laccase, 4.9 U/mL MnP_{270} , 0.96 U/mL of MnP_{610} , and 0.29 U/mL LiP indicating that this fungus is a good candidate for scaled up production of LME for different biotechnological applications.

Keywords: Basidiomycetes, *Cerrena unicolor*, Submerged fermentation, Lignin-modifying enzymes, Mandarin peels, Biotechnological applications.

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1. Introduction

Wood-degrading basidiomycetes are the only organisms capable of completely mineralizing lignocellulose, the most abundant recalcitrant renewable material available in the nature due to their ability to synthesize relevant enzymes for breaking down cellulose, hemicellulose, and lignin, a natural aromatic polymer [1]. The white-rot basidiomycetes (WRB) secrete one or more of four extracellular lignin-modifying enzymes (LME) that are essential for lignin degradation: lignin peroxidase (LiP, EC 1.11.1.14), manganese dependent peroxidase (MnP , EC 1.11.1.13), versatile peroxidase (VP, EC 1.11.1.16) and laccase (EC 1.10.3.2). The LME are of fundamental importance for the efficient bioconversion of plant residues and they are prospec-

tive for a large number of biotechnological applications in chemical, fuel, pulp and paper, food, textile, dye, and cosmetic industries as well as in agriculture. Moreover, these enzymes are directly involved in the soil and water bioremediation processes degrading various xenobiotic compounds (polycyclic aromatic hydrocarbons, dioxins, explosives, synthetic dyes, micropollutants and many other) [2-4].

It is evident that the growing market satisfaction for LME applications in industrial and environmental technologies and processes require huge amounts of this enzyme at low cost. The main issue delaying wide implementation of LME at industrial scale is their too high cost because of comparatively low enzyme yield in most tested fungi and limited fundamental knowledge on their physiology and biochemistry. Although many recombinant organisms

efficiently overproduce other industrial enzymes, high expression of LME in heterologous systems has not been achieved [5, 6]. Therefore, various approaches and strategies, including search for new enzyme producers, exploitation of cheap plant raw materials as growth substrates, optimization of fermentation media and cultivation conditions, usage of effective inducers, surfactants, development of better bioprocess technologies, etc. [7–9] have been exploited. Nevertheless, analysis of literature data shows that there is an increased demand for fungi with increased growth rate and, at the same time, with an increased enzyme production during the primary metabolism. Moreover, current knowledge on the physiology and biochemistry of WRB is still limited in order to realize their biotechnological potential on industrial level. In particular, very little is known about mechanisms up- and down-regulating oxidative enzyme synthesis in the fermentation of plant raw materials. It is clear that to develop strategies and technologies for LME overproduction a deeper understanding of mechanisms of individual LME synthesis regulation under specific growth conditions is a critical task.

The aim of this study was to elucidate the nutritional requirements of *Cerrena unicolor* BCC306 providing enhanced production of LME by this new potent laccase producer.

2. Materials and Methods

2.1. Organism and inoculum preparation

C. unicolor BCC306 was isolated from the forest of Eastern Georgia and properly deposited in the basidiomycetes' culture collection of the Agricultural University of Georgia. The fungal inoculum was prepared by growing mycelium taken from agar slants on a rotary shaker in 250 mL flasks containing 100 mL of the basal medium (per L): 15 g glucose, 3 g peptone, 1 g KH_2PO_4 , 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 3 g yeast extract. The inoculum cultivation was performed at 27°C and 150 rpm for 7 days, then the grown fungal biomasses were homogenized in a Waring laboratory blender and used as an inoculum for submerged cultures.

2.2. Lignocellulosic materials

The following available in large amounts in Georgia plant raw materials were tested as growth substrates in order to establish their impact to *C.*

unicolor BCC306 enzyme activity: wheat bran, mandarin peels, residue after ethanol production from the wheat grains (EPR), pomegranate, and wheat straw. All plant residues were oven-dried at 50°C and ground to powder in a laboratory mill prior to addition to the nutrient medium.

2.3. Shake-flask cultivation conditions

The submerged cultivation of fungi was conducted in the rotary Innova 44 shakers (New Brunswick Scientific, USA) at 27°C and 160 rpm. The homogenized mycelium (5 mL) was used to inoculate the 250-mL flasks containing 50 mL of the basal medium supplemented with 1.0 mM $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The above-mentioned lignocellulosic materials in an amount of 40 g/L were used as growth substrates instead glucose. Several organic and inorganic nitrogen sources at an equal concentration of 20 mM as nitrogen were tested to optimize LME production by *C. unicolor* BCC306.

The pH of all media was adjusted to 5.0 prior to sterilization to create optimal conditions for lignocellulosic polysaccharide hydrolysis and all submerged cultivations were carried for 10 days. At predetermined time intervals, 1 mL of culture was sampled and solids were separated by centrifugation (Eppendorf 5417R, Hamburg, Germany) at 10,000 g for 5 min at 4 °C. The supernatants were analyzed for pH, reducing sugars and enzyme activities.

All experiments were performed twice using three replicates at each time point. All results were expressed as the mean \pm SD with only $p \leq 0.05$ considered as statistically significant.

2.4. Cultivation in bioreactor

To scale up the LME production, the cultivation of *C. unicolor* BCC306 was performed in the 7 L fermenter LILFUS GX (Incheon, South Korea) with three Rushton impellers. The fermenter was filled with 5 L of the optimized medium (per L): 40 g mandarin peels, 1 g KH_2PO_4 , 5 g peptone, 3 g yeast extract, 0.5 g MgSO_4 , 1.0 mM CuSO_4 and 3 mL polypropylene glycol 2000 as an antifoam. The fermenter equipped with pH, temperature and pO_2 probes was sterilized (121°C, 40 min) and inoculated with 500 mL of homogenized mycelium. Fermentation was carried out without baffles at 27°C and at the constant airflow rate of 1 v/v/min. The medium pH was controlled at 5.3 during initial 5 days of fermentation to provide optimal conditions for the mandarin peels polysaccharide hydrolysis, then it was increased to 6.2 where the synthesized

enzymes are most stable. The agitation rate was 200 rpm during 3 days, then it was increased to 300 rpm and after 7 days decreased to 200 rpm till the end of fermentation. During the fermentation process, samples were collected daily and immediately analyzed for enzyme activity. After 9 days of fermentation, fungal biomass was separated from culture liquid by successive filtration and centrifugation at 5,400 g for 15 min. Enzyme preparation was isolated from the culture liquid by precipitation with $(\text{NH}_4)_2\text{SO}_4$ at 70% saturation and the precipitate was dissolved in 0.05 M phosphate buffer (pH 6.0).

2.5. Enzyme activity assay

Laccase activity was determined spectrophotometrically (Camspec M501, Cambridge, UK) at 420 nm as the rate of 0.25 mM ABTS (2,2'-azino-bis-(3-ethylthiazoline-6-sulfonate)) oxidation in 50 mM Na-acetate buffer (pH 3.8) at room temperature [10]. MnP activity was measured at 270 nm by following the formation of a Mn^{3+} -malonate-complex [11] and by oxidation of Phenol Red [12] in the presence of 0.1 mM H_2O_2 . LiP activity was determined spectrophotometrically at 310 nm by the rate of oxidation of 2 mM veratryl alcohol in 0.1 M sodium tartrate buffer (pH 3.0) with 0.2 mM hydrogen peroxide [13]. To establish true peroxidase activity, activities in the absence of H_2O_2 were subtracted from the values obtained in the presence of hydrogen peroxide. One unit (U) of LME activity was defined as the amount of enzyme that oxidized 1 μmol of substrate per minute.

3. Results

3.1. Effect of lignocellulosic growth substrate

To obtain an insight into physiology of the *C. unicolor* BCC306 LME activities regulation, initially, the effect of selected plant raw materials on the fungus enzyme activity was evaluated. All materials supported equally good fungal growth in form of small pellets. However, the values for individual oxidases differed significantly depending on growth substrates used. Results given in Table 1 indicate that in the submerged cultivation in the absence of lignocellulosic material the fungus secreted significant activities of laccase and MnP but supplementation of this medium with lignocellulosic growth substrates highly enhanced the target enzyme production. In particular, *C. unicolor* 306 secreted the highest laccase activity in the mandarin peels fermentation followed by the wheat bran and pomegranate residues. Specifically, supplementation of the control medium with mandarin peels caused 31-fold increase in the *C. unicolor* BCC306 laccase activity. Other tested lignocellulosic materials, including the lignified wheat straw, also provided accumulation of comparatively high laccase activity.

Unlike laccase, the MnP activity significantly varied depending on the growth substrate used. Among them, only mandarin peels supplementation to the basal medium more than 3-fold increased the MnP_{270} activity whereas addition of pomegranate or wheat straw 5- and 11-fold decreased this enzyme activity, respectively. The measurement of phenol red-oxidizing activity revealed that the submerged

Table 1. Effect of growth substrates on the *C. unicolor* 306 lignin-modifying enzyme activity

Growth substrates	Laccase (U/mL)	MnP_{270} (U/mL)	MnP_{610} (U/mL)	LiP (U/mL)
Control	$5.7 \pm 0.3^{(7)}$	$0.69 \pm 0.10^{(4)}$	$0.18 \pm 0.02^{(4)}$	0
EPR	$43.8 \pm 4.2^{(9)}$	$0.62 \pm 0.09^{(7)}$	$0.37 \pm 0.03^{(7)}$	$0.12 \pm 0.02^{(9)}$
Mandarin peels	$179.1 \pm 14.0^{(7)}$	$2.32 \pm 0.30^{(7)}$	$0.93 \pm 0.07^{(4)}$	$0.35 \pm 0.04^{(9)}$
Pomegranate	$103.4 \pm 9.1^{(9)}$	$0.13 \pm 0.01^{(7)}$	$0.57 \pm 0.04^{(7)}$	$0.27 \pm 0.03^{(9)}$
Wheat bran	$108.5 \pm 13.2^{(9)}$	$0.59 \pm 0.05^{(7)}$	$0.88 \pm 0.08^{(4)}$	0
Wheat straw	$40.1 \pm 3.7^{(12)}$	$0.06 \pm 0.01^{(4)}$	$1.09 \pm 0.12^{(7)}$	$0.11 \pm 0.01^{(9)}$

fermentation of all used lignocellulosic materials 2-6-fold stimulated secretion of MnP_{610} as compared with the control medium. Especially, wheat straw appeared to be the most appropriate growth substrate for this enzyme production. Finally, the data received show that *C. unicolor* BCC306 did not produce LiP in the synthetic medium without lignocellulosic material. Moreover, wheat bran did not provide this enzyme expression. Of plant raw materials used, mandarin peels and pomegranate ensured accumulation of appreciated levels of extracellular LiP.

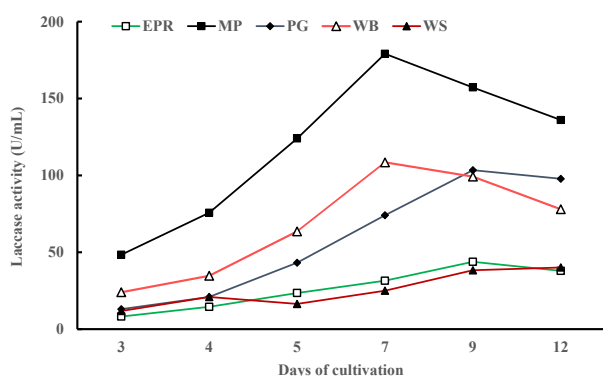


Fig. 1. Profiles of the *C. unicolor* 306 laccase activity accumulation in dependence on lignocellulosic growth substrate. EPR – ethanol production residue, MP – mandarin peels, PG – pomegranate, WB – wheat bran, WS – wheat straw.

Analysis of kinetics of LME accumulation in the submerged fermentation of plant raw materials showed that not only enzyme yield but profiles of enzyme activity depend on the growth substrate used. Thus, in the fermentation of mandarin peels and wheat bran laccase activity rapidly accumulated and reached maximum on the day 7 while in the fungus cultivation in presence of pomegranate and ethanol production residue the laccase peak activity was achieved after 9 days of fermentation (Fig. 1).

Moreover, in the fermentation of wheat straw, small laccase activity peak was revealed after 3 days of the cultivation, then it slightly decreased and after that gradually increased till the end of experiment. Kinetics of MnP activity accumulation also depended on lignocellulosic material; however, maximum of this enzyme activity was detected after 4 and 7 days of cultivation (Table 1). At the same time, the period of achieving of LiP activity maximum did not depend on the used growth substrate.

3.2. Effect of mandarin peels concentration

Subsequently, the effect of growth substrate concentration on LME production by *C. unicolor* 306 was evaluated. The data presented in Table 2 indicate that in the fermentation of mandarin peels the higher was this material concentration the higher was the laccase yield. The highest laccase activity was revealed in the fungus cultivation in media containing 5 and 6% of the growth substrate; however, the peak of the laccase activity was achieved after 7 and 10 days of cultivation, respectively. The same regularities were revealed in the measurement of MnP activities. In particular, *C. unicolor* 306 MnP_{270} activity gradually increased with elevation of growth substrate concentration from 0 to 6% reaching very high value of 6.56 U/mL after 10 days' fermentation while the maximum of MnP_{270} was achieved in the medium containing 5% of mandarin peels. It worth noting that exploitation of media with high concentration of mandarin peels shifted the maxima of MnP activities to later period of cultivation. The data received show that as compared with laccase and MnP activities expression, *C. unicolor* 306 is comparatively poor producer of LiP. This enzyme activity gradually increased only with an elevation of mandarin peels concentration to 4%, higher concentrations of the growth substrate negatively affected the enzyme synthesis.

Table 2. Effect of mandarin peels concentration on the *C. unicolor* 306 lignin-modifying enzyme activity

Concentration (%)	Laccase (U/mL)	MnP_{270} (U/mL)	MnP_{610} (U/mL)	LiP (U/mL)
Control	$6.1 \pm 0.5^{(7)}$	$0.54 \pm 0.06^{(4)}$	$0.14 \pm 0.02^{(4)}$	0
2.0	$15.8 \pm 1.4^{(7)}$	$0.66 \pm 0.06^{(7)}$	$0.27 \pm 0.03^{(4)}$	$0.07 \pm 0.01^{(7)}$
3.0	$57.5 \pm 7.3^{(7)}$	$1.88 \pm 0.23^{(4)}$	$0.76 \pm 0.07^{(4)}$	$0.21 \pm 0.03^{(10)}$
4.0	$182.0 \pm 19.8^{(7)}$	$3.13 \pm 0.41^{(4)}$	$0.97 \pm 0.13^{(7)}$	$0.32 \pm 0.03^{(10)}$
5.0	$246.5 \pm 30.2^{(7)}$	$4.69 \pm 0.55^{(7)}$	$1.23 \pm 0.14^{(7)}$	$0.31 \pm 0.04^{(10)}$
6.0	$254.1 \pm 33.7^{(10)}$	$6.56 \pm 0.81^{(10)}$	$1.16 \pm 0.15^{(10)}$	$0.24 \pm 0.03^{(10)}$

Table 3. Effect of nitrogen sources on the *C. unicolor* 306 lignin-modifying enzyme activity

Nitrogen sources	Final pH	Laccase (U/mL)	MnP ₂₇₀ (U/mL)	MnP ₆₁₀ (U/mL)	LiP (U/mL)
Control	5.0	138.6 ± 11.7 ⁽⁷⁾	2.34 ± 0.26 ⁽⁷⁾	1.09 ± 0.12 ⁽⁷⁾	0.14 ± 0.02 ⁽¹⁰⁾
KNO ₃	7.0	230.7 ± 21.5 ⁽¹⁰⁾	1.90 ± 0.25 ⁽⁴⁾	0.78 ± 0.07 ⁽⁴⁾	0.39 ± 0.04 ⁽¹⁰⁾
NH ₄ NO ₃	6.0	229.0 ± 27.0 ⁽¹⁰⁾	1.34 ± 0.20 ⁽⁷⁾	0.47 ± 0.06 ⁽⁴⁾	0.16 ± 0.03 ⁽¹⁰⁾
(NH ₄) ₂ SO ₄	5.7	238.3 ± 21.0 ⁽¹⁰⁾	1.67 ± 0.21 ⁽⁴⁾	0.57 ± 0.06 ⁽⁴⁾	0.18 ± 0.02 ⁽¹⁰⁾
Peptone	6.5	298.2 ± 26.3 ⁽¹⁰⁾	6.46 ± 0.59 ⁽⁷⁾	1.14 ± 0.10 ⁽⁷⁾	0.24 ± 0.03 ⁽¹⁰⁾
Casein hydrolysate	6.6	278.8 ± 30.27 ⁽¹⁰⁾	4.67 ± 0.50 ⁽⁷⁾	1.10 ± 0.11 ⁽⁷⁾	0.27 ± 0.03 ⁽¹⁰⁾

3.3. Effect of nitrogen sources

To optimize the cultivation conditions favoring the target enzyme production effect of an additional nitrogen source was investigated. The data received evidence that the fungus effectively utilize the nitrogen available in the lignocellulosic material and in the yeast extract for the laccase production (Table 3). Specifically, the medium without an additional nitrogen ensured accumulation of 138.6 U/mL laccase, 2.34 U/mL MnP₂₇₀, 1.09 U/mL MnP₆₁₀, and 0.14 U/mL LiP. However, all the tested additional sources of nitrogen favored accumulation of laccase in the culture liquid. Among them, organic compounds, peptone and casein hydrolysate appeared especially appropriate for the laccase secretion providing 2-fold increase in *C. unicolor* 306 laccase activity as compared with the control medium.

The measurement of the MnP activities again showed high productivity of *C. unicolor* 306 in the control medium and in the media containing inorganic and organic compounds. However, none of them increased MnP₆₁₀ activity and only peptone and casein hydrolysate enhanced the MnP₂₇₀ activity. On the contrary, supplementation of the control medium with several nitrogen containing compounds inhibited the MnP activity expression. Finally, like in the previous experiments, *C. unicolor* 306 expressed comparatively low lignin peroxidase activity, peptone and casein hydrolysate rather promoted LiP accumulation, but the highest enzyme activity was revealed during the fungus cultivation in the medium supplemented with KNO₃ as an additional nitrogen source.

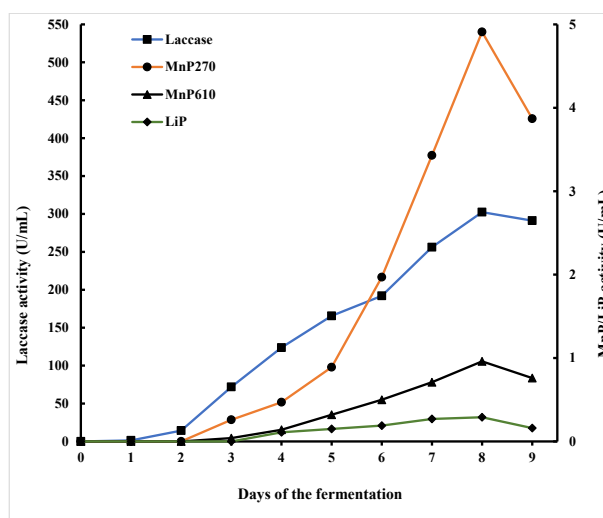


Fig. 2. Profiles of laccase, MnP, and LiP accumulation in the fermentation of mandarin peels by *C. unicolor* 306.

3.4. Scaled up LME production in bioreactor

To scale up the LME production by *C. unicolor* 306 the fungus cultivation was carried out in a fermenter filled with an optimized medium, taking into account the results obtained in agitated flask cultures. A stirred-tank bioreactor was employed to efficiently produce the target enzyme since it provides better mixing of medium and the fungus growth in form of pellets of desired size. The medium pH was controlled at 5.3 during initial 5 days of fermentation to provide optimal conditions for the mandarin peels polysaccharide hydrolysis and to steadily supply the growing culture with carbon and energy sources, then it was increased to 6.2 where the synthesized enzymes are most stable.

As shown in Figure 2, the presence of *C. unicolor* 306 laccase activity was detected after the first day of fermentation, with a gradual increase in its activity throughout the cultivation time, reaching the maximum on day eight (302.4 U/mL) followed by slight decrease of the enzyme activity. An appreciable amount of MnP₂₇₀ activity was released by the third day of fermentation with the maximum activity detected on day 8 (4.9 U/mL) followed by the sharp decrease on the next day of cultivation. Low MnP₆₁₀ activity was also detected after three days of fermentation, the enzyme activity consistently increased till day 7 of fermentation, after that it gradually decreased. LiP activity was detected later all other enzymes, after four days of fermentation, reaching its maximum (0.29 U/mL) after eight days of cultivation. The isolated and concentrated (158 mL) enzyme preparation from *C. unicolor* 306 contained 8625 U/mL of laccase activity, 112 U/mL of MnP₂₇₀ activity, 16 U/mL of MnP₆₁₀ activity, and 4.7 U/mL of LiP activity.

Discussion

Like in earlier studies with *C. unicolor* strains [7, 14], the recently isolated WRB *C. unicolor* 306 has proven to be a very promising producer of LME, especially laccase and MnP. Therefore, establishment of optimal cultivation conditions for this industrially important fungus was of high practical value. The utilization of industrial and agricultural wastes or byproducts for LME production is a common approach and an effective way to promote enzyme synthesis and reduce the cost of fermentation [1, 7, 15, 16]. Thus, significant levels of *Pleurotus sajjar-caju* laccase and MnP production was recorded in the mushroom cultivation in media containing oil palm waste followed by banana residues and cotton stalks [Thiribhuvanamala]. Among various types of agro-industrial wastes, orange peel was the best inducer of *Pleurotus ostreatus* laccase [17]. The results described in this study demonstrate a clear regulatory role and significant stimulation of LME activity by several plant raw materials tested. Among them, mandarin peels containing significant concentrations of soluble carbohydrates and other nutrients for an abundant growth of fungi as well as flavonoids and phenolic compounds [9, 18] ensured simultaneous and exceptional production of laccase, MnP, and LiP by *C. unicolor* 306. It is interesting that this fungus was capable to secrete significant levels of laccase and especially MnP₆₁₀ activities in

the fermentation of wheat straw. This observation is consistent with the results of Munir et al. [15] and Kachlishvili et al. [18]. The most important finding of this study is that *C. unicolor* 306 is capable to constitutively produce laccase and MnP in the control medium, although it is not excluded these enzymes production occurred due to the availability in the yeast extract of a pool of specific amino acids. On the contrary, *C. unicolor* 306 does not express LiP activity in the synthetic medium and an availability of lignocellulose in nutrient medium is a prerequisite for this production by the fungus.

Supplementation of all lignocellulosic materials to the control medium manifold improved the fungus laccase activity but with a different extent. It is evident that the growth substrates used have different chemical compositions and differ in aromatic compounds content that may be released to the liquid medium during sterilization and fungal growth. It is also possible that new aromatic compounds appeared during lignocellulose metabolism, enriching the pool of new LME inducers. Obviously, this circumstance is a main reason of significant increase of laccase activity in parallel with gradual increase of mandarin peels concentration. This finding is in good agreement with observations of other researchers. Thus, Zhao et al. [17] showed that the more orange peel was added to the culture of *Pleurotus ostreatus*, the better was the laccase production by this mushroom.

It is well known that the source and concentration of nitrogen in cultivation media affect LME production [8, 15, 19]. However, some studies have reported increased enzyme activity under non-limiting nitrogen conditions, while others reported opposite results [20, 21]. In this study, supplementation of the control medium with all additional nitrogen sources resulted in increase of *C. unicolor* 306 laccase activity. It can be assumed that the higher enzyme activity was simply due to a higher biomass production in the media supplemented with nitrogen sources. Nevertheless, unlike laccase, the fungus MnP activity decreased in the presence of inorganic salts added to the control medium at concentration of 20 mM as nitrogen. At the same time, organic nitrogen sources promoted the MnP accumulation. We can assume that these compounds contain aromatic amino acids which cause stimulating effect on the enzyme synthesis. It is worth noting that supplementation of the control medium with organic nitrogen source and KNO₃ favored the increase of the media pH and it is possible that this circumstance

in turn favored the enzyme secretion by the tested fungus. However, this hypothesis need testing.

Finally, based on the data obtained in this study and several approaches, LME production by *C. unicolor* 306 was performed in 7 L laboratory fermenter indicating that these enzymes production with a high yield can be successfully scaled up for different biotechnological applications. Undoubtedly, *C. unicolor* 306 is a good candidate for these purposes. However, more detailed information on regulation of each individual LME synthesis by this fungal species is required for the development of cost-effective production and application technologies.

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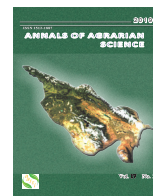
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Typology and distribution regularities of oriental hornbeam shrubberies (*Carpineta orientalis*) in Tbilisi environs

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ABSTRACT

Oriental hornbeam formation (*Carpineta orientalis*) of Tbilisi environs is studied for the first time. Oriental hornbeam shrubbery is one of the characteristic and prevalent formations of Tbilisi environs. Tbilisi environs area of oriental hornbeam formation mostly include foothills and lower mountain belt at about 600 to 1000(1100) m above s.l., rarely is in middle mountain belt (1100-1300 m above s.l.). Its plant communities with different plots area are fragmentary spread mostly on Saguramo-Ialno ridge and east endings of Trialeti ridge; they also meet on Skhaltba low range. Plant communities are developed on slopes with various exposure and inclination, on the cinnamonic and brown forest soils. Sometimes soil is thin and skeleton, rarely with bare mother rocks. Formation is characterised by rich typological composition. 8 plant communities were identified by us: (1) Querceto-Carpinetum graminoso-mixtoherbosum, (2) Carpinetum graminoso-mixtoherbosum, (3) Carpinetum mixtoherbosum, (4) Carpinetum poosum nemoralis, (5) Carpinetum pooso-caricosum, (6) Carpinetum caricosum humilis, (7) Carpinetum caricosum digitatae, (8) Carpinetum ruscosum. Their phytocoenological characteristics are presented. Geo-botanical descriptions are represented for each community in the form of consolidated table, in which are given general geo-botanical characteristics (general projective coverage, sodding degree, density, projective coverage, distribution and average height of each layer, floristic composition, coenetic role of each species - projective coverage, and etc.). Physical-geographical conditions (altitude, relief, exposure, inclination) are also given. In Tbilisi environs oriental hornbeam plant communities are secondary origin and derived as a result of digressive successions of oak forest (*Querceta iberici*).

Keywords: *Carpinus orientalis*, Plant community, Typology, Geo-botanical characteristics, Xeromesophilous shrubberies, Tbilisi environs.

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Introduction

Oriental hornbeam formation (*Carpineta orientalis*) relates to xeromesophilous shrubberies. Oriental hornbeam shrubbery is one of the characteristic and prevalent formations of Tbilisi environs [1, 2]. They mostly are secondary origins and derived as a result of digressive successions of oak forest (*Querceta iberici*). They relate to I stage of post-forest vegetation succession [3, 4]. At the same time, oriental hornbeam formation distributed in the other regions of South Caucasus [5-7, and etc.]. In spite of this, literary data is very scanty – only typological composition is given in Lachashvili et al. [1].

Objectives and Methods

The object of research is oriental hornbeam formation (*Carpineta orientalis*) of Tbilisi environs. The aim of our research was to establish typological composition and distributed regularities of oriental hornbeam formation in Tbilisi environs; determine phytosociological structure for each distinguished plant communities which would be reflected in the consolidated geo-botanical tables.

Phytosociological data was obtained by the route method in long period (2008-2015). 70 geo-botanical surveys (releve) were made. Geo-botanical

surveys were carrying out on 25 m² plots. During the geo-botanical surveys, studying the structure of phytocoenoses and identification of syntaxa, we were guided by the traditional geo-botanical methods [8-14]. Instead of the term “association” that is observed in soviet literature, we use the term “plant community” that is recognized through the Europe.

Life forms of the plants are separated on the basis of C. Raunkiaer [15] and I. Serebriakov classifications [16].

Soil types are founded on the modern classifications [17-19].

Results and Analysis

1. Areal and short physical-geographical characteristics

This formation is one of the characteristic for vegetation cover of Tbilisi surroundings. In Tbilisi environs oriental hornbeam communities are distributed on Saguramo-Ialno ridge and east endings of Trialeti ridge. Their altitudinal range is from 500 to 1200 (1300) m above s.l., however, the main area is in foothills and lower mountain belt, approximately 600-1000 m above s.l..

Oriental hornbeam communities in Tbilisi environs mainly are spread in moderately humid climate with moderately warm long summer and moderately cold snowy winter. In the distribution area of oriental hornbeam formation average annual temperature is 7.4°-11°C; mean annual precipitation is 550-800 (900) mm; precipitation-evaporation ratio is within the range 1 [20, 21].

Oriental hornbeam communities are developed on the cinnamonic and brown forest soils. Sometimes soil is thin and skeleton, rarely with bare mother rocks.

2. Typological composition and geo-botanical characteristic

Oriental hornbeam shrubberies are distinguished by typological variety. In Tbilisi environs within formation 8 plant communities were identified by us. They are: (1) Querceto-Carpinetum graminoso-mixtoherbosum, (2) Carpinetum graminoso-mixtoherbosum, (3) Carpinetum mixtoherbosum, (4) Carpinetum poosum nemoralis, (5) Carpinetum pooso-caricosum, (6) Carpinetum caricosum humilis, (7) Carpinetum caricosum digitatae, (8) Carpinetum ruscosum.

The distribution area in the environs of Tbilisi and consolidated geo-botanical tables for each distinguished plant communities are given below.

Abbreviations:

m – meter cm – centimeter s. – specimen

N – North S – South W – West

Th – Therophyte H – Hemicryptophyte

G – Geophyte Ch – Chamaephyte

Ph – Phanerophyte

1. Querceto-Carpinetum graminoso-mixtoherbosum

This plant community belongs to first stage of digressive successive of oak forests. They are characterized by the participation of oaks in the arborescent stratum. Oaks density is within 0,1-0,2. In the composition of both shrubs and grasses are good preserved forests species which make the core of floristic composition. However, floristic composition is enriched with non-characteristic species of forests.

Distribution in Tbilisi environs: Teleti, Mamadaviti and Mskhaldidi ridges; Altitude (m): 950-1050; Topography: slopes; Exposure (macro): N-E, S, W; Exposure (micro): N-E, N-W, E; Inclination: 27°-35°, rarely 13°-15°; Soil: cinnamonic and brown forest;

Table 1. Querceto-Carpinetum graminoso-mixtoherbosum

<i>Cotoneaster morulus</i>	-	-	-	-	+	-	-	-
<i>Crataegus kyrtosyla</i>	2-3	+	-	-	-	-	-	1
<i>Euonymus leiophloeus</i>	+	-	-	-	+	-	+	-
<i>Euonymus verrucosus</i>	2-3	+	-	-	-	-	+	-

<i>Juniperus communis</i> subsp. <i>oblonga</i>	-	-	-	-	+	-	-	-
<i>Ligustrum vulgare</i>	+ 1c.	-	-	-	-	-	-	+
<i>Lonicera caprifolium</i>	+	-	-	+	+	-	+	-
<i>Lonicera caucasica</i>	1-2	+	+	-	-	-	-	+
<i>Swida australis</i>	+	+	+	-	-	-	+	-
Semishrubs & dwarf semishrubs (Ch)								
<i>Teucrium nuchense</i> (T. <i>chamaedrys</i> subsp. <i>nuchense</i>)	-	-	+	-	-	-	-	-
Perennial herbs (H)								
<i>Achillea bisserrata</i>	-	-	-	+	+	-	+	-
<i>Aegonychon purpurea-coeruleum</i>	-	-	+	-	-	-	+	-
<i>Albovia tripartita</i>	-	-	-	-	+	+	-	+
<i>Alliaria petiolata</i>	-	+	-	-	-	-	+	-
<i>Alyssum murale</i>	-	-	-	+	+	-	-	-
<i>Anthriscus nemorosa</i>	-	+	-	-	-	-	-	+
<i>Asplenium adiantum-nigrum</i>	-	-	-	-	+	-	-	-
<i>Asplenium trichomanes</i>	-	-	-	-	+	-	-	-
<i>Brachypodium sylvaticum</i>	+	-	-	-	-	-	+	-
<i>Campanula alliarifolia</i>	-	-	-	-	-	+	-	-
<i>Campanula rapunculoides</i>	+	+	0.5	2	+	1	2	+
<i>Carex digitata</i>	-	-	6-7	12	+	6-7	-	4-5
<i>Carex pallescens</i>	6	4	+	-	-	+	2	-
<i>Clinopodium vulgare</i>	-	-	4-5	4	+	-	1-2	-
<i>Cruciata laevipes</i>	+	-	-	-	-	-	-	-
<i>Dactylis glomerata</i>	-	-	+	+	+	+	-	+
<i>Dictamnus albus</i>	-	+	-	-	-	-	-	-
<i>Galium album</i>	-	-	1-2	5	+	+	1	-
<i>Galium spurium</i> (G. <i>vaillantii</i>)	-	-	+	5	+	-	1	-
<i>Galium tricorutum</i>	-	-	+	+	+	-	-	+
<i>Hieracium auriculatum</i>	-	-	-	2	+	1-2	-	+
<i>Hypericum perforatum</i>	-	-	+	-	-	-	-	-
<i>Lapsana grandiflora</i> (L. <i>communis</i> subsp. <i>grandiflora</i>)	-	-	-	-	-	+	+	-
<i>Lathyrus laxiflorus</i> (<i>Orobus hirsutus</i>)	+	-	-	-	-	-	+	-
<i>Lathyrus roseus</i>	-	+	-	-	+	-	-	+
<i>Myosotis sylvatica</i>	-	-	+	+	+	+	+	-
<i>Physospermum cornubiense</i>	1-2	0.5	+	-	-	-	-	+
<i>Poa nemoralis</i>	3-4	+	9-10	7	+	5-6	3-4	4-5
<i>Polypodium vulgare</i>	5	-	1	2	+	6-7	+	2-3

<i>Primula veris</i> subsp. <i>macrocalyx</i>	1	+	3	2	-	-	-	2
<i>Silene italica</i>	+	-	2-3	1	-	1	2-3	2
<i>Thalictrum collinum</i>	+	-	-	-	-	-	-	-
<i>Trifolium pratense</i>	-	-	+	-	-	+	-	-
<i>Turritis glabra</i>	-	-	+	+	+	+	+	-
<i>Veronica peduncularis</i>	-	0.5	3-4	4	+	1-2	3	2
<i>Vicia truncatula</i>	+	+	+	+	+	-	+	+
<i>Viola alba</i>	+	7-8	1	-	+	1	3-4	2
<i>Viola odorata</i>	-	1	-	-	-	-	-	-
<i>Viola reichenbachiana</i>	-	-	-	+	-	-	-	-
Perennial herbs (G)								
<i>Allium pseudoflavum</i>	-	-	-	+	+	-	-	-
<i>Epipactis helleborine</i>	-	-	+	-	-	+	-	-
<i>Orchis purpurea</i> subsp. <i>caucasica</i>	-	-	-	+	-	-	-	-
<i>Platanthera chlorantha</i>	-	-	-	+	+	-	-	+
<i>Polygonatum glaberrimum</i>	2	-	-	-	+	-	+	-
<i>Sedum maximum</i> subsp. <i>ruprechtii</i> (<i>S. caucasicum</i>)	-	-	-	-	+	-	-	-
Annual plants (Th)								
<i>Alyssum alyssoides</i>	-	-	+	-	-	-	-	-
<i>Arabis nova</i> (<i>A. auriculata</i>)	-	-	-	-	+	-	+	-
<i>Asperula arvensis</i>	-	-	+	+	-	-	-	+
<i>Lapsana communis</i>	-	-	3	-	-	-	-	+
<i>Moehringia trinervia</i>	-	-	+	+	-	-	+	-
<i>Sedum hispanicum</i>	-	-	+	-	+	-	-	-
<i>Thlaspi orbiculatum</i>	-	-	+	+	+	+	+	+
<i>Torilis japonica</i>	-	-	+	+	+	-	-	+
<i>Trifolium aureum</i>	-	-	-	-	-	+	-	-

2. *Carpinetum graminoso-mixtoherbosum*

Plant community is represented by two ecological variant. First variant subsequently of digressive succession of forests were formed early case and now mostly is created with low height (2,5-3,5 m) oriental hornbeams. 4-4,5 m height plant communities are rare. Structural characteristics of forests in their structure are noticeably decreased – floristic composition both shrubs and grasses are much reduced. Floristic composition is enriched with non-characteristic species of forests.

The second variant was formed relatively recently and therein forest structure is good preserved. Average height of their arborescent stratum is considerably high – mostly within 6-7(8) m. Characteristic

species of forests make the basis of floristic composition. These plant communities are one of first stage of digressive successive of oak forests [3, 4]. Because of such important structural differences, their geo-botanical tables are given severally. First variant is met relatively frequently.

First variant

Distribution in Tbilisi environs: foothills of Armazi, Skhaltba and Saguramo ridges; Altitude (m): 500-800; Topography: slopes; Exposure (macro): S, N, W; Exposure (micro): S-W, N, W, N-W; Inclination: 1°-2° to 35°-37°, rarely 1°-7°; Soil: cinnamonic and brown forest;

Table 2. *Carpinetum graminoso-mixtoherbosum* (first variant)

Surveys	1	2	3	4	5	6	7	8	9	10
Date	14.07.10.	03.07.11.	06.07.11.	06.07.11.	06.07.11.	15.07.11.	15.07.11.	15.07.11.	17.07.13.	10.07.14.
Altitude (m)	560	765	528	546	562	685	683	680	655	705
Exposure (macro)	S	S	W	W	W	N	N	N	N	W
Exposure (micro)	S-W	W	W	N-W	W	N	N	N	N	W
Inclination	30°-33°	7°	35°	35°-37°	35°-36°	2°	1°-2°	2°	20°-22°	10°-12°
I layer (Arborescent stratum)										
Density of canopy	0,75-0,8	0,8-0,9	0,8	0,75-0,8	0,75-0,8	90	75-80	80-85	0,8-0,9	0,9
Average height (m)	2,5	4-4,5	2,2	3	2,5	2,5-3	2,5-3	2,7-3	2,5	2,8-3
Maximum height (m)	3	5	3	3,5	3,3	4	3,5	4	3,5	3,5
II layer (Grass cover)										
Projective coverage (%)	9-10	3-5	7-8	9-10	8-10	15-17	20	15-17	6-7	5-6
Average height (cm)	15-17	12-15	20	17-20	17-20	15	22-25	20	18-20	16-18
III layer (Moss cover)										
Projective coverage (%)	+	-	-	+	+	+	+	+	-	+
Litter										
Projective coverage (%)	92-95	95	85-90	90-95	90-95	90-95	90	90-95	87-90	90-95
Depth (cm)	2-3	1-5	2-3	2-3	3-5	5	4-5	5	3-4	3
Floristic composition										
Trees (Ph)										
<i>Quercus iberica</i>	+1 s.	-	-	+1 s.	+1 s.	-	+ 2 s.	+1 s.	-	+1 s.
Shrubs (Ph)										
<i>Carpinus orientalis</i>	75-80	80-90	80	75-80	75-80	90	75	75-80	80-90	90
<i>Cotoneaster morulus</i>	+	-	+	+	-	-	-	-	-	-
<i>Prunus divaricata</i>	-	+	-	-	-	-	-	-	-	-
<i>Rosa canina</i>	-	-	-	-	-	+	-	-	-	+
<i>Swida australis</i>	-	+	-	-	-	-	-	-	+	-
Perennial herbs (H)										
<i>Aegonychon purpurea-coeruleum</i>	2	+	-	-	-	1-2	+	1-2	1	+
<i>Carex digitata</i>	-	-	-	-	-	1	2-3	-	-	-
<i>Carex halleriana</i>	-	-	+	+	+	-	-	-	-	-
<i>Carex humilis</i>	-	+	-	-	-	-	-	-	-	+
<i>Clinopodium vulgare</i>	+	-	-	-	-	-	-	-	+	-
<i>Dactylis glomerata</i>		-	4	4	4-5	-	-	+		
<i>Dictamnus albus</i>		+	-	-	-	-	-	-		
<i>Filipendula vulgaris</i>	-	-	-	-	-	-	-	-	+	-
<i>Galium album</i>	+	-	+	+	+	-	-	-	+	-
<i>Klasea quinquefolia</i>	-	+	-	-	-	-	-	-	-	-
<i>Melica uniflora</i>	1	-	-	-	-	7	7-8	5	-	-
<i>Myosotis sylvatica</i>	+	-	+	-	+	-	-	-	-	-
<i>Poa nemoralis</i>	-	0,5	-	-	-	-	6-7	+		2
<i>Psephellus carthalinicus</i>	-	-	+	-	-	-	-	-	-	+

<i>Pyrethrum corymbosum</i>	-	+	-	-	-	-	-	-	+	-
<i>Silene italica</i>	4-5	-	4	6-7	5-6	2	+	3	3-4	2-3
<i>Vincetoxicum amplifolium</i> (V. <i>scandens</i>)	+	-	-	-	-	-	+	+	-	-
<i>Viola alba</i>	2	1	+	+	+	6-7	5	6	2-3	1
Perennial herbs (G)										
<i>Cephalanthera damasonium</i>	-	+ 1 s.	-	-	-	-	-	-	-	-
<i>Muscari szovitsianum</i>	+	-	-	-	-	+	-	+	-	-
Biannual plants (H)										
<i>Campanula sibirica</i> subsp. <i>hohenackeri</i>	+ 1 s.	-	-	-	-	-	-	-	-	-
Annual plants (Th)										
<i>Asperula arvensis</i>	-	-	-	+	+	-	-	-	-	+
				1-2 s.	2 s.					
<i>Bromus japonicus</i>	-	-	+	+	+	-	-	-	-	-

Second variant

Distribution in Tbilisi environs: Mamadaviti, Saguramo ridge (surroundings of vil. Tsitsamuri) and Mskhaldidi-Lisi ridges (surroundings of vil. Tsod-

oreti); **Altitude (m):** 600-1020; **Topography:** slopes, rarely plane place; **Exposure (macro):** N, E, S-W; **Exposure (micro):** N-E, N-W, N, S-W; **Inclination:** 13°-15° to 20°-35°, rarely 3°-4°; **Soil:** cinnamonic and brown forest;

Table 3. *Carpinetum graminoso-mixtoherbosum* (second variant)

Surveys	1	2	3	4	5	6	7
Date	12.07.08.	15.07.08.	15.07.08.	07.07.09.	07.07.09.	16.07.11.	11.07.15.
Altitude (m)	810	888	928	996	1007	620	780
Exposure (macro)	N	N	N	E	N	S-W	N
Exposure (micro)	N-W	N	N	N-E	N-E	S-W	N-W
Inclination	25°	13°-15°	15°-17°	20°	32°-35°	3°-4°	18°-20°
I layer (Arborescent stratum)							
Density of canopy	0.7-0.75	0.7	0.75	0.6	0.5-0.6	0.7	0,6-0,7
Average height (m)	6	6	4-5	6-7	4,5-5	8	6-7
Maximum height (m)	7	7	6	8	6,5	9	8
II layer (Grass cover)							
Projective coverage (%)	3-5	2-3	3-5	45	18-20	20	14-16
Average height (cm)	17-20	20	15	30	32-37	20	20-22
III layer (Moss cover)							
Projective coverage (%)	+	-	2-3	-	-	+	+
Moss							
Projective coverage (%)	+	-	2-3	-	-	-	+
Lichen							
Projective coverage (%)	+	-	+	-	-	-	-
Litter							
Projective coverage (%)	95-96	95-97	75	92-93	90-92	90	88-90
Depth (cm)	2-4	2-6	1-3	2-4	2-4	2-3	1-3

Floristic composition							
Trees (Ph)							
<i>Acer cappadocicum</i>	-	-	-	-	+ 1s.	-	-
<i>Fraxinus excelsior</i>	-	-	-	+	+ 1s.	-	+ 1s.
<i>Quercus iberica</i>	-	+	-	-	+	-	+ 1s.
<i>Sorbus torminalis</i>	-	-	-	+	-	-	-
Shrubs (Ph)							
<i>Carpinus orientalis</i>	70-75	70	70	60	50-60	70	60-70
<i>Cornus mas</i>	+	-	-	+	-	+	+
<i>Cotoneaster morulus</i>	+ 1 s.	-	+ 1 s.	-	-	-	-
<i>Crataegus kyrtostyla</i>	+	+	+	+	-	+	+
<i>Euonymus leiophloeus</i>	-	-	-	-	+	-	-
<i>Euonymus verrucosus</i>	-	15-16	7-8	+	-	+	+
<i>Hedera helix</i>	-	-	-	-	-	+	-
<i>Ligustrum vulgare</i>	3	+	-	-	-	+	+
<i>Lonicera caprifolium</i>	2	15-16	1	2	3	20	3-4
<i>Lonicera caucasica</i>	-	-	-	+	-	+	+
<i>Prunus divaricata</i>	+ 1 s.	-	-	-	-	-	+ 1 s.
<i>Rhamnus cathartica</i>	-	-	+	-	-	-	-
<i>Swida australis</i>	-	+	-	+	-	+	-
<i>Viburnum lantana</i>	-	-	-	-	+	-	-
Perennial herbs (H)							
<i>Achillea biserrata</i>	-	-	-	-	-	1-2	-
<i>Aegonychon purpurea-coeruleum</i>	-	+	-	-	-	+	-
<i>Albovia tripartita</i>	-	-	-	1-2	7-8	-	3-4
<i>Alliaria petiolata</i>	+	+	+	+	6-7	-	2-3
<i>Asplenium adiantum-nigrum</i>	-	-	-	+	-	-	+
<i>Asplenium trichomanes</i>	-	-	-	+	-	-	-
<i>Brachypodium sylvaticum</i>	-	-	-	-	-	2	-
<i>Campanula rapunculoides</i>	-	+	+	4	+	+	1-2
<i>Carex humilis</i>	1	-	+	-	-	-	-
<i>Carex pallescens</i>	+	+	+	-	-	-	+
<i>Clinopodium vulgare</i>	+	-	-	+	-	+	-
<i>Cruciata laevipes</i>	-	-	+	-	-	-	-
<i>Festuca drymaja</i>	-	-	-	+	-	+	-
<i>Filipendula vulgaris</i>	+ 1 s.	-	-	-	-	-	-
<i>Galium spurium (G. vaillantii)</i>	-	-	-	-	5	-	-
<i>Geum urbanum</i>	-	+	+	+	-	+	+
<i>Helleborus caucasicus</i>	-	-	-	1	+	-	-
<i>Hypericum perforatum</i>	-	-	-	+	-	-	-
<i>Klasea quinquefolia</i>	-	-	-	+	-	2-3	1-2
<i>Laser trilobum</i>	-	-	-	+	-	+	+
<i>Lathyrus laxiflorus (Orobis hirsutus)</i>	-	-	-	+	-	8-9	-
<i>Luzula multiflora</i>	-	-	-	-	+	-	-
<i>Melica picta</i>	-	-	-	-	-	+	+
<i>Melica uniflora</i>	-	-	-	4	-	8	-

<i>Physospermum cornubiense</i>	-	-	-	16	-	+	1
<i>Poa nemoralis</i>	1	+	+	17	3-4	-	-
<i>Polypodium vulgare</i>	+	-	+	+	+	-	+
<i>Primula veris subsp. macrocalyx</i>	-	+	+	6	-	+	2-3
<i>Primula woronowii</i>	-	-	-	+	-	-	+
<i>Pyrethrum corymbosum</i>	-	+	-	+	-	-	-
<i>Seseli peucedanoides</i>	-	-	-	+	-	-	-
<i>Solidago virgaurea</i>	-	-	-	+	-	+	-
<i>Thalictrum collinum</i>	+ 1 s.	+	+	-	-	-	-
<i>Valeriana officinalis</i>	-	-	-	+	-	-	-
<i>Veronica peduncularis</i>	-	-	-	1	+	-	+
<i>Vicia truncatula</i>	-	+	-	+	+	-	-
<i>Vincetoxicum amplifolium</i> (V. scandens)	-	-	-	-	-	+	-
<i>Viola alba</i>	1-2	+	1	2-3	+	+	3
<i>Viola odorata</i>	-	+	+	-	-	-	+
<i>Viola reichenbachiana</i>	-	-	-	-	+	+	-
Perennial herbs (G)							
<i>Cephalanthera rubra</i>	-	-	-	-	-	+	-
<i>Dioscorea communis</i> (<i>Tamus communis</i>)	+ 1 s.	-	-	-	-	-	+
<i>Platanthera chlorantha</i>	-	-	-	-	+	-	+
<i>Polygonatum glaberrimum</i>	-	-	+	2	2-3	+	1
Biennial plants (H)							
<i>Campanula sibirica subsp. hohenackeri</i>	+ 1 s.	-	-	-	-	-	-
<i>Lactuca quercina subsp. wilhelmsiana</i>	-	-	-	-	+	-	-
Annual plants (Th)							
<i>Thlaspi orbiculatum</i>	-	-	+	+	+	-	+
<i>Geranium lucidum</i>	-	-	+	+	3-4	-	-
<i>Geranium robertianum</i>	-	-	+	-	-	-	-

3. *Carpinetum mixtoherbosum*

Plant community is very rare and has limited distribution area.

Distribution in Tbilisi environs: foothills of Sa-

guramo ridge (surroundings of vil. Tsitsamuri); Altitude (m): 600-700; Topography: slopes, rarely plane place; Exposure (macro): N; Exposure (micro): N, N-W; Inclination: 20°-30°, rarely 1°-2° to 8°; Soil: cinnamonic and brown forest;

Table 4. *Carpinetum mixtoherbosum*

Surveys	1	2	3	4	5
Date	16.07.11.	16.07.11.	16.07.11.	16.07.11.	16.07.11.
Altitude (m)	615	649	657	667	633
Exposure (macro)	N	N	N	N	N
Exposure (micro)	N	N-W	N	N	N-W
Inclination	30°	18°-20°	1°-2°	20°-25°	25°-27°
I layer (Arborescent stratum)					
Density of canopy	0.6-0.7	0.7-0.75	0.7-0.75	0.7	0,7
Average height (m)	6	5-7	5-6	7	6-7
Maximum height (m)	9	8	7	8	8
II layer (Grass cover)					
Projective coverage (%)	20	22-25	12	6-7	15-17
Average height (cm)	17-20	22-25	17-20	20	18-20
III layer (Moss cover))					
Projective coverage (%)	2-3	+	-	-	+
Litter					
Projective coverage (%)	75-80	80	90	95	85
Depth (cm)	2-3	3-4	3-5	3-5	3-5
floristuli Semadgenloba					
Trees (Ph)					
<i>Quercus iberica</i>	-	-	+ 1 s.	-	+ 1 s.
<i>Sorbus torminalis</i>	1	-	-	-	-
Shrubs (Ph)					
<i>Carpinus orientalis</i>	60-70	70-75	70-75	70	70
<i>Citrus caucasicus</i>	+	-	-	-	-
<i>Cornus mas</i>	+	15	1	+	+
<i>Crataegus kyrtostyla</i>	+	-	+	-	+
<i>Euonymus europaeus</i>	+	+	-	-	-
<i>Euonymus verrucosus</i>	-	+	-	-	+
<i>Hedera helix</i>	-	+	-	-	-
<i>Ligustrum vulgare</i>	-	-	+	-	+
<i>Lonicera caprifolium</i>	4-5	15	+	+	+
<i>Lonicera caucasica</i>	-	+	-	-	+
<i>Mespilus germanica</i>	+	-	-	-	-
<i>Ruscus aculeatus</i>	+	-	-	-	-
<i>Swida australis</i>	-	-	5	3-5	-
Perennial herbs (H)					
<i>Achillea biserrata</i>	+	+	+	+	+
<i>Campanula rapunculoides</i>	3	2	+	+	2-3
<i>Carex digitata</i>	+	+	+	+	+
<i>Carex humilis</i>	3-4	-	-	-	-
<i>Klasea quinquefolia</i>	-	12-15	7	+	4-5
<i>Laser trilobum</i>	1-2	+	-	-	1-2
<i>Lathyrus laxiflorus</i> (<i>Orobus hirsutus</i>)	1-2	-	+	-	-
<i>Lathyrus roseus</i>	1-2	-	-	-	1
<i>Melica uniflora</i>	+	+	+	+	+
<i>Physospermum cornubiense</i>	+	+	+	3	1-2
<i>Primula veris</i> subsp. <i>macrocalyx</i>	6-7	+	1	1	2-3
<i>Pyrethrum corymbosum</i>	-	-	-	+	-
<i>Vicia truncatula</i>	-	+	+	-	+
<i>Vincetoxicum amplifolium</i> (<i>V. scandens</i>)	-	+	+	+	+
<i>Viola alba</i>	2	4-5	+	2	4
<i>Viola reichenbachiana</i>	-	3-5	5-6	3	-
Perennial herbs (G)					
<i>Cephalanthera rubra</i>	-	-	-	+	-
<i>Helleborus caucasicus</i>	-	+	-	-	-
<i>Polygonatum glaberrimum</i>	3	-	-	-	+

4. *Carpinetum poosum nemoralis*

This plant community is one of more widely distributed.

Distribution in Tbilisi environs: TeleTi ridge (surroundings of vil. Tabakhmela), Mamadaviti

ridge (surroundings of Lake Kustba and vil. Tsavkisi), Mskhaldidi ridge (surroundings of vil. Tsodreti) and etc.; Altitude (m): 800-1400; Topography: slopes; Exposure (macro): N, S, S-E, W; Exposure (micro): N, N-E, S-E, E, W; Inclination: 13°-15° to 30°-35°; Soil: cinnamonic and brown forest;

Table 5. *Carpinetum poosum nemoralis*

Surveys	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Date	12.07.08.	13.07.08.	13.07.08.	16.07.08.	16.07.08.	16.07.08.	19.07.08.	19.07.08.	19.07.08.	08.07.09.	08.07.09.	08.07.09.	06.07.09.	06.07.09.
Altitude (m)	808	810	877	1360	1236	1233	1038	1035	1022	959	967	970	995	1005
Exposure (macro)	N	N	N	S-E	S-E	S-E	S	S	S	N	N	N	W	W
Exposure (micro)	N	N-E	N	S-E	S-E	S-E	E	E	E	N	N	N	W	W
Inclination	30-32°	20-22°	25-27°	18°-20°	14°-15°	13°-15°	16°-18°	15°-16°	15°	25°	20-22°	18°-20°	28-30°	25°
I layer (Arborescent stratum)														
Density of canopy	0.7-0.75	0.6	0.7	0.7	0.6	0.75-0.8	0.8	0.8	0.8	0.5	0.5-0.6	0.45-0.5	0.7-0.8	0.7-0.8
Average height (m)	6	5-6	5	7-8	5-6	5-6	3-4	3-4	4-5	3-4	4-5	4-5	5	5-6
Maximum height (m)	7	8	6	10	7	7-8	5	6	6,5	4,5	6,5	5,5	6	7
II layer (Grass cover)														
Projective coverage (%)	20	5-6	7-8	60	52-55	38-40	10	48-50	40	50-52	35-38	33-35	37-40	25-28
Average height (cm)	23-28	23-28	23-28	25-30	22-25	25-30	20	30-35	32-37	35-40	40-45	45-50	42-47	42-47
III layer (Moss cover)														
Projective coverage (%)	+	+	-	-	+	-	-	-	-	1-2	-	-	3-5	5-6
Moss														
Projective coverage (%)	+	+	-	-	+	-	-	-	-	1-2	-	-	3-5	5-6
Lichen														
Projective coverage (%)	+	+	-	-	-	-	-	-	-	-	-	-	+	-
Litter														
Projective coverage (%)	70	95-96	95-96	90-95	80-85	92-93	75-80	80	80-85	92-95	85-90	90	75-80	80-85
Depth (cm)	1-2	2-3-5	2-4-6	2-4-5	2-3-5	2-4	1-3	1-3	1-3	5-8	3-5	3-5	1-3	3-4
Floristic composition														
Trees (Ph)														
<i>Carpinus caucasica</i>	-	-	-	+1 s.	+1 s.	-	-	-	-	+	-	-	-	-
<i>Fraxinus excelsior</i>	-	-	-	-	-	-	-	-	-	+1 s.	-	+	-	-
<i>Pyrus caucasica</i>	-	-	-	-	-	-	+1 s.	-	10 s.	-	-	-	-	-
<i>Quercus iberica</i>	-	-	+	-	+	-	+1 s.	-	15	+	+1 s.	-	0.2	0.2
Shrubs (Ph)														

<i>Carpinus orientalis</i>	70-75	50	70	70	60	75-80	65-70	65-70	45-50	40-50	50-60	40-50	05.-06.	70
<i>Cornus mas</i>	-	+	-	-	-	+	+	8	5	-	-	-	-	-
<i>Cotoneaster morulus</i>	-	-	+	-	-	-	+	-	-	-	-	-	-	-
<i>Crataegus kyrtostila</i>	-	+	-	-	+	+ 1 s.	5-7	4-5	5	-	+	-	-	-
<i>Euonymus leiophloa</i>	+	-	-	-	-	-	-	-	-	+	+	-	-	+
<i>Euonymus verrucosus</i>	-	+	+	-	-	-	-	+	-	+	-	-	-	-
<i>Juniperus oblonga</i>	-	-	-	-	-	-	-	-	-	+	+	-	-	-
<i>Ligustrum vulgare</i>	-	-	+	-	-	-	-	+	-	+	-	-	-	-
<i>Lonicera caprifolia</i>	-	13-15	-	+	+	-	2-3	+	-	15-20	+	9-10	-	+
<i>Lonicera caucasica</i>	-	-	30-32	+	-	-	+	-	+	-	-	-	-	+
<i>Mespilus germanica</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Prunus divaricata</i>	-	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>Rhamnus cathartica</i>	-	-	+ 1 s.	-	-	-	-	-	-	-	-	-	-	-
<i>Rosa canina</i>	-	-	-	-	+	-	-	-	+	-	-	-	-	-
<i>Swida australis</i>	-	10	-	-	-	-	5-7	-	+	-	-	-	-	+
<i>Viburnum lantana</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Perennial herbs (H)														
<i>Achillea biserrata</i>	-	-	-	3	-	-	-	-	-	+	+	+	+	+
<i>Aegonychon purpurea-coeruleum</i>	-	+	-	-	-	-	-	-	-	-	+	-	-	-
<i>Albovia tripartita</i>	-	-	-	4	-	-	-	-	-	-	1	+	+	3-5
<i>Alliaria petiolata</i>	2-3	-	1	+	-	-	+	+	-	+	-	-	-	-
<i>Anthriscus nemorosa</i>	-	-	-	+	-	-	+	-	-	-	-	-	-	-
<i>Asplenium adiantum-nigrum</i>	-	-	-	-	-	-	-	-	-	-	+	-	-	+
<i>Asplenium trichomanes</i>	-	-	-	-	-	-	-	-	-	-	+	-	+	+
<i>Brachypodium sylvaticum</i>	-	+	-	6-7	-	-	+	-	-	-	-	-	-	-
<i>Campanula rapunculoides</i>	1	-	+	+	+	0.5	+	3-4	1	+	1-2	4	3	4-5
<i>Carex digitata</i>	-	-	-	-	-	-	-	-	-	+	-	-	2-3	+
<i>Carex humilis</i>	2-3	-	0.5	-	-	1	-	-	-	-	-	-	-	-
<i>Carex pallescens</i>	-	+	-	-	1-2	+	0.5	5	1	+	1	+	2	+
<i>Clinopodium vulgare</i>	-	-	-	+	-	+	0.5	-	-	-	+	-	1	1
<i>Cruciata laevipes</i>	-	+	-	+	+	-	-	-	-	-	-	-	-	-
<i>Dactylis glomerata</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
<i>Dictamnus albus</i>	-	-	-	-	-	-	-	+	-	-	-	-	-	-
<i>Fragaria vesca</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-
<i>Galium album</i>	-	-	-	-	-	-	-	-	-	+	-	-	1-2	-
<i>Galium humifusum</i>	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Galium rivale</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Galium tricorntum</i>	-	-	-	-	-	-	-	-	-	8-9	1	-	+	1
<i>Geum urbanum</i>	+ 1 s.	+	-	+	-	-	-	+	-	-	-	-	-	-
<i>Lapsana grandiflora (L. communis subsp. grandiflora)</i>	-	-	-	+	-	-	-	-	-	-	-	-	-	-
<i>Laser trilobum</i>	-	-	-	-	-	-	-	-	-	-	-	+	-	+
<i>Lathyrus laxiflorus (Orobis hirsutus)</i>	-	-	-	1	-	-	-	-	-	-	-	-	-	+

<i>Lathyrus roseus</i>	-	-	-	-	-	-	+	3-4	18-20	-	+	-	-	+
<i>Melica picta</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>Melica uniflora</i>	-	-	-	-	-	-	-	-	-	+	2	+	-	-
<i>Physospermum cornubiense</i>	-	+	-	-	+	1	+	+	+	6-7	6	5	-	+
<i>Poa nemoralis</i>	12-13	4	5	40	50-52	33-35	6-7	40	17-18	25	22-24	20-22	22-25	12-15
<i>Polypodium vulgare</i>	1	-	2	-	-	-	-	-	-	5	2	-	1	+
<i>Primula veris</i> subsp. <i>macrocalyx</i>	1	-	+	1	-	-	1	+	1-2	+	1	+	1-2	+
<i>Pyrethrum corymbosum</i>	-	+	+	-	-	-	-	-	-	+	+	-	-	-
<i>Silene italica</i>	-	-	-	-	+	-	1	+	-	1-2	+	+	-	-
<i>Stachys iberica</i>	-	-	-	-	+	-	-	-	-	-	-	-	-	-
<i>Thalictrum collinum</i>	+ 1c.	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Turritis glabra</i>	-	-	-	-	-	-	-	-	-	+	+	+	-	-
<i>Valeriana officinalis</i>	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<i>Veronica peduncularis</i>	-	-	-	3	+	3	3-4	+	0.5	+	-	-	7-8	3-5
<i>Vicia truncatula</i>	-	-	+	-	+	+	+	+	+	+	+	+	-	+
<i>Vincetoxicum amplifolium</i> (<i>V. scandens</i>)	-	-	-	-	-	-	-	-	+	-	-	-	-	-
<i>Viola alba</i>	+	-	0.5	4-5	+	4	+	+	0.5	+	-	1	-	-
<i>Viola odorata</i>	-	+	-	-	-	-	+	+	-	+	+	-	2	+
<i>Viola reichenbachiana</i>	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Perennial herbs (G)														
<i>Helleborus caucasicus</i>	-	-	-	-	-	-	-	-	-	-	+	-	-	-
<i>Polygonatum glaberrimum</i>	-	-	-	2	2-3	-	-	-	+ 1 s.	7	1	7-8	1	4-5
<i>Sedum maximum</i> subsp. <i>ruprechtii</i> (<i>S. caucasicum</i>)	+	-	-	-	-	-	-	-	-	-	+	-	-	-
Biennial plants (H)														
<i>Lactuca quercina</i> subsp. <i>wilhelmsiana</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Annual plants (Th)														
<i>Arabis nova</i> (<i>A. auriculata</i>)	-	-	-	-	-	-	-	-	-	-	-	-	+	+
<i>Geranium lucidum</i>	+	+	-	-	-	-	-	-	-	1	-	-	-	-
<i>Thlaspi orbiculatum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	+
<i>Torilis japonica</i>	-	-	-	+	-	-	-	-	-	-	-	-	+	+

5. *Carpinetum pooso-caricosum*

This plant community has relatively limited distribution area.

Distribution in Tbilisi environs: Mamadaviti and

TeleTi ridges; Altitude (m): 800-1050; Topography: slopes; Exposure (macro): N, rarely S; Exposure (micro): N, N-W, N-E, E, rarely S-E; Inclination: 13°-15° to 30°-32°; Soil: cinnamonic and brown forest;

Table 6. *Carpinetum mixtoherbosum*

Surveys	1	2	3	4	5	6	7	8
Date	12.07.08.	12.07.08.	12.07.08.	13.07.08.	13.07.08.	13.07.08.	19.07.08.	15.07.14.
Altitude (m)	810	816	813	810	822	848	1040	840
Exposure (macro)	N	N	N	N	N	N	S	N
Exposure (micro)	N-W	N	N	N-E	E	N-E	S-E	N
Inclination	°25°	22°-25°	30°-32°	17°-18°	13°-15°	25°-27°	22°-25°	20°-22°
I layer (Arborescent stratum)								
Density of canopy	0,6	0.6-0.7	0.7-0.75	0.7-0.75	0.6	0.7-0.75	0.6-0.7	0,7
Average height (m)	6	5-6	6-7	6	6-6.5	4-5	5-6	5-6
Maximum height (m)	7	8	7.5-8	8	8-9	6	7	7
II layer (Grass cover)								
Projective coverage (%)	45	20-22	28-30	8-10	33-35	36-38	10-12	23-25
Average height (cm)	20	22-25	25-30	17-20	25-28	22-25	25	20-22
III layer (Moss & lichen cover)								
Projective coverage (%)	+	-	+	-	+	-	-	+
Moss								
Projective coverage (%)	+	-	+	-	+	-	-	+
Lichen								
Projective coverage (%)	-	-	+	-	-	-	-	+
Litter								
Projective coverage (%)	80-85	85-90	85-90	90	90-92	85-90	90	85-88
Depth (cm)	1-3	1-2	2-3	2-4	1-3	3-5	2-3	1-2
Floristic composition								
Trees (Ph)								
<i>Quercus iberica</i>	-	+ 1 s.	+ 1 s.	+ 1 s.	-	+ 2 s.	-	+ 1 s.
Shrubs (Ph)								
<i>Carpinus orientalis</i>	60	60-70	70-75	70-75	60	70-75	60-65	70
<i>Cornus mas</i>	-	-	-	+	-	-	+	+
<i>Cotoneaster morulus</i>	+	+	+	-	+	-	+	-
<i>Crataegus kyrtostyla</i>	+	-	-	+	+	-	5-6	+
<i>Euonymus leiophloeus</i>	-	+	-	-	-	-	-	-
<i>Euonymus verrucosus</i>	+	-	-	-	+	+	-	+
<i>Jasminum fruticans</i>	-	-	+	-	+	-	+	-
<i>Ligustrum vulgare</i>	-	+	4-5	+	+ 1 s.	+	-	+
<i>Lonicera caprifolium</i>	+	-	5-6	4-5	+	+	-	+
<i>Rosa canina</i>	+ 1 s.	-	-	-	-	-	-	-
<i>Spiraea hypericifolia</i>	+ 1 s.	-	+	-	-	-	-	-
Perennial herbs (H)								
<i>Aegonychon purpurea-coeruleum</i>	-	+	-	+	+	-	-	+
<i>Albovia tripartita</i>	-	-	-	-	-	+	-	-
<i>Alliaria petiolata</i>	+	4	1-2	-	-	+	-	-
<i>Anthriscus nemorosa</i>	-	-	-	-	-	-	+	-

<i>Campanula rapunculoides</i>	-	-	+	-	-	+	1	+
<i>Carex humilis</i>	10	2-3	10-12	-	3-4	4	+	4-5
<i>Carex pallescens</i>	30	7-8	7-8	4-5	10-12	5	3-4	5-6
<i>Clinopodium vulgare</i>	2-3	-	-	-	-	-	0.5	-
<i>Cruciata laevipes</i>	-	-	-	-	+	+	-	-
<i>Dactylis glomerata</i>	-	-	-	-	+	-	+	-
<i>Dictamnus albus</i>	-	-	-	-	-	-	+	-
<i>Fragaria vesca</i>	-	-	-	-	-	-	+	-
<i>Galium spurium</i> (G. vaillantii)	-	-	-	-	-	-	+	-
<i>Geum urbanum</i>	+	-	+	-	+	+	+	-
<i>Klasea quinquefolia</i>	-	-	-	+	-	-	-	+
<i>Melica picta</i>	-	-	-	-	+	-	-	-
<i>Melica uniflora</i>	-	+ 1 s.	-	-	-	6-7	-	-
<i>Physospermum cornubiense</i>	-	-	-	+	-	-	-	+
<i>Poa nemoralis</i>	5	5	8-9	4-5	22-25	18-19	7-8	10-12
<i>Polypodium vulgare</i>	-	-	+	-	-	-	-	-
<i>Primula veris</i> subsp. <i>macrocalyx</i>	+	-	+	-	-	2-3	+	1
<i>Silene italica</i>	+	+	+	+	+	-	-	+
<i>Thalictrum collinum</i>	+	-	+	-	-	+	-	-
<i>Valeriana officinalis</i>	-	+	+	-	-	-	-	-
<i>Vicia truncatula</i>	-	-	-	+	-	-	-	-
<i>Vinca herbacea</i>	-	-	-	-	-	-	+	-
<i>Viola alba</i>	1-2	1	+	+	+	4	0.5	3
<i>Viola odorata</i>	-	-	-	+	+	+	+	-
Perennial herbs (G)								
<i>Polygonatum glaberrimum</i>	-	-	-	-	-	+	-	-
<i>Sedum maximum</i> subsp. <i>ruprechtii</i> (S. <i>caucasicum</i>)	+	-	-	-	-	+	-	-
Annual plants (Th)								
<i>Geranium lucidum</i>	-	2-3	2	-	+	+	-	+

6. *Carpinetum caricosum humilis*

Distribution in Tbilisi environs: Saguramo-Ialno and Mskhaldidi-Lisi ridges; Altitude (m): 600-1100;

Topography: slopes; Exposure (macro): S, N, E; Exposure (micro): S-W, N-W, E; Inclination: 15°-35°, rarely 8°-10°; Soil: cinnamonic and brown forest;

Table 6. *Carpinetum caricosum humilis*

Surveys	1	6	2	3	4	5	7
Date	16.07.11.	12.07.12.	16.07.12.	16.07.12.	16.07.12.	16.07.12.	14.07.14.
Altitude (m)	615	1096	850	868	875	878	1060
Exposure (macro)	N	E	S	S	S	S	S
Exposure (micro)	N-W	E	S-W	S-W	S-W	S	S-W
Inclination	15°-18°	8°-10°	25°	15°	30°-32°	25°-27°	20°-22°
I layer (Arborescent stratum)							
Density of canopy	0.7-0.8	0,5-0,6	0.6-0.7	0.7-0.75	0.7-0.75	0,5-0,6	0,7
Average height (m)	7	3,5-4	5-6	7-8	6	5	5-6
Maximum height (m)	9	5	7	9	7.5	6	7,5
II layer (Grass cover)							
Projective coverage (%)	30	35-40	35-40	45	40-42	37-40	35-38
Average height (cm)	17-20	22-25	22-25	17-22	22-25	25	20-23

III layer (Moss cover)							
Projective coverage (%)	+	-	1-2	-	-	-	+
Litter							
Projective coverage (%)	50	80	95	95	95	95-96	90
Depth (cm)	2-4	1-3	1-4	2-5	3-5	1-6	2-3
Floristic composition							
Trees (Ph)							
<i>Fraxinus excelsior</i>	+ 1 s.	-	+ 1 s.	+ 1 s.	0.1	0,1	+ 1 s.
<i>Quercus iberica</i>	-	-	0.1	-	+ 1 s.	-	-
<i>Sorbus torminalis</i>	+	-	-	-	-	-	+
<i>Ulmus minor</i>	-	-	-	-	+	-	-
Shrubs (Ph)							
<i>Carpinus orientalis</i>	70-80	50-60	60	70-75	70-75	50-60	70
<i>Berberis vulgaris</i>	-	-	+	+	+	-	-
<i>Cornus mas</i>	+	+	20	+	+	5	+
<i>Cotoneaster meyeri</i>	-	-	-	-	+	-	-
<i>Cotoneaster morulus</i>	+	+	-	-	-	-	-
<i>Crataegus kyrtostyla</i>	-	+	-	-	-	-	+
<i>Cytisus caucasicus</i>	-	-	-	+	-	-	-
<i>Euonymus europaeus</i>	-	-	+	-	-	-	-
<i>Euonymus verrucosus</i>	-	-	-	-	+	+	+
<i>Juniperus communis</i> <i>subsp. oblonga</i>	+	-	1-2	+	6	3	-
<i>Ligustrum vulgare</i>	-	-	3-4	+	+	5-6	+
<i>Lonicera caprifolium</i>	+	-	-	-	-	+	+
<i>Lonicera caucasica</i>	-	-	+	-	+	-	-
<i>Paliurus spina-christi</i>	-	-	-	+ 1 s.	+ 1 s.	-	-
<i>Viburnum lantana</i>	-	-	+	+	+	2-3	+
Semishrubs & dwarf semishrubs (Ch)							
<i>Teucrium nuchense</i> (<i>T. chamaedrys</i> subsp. <i>nuchense</i>)	-	+	-	-	-	-	-
Perennial herbs (H)							
<i>Achillea biserrata</i>	+	-	-	-	-	-	-
<i>Aegonychon purpurea-coeruleum</i>	+	+	4	3	1-2	+	1
<i>Albovia tripartita</i>	-	+	+	-	-	-	-
<i>Asplenium adiantum-nigrum</i>	+	-	-	-	-	-	-
<i>Brachypodium sylvaticum</i>	-	-	+	8	2	5	-
<i>Briza media</i>	-	+	-	-	-	-	-
<i>Campanula rapunculoides</i>	+	+	3-4	2	1	+	2-3
<i>Carex digitata</i>	+	-	-	1	-	1-2	-
<i>Carex flacca</i> subsp. <i>erythrostachys</i> (<i>C. cuspidata</i>)	-	-	+	+	1	-	+
<i>Carex humilis</i>	20	35	25	20-22	30	25-27	25-28
<i>Dactylis glomerata</i>	-	+	-	-	-	-	-
<i>Falcaria vulgaris</i>	-	+	-	-	-	-	-
<i>Fragaria vesca</i>	-	+	-	-	-	-	-
<i>Galium album</i>	-	-	+ 1 s.	+	-	-	+ 1 s.
<i>Galium humifusum</i>	+	+	-	-	-	+	-
<i>Klasea quinquefolia</i>	-	-	-	1	-	+	+
<i>Laser trilobum</i>	-	-	4-5	+	8	5-6	1-2
<i>Lathyrus laxiflorus</i> (<i>Orobanchus hirsutus</i>)	+	+	-	-	-	-	-
<i>Lathyrus roseus</i>	6	-	-	-	-	-	+
<i>Melica uniflora</i>	1	-	-	-	-	-	-

<i>Physospermum cornubiense</i>	+	-	+	6	+	+	2
<i>Poa nemoralis</i>	-	+	-	-	-	-	-
<i>Potentilla recta</i>	-	+	-	-	-	-	-
<i>Primula veris</i> subsp. <i>macrocalyx</i>	+	-	-	-	-	-	+
<i>Primula woronowii</i>	-	-	-	-	-	+	-
<i>Vincetoxicum amplifolium</i> (V. <i>scandens</i>)	-	-	-	+	+	+	+
<i>Viola alba</i>	-	+	2-3	5-6	1-2	7-8	3-4
Perennial herbs (G)							
<i>Cephalanthera damasonium</i>	-	+	+	1	-	-	-
<i>Cephalanthera rubra</i>	+	-	-	-	-	-	-
<i>Dioscorea communis</i> (<i>Tamus communis</i>)	-	-	+	+	-	-	-
<i>Limodorum abortivum</i>	-	-	+	-	-	-	-
<i>Polygonatum glaberrimum</i>	4	-	+	-	-	-	+

7. *Carpinetum caricosum digitatae*

This plant community is rare and has limited distribution area.

Distribution in Tbilisi environs: Skhaltba low

range and east foothills of Armazi ridge; Altitude (m): 650-800; Topography: slopes; Exposure (macro): W, N-W, N; Exposure (micro): W, N-W, N; Inclination: 5-15°; Soil: cinnamonic and brown forest;

Table 7. *Carpinetum caricosum digitatae*

Surveys	1	2	3	4
Date	03.07.11.	03.07.11.	03.07.11.	15.07.11.
Altitude (m)	756	762	764	684
Exposure (macro)	W	W	W, N-W	N
Exposure (micro)	W	W	N-W	N
Inclination	10°	7°	10°-12°	6°-7°
I layer (Arborescent stratum)				
Density of canopy	0,8	0,8-0,9	0,75-0,8	0,75
Average height (m)	4	5	4.5-5	2,5
Maximum height (m)	5	6	6	3
II layer (Grass cover)				
Projective coverage (%)	37-40	20	23-25	60-63
Average height (cm)	17-20	17-20	25	35
III layer (Moss & lichen cover)				
Projective coverage (%)	-	-	-	
Litter				
Projective coverage (%)	80	95	80	80
Depth (cm)	0,5-1,5	1-5	1-5	3,5
Floristic composition				
Trees (Ph)				
<i>Quercus iberica</i>	-	-	-	+
Shrubs (Ph)				
<i>Carpinus orientalis</i>	35-40	80-90	75-80	75
<i>Euonymus verrucosus</i>	-	-	+	-
<i>Juniperus foetidissima</i>	+	+	+	-
<i>Juniperus oxycedrus</i>	+	-	+	
<i>Lonicera caprifolium</i>	-	-	+	-
<i>Prunus divaricata</i>	-	+	-	-
<i>Ruscus aculeatus</i>	-	-	+	-

<i>Spiraea hypericifolia</i>	-	-	+	-
<i>Swida australis</i>	2	+	+	-
Perennial herbs (H)				
<i>Aegonychon purpurea-coeruleum</i>	2	+	-	-
<i>Campanula rapunculoides</i>	+	-	+	+
<i>Carex digitata</i>	32	16-18	20-22	42-45
<i>Clinopodium vulgare</i>	+	-	-	-
<i>Dictamnus albus</i>	-	+	-	-
<i>Falcaria vulgaris</i>	+	-	-	-
<i>Fragaria vesca</i>	1	-	+	-
<i>Klasea quinquefolia</i>	-	+	-	-
<i>Melica uniflora</i>	5	1-2	2-3	15-16
<i>Poa nemoralis</i>	-	-	-	2
<i>Psephellus carthalinicus</i>	+	-	-	-
<i>Pyrethrum corymbosum</i>	-	+	+	-
<i>Silene italica</i>	+	-	+	+
<i>Viola alba</i>	2	1	0,5	1
Perennial herbs (G)				
<i>Asparagus verticillatus</i>	-	-	+	-
<i>Cephalanthera damasonium</i>	+	+	+	-
<i>Muscari szovitsianum</i>	+	-	-	+
<i>Sedum maximum</i> subsp. <i>ruprechtii</i> (<i>S. caucasicum</i>)	-	-	-	+

8. *Carpinetum ruscosum*

This plant community has relatively limited distribution area.

Distribution in Tbilisi environs: Saguramo ridge; Altitude (m): 600-800; Topography: slopes; Expo-

sure (macro): E, W, S; Exposure (micro): E, W, rarely S-W, N-E and S; Inclination: 5°-10° to 30°-40°(45°); Soil: cinnamonic and brown forest, thin or middle depth, often with stones, sometimes denuded bedrocks are observed;

Table 8. *Carpinetum ruscosum*

Surveys	1	2	3	4	5	6	7
Date	06.10.12.	10.09.15.	20.07.15.	28.07.16	28.07.16	19.07.17.	24.07.17.
Altitude (m)	638	725	715	780	626	795	630
Exposure (macro)	E	E	W	S	E	W	W
Exposure (micro)	E	N-E	S-W	S-E	E	W	W
Inclination	12°-15°	5°-8°	8°-10°	10°-12°	30°-32°	15°-17°	30°-35°
I layer (Arborescent stratum)							
Density of canopy	0,55-0,6	0,6	0,6-0,65	0,55-0,6	0,5-0,6	0,65-0,7	0,7-0,8
Average height (m)	4,5-5	4-5	3-3,5	4-4,5	3,5	4-5	3-3,5
Maximum height (m)	6	5,5	5	5,5	5	6	4
II layer (<i>Ruscus ponticus</i>)							
Projective coverage (%)	55-60	75-80	45-50	20-23	65	85-90	35-40
Average height (cm)	90-100	45-50	50-55	40-45	55-65	60-70	40-50
III layer (Grass cover)							
Projective coverage (%)	20-22	7-8	21-23	32-34	15-17	6-8	18-20
Average height (cm)	22-25	20-22	25	25-27	10-12	12-15	20
IV layer (Moss)							
Projective coverage (%)	4-5	+	-	+	-	+	6
Litter							
Projective coverage (%)	20-22	12-15	35-40	70-72	27-30	10	50-52
Depth (cm)	0,5-1	1	1-1,5	0,5-1	0,5-1	1-2	1-2
Floristic composition							
Trees (Ph)							

<i>Quercus iberica</i>	+ 1 s..	+	+	-	-	+ 1 s..	-
Shrubs (Ph)							
<i>Carpinus orientalis</i>	55-60	60	60-65	50-55	50-60	65-70	70-80
<i>Cornus mas</i>	-	+	-	-	-	-	-
<i>Cotoneaster morulus</i>	+	-	-	-	+	-	+
<i>Cotoneaster saxatilis</i>	-	+	-	+	-	+	-
<i>Crateagus kyrtostyla</i>	+	+	-	+	-	+	-
<i>Cytisus caucasicus</i>	-	-	-	-	+	-	+
<i>Euonymus leiophloeus</i>	-	+	-	-	-	-	-
<i>Euonymus verrucosus</i>	+	+	-	-	-	-	-
<i>Jasminum fruticans</i>	-	-	-	+	+	-	-
<i>Juniperus oxycedrus</i>	-	-	-	+	-	-	+
<i>Ligustrum vulgare</i>	+	-	+	-	+	+	-
<i>Lonicera caprifolium</i>	-	+	-	-	-	-	-
<i>Paliurus spina-christi</i>	-	-	-	+	-	-	-
<i>Prunus divaricata</i>	+	-	-	-	-	-	-
<i>Rosa canina</i>	+	-	-	-	-	-	-
<i>Spiraea hypericifolia</i>	-	-	-	-	+	-	+
<i>Swida australis</i>	+	-	+	-	-	+	-
Perennial herbs (H)							
<i>Aegonychon purpurea-coeruleum</i>	4	2-3	2	3-4	3	-	2-3
<i>Albovia tripartita</i>	-	+	-	-	-	-	-
<i>Alliaria petiolata</i>	-	1	0,5	-	-	+	-
<i>Anthriscus nemorosa</i>	3-4	-	-	-	-	2	-
<i>Brachypodium pinnatum</i>	-	-	2	-	-	-	-
<i>Brachypodium sylvaticum</i>	-	1	-	-	-	2	-
<i>Campanula rapunculoides</i>	1	+	-	-	-	+	+
<i>Carex humilis</i>	+	-	-	-	-	-	1
<i>Clinopodium vulgare</i>	-	-	1-2	2-3	3	-	2-3
<i>Cynosurus echinatus</i>	-	-	-	-	+	-	+
<i>Dactylis glomerata</i>	-	-	-	4-5	3	-	3-4
<i>Fragaria vesca</i>	-	-	-	3-4	3-4	-	4-5
<i>Galium spurium</i> (G. vaillantii)	+	-	-	+	1-2	-	-
<i>Geum urbanum</i>	-	+	-	-	-	+	-
<i>Klasea quinquefolia</i>	-	+	-	-	-	-	-
<i>Laser trilobum</i>	+	-	-	-	-	-	-
<i>Melica uniflora</i>	2-3	1-2	-	-	-	1-2	-
<i>Physospermum cornubiense</i>	-	-	+	-	-	-	-
<i>Poa nemoralis</i>	+	-	3-4	-	-	-	1-2
<i>Polypodium vulgare</i>	-	+	-	-	-	-	-
<i>Primula veris</i> subsp. macrocalyx	-	+	-	-	-	-	-
<i>Silene italica</i>	3-4	-	3-4	6-7	-	1-2	-
<i>Thalictrum collinum</i>	-	-	-	+	+	-	-
<i>Vicia truncatula</i>	+	-	-	-	-	-	-
<i>Vincetoxicum amplifolium</i> (V. scandens)	-	-	+	-	-	-	-
<i>Viola alba</i>	5	-	6-7	8-9	5	2-3	5-6
<i>Viola odorata</i>	-	1-2	-	-	-	-	-
Perennial herbs (H)							
<i>Asparagus verticillatus</i>	-	-	-	+	-	-	-
<i>Cyclamen vernum</i> (C. coum subsp. caucasicum)	2	-	1-2	-	-	+	-
<i>Helleborus caucasicus</i>	-	-	-	3-4	-	-	-
<i>Muscari szovitsianum</i>	-	-	-	+	-	-	-

<i>Scilla siberica</i>	-	+	-	-	-	-	-
Annual plants (Th)							
<i>Geranium lucidum</i>	0,5	-	1	-	-	+	-
<i>Thlaspi orbiculatum</i>	-	+	-	-	-	-	-
<i>Torilis japonica</i>	-	-	-	-	-	0,5	-

Renewal of plant communities

Seedlings (5-15 cm height) of oriental hornbeam with various numbers are in the all described plots. Participation of seedlings of main dominants of forest vegetation – *Quercus iberica* and *Fraxinus excelsior* are important. Their seedlings and young individuals (5-30 cm height) were recorded almost in the all plots. In the several plots also are seedlings and young individuals of characteristic woody plants of forests. They are: *Acer campestre*, *Acer cappadocicum*, *Sorbus torminalis*, *Tilia begoniifolia*.

Wide participation of seedlings and young individuals of characteristic woody plants of forests indicate that oriental hornbeam formation of Tbilisi environs is related with forests of foothills and lower mountain belt.

Conclusion

Main area of oriental hornbeam formation (*Carpineta orientalis*) in Tbilisi environs is in foothills and lower mountain belt, approximately 600-1000 (1100) m above s.l. It is rare in middle mountain belt (1100-1300 m above s.l.). Plant communities of formation are fragmentary distributed. They are developed on slopes with various exposure and inclination, on the cinnamonic and brown forest soils. Often soils are skeletal. In Tbilisi environs oriental hornbeam plant communities are secondary origin and derived as a result of digressive successions of oak forest (*Querceta iberici*).

Formation is characterised by rich typological composition. 8 plant communities were identified in Tbilisi environs by us: (1) *Querceto-Carpinetum graminoso-mixtoherbosum*, (2) *Carpinetum graminoso-mixtoherbosum*, (3) *Carpinetum mixtoherbosum*, (4) *Carpinetum poosum nemoralis*, (5) *Carpinetum pooso-caricosum*, (6) *Carpinetum caricosum humilis*, (7) *Carpinetum caricosum digitatae*, (8) *Carpinetum ruscum*. From them *Carpinetum poosum nemoralis* and *Carpinetum graminoso-mixtoherbosum* are comparatively widespread and others are relatively rare.

In the phytocenological structure of plant communities 2 layer are sharply expressed: I layer - arborescent stratum, II layer – grass cover. Moss

and lichen cover is not always developed. Average height of arborescent stratum vary in (3,5)4-5(7) m and density of canopy from 0,4-05 to 0,6-0,7(0,8-0,9) [40-50% to 60-70%(80-90%)].

Floristic composition is complex. Participation of main dominants of forests (*Quercus iberica*, *Fraxinus excelsior*) is not rare, but they in most cases are presented with 1-2 specimens. Majority of woody plants are characteristic species of forests and elements of shibliak and xerophytic forests are rare. Core of grass cover composition in the same way is created by forest components. Part of them is dominants of grass cover. Composition of herbs is enriched with characteristic plants of different types of shrubberies and, partially, of dry meadows and steppe-meadows, but they are not appertained to constant species.

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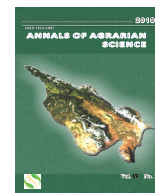
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The influence of norms, forms and input rules of nitrogen fertilizers upon NO_3 consistency in orange and lemon crops

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ABSTRACT

The paper overviews what is the influence of norms and forms of nitrogen-based fertilizers on the nitrate accumulation in the fruits of Washington Navel orange and Meyer lemon. Researches took place in Anaseuli on red and yellow soils of the experimental plots of subtropical crops and tea industry institutes of Georgian Agricultural University. In order to determine the impact of nitrogen fertilizer norms on nitrate accumulation in Washington Navel orange, ammonium sulfate and ammonium nitrate have been used with the doses-75, 150, 300, 450 g/per tree on the background of P,K,Ca,Mg in accordance with agricultural laws. As for the nitrate accumulation from the nitrogen fertilizer forms, we used ammonium sulfate, ammonium nitrate, ammonium sulfate, ammonium nitrate, carbamide, montanus nitrate and sodium nitrate on the background of P,K,Ca,Mg. In order to accumulate nitrates in Meyer lemon fruits in condition of yellow soil, single and twofold norms of carbamide and montanus nitrate of nitrogen fertilizers (150 and 300 g/per tree), various rules of input and organic fertilizers such as N:P:K have been applied in different ratios. It has been investigated that there is slight impact of nitrogen fertilizers on nitrate deposition in orange fruits and formed fruits are ecologically clean. The same relation has been identified in lemon fruits while using nitrogen fertilizers. Taking into account all above mentioned things we can figure out that the most efficient and pertinent nitrogen fertilizer norm to orange and lemon in case of having the red and yellow soil 150 g/per tree, for orange it is $\text{P}_2\text{O}_5_{300} \text{K}_2\text{O}_{200}$ and for lemon- $\text{P}_2\text{O}_5_{150} \text{K}_2\text{O}_{100}$. As for nitrate accumulation in Meyer lemon preserving all nitrogen fertilizer application rules, growing tendency of NO_3 is obvious, but it does not exceed permissible limit (50 mg/kg).

Keywords: Soil, Fertilizers, Washington Navel orange, Meyer lemon, Fruits, Nitrates.

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Introduction

The role of organic and mineral fertilizers in oranges and lemons is especially important as it significantly increases their crop level and also strengthens plants to successfully cope with different diseases and extremely low temperature [1-5]. Undoubtedly, nitrogen fertilizer norms are very efficient guaranteeing very high level of productivity.

In order to get the high level of plant provision through the help of nutrition elements, plant diagnostics should be implemented. In addition, extreme attention is paid not to particular elements but to their merging antagonism and ion synergism abroad [6]. The nutrition optimization of all agricultural plants and particularly of orange and lemon should enhance the level of crop and provide ecologically clean crop respectively.

It can be concluded that we have studied the Washington Navel orange and Meyer lemon nitrate consistency, their norms, forms and input rules when applying nitrogen fertilizers in perennial field experiments.

Objects and Methods

Scientific researches regarding the influence of nitrogen fertilizer forms, norms and input rules upon red soil Washington Navel orange and yellow soil Meyer lemon fruits on NO_3 consistency has been conducted in the west subtropical zone of Georgia. Soil agrochemical indicators prior to conducting the experiment are demonstrated in table#1.

Field experiment on orange has been laid out on the red soil. Replication of the experiment

is possible six more time. There are six plants in each division, two of which are protective and four accounting units. All in all, in every variant there are 24 accounting plants. Plant feeding area covers 2.2x3.0 m. Fertilizers were delivered following the agricultural rules and calcareous fertilizers based on one exchange acidity. 20 kg manure per tree has been used on the background of P_2O_5 300 K_2O once in two years. The single norm of nitrogen fertilizers amounted 150 g/per tree.

Field experiment on yellow soil lemon on two-year seedlings. Plant spacing on 1.5x2.2 m, nutrition area 3.3 m². Meyer lemon can be applied in the experiment for five times, there are six plants on each division, two of them protective and the rest accounting ones. Every variant amounts 20 accounting plants. Fertilizers have been delivered $N_{150}P_2O_5$ 150 K_2O 100 g/per tree. Organic fertilizer-manure 15 kg/per tree. Calcareous fertilizer with one exchange acidity. In the process of experiment we have applied the peat on the whole area as well. Agricultural laws have been preserved when taking care of the plants [7]. Before conducting the experiment, total humus of sample soils has been determined through the Turin method by Nikitin modification

[8], total N- by Kheldahl method, pH (H_2O , KC), total sum of absorbed roots has been determined by Capen and Glikovits method, P_2O_5 and K_2O by Oniani method, hydrolyzed N-by Turin and Kononova method [9,10]. Also, we have determined NO_3 through the ion-selective method [11, 12].

Results and analysis

In the process of citrus cultivation and caring, significant attention is focused on relevance of above mentioned indicators with standards, one of the most key regulatory roles of which is represented by mineral treatment [5, 13-15]. Thereafter, it must result in getting ecologically clean product, not containing excessive amount of harmful toxic substances (heavy metals, nitrates, fluorine, chlorine) [16].

Mineral fertilizers and particularly nitrogen fertilizers greatly influence upon the nitrogen consistency in separate parts of citrus plants, including their fruits too [17]. Therefore, we have been tasked to identify NO_3 in pulp and skin of fruits, results are demonstrated in table#2.

According to researches conducted by O. Zardalishvili [18] we can conclude that fertilizer forms have a considerable impact on nitrate deposition in plants.

Table 1. Soil agrochemical indicators before conducting the experiment

Type of experiment	Soil Types	Soil Depth/ cm	Total Humus %	Total Nitrogen%	Acidity forms Mg. equivalent /100g		pH _{KCl}	Moving mixtures mg/100g soil		
					Exchange	Hydrolyzed		Hydrolyzed Nitrogen	P ₂ O ₅	K ₂ O
Field experiment on orange	Red soil	0-15	4.69	0.20	2.10	7.56	4.92	10.6	74.0	56.0
		15-30	3.11	0.18	3.51	7.86	4.62	9.0	27.0	49.0
Field experiment on lemon	Yellow soil	0-15	2.58	0.18	1.12	8.54	5.31	12.8	33.0	16.50
		15-30	1.60	0.16	2.43	9.50	4.90	8.3	18.0	21.70

Table 2. On NO_3 consistency in Washington Navel orange fruit

Nitrogen fertilizer norms and forms on the background of PKCaMg (g/per tree)	Mg/100g	%	mg/1kg in fruit

Nitrogen fertilizer norms			
PKCaMg-background	0.71	0.0007	7.1
(NH ₄) ₂ SO ₄ 75	0.56	0.0005	5.6
(NH ₄) ₂ SO ₄ 150	0.44	0.0004	4.4
(NH ₄) ₂ SO ₄ 300	0.43	0.0004	4.3
(NH ₄) ₂ SO ₄ 450	0.55	0.0005	5.5
NH ₄ NO ₃ 75	0.91	0.0009	5.1
NH ₄ NO ₃ 150	0.60	0.0006	6.0
NH ₄ NO ₃ 300	0.42	0.0004	4.2
NH ₄ NO ₃ 450	0.38	0.0004	3.8
Nitrogen fertilizer forms			
Unfertilized	0.69	0.0007	6.9
PKCaMg-background	0.56	0.0005	5.6
(NH ₄) ₂ SO ₄	0.63	0.0006	6.3
(NH ₄) ₂ SO ₄ 60% NH ₄ NO ₃ 40%	0.40	0.0004	4.0
NH ₄ NO ₃	0.69	0.0007	6.9
(NH ₂) ₂ CO	0.65	0.0006	6.5
NaNO ₃	0.63	0.0006	6.3

Nitrate consistency can be reduced by applying slow-release nitrogen fertilizers in the plants. Hence, the influence of nitrogen fertilizer norms and forms upon Washington Navel orange fruit on NO₃ consistency is represented in Table#2, which clearly demonstrates there is no substantial difference among nitrogen fertilizer norms in this prism. Slightly exceeds NO₃ consistency (0,1-0,35 mg 100g/juice) in fruits formed by the impact of Ammonium nitrate norms (75-150 g/per tree). In case of having higher norms, NO₃ consistency exceeds ammonium sulfate variant in comparison with ammonium nitrate variant. As for the influence of nitrogen fertilizer norms on orange fruit nitrate consistency, there was no considerable difference between them if not taking into account ammonium

nitrate variant. Based on our result we can conclude that orange fruits belong to ecologically clean product, as 1 kg citrus should not consist more than 50 mg/kg NO₃ [11, 19].

The quality of Meyer lemon is determined by nitrate consistency as well, food element ratio and input timeframe violation in mineral fertilizers greatly impacts on it, resulting in nitrate accumulation in agricultural crop. Nitrates which are in reaction with secondary amines in acidic area, develop nitrosamines. These compounds are hazardous because they own carcinogenic and mutagenic defects [20].

In order to identify what is the Meyer lemon fruit (pulp, juice) consistency with nitrates, we have decided to examine what is the nitrate content in crop got from perennial field experiment in accordance

with separate variants. Results are represented in Table# 3. According to Table# 3, we can figure out that the nitrogen fertilizers' usage does not cause the harmful nitrate consistency in lemon fruits and ranges within 18-26 mg/kg, which is by 24-32 mg/kg less than the standardized 50 mg/kg [19]. So, it is clear that the product obtained in conditions of our experiment is ecologically clean. If we compare separate variants with each other, we will see that the high nitrate level is vivid in the variant where phosphorous fertilizer has twofold agro technic norm ($N_1P_2K_1$) and in 100 g pulp and juice reaches 1.44-1.41 mg. Almost in every version NO_3 consistency in pulp is by 0,01-0,05 mg more compared to juice. There is no vivid difference between nitrogen fertilizer forms, particularly in fractional input versions we can note the nitrate growing tendency in fruit pulp and juice in comparison with simultaneous input, which can be prescribed to input timeframes and N:P:K correlation. We can sum up that Meyer lemon fruit is ecologically clean and the most optimal norm for it is carbamide and ammonium saltpeter norm N.150g/per tree on the background of single agro technic norm (P_2O_5 150 K_2O 100) of phosphorus and potassium.

Conclusion

Researches carried out on Washington Navel red and Meyer lemon yellow soil gardens in the west

subtropical zone of Georgia concerning deviations caused by applying the nitrogen fertilizer norms, forms and input rules in citrus plants on nitrate consistency, we can make the following conclusions:

1. Norms and forms of nitrogen fertilizers do not have a strong influence upon NO_3 accumulation in orange fruits, slight advantage is noted on ammonium nitrate version (150 kg/per tree). In case of slow-release nitrogen fertilizers, reduction of NO_3 accumulation takes place;
2. Accumulation of NO_3 in lemon fruits is not caused by nitrogen fertilizer norms, forms and input rules. The only clear thing is that the nitrate level increases on the twofold agro technic background of phosphorus fertilizers, but it does not exceed the acceptable norm (50 mg/kg);
3. Norms, forms and input rules of nitrogen fertilizers (150 g/ per tree) in condition of yellow/red soils of subtropical zone of Georgia does not cause the nitrate accumulation in lemon fruits and as a result, we get ecologically clean product.

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Table 3. On NO_3 consistency in Meyer lemon fruit

Experiment Scheme	mg/100g		total mg/kg in fruit
	Pulp	Juice	
Unfertilized	1.25	1.22	24,7
P_1K_1	1.83	0.79	26,2
$N_1P_1-(NH_2)_2CO^x$	1.35	1.35	27,0
$N_1K_1-(NH_2)_2CO^x$	0.91	0.89	18,0
$N_1P_1K_1-(NH_2)_2CO^x$	1.00	1.00	20,0
$N_1P_1K_1-(NH_2)_2CO^{xx}$	1.12	1.07	21,9
$N_1P_1K_1-NH_4NO_3^x$	1.23	1.23	24,6
$N_1P_1K_1-NH_4NO_3^{xx}$	1.11	1.12	22,3
$N_1P_2K_1-(NH_2)_2CO^x$	1.44	1.41	26,5
$N_1P_1K_1-(NH_2)_2CO^x+manure$	1.02	1.02	20,2
$N_2P_2K_2-(NH_2)_2CO^x+manure$	1.20	1.20	24,0
$N_2P_1K_1-(NH_2)_2CO^x$	1.18	1.18	23,6
$N_2P_2K_2-(NH_2)_2CO^{xx}$	1.26	1.29	25,5

X- simultaneous input

XX- fractional input

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Pseudoprotein-based nanoparticles show promise as carriers for ophthalmic drug delivery

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ABSTRACT

Drug delivery used to treat ocular disease still poses a challenge to modern ophthalmology. Well-established intravitreal injections imply discomfort to the patients and risk of ocular complications. Therefore, opportunities to deliver drugs by topical administration are investigated thoroughly. Despite its seemingly easy accessibility, the eye is well protected by efficient mechanisms that rapidly remove drugs after instillation on the eye surface. Hence, eye drops are less effective for the treatment of various diseases, which necessitates a risk-containing procedure of intravitreal injection. One of the rational ways to overcome the problem is the application of drug-loaded polymeric nanoparticles (NPs) that are able to penetrate through ocular barriers when administered topically. Pseudo-proteins (PPs) - amino acid-based biodegradable polymers are one of the most suitable materials for the design of drug delivering NPs. One of the most important features of such kind of nanovehicles is “disappearance” from the body after their function is fulfilled. We have prepared biodegradable NPs of various types by nanoprecipitation of the PEA-class of PP composed of L-leucine, 1,6-hexanediol and sebacic acid (8L6). The originally designed arginine-based cationic PEA and comb-like PEA containing lateral PEG-2000 chains along with 8L6 anchoring fragments in the backbones were used to construct positively charged and PEGylated NPs. The NPs were loaded with fluorescein diacetate (FDA) as a fluorescent probe to detect if the NP penetrated through the ocular barriers. A preliminary *in vivo* study on intraocular infiltration of the NPs has been done using wild-type C57BL/6 mice. After penetrating into the cellular lysosomes, FDA probes became visible due to the hydrolysis of the diacetate groups, thus allowing for the detection of the NPs as tiny fluorescent spots inside the tissues. One day after administration, fluorescent dots were found at various sites - always in the peripheral cornea and the sclera, and in different layers of the outer retina depending on the type of NPs used. Four days after administration, fluorescent dots were still visible in the peripheral cornea and the sclera with some of the NPs. These results show that the new type of NPs infiltrate the ocular tissues after topical administration and are taken up by the cells. This raises hope that the NPs may be useful carriers for ocular delivery of therapeutic agents.

Keywords: Biodegradable polymers, Pseudo-proteins, Nanoparticles, Biodegradable surfactant, PEGylation, Ocular penetration.

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1. Introduction

Most of the diseases affecting the posterior segment of the eye are related with visual impairment and blindness. The effective treatment of these pa-

thologies is one of the major challenges in drug delivery as most of them are chronic and multifactorial. Among them, aged related macular degeneration, diabetic retinopathies and glaucoma produce irreversible visual damage and blindness [1]. These

diseases are becoming more and more prevalent in the aging populations, and nowadays tens of millions of patients are affected worldwide. Depending on the disease, the medications should be delivered to the retinal cells, retinal pigment epithelium or choroid. Furthermore, therapeutic concentrations of the active substance in the intraocular target site have to be maintained during a long period of time.

Due to the ocular barriers, it is difficult to deliver effective drug concentrations to the posterior tissues of the eye using non-invasive routes such as topical or systemic administration [2]. It is well known that after topical administration only very low drug concentrations are reached in the retina and choroid [3]. This is due to the obstacles of drug penetration that include the short residence time of formulations on the ocular surface, the presence of tissue barriers (cornea, lens, conjunctiva, sclera), and flow mediated drug loss factors (conjunctival blood flow, aqueous humor flow) that limit the drug access to the retina and choroid. Although systemic administration is used to deliver some drugs to the eye (e.g., corticosteroids), this route is restricted by the systemic toxicity of the drugs and reduced access to the target site, mainly due to the blood-aqueous and blood-retinal barriers [2]. The most effective method of drug delivery to the back of the eye is through intraocular administrations, mainly intravitreal injections. However, intravitreal administration is an invasive mode of drug delivery and it is sometimes associated with adverse effects (endophthalmitis, hemorrhages, ocular hypertension, damage of lens or retinal detachment) and it requires frequent visits of the patients to clinics. For this reason, ophthalmic drug delivery is one of the most challenging endeavours facing the ocular pharmacologists. A major challenge is to get over the ocular barriers and reach the tissue target. Controlled drug delivery systems able to release and maintain effective active substance levels over long periods of time, would prolong the dosing interval to months [4,5]. Therefore, the intraocular administration of drugs using sustained/controlled drug delivering (SCD) beads looks more promising.

Microspheres (MSs) are emerging therapeutic tools for SCD as they can be administered as a conventional injection by periocular and intraocular routes. MSs made of biodegradable polymers (BPs) are by far more promising since they can be cleared from the site of administration over time. Ophthalmic drug delivery systems can be made with a variety of biodegradable materials such as polyesters

(lactide and glycolide copolymers (PLGA), polycaprolactones, poly(β -hydroxybutyrate)), polyamides (including natural polymers such as collagen, gelatine and albumin), heteropolysaccharides (chitosan). Synthetic polymers have an advantage over the naturally occurring ones since they reveal from low to zero immunogenicity.

The obvious advantages of biodegradable implants over the non-degradable devices in the clinical practice have promoted the interest in novel BPs adequate for intraocular drug delivery purposes. However, biodegradable injectable implants mostly made of poly- α -hydroxy acids - lactide/glycolide polyesters (PEs) and co-PEs, degrade and release drugs in non-zero order kinetics, which is inefficient for long-term sustained drug release [6,7]. The degradation rate of the aliphatic PEs cannot be tuned easily as most of these polymers are synthesized from single monomers. Attempts to tune the degradation rate often involves copolymerization or blending with other polymers that are not always successful [8]. Besides, the PEs release acidic products upon biodegradation that are considered to be toxic to some cells causing undesired phenotype modulations that limits their biomedical applications [9-13]. In addition, the acidic products transiently decrease vitreous humour to pH 7 and increases the risk of inflammation before being metabolized. It should also be noted that lactide/glycolide polymers show low compatibility with acid sensitive bio-pharmaceuticals [6].

More promising for sophisticated biomedical applications look BPs made of naturally occurring α -amino acids, fatty diols and dicarboxylic acids, so called pseudo-proteins (PPs) [14-16]. These relatively new family of BPs in most cases release low acidic products, and some of them show self-buffering property thus preventing significant pH drop during the degradation process; in other words, no local acidic environment causing inflammation is built up upon the biodegradation of the PPs. The PP of co-poly(ester amide) (**co-PEA**) class, originally developed by Katsarava et al. [17,18], was successfully used recently by the team of Royal DSM for developing micronized particles for intraocular delivery of dexamethasone (DEX) - microfibriles [6] and microsphere [7]. The co-PEA based micronized devices showed excellent biocompatibility when used for intraocular purposes [6,7]. The use of PP-based micronized particles is a progressive method for the delivery drugs into the ocular tissues, however, also needs, though less often, painful and undesirable intraocular injection.

To overcome the problems, nanotechnology involving drug-loaded polymeric nanoparticles (NPs), has been proposed as ophthalmic drug delivery systems that may control drug release and maintain therapeutic levels over a prolonged period of time. The use of NPs, designed in due manner, can provide high corneal penetration of drugs and exclude the need of painful injections. In addition, NPs were shown to adhere preferentially to inflamed precorneal tissues of the eye. This seems to be an effective targeting of drugs to inflamed parts of the eye and might be a promising application for nanoformulations [19].

Various NPs-based ophthalmic drug delivery systems have been offered: liposomes, dendimers, β -cyclodextrine, various emulsions, and nanospheres [20]. Liposomes and emulsions liposomes are less popular because of their short shelf life, limited drug capacity, use of aggressive conditions for preparation and problems in sterilization [21]. More promising for ophthalmic drug delivery are NPs [20]. Various non-degradable and degradable polymers of both natural and synthetic origin were used in ophthalmic drug nanoformulations [22-24]. Synthetic BPs are more suitable for drug delivery purposes since they can be cleared from the body after their function is fulfilled. The most usable to date are PEs such as lactide/glycolide polymers/copolymers. The serious drawbacks of the PEs limiting their biomedical applications were discussed above (see Refs. [6-13]).

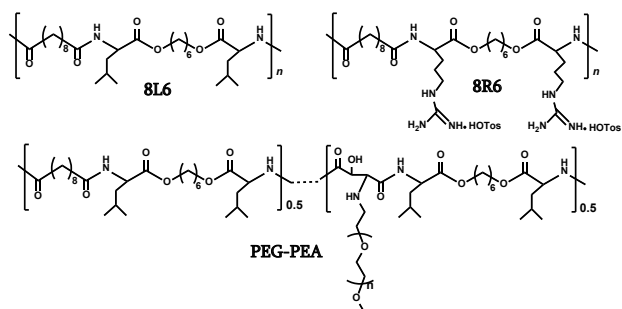
Pseudo-proteins (PPs) - a new family of amino acid based synthetic analogous of proteins, merits of which were discussed in brief above, look more promising for constructing the drug delivery NPs. Three basic classes of the PPs obtained are – poly(ester amide)s (PEAs), poly(ester urethane)s, and poly(ester urea)s [14-16].

For constructing nanosized drug delivery vehicles we have selected PP-PEAs. Namely, we used the *homo*-PEA labelled as 8L6 (Scheme 1), composed of sebacic acid (8), L-leucine (L) and 1,6-hexanediol (6), 8L6. The selected PEA 8L6 represents a fragment of the highly biocompatible co-PEA of complex structure used by Royal-DSM (DSM-PEA) as a drug delivery platform [6,7]. The DSM-PEA labelled as $[8L6]_{0.30}$ - $[8LDAS]_{0.45}$ - $[8K(Bn)]_{0.25}$, is made of amino acid based three monomers: bis-(L-leucine)-1,6-hexanediol diester, L6 (30 mol%), bis-(L-leucine)-1,4-dianhydrosorbitol diester, LDAS (45 mol%), and lysine benzyl-ester, KBN (25 mol%) originally designed previously [17,18] and obtained *via* solution active polycon-

densation (SAP). The selected PP-PEA 8L6 is by far more simple than DSM-PEA (that means its biodegradation products are more definite and predictable) and was obtained *via* very fast (lasts 15-20 min) interfacial polycondensation (IP), in contrast to SAP which lasts 16 h. The polymer was synthesized using one amino acid based monomer - L6, by its IP with sebacoyl chloride [25], which is a cheap and purchasable product. Hence, the PEA 8L6 is by far simple and cost-effective as compared with the DSM-PEA.

Previous systematic study of four PPs of various structures in terms of particles size, stability and cell compatibility showed the PP-PEA 8L6 was the best [26]. Along with 8L6 in the present study we used also cationic PP-PEA 8R6 [27] (Scheme 1) for imparting positive charge to the NPs by blending with neutral 8L6, and an originally designed PP-surfactant – PEG-attached co-PEA (PEG-PEA) [28] which represents at the same time NPs' PEGylating agent.

Positive surface charge (positive zeta-potential) is favourable for penetration of NPs through biological (ophthalmic) barriers such as cornea, lens, etc. It is known that a positive charge helps with the NPs adhesion to the surface of cells and stimulates penetration into the cells *via* endocytosis [29,30]. Surface PEGylation decreases the affinity plasma proteins (opsonins) for adsorption on NPs and in that way suppresses phagocytosis [31,32]. Along with the protection of the NPs from phagocytosis the PEGylation increases ocular drug bioavailability of NPs [33].



Scheme 1. PP-PEAs used for fabricating NPs.

In the present study we have prepared NPs of various types loaded with fluorescent probe - fluorescein diacetate (FDA) which is non-fluorescent and become fluorescent after penetrating into cells and enzymatic hydrolysis of ester groups [34]. A preliminary study of permeability of the obtained NPs through the ocular barriers was done using wild-type C57BL/6 mice.

2. Materials and Methods

2.1. Materials

Surfactant Tween 20, Sorbitanmonolaurate (MW 1,228), was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methoxy-PEG-amine with average molecular weight 2,000 Da (mPEG-amine-2000) was purchased from Laysan Bio. Fluorescent probe – fluorescein diacetate was purchased from Santa Cruz Biotechnology. Organic solvent - Dimethyl sulfoxide (DMSO) was purchased from Carl Roth (Karlsruhe, Germany). All the chemicals were used as received. The dialysis bag (MWCO 25 kDa) was purchased from Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA. The PP-PEAs, selected for the proposed study, were originally synthesized as reported previously: the leucine (**L**) based PP-PEA 8L6 *via* the Interfacial Polycondensation (IP) [25,35], and the arginine (**R**) based biodegradable cationic PP-PEA 8R6 - *via* Solution Active Polycondensation (SAP) [27]. The new functional PP - surfactant/PEGylating agent composed of amino acid **L** and containing lateral PEG chains, PEG-PEA, was synthesized by interaction of epoxy-co-PEA [8L6]_{0.5}-[tES-L6]_{0.5} with mPEG-amine-2000 as reported previously [28].

For the *in vivo* experiments, we used adult wild-type C57BL/6J mice. They were held in ventilated cages in our own animal facility at 12 hours/12 hours light/dark cycle, with standard food and drinking water *ad libitum*. The experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the EU directive 2010/63/EU and were approved by the local authorities (LANUV, Recklinghausen, Germany, file number 84-02.04.2018.A175).

2.2. Characterization of polymers

The number-average (M_n), and weight-average (M_w) molecular weights (MWs), and dispersity (D) of the polymers were determined using the GPC. The MWs of the PPs - 8L6 and [8L6]_{0.5}-[tES-L6]_{0.5} were determined on a machine of Waters Associates, Inc., Milford, MA, USA, equipped with Styragel columns in DMF: HR4, HR3, HR0.5 (all 7.8 mm × 300 mm), a high-pressure liquid chromatography pump (Waters 1525 Binary HPLC) and a Waters refractive index detector 2414 and UV-detector (Waters 2487 dual absorbance detector, $\lambda = 240$ nm). A solution of LiBr (0.1 M) in DMF was used

as an eluent. Injected volume was 100 μ L, the sample concentration 5.0 mg/mL, flow rate 1.0 mL/min and temperature 35 °C. The columns were calibrated with PMMA standards. The MW of the cationic polymer 8R6 was determined on a Shimadzu GPC machine, model LC-8A equipped with an Empower computer program (Waters), a PL HFIP gel column (Polymer Lab, Theale, Berkshire, UK) and a refractive index detector (Shimadzu RID-10A, Shimadzu Scientific Instruments, Columbia, MD, USA). The polymer 8R6 was dissolved in and eluted with HFIP containing CF₃COONa (0.05 M, to suppress polyelectrolyte effects). The injected volume was 100 μ L, the sample concentration 2.0 mg/mL, and the flow rate 0.5 mL/min. The columns were calibrated with PMMA standards.

2.3. Preparation of various types of fluorescently-tagged NPs

Different types of fluorescently-tagged NPs were prepared according to the polymer deposition/solvent displacement (nanoprecipitation) method under the optimal conditions previously established for PPs - amino acid based biodegradable ester polymers [26].

Non PEGylated NPs (labelled as TK-1 and TK-5 NPs) were prepared as follows: 6.0 mg of PEA 8L6 (in case of TK-1 NPs) or the 70/30 mixture of PEAs 8L6/8R6 (i.e. 4.2 mg of PEA 8L6 and 1.8 mg of cationic PEA 8R6) (in case of TK-5 NPs) was dissolved in 1.0 mL of DMSO (organic phase) along with the 6.0 μ g of fluorescein diacetate (i.e. 0.1 w% from the polymer weight) and dropwise added (dropping rate 12 drops/min) to 10.0 mL of water (inorganic phase) containing 50.0 mg of the surfactant Tween 20 (organic/water phases ratio 1:10 v/v) at a stirring rate of 700 rpm using a magnetic stirrer. All manipulations were done at room temperature.

The surface PEGylated NPs (samples TK-2 and TK-6 NPs) were prepared as follows: 6.0 mg of PEA 8L6 (in case of TK-2 NPs) or the 70/30 mixture of PEAs 8L6/8R6 (in case of TK-6 NPs) was dissolved in 1.0 mL of DMSO (organic phase) along with the 6.0 μ g of fluorescein diacetate (i.e. 0.1 w% from the polymer weight) and dropwise added (dropping rate 12 drops/min) to 10.0 mL of water (inorganic phase) containing 25.0 mg of the surfactant Tween 20 and 25.0 mg of biodegradable surfactant PEG-PEA (organic/water phases ratio 1:10 v/v) at a stirring rate of 700 rpm using a magnetic stirrer.

Other two types of surface PEGylated NPs (sam-

ples TK-3 and TK-7 NPs) were prepared without using the surfactant Tween 20. Manipulations were done as follows: 6.0 mg of PEA 8L6 (in case of TK-3 NPs) or the 70/30 mixture of PEAs 8L6/8R6 (in case of TK-7 NPs) was dissolved in 1.0 mL of DMSO (organic phase) along with the 6.0 µg of fluorescein diacetate (i.e. 0.1 w% from the polymer weight) and dropwise added (dropping rate 12 drops/min) to 10.0 mL of water (inorganic phase) containing 50.0 mg of the biodegradable surfactant PEG-PEA (organic/water phases ratio 1:10 v/v) at a stirring rate of 700 rpm using a magnetic stirrer.

The surface and bulk PEGylated NPs (samples TK-4 and TK-8 NPs) were prepared using so called modified nanoprecipitation method [26]. Manipulations were done as follows: 6.0 mg of PEA 8L6 (in case of TK-4 NPs) or the 70/30 mixture of PEAs 8L6/8R6 (in case of TK-8 NPs) was dissolved in 1.0 mL of DMSO (organic phase) along with the 6.0 µg of fluorescein diacetate (i.e. 0.1 w% from the polymer weight). A half portion of the biodegradable surfactant PEG-PEA (25.0 mg) was also dissolved in the organic phase. Afterwards, the prepared organic phase was added dropwise (dropping rate 12 drops/min) to 10.0 mL of water (inorganic phase) containing another half of the surfactant PEG-PEA (25.0 mg) at a stirring rate of 700 rpm using a magnetic stirrer. Note, that in case of TK-4 and TK-8 NPs the surfactant PEG-PEA was equally distributed between organic and water phases.

In all cases, after adding the organic phase, the aqueous phase became turbid indicating formation of NPs. The suspensions of the NPs, obtained after the complete addition of the organic phase, were stirred for 10-15 min and then dialyzed against distilled water for 2 h using the dialysis bag with MWCO 25 kDa to remove the organic solvent and residual surfactant. After dialysis the volume of suspension was reduced to 10.0 mL by evaporating water on a rotary evaporator under reduced pressure. The obtained nanosuspensions were stored in a refrigerator at 4-5°C.

2.4. NPs' size, size distribution and zeta-potential

The obtained PEGylated NPs were characterized by size (Average Diameter - AD), size distribution (Polydispersity Index - PDI), and zeta-potential (ZP), which were determined by dynamic light scattering (DLS) using a particle size analyzer (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK) at 25 °C. The AD and PDI are presented as an average

of five measurements ± standard deviation (SD). The PDI < 0.04 corresponds to a narrow distribution, $0.04 \leq \text{PDI} \leq 0.16$ – to a mean distribution, and PDI > 0.16 – to a wide distribution.

2.5. Application of NPs onto the murine eyes and analysis of results

3. 10 m Results and discussion

3.1. Selection of the PP-PEAs

The PP-PEA 8L6 composed of L-leucine (**L**), 1,6-hexanediol (**6**) and sebacic acid (**8**) was selected as a basic polymer for preparing the various types of NPs (non PEGylated and PEGylated). We have found this PP-PEA as an optimal for fabricating resorbable NPs in terms of storage and cell compatibility [26]. For imparting a positive charge to the NPs that enhances both their stability and cellular uptake [29,30], arginine-based cationic PP-PEA 8R6 was used. Among recently designed arginine-based PP-PEAs [27] 8R6 showed desirable hydrophobicity - it dissolved in water only upon heating to 60–70 °C and precipitated when cooled to r.t. We assumed it would be retained by the NPs, i.e. would not easily be washed out from the NPs in the water phase. Note, the new biodegradable PP-surfactant PEG-PEA that at the same time represents the PEGylating agent was selected for preparing both the surface and bulk PEGylated NPs. The selected PEG-PEA contains backbone fragments similar to the backbones of 8L6 and 8R6 that provides a high affinity between these polymers, that in turn, should provide a firm anchoring of the PEG-PEA with NPs made of the 8L6 or 8L6/8R6 blend. The structures of the selected PEAs are depicted in Scheme 1, their MWs determined earlier and reported in our previous work [28], are given in Table 1.

Table 1. MW characteristics of the PEAs

Polymer	M _w	M _n	D
8L6	76,100	44,200	1.72
8R6	17,500	7,200	2.43
PEG-PEA	36,800	28,400	2.58

3.2. Fabrication of various types of fluorescently-tagged NPs

As noted above in the section 2 the different types of fluorescently-tagged NPs have been prepared us-

ing the nanoprecipitation method. All eight types of the obtained NPs labelled as TK-1 NPs, TK-2 NPs, etc. (see Table 2) can be divided into two groups – negatively charged NPs (from TK-1 to TK-4 NPs) and positively charged NPs (from TK-5 to TK-8 NPs). The negatively charged NPs were prepared on the basis of PP-PEA 8L6 whereas the positively charged NPs were fabricated on the basis of mixture of PP-PEAs 8L6/8R6 (70/30 w%). We suppose that the negative charge (i.e. negative Zeta-Potential, ZP) of the NPs is caused by a partial hydrolysis of the ester links of the PEAs generating free carboxyl groups (carboxylate anions $-\text{COO}^-$). As regards the positive charge of the NPs, it is provided by guanidine groups of the cationic PP-PEA 8R6.

The results given in Table 2 show that the size (Average Diameter, AD) of the obtained NPs varies within 68.5 – 130.2 nm. AD of the negatively charged NPs (samples from TK-1 to TK-4 NPs) is smaller than the AD of the positively charged NPs (samples from TK-5 to TK-8 NPs) - the AD of negatively charged NPs varies from 68.5 to 97.6 nm whilst the AD of positively charged NPs varies from

115.2 to 130.2 nm. With regard to the particle size distribution as we can see from Table 2, obtained fluorescently-tagged NPs showed mean ($0.04 \leq \text{PDI} \leq 0.16$) to wide size distribution ($\text{PDI} > 0.16$).

3.3. Stability of the NPs

All types of the obtained fluorescently tagged NPs were studied for stability upon storage at low temperature. The NPs' AD and PDI were measured right after the fabrication and then the NPs' suspensions were stored refrigerated at 4-5 °C. After pre-determined time (30, 60, and 90 days), the suspensions were thoroughly shaken and analysed for the AD and PDI.

The results, listed in Table 3, show that the fabricated NPs were highly stable – no substantial change of the AD and PDI, or aggregation is observed after 90 days of storage. Along with the standard surfactant Tween 20 the new biodegradable PEG-PEA provided good stabilization of the NPs as well.

Table 2. *The obtained NPs and their parameters*

Type of NPs	AD (nm) \pm SD	PDI \pm SD	ZP (mV) \pm SD
TK-1 NPs (Non PEGylated)	68,5 \pm 3,1	0,228 \pm 0,006	-24,2 \pm 1,7
TK-2 NPs (Surface PEGylated)	73,4 \pm 7,2	0,200 \pm 0,012	-19,0 \pm 0,4
TK-3 NPs (Surface PEGylated)	70,1 \pm 2,3	0,188 \pm 0,002	-14,5 \pm 1,2
TK-4 NPs (Surface and bulk PEGylated)	97,6 \pm 2,6	0,112 \pm 0,008	-14,7 \pm 1,1
TK-5 NPs (Non PEGylated)	115,2 \pm 3,8	0,117 \pm 0,009	+23,2 \pm 1,3
TK-6 NPs (Surface PEGylated)	118,3 \pm 4,1	0,142 \pm 0,013	+15,5 \pm 1,1
TK-7 NPs (Surface PEGylated)	125,7 \pm 4,3	0,221 \pm 0,014	+6,9 \pm 1,2
TK-8 NPs (Surface and bulk PEGylated)	130,2 \pm 3,8	0,143 \pm 0,011	+7,5 \pm 0,4

Table 3. *The stability of the prepared NPs upon storage at 4-5°C*

Type of NPs	Time			
	Freshly prepared	After 30 days	After 60 days	After 90 days
AD (nm) \pm SD				
	[PDI \pm SD]			
TK-1 NPs	68.5 \pm 3.1 [0.228 \pm 0.006]	69.1 \pm 2.3 [0.221 \pm 0.012]	72.3 \pm 4.8 [0.218 \pm 0.007]	71.3 \pm 2.4 [0.219 \pm 0.013]
TK-2 NPs	73.4 \pm 7.2 [0.200 \pm 0.012]	75.1 \pm 2.3 [0.199 \pm 0.009]	74.0 \pm 2.1 [0.203 \pm 0.011]	74.8 \pm 1.2 [0.200 \pm 0.012]

TK-3 NPs	70.1 ± 2.3 [0.188 ± 0.002]	72.2 ± 1.3 [0.181 ± 0.006]	70.4 ± 1.9 [0.179 ± 0.005]	71.8 ± 2.3 [0.178 ± 0.009]
TK-4 NPs	97.6 ± 2.6 [0.112 ± 0.008]	99.2 ± 3.2 [0.119 ± 0.006]	95.8 ± 3.4 [0.129 ± 0.012]	98.3 ± 2.8 [0.121 ± 0.011]
TK-5 NPs	115.2 ± 3.8 [0.117 ± 0.009]	113.6 ± 3.4 [0.119 ± 0.007]	118.2 ± 2.7 [0.122 ± 0.008]	116.1 ± 3.6 [0.124 ± 0.010]
TK-6 NPs	118.3 ± 4.1 [0.142 ± 0.013]	110.5 ± 1.9 [0.139 ± 0.012]	115.6 ± 2.1 [0.141 ± 0.011]	108.3 ± 1.2 [0.149 ± 0.013]
TK-7 NPs	125.7 ± 4.3 [0.221 ± 0.014]	118.4 ± 5.1 [0.229 ± 0.012]	121.9 ± 3.8 [0.219 ± 0.009]	119.2 ± 4.1 [0.218 ± 0.006]
TK-8 NPs	130.2 ± 3.8 [0.143 ± 0.011]	131.4 ± 3.1 [0.151 ± 0.016]	128.7 ± 4.2 [0.140 ± 0.009]	129.3 ± 4.4 [0.136 ± 0.010]

3.4. Penetration of the NPs into murine eyes

After giving drops of the NPs onto ocular surface of the mice, penetration of NPs into the eyes was checked by inspecting fluorescence at 488 nm in the frozen sections. As fluorescence becomes visible only after cleavage of the ester bond in the FDA molecule, only those NPs can be visualized that were taken up and digested by cells in the ocular tissues. We checked the different parts of the eye for fluorescent dots, which would indicate phagocytosed NPs. Results of a semi-quantitative evaluation of the extent of appearance of fluorescent dots are shown in Table 4.

We first inspected the cornea of the treated eyes, as localization of NPs would be anticipated there in the first instance. Fluorescence microscopy images of sections of the cornea are shown in Fig. 1. Besides a faint autofluorescence of the ocular tissue, some scattered bright dots are visible in the cornea (arrowheads in Fig. 1). Such dots do not appear in sections of eyes without NPs. These dots are located in the corneal epithelium and also in the stroma. Notably, they can be found only in the vicinity of the limbus between the cornea and the sclera and not in the central parts of the cornea.

Table 4. The obtained NPs and their parameters

Frozen section	Marginal Cornea	Central Cornea	Lens	OS/IS	Nearby OPL	Sclera
TK-1 NPs	++	-	-	+	++++	++
TK-2 NPs	+	-	-	+	-	+
TK-3 NPs	+	-	-	+	+	+
TK-4 NPs	+	-	-	++++	-	+
TK-5 NPs	++	-	-	+	+	+
TK-6 NPs	+	-	-	+++	?	+
TK-7 NPs	+	-	-	+	+	++
TK-8 NPs	+	-	-	+++	-	+

Symbols: + ...++++ NPs are present to a different degree; - no NPs are visible; ? NPs not clearly visible.

Fluorescent dots were found also deeper in the eye, in the retina. Again, the tissue shows a faint autofluorescence, except for the photoreceptor inner segments and outer segments that show a higher autofluorescence due to rhodopsin, which is present there. Whereas all eight kinds of NPs were found to a similar extent in the cornea, there are clear dif-

ferences between the types of NPs regarding their presence in the retina. Most fluorescent dots were seen when the NP TK4 were used, and such dots were also seen if TK6 or TK8 were applied (arrowheads in Fig. 2). Some more fluorescent dots were visible in the outer plexiform layer and, in a few cases, in the inner retina.

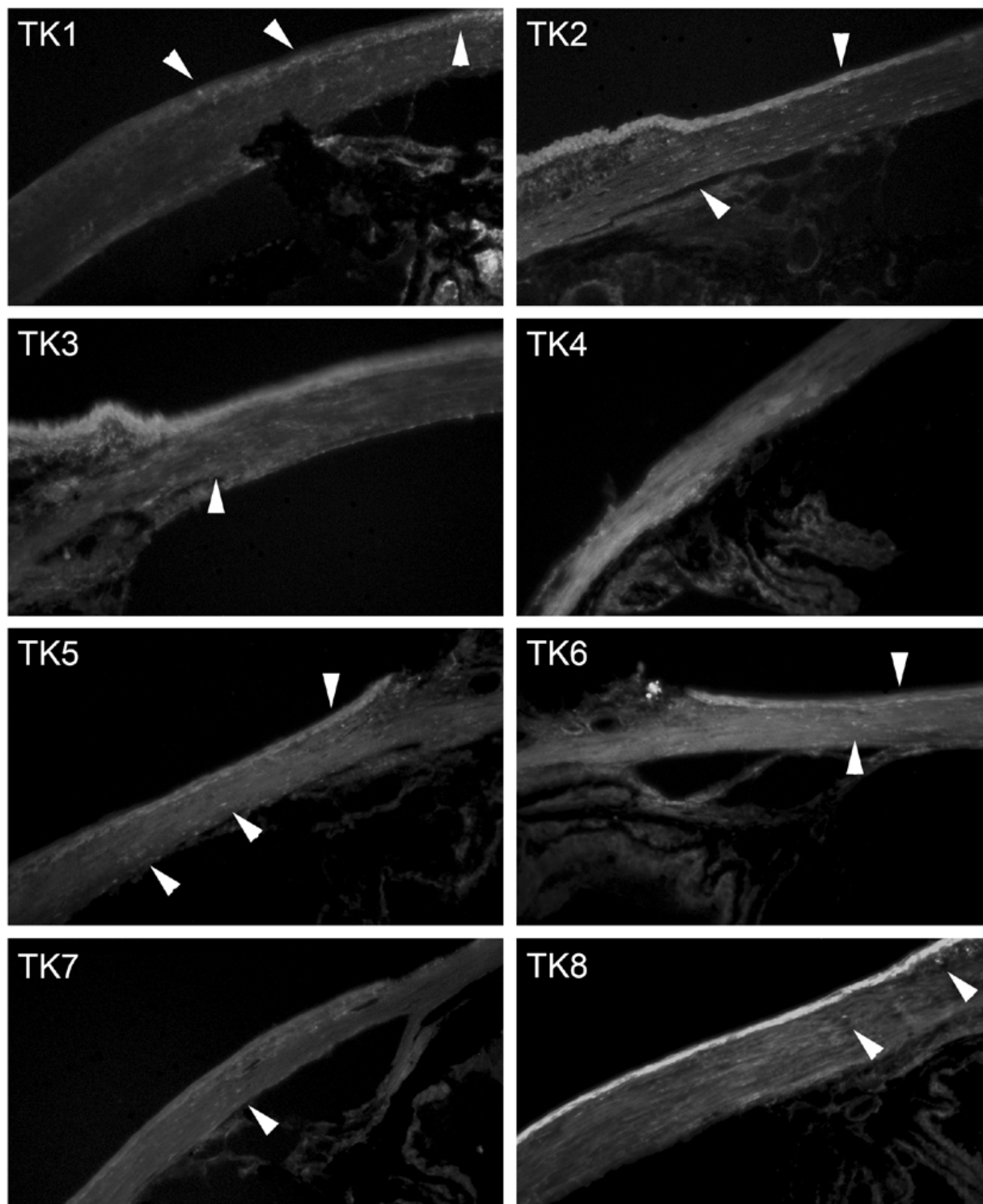


Fig. 1. Fluorescence images of the cornea nearby the limbus in frozen sections of murine eyes after topical administration of NPs as indicated. Arrowheads point to fluorescent dots. Scale bar: 100 μ m.

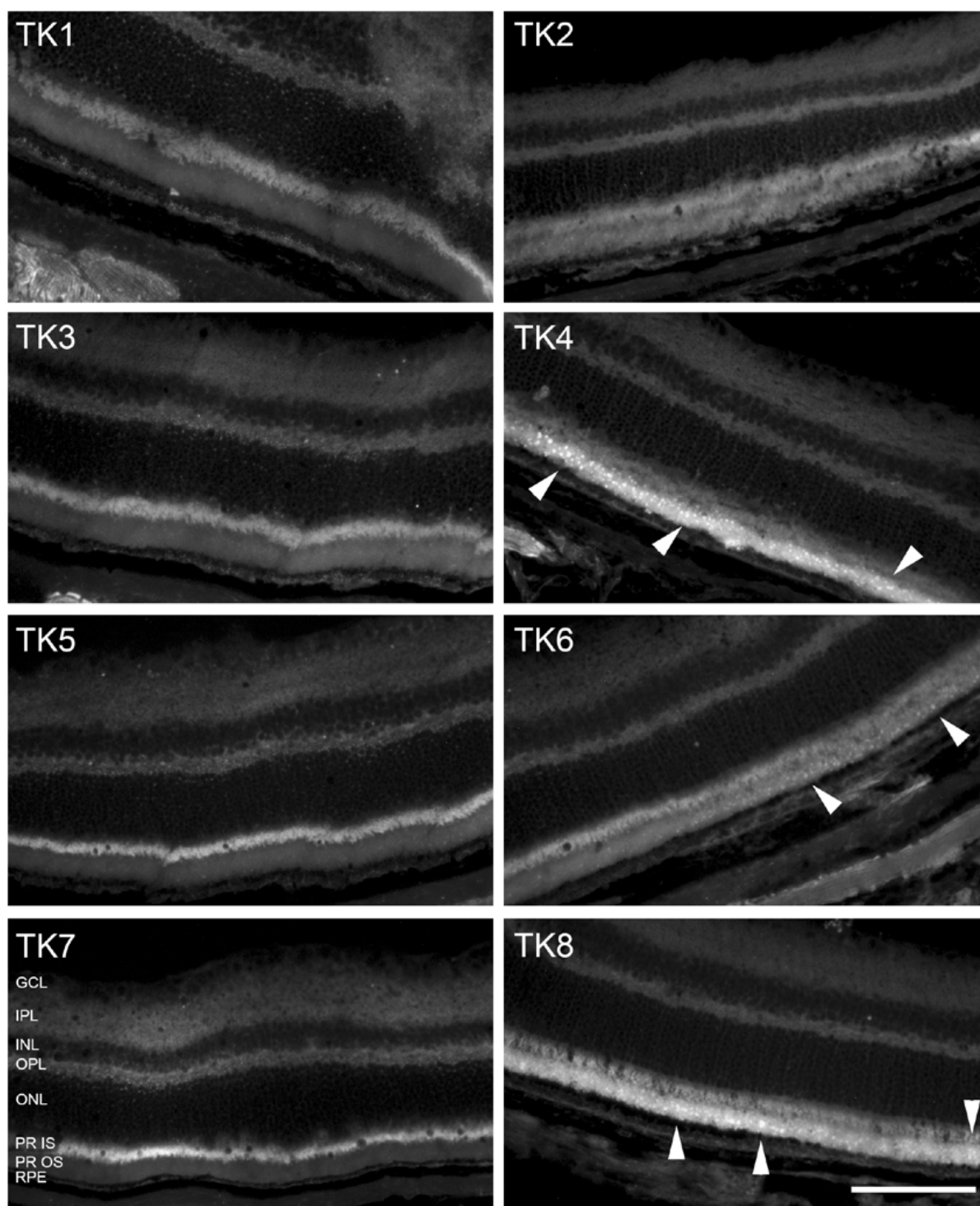


Fig. 2. Fluorescence images of the retina in frozen sections of murine eyes after topical administration of NPs as indicated. Arrowheads point to fluorescent dots. Scale bar: 100 μ m. Layers of the retina in a histological section: GCL – ganglion cell layer; IPL – inner plexiform layer; INL – inner nuclear layer; OPL – outer plexiform layer; ONL – outer nuclear layer; PR IS – photoreceptor inner segment; PR OS – photoreceptor outer segment; RPE – retinal pigment epithelium.

4. Conclusion

Eight different types of PP-based NPs –negatively/positively charged and PEGylated/non-PEGylated ones, loaded with fluorescein diacetate as a fluorescent probe were successfully obtained. All

the NPs were studied for the penetration through the ocular barriers using adult wild-type C57BL/6J mice. The fluorescent dots were found in the sclera after topical application of the NPs. Whereas this appears to be intelligible, the appearance of such dots within the eye, in the retina, was surprising.

At the moment, we cannot explain mechanisms by which the NPs may enter the eyes. Further studies will be performed using different fluorescent dyes to be able to trace the administered NPs within the eyes. The present results raise the hope that the used NPs can be suitable in the future to deliver ocular drugs to the diseased eye.

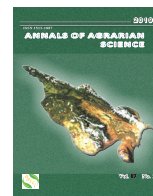
Acknowledgments

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Intensified Technology of the New Type Potassium Fertilizer Production

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ABSTRACT

Soil is the important component of the ecological system on the basis of which plants grow making in their turn an important link in the food chain. In restoration of soil fertility the various fertilizers are used among which potassium fertilizers are important.

We have researched the method of potassium fertilizer production with use of wasteless, ecologically poor technology. The fertilizer is produced with the non-traditional, combined sorption and membrane method, where the Georgian natural zeolite - clinoptilolite is used as substrate and the Black sea water as a potassium ions source. Also we used electro dialysis for preliminary concentration of seawater. We have matched and developed the optimal parameters of operation of electro dialysis apparatus providing excess of potassium concentration indexes over the same for the other ions. The method is prospective for the countries of the Black sea region with deficit of potassium fertilizers and availability of the recourses of zeolites.

Keywords: Potassium fertilizer, Ion-exchange sorption, Electro dialysis concentration, Seawater, Georgian natural zeolites, Zeoponics.

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Introduction

Welfare of human society depends to a large extent on state of land resources and its fertility. Soil gradually loses the micro- and macronutrient elements, but their recourses in the oceans grow. Such growing is caused with soil erosion, as well as rivers flowing thereto and atmospheric precipitations.

One of the variants of elimination of micro- and macroelements deficit includes processing of the world ocean recourses. The scientists noticed possibility of extraction of rare metals from seawater. The rare metals contain in seawater in ionic state, what simplifies their extraction.

The Georgian and Russian scientists experimentally proved possibility of extraction of poor magnesium, potassium, sodium, bromine, lithium and the other valuable microelements [1-8].

At the same time, a new generation fertilizers attract more and more attention. It is so called meliorants – zeoponics, a kind of zeolite enriched with potassium and the other macro- and micronutrients.

Their production technology is based on dependence of sorption processes on temperature [9-13].

The new type fertilizer rules out accumulation of nitrates in plants and their damage with the pathogenic microbes; it improves the biochemical processes in plants, simultaneously decreases possibility of accumulation of toxic substances in plants and further in food. Potassium-enriched products may be used separately as a full-value fertilizer or as a part of combined fertilizers. It improves also structure of soil [14-17].

A number of works covers development of sorption technology and research of physicochemical regularity [1, 3, 9, 11]. It is established that change of temperature of sorption causes sharp change of selectivity of sorbent to metal ions. The experiments prove the selectiveness series given in the writings: $Cs > K > Sr = Ba > Ca >> Na > Li$ [1, 3, 4, 10].

The essence of the method includes use of the cheap natural sorbents with capacity of selective extraction of the ions of alkaline and alkaline-earth metals. Separation of seawater components for pro-

duction of potassium-enriched solution is provided on clinoptilolite by so called dual temperature ion-exchange method. Kinetics of metal ion sorption is researched in static [4] and further in the dynamic conditions [10, 18]. The experiment proves that the process runs by the way of replace of one kind of ion by the other at change of temperature.

Specifically: at low temperature K^+ retains at clinoptilolite, while Ca^{+2} and Mg^{+2} move into filtrate and vice versa, at high temperature Ca^{+2} and Mg^{+2} retain at clinoptilolite, but the potassium ions move into filtrates.

It is known that in comparison with the other waters, in the Black sea these components including potassium are twice less. So, we have to process a great amount of seawater to receive the final product. Aiming this, we have researched combination of the sorption method with the method of electro-dialysis concentration.

We modified the process of sorption of potassium on clinoptilolite: aiming intensification, we used

seawater concentrate in sorption on the model electro-dialysis plant and aiming to grow concentration of potassium in the concentrate, provided desorption of clinoptilolite enriched with potassium with hot sea water (60-70°C). Resulted this, we received the solution with concentration of potassium higher than the same of sodium.

Objects and methods

In the experiments we used clinoptilolite from Dzegvi mine. Its percent content is: SiO_2 -60.1%, Al_2O_3 -12.86%, Fe_2O_3 - 1.57%, MgO - 1.58%, CaO - 4.54%, Na_2O -1.68%, K_2O -1.38%, H_2O -16.3%, Si/Al -4.13%. Exchange capacity of clinoptilolite is established to be $C_{\Sigma} = 1.91$ mg-eq/g

We fractionated the grained clinoptilolite according to the particles sizes running them through the screens and separated the fraction of 2.0 – 2.5 mm.

We used seawater taken from Batumi harborage. Its Chemical content shown in the table below:

Table. Chemical content (g/l) of seawater used in the experiment.

Initial Sea Water	Na^+	Mg^{+2}	Ca^{+2}	K^+	Cl^-	SO_4^{-2}
Sea Water 1	5,7	0,729	0,200	0,23	11, 224	2,1
Sea Water 2	6,8	0,608	0,240	0,31	9, 184	1,9
Sea Water 3	8,7	0,630	0,340	0,20	9, 999	1,47

We performed seawater concentration experiment on the model electro-dialysis plant which hydraulic scheme is given on Fig. 1

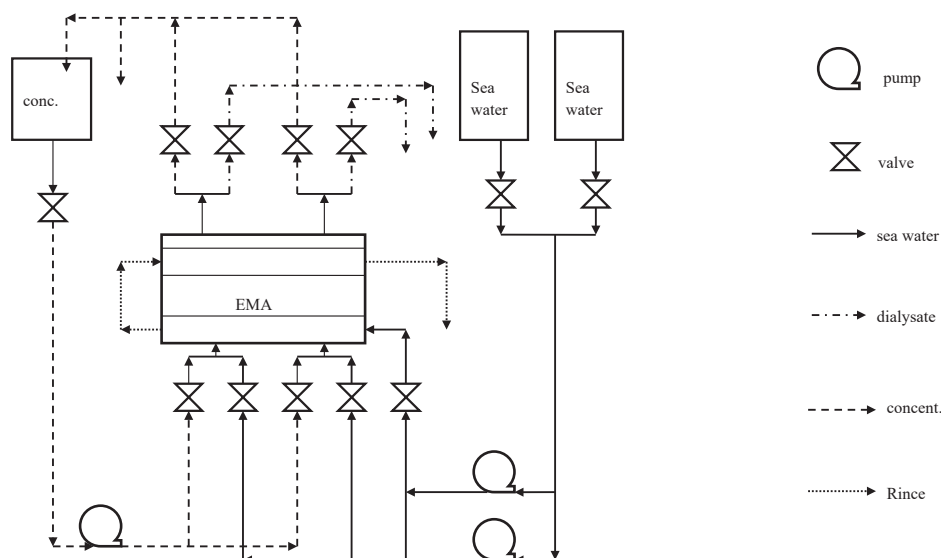


Fig. 1. Hydraulic scheme of the model electro-dialysis plant (Remark: EMA – electromembrane apparatus).

Operational package of the model electro dialysis plant includes 30 dialysates and 30 concentration chambers with parallel-serial scheme and 7 sections.

Electrodialysis plant complex includes:

- two platinum-covered titanium electrodes;
- polypropylene frames – 61 pieces

10 cycles of seawater concentration were performed. In the course of the process dialysate flowed directly, but the concentrate was run by circulation. After each cycle of concentration we changed polarity and hydraulic stream direction on the electrodes in the operational chamber of electro dialysis plant aiming to avoid deposition on electrolytes and membranes. Current strength $I = 3,8 - 6.2A$ (according to temperature), voltage 1 V. On this model plant specific output (μ) was $4.9 \text{ l/m}^2 \text{ h}$. for concentrate. Power consumption for concentration process was $5.3W$ per liter of concentrate.

We researched sorption of potassium ions from seawater concentrate in dynamic conditions in the thermostatic glass columns containing 200g of clinoptilolite ($S=130 \text{ cm}^2$, $h=30\text{cm}$). First we ran it through the first column clinoptilolite at T_1 temperature ($T_1=13-15^\circ\text{C}$), until content of K^+ in filtrate gets equal to the initial content.

On the 2nd stage (desorption of potassium) we used natural seawater. This time, considerable amount of sodium retained on clinoptilolite. We ran hot seawater at T_2 temperature ($T_2= 80-85^\circ \text{C}$) through the column. After that we cooled the concentrate received from the column down to temperature T_1 and ran it through clinoptilolite contained in the 2nd column, where we received the potassium-enriched product.

In research of sorption-desorption processes we determined content of K^+ and Na^+ by the flame-ionization photometry (we used Jenwey flame-ionization photometer, model PFP7), but content of Ca^{+2} and Mg^{+2} – by titrimetric method.

Concentration of the components on sorbent in ion-exchange phase was calculated by the formula:

$$\bar{C}_i = \frac{C_{0,i}(V_n - V_0) - \sum_{i=1}^{i=n} C_i V_i}{m}$$

where $C_{0,i}$ – initial concentration of solution, mg.eq./ml, V_n – volume of solution passed through the column before coming of n fraction (ml), V_0 – free volume in the column determining by solution under sorbent layer in the porous space of the layer, C_i – concentration of i - fraction, m – mass of sorbent (g).

After processing of the results we drew up the tables and the made drawings completely reflecting our results.

Results and Discussions

The results clearly show specificity of change of concentration of the various elements in seawater in the process of electro dialysis. According to the data, concentration coefficient $\beta = C \text{ conc}/C \text{ initial}$ equals to: in the first example (cycle N1) – $\beta=4.35$ for potassium, $\beta=2.59$ for sodium; in the second example (cycle N2) – $\beta=4.4$ for potassium, $\beta=2.87$ for sodium; in the third example (cycle N7) – $\beta=3.48$ for potassium, $\beta=2.6$ for sodium. Concentration coefficient for sulphate ion is 1.9.

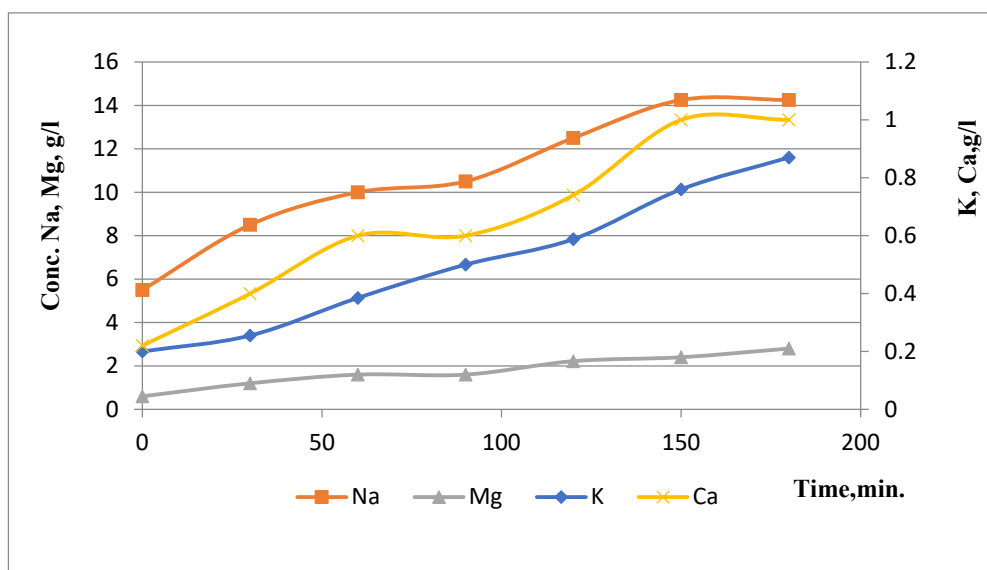


Fig. 2. Electrolysis Concentration of Seawater; cycle N1.

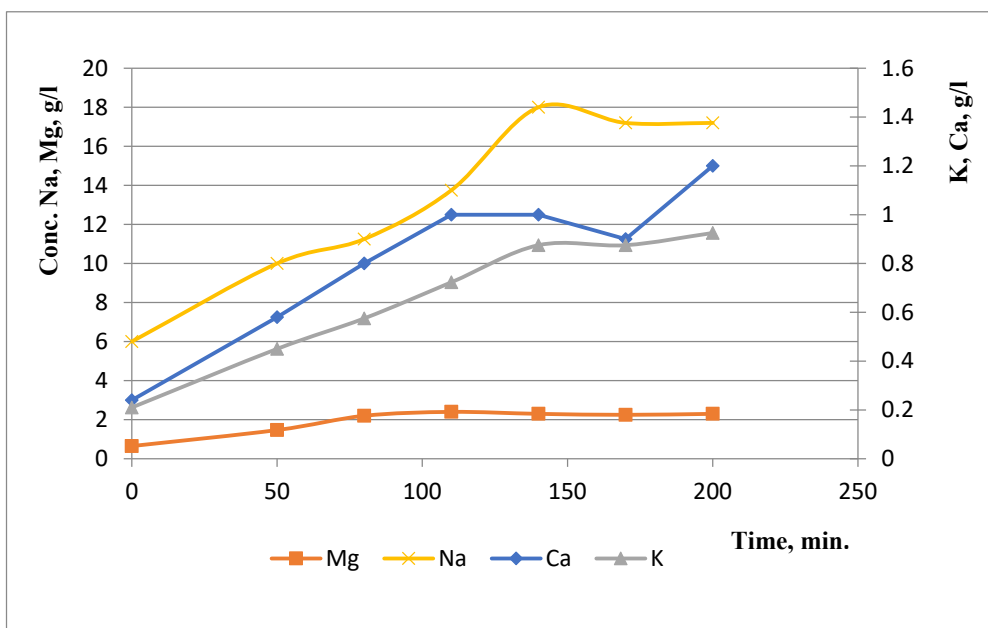


Fig. 3. Electrolysis Concentration of Seawater, cycle N2
 The experiment proved irregularity of the indexes received for concentration of sulphate ions.

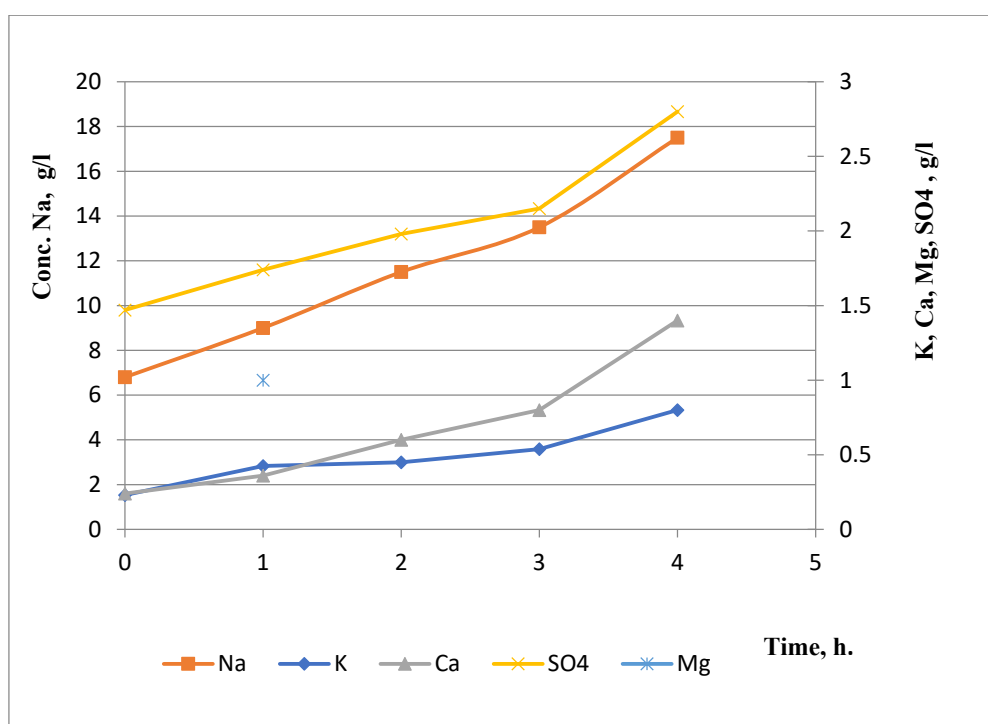


Fig. 4. Electrolysis Concentration of Seawater, cycle N7

In the course of the both concentration cycles $\beta = C_{conc} / C_{initial}$ was 3.8-4.4 for potassium ions and 1.87-2.87 for sodium ions, i.e. potassium ions concentration index was twice more than sodium ions concentration index.

The sorption-desorption dual temperature ion-exchange mode of ions separation showed the regularity according to which, the process of

potassium ions sorption was accompanied with desorption of calcium ions, light desorption of magnesium ions, but sodium ions concentration practically remained unchanged. Fig. 5 shows change of quantity of potassium (mg) retained on clinoptilolite together with change of potassium ions concentration (mg/l) in filtrate in the process of sorption from electro dialysis concentrate

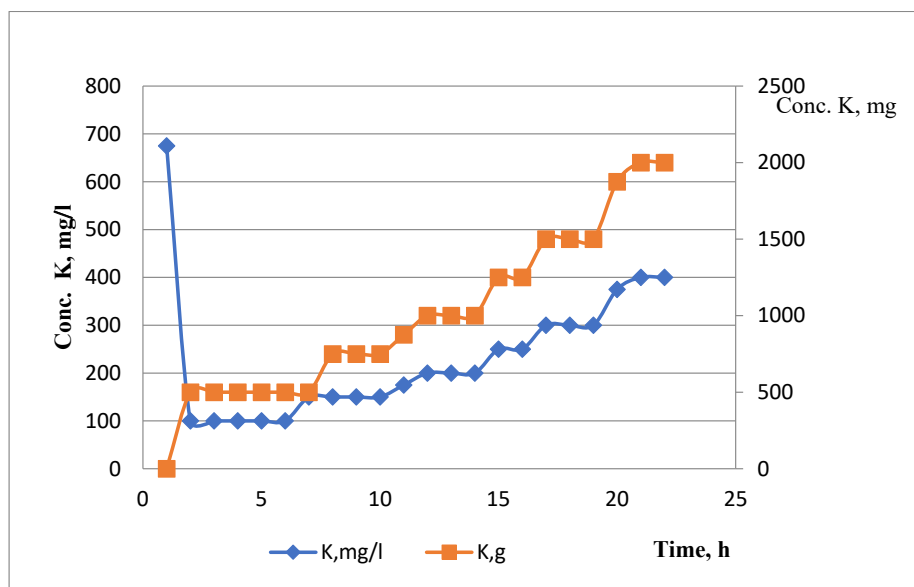


Fig. 5. Change of potassium ions concentration (mg/l) in the process of sorption from electro dialysis concentrate.

Difference was revealed also in the process of desorption with hot seawater: potassium desorption was accompanied with the bivalent ions sorption, but sodium concentration remained unchanged (Fig. 6)

Processing of potassium-enriched clinoptilolite allowed ions separation with twentyfold volume hot seawater. In case of seawater general salinity 17g/l we received the concentrate with potassium concentration of 0.6 g/l and calcium concentration of 0.1g/l what triply exceeds the results of the last research. According to the practical considerations, continuation of the process up to constant meaning of potassium ions concentration is not purposeful. Maximum concentration of retained potassium on clinoptilolite in the balanced condition equals to 4%.

lomite in the balanced condition equals to 4%.

The experiment was performed both in the laboratorial conditions and with the experimental model with production of the sample of zeolite fertilizer.

Conclusion

Thus, on the basis of the natural zeolite (clinoptilolite) we produced the potassium –enriched fertilizer as nitrate-free, ecologically poor product. It has prolonged action, i.e. capacity of long-time regulation of ion-exchange processes.

We have matched electro dialysis membranes (MK-40 and MA-40), determined the parameters of

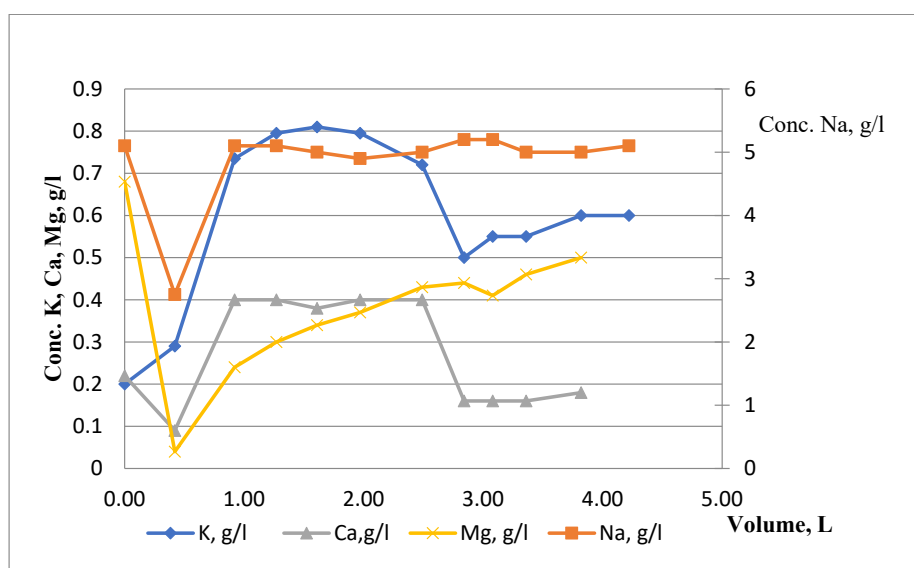


Fig. 6. Change of ions concentration in desorption with hot seawater

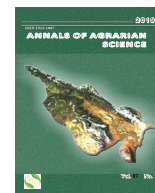
technological process providing excess of concentration indexes of potassium ions over the other ions concentration indexes in seawater concentrate. Use of electro dialysis in the process allowed to make concentration of potassium ions in seawater 4 times more, resulting intensification of the process.

Cheapness and availability of the raw material (the natural zeolite, clinoptilolite from Dzegvi and Tedzami mines) is used as a substrate and seawater – as a source of potassium ions) make this method very interesting and prospective for the countries with clinoptilolite stocks, located in the seaside regions and engaged in agrarian industry (Georgia, Greece, Romania, Bulgaria).

These products may be consumed by small or large scale factory-farm enterprises or private farms.

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Scale insects (Hemiptera: Coccoomorpha) on apple and neighbouring plants in Eastern Georgia (Sakartvelo)

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ABSTRACT

Surveys of the scale insect fauna of East Georgia were carried out in vegetation season (from March to November) during the years 2017 and 2018. A total of fifty-five apple orchards were studied in Kartli and Kakheti regions; 7 of them in Kakheti region and 48 in Shida Kartli region (Gori, Kareli). In Shida Kartli, most apple orchards had been sprayed heavily with chemicals (14 to 22 times). In 2 unsprayed orchards, intensive settlements (level 3) of San Jose scale, *Comstockaspis perniciosus* (Comstock) (Hemiptera: Diaspididae) was noted – Kheltubani (42°04'02"N, 44°09'42"E) and Kere (42°10'31"N, 44°04'51"E). *Comstockaspis perniciosus* was found in 50 % of the branches and twigs and also on the leaf petioles. As for the remaining orchards, the scale insect infestations in Sasireti and Gombori were less intense (level 2), whilst in 19 apple orchards the infestations were very low (level 1). Additionally, neighbouring fields were searched for scale insects. All plants around the apple orchards were searched and checked visually. In total, 51 species of scale insects were recorded: Acanthococcidae (2 spp.), Asterolecaniidae (1 sp.), Coccidae (13 spp.), Cryptococcidae (1 sp.), Diaspididae (18 spp.), Eriococcidae (2 spp.), Kermesidae (1 sp.), and Pseudococcidae (13 spp.).

Keywords: Diaspididae, Asterolecaniidae, Pseudococcidae, Coccidae, Kermesidae Apple orchard.

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Introduction

Scale insects (Hemiptera: Coccoomorpha) are small and hardly visible sap-sucking insects [1, 2, 3]. There are about 8000 species in the world [11]. They have soft bodies covered with a waxy test or wax like filaments [1]. The waxy test protects the insect from drying, and unfavorable environmental conditions and from pesticides [1, 2, 3]. Scale insects have well pronounced sexual dimorphism [1]. Males are more typical insects-like, with wings and legs, whereas females have no wings at all and are often legless [1, 2, 3]. Male scale insects have complete metamorphosis, whereas female development is paedomorphic (adults resemble nymphs) [1]. Some scale insects have remarkable diversity in their genetic systems (e.g., parthenogenesis, hermaphroditism, and paternal genome elimination),

chromosome number, sperm structure, and types of endosymbiosis [2]. The female lays eggs (oviparity) in a cavity under her body or within a waxy covering (ovisac) that may be attached to her body, or the eggs may be retained in the reproductive tract until they are ready to hatch (ovoviviparity) [1, 2, 3]. The mobile first-instar nymphs, called crawlers, are the main dispersal agents for scale insects; other immature instars generally are sessile [1, 2, 3]. The number of generations a year varies often within species and ranges from one to up to seven or eight [1]. Scale insects feed from the phloem or parenchyma, and their host associations range from monophagous to polyphagous [1, 2, 3]. Sap removal is the main cause of plant damage, but a few species of mealybugs and armored scales also transmit plant pathogens or toxins that may further reduce plant vigor and eventually kill the host [1, 2, 3]. They are

pests of fruit trees, vineyards, and ornamental plants [1, 2, 3]. Mealybugs and soft scales excrete honeydew, which is consumed by ants and the ants protect them from parasites and predators in exchange, i.e. have a mutualistic relationship [2, 3]. Honeydew often accumulates on leaves and fruit where saprophytic sooty molds grow, causing the blackness [4].

Many scale insects are economically important pests of agriculture, horticulture, and forestry [1]. The major scale insect pests worldwide are mealybugs (Pseudococcidae), but other serious pests include some armoured scales (Diaspididae), soft scales (Coccidae), lac insects (Kerriidae), and ice-ryines (Monophlebidae) [1, 2]. Many scale insects have been considered to be important pests for Georgian agriculture [4]. Scale insects have a considerable number of natural enemies. The most important predators of scale insects are ladybird beetles (Coleoptera: Coccinellidae); especially species of *Rodolia* spp, *Chilocorus* spp, and *Cryptolaemus* spp. [1]. The main parasitoids of scale insects are chalcidoid wasps, especially species of Aphelinidae (e.g., species of *Aphytis*, *Encarsia*, and *Coccophagus*) and Encyrtidae (e.g., species of *Anagyrus*, *Microterys*, *Leptomastix*, and *Metaphycus*), although some scale insects are attacked by flies that may be either parasitic (e.g., Cryptochaetidae) or egg predators (e.g., a few Cecidomyiidae) [2, 3]. The parasitoid complex of various scale insects had been studied from Georgia [5, 6, 7, 8, 9], however many pestiferous scales still remain poorly studied, in terms of their natural enemies.

Scale insects may act as pests to any kind of the plants [10]. In the case of apple, they settle on trunk, branches, twigs, leaves and fruits [10]. Both armoured scales and soft scales settle on the surface of the apple fruit [10]. Yellow, red and brown gouts appear on the damaged skin of the fruit and therefore, such product loses visual effect [4]. Apple is a very important crop because its fruit contains many minerals and vitamins thus, it has curative traits [11]. Out of various types of fruits, apple takes the biggest part in human nutrition ration [11]. Apple is the most cultivated crop in the Eastern part of Georgia (mainly in Kartli region), but apple gardens and isolated trees can be seen in the western part as well, such as Racha and Imereti regions [personal observation]. According to the FAO data 68.600 tons of apples were harvested in Georgia in 2013 [12]. Apple productivity can be reduced because of diseases and pest insects, particularly the scale insects. In the surroundings of intensive apple production region

(Kartli) there is a diverse composition of agricultural and wild plants, such as cherry, apricot, plum, willow, poplar and wild apple.

In Georgia are three main groups of scale insects: armored scale insects (Diaspididae), which are represented by 90 species belonging to 38 genera, mealybugs (Pseudococcidae) with 71 species belonging to 31 genera and the soft scale insects (Coccidae) with 43 species belonging to 23 genera [13].

Twenty seven scale insect species related to apple (*Malus domestica* L. and *Malus orientalis* L., Rosaceae) have been recorded around the world [14]. According to the literature data of Hadzibeyli [4], 15 apple (*Malus* sp.) related species have been revealed in Georgia. Japoshvili [6] recorded three species of coccids on apple: *Lepidosaphes ulmi*, *Comstockaspis perniciosus* (as *Quadraspidiotus perniciosus*) and *Phenacoccus* sp. (i.e, *Ph. mespili*).

In this study our goal was to determine the scale insect fauna of the apple orchards and its neighboring plants in East Georgia, to investigate their current composition, conditions and importance.

Materials and methods

Surveys of the scale insect fauna of East Georgia were carried out during 2017 and 2018 vegetation seasons. Fifty-five apple orchards had been studied in Kartli and Kakheti regions. Field trips started on 7th of May and end on 23 of September. There were 10 trips, each for 3 days. Fifteen random apple trees were studied in each garden and neighboring plants around apple orchards.

Invasion intensity of each tree was done by 5 point system, 0 points means that there are no pests on the plant, 1 point – there are separate pest units on leaves, branches or trunk, 2 points – 25% of leaves and branches are populated with scale insects, 3 points – scale insects are present on 50% of the leaves, and also on fruits and branches, 4 points – the plant is almost entirely covered with scale insects, fall of leaves and fruits, withering of the branches is also visible [4].

From each damaged tree 5 cm² of the damaged bark was taken. Damaged 10 cm long twigs and fruits were collected and placed in packages, which were labelled. In the laboratory, a few specimens (about 20 individuals) were taken from the plant carefully. Specimens were stored in 70% ethanol (for morphological studies). Slide mounting was done according to Wilkey's method [1]. Species identification were done by using different keys [1, 15, 16].

Apple (*Malus* sp.) pests are indicated with one asterisk in the text. New records for Georgia are indicated with two asterisks. The numbers of slide mounted material are shown in brackets.

Results

We investigated 55 apple orchards, 7 of them are in Kakheti region and 48 in Shida Kartli region (Gori, Kareli). In Shida Kartli most apple orchards were sprayed by chemicals. Intensive infestations of scale insects (3 points - scale insects were on 50% of the branches, twigs and also on leaf petioles) were found in 2 orchards at Kheltubani (42°04'02"N, 44°09'42"E) and Kere (42°10'31"N, 44°04'51"E). These orchards were not sprayed with oil-based reagents. As for the rest of the orchards, in Sasireti and Gombori scale insect infestations were 2 points, 19 apple orchards have a 1 point infestation, and the remaining 32 apple orchards there were none, or they were found only on separate trees. See diagram below.

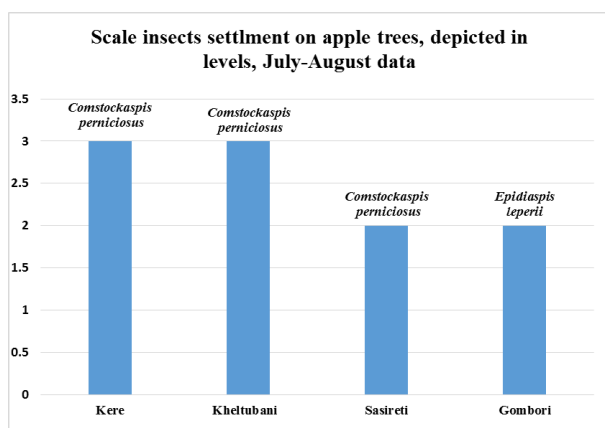


Fig. 1. Scale insect settlement on apple trees. On the graph blue colored columns show points of species settlement in the orchards.

List of species of scale insects in Kartli and Kakheti region:

Family- Acanthococcidae

Genus- Acanthococcus Signoret

1. *Acanthococcus aceris* Signoret

Material examined: 1 ♀, Khintsvi, *Acer* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, 9.VIII.2017, Coll. No: 86. 3 ♀♀, Tsemi (Borjomi), 28.VIII.2017, *Acer* sp., Leg. M. Batsankalashvili, Coll. No: 166. Rusiani, 15.VIII.2017, *Acer* sp., M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 131.

Genus- *Anophococcus* (Signoret)

2. *Anophococcus agropyri* (Borchsenius)

Material examined: 5 ♀♀, Kintsvi, 9.VIII.2017, Poaceae, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 80. 2 ♀♀, Vaziani-Gombori-Telavi road (41°44.23'N, 45°7.403'E), 15.VIII.2017, *Agropyron* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 120.

Family- *Asterolecaniidae*

Genus- *Bambusaspis* Cockerell

3. *Bambusaspis bambusae* (Boisduval)

Material examined: 2 ♀♀, Botanical garden of Tbilisi, *Bambusa* sp. 3.VIII.2017, M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 23.

Family- *Coccidae*

Genus- *Ceroplastes* Gray

4. *Ceroplastes floridensis* (Comstock)**

Material examined: 1 ♀, Botanical garden of Tbilisi, 3.VIII.2017, *Eriobotrya japonica*, M. B. Kaydan, G. Japoshvili, M. Batsankalashvili Coll. No: 32. 2 ♀♀, Botanical garden of Tbilisi, *Laurus* sp., 3.VIII.2017, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili Coll. No: 37; 3 ♀♀, Isani, 12.VIII.2017, *Laurus nobilis*, M. B. Kaydan, G. Japoshvili, M. Batsankalashvili Coll. No: 102.

5. *Ceroplastes rusci* (Linnaeus)**

Material examined: 4 ♀♀, Tbilisi, Abanotubani, 3.VIII.2017, *Prunus laurocerasus*, Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No: 31.

Genus- *Coccus* Linnaeus

6. *Coccus hesperidum* (Linnaeus)

Material examined: 4 ♀♀, Tbilisi, Rustaveli Avenue, 1.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, Kirkitadze, Coll. No: 11. 3 ♀♀, Botanical garden of Tbilisi, 3.VIII.2017, *Laurus* sp.; Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 37. 2 ♀♀, Abanotubani, 3.VIII.2017, *Magnolia grandiflora*, Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No: 49.

7. *Coccus pseudomagnoliarum* (Kuwana)

Material examined: 1 ♀, Mtskheta, 1.VIII.2017, *Celtis* sp., Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No: 43.

Genus- *Eriopeltis* Signoret

8. *Eriopeltis festucae* (Boyer de Fonscolombe)

Material examined: 2 ♀♀, Vaziani-Gombori-Telavi road (41°44'13.80"N, 45°7'24.18"E), 15.08.2017, *Agropyron* sp., Leg.

- M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 120. 2 ♀♀, On the road between Ujarma and Paldo (41°48'18.36"N, 45°08'54.12"E), *Agropyron* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 125.
Genus- *Neopulvinaria* Hadzibeyli
9. *Neopulvinaria innumerabilis* (Rathvon)
Material examined: 1 ♀, Tbilisi, Rustaveli Avenue, *Fraxinus* sp., 1.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 12.
Genus- *Parthenolecanium* Sulc
10. *Parthenolecanium corni* (Bouche)
Material examined: 3 ♀♀, Tbilisi, Vake Park, *Quercus* sp., 1.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 5. 1 ♀, Vera Park, 1.VIII.2017, *Celtis* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 16; 1 ♀, Botanical garden of Tbilisi, *Fraxinus* sp., Leg. M. B. Kaydan, M. Batsankalashvili Coll. No: 51. 1 ♀, Sasireti, 8.VIII.17, *Cydonia oblonga*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 64; 1 ♀, Doesi, 8.VIII.17, *Prunus persica*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 68. Kareli, 9.VIII.17, *Prunus divaricata*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 69; 2 ♀♀, Doesi, (41°56'4.14"N, 44°13'43.56"E), *Prunus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 72; 3 ♀♀, Kintsvisi, 9.VIII.17, *Corylus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 85; 1 ♀, Karaleti, 9.VIII.17, *Cornus sanguinea* subsp. *australis* Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 90; 1 ♀, Near to Mukhrovani (41°47'01.80"N, 45°09'23.82"E) 15.VIII.2017, *Crataegus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 112. 2 ♀♀, Road between Vaziani and Mukhrovani (41°44'13.80"N, 45°07'24.18"E), 15.VIII.2017, *Prunus armeniaca*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 122. 2 ♀♀, Tianeti, 16.VIII.2017, *Fraxinus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 140;
Genus- *Physokermes* Targioni Tozzetti
11. *Physokermes piceae* (Schrank)
Material examined: 1 ♀, Kintsvisi, 9.VIII.2017, *Picea* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 82.
Genus- *Pulvinaria* Targioni Tozzetti
12. *Pulvinaria floccifera* (Westwood)
Material examined: 1 ♀, 3 ♀♀, Botanical garden of Tbilisi, 3.VIII.2017, *Taxus* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 38, 39. 3 ♀♀, Gori, 8.VIII.2017, *Tilia* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 60.
13. *Pulvinaria juglandii* Hadzibeyli
Material examined: 1 ♀, Tiniskhidi, 9.VIII.2017, *Juglans regia*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 56.
14. *Pulvinaria peregrina* Ben-Dov
Material examined: 2 ♀♀, Gori, 8.VIII.2017, *Platanus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 81. 3 ♀♀, Telavi, 16. VIII.2017, *Platanus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 136). Telavi, 16.VIII.2017, *Tilia* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 137.
15. *Pulvinaria vitis* (Linnaeus)
Material examined: 3 ♀♀, Sasireti, 8.VIII.2017, *Vitis vinifera*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 65.
Genus- *Shpaerolecanium* Sulc
16. *Shpaerolecanium prunastri* (Boyer de Fonscolombe)
Material examined: 3 ♀♀, Karaleti, 9.VIII.2017, *Prunus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 83. 1 ♀, Karaleti, 9.VIII.2017, *Prunus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 87; 2 ♀♀, Kareli, 9.VIII.2017, *Prunus divaricata*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 88.
Family- Cryptococcidae
Genus- *Cryptococcus* Douglas
17. *Cryptococcus fagisuga* Lindinger
Material examined: 1 ♀, Kintsvisi, 9.VIII.2017, *Fagus orientalis*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 79.
Family- Diaspididae
Genus- *Carulaspis* MacGillivray
18. *Carulaspis carueli* (Signoret)
Material examined: 2 ♀♀, Tbilisi, Rustaveli Avenue, 1.VIII.2017, *Juniperus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 7; 1 ♀, Vera Park, 1.VIII.2017,

- Cupressus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 8; 2 ♀♀, Vera Park, 1.VIII.2017, *Juniperus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 17.
Genus- *Chionaspis* Signoret
19. *Chionaspis salicis* (Linnaeus)
Material examined: 2 ♀♀, Near to Tetrtsklebi (41°51'53.82"N, 45°17'21.12"E), *Salix* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 129.
Genus- *Chrysomphalus* Ashmead
20. *Chrysomphalus dictyospermi*
Material examined: Tbilisi, Agricultural University of Georgia, Leg. M. Batsankalashvili (150).
Genus- *Comstockaspis* MacGillivray
21. *Comstockaspis perniciosus* (Comstock)*
Material examined: 3 ♀♀, Ateni, 30.VIII.2017, *Malus domestica*, Leg. M. Batsankalashvili Coll. No: 167. 3 ♀♀, Variani, 1.VIII.2017, *Malus domestica*, Leg. M. Batsankalashvili Coll. No: 168; 1 ♀, Tbilisi: Vera Park, 1.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 10. 3 ♀♀, Sasireti, 8.VIII.2017, *Prunus persica*, M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 63. 1 ♀, Sasireti, 8.VIII.2017, *Cydonia oblonga*, M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 64. 3 ♀♀, Doesi, 8.VIII.2017, *Prunus persica*, M.B.Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 68. 2 ♀♀, Sasadilo (41°52'20.34"N, 45°08'18.72"E), 16.VIII.2017, *Pyrus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 141. 1 ♀, Between Tetrtsklebi and Telavi (41°53'12.24"N, 45°21'47.40"E), 15.VIII.2017, *Elaeagnus rhamnoides*, Leg. M.B.Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll.No: 116; 1 ♀, Between Mejriskhevi and Kvarkheti, 31.VIII.2017, *Malus domestica*, Leg. M. Batsankalashvili, Coll. No: 169. 1 ♀, Kheltubani (42°04'1.68"N, 44°09'41.70"E), 30.VIII.2017, *Prunus persica*, Leg. M. Batsankalashvili; Coll. No: 172; 1 ♀, Kheltubani (42°04'1.68"N, 44°09'41.70"E), 30.VIII.2017, *Malus domestica*, Leg. M. Batsankalashvili, Coll. No: 173; 3 ♀♀, Kere (42°10'31.08"N, 44°04'50.58"E), 22.IX.2017, *Prunus persica*, Leg. M. Batsankalashvili, G. Kirkitadze, Coll. No: 182; 1 ♀, Betlemi (41°59'2.94"N, 44°00'1.86"E), 22.IX.2017, *Malus domestica*, Leg. M. Batsankalashvili, G. Kirkitadze, Coll. No: 187; 2 ♀♀, Sasireti (41°55'35.22"N, 44°18'23.10"E), 8.VIII.2017, apple orchard, *Malus domestica*, Leg. M.B.Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 200; 2 ♀♀, Doesi, (41°56'4.14"N, 44°13'43.56"E), 8.05.2017, apple orchard, *Malus domestica*, Leg. M. Batsankalashvili, G. Kirkitadze Coll. No: 212; 1 ♀, Tiniskhidi (41°59'24.96"N, 44°04'18.42"E), 7.05.2017, apple orchard, *Malus domestica*, Leg. M. Batsankalashvili, G. Kirkitadze Coll. No: 213.
Genus- *Diaspidiotus* Berlese
22. *Diaspidiotus marani* (Zahradnik)
Material examined: 2 ♀♀, Tbilisi, Vake Park, 1.VIII.2017, *Fraxinus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 19; 1 ♀, Sasireti, 8.VIII.2017, *Juglans regia*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze Coll. No: 67.
23. *Diaspidiotus ostreaeformis* (Curtis)
Material examined: 1 ♀, Tiniskhidi (41°59'24.96"N, 44°04'18.42"E), 8.VIII.2017, *Prunus domestica*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 62.
24. *Diaspidiotus uvae* (Comstock)**
Material examined: 3 ♀♀, Tiniskhidi, 9.VIII.2017, 41°59'24.96"N, 44°04'18.42"E, *Juglans regia*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 56.
Genus- *Epidiaspis* Cockerell
25. *Epidiaspis leperii* (Signoret)*
Material examined: 3 ♀♀, Tbilisi, Vake Park, *Malus sylvestris*, 1.VIII. 2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 13. 3 ♀♀, Rusiani, *Malus domestica*, 15. VIII.2017, Leg. M.B.Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 132; 2 ♀♀, Sasadilo (41°52'20.34"N, 45°08'18.72"E), *Pyrus* sp., 16.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 141; 2 ♀♀, Askilauri, apple orchard (41°51'58.74"N, 45°10'5.64"E), 3.VI.2017, *Malus domestica*, Leg. M. Batsankalashvili, G. Kirkitadze, Coll. No: 202; 3 ♀♀, Zemo Chocheti, apple orchard, (41°54'29.82"N, 44°20'4.20"E), 7.V.2017, *Malus domestica*, Leg. M. Batsankalashvili, G. Kirkitadze, Coll. No: 216.
Genus – *Kuwanaspis* MacGillivray
26. *Kuwanaspis pseudoleucaspis* (Kuwana)
Material examined: 3 ♀♀, Botanical garden

- of Tbilisi, 3.VIII.2017, Bambusoideae, M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 24.
Genus- *Lepidosaphes* Shimer
27. *Lepidosaphes malicola* (Borchsenius)
Material examined: 7 ♀♀, Kareli, 9.VIII.2017, *Juglans regia*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 70; 4 ♀♀, Vaziani-Gombori-Telavi road (41°44'13.80"N, 45°07'24.18"E), 15.VIII.2017, *Populus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 117; 3 ♀♀, Tianeti, 16.VIII.2017, *Fraxinus*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 140.
28. *Lepidosaphes ulmi* Linnaeus*
Material examined: 5 ♀♀, Tbilisi, Isani, 12.VIII.2017, *Vitis vinifera*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 97; 1 ♀, Apple orchard of Tianeti, 16.VIII.2017, *Malus domestica*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 152.
29. *Lepidosaphes conchiformis*
Material examined: 2 ♀♀, Tbilisi, Vake Park, 1.VIII.2017, *Quercus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 5
Genus- *Leucaspis* Signoret
30. *Leucaspis pusilla* Law
Material examined: 2 ♀♀, Tbilisi, Vake Park, 1.VIII.2017, *Pinus nigra*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 22; 4 ♀♀, Botanical garden of Tbilisi, 3.VIII.2017, *Pinus nigra*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 33.
Genus- *Mercetaspis* Gomez-Menor Ortega
31. *Mercetaspis sureyana* (Bodenheimer)
Material examined: 5 ♀♀, Gori, 9.VIII.2017, *Astragalus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 73.
Genus- *Parlatoria* Targioni Tozzetti
32. *Parlatoria oleae* (Colvee)
Material examined: 2 ♀♀, Tbilisi, Vera Park, 1.VIII.2017, *Crataegus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 9; 4 ♀♀, Vake Park, near to Turtle Lake, 1.VIII.2017, *Rhamnus pallasii*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 20; 2 ♀♀, Uplistsikhe, 9.VIII.2017, *Prunus armeniaca*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 66; 4 ♀♀, Uplistsikhe, 9.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 77; 2 ♀♀, Tbilisi, Isani, 12.VIII.2017, *Prunus domestica*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 94; 2 ♀♀, Tbilisi, Isani, 12.VIII.2017, *Rosa canina*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 101; 1 ♀, Apple orchard of Zemo Choche-ti, 8.VIII.2017, *Prunus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 103; 1 ♀, Telavi, 16.VIII.2017, *Mespilus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 144.
Genus- *Pseudaulacaspis* MacGillivray
33. *Pseudaulacaspis pentagona* (Targioni Tozzetti)
Material examined: 1 ♀, Tbilisi, Isani, 12.VIII.2017, *Ribes* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 99.
Genus- *Salicicola* Lindinger
34. *Salicicola archangelskyae* (Archangelskaya)
Material examined: 6 ♀♀, Tbilisi, Rustaveli avenue, 1.VIII.2017, *Fraxinus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 12; 3 ♀♀, Botanical garden of Tbilisi, 1.VIII.2017, *Fraxinus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No: 51; 5 ♀♀, Tbilisi, Isani, 12.VIII.2017, *Syringa vulgaris*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 95.
Genus- *Unaspis* McGillivray
35. *Unaspis euonymii* (Comstock)
Material examined: 2 ♀♀, Mtskheta, Armazi, 18.VII.2017, *Euonymus* sp., Leg. M. Batsankalashvili, Coll. No: 194.
Family- Eriococcidae
Genus- *Eriococcus* Targioni Tozzetti
36. *Eriococcus williamsi* Danzig
Material examined: 2 ♀♀, Botanical garden of Tbilisi, 3.VIII.2017, *Buxus balearica*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 29; 4 ♀♀, Tkibuli, 13.VIII.2017, *Buxus* sp., Leg. G. Kirkitadze, Coll. No: 159.
Genus- *Rhizococcus*
37. *Rhizococcus lactucae* (Borchsenius) **
Material examined: 1 ♀, Near to Mukhrovani (41°47'1.80"N, 45°09'23.82"E), 15.VIII.2017, *Centaurea* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 108.
Family- Kermesidae
Genus- *Kermes* Boitard

38. *Kermes vermilio* (Planchon)**
 Material examined: 5 First instar larvae, Telavi, 16.VIII.2017, *Quercus ilex*, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze (138).
 Family- **Pseudococcidae**
 Genus- *Antonina* Signoret
39. *Antonina crawi* Cockerell
 Material examined: 3 ♀♀, Botanical garden of Tbilisi, 3.VIII.2017, *Bambusa* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 24.
 Genus- *Dysmicoccus* Ferris
40. *Dysmicoccus angustifrons* (Hall)
 Material examined: 2 ♀♀, Near to Mukhrovani (41°47'1.80"N, 45°09'23.82"E), 15.VIII.2017, *Centaurea* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 108; 1 ♀, Vaziani-Gombori-Telavi road (41°44'13.80"N, 45° 7'24.18"E), 15.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 118.
 Genus- *Heliococcus* Sulc
41. *Heliococcus glacialis* (Newstead)
 Material examined: 1 ♀, Askilauri, 15.VIII.2017, *Melilotus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 105.
42. *Heliococcus sulcii* Goux
 Material examined: 3 ♀♀, Tbilisi, Rustaveli avenue, 1.VIII.2017, *Berberis* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No: 6; 1 ♀, Abanotubani, 03.VIII.2017, *Berberis soulieana*, Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No: 30.
43. *Heliococcus bohemicus* (Sulc)
 Material examined: 3 ♀♀, Gori, 9.VIII.2017, Poaceae, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:75.
 Genus- *Phenacoccus* Cockerell
44. *Phenacoccus pumilus* Kiritshenko
 Material examined: 2 ♀♀, Gori, 9.VIII.2017, *Thymus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:55; 2 ♀♀, Near to Mukhrovani (41°47'1.80"N, 45° 9'23.82"E), 15.VIII.2017, Apiaceae (Umbellifera), Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:110; 3 ♀♀, Vaziani-Gombori-Telavi road (41°53'12.24"N, 45°21'47.40"E), Apiaceae, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:113; 4 ♀♀, Vaziani-Gombori-Telavi road (41°53'12.24"N, 45°21'47.40"E), 15.VIII.2017, herbaceous plant, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:114; 2 ♀♀, Vaziani-Gombori-Telavi road (41°53'12.24"N, 45°21'47.40"E), 15.VIII.2017, Apiaceae, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:115.
45. *Phenacoccus tergrigiorianae* Borchsenius **
 Material examined: 1 ♀, Botanical garden of Tbilisi, 3.VIII.2017, *Amaranthus retroflexus*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:47; 1 ♀, Askilauri, 15.VIII.2017, *Melilotus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:105; 1 ♀, Bochorma (41°54'54.48"N, 45°07'21.12"E), 15.VIII.2017, Herbaceous plant, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:106; 2 ♀♀, Near to Mukhrovani (41°47'01.80"N, 45°09'23.82"E), 15.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:109; 3 ♀♀, Vaziani-Gombori-Telavi road (41°44'13.80"N, 45°07'24.18"E), 15.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:119; 1 ♀, Bochorma (41°54'54.48"N, 45° 7'21.12"E), 16.VIII.2017, Brassicaceae, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:123; 3 ♀♀, Bochorma (41°54'54.48"N, 45° 7'21.18"E), 16.VIII.2017, Brassicaceae, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:124; 2 ♀♀, Bochorma (41°54'54.48"N, 45°07'21.18"E), 16.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:127; 3 ♀♀, Tianeti-Zaridzeebi-Zhinali road (42°08'18.96"N, 44°48'54.06"E), 16.VIII.2017, Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:128; 5 ♀♀, Tbilisi, Isani, *Daucus sativus*, 19.VIII.2017, G. Kirkitadze, Coll. No: 162; 1 ♀, Botanical garden of Tbilisi, 3.VIII.2017, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:164.
46. *Phenacoccus transcausicus* Hadzibeyli*
 Material examined: 3 ♀♀, Tbilisi, Vake Park, 1.VIII.2017, *Buxus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:14; 2 ♀♀, Botanical Garden of Tbilisi, 03.08.2017, *Fraxinus* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:45; 5 ♀♀, Sasireti, 8.VIII.2017, *Prunus persica*, Leg. M.

B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:63; 4 ♀♀, Isani, 12.VIII.2017, *Prunus persica*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:100. 3 ♀♀, Tbilisi, Apple orchard of Agricultural University of Georgia, 19.VIII.2017, *Malus domestica*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:149.

Genus- *Planococcus* Ferris

47. *Planococcus ficus* (Signoret)

Material examined: 4 ♀♀, Abanotubani, 3.VIII.2017, *Platanus* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:44; 2 ♀♀, Tbilisi: Isani, 12.VIII.2017, *Punica granatum*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No: 92; 3 ♀♀, Tbilisi: Isani, 12.VIII.2017, *Ficus carica*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:98.

48. *Planococcus vovae* (Nasonov)

Material examined: 3 ♀♀, Tbilisi, Rustaveli avenue, 1.VIII.2017, *Juniperus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:7; 5 ♀♀, Vake Park, 1.VIII.2017, *Cupressus* sp., Leg. M. B. Kaydan, M. Batsankalashvili, G. Kirkitadze, Coll. No:21; 4 ♀♀, Botanical garden of Tbilisi, 3.VIII.2017, *Cupressus* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:34; 3 ♀♀, Botanical garden of Tbilisi, *Cupressus sempervirens* f. *pyramidalis*, Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili, Coll. No:35.

Genus- *Pseudococcus* Westwood

49. *Pseudococcus comstocki* (Kuwana)

Material examined: 3 ♀♀, Mtskheta, 2.XI.2017, *Morus alba*, Leg. M. Batsankalashvili, Coll. No: 196; 3 ♀♀, Tbilisi, David Agmashenebeli avenue, 22.VIII.2017, *Catalpa*, Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No:161.

50. *Pseudococcus longispinus* (Targioni Tozzetti)

Material examined: 3 ♀♀, Botanical garden of Tbilisi: Orangery, 3.VIII.2017, *Dracaena* sp., Leg. M. B. Kaydan, G. Japoshvili, M. Batsankalashvili Coll. No:25.

51. *Pseudococcus viburni*

Material examined: 3 ♀♀, Tbilisi, Abanotubani, 3.VIII.2017, *Prunus laurocerasus*, Leg. M. B. Kaydan, M. Batsankalashvili, Coll. No:31.

As a result, it was found out that in Kartli gardens is commonly widespread *Comstockaspis perniciosus*.

Altogether 51 scale insect species were collected. 6 species (3, 06 %) proved to be new to the Georgian fauna: *Ceroplastes floridensis* (Comstock), *Ceroplastes rusci* (Linnaeus), *Diastpidiotus uvae* (Comstock), *Rhizococcus lactucae* (Borchsenius), *Kermes vermilio* (Planchon), *Phenacoccus tergrigorianae* Borchsenius.

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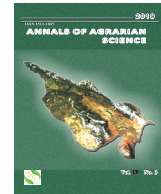
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Patterns of religiosity and modernization in Georgia - rural urban dimensions

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ABSTRACT

The general objective of the presented research is to determine and show trends in secularism through the rural – urban prism. In this paper we have examined secularization at two levels: 1) individual religious beliefs and practice and 2) separation between church and civic domain, such as politics. For decades, sociologists have believed that due to the rational, liberal nature of the city the degree of religiosity and consequently the rate of secularism would fall. Although in Georgian context study has shown the extent of religiosity in the city areas is significantly higher than religiosity in the rural residents. For measure of religiosity has been used The Centrality of Religiosity Scale (CRS) - a differentiated model by Huber, which is constituted by five core dimensions: public practice, private practice, religious experience, ideology and the intellectual dimensions. The findings of the research has shown that there is a significant difference in all dimensions of religiosity between rural and urban residents (except private practice) and the level of these sub-dimensions in urban residents is higher. However, The findings of the research show that Tbilisi (the capital) even though comparatively higher rate of religiosity (the first level of secularization) is considerably more secular in terms of the relationship between politics and religion (the second level of secularization).

Keywords: Religiosity, Modernization, Secularization, Politics, Rural, Urban.

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1. Introduction

The aim of this paper is to examine if religious “revival” somehow implies threat to modernity. What is the specifics of religiosity in modern Georgia? We would like to understand the nature of religious revival in relation to modernity and secularization theories (we will examine secularization on individual and public levels). Since urbanization has been one of the core constituent of modernization [1] we will try to assess religiosity through rural - urban prisms, what kind of differences occur between rural and urban citizens in: overall religiosity, attitudes toward secular and religious interference, also some correlated demographic features as well.

By the end of the last century dramatic religious growth had occurred throughout the former Soviet Union countries [2,3] and Georgia was not an excep-

tion. After the collapse of the Soviet regime, intense national - religious discourses appeared on Georgian political arena. The struggle for independence and the transition period from the communist rule to liberal democracy “was steered by national idea and return to the God’s province, as embodied by Georgian Orthodox Church” [4]. Eventually, social power of the religious institution and authorities sharply increased. On the one hand, the religious institution was the only social actor who could ideologically unify disintegrated (socially as well as politically) citizens, and, on the other hand, the ideological vacuum created by the transition was tried to be partially filled up with traditional religion [4].

After almost three decades Georgian Orthodox Church still maintains strong social influence, for instance, trust in religious institutions is much higher than trust in any political or civic institution

[5]. The process of resurrection of public religion has emerged multitude of studies. Religion has been researched from different disciplines and perspectives (psychology, sociology, political and history studies). Most of them relate relationship between state/nation and religion [6-10]. Some studies focus on religion and education [11], everyday life [12], Trust in Religious Institutions [5], correlation studies of religiosity [13], secular religion [4] and so on.

2. Modernity, religion and secularization:

After the religious revival throughout the world, starting up from the 1990-ies, brought about the necessity to rethink certain theory in social sciences. Namely, secularization theory (see e.g. McLeod) has predicted that secularization and modernization go hand in hand, advocates of secularization theories were arguing that religious beliefs as well behaviors would take the peripheral place in modern society; For Giddens “most of the situations of modern social life are manifestly incompatible with religion as a pervasive influence upon day-to-day life. Religious cosmology is supplanted by reflexively organised knowledge, governed by empirical observation and logical thought, and focused upon material technology and socially applied codes. Religion and tradition has been always closely linked, and the latter is even more thoroughly undermined than the former by the reflexivity of modern social life, which stands in direct opposition to it” [14]. “For nearly three centuries, social scientists and assorted western intellectuals have been promising the end of religion” [15], however, there is no clear evidence regarding deprivation of religion (in some cases we are the witnesses of uprising of religiosity: for instance, post Soviet countries, Islamic states, Latin America or the USA), and this way, legitimate question occurs: is the formation of modern nations directly linked to the parallel and mutually dependent processes of secularization and modernization? [6].

In the debate on usefulness of secularization thesis, Stark states that secularization theory is “useless as a hotel elevator that only goes down”. Stark emphasizes that perhaps religion one day will have been expelled from social space but not because of modernity [15]. To the question whether and how secularization is taking place, Peter Berger [16] says:

I think what I and most other sociologists of religion wrote in the 1960s about secularization was a mistake. Our underlying argument was that secularization and modernity go hand in hand. With

more modernization comes more secularization. It wasn't a crazy theory. There was some evidence for it. But I think it's basically wrong. Most of the world today is certainly not secular. It's very religious.

As it seems secularization theory was simply an illusion of Enlightenment. The “illusion” of secularization has been reinforced by exaggerated perceptions of past religiousness as well [15].

Furthermore, secularization as a notion can mean a number of different things, which can cause additional ambiguities. For instance, MacLeod [17; 18] distinguishes secularization at three levels: the first level involves individual belief and practice, which means to what extent has there been a decline in the proportion of the population having a religious view of the world, belonging to religious organizations or engaging in religious rites? The second level stresses the role of religion in public institutions - the extent of separation between church and state, or church and civic domain (e.g., education system), and the third cultural level, which is less clearly defined and relatively neglected [17]. Similarly, modernity in classical sociological theories has not been defined univocally. For instance Weber stresses on rationality as a main feature of modernity, for Durkheim it is stratification, Simmel emphasizes urbanization, city life and economy. In addition, all this classic theorists, even if only implicitly, saw modernity as Western project: “Many of the movements that developed in non-Western societies articulated strong anti-Western or even anti modern themes, yet all were distinctively modern” [19]. The notion of multiple modernities suggests an alternative project of modernization. In this framework religious movements (even fundamentalistic) are considered in the boundaries of modernity [20].

According to classical theories, urbanization strongly predicts decrease in religiosity (the first level of secularization). Rural residents tend to be more conservative and orthodox in their beliefs [21]. But, on the other hand, Religious Market Theory [22] suggests that religiosity (including participation) increases with market density, the more competition there is, the more religious people there are. In urban areas religious pluralism and diversity is higher, hence theory predicts higher religious adherence in cities [23].

2.1. Operationalization of religiosity

For comprehensive analysis of religiosity Stefan Huber suggests a differentiated model of religiosity

- The Centrality of Religiosity Scale (CRS), constituted by five core dimensions: public practice, private practice, religious experience, ideology and the intellectual dimensions [24,25]. The model integrates theoretical concepts of religion from sociological, psychological and theological perspectives. From a sociological perspective CRS's five core dimensions derives from Charles Y. Glock's [26] multidimensional model of religion. From a psychological perspective, Huber's model of religiosity (centrality of religion) is theoretically connected with W. Allport's [27] ideas of religiosity. The core-dimensions, from psychological approach, "can be seen as channels or modes in which personal religious constructs are shaped and activated" [25]. The five core dimensions could be described as follows:

- Public practice - ritualistic action, public participation in religious rituals: church attendance, taking part in religious services, etc.
- Private practice - religious activities and rituals in private space, such as prayer or meditation.
- Religious experience - perception of transcendent reality, feeling contact with something divine.
- Ideology - beliefs regarding the existence of a transcendent reality.
- Intellectual - knowledge, concerning religion.

3. Methodology

This paper is based on the data obtained through large-scaled nationally representative population survey that was conducted in autumn of 2017 in frame of the research project "Patterns of religiosity and modernization in Georgia" funded by Shota Rustaveli National Science Foundation of Georgia.

All regions of the country are covered with the exception of areas not controlled by Georgian authorities since 1990s (Abkhazia and former South-Ossetia autonomies) and some surrounding territories currently occupied by Russian troops. The total population in these excluded areas is about 5%. From the sampling frames also were excluded some remote villages in mountain area where less than 10 person are living and comprising 0.3% of recent Georgian population (0.4% of total number of households residing in Georgia).

Applying a multistage stratified random sampling procedure based on Georgian population census of 2014 about 2000 households around whole Georgia were randomly chosen. Only one respon-

dent has been interviewed in each household and interviewed by face-to-face using a standardized questionnaire related to religious issues.

The survey's questionnaire contained several instruments from the measurement of Centrality of Religiosity (CRS) by Huber [24,25]. Seven instruments derived from the model were included in the questionnaire:

- ✓ Centrality of Religiosity - 7 items
- ✓ Religious and spiritual self-concept - 2 items
- ✓ Interreligious Perception - 6 items
- ✓ Religious Fundamentalism - 6 items
- ✓ Religious Pluralism - 3 items
- ✓ Religious Reflexivity - 3 items
- ✓ Atheism - 3 items

Questionnaires also included some items based on measurement of modernization from European Value Surveys, namely those referring to the separation of religion and politics, measured on a five point scale, from "strongly agree" to "strongly disagree":

"How much do you agree or disagree with each of the following..."

Item 1. "Politicians who do not believe in God are unfit for public office"

Item 2. "Religious leaders should not influence how people vote in elections"

Item 3. "It would be better for... (your country) if more people with strong religious beliefs held public office"

Item 4. "Religious leaders should not influence government decisions"

Statistical analysis of abovementioned religiosity's measures of combined with detailed socio-demographic characteristics of respondents widely presented in our questionnaire gave us possibility to analyse different aspects of religiosity in Georgia and nature of its differentiations among urban and rural population.

4. Findings

4.1. Religiosity (secularization first level)

The score of religiosity (CRS's score) ranges between 1.0 and 5.0 (In the calculation of the CRS score, the item sum score is divided through the number of scored scale items). For the categorization of the groups of the "highly-religious" "religious" and "non-religious" Huber proposes the following thresholds: 1.0 to 2.0 - not-religious; 2.1 to 3.9 – religious; 4.0 to 5.0 - highly-religious [25] (Table1).

Table 1. *Percentile Rank of CRS (Capital, Urban and Rural)*

Settlement type		Capital	Other Urban	Rural
N		558	558	704
CRS Score		<i>Percentile Rank</i>		
not - religious	1.00	0		0
	1.20	0		1
	1.40	1		1
	1.60	1	0	1
	1.80	1	0	2
	2.00	3	1	3
religious	2.20	4	3	6
	2.40	7	5	9
	2.60	10	11	15
	2.80	14	17	23
	3.00	20	25	31
	3.20	25	33	40
	3.40	34	43	51
	3.60	46	53	61
3.80	56	66	71	
highly religious	4.00	69	74	80
	4.20	79	84	86
	4.40	87	92	92
	4.60	93	96	97
	4.80	98	97	99
	5.00	100	100	100
mean		3.71	3.60	3.46
SD		.73	0.68	0.73

According to abovementioned scale in Tbilisi 31% of respondents might be considered to be highly religious, in other urban areas of Georgia the share of highly religious people is a bit lower - 26%. However, only 20% of rural inhabitants belong to the category of highly religious.

These findings are further confirming by more specified statistical analysis presented below.

Statistical analysis shows that the average reli-

giosity (the mean score of CRS) of people in urban areas was 3.65 and in rural areas - 3.46, which is in line with conclusion above. In further analysis we have tested statistical significance of the difference in religiosity between urban and rural population of Georgia. The data on t-test presented in the Table 2 shows that the level of religiosity of urban residents is significantly higher than that of rural residents $t(1818)=5.47, p<.001$.

Table 2. *The Centrality of Religiosity Scale. Urban Rural*

	Location	Mean	df	t rate	P value
CRS	Urban	3.65	1818	5.47	.000
	Rural	3.46			

Furthermore, we applying to statistical methods examined whether capital city, Tbilisi, is distinctive from the other sub-samples of respondents from the point of view its population’s level of religiosity. Table 3 shows that level of religiosity residents of Tbilisi (M=3.71, SD= 0.73) is significantly higher than in rural (M=3.46, SD= 0.73) area and even that of urban (M=3.60, SD= 0.68) area of Georgia: $F(2, 1817) = 17.94, p < .001$.

In the Table 4 we present the corresponding statistics for distinctive dimensions of religiosity constituting the Huber’s scale, which also shows that mean scores of CRS’s dimensions in urban settlements are significantly higher than in rural:

Public practice - Urban respondents manifest significantly higher mean scores of taking part in religious services than do their counterparts residing in rural areas: $t(1434)=4.50, p < .001$

Similar results are found for **Ideological** dimen-

sion: urban residents are more likely to believe that god or something divine exists than rural citizens: $t(1531)=3.04, p=.002$

Statistical analysis shows that the mean score of **Intellectual** dimension (How often person thinks about religious issues) in urban areas are significantly higher than in rural settlements: $t(1908)=5.37, p < .001$

Mean score of **Experiential** dimension (as measured by feelings that God or something divine intervenes in respondents’ life) is higher in urban areas as well, and the difference is statistically significant: $t(1446)=4.73, p < .001$

Private practice tends to be an exception from this point of view: statistically significant difference between urban and rural residents has not been found for frequency of prayer. Though level of this dimension in Tbilisi is significantly higher than in rural or in other urban settlements: $F(2, 1901)=6.07, p=.002$.

Table 3. ANOVA Comparisons of CRS. Capital, Urban, Rural

Group	n	Mean	SD	Tukey’s HSD Comparisons	
				Tbilisi	Urban
Tbilisi	558	3.71	0.73		
Urban	558	3.60	0.68	.041	
Rural	704	3.46	0.73	< .001	.002

Table 4. CRS dimensions. Rural - Urban

Dimensions of CRS	Location	Mean	df	t rate	P value
Public practice	Urban	3.15	1434	4.50	.000
	Rural	2.89			
Private practice	Urban	3.84	1462	.084	3.97
	Rural	3.78			
Experience	Urban	3.56	1446	4.73	.000
	Rural	3.33			
Ideology	Urban	4.13	1531	3.04	.002
	Rural	4.02			
Intellect	Urban	3.48	1908	5.37	.000
	Rural	3.23			

Statistical analysis shows that impact of religious socialization on religiosity is significant. Religiosity is significantly higher in those cases where religion has been part of respondents' upbringing ($t(1679)=12.6, p<.001$).

4.2 Separation of religion and politics. (Second level of secularization)

Four statements have been examined to understand separation between religion and politics. Statistical analysis shows that the capital tends to be more secular: level of secularization (at public level) in Tbilisi is significantly higher (see Table 5, 6, 7 and 8-higher scores indicate disagreement with statements.) than other areas.

Tables 5 and 7 shows that respondents from Tbilisi less agree with following statements: 5) *Politicians who do not believe in God are unfit for public office* and 7) *It would be better for Georgia if more people with strong religious beliefs held public office*; Therefore they tend to be more secular.

Findings presented in tables N6 and N8 shows that citizens from Tbilisi are more agree with following statements: 6) *Religious leaders should not influence how people vote in elections* and 8) *Religious leaders should not influence government decisions*. Thus respondents from Tbilisi are more secular and as post hoc test shows the difference is statistically significant.

Table 5. *Politicians who do not believe in God are unfit for public office*

Group	n	Mean	SD	Tukey's HSD Comparisons	
				Tbilisi	Urban
Tbilisi	568	2.79	1.29		
Urban	558	2.44	1.29	< .001	
Rural	705	2.20	1.23	< .001	.002

Table 6. *Religious leaders should not influence how people vote in elections*

Group	n	Mean	SD	Tukey's HSD Comparisons	
				Tbilisi	Urban
Tbilisi	590	1.89	0.99		
Urban	574	2.28	1.26	< .001	
Rural	713	2.12	1.21	.001	.046

Table 7. *It would be better for Georgia if more people with strong religious beliefs held public office*

Group	n	Mean	SD	Tukey's HSD Comparisons	
				Tbilisi	Urban
Tbilisi	583	2.80	1.20		
Urban	554	2.42	1.21	< .001	
Rural	718	2.06	1.07	< .001	< .001

Table 8. *Religious leaders should not influence government decisions*

Group	<i>n</i>	Mean	<i>SD</i>	Tukey's HSD Comparisons	
				Tbilisi	Urban
Tbilisi	578	2.15	0.99		
Urban	561	2.39	1.25	.002	
Rural	717	2.45	1.29	< .001	.649

5. Conclusions and discussion

The revival of religion in the modern world has questioned the classical theories of the secularization, which made scholars from different disciplines to rethink the existing relationship between religion and modernity, as well as secularization as the centrality of the notion in the modern world. In this study of Georgian context, we tried to show trends in secularism through in the rural – urban prism. Classical theories (see Durkheim, Simmel) considered that due to the rational, liberal nature of the city the degree of religiosity and consequently the rate of secularism would fall. Although study has shown the extent of religiosity (CRS) in the city areas is statistically significantly higher than religiosity in the rural residents. However, city areas are considerably more secular in terms of the relationship between politics and religion.

The high extent of religious beliefs in the urban areas are tried to be explained by religious market theory [22]. Urban areas are expected to be more religiously pluralistic, although the theory in the Georgian context does not have explanation value due to the monopolistic position of the Georgian Orthodox Church. Process of religious socialization, as well as, the tendency of spatial development of religious institutions carries greater explanation value. The study has shown that the influence of religion in upbringing in urban areas is even sharper than in the rural areas of the country, which may have an impact on higher religiosity in urban area. As for the influence of religious institutions, the Georgian Orthodox church started gaining social power from the capital in the late 90's and spread its influence later in the periphery. Still rural areas remain having less developed religion institutions (churches, priests) and therefore less access to religious services and theological teaching. Interestingly, there is no significant dif-

ference between rural and urban areas on the *Private practice* dimension, where the role and influence of the religious institution is logical to be the least.

Finally, in terms of the separation of religion and politics, the capital is the most secular in the country, which may indicate that the tendency of two levels of secularization (private and public) are mutually independent dimensions.

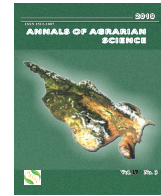
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Radiobiological Method of Studying Sustainability of Grafted Grape Georgian Varieties towards Extreme Factors

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ABSTRACT

Prognosis of the grape endemic varieties sustainability represents an important scientific-practical task for Georgia. This is conditioned by the circumstances that more than 500 endemic varieties of grape grow in different climatic zones of Georgia. Especially noteworthy is problem of sustainability towards high temperatures of endemic grape grafting with regard to expected global-climatic processes. The present work deals with the radiobiological method of studying sustainability of grafted grape endemic varieties based on several radiobiological and radiological methods. In particular, using the primary fluorescence of grape tissues by the high quality accuracy can be determined structural-functional condition of the grafted grape accretion zone. In addition, by examining the xylem transport of graft, it's possible to test stability of grape in condition of optimum and extreme temperature regime. Overall, conducted research allows complex diagnostics of accretion quality of plant transplants and their sustainability level prediction towards unfavorable environmental factors.

Keywords: Georgian grape, Radioisotopic method, Grafting sustainability, High temperature, Radiological studies, Antigenic structure.

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Introduction

The central task of live nature protection represents to maintain biological diversity. Based on the data of International - World Wildlife Fund, biological diversity is defined as the diversity of all forms of life on earth. These include: millions species of plant, animal and microorganism and their gene sets in complex ecosystems, which form the living nature [1-3]. At the same time, in relation to agricultural plants, the problem involves not only the protection issues of genetically unique organisms, but also the mission of creating favorable conditions for their real use in agricultural production. This applies to many rare, endemic varieties and species. In this aspect very important is the circumstance, that cultivation of perennial plants requires a number of practical tasks to be solved, in particular, to obtain high quality grafting material.

In many countries of the world the development of viticulture as a field depends on the supply of

farmland by quality seedling materials [4-7]. Apart from this different problems arises to Georgian winegrowers caused by the large number of endemic varieties of Georgian grape and their agrobiological peculiarities [8,9].

Therefore, the agenda is not only the issue of providing farmland healthy seedling material, but also the necessity of survival and distribution of endemic varieties. In biological view, grafted plant is a unique organism which combines two genetically different individuals. If we consider that each grafting component for it's partner is different and often carry "foreign" genetic information during grafting period, then the diversity of reactions which hinder as the accretion process of plant transplants as well as their viability will be clear [10,11,12]. It is clear that the specific of the biological incompatibility mechanism of the tissues is determined by the taxonomic location of the organism in the hierarchy. If we consider this issue in the area of stock and grafting tissues incompatibility, there appear a number of

different processes: Anatomical incompatibility of the stock and grafting structures, different dynamics of callusogenesis, antigenic structures incompatibility of the plant transplants and many other physiological-biochemical processes, so biological incompatibility of the tissues is based a combination of events on various structural-functional levels.

Today is successfully implemented different settings by affinity of stock and grafting through many forms of stock. Nevertheless, in most cases, certain types of grapes demote ability to overcome environmental adverse conditions, which is due to the imperfect conductive system restored by differentiation of callus tissue between stock and grafting. In such situation, during optimal conditions for a long time fully retains normally parameters characteristic of its viability, but in case of extreme conditions of environment (eg. extreme temperature), due to the demote ability of conductive system restoration develops not only the negative changes of the physiological-biochemical parameters of the plant, but also partial necrotization of the grafting zone of tissues [13,14,15,16]. Therefore, there is necessity for effective methodologies to be developed, able to assess the accretion degree of the graft by considering their further use in plantation development. At the same time it is necessary to take into account the structural-functional condition of the plants not only for constitution of accretion quality, but also to predict their viability for a long time. The present work presents the methods of determining qualitative indicators of grafting that are based on the results of radiological studies.

Objectives and methods

Quality indicators of grafting are basically defined by two parameters - functional condition of tissues in the accretion zone and the post transplantation restore level of conductive system. The functional condition assessment of the tissues was based on the primary fluorescence of the tissues. For induction of primary fluorescence of tissues as a source ultraviolet radiation (Ultraviolet Mercury-quartz lamp of high pressure (250 Watt)) was used. Induction factor of lighting was a blue part of the spectrum, which was obtained by combination of violet and blue filters of different width. The “locking” yellow filter provided a visibility of reflected wave, excited by ultraviolet irradiation. Observation on microtome slices of accretion zone of grafting was carried out with a standard light microscope.

To study the process of intergrowth of grape grafting and the quality of the resulting seedlings, we carried out various experiments using the radioisotope method. Studies were carried out at various stages of the formation of experimental grafting. The study of the regeneration process of the conducting system during the stratification of graftings was carried out in two ways. The first method consisted in analyzing the “flow” of the radioactive label from the stock to the graft part during the stratification on an isotopic solution. The second is when the cuttings are saturated with an isotopic solution and the stratification on the water. After grafting they were placed on an open stratification into a climatic chamber with moistened air at a temperature of 28-30°C. The basal ends of the grafting were kept in the water at all times with a radioindicator. In experiments, sodium phosphate orthophosphate was used. The radioactivity of the solution used in the work was 2.5 millicuries per liter. After stratification, grafting was divided into parts and the total content of labels in different parts of the grafting was determined.

Results and discussion

Primary fluorescence of living tissue, due to its potential capabilities, has advantages compared to other research methods. Such capabilities can be attributed to the observation of living cells and tissues, without fixation and processing with specific reagents. A practical solution to this approach is appropriate that unlike other plants, grape is characterized by a good ability of primary fluorescence. 15-20 micron thicknesses slices were obtained on microtome for analysis of primary fluorescence of grape tissue. During the ultraviolet irradiation of the slice, various tissues gave a different fluorescence; In particular: The fluorescence of the xylem cells was dark green when the cells of the walls were fluorescent by pale yellow color. Different picture of the primary fluorescence was obtained in case of irradiation already formed healing zone of graftings. The preliminary examination of the function of the grafting components was carried out by the intensity criterion of callus formation. Endemic varieties of grape characteristic to different zones of Georgia were used in the work. As the first picture shows, most of them are characterized by the specifications of callusogenesis (Fig.1).

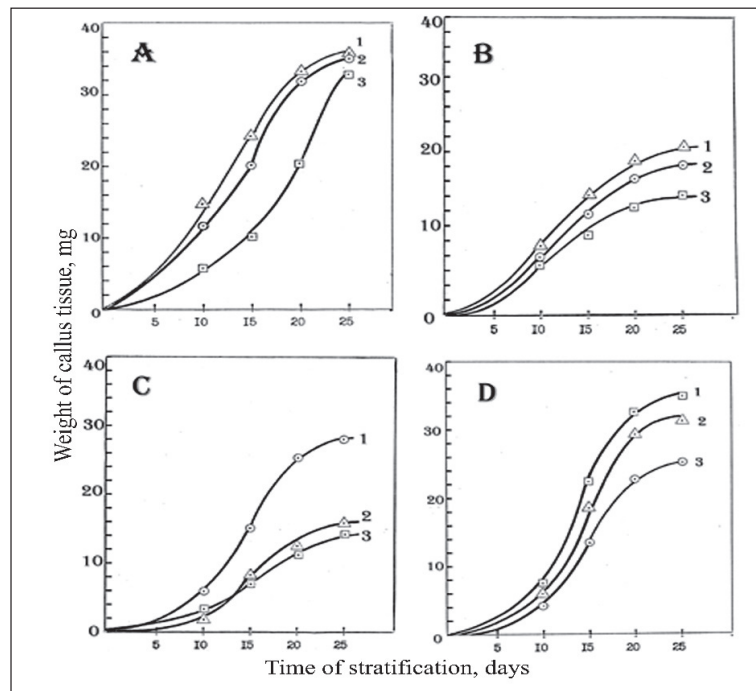


Fig. 1. *S*Callusogenesis dynamics of Georgian grape varieties

A-Kakhetian varieties: 1- kharistvala black, 2- Kisi, 3-Vardisperi;

B- Kartli varieties: 1-Asuretuli, 2-Gorula, 3-Ananura;

C- Racha-Lechkhumi varieties: 1-Alexandrouli, 2-Green from Oni, 3- Usaxelouri;

D-Megrelian varieties: 1-Paneshi pcoxos, 2-Ojaleshi, 3- Zerdagi

Therefore, the cause of the detection of accretion hidden defects during the grafting can be differences in the dynamics of the callusogenesis of stock and graftings.

After that, grafts were adopted by the so-called omega and indirect copulation method.

The results showed that a healthy, normal functioning lignified tissue causes a sharp, yellow fluorescent light. Non viable areas, lesions, necrotic areas and other anatomical and functional defects of accretion are less fluorescence. The full fluorescence of tissue was observed in the accretion zone of grape grafting, which indicating the complete restoration of the damaged tissues and all tissues were functioning normally in the given grafting. In second case we got another picture, when on healing area was observed a dark zones, indicating the presence of necrosis (Fig.2).

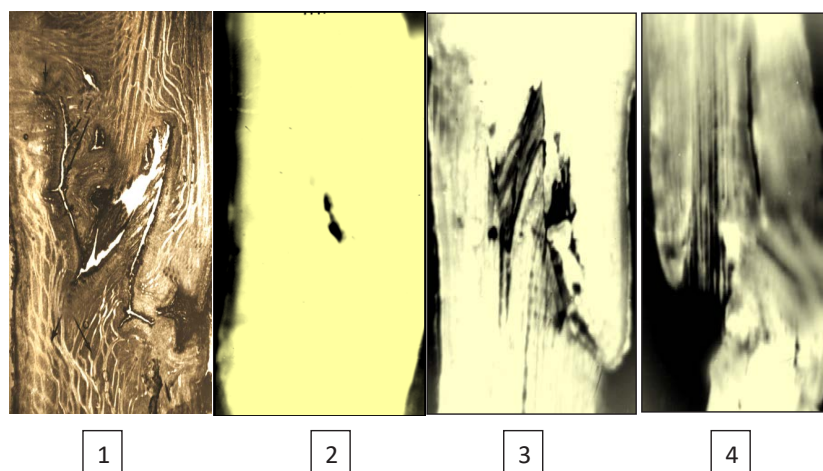


Fig. 2. *S*Structural-functional condition of tissue of grape grafting accretion zone

1- Histological picture of grafting accretion zone (light microscope); 2- First ultraviolet light of fully recovered tissues of grafting; 3-Average structural-functional defects of grafting accretion zones (ultraviolet microscopy); 4-Strong structural-functional defects of grafting accretion zones (ultraviolet microscopy).

The sharp fluorescent light of graftings in graft indicates the viability of these tissues, which means, that conductive system was fully recovered between the grafting components. During the analysis, there were detect non-fluorescent dark areas, where the wide necrotic zone was fully isolated, which led the reduction of nutrients in grafting area. Such graftings, as a rule, are characterized by low ability of growth-development and are unfit for cultivating plantation. In many cases the grafts were found, in which, at first glance, the grafting components were perfectly combined and the healing zone was flourished fluorescent, but in the grafting area of the graft, there was a weak fluorescence and the sharp expressed border between the stock and grafting. This picture indicates a low level of vitality which is the result of weak differentiation between the grafting components. It is noteworthy that the fluorescence analysis method can be used during early stage-stratification of grafting formation. In this case, we can discuss about normal course of accretion processes by the level of tracheid strand creation. Ultraviolet irradiation with blue-ultraviolet spectrum causes yellow flashes when non-differentiated callus mass is not fluorescent. If stratification is going on light, due to the formed chlorophyll, callus acquires red, but tracheid strand yellow color. In order to check adequacy of the treated method we had used labeled atoms method (saturated solution by ^{32}P isotope). Isotope migration was observed by radioautography.

The use of radiation methods for various types of tissue incompatibility basically reduces to the

analysis of primary contacts caused by the processes of recognition at the cellular level of transplants. At the heart of the proposed method is the analysis of transport of radioactive phosphorus from a stock saturated with radioactive solution into an unsaturated grafting. Thus, the parameters of the investigated processes involve both the time characteristics of the primary contact and the beginning of the functioning of the joint conductive system that formed as a result of cytodifferentiation of the accretion callus tissues. In order to characterize the dynamics of redistribution of radioactive phosphorus in a stock-graft combination of grafting under study, we analyzed the isotope label localization zones using the radioautography method. The radioautoradiographs shown in Fig.1 testify that the pattern of localization of the isotope label at various times of the stratification period varies significantly. So, on the radioautogram of the grape grafting indicates, that 5 days after the onset of stratification, main zone of radioactive label localization is at stock (Fig.3-1). Non-luminescence zone of the grafting may indicate that active processes, associated with the formation of callus cells are taking place below the accretion zone, but the accretion of callus and the restoration of the conduction system has not yet occurred.

A somewhat different picture of the localization of radiophosphorus in different areas of grafting was observed 10 days after the beginning of the stratification (Fig. 3-2).

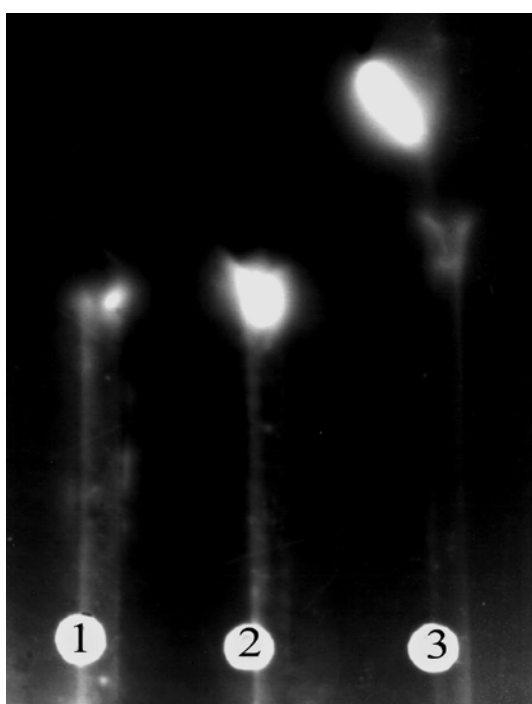


Fig. 3. Localization of radioactive phosphorus in various zones of the grafted plant

1-on the 5th day of the stratification period;
2-on the 10th day; 3-on the 15th day.

The similar to 5th day of stratification, there is no fluorescence of grafting zone; This is an indication that the entry of a radioactive label into this part of the grafting has not yet begun because of a dense physiological barrier in the form of undifferentiated callus. At the same time, it should be noted that the luminescence caused by the action of the radioisotope on the photographic plate has become more intense. In interpreting these data, it can be concluded that a conglutination of wound callus of the grafted components occurred and a solution with a radioisotope label entered the basal part of the graft adjacent to the accretion zone.

An even more significant change in the pattern of radiophosphorus localization in grape grafting was observed on the 15th day of the stratification period (Fig. 3-3). Here the main zone of localization was in the developing bud of grafting. At the same time, the radioactivity of the accretion zone itself decreased markedly, which was manifested in a decrease of the luminescence in accretion zone. The appearance of a significant amount of radiophosphorus in the grafting part of the graft is an indication that the conductive system is restored and the active xylem transport between the stock and the graft part is started.

The analysis of the change in the distribution of radiophosphorus label in grafting at various stages of stratification showed the importance of changes in the dynamics of accumulation of radiophosphorus in the accretion zone when the structural integrity and functional activity of the conducting system are restored.

In recent years, regarding to the risks of global climate changes, the issue of sustainability of endemic species towards the high temperature regime has become especially actual. In addition, if the research direction in relation to various agricultural plants can be achieved through the selection of genetic material, depending on the need to maintain genetic diversity while working with endemic varieties, it is impossible to use the methods of selection. In this case, for sustainability analysis of grape plant grafting towards high temperature, the leading factor is grafting adaptation level with the xylem transport intensity, which is provided by stock part of the transplant. In this aspect, the recovery rate of accretion zone tissues provide active xylem transport, which is the criterion of sustainability towards the high temperature regime of the grafted plant. In order to study the restoration level of transplants accretion zone, the method of labeled atoms was used

by us. In particular, the xylem transport intensity was studied during optimum and extreme temperatures. As radioautography showed, the last place of xylem transport is the leaf surface tissue, which indicates the radioactive label localization (Picture 4–A). Naturally, in case of temperature regime increases, transpiration intensity allows to prevent the leaves surface overheating, which in total ensures the sustainability of the grafted plant. For the study of the importance of tissue restoration levels, experiments were done on such grafts which were characterized by different structural-functional conditions of the accretion zone tissue. As the graph shows (Fig. 4-B), plant transplant different by restoration level indicators of accretion zone tissues were characterized by different intensity of xylem transport. Difference in transition intensity of radioactive label in grafting area of grafts, in case of high and relatively low recovery levels indicators of tissues in the accretion zone was 29,5%. It's no less important how the same characteristic is changing in relation to extreme temperature regime. It is possible that the temperature factor is the criterion of sustainability of the tissue with respect to different levels of tissue restoration. As shown in 4B-3 and 4B-4 graphs, in condition of extreme temperature regime, in both versions are stated a high rate of radioactive label overflow in the accretion zone; However, quantitatively among the variants are stated- Increase by 14% within a good accretion ability variants, but 6.8% in grafting of low-accretion ability.

Based on the obtained results was shown, by means of structural-functional analysis of grafting components It's possible to monitor cytodifferentiation process of callus tissue, which, in turn, helps to assess the active transport of conductive tissues, and the latter is a precondition to increase the sustainability of plant towards high temperature. It should be noted, that the approach offered by us is noteworthy when the researcher has a goal of maintaining the biodiversity of the endemic varieties with respect to global warming. If sustainability of non-endemic perennial agricultural plants towards high temperature is resolved through the selection of new drought resistant varieties, this methodological approach is not acceptable regarding to endemic varieties, because of phenomenon of genetic purity should be considered.

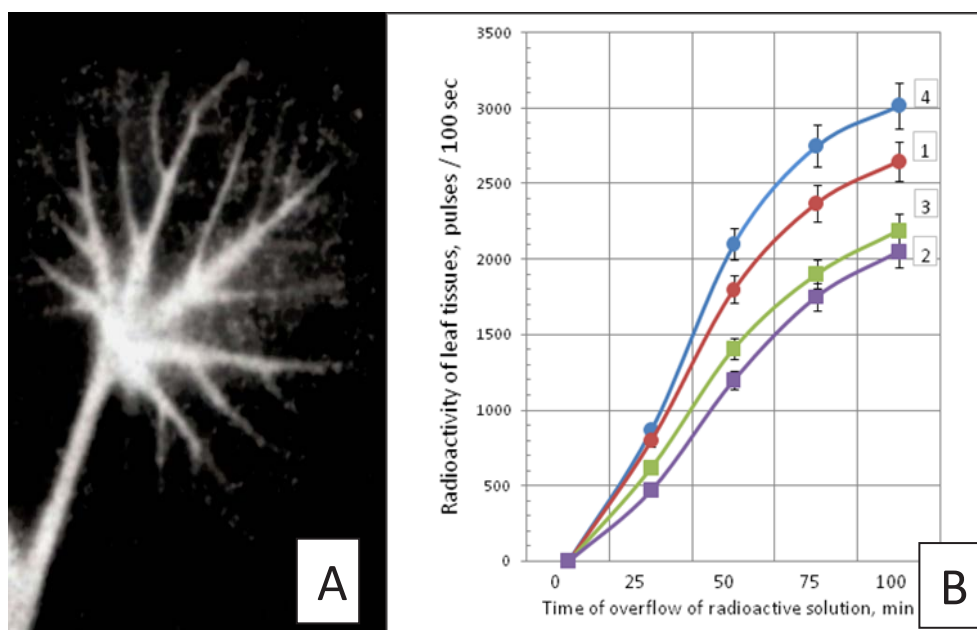


Fig. 4. Radioisotope (^{32}P) solution transport in stock-grafting system of Grafted grape
 A-Grape leaf Radioautograph; B-Dynamics of radiophosphorus movement in the grafting,
 1- Intensity of radiophosphorus transport (25°C) in stock-grafting system of high quality grafting, 4- Same, in condition of extreme ($40\text{--}45^{\circ}\text{C}$) temperature; 2- Intensity of radiophosphorus transport (25°C) in stock-grafting system of incomplete accretion grafting, 3- Same, in condition of extreme ($40\text{--}45^{\circ}\text{C}$) temperature.

Conclusion

Obtained results received on the basis of radioisotopic method, were adequate to the primary fluorescence picture, which indicates high efficiency of the treated method. It is noteworthy, that proposed approach is especially important when are dealing with grafting components having various affinity. Conclusion can be made based on the performed work, that the method of structural-functional analysis of grafting, based on the first fluorescence of the tissues, can be used as an efficient method of early diagnostic of grafting quality. The use of this method will enable us to enrich the arsenal of the methods to verify the large party of grafting for the purpose of planting the grape.

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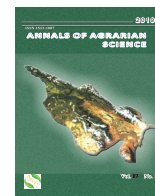
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In Urboecosystems (in Example of Baku City)

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ABSTRACT

The present work is devoted to the assessment of mycological safety according to conditional pathogenic fungus urboecosystem. It was determined that the composition of dominant nucleus of aeromycobiotic system directly formed in urban ecosystems depends on the humidity of the urban environment. It was revealed that if the humidity in the urban environment is more than 70%, the number of conditional pathogen fungi inside the aeromycobiota is rising and the risk factor among the population is increasing.

Keywords: urboecosystem, aeromycobiota, conditional pathogenic, mycological safety, dominant nucleus, humidity, risk factor.

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Introduction

Recently the central cities of the world are characterized by the increasing dynamic of population. The increase in the number of urban population and their negative anthropogenic impact on the environment has led an increase in the strengthening of the risk factor. In other words, the rapid development of urbanization also became a source of “the infection of civilization” [1;2]. Microscopic fungi caused serious global medical and social problems in modern age. It must be noted that destruction of the state of equilibrium between different components of urban ecosystem has led to an increase of biological activity of microscopic fungi, especially opportunistic and allergen ones. Therefore a tendency of increasing allergic diseases of different origin is being observed [3;4]. It is known that in committing allergic diseases mold also take place, this is associated with the role of humidity factor in the development of opportunistic or allergic fungus.

It must be noted that even if the humidity is not manifested in open systems or urban environment in water vapor form, in closed systems or on the walls of housing buildings it appears on the form of rot. So the majority of micromycetes spread either in open or in closed condition show pathogenic features under the influence of humidity factor. Therefore, those become agents of different mycot-

ic diseases in human like exogenous allergens or endogenous opportunists [5;6]. It must be determined that the composition of aeromycocomplexes formed by microscopic fungi of urban areas is still learned in closed spaces, in other words in the interior air of buildings. However, the composition of mycobiota formed in open urban ecosystems, in other words in the city air is practically almost not studied [7;8].

The aim of the presented study is to assess micromycetes in the air of Baku city scoring by conditional pathogen fungus.

Material and methods

The studies were carried out in the territory of Yasamal and Sabail districts of Baku city. As the object of the research samples taken from the different atmospheric air. Examples were taken from the air at 0,2m above the land and from a height corresponding to the average height of human beings, in other words from 1,5m height which is close to the respiratory system of people. In this case, aspiration and sedimentation techniques were used. In aspiration method sampling was carried out with the help of ПИY-1Б aspirators. In sedimentation method Petri dishes are held open in 1,5m height atmosphere from the land for 30 minutes and micromycetes spontaneously settle on medium malt agar. Tak-

en samples were incubated in 22-25°C for 7 days. After that the colonies were identified by being analyzed due to the cultural morphological features using microscope [9;10].

Results and discussion

It was determined that ecological factors in urban ecosystem, in other words humidity and temperature regimes if characterized by optimal parameters, species appear which belong to the first group of conditional pathogenic fungi according the BSL (Biological Safety Levels) scale. The studies held in different micro districts and residential areas, as well as parks and gardens of Baku were carried out in open air. It was revealed that if humidity in the urboecosystem is less 70% in this case the climatic conditions of the urban environment considered to be favorable then and the generated aeromycobiota has species diversity.

The analyses of the species composition of aeromycobiota show that micromycetes having dark-colored melanin containing spores take dominant position. From this fungus species of *Alternaria* genus -*A.alternata*, *A.tenuissima*, *A.chartarum*, *A.longipes*, *A.consortiale*, *A.radicina* and species of

Cladosporium genus - *C.cladosporioides*, *C.herbarum*, *C.sphaerospermum*, *C.elatum*, *C.variable* are represented more (Fig. 1 and 2)

It must be noted that spores of these fungi are nearly equal to 75 µm. Studies carried out at different times of the year show that in summer and autumn the urban environment is characterized by richer aeromycobiota. In this period the density of opportunist and allergen fungi included at BSL-1 group in the air is not so great, equal to 600 CFU/m³ this case shows that infection sources are potential in the urban environment and proves the relative stability of mycological safety.

But environmental disruption in urboecosystem or the increasing indicators of humidity and temperature causes major qualitative changes in aeromycobiota. It was determined that when humidity level in the city is more 70% the number of micromycetes spread in the atmosphere air increase differential to 1500 CFU/m³ and conventional pathogenic fungi of BSL-2 group appear inside mycobiota. It must be noted that in such the number of fungi belonging to *Alternaria* and *Cladosporium* genus is not decreasing and in aeromycobiota moisture-loving fungi, including species of *Paecilomyces* genus- *P.inflatus*, *P.carneus*, *P.aeruginus*, *P.variotii* dominate.

Table. Taxonomic structure of aeromycobiota of Baku

№	Genus	Species
1.	<i>Alternaria</i>	<i>Alternaria alternata</i> (Fr.)Keissl; <i>A.consortiale</i> (J.W.Groves) <i>A.chartarum</i> Preuss <i>A.longipes</i> E.W. Mason <i>A.radicina</i> Meier <i>A.tenuissima</i> (Kinze:Fr)Wilt..
2.	<i>Aspergillus</i>	<i>Aspergillus glaucus</i> Link, <i>A.candidus</i> Link.: Fr, <i>A.flavus</i> Link:Fr; <i>A.fumigatus</i> Fresen; <i>A.niger</i> Tiegh;; <i>A.ochraceus</i> K.Wilh, <i>A.terreus</i> Thom, <i>A.versicolor</i> (Vuill.)Tirab
3.	<i>Aureobasidium</i>	<i>Aureobasidium pullulans</i> Arnaud
4.	<i>Botrytis</i>	<i>Botrytis cinerea</i> Pens:Fr.,
5.	<i>Cladosporium</i>	<i>Cladosporium cladosporioides</i> G.A. de Vries, <i>C.elatum</i> Nannf, <i>C.herbarum</i> (Pers) Link, <i>C.sphaerospermum</i> Penz, <i>C.variable</i> G.A. de Vries
6.	<i>Fusarium</i>	<i>Fusarium moniliforme</i> Sheld; <i>F.sambucinum</i> Fuckel; <i>F.heterosporum</i> Nees
7.	<i>Phoma</i>	<i>Phoma lingam</i> (Tode) Desm
8.	<i>Monilia</i>	<i>Monilia digitata</i> Pers
9.	<i>Mucor</i>	<i>Mucor circinelloides</i> Tiegh., <i>M.hiemalis</i> Wehmer., <i>M.racemosus</i> Fresen
10.	<i>Paecilomyces</i>	<i>Paecilomyces aerugenus</i> Samson; <i>P.carneus</i> (Duche & R.Heim), <i>P.inflatus</i> (Burnside); <i>P.variotii</i> Bainier;
11.	<i>Stachybotrys</i>	<i>Stachybotrys atra</i> ; <i>St.cylindrospora</i> C.N.Jensen; <i>S.chartarum</i> Hughes.
12.	<i>Verticillium</i>	<i>Verticillium cephalosporium</i> W.Gams; <i>V.tenerum</i> Nees.
13.	<i>Ulocladium</i>	<i>Ulocladium atrum</i> Preuss; <i>U.chartarum</i> (Preuss) Simmons.



Fig. 1. Colonies (a) and conidia (b) of *Alternaria*

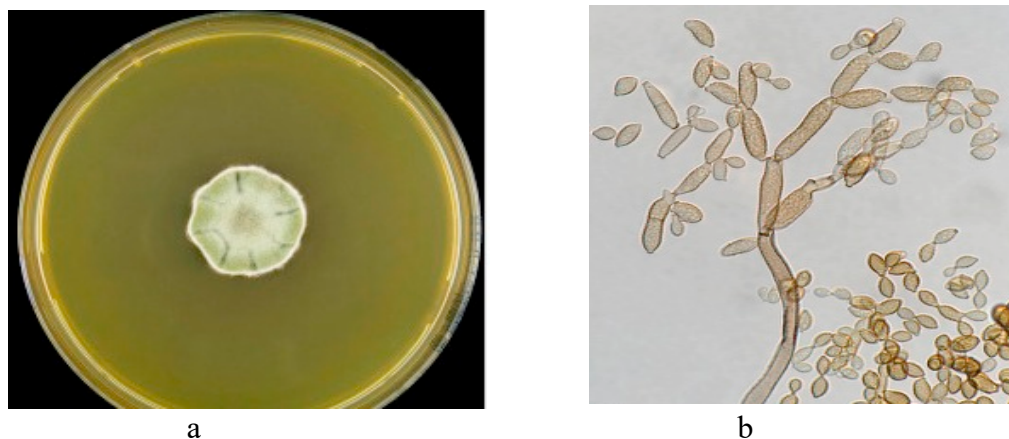


Fig. 2. Colonia (a) and conidia (b) of *Cladosporium*

Occurrence these fungi in urboecosystem variates between 40,5- 63,2% which once again confirms that they dominate in humid period of the city. At the same time the increase of other species of mycocomplex formed in urban ecosystem including *F.moniliforme*, *F.sambucinum*, *F.heterosporum* of Fusarium genus, *C.cladosporioides*, *C.herbarum* species of Cladosporium genus, *A.glaucus*, *A.niger*, *A.candidus*, *A.flavus*, *A.fumigatus*, *A.ochraceus*, *A.terreus*, *A.versicolor* species of Aspergillus genus, *A.pullulans* of Aerobasidium genus, *V.cephalosporium*, *V.tenerum* species of Verticillium genus prevalence rate increases up to 40,2%.

In addition, in the wet autumn of 2015 as a result of research conducted in an urban environment *B.cinerea* species of Botrytis genus, *M.digitata* species of Monilia genus and *Ph.lingam* of Phoma genus have been found which are considered to be rare to urban ecosystems this fungi occurrence was equal to 10,3% respectively. It should be noted that the increase of ecological factors, especially humidity in environment has resulted in appearing of all the categories of fungi including dominant, often found and rare species, as well as their opportunist and allergen types.

Mycological analyses were held in the air of “Old city”, antral part of Baku built in special historical and architectural style and characterized by high rates of ecological factors. It was revealed that “Old city” has a special aeromycobiota and it has such potential pathogen species of Mucor genus like *M.circinelloides*, *M.hiemalis*, *M.racemosus*, from Stachybotrys genus *S.atra*, *S.cylindrospora*, *S.chartarum*, from Ulocladium genus *U.atrium*, *U.chartarum*.

Conclusion

A result of held researches it was revealed that depending on how the year was the humidity in the urban environment is considered to be the main factor in the formation of aeromycobiota in urban ecosystem. As a result of researches it was revealed that there exist close correlation between humidity level of urban environment and dominant nucleus of aeromycobiota formed in ubroecosystem. Note that the entrance of opportunist or allergen species of microscopic fungi to the dominant nucleus of aeromycobiota formed in ubroecosystem causes

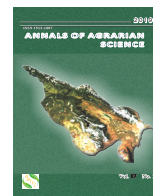
the increase of risk among population and leads to breach of mycological security. This is considered to be potentially dangerous situation the population having low immune status and children.

It was revealed that aeromycobiota formed in the urban environment is completely different from mycocomplexes in environment natural biocenosis. In terms of composition and to the number of opportunistic fungi.

It was also determined that depending on the ecological situation of urboecosystems the registration of opportunist and allergen fungi species in the air is closely connected to the humidity level of aeromycobiota formed in city environment.

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The Plum (*Prunus domestica*) Cuticle, its components and their impact on the drying process

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ABSTRACT

The Main aim of this research is understanding how to carried our drying process for the two varieties of plum: “Shavkliava” (local) and “Stanley” (introduced) according of cuticle performance. The four fractions was take out from the isolated cuticle: epicuticular wax, intracuticular wax, triterpene acids, and cutin. All these have been analyzed. Obtained data according to the varieties is following: ‘Shavkliava’ - 370-478-594-980 $\mu\text{g}/\text{cm}^2$ and ‘Stanley’ is 625-440-770-1100 $\mu\text{g}/\text{cm}^2$. The optimal parameters of the blanching was established: ‘Shavkliava’ NaOH concentration 0.5%, 20-30 seconds of exposure, temperature 90°C; ‘Stanley’ 0.5 % - 30 seconds 95°C respectively. In such conditions only epicuticular wax is moved off. When there is a high concentration – 1 % NaOH, the intracuticular wax, the total number of phenols and terpene acids decreased, which significantly reduces the dry product’s quality parameters. Treatment involves stopping the oxidation process temporarily. In connection with this, it determined that the phenol compounds in the initial samples and after drying. With the impact of optimal conditions for treatment, the total number of common phenols is significantly more and consists 35.5% for ‘Shavkliava’, 30 % for ‘Stanley’. Drying was carried out in three temperature conditions gradually 40-45°C, 60-65°C, 75-85°C.

Keywords: Variety, Epicuticular wax, Blanching, Triterpene acids, Temperature, Common phenols

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1. Introduction

The demand on the dry plum increased significantly because of its useful properties. According to the existing information, by proved high confidence, it is considered as a product having not only important nutritional but functional features as well. This is one of the important product which is characterized by high antioxidant content. This feature highly depend on the content of phenolic compounds. It was established by research that this is basically Hydroxycinnamic acids like – neo-chlorogenic and chlorogenic acid [1-3]. In this regard, well known that the skin of plums is especially distinguished [4,5].

Actually, Triterpenoids are counted as biologically active substances, which are part of the cuticle. It is considered that they represent essential

components for human health and have various biological effects: they can reduce the risk of chronic diseases; at the same time, they have antiviral, antibacterial and anti-carcinogenic action; they could lower cholesterol level as well [6- 8].

The primary purpose during of regular drying process is the maximum keeping of phytochemical ingredients and reduction of the quantity of water to the level, which leads to long and proper storage.

During the drying process, the water from the cells moves to the cuticle, but epicuticular wax prevents the evaporation process - to accelerate this process it is necessary to remove this barrier.

Fruit species and varieties highly differ according to cuticle contents and types, and it is necessary to provide the specific procedures before of start drying process. For instance, For Tart cherry treatment, it is recommended to use 2 % of oleic acid

ethyl ester; the temperature must be 23°C, during of 1 min. According of literature data Exposure by high temperature around 60°C, in this case has a negative impact. [9].

For the plum, it is used 4 % etiolate, 1% NaOH, in two temperature regimes -23°C and 60°C. Better results will be obtained, in case of treatment with 1 % KOH solution, by temperature 60°C, 1 min of exposure [10]. Treatment of grapes appeared effective with 6% K₂CO₃ solution, in temperature 50°C, 2 min of exposure [11]. The experiments showed that the treatment of plum is recommended with 1 % NaOH solution, in the temperature 85°C [12]. For blueberry treatment, it is used 0.1 % n NaOH solution, temperature 70°C [13]. Based on the analysis it is revealed that the pretreatment procedures significantly reduce drying time and decreases accordingly energy consumption [14,15].

Based on this review is cleared that the data are quite differ according to biological and genetic properties of fruits. Therefore, it is necessary, in each cases for each fruits should be selected that the proper substances and the optimal parameters for treatment.

The goal of the research is to determine the quantitative indicators of the cuticle components of two plum varieties, as well the selection the parameters for blanching and study the impact of various types of pretreatment on the cuticle components and the oxidation intensity of the phenol compounds during drying process.

2. Materials and methods

The two widespread plum varieties of ‘Shavkliava’ (local) and ‘Stanley’ (introduced) were selected for given research. The experimental samples were taken from SRCA jigaura research station in the period of optimal ripening phases of these varieties – they has the characteristic color of the variety, aroma, taste; it is slightly soft with the touch of the finger.

The efficiency of drying process is affected by the pretreatment procedures, which are significantly related to the size of cuticle’s membrane. In this regard, the components of the cuticle are determined: epicuticular wax, intracuticular wax, triterpene acids, and cutin.

For the take out the cuticle, the skin of the plum is placed in ammonia solution of the oxalic acid, whose pH is 4,0, within of 40 hours, at temperature - 37°C. The received material is cleaned with water, dried and packed in a filter paper and is placed in

Soxhlet apparatus. The following scheme was apply for the fractionation of the cuticle (Fig. 1).

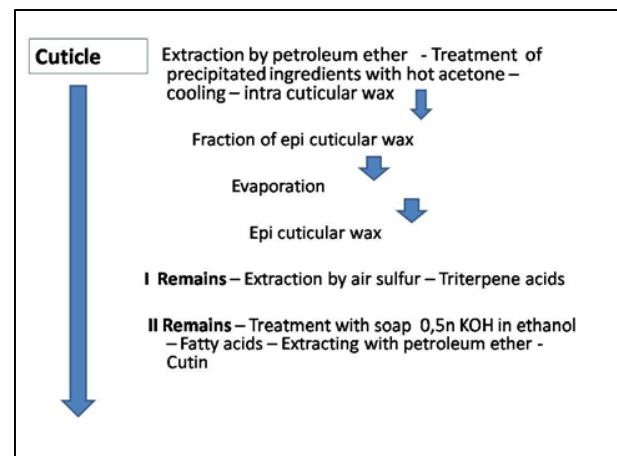


Fig. 1. Scheme of fractionation of the cuticle

In the experimental samples of phenol compounds, the total number is determined by Folin-Chiokalte method, on the spectrometer, on the wavelength of 765 nm. The standard solution is prepared by using Gallic acid.

It is well known, that in the drying technology pretreatment (blanching) is essential, which aims to increase the drying speed. Also, it provides for a reduction-oxidation process at a high temperature by inactivation of the enzyme.

In this regard, 0.5 and 1 % NAOH solution were used. In both cases, exposure was 20-30 sec., the blanching temperature were differ according of varieties for ‘Shavkliava’ - 90°C, for ‘Stanley’ - 95°C.

Plum drying is carried out in three stages under various temperature conditions: A - 40-45 °C, 6 hours of exposure, B - 60-65 °C, 4 hours of exposure; C - 75-85 °C, drying continues until a water content is decreasing till of 25 %.

3. Results and discussions

According of Performed research revealed that the plum cuticle data varies significantly according to the epicuticular wax and triterpene acid content for the different varieties: ‘Shavkliava’ - 370-594 µg /cm² and ‘Stanley’ - 625-770 µg /cm².

Intracuticular wax content data is almost the same, there is no change regarding cutin content (Table.1).

Table 1. Plum cuticle components, $\mu\text{g}/\text{cm}^2$.

Variety	Epi- cuticular	Intracuticular	Terpene Acid	Cutin
'Shavkliava'	370	478	594	986
'Stanley'	625	440	770	1100

It is very important that Wax is well dissolved in alkaline solution, and this is considered during plum drying process – epicuticular wax becomes separate from cuticle and water starting intensively evaporate. Other hand, the concentration is effected on this process as well and has vital importance.

During of 1% NaOH solution treatment, when the duration 20-30 seconds, the 'Shavkliava' temperature is 90 °C and 'Stanley' temperature is 95 °C, the quite thin net cover is formed on the skin. Other damage, which might affect the drying process,

have not been observed visually, the Only difference occurs for the 'Shavkliava' on 95°C, when juice is starting leaching.

The above analysis showed that quantitative indicator of cuticle components had changed significantly. Epicuticular wax is eradicated, intracuticular wax and triterpene acids-decreased (Table 2).

It is very important to note, that the change is more higher in the case of 'Shavkliava': intracuticular wax - 29.3%, triterpenes acids - 21.7%. In the case of 'Stanley' is only - 17.2% and 13.0% (Fig. 2.)

Table 2. Plum cuticle components after initial treatment, $\mu\text{g}/\text{cm}^2$

Temperature, °C	NaOH Solution Concentration, %	Epicuticular Wax	Intracuticular Wax	Triterpene Acids	Cutin
'Shavkliava'					
90	0.5	0	467.0	573.0	962.0
90	1.0	0	338.0	465.0	980.0
'Stanley'					
95	0.5	0	470	752	1120.0
95	1.0	0	364	670	1097.0

* Exposition 20-30 seconds

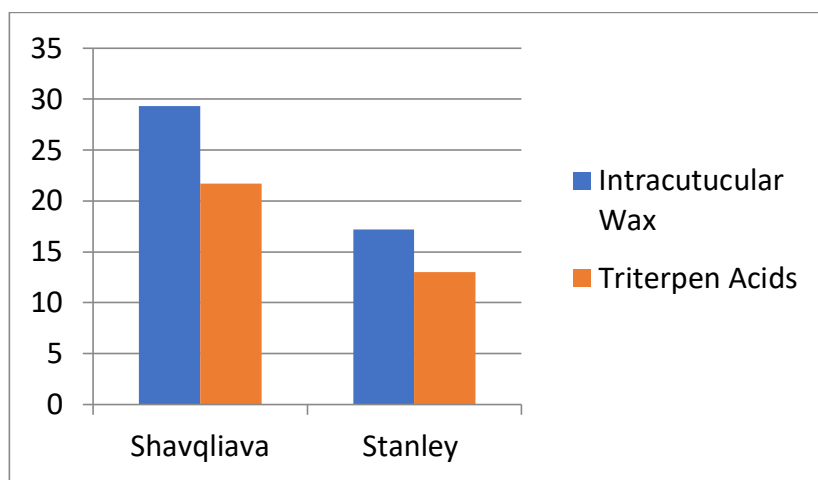


Fig. 2. Rates of Epicuticular wax and triterpene acids in conditions of 1.0% NaOH solution treatment.

It was found that Only epicuticular wax was removed after treatment with relatively low 0.5% NaOH solution. The results show that correct selection of pre-treatment (blanching) parameters is vitally important, which increases drying speed. Also, it considers reducing oxidation process within high-temperature conditions by enzyme inactivation.

The data of the total amount of phenols is represented in initial samples and after the drying process. They are decreased in case of treatment with 1.0% NaOH solution. (Table 3).

As determined, The activation of oxidation process is caused by decreasing the number of intracuticular wax and triterpene acid by increasing penetration of oxygen. According of data, in the case of ‘Shavkliava’ it is reduced by 30% and by 35.5% in case of ‘Stanley’ (Fig. 3).

It should be noted that the removal of the intracuticular wax reduces the quality of dried plum-it is less elastic and less shining.

The differentiation of drying temperature has some theoretical explanation – on 40-45 °C temperature the enzyme-invertase is activated inside the pulp of the plum, as a result of this process the hydrolysis of sucrose is taking place; Monosaccharide’s are getting into non enzymatic reaction with

amino acids on 60-65°C temperature and as a result the melanoidins are formed, which could increase the antioxidant activity [16].

It is interesting that in the 75-85°C temperature conditions, formation of dark pigments and aromatic substances is continued, intracuticular wax is takes out, as a consequence of this process dry plum fruit obtains required specific taste, aroma, color, and shining.

4. Conclusion

It is very important to perform initial treatment correctly during of plum drying technology: appropriately designated concentration, exposition, and temperature of used substances. All this approaches determines biologically active elements - phenolic compounds, well-preserved triterpene acids, and suitable market appearance.

The optimal parameters of the blanching was established: ‘Shavkliava’ NaOH concentration 0.5%, 20-30 seconds of exposure, temperature 90°C; ‘Stanley’ 0.5 % - 30 seconds 95°C respectively. In such conditions only epicuticular wax is moved off. When there is a high concentration – 1 % NaOH,

Table 3. Change of phenolic compounds during the plum drying process, µg /100g.

Variety	Initial	Dried Product	
		I version*	II version**
‘Shavkliava’	172	409	280
Stanely	210	580	374

I version* - 0.5% NaOH Solution Treatment

II version** - 1.0% NaOH Solution Treatment

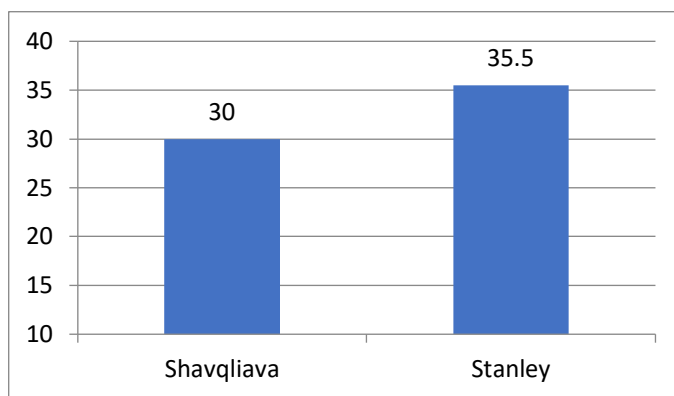


Fig. 3. decreasing rate of phenolic compounds in the drying process during 1.0% NaOH solution treatment, %

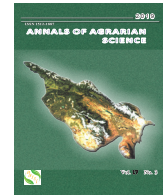
the intracuticular wax, the total number of phenols and terpene acids decreased, which significantly reduces the dry product's quality parameters

5. Acknowledgement

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Isolation of Some different Fungi from the Larvae of the European Cockchafer *Melolontha melolontha* L. (Col: Scarabaeidae) at Erzincan Province in Turkey

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ABSTRACT

The European cockchafer (*Melolontha melolontha*, L., (coleoptera: scarabaeidae) is a widely distributed pest throughout turkey and damage many plant species and cause substantial losses in meadows, pasture, horticulture, forest and other production. In our studies, we focused on the isolation of entomopathogenic fungi for *M. melolontha*. The larvae were collected from the ploughing fields in 3 locations (merkez-ekşisu, geyikli village, buğdaylı village; üzümlü-çermik village; kemah) in erzincan province at the end of april 2015. The most abundant species was found *Beauveria bassiana* (53.3% of samples). Two isolates *Olfecanicillum* sp. (13.3 %) and *Fusarium* sp. (13.3%) were also detected. No entomopathogenic fungi were isolated from 3 of the samples (20%).

Keywords: European Cockchafer, *Melolontha melolontha*, *Beauveria bassiana*, *Lecanicillum* sp., *Fusarium* sp. Chemical insecticides

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1. Introduction

The European cockchafer is widely distributed harmful pest, over the world and Turkey as well. The insect, both larvae and adult are destructive for vegetation plant. In the last 25 years, *M. melolontha* extensive and lethal damage to the roots of meadows and ornamental plants, vegetable crops and young trees, causes economic losses of cultivated plants and forest production [1-15]. Turkey and other country use of chemical insecticides to control *M. melolontha* and these insecticides have been considerably reduced due to environmental and public health concerns [7]. It seems that population density of *M. melolontha* will increase so that it becomes abundant and important pest species. Entomopathogenic organisms are the most promising control agents. There are numerous papers on entomopathogens of *M. Melolontha*, such as viruses, rickettsia, bacteria, nematodes and fungi [8-19], but a few records of pathogens causing diseases in *M. melolontha* populations in Turkey. Some research-

er tested entomopathogenic bioproduct based on *Beauveria brongniartii* on *M. melolontha* larvae. According to this study, the cumulative effect of the biological product provided a decreased density of larval populations under economic threshold level [20]. Some scientist studied on the possible joint action of microorganisms pathogenic for *M. melolontha*. For this, they determined the effects of simultaneous or consecutive contamination of the larvae of *M. melolontha* with entomopathogenic organisms, *Beauveria tenella*[21]. Successful use of entomopathogenic fungi as microbial control agents of insects will ultimately depend on how well the strains are selected. For the development of mycoinsecticides based on entomopathogenic fungi, this research was conducted in the province of Erzincan province in years 2015. Our aim was to isolate the insect pathogenic fungi found in agricultural land and urban pests.

2. Materials and methods

2.1. Information About the Research Area

The province of Erzincan (39°02'n to 40°05'n, 38°16e to 40°45'e) covers ca. 11,900 km² of turkey and is located in the eastern part of Anatolia, which has a continental climate. Soil samples were collected from different geographical sites distributed through the Erzincan province (Merkez-ekşisu, ge-yikli village, buğdaylı village; üzümlü-çermik vil-lage; Kemah,). The definition «Mountains around and vineyards in the centre» made by the people for Erzincan indicate the geographical position of the city. The province is surrounded by mountain ranges in the south and in the north. There are very fertile lands in the river basins between these moun-tains. The Erzincan plain, where Erzincan province is located is covered with vineyards and has been the cradle of the great civilizations since the early ages of history. The karaparmak grapes from the Er-zincan vineyards are famous.

2.2. Exploration of Entomopathogenic Fungi

The method by collecting insects were sick or dead due to fungus infection [22]. The infected in-sects that showed symptoms of dry body and the presence of conidia and fungal conidia, white or green body of the larvae were isolated. Then, the fungus-infected insects were isolated in the labora-tory at a cabinet of laminar air flow that had been sterilized by 70% alcohol.

2.3. Isolation and Identification of Fungi from Insects

we focused on the isolation of entomopathogenic fungi for *M. melolontha* from soil. The larvae were collected from the plowing fields in 3 locations (Merkez-Ekşisu, Üzümlü-Çermik village and Ke-mah) in Erzincan province at the end of April 2015. Isolation of entomopathogenic fungi used methods of Herlinda [23]. The fungus-infected insects and caterpillars were sterilized with 1% sodium hypo-chlorite or 70% alcohol for three minutes. Then in-sects were rinsed with sterile water three times, and dried on top of sterile filter paper. Then, they were placed in a petri dish (diameter 9 cm) containing moist sterile paper and incubated to stimulate co-nidial germination. Fungi were isolated, cultured on

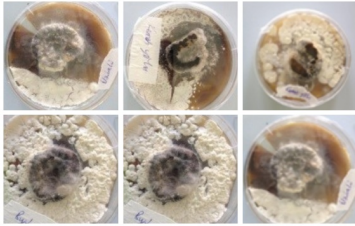
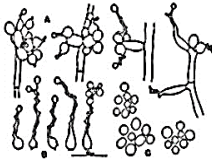
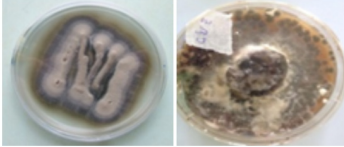

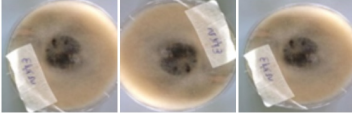

Saborroud Dextrose Agar (SDA) medium, and incu-bated for seven days at 25-27 C and relative humidity (RH) 80-85% 12-14 days pure culture of fungi were identified by using reference of Toledo [24].

3. Results and discussions

Isolation fungi from the larvae of European cockchafer; The most abundant species was found *B. bassiana* (53.3% of samples). Two isolates of *Lecanicillum sp.* (13.3 %) and *Fusarium sp.* (13.3%) were also detected. No entomopathogenic fungi were isolated from 3 of the samples (20%) (Ta-ble 1). Entomopathogens fungi cause disease in *M. melolontha* is of interest as agents for natural control of this pests. *B. bassiana* and *Lecanicillum spp* are entomopathogenic fungi on insects. Some researcher reported that *Fusarium spp.* has got the ability of pathogenic on insects [25]. Especially *B. bassiana* of these fungi is originated from soil and can infected *M. melolontha*. Because *B. bas-siana* has got wide host species. The fungi species isolate from *M.melolontha* population in Erzincan Province, belonged to Ascomycota division. *Beau-veria bassiana* and *Lecanicillum* spare from family Cordycipitaceae, *Fusarium* from Nectriaceae [26]. The main route of entrance of the entomopathogen is through integument and it may also infect the in-sect by ingestion method or through the wounds or trachea the main route of an entrance of the ento-mopathogen is through integument and it may also infect the insect by ingestion method or through the wounds or trachea [27]. At recent times, about 90 genera and almost above 700 species are considered as insect infecting fungi that represent about all the major classes of fungi [28,29]. There is a new inter-est in using entomopathogens for biological control of plant pest insects in Turkey. Despite Turkey has potential to find and develop entomopathogens fun-gi species, studies on entomopathogenic organisms infecting *M. melolontha* has limited. In this paper, entomopathogenic fungi infecting *M. melolontha* is presented to stimulate.

In conclusion, several side effects of chemical pesticides such as the development of resistance and the negative impact on the environment have encouraged several researchers to investigate alter-native control methods on important agricultural and urban pests. In consequence, the development of biopesticides that are effective, biodegradable and no harmful side effect on the environment, turn out to be a priority of these studies. Our isolates of

Table. *Isoaltion fungi from the larvae of European cockchafer*

Fungus Species	Number of Isolated	Original Picture	Original Figure
<i>Beuveria bassiana</i> (Hypocreales: Cordycipitaceae)	7		
<i>Lecanicillum sp.</i> (Hypocreales: Cordycipitaceae)	3		
<i>Fusarium sp.</i> (Hypocreales: Nectriaceae)	3		

this fungus species, especially *B. bassiana* and *Lecanicillum* sp. reported not only in agriculturally harmful insects but also in a variety of sources for the use of tick and mosquitoes in the human and animal health. If we want to develop an alternative biological control agent to chemical pesticides and developed good biocontrol agent against any pests, it should be noted that the first work, we have to make the isolate of entomopathogenic fungus that is the biological control agent.

4. Acknowledgements

The entomopathogenic fungus isolation of this study was supported by the Erzincan Binali Yıldırım University Research Foundation (FEN-A-300614-0104). In addition, in the identification of fungus species and other studies that helps Özlem GÜVEN KALKAR (Isparta Suleyman Demirel University, Plant Protection) and Mariam CHUBINISVILI (NLE Agricultural University of Georgia).

5. References

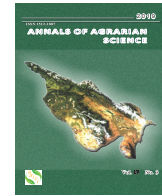
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The Influence of the Weather Conditions on Biological Soil Activity and Maize Productivity

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ABSTRACT

Article presents the results of studies of the influence of weather conditions on the activity of maize microbiocenosis, plant green mass productivity, grain yield, the spread and development of diseases in agrocenosis. Methods. Field experiment, rehydration, adsorption, phytopathological assessment of plants. Results. Weather conditions during the research period were characterized by an increased temperature regime, insufficient and uneven distribution of precipitation during the growing season, which significantly affected the growth, development and maize productivity. The increase of the grain yield of maize (by 3–4 %) compared with the best year due to weather conditions, with a clearly reduced amount (by 3.5–13.7 %) of the formed green mass through the lack of moisture was noted. In conditions of overwetting (precipitation is more than 48 mm than the average annual and an increased average monthly air temperature of 3–5°C) compared with similar hot and dry (arid) periods (rainfall is 15–45 mm lower average annual), in gray forest loamy soil an increase in total microbial biomass by 12–108 % and carbon dioxide emission by 9–12 % increased the synthesis of humic substances by 15–39 % was noted. The decrease by 1.5 time in the development of root rot in maize plants and by 6–13 % of their spread was noted. Conclusions. Thus, changes in weather conditions (increase in average monthly temperatures by 3–5°C and increase in precipitation by 48 mm / decrease in precipitation by 15–45 mm) did not have a clear effect on the functioning and activity of microbiocenosis and soil fertility.

Keywords: *Zea mays* L., Carbon dioxide emission, Development of diseases, Biomass of microorganisms, Spread of diseases, Hydrothermal regime.

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Introduction

Ukraine is one of the five largest exporters of maize in the world. The area under maize crops increased by 3.7 times from 1990 to 2018. Such a significant increase in cultivated areas of the crop caused gross disturbances in crop rotation, an increase in the number of pathogenic microorganisms, harmful insects and weeds in agrophytocenoses, and an increase in the biological and chemical pollution of agroecosystems due to an increase in the use of plant protection chemicals [1]. Irrational and irresponsible use of arable land leads to the loss of organic matter, which in turn turns the agroecosystem into a powerful source of greenhouse gas (CO₂) [2, 3]. A sensitive indicator of changes in the state of biotic and abiotic components of the soil, which are caused by natural and anthropogenic factors, is the

activity of microbiocenosis.

Changes in climatic parameters (temperature, precipitation, increase CO₂ concentration, etc.) have both direct and indirect effects on soil microorganisms through changes in physiological and biochemical processes in plant organisms, which initially causes changes in the trophic chains of microorganisms, and further in the structural and functional organization of the entire microbiocenosis.

During various studies [4–7] the most frequently used indicators of the rate of production of CO₂ and microbial biomass, which allow us to assess the state of microbiocenosis of the soil and agroecosystem as a whole. It is known that an increase in the concentration of CO₂ in the atmosphere usually stimulates the influx of organic carbon into the soil system, increasing the activity of the root system

and exudation, while the quality of the exudates produced by the plant decreases. It is known that an increase in CO₂ concentration reduces the nitrate content in the soil, while the spread of mycorrhizal organisms increases by 47%, and the number of nitrogen-fixing bacteria also increases [8]. It is proved that higher plants are the main factor determining the composition, abundance and activity of microorganisms in the soil [9]. It should be noted that carbon cycle reactions are highly sensitive to the temperature factor. Minor changes, which can contribute to significant emissions of carbon from the soil back into the atmosphere. The indirect effect of climate change on microorganisms, i.e indirectly through plants, it can be stronger than with the direct action of the temperature factor on the formation of the composition, bioecological properties and microbial community functions [10-12]. Naturally, climate change also poses a new problem for maize producers and requires a rethinking and improvement of each element of agricultural technology. Since in different years the amount of moisture and the distribution of precipitation during the growing season significantly affect the yield and quality of maize grain [13,14].

Based on the foregoing, the aim of our work was to study the influence of weather conditions (air temperature, rainfall) on the change in the main indicators of soil microbiocenosis activity, the phytopathological situation in agrocenosis and maize productivity.

Objects and Methods

The studies were conducted on the experimental fields of the Institute of Forage and Agriculture of Podillya of National Academy of Agrarian Sciences of Ukraine during 2011-2013. Soil sampling was

carried out by the standard method [15] in long-term field experiments, the characteristics of which are shown in Table 1. Soil samples from maize agroecosystems were taken from the plant layer 0-20 cm from May to September, when the microbiocenosis reached its stable, balanced state. All samples were prepared by a single method: they were dried in air and crushed to a size of < 3 mm; visible plant debris and mesofauna were removed. The experiments were carried out in five repetitions.

To characterize the hydrothermal regime during the study, the average values of monthly air temperatures and precipitation for the field test area were used. For a more complete analysis of heat resources and precipitation, we calculated the hydro-thermal coefficient of Selyaninov (HTC), which is adequate to the ratio of precipitation to 0.1 sum of temperatures for the study period with values over 10°C [16,17]. To assess hydrothermal conditions by the HTC parameter, the following gradation of parameters was adopted: HTC below 0.5 – a sharp deficit of precipitation (severe drought); 0.6–0.7 – insufficient humidity (very dry) 0.8–0.9 – drought (drought is not intense), 1.0–1.2 – insufficient humidity, 1.3–1.6 – moderate humidity, > 1.7 – excess humidity, HTC > 2.0 – strong excess humidity [18]. In experiments, a hybrid of maize Krasilov 327 MB FAO 350 was grown with a sowing rate of 5.5 million viable seeds per hectare. The experimental area was 30 m² with a row spacing of 0.7 m. Field trials included three blocks. Plant growing practice was standard for the conditions of the Right Bank Forest-Steppe Zone of Ukraine. In each year of research, maize was sown in the last ten days of April and harvested in the first ten days of October. The agrochemical parameters of the soil were determined according to standard methods [19], the research results are shown in Table 1.

Table 1. Agrochemical characteristics of the soil of the experimental fields, 0–20 cm

Soil type	pH	Humus %	Concentration, mg/kg soil		
			Nitrogen easily hydrolyzed compounds	Mobile phosphorus	Exchange potassium
Gray forest medium loamy	4,2	2,06	74,2	174	115

The activity of microbiological processes occurring in the soil of the maize root zone was determined three times during the growing season in the phase of 2–3 leaves, tillering and wax ripeness. The carbon content of microbial biomass (Smik) in the soil was determined by the rehydration method, by gently drying the samples at a temperature of 65–70°C for 24 hours with further extraction with a 0.5 M solution K_2SO_4 [20-22]. The intensity of carbon dioxide (CO_2) emission from the soil was carried out under controlled conditions (temperature, humidity) by the adsorption method after alkaline adsorption was determined by titration and conversion to the amount of CO_2 released from the soil [15,20,22]. To determine the complex damage of plants by root rot (average intensity of damage to plants,%), we used the four-point VIZR scale modified by V. Peresyphkin and V. Pidoplichko [19]. As a result of the experiment, a visual assessment of the damage to plants by root rot was carried out, where: 0 points – there were no signs of damage; 0.1 points – damages were noted in the form of separate brown or black dots on the roots, the underground internodes and the basal part of the stems; 0.5 points – dot damages of half of the underground internodes or roots; 1 point – slight browning or blackening of the underground internodes, the base of the stem and root system in the form of separate strokes; 2 points – a strong browning of the underground internodes and roots on the basis of the stem brown or black stripes; 3 points – strong and continuous browning of the base of the stem and the underground internode, more than half of the roots have died out; 4 points – the plants died. The spread of diseases is the number of affected plants or their individual organs as a percentage of the total number examined on the area or field, was determined by calculation [19], according to the formula:

$$P = \frac{n \cdot 100}{N} ,$$

where P is the spread of diseases,%; n is the number of affected plants (plant organs) in the sample; N is the total number of examined plants (organs) in the sample. The direction of microbiological processes in the soil (mineralization coefficient, humus accumulation coefficient) was determined by E. Andreevuk and G. Iutinskaya [23]. Statistical processing of experimental results was performed in Microsoft Excel. The mean values (\bar{x}) and their standard deviations (SD) were determined. The significance level selected for the study was $P < 0.05$.

Results and Discussion

During three years of field research, the temperature regime of the first half of the maize vegetation was characterized by an excess of long-term average values of 1–5°C, especially in June–July (Fig. 1).

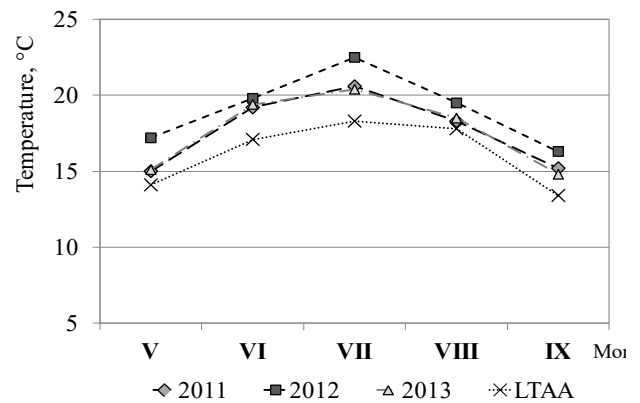


Fig. 1. Dynamics of average monthly temperature during the maize growing season

Monthly temperatures exceeding 3–5°C continued throughout the growing season – from May to September 2012. These months in 2011 and 2013 were characterized by significant amount of precipitation, whereas the entire vegetation period of 2012 was observed to be drought (Fig. 2).

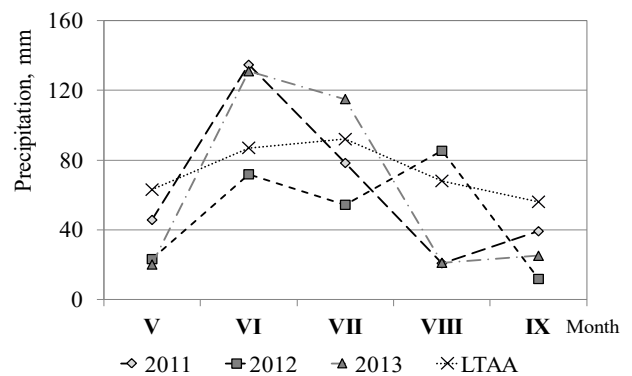


Fig. 2. Dynamics of monthly precipitation during the maize growing season

Maize is a heat loving and drought tolerant crop, which requires an average daily temperature of +25°C for growth and development. This culture tolerates drought well until the tube exit phase. From the throwing panicles moment its moisture need is the most significant [24]. A lack of moisture during this critical period causes withering of the plants, drying of the leaves, decreased photosynthetic activity and pollen viability, and this leads to impaired

fertilization and subsequent formation of grain [25].

Thus, comparing the values of the hydro-thermal coefficient (HTC) of each year of research and the average long-term indicators (ALTI), it follows that the weather conditions of 2013 were close to optimal for the development of plants and the formation of a maize crop (Fig. 3). ALTI data for 2013 have an advantage over others and characterizes not only the incoming part of the water balance (precipitation), but also the unproductive discharge of moisture (evaporation) from the surface of the soil or plants and allows to evaluate the influence of two abiotic factors at the same time [6].

The first half of the 2011 year vegetation season was favorable for the level of HTC, and since August there was a lack of precipitation and drought, which negatively affected the formation of ears of corn and grain. In 2012, the level of HTC during the entire growing season was very low, which indicates the extreme conditions for the development of plants and the accumulation of green mass of maize, because the long period (May–July) with abnormal heat and drought was observed.

Comparison of agrometeorological indicators in the researched years with the obtained crop showed that the range of differences in weather conditions during the growing season of maize significantly influenced the biological activity of microbiocenosis (Table 2), the yield of green mass and grain (Fig. 4).

The lack of precipitation in August leveled out favorable weather conditions for plants of the first half of the maize vegetation, which did not allow the plants to form a high yield due to the use of nutrient reserves accumulated in the green mass. The initial phases of maize ontogenesis (May) were characterized by an insufficient amount of precipitation, respectively, and moisture in the soil, which most likely restrained the development diseases of young plants' roots. According to the results of phytopathological evaluation, the distribution of root rot of maize was 56 % (Table. 2). Confirmation of the average level of disease development is the content of microbial biomass at the level of 112.3 $\mu\text{g C / g}$ of soil and carbon dioxide emissions in the range of 32.8 mg of CO_2 / kg of soil. Thus, it can be argued that the bacterial component, rather than the

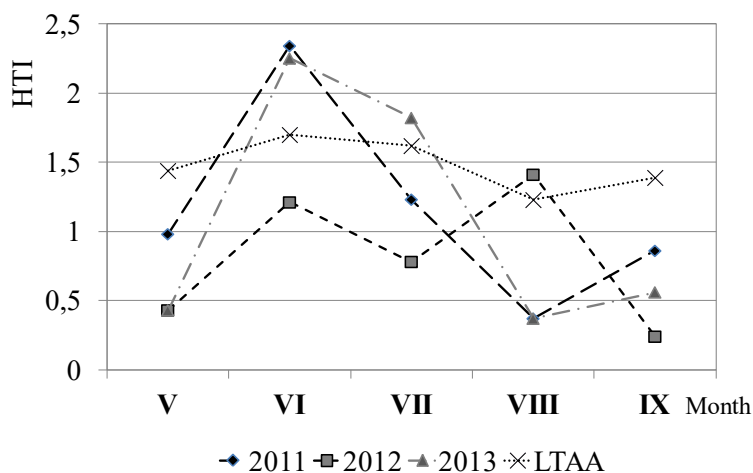


Fig. 3. Dynamics of HTC during the maize growing season

Table 2. Indicators of the biological activity of microbiocenosis and phytopathological evaluation of maize plants

Test indicator	Researches period, year		
	2011	2012	2013
Microorganisms biomass, $\mu\text{g C / g}$ of soil	112,3±5,17	100,6±3,82	208,3±8,75
CO ₂ emission, mg CO ₂ / kg of soil	32,8±0,95	29,2±1,02	32,0±1,06
The spread of root rot, %	56,0±4,2	62±2,3	49±3,1
The development of diseases, %	2,1±0,5	3,3±0,4	2,0±0,5

fungal one, dominated in the microbial community of the soil [26], as the fungal component forms a significant part of the biomass of soil microbiocenosis [27; 28]. The activity of synthesis processes in the soil was positively affected by conditions of elevated temperatures and humidity, typical for the period May–July 2011, as evidenced by the values of the mineralization and humus accumulation coefficients (1.32 and 1.28 respectively).

Moisture deficiency and increased temperature regime during May–July 2012 caused a significant decrease in the formation of green mass of maize (Fig. 4).

As a result, the productivity indicators of the green mass of plants amounted to 57.9 t ha^{-1} , which are $7.3\text{--}9.2 \text{ t ha}^{-1}$ less than in other years. At the same time, the grain maize yield was at a level close to the crop with optimal weather conditions – 6.37 t ha^{-1} . This effect is due to the drought tolerance of the culture. It also contributed to obtaining a relatively high grain yield in a dry year and an increased average monthly temperature, because under such conditions, plants of type C4 increase the efficiency of nitrogen use in photosynthetic structures, to which this crop belongs [5]. Another physiological feature of maize plants provides its resistance to drought – it has a very low transpiration coefficient, because plants use carbon dioxide more economically with minimal water losses [29]. However, a high level of solar insolation and a significant precipitation deficit during certain interphase periods of the vegetation affected plant incidence with dis-

eases. The prevalence of root rot in agrophytocenosis was fixed at the level of 62 %, and the development of diseases was 3.3 %, which was 1.6 times higher than in other researched periods (Table 2). It is known that the defeat of maize (fusarium root and stem rot) contributes to the weakening of plants due to violations of the soil water regime and other weather stresses [30]. Therefore, it can be assumed that the unstable distribution of precipitation during the growing season and the excess rainfall in April before sowing contributed to the development of the disease.

It is known that temperature is more significant influencing factor of the functional structure of soil microbiocenosis. Scientific research [24] proved that temperature affects the population density of bacteria with an eutrophic type of nutrition and is 86.2 %, streptomycetes – 22.1 %, micromycetes – 18.5 %. The simultaneous combination of temperature and soil moisture factors contributes to growth in the influence of the latter on the number of streptomycetes by 3 times, soil micromycetes and oligotrophic bacteria – by 4 times, bacteria immobilizing nitrogen of mineral compounds – by 8 times.

Abnormal weather conditions in 2012 (exceeding average monthly temperatures by $3\text{--}5^\circ\text{C}$ and a reduced amount of precipitation by $15\text{--}45 \text{ mm}$ depending on the study period) also affected the biological activity of the soil. The content of microbial biomass in maize agrocenosis was $100.6 \mu\text{g C} / \text{g}$ of soil, which is 2 times lower than the data for the optimal weather year (2013). Also the anal-

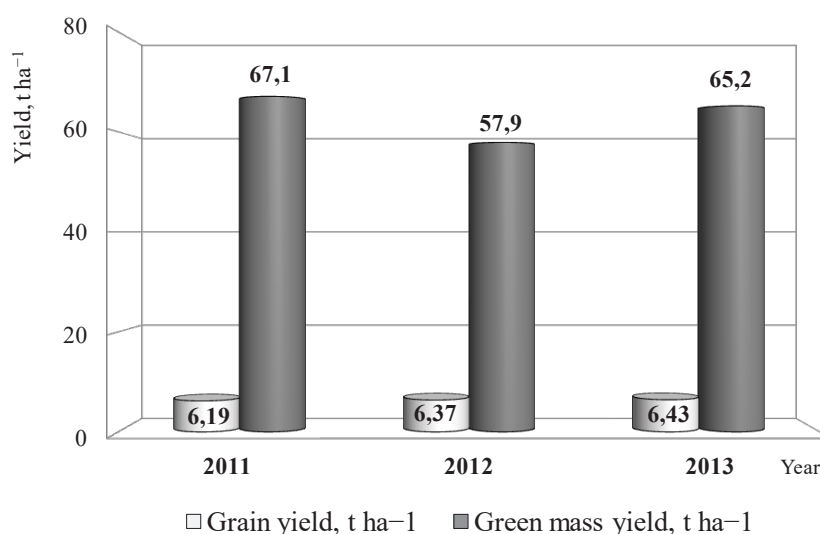


Fig. 3. Maize yield, t ha^{-1}

* Yield t ha^{-1} , $\text{SSD}_{2011-2013}$: 0,08; green mass t ha^{-1} , $\text{SSD}_{2011-2013}$: 1,5

ysis of the indicators of biological activity of the soil showed that an increase in temperature and the reduction amount of precipitation negatively affects the processes of humus accumulation in the soil ($K_{\text{hum}} = 0.92$).

If the initial stage of the growing season was characterized by low rainfall, the temperature regime was optimal for the development of maize plants and the accumulation of phytomass, which promotes the development of the powerful root system that permits deep into the substrates of the soil. This, in its turn, favorably influence the formation of a high yield in conditions of insufficient moisture supply. That is why in 2013, despite a very low rainfall in May and August, a high yield of green mass and maize grain was obtained, respectively 65.2 and 6.43 t ha⁻¹ (Fig. 4). Maize plants at the end of the growing season show a high need for nutrients for grain formation.

In 2013 the phytosanitary condition of maize crops was the best. Diseases affected up to 49 % of crops with its development in only 2 % of plants. It should be noted that there were no objective reasons for the development of fungal diseases associated with weather conditions, since during the period of sowing and the initial growth and development of maize plants, an increase in air temperature and a small amount of precipitation was observed. Probably, the main sources of infection of maize plants were root rot pathogens located in the soil or on the seed surface. Indicators of microbial biomass and carbon dioxide emissions were 208.6 µg C / g of soil and 32.0 mg CO₂ / kg of soil, respectively (Table 2). For hydrothermal conditions 2013 approached to the long-term average data; during this period the balance of the processes of humus accumulation was noted ($K_{\text{hum}} = 1.06$). Thus, the obtained results indicate that in 2013 there were optimal conditions for the functioning of the soil microbiocenosis in maize crops.

The influence of the weather on yield and grain quality is also confirmed by researching [13, 31]. For example, the maize crop under optimal weather conditions (HTC 1.01) amounted to 1.05 t ha⁻¹ with the maximum efficiency of the plant protection system. Under conditions of heat and moisture deficit (HTC 0.56), 0.43 t ha⁻¹ of maize was harvested and a low level of chemical protection of plants was recorded [31]. Accordingly, contrasting weather conditions during the researched period had a significant impact on the effectiveness of the herbicides and the number of weeds, and became a determining factor in the maize yield.

Conclusion

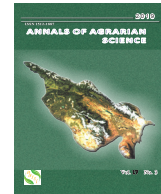
It has been established that in years with an extremely high temperature and arid conditions (exceeding average monthly temperatures by 3–5°C and a reduced amount of precipitation by 15–45 mm, depending on the month of research), it is possible to obtain a quite high yield of maize grain at the level of 6.37 t ha⁻¹, but with losses in green mass productivity (in the range of 7–9.5 t ha⁻¹). It should be noted that the uneven distribution of precipitation during the growing season and the excessive amount of precipitation before sowing (exceeding the norm by 48 mm) contributed to the development of plant diseases by 1.6 times and an increase in the spread of diseases by 26 % compared to favorable weather conditions. Consequently, during the critical temperature regime, but with excess humidity in the first half of the growing season, high rates of root rot prevalence (56 %), which caused the decrease in the grain yield to the level of 6.1 t ha⁻¹ were observed. However, a high level of humidity has contributed to the accumulation of the large amount of green mass for plants (67.1 t ha⁻¹), which is 9.2 t ha⁻¹ more than in adverse year conditions. On the other hand, during extreme periods (excessive humidity and a monthly average temperature increase of 2–5°C) in comparison with the same hot but arid periods, the amount of total microbial biomass in the soil increases (by 12–108 %) and the synthesis processes increase humic substances (by 15–39 %) and carbon dioxide emission is activated (by 9–12 %). Therefore, such weather conditions contribute not only the increasing of the yield of maize green mass but also preservation of soil fertility.

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Classification and Nomenclature of Soil Subtypes of the Republic of Armenia According to International Standards

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ABSTRACT

The Republic of Armenia is a small mountainous country with vividly expressed vertical zoning and scarce in soil, where among all the components of the landscape soil cover is also exposed to zoning patterns typical of mountainous countries.

The classification of all genetic soil types and subtypes separated in the territory of the republic is based on the criteria presented in International World Reference Base for Soil Resources (WRB).

Keywords: Vertical zoning, Soil type, Subtype, International classification, Genetic horizon, Structure of soil profile.

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Introduction

Complex mountainous relief, diversity of surface forms, strict ruggedness, spotted geological structure, heterogeneous nature of soil-forming mother-types, abrupt fluctuations in altitude, as well as the diversity of climatic conditions contributed to the formation of a spotted soil cover. For that reason, almost all soil types that are common in Europe are available in the small territory of the republic.

Depending on the specific physical-geographical conditions of each vertical zone, quite different genetic types, subtypes have been formed and evolved, which are quite different in their origin, composition, structure and agro-industrial properties, which replace each other from lowlands to mountain peak.

14 genetic soil types existing in the territory of the Republic of Armenia have been subdivided into 27 subtypes [1-3].

The material, object and research methods

The compliance of the names of all soil types and subtypes existing in the territory of the Republic of Armenia with the standards of international

WRB system served as research material. Soil cover of the republic served as the object of the research. Research has been carried out according to the criteria adopted by the WRB system.

Research results

Taking into account conditions of the subtype, the structure of soil profile and the main properties of individual genetic horizons, most acute expression of any factor of soil formation processes, the soil type has been divided into different subtypes.

Based on the above considerations, especially WRB system standards, the soil types existing in the Republic of Armenia have been divided into the following subtypes:

Calcisols – As semi-desert brown soils were formed within the borders of Ararat Basin which has homogeneous climatic conditions, mother-types and plant coverage, only one brown typical subtype was separated within the mentioned soils, which has received the name Typical fulvous according to the International Classification of soils.

Anthrosols – Based on the structure of soil profile, the properties of individual genetic horizons, ground humidification and age-old use of soils, the

following subtypes are separated within the soil type Anthrosols, which has received the following names according to the international classification: Humid-meadow, Meadow fulvous irrigated and Residual. Soils of Humid-meadow subtype occupy a relatively small space. They mainly develop in areas where the ground water level is very high (up to 1.5 m) and generally have a capillary nutrition. Ground waters are either lightly mineralized or freshwater. According to depth, buried or illuvial-humus horizons are met here, that have been formed as a result of regularly rinsing of soils in the past. Besides, carbonate horizon is also met in the lower layers of soil profile, which is caused by prolonged impact of ground water. Salinized and alkalized horizon is formed in the middle, very often also in the lower layers of profile in the distribution area of mineralized ground waters [4-5].

Soils of Meadow fulvous irrigated subtype have a large areal. This subtype was formed both in the conditions of ground water nutrition and the humidification of irrigation water.

In such areas soil formation processes occur in a relatively weak ground nutrition conditions.

The depth of ground waters varies between 1,5-4,0 m. Usually they are freshwater or lightly mineralized.

One of the most important features of soils of Meadow fulvous irrigated subtype is that it does not have carbonate profile.

The soils are characterized by dark cinnamonic color of upper horizons and by dark brown color of lower horizons. The capacity ranges from 40-60, sometimes up to 70 cm [6-7].

Residual subtype was formed on the old terrace of Aras River. Initially soil formation took place here in the conditions of capillary ground humidification, too. But now the ground water level has dropped and they are on 5-6 m and greater depths, as result of which capillary nutrition is cut from soil horizons, though the signs of ancient hydromorphism are still preserved and have a residual character. Currently the formation of these soils occurs under the influence of automorphic processes, in the conditions of irrigation and intensive cultivation.

Soils of Residual subtype are characterized by dark brown color of upper horizons and by dark brown color of lower horizons. One of the characteristic features of the soils is that there is more than 70 cm, powerful agro-irrigation horizon and weak discrimination of genetic horizons of the profile here.

Antrosols (Fluvisols) soil type is divided into two subtypes: Floodland-terraced, marsh and Floodland-terraced, meadow [8-9].

Flood land-terraced, marsh soils are disseminated in lower terraces of river valleys, where there is excess of moisture. The mentioned soils are mainly located in those distribution areas of Floodland-terraced, meadow soils where ground water level is higher than 1 m and close to the ground surface. Here, the upper horizon of soils is rich in peaty, dilapidated organic materials and finely dispersed sludge particles where humus content sometimes reaches up to 17%. Ground waters are not mineralized and do not contain water-soluble salts with qualitative composition and dangerous quantities for optimal growth and development of crops.

Soils of Flood land-terraced, meadow subtype are disseminated in those parts of river valleys that are exposed to the influence of alluvial horizons. That's why it becomes clear from the study of the morphological features of the soils that there is vividly expressed streaked structure of the profile and spotted mechanical structure here. Diversity is also observed in terms of the content of humus and carbonates.

Solonetz-Solonchaks soil type is divided into the following two subtypes: Saline alkali meadow-marsh and Saline alkali meadow [10].

Soils of Saline alkali meadow-marsh subtype have limited distribution and are met in the form of islets along Metsamor River, in the borders of the delta of Hrazdan River and peripheral regions of Yeraskh steppe.

Saline alkali meadow subtype occupies quite a lot of space (around 80%). They are met in Armavir, Masis, Ararat regions with large masses.

Solonetz – This soil type has only one subtype - Saline paleo-hydromorphic.

Saline paleo-hydromorphic soils are characterized by a homogeneous structure of the profile, by weak discerning of the genetic horizons, by red or yellow color, by loamy or heavy loamy-sandy mechanical composition. The soils are mainly low-powered, containing gypsum, equally carbonatized, coherent, solid and cracked.

Umbrisols, (Leptosols) – As a result of field and laboratory research Umbrisols, (Leptosols) soil type has been divided into three subtypes: Turfy-terrace, Turfy (brown), Weak turfy (dark colored) [11].

Turfy-terrace subtype soils develop in alpine zone within the range of 2700-3600 m altitude above sea level, on the slopes having different cur-

vature and location. They occupy a small space where many exits of radical rocks are very often observed.

The most important morphological features of Turfy-terrace soils are the presence of peaty horizon of 5-10 cm power, brown, sometimes dark brown color of humus horizons, medium strength, strong rockiness, especially in the lower horizons, loamy-sandy, rarely also light loamy-sand mechanical composition, weak discerning of the genetic horizons and the absence of carbonates throughout the profile.

Turfy (brown) soils are formed in the lower parts of alpine zone and in the upper parts of sub-alpine zone within the range of 2700-3500 m altitude above sea level, on the slopes having different curvature and location.

The most important morphological features of Turfy (brown) soils are brown, dark brown, sometimes black color of humus horizons, well expressed waterproof granular structure, the absence of carbonates throughout the profile, loamy-sandy mechanical composition, weak discerning of the genetic horizons. The capacity of humus horizons is not large, relatively powerful soils are met in low-lying areas of the relief. Humus content can reach up to 16%.

Soils of Weak turf (dark colored) subtype develop under short and dense alpine plant communities. They are distinguished by black or brown-black color, by small granular structure of upper horizons, large capacity (75 cm) of humus horizons, acid reaction, considerable saturation of absorbing complex, significant content of exchangeable aluminum and high content of nitrogen and humus.

Phaeozems soil type is divided into Black-earth and Typical subtypes.

Soils of Black-earth subtype occupy a large area in Sevan basin, central Armenia and in Shirak region. The mentioned soils occupy the upper part of meadow steppe zone where climatic conditions are severe.

Flora is fairly thick and lush here, steppe and meadow communities of mesophilic nature are largely dominate here. Humus content sometimes reaches up to 18%.

One of the most important morphological features of the soils of Black-earth subtype is black or brown color of humus horizons and light brown color of the lower horizons.

Typical subtype occupies large areas in southern, southwestern, and sometime in southeastern loca-

tions of arid slopes of Southern and Central Armenia. The mentioned soils have intermediate position between Kastanozems soils and Acrisols soils. Here humus horizons of the soils have light color compared to the previous subtype due to the lower humus content (9-10%).

Acrisols (Cambisols) soil type is divided into Leached, Typical, Carbonate subtype [11- 13].

Leached subtype is the most disseminated and the soils of Carbonate subtype occupy the smallest area. Leached soils are mainly disseminated in the middle and upper parts of the forest zone. They develop in relatively more humid areas than the soils of Carbonate Typical subtype. Main morphological features of Leached soils are gray or brown color of the whole profile, full- washing of carbonates, weak discerning of the genetic horizons, nutty structure, high humus content (7-12%) in the upper horizons, neutral or weak acid reaction, high absorption capacity, the presence of exchangeable acidity in the upper parts of the zone, clayization of middle and lower part of the profile, the lack of the phenomenon podzolics.

Typical soils have intermediate position between soils of Leached and Carbonate subtypes and are mainly disseminated in middle and lower parts of the forest zone. One of the important features of these soils is refining upper and middle horizons from calx, cinnamonic or dark cinnamonic color, nutty structure and clayization of the profile.

Soils of Typical subtype are mainly used in agricultural production. There have been significant changes in soil profile as a result of lengthy and uncoordinated use. In particular, structure considered valuable from agronomic point of view has disintegrate, the amount of water-resistant aggregates has reduced, humus content has decreased, etc.

Carbonate soils develop in the lower part of the forest zone on southern and eastern slopes. From morphological point of view, these soils have a profile rich in carbonates, dark brown color of humus horizons, granular structure sometimes also nutty structure of humus horizons, loamy-sandy sometimes also light loamy mechanical composition and some cementation of the whole profile especially in middle horizons. The presence of illuvial carbonate horizon is also typical of these soils.

Chernozems - Depending on hydrothermic regime of the zone, the nature of the relief, maternal rocks and other conditions of soil formation, soils are divided into Leached, Typical and Carbonate subtypes [14].

Humus horizons are washed from carbonates in the soils of Leached subtype, carbon content starts from B horizon in case of Typical subtype, and these are available in all soil profile in Carbonate soils.

Leached subtype is met with large areas in Northern Armenia, especially in Ashotsk plateau and Lori field. The mentioned soils were formed on the weathered soft mass of andesite-basalt, Liparit-dacins, porphyrites, tofo lavas.

Humus horizons are characterized by dark gray or dark brown color, carbonates are only in the soil-forming maternal rocks and there is some accumulation of silt fraction in the middle and upper horizons of soils. Soils of Typical subtype are prevalent in Lake Sevan basin, Aparan-Hrazdan regions. Weathered materials of andesite-basalt, porphyrites, tofo lavas serve as soil-forming mother-types. Discerning of the genetic horizons is vividly expressed, where A horizon is distinguished by relatively fragile structure and B or BC horizons by solid structure. There is some accumulation of silt fraction in the middle and upper horizons of the mentioned soils, too.

Soils of Carbonate subtype are mainly disseminated in the field of Shirak and in Central Armenia. Soil-forming mother-types are represented by weathered materials of volcanic and sedimentary rocks, where the content of carbonates reaches up to 30-35%. Discerning of the genetic horizons is well expressed in soil profile, there is no accumulation of silt fraction in the middle and upper horizons here.

Kastanozems - Dry steppe soil zone is distinguished by the variety of climatic conditions. Kastanozems soil type specified in this zone is divided into Dark chestnut and Light chestnut subtypes.

Soils of Dark chestnut subtype with small surfaces are disseminated in the upper areas of dry steppe natural-soil zone and are mostly bordered by the soils of Chernozems Carbonate subtype. The content of humus ranges from 3,0-4,2% in the upper humus accumulating horizons. This subtype is disseminated in relatively flat and lightly fragmented areas of the relief, for that reason they are distinguished by a relatively large capacity, fragile structure, homogeneous color of horizons and low content of carbonates.

Soils of Light chestnut subtype are disseminated in low-lying areas of the natural-soil zone. Humus content is about 3.0% in humus accumulating horizons. Light chestnut soils are rocky, carbonate, not alkalized, low-powered and medium powered and considerably solid. Unlike previous subtypes, these soils are lightly aggregated and contain less amount of water-resistant aggregates [15].

Conclusion

According to the international soil classification system, as a result of grouping and new naming, main soil types and subtypes of the territory of the Republic of Armenia have received the following names:

Semidesert brown soils – (Calcisols) - Typical fulvous.

Irrigated meadow brown soils, flood-plain-terraced soils - (Anthrosols) 1. Humid-meadow, 2. Meadow fulvous, irrigated, 3. Residual, 4. Floodland-terraced, marsh, 5. Floodland-terraced, meadow.

Hydromorphone saline-alkaline soils and Paleohydromorphic connected alkaline soils - (Solonetz-Solonchaks) 1. Saline alkali meadow-marsh 2. Saline alkali meadow 3. Saline paleo-hydromorphic.

Brown soils or Brown chestnuts - (Kastanozems) – 1. Dark chestnut 2. Light chestnut

Mountainous black soils and meadow black soils - (Chernozems) 1. Leached 2. Typical 3. Carbonate

Cinnamonic forest, Forest sod – carbonate and Brown forest soils - (Acrisols, Cambisols) 1. Leached 2. Typical 3. Carbonate.

Meadow prairie soils - (Phaeozems)- 1. Black-earth 2. Typical

Mountainous-Meadow Soils - (Umbrisols, Lep-tosols) - 1. Turfy-terrace 2. Turfy (brown) 3. Weak turfy (dark colored).

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