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Aims and Scope

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Method of laser diffraction spectra's deconvolution for characteristics strength of soil microaggregates

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A B S T R A C T

Laser diffractometry, yielding exactly, cannot replace the pipette analysis of the soils/sediment particle size distribution. But laser diffractometry can be used to improve soil physical knowledge related with soil structure stability, for example, to assess the degree of microaggregates preservation after their partial dispersion. For this purpose it is necessary to use deconvolution of the laser diffraction curves, with the following analysis of the number of LD-fractions and their properties. It was pproposed 4 indicators of LD-fractions: theoretical number of fractions (N), the real number of fractions (n), period of fractions (T), and their dispersion (D). Introduced a new indicator of the aggregates strength (RMA), taking into account the role of only 3 independent criteria: N, D and T. According to RMA-indicator, the aggregates in paleosols are stronger than aggregates in recent andosols.

Keywords: Degree of microaggregates preservation, Soil structure stability, LD-fractions. Laser diffractometry, Real number of fraction, Dispersion if fraction.

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Introduction

At present, two methods for analyzing the grain size composition of soils and sediments are common. The older method, a sieve analysis combined with a thin-particle pipette analysis (SPA), 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, 3]. Laser diffractometer is provide rapid analysis and do not need a larger sample mass. Another important LDA advantage is the obtaining of a practically continuous spectrum of the dependence of the particle content in % on their size. On the graph of the LD-function, a sequence of minima and maxima is formed. They are interpreted as follows: the position of the minima on the particle diameter axis is the boundary between the fractions, and the magnitude of the (maximum) is proportional to the fraction part in these boundaries.

But the LDA has a serious flaw. Numerous tests have shown that usually the grain size composition determined by the LDA method differs significantly from the composition determined by the SPA method [2, 3, 4-7]. The LDA method is not capable

of taking into account the deviation of thin clay particles from the spherical shape [4, 2]. But there is an error in the content of sand particles, if their shape is very different from the ball [5]. In addition, an error makes a difference in the density of minerals within a single sample. As a result, the composition determined by the LDA method may be very different from the true grain size composition determined by the SPA.

To use laser diffraction to determine the grain size composition, much work is being done to align the LDA and SPA data. To do this, they determine the conversion factors for the content of each particle fraction [6]. In this case, the correction factors are valid only for a given series of samples. In the absence of a reliable statistical relationship between the content of the same fractions the determination of correction factors becomes impossible, determined by two methods [6]. This led to limitations on the use [5] and the realization that it is not possible to determine the grain size composition by the LDA method [2].

In general, except for rare cases of accidental coincidence, the LDA method can not determine the grain size composition of soil/sediment. In order to distinguish from the grain size fractions, we will name the fractions revealed by the laser diffraction method as "LDA-fractions". But then the question arises: what can be the use of the LDA method? We will try to answer this question.

We'll give a quote from the very important paper of Buurman et al. “Grain-sizing by laser diffraction cannot replace the classical combination of sieving and sedimentation as long as correlations between the methods have not been established for many populations of samples. Nevertheless, grain-size determination by laser diffraction has a great potential for use in soil science, e.g., for detailed comparison of samples from the same origin to establish homogeneity of parent materials, for the study of texture changes caused by weathering, and for changes in aggregation” [8].

Probably, the significance of this rapid analysis is the characterization of the relative dispergation of soils and sediments. With the new approach, because of the conventionality of the absolute indices of LDA fractions, such as the average particle diameter (d_{aver}) and their content n, the authors use relative dispersity indices.

Let's pay attention to the studies, where relative LDA indicators are used. At the same time, the authors exclude from consideration such absolute indicators of LDA fractions as the average particle diameter (d_{aver}) and their content n. Thus, the advantage of LDA is used as an express analysis of the relative characteristics of different systems.

For example, the difference in loess particle sizes obtained from two models of the LDA function analysis is used: Freinhofer (FH) and Mie (Mie) [9]. Then the relative particle dispersity index ΔGSD is calculated:

$$\Delta GSD = GSD_{FH} - GSD_{Mie}$$

A strong disagreement between the results of the two methods of analysis (SPA and LDA) is noted by many authors [2, 10]. Thus, the composition of loess soils by LDA is strongly depleted of silty particles <2 μm and enriched in dust particles of 2-20 μm [10]. In paper [2], a regression equation was given for 158 samples that bound the content of clay particles thinner than 2 μm, determined by the laser diffraction (Y) method with their content from the pipette analysis (X):

$$Y = 0.361\ X - 0.232.$$

As can be seen, on average the sludge content, determined by the LDA, is 2.8 times less than in the classical pipet-analysis. In the same paper [2] on certified samples it was shown that the amount of silt, according to the LDA, is 2/3 below its actual content.

Data on the extremely low shortage in the content of clay particles by the LDA method in one of the samples are given: the content of these particles was 5.5 times lower than for the pipet-method [3].

As can be seen, using the relative characteristics of LD-fractions, new information on the properties of disperse systems is obtained. The relative characteristics of LD-fractions can also be used to solve some soil problems.

One of the problems facing the soil scientist is the need for an operational control of the conservation of soil microaggregates, after chemical preparation for grain size analysis. The degree of safety of microaggregates with the selected preparation for LDA reflects the genetic features of soils.

The most durable microaggregates are formed in volcanic-ash soil, taxonomically Andosol [11, 12]. In Andosol are formed the organo-mineral associations occurring at smaller spatial scales because this soil type holds much higher amounts

of organic matter and short-range-order minerals compared to any other soil types [13].

At present, the completeness of the dispergation of microaggregates is estimated by a rather complicated method: using microscopy methods, including expensive electron microscopy. At the same time, a detailed analysis of the LDA-spectra can give an answer to the completeness of the microaggregates dispergation. The fact is that the grain size fractions of elementary particles of soils and sediments have a number of characteristic features that differ from the properties of microaggregates. If the LDA-spectra deviate from standards (extremely dispersed samples), we can speak of the preservation of microaggregates, that is, of incomplete soil dispergation.

But it is not possible to determine directly the fractal indices from the visual analysis of the LDA-spectra. This requires splitting the function of the LD-spectra. The procedure for splitting the spectrum is called as "deconvolution".

Objective: to use deconvolution of laser diffraction spectra as a method of controlling the preservation of microaggregates after chemical preparation of soils.

The method of deconvolution

The meaning of deconvolution (with respect to decoding the energy dispersive X-ray fluorescence spectrum) is described in detail by Savichev and Stepanov [14]. Let us apply this technique for the deconvolution of the grain size spectra of soils.

Since the particle size varies over a wide range, the logarithms of the particle diameters are plotted along the abscissa axis, i.e. spectra are displayed on a semilogarithmic scale. The base of the logarithm does not matter much, it is important that it is greater than one. We adopted the construction of Gaussian curves in natural logarithms. Then the initial distribution looks like this:

$$Y(\mathrm{lnd}), \qquad (1)$$

Where d is the particle diameter. The initial distribution represents the sum of the individual lines whose wings are superimposed on each other. Let us assume that the true contours of the lines have a Gaussian shape. We construct a model distribution representing the Gaussian curves sum:

$$Y_{mod}(\mathrm{lnd}) = \sum_{i=1}^{N} A_j \cdot \exp\{-[(\mathrm{lnd}_j - \mathrm{lnd})/D_j]^2\}. \qquad (2)$$

Here, the index of the line number j runs through the values 1, 2, 3, ..., K, where K is the quantity of lines, A_j is the amplitude of the j-th Gaussian curve, d_j is the position of the j-th line vertex on the abscissa (μm), D_j is the dispersion of j- th Gaussian curve.

The transition to a discrete distribution for given diameters d_i yields expressions (1) and (2) to the form:

$$Y(\mathrm{lnd}_i) = Y_i, \qquad (3)$$

and

$$Y_{mod}(\mathrm{lnd}_i) = Y_{mod\ i}. \qquad (4)$$

Here the index i runs through the values 1, 2, 3, ..., N, where N is the quantity of measurement points.

The model distribution is found from the condition for minimizing the deviation of the model distribution from the initial one:

$$\sum_{i=1}^{N} (Y_i - Y_{mod\ i})^2 = \min. \qquad (5)$$

However, expression (5) assumes identical error prices at small and large values, although for small values the error price is higher. To take this into account, we introduce the statistical weight of ordinates:

$$G_i = 10/(Y_i + 1)^{1/2}, \qquad (6)$$

and we search for the optimal model distribution from the minimization of the function:

$$\sum_{i=1}^{N} G_i (Y_i - Y_{mod\ i})^2 = \min. \qquad (7)$$

From condition (7) we search for all the exponents of each of the Gaussian curves: the amplitude A_j, the position of the vertex d_j (d_{aver}) and the dispersion value D_j.

The problem is linear only in the amplitudes A, but non-linear in the diameters of the maxima d_{aver} and the dispersions D. Therefore, it is necessary to go over the positions of the maxima and dispersions with a small step and to calculate the amplitudes at each step.

The most insignificant fractions with a share of <1%, as unreliable, are excluded from consideration. Examples of two deconvolution LD-spectras are shown in figures 1 and 2.

Objects

We studied recent soils and paleosols in quaternary fluvio-lake sediments in the basin of Bozhano in northern Italy, described in the paper [15]. 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 strongly enriched in carbonates.

Recent soils are referred to as 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, in varying degrees of erosion.

Before the grain size analysis, for dispersing the microaggregates samples <2 mm were treated with H_2O_2 to oxidize the organic matter, and then dithionite-citrate-bicarbonate (DCB) to dissolve the glandular cement. In carbonate rock samples underlying the recent soil (RS-CI and RS-C2 samples), the carbonates were removed by treatment with Na acetate buffered at pH 5.

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 [15]. The obtained spectra of particle distribution were deciphered by deconvolution.

Calculations of LD-fractions

Main indicators of LD-fractions are their real content in this sample (n), average diameter $d_{average}$, period T, and dispersion (D).

The average diameter (d_{aver}) of all fractions allows us to determine the period of the function T:

$$T = (d_{aver})_{i+1} : (d_{aver})_i \quad (8)$$

This makes it possible to compare the T values of the soils with the periodicity of the sediment fractions: T = 3-4 [16, 17]. For vrtisols period received us is T ~ 3.3 [18]. The larger the value of T, the stronger the fractions are differentiated.

Another important indicator of the differentiation of LDA fractions: the dispersion of Gaussian curves D. The smaller the value of D, the more pronounced the differentiation of the fraction. By the value of D, one can judge the homogeneity of the particle distribution in a given fraction.

As additional characteristics of LD-fractions, you must enter the new indicators; one of them is the theoretical number of LD-fractions (N). In the range 0-2000 μm, it is allocated 9 classic size fractions of differing mineralogy of the dominant particles. Each faction is marked the index. The faction fc: 0-0.2 μm; mC: 02-0.63 μm; cC: 0.63-2.0 μm; fSi: 2.0-6.3; mSi: 6.3-20 μm; cSi: 20-63 μm; fS: 63-200 μm, mS: 200-630 μm and cC: 630-2000 μm [9]. Similarly it is calculated the period E, based on the borders between the factions. According to [9] the period of classical size fractions T ~ 3.1.

Thus, each of the groups: clay (c), silt (Si) and sand (S) are subdivided into 3 factions: fine (f), medium (m) and large (c), total 9 classic fractions. It is possible that each grain fraction is meet a real set of particles, whose distribution is described by the Gauss-curve. The reality of individual solid fractions is confirmed by the results of rock crushing. The close number of factions was identified with the spraying rocks by Sadovsky [17, 14]. It is proved that when the dispersion of particles is formed are not arbitrary, but quite specific sizes. In the interval 0 - 2000 μm are identified 8 fractions. A small difference among the factions is so classic width slightly narrower than fractions resulting after the experimental crushing of rocks. The proximity of a number of theoretical and a number of real-world factions is confirm the possibility of using borders classic fractions to analyze fractions identified in the result of LD-deconvolution.

Thus, the average diameter (d_{aver}) of the LD-fractions, you can identify this LD-fractions by placing this faction in the classical borders of one of the 9 groups of clay, silt and sand. This will examine the nature of distribution of LD-fractions within the classical scale size [9].

For example, in the sample of RS-A were identified four factions, n = 4. In accordance their average diameters (d_{aver}) the factions are fall into the following classic groups: cC (coarse clay), mSi (middle silt), cSi (coarse silt) and mS (middle send). Will be denoted by symbol "N" is the total number of classic fractions ranging from very thin to very large fraction of the sample. As you can see, the sample RS-A in the interval from cC to mS are contains six classic fractions, then N = 6. The

difference: N-n = 6-4 = 2, this means two spaces in the distribution of LD-fractions of sample RS-A.

In the future it will be shown that in all samples of Italian andosols and paleosols: N > n. The inequality (N > n) is the important difference of aggregates andosols and paleosols from the grain-size composition of previously studied soils. For them equality (N = n), then there are no gaps in the distribution of graine size fractions on the scale of classical groups. Thus, there is a possibility to identify gaps in continuum of classic size groups. In the case of incomplete soil dispersion, the gap reflects the increased aggregation of related, coarser, aggregates.

Results and discussion

The number and properties of LD-fractions

Insufficiently complete energy impact in the preparation of soils for LDA appears as a dignity, allowing to assess the strength of the surviving micro-aggregates.

We propose several indicators of the microaggregates safety, which can be derived from the LD-spectra. This is the number of fractions of N and their characteristics. Comparing the data on the number of LD-fractions and their properties in a given soil with data on LD-fractions of reference samples that are known to be completely dispersed, it is possible to determine the strength of the surviving microaggregates.

Absolute indices of the fractions determined by the LDA, such as the average particle diameter (d_{aver}) and their content, have no physical meaning, due to the already mentioned deficiencies of the LDA. Repeat that only relative (dimensionless) indices have meaning: 1) the real number of fractions (n), 2) the theoretical number of fractions (N), 3) period (T) of the function n = f (d), and 4) the dispersion of an fraction (D).

The results of counting these five criteria are given in the table. 1.

The number of LD-fractions. The real number of LDA fractions (n) in andosol and in the paleosols of the fluvio-lake sediments of northern Italy is from 2 to 4.

In reference soils and rocks, the real number of LDA fractions in the range 0-2000 μm, is n = 8 [10, 11]. A small number of fractions (n = 2-4) mean that the processing of DCB did not completely disintegrate the recent andosols and paleosols of northern Italy.

Maximum theoretical number of LD-fractions is detected the particles or aggregates with size medium from clay (mC) to the large sand (cS), i.e., Nmax = 8. For our andosols and paleosols are N = 3-6. All samples have the difference: (N-n) = 1-2. Inequality (N > n) is due to omissions in the content of 1-2 classic fractions.

Reasons for skipping of the fractions: 1) natural heterogeneity in composition of sedimentary rocks, 2) preservation aggregates due to incomplete dispersion of soil or sediment. It should be noted that previously studied vertisols in Stavropol (South Russia) after processing the ultrasound respected equality: N = n [18]. When the same processing in some specimens vertisols in Texas (USA) (N-n) = 1, while most samples (N = n).

Thus, all specimens of Italian andosols have inequality (N > n) occurs due to omissions in the content of 1 or even 2 fractions. One possible reason is preservation of aggregates due to incomplete dispersion of soil.

Characteristics of the preserved microaggregates: T and D. Another relative index of soils is the period T of adjacent fractions (table 1). The average values of the period T for six samples vary from 5.5 to 13.3. Meanwhile, according to Sadovsky average values of T for rocks 3-4 [16, 17], and according to our data for vertisols $T_{(aver)}$ = 3.3 [18]. Explicitly overestimated values of T in Italian andosols indicate an incomplete dispersion of microaggregates. There are especially large periods T in the samples of vertisols (Stavropol and Texas) [18].

The next relative index of LD-fractions is dispersion D. The studied Italian andosols and paleosols differ by an increased dispersion of the fractions: the D value reaches 1.5, while the dispersion of the fractions after complete dispersion of the samples of vertisols $D_{(aver)}$ = 0.6 [18]. Very high values of the variance indicate the heterogeneity of the LD-fractions in the studied soils, possibly including particles of different sizes. Thus, an abnormally high dispersion also indicates the preservation of microaggregates. The safety of microaggregates makes it possible to assess the strength of cement in different layers of this profile.

Micro-aggregates strength criteria, reflecting both: the content preserved units and their relative strength, have five LD-fractions indicators. For some indicators maximum strength is translated into maximum values (D) and (T), for other indicators maximum strength is translated into minimum values (N and n). Specific extreme values of LD-fractions and soil indexes, are given in the table. 2.

As you can see, the strongest aggregates have the paleosol SIV-1 with extreme values of the three indicators (N, N, and D). The strength aggregates have the paleosol SIII-1 with two indicators (N and n). Also the strength aggregates have the paleosols: SI-1 (with H) и SII-1 (with T).

Since the LD-factions depend on each other, their informative value is not the same. Most high informative value has the criteria that correlate with other related very weakly.

Statistical connection with indexes. We have calculated the correlation coefficients between pair of the five indicators: n, N, D and T (table 3).

Then we are calculated the average value of the correlation coefficient excluding characters on r-coefficients. The average values of the r-coefficient are increase in the sequence: 0.39 (T) < 0.45 (N) < 0.54 (D) < 0.68 (n). Most high information has first three indexes (T, N and D). These three indexes were used to create a new, integral indicator of the strength of the persevered resistance of micro-aggregates (RMA).

It is the averaged sum of the ratios of the three indicators of the LD-fractions in the given soil in comparison with the parameters of the reference LD-fractions obtained after complete dispersal of the microaggregates; N = 8, D = 0.6 and T = 3.3. This RMA index is determined from the expression:

$$RMA = [(8/N) + (D_{aver}/0.6) + (T_{aver}/3.3)] : 3 \quad (9)$$

Knowing this index, we determine the total strength of microaggregates that survived after a given chemical dispergation. In a fully dispersed sample RMA is 1. But if the aggregates are preserved the value RMA is higher. We will consider samples with RMA = 1-2 as containing aggregates of low strength, and samples with RMA > 2 with aggregates of high strength.

On average, in recent andosols RMA = 1.91 ± 0.20, while in paleosols, the average RMA index rises to 2.28 ± 0.10, the difference in the mean is significant at P = 80%. Thus, in paleosols the strength of microaggregates is higher than that of recent andosols. Probably, in the course of time, the strength of cement cementing the particles of the pyroclastic material of andosols increases. The strongest of aggregate are designs in paleosols: SIV-1 (RMA = 2.56) and SII-1 (RMA = 2.50).

The reasons for preserving part of the microaggregates in andosol

Strong micro aggregates are cemented with cement, for the destruction of which the application of considerable energy is required. Microaggregates are destroyed by physical or chemical methods. Ultrasound is one of the most often method used; consumable energy depends on the power of the dispergator and the time of exposure.

It is more difficult to estimate the energy consumed during chemical dispergation of microaggregates. Most often perhydrol for oxidation of humus is used and dithionite is used to reduction Fe^{3+} in the composition Fe-(hydr)oxides to Fe^{2+}. In the first case, the released energy can be calculated by the reaction of the decomposition of H_2O_2, in the second case the energy for the decay reaction of dithionite. In the surviving microaggregates, the energy necessary for the destruction of cement exceeds the energy spent on dispergation.

The mineralogical composition of andosols in a neutral medium is determined by the hydrolysis of volcanic glass [19]. This leads to the formation of typical minerals of andosols: clay minerals (allophane and imogolite) and Fe-hydroxide: ferrihydrate $2Fe_2O_3 \cdot FeOOH \cdot 4H_2O$) [19]. The role of mineral cement in andosols is performed by active particles of allophone, imogolite and ferrihydrate, connected with organic matter [11].

Allophane and imogolite are nano-sized aluminosilicate with hollow spherule and tube structures with the diameter varies from 3 to 5.5 nm and have high cation/anion exchange capacity as well as extensive, variable-charged surfaces [11, 12]. Together with Fe-bearing short-range-order minerals which can easily dissolve and precipitate upon redox changes, these nano-sized short-range-order minerals and the organic matter bound to them may act as strong binding agent for aggregate formation. 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 Andisols [11, 12].

DCB effectively dissolves Fe-cement in strongly ferruginized soils, for example, in ferrallitic soils [20]. Feature of these soils is high content of gross iron, mach more above the Clarke of the lithosphere - 6.2% [21]. But the gross iron content in the Italian andosols and paleosols is very low: 0.7-2.8%. This already indicates a weak ferrigination of andosols.

The most of thin microaggregates of Andosol have very high strength. Maximum dispersion was achieved only after sodium saturation pretreatment followed by the sonication at the energy level 5–10 folds higher than normally required for non-volcanic soils (Alfisol, Molisol, Oxisol and other) and reaches – 5 kJ /mL [11]. It is obvious that DCB is not able to completely destroy solid microaggregates of Andosols.

A thorough research carried out using near-edge X-ray absorption fine structure was shown that organic matter forms a weak connection with the smooth surface of large crystals [12]. This explains the reason not to attend such minerals like kaolinite, hematite, goethite in the formation of microaggregates in andosol. But organic matter is formed a strong connection with unordered minerals such as allophone, imogolite and ferrihydrite [12].

In [15], the authors randomly used a reagent (DCB) that is not very selective to andosols. But this allowed us to obtain new information on the composition of soil microaggregates. In the future, it is possible to deliberately apply soft processing to obtain information about firmly connected soil microaggregates, not only in Andosol.

Conclusion

Andosols have possessed special properties which distinguish them from the many other soils. The role of durable cement in andosol is not Fe-oxides (as in oxysol), but organo-mineral complexes based on short-range-order minerals (allophone, imogolite and ferrihydrite). This is not to prejudge the effectiveness of DCB as a chemical to destroy solid microaggregate in andosol. This leads to the conservation of the andosols aggregates after processing by DCB.

For the determination of soil grain size and micro aggregates are using 2 methods: classic pipet-analysis and a new more powerful analysis using laser diffraction. Recent studies have shown that the composition determined by the laser diffraction method differs greatly from the grain size composition according to the pipet method.

But the laser diffraction data is possible to use for another purpose: to characterize strength of microaggregates preserved after chemical treatment of soils. For this purpose it is necessary to use deconvolution of the laser diffraction curves, with the following analysis of the number of LD-fractions and their properties. It was pproposed 4 indicators of LD- fractions: theoretical number of fractions (N), the real number of fractions (n), period of fractions (T), and their dispersion (D).

We analyzed recent andosols and paleosols in northern Italy, which studied be Colombo et al. [15]. The theoretical number of andosols and paleosols fractions is N = 3-6, whereas in standard, completely dispergated sedimentary rocks, the number of grain size fractions in the range up to 2000 μm is higher and is 8. The real number of LD-fractions in andosols and paleosols is n = 2-4, whereas in standard, completely dispergated vertisois and oxisols n = 4-5.

Additional information on the degree of dispersion of the samples is given by the period T of adjacent fractions. In Italian andosols and paleosols T = 12-14, which is much higher than the values of T for sedimentary rocks (3-4) and for soils T ~ 3. In addition, the fractions of Italian andosols and paleosols are characterized by increased dispergation, the D value reaches 1.5, whereas the dispersion of the granulometric fractions after complete dispergation of the samples is usually below: ~0.5. All this indicates partial preservation of microaggregates after treatment of soils with dithionite-citrate-bicarbonate.

Introduced a new indicator of the aggregates strength (RMA), taking into account the role of only 3 independent criteria: N, H and T. According to RMA, the aggregates in paleosols are stronger than aggregates in recent andosols. The strongest of aggregate are designs in solums: 1-SIII and SIV-1.

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Improving of Copper (II)-Ions Phytoextraction by Using Glycolipid Biosurfactants

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A B S T R A C T

Pollution of soil with heavy metals is a dangerous issue that endangers both the environment and human health. Phytoextraction, that implies planting contaminated area by previously selected species of plants having the potential to extract heavy metals from the soil, is common used technology. Some limitations of this technology revealed in difficulty to absorb the heavy metals, usually being in form of insoluble. For aim to increase the phytoextraction efficiency it is necessary to use agents able of solubilization of heavy metals. The aim of presented work is to test some biosurfactants for enhancing copper(II) phytoextraction by different plant species. For this purpose, the model experiments for cleaning of water and soils artificially contaminated with copper(II) ions have been carried out. The obtained results show that rhamnolipids and trehalose lipids increase the phytoextraction effectiveness significantly (2-3 times). Alfalfa with trehalose lipids are the most effective tools for cleaning soils contaminated with copper(II) ions. In this case removal of heavy metal from soil is achieved by 75%, and main part of removed copper(II) ions (approximately 60%) is translocated in upper ground parts of plant that is very important for performing phytoextraction process successfully.

Keywords: Heavy metals, Copper, Phytoremediation, Phytoextraction, Biosurfactants, Pollution

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Introduction

Heavy metals existing in environment cause the gross violation of natural equilibrium and processes of contamination of life importance ecosystems – ground and surface waters, soils, atmospheric air, vegetation (including cultivated verdure) get irreversible character, which reflects directly on the condition of health of population.

Especially dangerous and complex are the contaminations with persistent toxic substances, among which are petroleum products, heavy metals, chlorinated organic substances etc. [1]. They cause a violation of the structure, aeration, water exchange in soils, lead to changes in ecosystems and impossibility of their use in industry and agriculture.

Biological methods of restoration the environment (bioremediation, phytoremediation), that is the treatment with microorganisms and plants, are priority ones among the most promising and environmentally acceptable methods. Due to the great power of natural detoxification processes, interest in the ecological potential of microorganisms and plants has increased in the

last decades [2-6]. Microorganisms that transform organics play an important role in maintaining the ecological balance in various ecosystems and, due to their high degradation and transformation power, are successfully used for sewage and soil purification. Plants actively participate in soil and air remediation processes. Plants and microorganisms, together or individually, mainly through their powerful oxidative enzyme systems are capable to remediate environment polluted by a wide spectrum of contaminants.

Phytoremediation is a unique cleanup strategy [3-6]. The realization of phytoremediation technologies for cleaning of environment polluted with heavy metals (phytoextraction) implies planting contaminated area by previously selected species of plants having the potential to extract heavy metals from the soil. Some limitations of this technology revealed in difficulty to absorb the heavy metals, usually being in form of insoluble. For increasing efficiency of phytoextraction, it is necessary to use agents able of solubilization of heavy metals and improve their absorption by plants. Some preparations of biological origin, such as surface-active compounds bacterial origin (biosurfactants) possess metal-chelating properties that enable their application in phytoextraction of heavy metals [7].

The aim of presented work is to test some glycolipid biosurfactants for enhancing copper(II) phytoextraction by different plant species.

Materials and methods

Microbial synthesis of biosurfactants was conducted using the strains-producers of the genera *Pseudomonas, Rhodococcus and Bacillus* on optimized liquid medium with glycerol, mannitol, hexadecane and plant oil industry wastes (20 g/l) [8-11]. Monocolonial selection of the strains has been carried out for the enhancement of their activity and the improvement of the surfactant synthesis. Rhamnolipids were isolated from cultural broth via acid precipitation (10% HCl to pH 3) and following extraction of the obtained precipitate with Folch mixture (chloroform : isopropanol – 2 : 1). Trehalose lipids were isolated with the same extragent from cell mass. Polysaccharides were isolated from cultural broth via precipitation with 2 volumes of ethanol. The solvent was evaporated under vacuum. The lipids were analyzed via thin layer chromatography and HPLC. The content of rhamnolipids was determined using orcinol method, trehalose lipids – using antron method.

The isolated biosurfactants we investigated by the following parameters: surface tension of solutions, critical micelle concentration (CMC), emulsification index (E24), thin layer chromatography, UV- and IR-spectroscopy.

The following preparations of biosurfactants were prepared:

- Rhamnolipids,
- Trehalose lipids,
- Rhamnolipid biocomplex PS,
- BR1 – complex of biosurfactants produced by strain of *Rhodococcus* sp. 50,
- BP1 – complex of biosurfactants produced by strain of *Pseudomonas* sp. 6R67,
- BB1 – complex of biosurfactants produced by strain of *Bacillus* sp. 3Zu9.

Plants. The following plant species: rape (*Brassica napus*), soybean (*Glycine max*) and alfalfa (*Medicago sativa*) were used as phytoremediators.

Model experiments. In the model experiments the soil samples contaminated artificially, as well as polluted as a result of oil pipeline accidental spills, were used.

The model experiments were carried out according to the following scheme: the suspension of microorganisms and solutions of biosurfactants was inoculated in the contaminated soil in the beginning of experiment. After different incubation times the plants were sowed in separate samples of soil. The conditions and details of each experiment are described in legends of figures.

Analysis of copper(II) ions. For analysis of Cu2+ content in plant and water samples have been determined sphectrophotometrically by measuring the absorption of the Cu(II)-dithizone (1,5-Diphenylthiocarbazone) complex at 553 nm, according to Kumar et al. [12].

Results and Discussions

For selection of biosurfactants and polysaccharides, chelating heavy metals to increase efficiency of their absorption by root

system and transportations in upper ground parts of plants, 7 preparations of biosurfactants were tested.

The following plants and biosurfactants were tested in the model experiments:

- Plants - soybean, alfalfa and rape (growing on hydroponic area);
- Copper(II) ions as model heavy metal (concentrations –100 and 200 ppm);
- Preparations of biosurfactants (concentration – 0.1 g/l):
  - o Rhamnolipids
  - o Trehalose lipids
  - o Rhamnolipid biocomplex PS
  - o BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50
  - o BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67 and
  - o BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9
  - o BB2 – complex of biosurfactants produced by strain of Baccilus spp.

For carrying out of experiments, seedlings of the plants were placed in special tubes on hydroponic media containing different concentrations of copper(II) ions and tested biosurfactants. After 7 days from the start of experiments, copper(II) contents were measured in growing medium and plant seedlings (separately in roots and upper parts). The obtained results are presented on Figures 1-4.

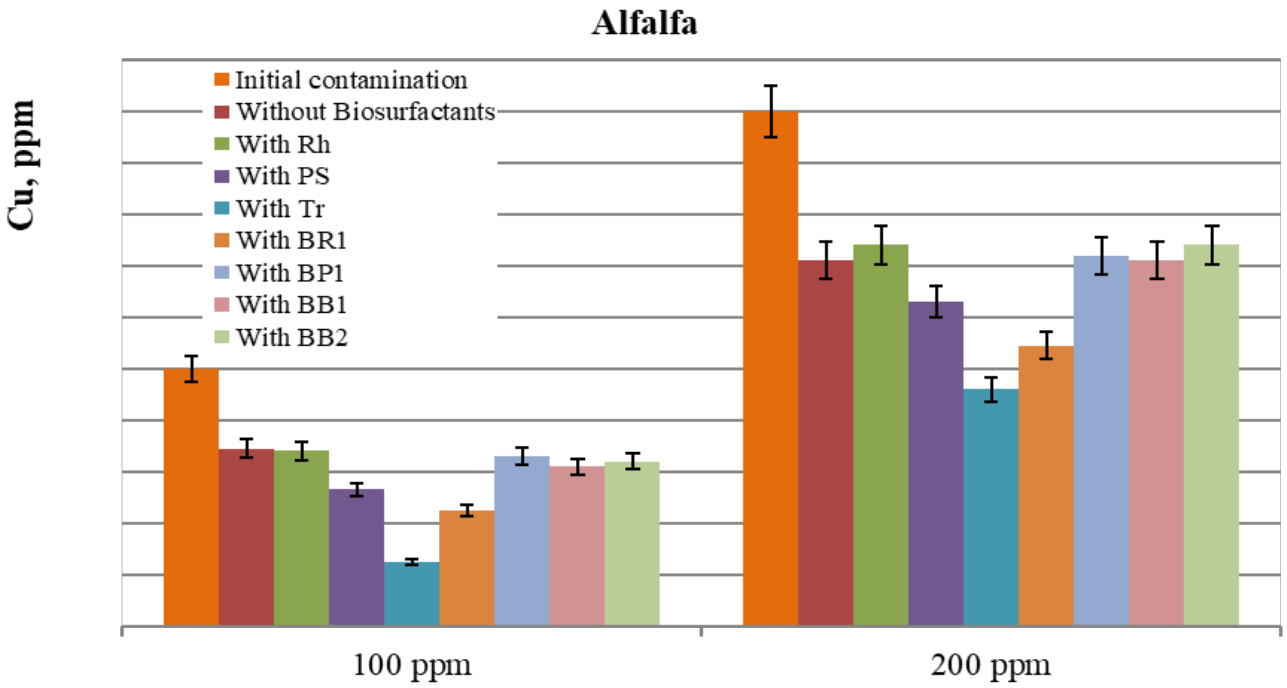

Fig. 1. The absorption of copper(II) ions from hydroponic media by alfalfa seedlings and the influence of different preparation on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

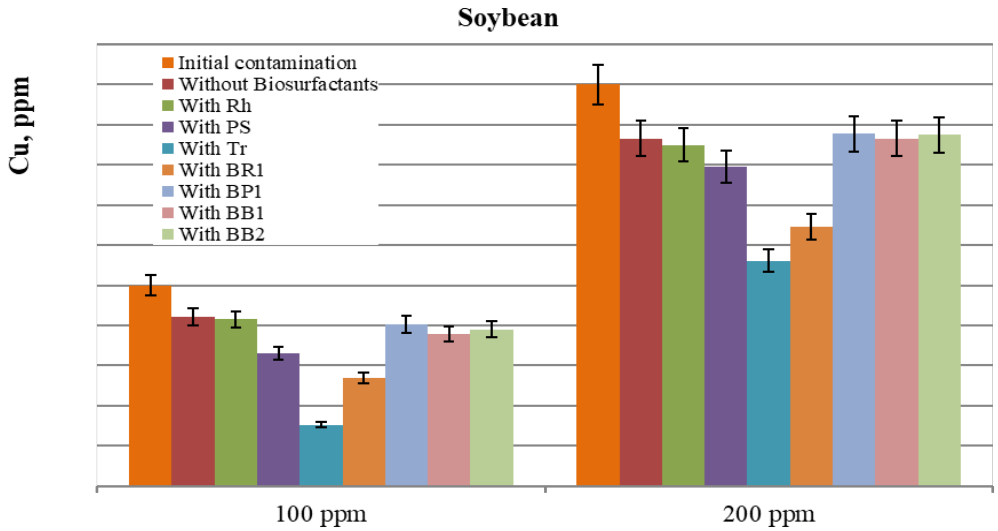

Fig. 2. The absorption of copper(II) ions from hydroponic media by soybean seedlings and the influence of different preparation on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

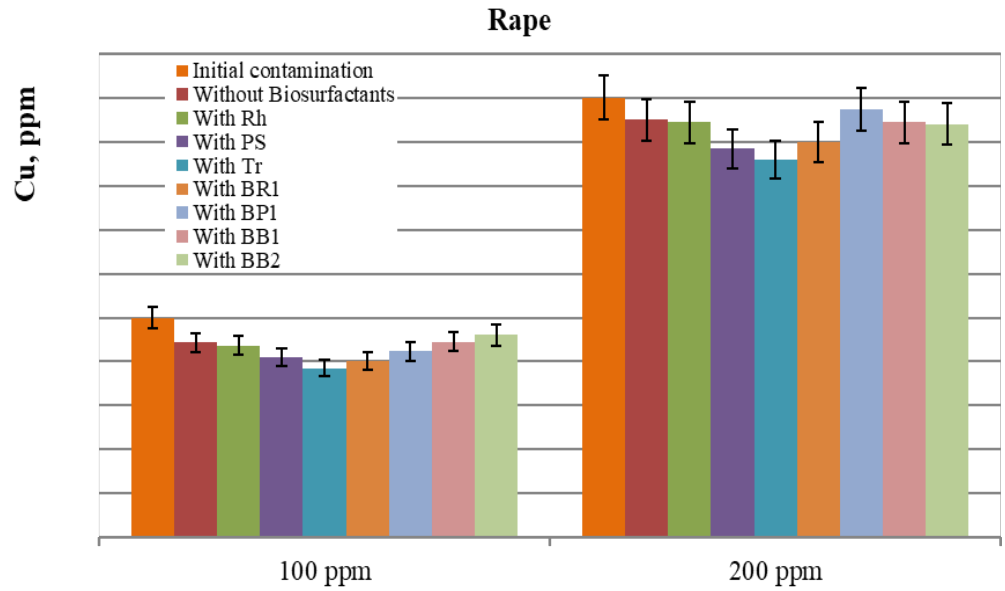

Fig. 3. The absorption of copper(II) ions from hydroponic media by rape seedlings and the influence of different preparation on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants p roduced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

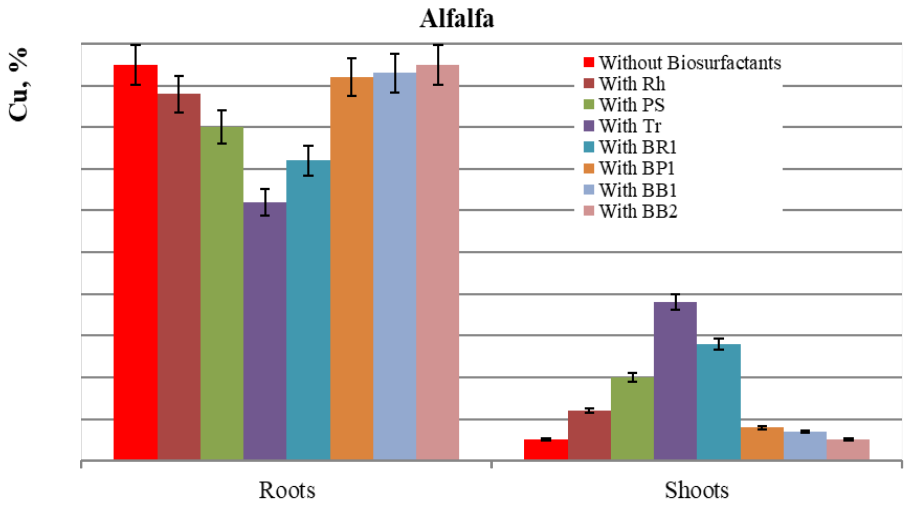

Fig. 4. The distribution of absorbed from hydroponic media copper(II) ions between roots and shoots of alfalfa seedlings and the influence of different preparations on this process. Preparations: Rh – Rhamnolipids; PS – Rhamnolipid biocomplex PS; Tr – Trehalose lipids; BR1 – complex of biosurfactants produced by strain of Rhodococcus sp. 50; BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67; BB1 – complex of biosurfactants produced by strain of Bacillus sp. 3Zu9; BB2 – complex of biosurfactants produced by strain of Baccilus spp. Initial concentration of Cu2+ – 100 and 200 ppm; concentration of preparations – 0.01%; duration of experiment – 7 days.

The obtained results show that after penetration in plants, copper(II) ions are located mainly in the roots of the plant. Among the tested plants, alfalfa revealed the highest ability to uptake copper(II) ions (coefficient of bioaccumulation equals 78 mg per kg of dry biomass). After alfalfa, came soybean (64 mg/kg) and rape (37 mg/kg).

Trehalose lipids sharply increase the process of translocation of copper(II) ions in the upper parts of plants, but in case of high concentrations of copper (100 and 200 ppm), so much quantity of heavy metal penetrates into plants that it becomes lethal to them. In case of using rhamnolipid biocomplex PS similar results are observed only at 200 ppm concentration of copper(II) ions. In case of biopreparation BP1 the increasing of uptake of copper(II) ions and intensification of their translocation in upper parts of plants is observed. Other 4 preparations do not affect absorption of copper(II) ions by plants and their translocation.

The optimal concentration of tested biosurfactants equals 0.1 g/l.

The model remediation experiments in laboratory and greenhouse conditions for cleaning of soils artificially contaminated with heavy metals (copper(II) ions) have been carried out.

The model experiments were carried out according to the following scheme:

1. The soil samples were dried, sieved and artificially contaminated with different concentrations of heavy metals (50 and 200 ppm according to content of metal ions in dried mass of soil). Heavy metals were added on soil in the form of copper(II) acetate water solution.
2. The plants (soybeen and alfalfa) were sowed in separate samples of soil.
3. For intensification of copper phytoextraction, after 3 weeks from the beginning of experiments, the following preparations were added to soil samples (concentration 0.1 g/l) as metal chelating agents:
   a. Rhamnolipid biocomplex PS
   b. Trehalose lipids
   c. Biopreparation BP1 (complex of biosurfactants produced by strain of Pseudomonas sp. 6R67)
   d. EDTA (for comparison of biopreparations effects with chemical chelating agent)

The model experiments were carried out during 30 days at greenhouse conditions (temperature 22-27°C). On 10th, 20th and 30th days of experiment the content of copper in soil samples was measured. After finishing the experiments, copper contents were measured also in plant seedlings (separately in roots and upper parts) (Table).

Table. *Concentration of copper(II) ions in soil during remediatin experiments by using alfalfa and different chelating agents. Initial concentration of copper(II) ions was equaled 50 ppm*

Chelating agent	Concentration of copper(II) ions in soil, ppm				Concentration of copper(II) ions in dried plant organs, ppm	
	Initial	After 10 days	After 20 days	After 30 days	In roots	In shoots
Control[1]	50	44	35	27	21	n.d.[2]
PS[3]		37	30	24	18	7
Tr[4]		18	15	12	11	16
BP1[5]		42	37	32	12	5
EDTA[6]		6	3	1	5	42

[1] – without chelating agents

[2] – n.d. – not detected

[3] – PS – Rhamnolipid biocomplex PS

[4] – Tr – Trehalose lipids

[5] – BP1 – complex of biosurfactants produced by strain of Pseudomonas sp. 6R67

[6] – EDTA – Ethylenediaminetetraacetic acid

As it seen from obtained results, using plants as phytoextractors without chelating agents has less effectiveness in case of contamination of soil with copper(II). Chelating agents increase the effectiveness of cleaning the soil from heavy metals significantly (2-3 times). The effectiveness of tested preparations for their application for enhancing of copper phytoextraction increases in the following order:

Biopreparation BP1 < Rhamnolipid biocomplex PS < Trehalose lipids < EDTA.

It is worth noting that tested biopreparations are much less effective than chemical chelating agent EDTA.

Alfalfa with biopreparation of trehalose lipids are the most effective tools for cleaning soils contaminated with copper(II) ions. In this case 75% removing of heavy metal from soil is achieved, and main part of removed copper(II) ions (approximately 60%) is translocated in upper ground parts of alfalfa.

Conclusion

The obtained results allow drawing following conclusions:

- Among the tested plants, alfalfa revealed the highest ability to uptake copper(II) ions (coefficient of bioaccumulation equals 78 mg per kg of dry biomass).
- Rhamnolipids and trehalose lipids increase the phytoextraction effectiveness of copper(II) ions significantly (2-3 times).
- Alfalfa with trehalose lipids are usable for cleaning soils contaminated with copper(II) ions, because effective phytoextraction of heavy metal from soil is achieved, and main part of removed copper(II) ions is translocated in upper ground parts of plant.

Acknowledgment

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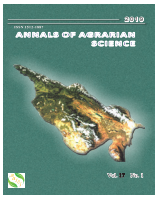

Major Agricultural Crops in the XVI Century Samtskhe-Javakheti

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A B S T R A C T

The paper represents a study of the XVI century historical document, known as the 'The Great Defter of Gurcistan Vilayet' and shows the agricultural image of the time. The document contains data describing Samtskhe-Javakheti region, which is located in the southern part of Georgia and unites six administrative municipalities. Above, mentioned historical source contains the information about the population, settlements, agricultural crops and taxes imposed on them. The paper focuses on the rye, millet, broad bean, cicer and lentil crops, which were widespread in described area, in the second half of the XVI century, but disappeared later. Research included land-use mapping, also creation of databases. The work also shows the results of the analysis of agricultural censuses from XX and XXI centuries, which revealed the major factors of disappearance of the crops.

Keywords: Retrospective cartography, Crops, Landscape change, Agricultural crops, Samtskhe- Javakheti, Georgia.

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Introduction

A crop diversity is a foundation for agricultural development of a country. To study and understand the traditional agricultural crops is strategically important to the states similar to Georgia. The existence of such crops in the area is an indicator of ancient traditions, maintenance and development of which can contribute to the food security and strengthening of agricultural sector. This can lead to the economic stability and empowerment of communities.

The officials of the Ottoman Empire prepared 'The Great Defter of Gurcistan Vilayet'in order to spread the influence over the territory of Georgia, and resulting from the developed political processes several historical regions of Georgia got under its command [1, 2]. The tax system of the Ottoman Empire itself implied the census and assessment of subjugated territories aimed to the tax collection. The imposed taxes entailed human custom duties (the military service of the Ottoman Empire) and a chief rent [1, 3, 4]. made in natural form, similar to the monetary one. The main concern of the Empire was to get the taxes. The way the goal would be reached did not matter. The region of Samtskhe-Javakheti was well developed from an agricultural standpoint. The tax data presented in 'The Great Defter of Gurcistan Vilayet' is the source that confirms the distribution of agricultural crops. The cereal crops are still in majority [5, 6]; The substantial part has disappeared from agricultural crops. In particular, this fact refers to millet and rye crops, as well as the legumes. Among the bean family, broad beans, lentil and cicer crops were traditional here and played quite an important role in the regional agriculture over the past centuries, however today a kidney bean replaced the other legumes.

By means of the historical materials, we have tried to study and assess the crops spread in the region and their state several centuries ago. Based

on the available basic sources (The Great Defter of Gurcistan Vilayet, results of 1923, 2002, 2014 agricultural inventories), we have assessed the distribution of the mentioned crops in the region and changes in the course of several centuries.

Objectives and methods

The 'Gurcistan vilayet' included the territory of modern Samtskhe-Javakheti, Kvabliani gorge adjacent to it and was extended to the territory of present-day Turkey, where it included the surroundings of Chrdili (Childir) lake, the Potskhovi River valley, as well as the upper part of Mtkvari River flowing through the Artaani plateau and the upper part of Oltisi River. Our research covers the part of the 'vilayet', which is in the limits of the modern Georgia that corresponds to the military-administrative unit of the present-day Samtskhe-Javakheti. The region under the study (Fig. 1.) includes six administrative units – Borjomi, Akhaltsikhe, Adigeni, Aspindza, Akhalkalaki and Ninotsminda municipalities [7]. The area of the region is 6412.8km2 [6]. Its population is 160, 5 thousand people [8]. The Didi Abuli Mountain (3301m) at the Samsari ridge is its highest point. The region is mountainous, with alternation of hollows, volcanic canyons, tablelands and mountain ranges. Here are represented Akhaltsikhe (Samtskhe) structural basin, the Lesser Caucasus mountain system, in a form of Arsiani, Adjara-Imereti and Trialeti ridges and volcanic highlands in form of Javakheti, Borjomi-Thori-Tsikhisjvari plateaus and Erusheti highland, as well as in form of Samsari and Javakheti ridges situated between volcanic plateaus, which borders with the region under study from the east. The regional climate transforms from moderate chilly subtropical one into a cold and the dry subtropical climate of mountain hollows, dry climate of mountain tablelands and cold climate of high mountains [9]. The amount of precipitation at the Javakheti tableland is within 500-600 mm, which is roughly similar within the limits of the hollow, while on the slopes of Trialeti, Arsiani and Adjara-Imereti ridges it increases and reaches the maximum of 1400-1500 mm in its upper part, approximately at 2200-2500 m height.

We have used the following materials to execute this work: 'The Great Defter of Gurcistan Vilayet', historical sources of the XVI-XX centuries, 1897 and 1923 census documents, as well as the results of a newest census in the form of the 2014 Geostat materials. As for cartographic documents, we made use of the map compiled by Jikia and Aslanikashvili in 1953, and large-scale 1:50000, 1:25000 maps and satellite images. In order to execute the work, we have used the method of retrospective mapping, the historiographic analysis and the statistical analysis, that assisted us in the manifestation of the situation revealed resulting from the processing of available archive documents and other sources.

The time interval of statistical materials is quite large. The numbers given in 'The Great Defter of Gurcistan Vilayet' providing us with information about what tax was imposed on different settlements; the equivalent of the chief rent was also calculated in kind, measured in weight and volume (so-called Qila), which slightly differed from each other in weight in case of various crops [1].

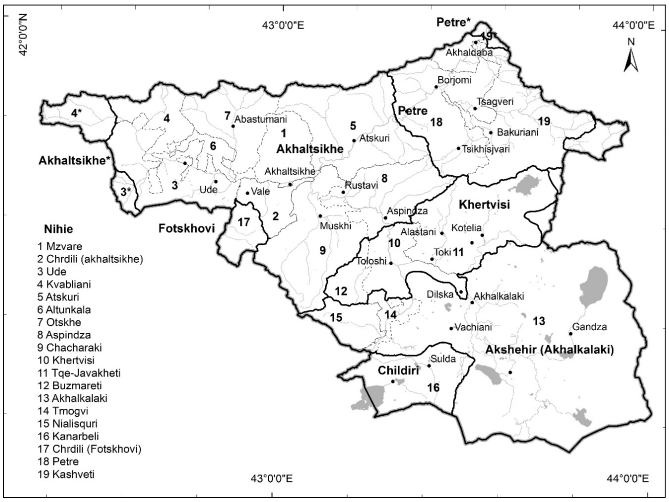

Fig. 1. *Map of the study area.*

The following materials were used as a basis of the map: large-scale 1:50000, 1:25000 maps and satellite images., By means of GIS-technology, we have selected the satellite images a zone of arable lands, which are suitable for agricultural crops, taking into account soils, slope inclinations, aspect and altitude above the sea level. We have composed GIS-layers of arable lands and perennial plantings, i.e. a zone, where the mentioned crops were cultivated.

The expedition method envisaged the visual study of land fund, directly in the field (on-site) and current state assessment. Field works foresaw an intensification of agricultural crop distribution area, and their verification with the outlines drawn from satellite images. We conducted GEO-information map compilation and analysis afterwards, when the tables calculated using Excel and reflecting the quantity of agricultural crops under study, sorted according the rural settlements, were transferred to GIS databases. The extrapolation was made for each settlement, according to arable lands. Thus, we got the map of the distribution of the above-mentioned crops for the second part of the XVI century, based on which we can conduct a comparative analysis and figure out what changes have happened during this time period and what trends exist as of today.

Results

Rye crop is still rare and occasionally found in Georgia. In the second part of the XVI century, the rye plantings were widely spread in the whole region under study. The five main areas of their concentration were identified, resulting from the mapping (Fig. 2.): 1. Samtskhe structural basin; 2. Uraveli gorge and Greli-Uraveli-Sakhudabeli band; 3. Javakheti highland; 4. Mtkvari valley from Aspindza to the south, in the Tolerta-Erkota-Atskvita band; 5. Niali fields, Buzmareti basin and its adjacent settlements, and according to administrative units of that time two peaks of their concentration are clear: Chacharaki district (Nihie) in Akhaltsikhe Liva (Sanjak) and Akhalkalaki district in the Liva (Sanjak) having the same name.

In total, the rye harvest was equal to 2356.96 tons, rye was sowed in 400 locations, other settlements were deprived of its plantings, including all those settlements, information on which is scarce. The zone of mentioned crops occupied far more areas than those where its actual plantings are distributed (Fig. 2.). Rye plantings occupy more than half of inhabited localities. However, today the plantings are available in singular experimental farms only.

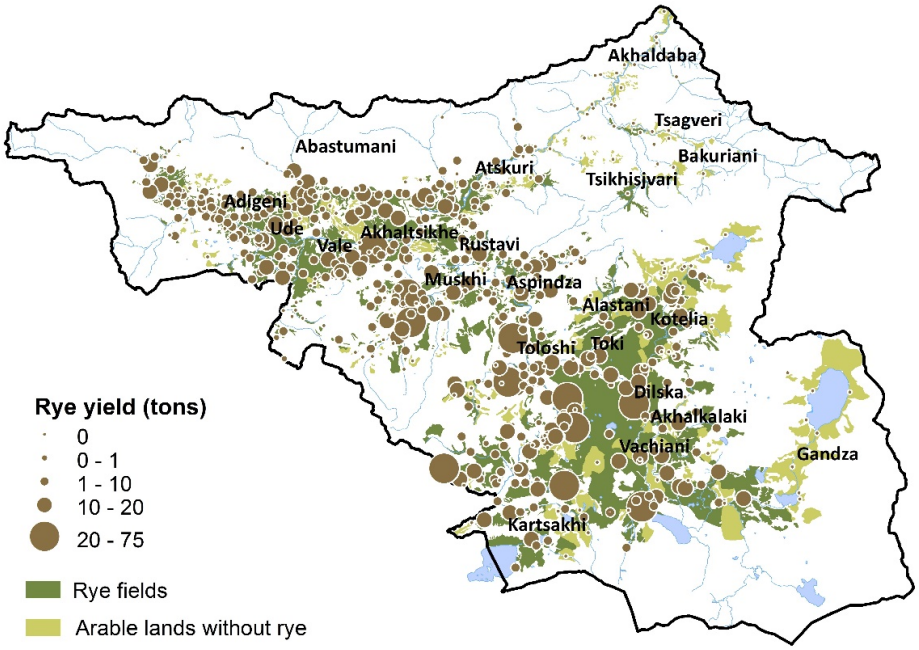

Fig. 2. *Rye plantings in Samtskhe-Javakheti in the second half of the XVI century.*

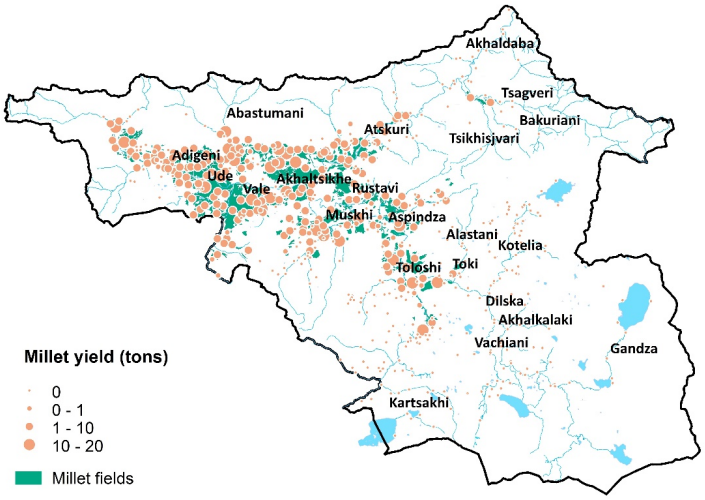

Fig. 3. *Millet plantings in the second halfof the XVI century.*

Millet plantings in the XVI century occupied far less areas, than in case of rye, since the millet was not the main food crop, as a wheat and barley. Its plantings ended at the approximately, 1500-1600 meters height above the sea level, which on one hand can be referred to its less productivity in highest belts, or else there could be another reason, e.g. that other crops were prioritized at the Javakheti plateau and Niala-Buzmareti volcanic fields, since it is not considered to be the best zone for millet plantings (Fig. 3.). The distinct concentration of their plantings in the millet distribution area is observed in the Samtskhe hollow; a relatively small area of its concentration is represented by Uraveli gorge. They are also concentrated on the Uraveli-Anda-Tskordza section and in the Aspindza-Saro-Nijgori-Toloshi band, from Tmogvi village to Nakalakevi. In total, 857.89 tons were harvested. The millet was sowed with 236 locations, other settlements were deprived of its plantings, including all settlements, information on which is scarce, since these villages were depopulated and their prescribed taxes, similar to rye plantings, are reflected in total monetary (Akce). The millet crop occupied a substantial sector in a third part of settlements identified in the area.

The millet plantings are concentrated in Udi, Mzvare and Chacharaki districts, while in the number of regions (Akhalkalaki, Tke-Javakheti, Kanarbeli, Nialiskuri, Kashveti, Kvabliani) its plantings are almost unavailable; The Kvabliani district is almost depopulated. The Kanarbeli, Nialiskuri, Akhalkalai and Tke-Javakheti districts avoided their sowing due to chilly climate, since in the Buzmareti district available settlements are almost in the same climate and edaphic conditions, as those of Nialiskuri and Kanarbeli. Millet plantings in small quantities were available there.

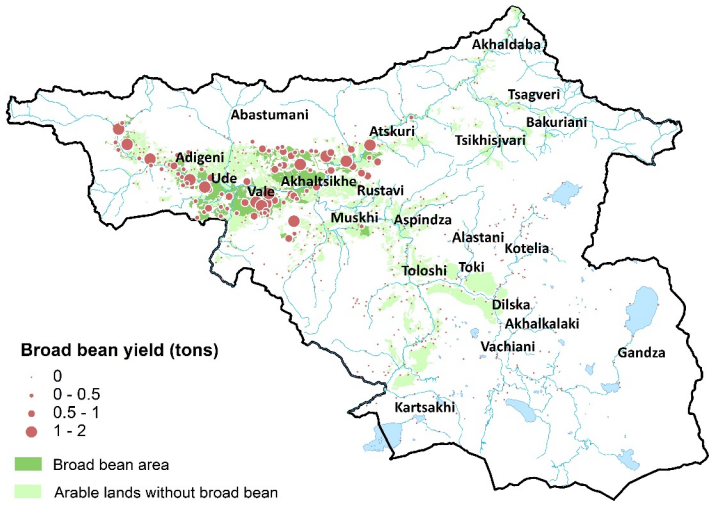

Fig. 4. *Broad bean plantings in the second part of the XVI century.*

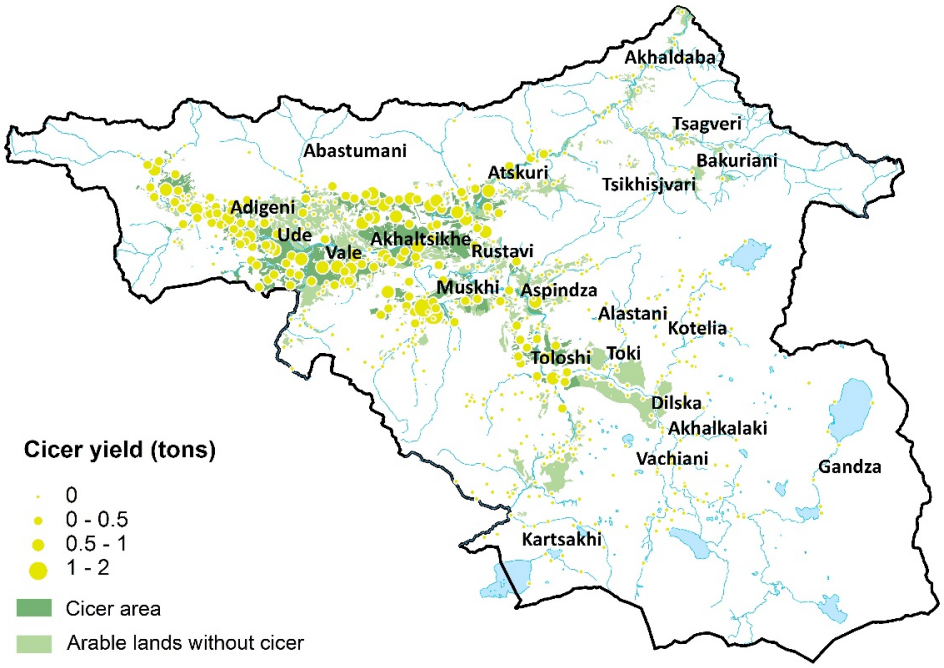

Fig. 5. *Cicer plantings in the second part of the XVI century.*

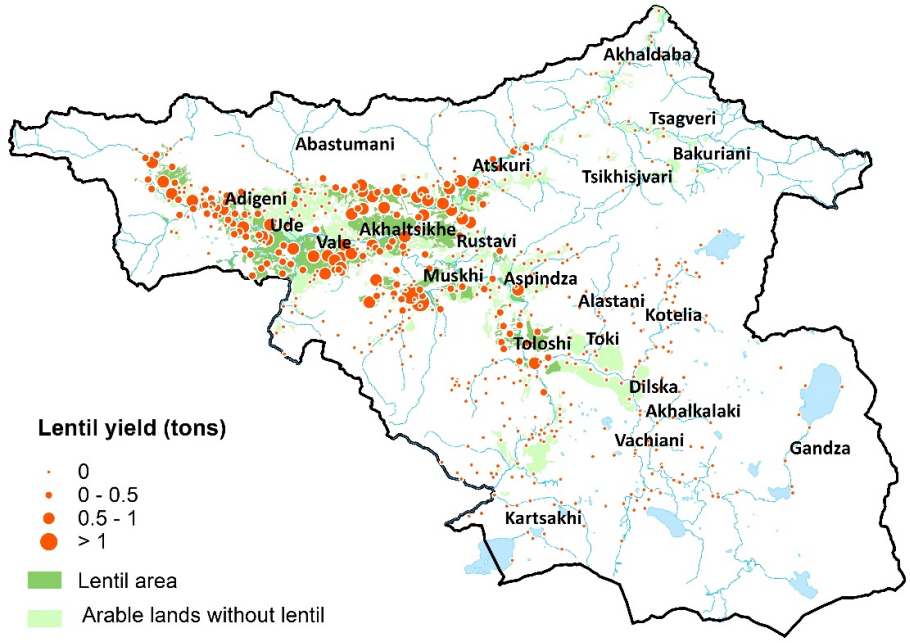

Fig. 6. *Lentil plantings in the second part of the XVI century.*

Broad bean (Fig. 4.) and cicer (Fig. 5.) plantings almost repeat the same zone, and their basic area coincides with each other as well as lentil (Fig. 6.) plantings repeat the distribution limits of other legume crops. The major part of them are, again, concentrated on the Samtskhe structural basin and in case of all three crops their harvest is getting smaller from the west of the hollow to its east. A top yield was obtained in Use district; the slightly less harvest is peculiar to Mzvare, Atskuri and Chacharaki districts; but the latter substantially lags behind other districts by broad bean plantings.

Table 1. *Administrative units entering into 'Gurcistan vilayet' at the present-day territory of Samtskhe-Javakheti and agricultural crops vanished today, as of the second part of the XVI century.*

Administrative units	Agricultural crops	Number of settlements with crops	Yield, Tons
Akhaltsikhe Liva			
Mzvare Nihie	Rye	18	97.9
	Millet	18	100.76
	Broad-bean	18	7.650
	Lentil	18	6.757,5
	Cicer	18	7.778
Chrdili (In Akhaltsikhe Liva)	Rye	22	113.52
	Millet	21	89.76
	Broad-bean	21	11.985
	Lentil	21	9.053
	Cicer	21	6.630
Ude	Rye	51	215.93
	Millet	51	171.27
	Broad-bean	39	16.575
	Lentil	50	15.81
	Cicer	50	13.388
Kvabliani	Rye	6	11.55
	Millet	1	2.2
	Broad-bean	0	0
	Lentil	1	0.765
	Cicer	1	0.255
Otskhe	Rye	18	113.3
	Millet	18	83.93
	Broad-bean	1	0.383
	Lentil	2	0.638
	Cicer	2	0.638
Atsk'uri	Rye	38	156.42
	Millet	26	77
	Broad-bean	14	8.16
	Lentil	21	9.18
	Cicer	21	7.65
Altunkala (Okrotsikhe)	Rye	14	87.45
	Millet	13	64.02
	Broad-bean	2	0.893
	Lentil	4	1.53
	Cicer	4	1.403
	Rye	35	147.18

Aspindza	Millet	25	45.98
	Broad-bean	1	0.255
	Lentil	7	2168
	Cicer	7	1785
Ch'ach'araki	Rye	40	326.15
	Millet	24	106.15
	Broad-bean	0	0
	Lentil	16	6.12
	Cicer	16	5.865
Total, in Akhaltsikhe Liva	Rye	242	1269.4
	Millet	197	741.07
	Broad-bean	96	45.9
	Lentil	140	52.02
	Cicer	140	45.392
Khertvisi Liva			
Khertvisi	Rye	29	242.11
	Millet	22	68.97
	Broad-bean	0	0
	Lentil	13	4.208
	Cicer	13	4.208
Tqe-Javakheti	Rye	35	226.6
	Millet	0	0
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Buzmareti	Rye	8	51.15
	Millet	0	0
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Total, in Khertvisi Liva	Rye	72	519.86
	Millet	22	68.97
	Broad-bean	0	0
	Lentil	13	4.208
	Cicer	13	4.208
Akshehir (Akhalkalaki) Liva			
Akshehir (Akhalkalaki)	Rye	43	355.3
	Millet	0	0
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Tmogvi	Rye	10	72.05
	Millet	2	12.1
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Nialisquri	Rye	15(5 Outside of Georgia)	96.8 (42.45 Outside of Georgia)
	Millet	0	0
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0

Total, in Akhalkalaki Liva	Rye	68	469.8
	Millet	2	12.1
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Childiri Liva			
Kanarbeli	Rye	15 (7 Outside of Georgia)	94.05 (37.95 Outside of Georgia)
	Millet	0	0
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Total, in Childiri Liva	Rye	15 (7 Outside of Georgia)	94.05 (37.95 Outside of Georgia)
	Millet	0	0
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Fotskhovi Liva			
Chrdili (In Fotskhovi Liva)	Rye	3 (9 Outside of Georgia)	3.85 (10.45 Outside of Georgia)
	Millet	12 (7 Outside of Georgia)	30.25 (27.5 Outside of Georgia)
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Total, in Fotskhovi Liva	Rye	3 (9 Outside of Georgia)	3.85 (10.45 Outside of Georgia)
	Millet	12 (7 Outside of Georgia)	30.25 (27.5 Outside of Georgia)
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Petre liva			
Petre	Rye	0	0
	Millet	2	5.5
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Kashveti	Rye	0	0
	Millet	1	2.75
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Total, in Petre Liva	Rye	0	0
	Millet	3	8.25
	Broad-bean	0	0
	Lentil	0	0
	Cicer	0	0
Total, in Region	Rye	400	2356.96
	Millet	236	857.89
	Broad-bean	96	45.901
	Lentil	153	56.23
	Cicer	153	49.6

The role and importance of the mentioned crops was high in the past, which was a good precondition for agriculture diversification and for a new introduction of a variety of crops (Table 1). In case, we compare the state of agricultural crops described in the 'Defter' with the current state, the change is obvious. It was interesting to figure out, what was the crucial moment, when the society stopped cultivating them. Was this fact related to any natural (climate, edaphic) or human factors?

According to the census conducted in 1923 (Table 2, 3), rye crop was still intensely cultivated in both Samtskhe structural basin and Javakheti plateau. The area under crops is small in Borjomi gorge. However, the reasons are different, from the present-day territory of Borjomi municipality is heavily devastated in the Defter, which cannot be said about the data of the 1923 census. The lack of arable lands, on one hand, and the functional change of this territory by the early XX century related to its use for recreation and health-

Table 2. *Agricultural plantings registered according to 1923 census [10]*

Mazra (administrative	N N	Community	Agriculture				
			Rye (ha)	Millet (ha)	Broad bean (ha)	Lentil (ha)	Cicer (ha)
Akhalkalaki	1	Alastani	108.56	0	1.853	0	0
	2	Baraleti	614.65	0	13.298	0.327	8.066
	3	Gorelovo	0	0	0	0	0
	4	Dilska	104.64	0	0	0	0
	5	Eshtia	5.886	0	0	0	0
	6	Kondura	85.238	0	0	0	0
	7	Kulikami	59.623	0	0	0	0
	8	Okami	251.57	0	0	0	0
	9	Sathkhe	26.596	0	0	0	0
	10	Khertvisi	105.95	0	0	0	0
		All	1362.7	0	15.151	0.327	8.066
Akhaltsikhe	1	Adigeni	32.373	2.507	0	0	0
	2	Ats'kuri	15.696	0.327	0	0	0
	3	Vale	86.219	0.327	0	0.109	0
	4	Varkhani	9.701	2.071	0	0	0
	5	Idumala	10.355	0.654	0	0	0
	6	Klde	1.635	0	0	0	0
	7	Lepisi	59.296	0	0	0	0
	8	Ude	14.933	1.308	0	0	0
	9	Uraveli	65.182	0	0	0	0
		All	295.39	7.194	0	0.109	0
Gori (Communities according of 'Gurjistan Villaiet' territory)	4	Akhaldaba	0	0	0	0	0
	7	Bakuriani	0	0	0	0	0
	8	Boriomi	0	1.962	0	0	0
	11	Guiareti	0	0	0	0	0
	12	Dviri	0	0	0	0	0
	32	Kvishkheti	0	0.327	0	0	0
	36	Tsagveri	1.09	0	0	0	0
		All	1.09	2.289	0	0	0
Total			1659.1	9.48	15.15	0.44	8.07

promoting purposes, on the other, are probable reasons for this fact. This region became the resort center of Romanovs' imperial family, and, respectively, the crops that took hold in the Russian Empire with high intensity (corn, potato) that was more rapidly distributed there. Distribution of potato has begun after 1840 and is chronologically related to the migration of different people groups of the Russian Empire (Dukhobors, Ukrainian, Polish and German migrants). The widespread replacement of local, traditional crops with the higher yielding and easy cultivated crops became a precondition for the encouragement towards the development of one-crop (monoculture) system in the Soviet Union and a major part of Javakheti was covered with potato plantings. Similar to the rye, the millet crop was of frequent occurrence in the Akhaltsikhe structural basin. The difference is that it had already been vanished from the Khertvisi community for this period. Roughly, the same took place regarding the lands of Borjomi Municipality.

Table 3. *The productivity of agricultural crops (in tons) registered according to the 1923 census [10]*

Mazra (administrative	N N	Community	Agriculture				
			Rye (ha)	Millet (ha)	Broad bean (ha)	Lentil (ha)	Cicer (ha)
Akhalkalaki	1	Alastani	271.41	0	3.706	0	0
	2	Baraleti	1536.62	0	26.596	0.654	16.132
	3	Gorelovo	0	0	0	0	0
	4	Dilska	261.6	0	0	0	0
	5	Eshtia	14.715	0	0	0	0
	6	Kondura	213.095	0	0	0	0
	7	Kulikami	149.057	0	0	0	0
	8	Okami	628.93	0	0	0	0
	9	Sathkhe	66.49	0	0	0	0
	10	Khertvisi	264.87	0	0	0	0
		All	3406.79	0	30.302	0.654	16.132
Akhaltsikhe	1	Adigeni	80.9325	6.2675	0	0	0
	2	Ats'kuri	39.24	0.8175	0	0	0
	3	Vale	215.547	0.8175	0	0.218	0
	4	Varkhani	24.2525	5.1775	0	0	0
	5	Idumala	25.8875	1.635	0	0	0
	6	Klde	4.0875	0	0	0	0
	7	Lepisi	148.24	0	0	0	0
	8	Ude	37.3325	3.27	0	0	0
	9	Uraveli	162.955	0	0	0	0
		All	738.475	17.985	0	0.218	0
Gori (Communities according of 'Gurjistan Villaiet' territory)	4	Akhaldaba	0	0	0	0	0
	7	Bakuriani	0	0	0	0	0
	8	Boriomi	0	4.905	0	0	0
	11	Gujareti	0	0	0	0	0
	12	Dviri	0	0	0	0	0
	32	Kvishkheti	0	0.8175	0	0	0
	36	Tsagveri	2.725	0	0	0	0
		All	2.725	5.7225	0	0	0
Total			4147.99	23.71	30.3	0.87	16.13

There was a very extensive and centralized agricultural policy of the USSR period. This policy was focused on the receipt of maximally abundant product and therefore the specialization envisaged growing such crops by 'Kollhozes' (collective farms) that could give higher yields. The development of this strategy caused the rooting of the crops that could bring higher yields for a specific territorial unit that is why those replaced traditional crops, which were distinguished by high crop yield and at the same time, which were more easily cultivated.

In the category of 'cereals and grain legume crops' of crop areas the legume crops were in the minority and mainly the kidney bean plantings are meant here, while the rest is occupied by the cereals. However, if we judge by the ensuing table (Table 4): rye and millet crops were no more sowed and, therefore, they had no part in the region everyday life, as agricultural crops [11].

Table 4. *Crop areas for cereals in the middle of the XX century (thousand hectares) [12]*

	Years		
Agricultural crops	1966	1968	1970
Autumn wheat	8.9	7.4	7
Autumn Barley	0.2	0.1	0.2
Spring Barley	19.6	16.3	15.8
Oats	1.1	1.4	2.2
Corn	3	2.4	1.9

These five agricultural crops had already been totally vanished from the explored region by that time. In addition, this process was developed just in several dozens of years. The crops that have thousand-year traditions disappeared in just dozens of years. The crops, which were successfully cultivated for thousand years, could not disappeared in several dozen years without an outside interference and this process is directly related to the agricultural policy pursued in the mentioned time period.

Conclusion

Based on the research outcomes, we can conclude the following: 1. Today, the presence of annual agricultural crops of the second part of the XVI century, except for cereals, cannot be felt in the region. Among traditionally existing cereal crops, only wheat and barley remain here; legume cultures are completely replaced by kidney beans; 2. The change of rules for the society and such distinct substitution of one agricultural crop by another could not have happened so simply, even due to recommendatory measures. The state-planned economy and collectivization of agriculture of the USSR period envisaged a centralization of farms and region's focus on a monoculture and planning of agricultural directions. These plans envisaged the receipt of maximally high yields emphasized the species that were aimed at quantitative indices and became a characteristic pattern of Soviet collective farms; 3. This trend caused an invasion of potato and corn crops in the 50-60s of the XX century and enlargement of their plantings at the expense of wheat, barley, rye, and millet plantings, as well. Today we reap the results of the mentioned state-planned economy that are expressed in the reduction of agricultural product diversity that makes up the traditional life and complicates the restoration-preservation of agricultural crops; 4. The comparative analysis conducted for the mentioned period gives us a good opportunity to be guided by the available information, should the local population, farmers and agribusiness sector of the mentioned branch of agriculture be interested in it. Since the traditional local agriculture, its crop species are part of regional traditions and culture, one of the forms of heritage, restoration and renewal of which is an important component of regional development.

Acknowledgements

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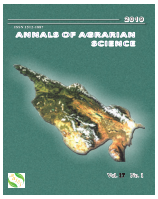

Flax Culture in the XVI Century Samtskhe-Javakheti

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A B S T R A C T

The work deals with the flax crop distribution in the second half of the XVI century. The study is based on the historic document: 'The Great Defter of Gurjistan Vilayet'. The research object is a flax crop, which was used in the past for oil manufacturing in the region under study. We have studied flax culture and have processed its data according to rural settlements and administrative units ('Liva' and 'Nihie') of the XVI century. We composed the map of the mentioned crop distribution, where the flax crops concentration areas are depicted. The comparative analyses of the conditions in the XVI and XXI centuries were conducted as well.

Keywords: Retrospective cartography, Flax, Landscape change, Agricultural crops, Samtskhe-Javakheti, Georgia.

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Introduction

Preservation and diversification of traditional agricultural crops are of great importance from both economic and cultural viewpoints. In the last century resulting from the Soviet state-planned economy the agricultural branch experienced a number of changes that were frequently related to the propagation of monocultures. This phenomenon became the reason for the disappearance of many agricultural crops that had a negative impact on both the diversity and competitiveness of the agricultural branches.

For Georgia as for the country with an ancient agricultural tradition, taking into account of the past experience is of great importance. There were such varieties of the agricultural crops here that are forgotten out and away. Fortunately, Georgia has a rich historiographic material in the form of documents, which confirm the existence of various agricultural crops and narrates us about their importance. Flax is directly among these crops.

From ancient times flax seeds were used as both feed and for curative purposes [1-5]. Oil – 'Bezir' was made of it. This oil was used not only for feeding and curative purposes, but also in the process of dyes and leathers manufacturing [6]. It was prepared by using the local special oil-pressing stones – 'Gelazi'. Some settlements were famous thanks to their 'Bezirkhanas', i.e. due to the local small enterprises, where the flax oil was manufactured [7, 8]. The information about rural settlements; also, the taxes levied is given in 'the Great Defter of Gurjistan Vilayet' [1, 2]. These taxes implied both money duty and its equivalent natural taxes [1, 3, 4].

Resulting from the study of the document we identified the flax distribution area, composed the maps that reflect the state existing in the XVI century.

Objects and methods

The region under study – Gurjistan Vilayet was spread on the territory of both modern Georgia and Republic of Turkey. The part of Gurjistan Vilayet located at the territory of Georgia mainly includes the administrative unit of Samtskhe-Javakheti. The study area is 6412.8 square kilometers. The population equals 160.5 thousand people; six municipalities – Akhaltsikhe, Adigeni, Borjomi, Akhalkalaki, Ninotsminda and Aspindza are combined in the region [9, 10]. The region is mountainous and is distinguished by alternation of structural basin, canyons, mountain chains and volcanic mountainous plateaus [11]. Samtskhe-Javakheti is located in the Mtkvari river valley, Akhaltsikhe structural basin and Javakheti plateau. In the past centuries the area was distinguished by flax plantings distribution.

The following archive sources, data and maps were used to conduct the research:

1. The main source was The Great Defter of Gurjistan Vilayet – a document drawn up by Ottoman Empire officials in the second half of the XVI century. The Georgian version of this document was prepared in three volumes by academician Sergi Jikia in the last century. We made use of the second and third volumes of the Defter, where the population and agricultural crops described in the settlements are given, as well as the taxes levied and the geographical locations of settlements are indicated, too, that was very important to their identification on the map;
2. The results of agricultural census carried out in 1923, in which the data on agricultural crops and areas under crop are given [12];
3. The results of 2004 and 2014 censuses that assisted us in the establishment of the modern pattern of flax culture distribution;
4. The map composed by Sergi Jikia and Alexander Aslanikashvili in 1953 based on 'The Great Defter of Gurjistan Vilayet' (1:330 000) [13];
5. Topographic maps (1:25000; 1:50000);
6. Satellite images;
7. Materials obtained in expeditions, notes and photos.

The following methods were used during our research:

a. The historiographic analysis methods, which envisage the processing and analysis of historical sources, as well as the study and analysis of works published by researchers in the last century;
b. Field research. We conducted the expeditions. In the course of expeditions, we became acquainted with the state of agricultural crops, the confirmed remnants of flax oil culture, which is kept in the form of residual elements of oil-pressing enterprises;
c. The mapping method, which envisages the identification of object locations and their plotting, marking out of distribution zones and the preparation of thematic maps;
d. Geo-information method, which implies preparation of digital layers and vast database using GIS-technologies, drawing of thematic maps on their basis, qualitative and quantitative analysis;
e. The method of data, comparative analysis, which implies the comparison of the state of the period under study with subsequent periods, including modern data that assist us to study the dynamic of the events and manifested tendencies.

Results

Flax plantings were distributed almost over all settlements both at the Javakheti plateau and in river valleys. There is observed, their concentration in the following sections: 1. Kvabliani river valley and Akhaltsikhe (Samtskhe) hollow; 2. Middle part of Uraveli river; 3. Mtkvari river valley at the Klde-Atskuri-Slesa section; 4. Mtkvari canyon at Margastani-Tmogvi-Vardzia section; 5. Buzmareti basin; 6. Javakheti plateau, volcanic tablelands, from where the flax plantings were distributed to the left side of Mtkvari canyon, on the Niala-Erusheti volcanic massif.

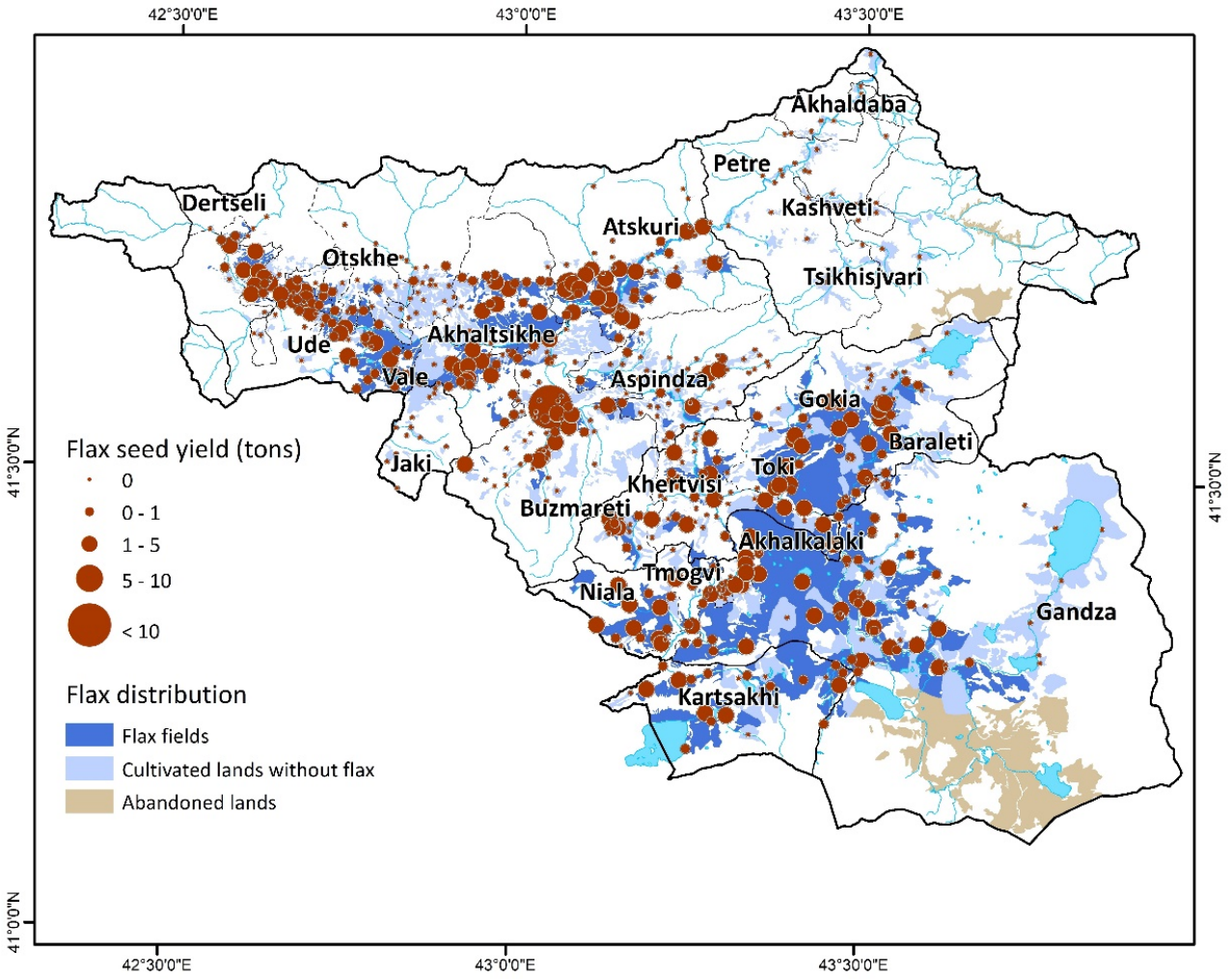

Fig. 1. *Flax crop in XVI century on the territory of Samtskhe-Javakheti*

There were not flax plantings in the Borjomi gorge (Petre Liva, Petre and Kashveti Nihies) that probably can be explained not only by shortage of lands but also by a small number of populations.

The flax was planted in the basin both at plain surfaces and artificial wall-less terraces – 'Laris'. We deem that especially favorable conditions for flax distribution were created at the volcanic plateau that can be explained by the following: despite the fact that the land fund was mainly used for cereal crops, flax crop played an important role here anyway. This can be confirmed by high indicators of its yield in Tke-Javakheti, Akshehiri (Akhalkalaki) and Qanarbeli Nihies. The high hypsometric range of its distribution is indicated also by the fact that flax was seeded starting with Atskuri Nihie to Nialiskuti, Akshehiri, Qanarbeli and Chacharaki Nihies. The highest yield of flax crop was characteristic also for villages located in Buzmareti Nihie, similar to Nihies, where the altitude of settlements hypsometrically reached 2100-2200 m and sometimes even exceeded it.

Table 1. *Flax seed yield (tons) at the territory of Samtskhe-Javakheti (according to administrative units entering the Gurjistan Vilayet)*

Administrative units (Liva, Nihie)	Number of settlements with flax crops	Total amount (tons)
Akhaltsikhe Liva (Sanjak)		
Mzvare	16	12.75
Chrdili (Akhaltsikhe)	21	19.76
Ude	45	39.27
Kvabliani	5	5.48
Otskhe	2	1.91
Atsk'uri	29	32.13
Altunkala (Okrotsikhe)	4	2.295
Aspindza	9	6.88
Ch'ach'araki	18	37.99
Total (within the Akhaltsikhe	149	158.465
Khertvisi Liva		
Khertvisi	16	15.43
Tqe-Javakheti	35	35.83
Buzmareti	8	8.03
Total (within the Khertvisi	59	59.29
Akshehir (Akhalkalaki) Liva		
Akshehir (Akhalkalaki)	43	42.33
Tmogvi	9	10.22
Nialisquri	20 (on the territory of Georgia - 15)	18.62 (on the territory of Georgia - 13.5)
Total (within the Akhalkalaki Liva)	72 (on the territory of Georgia - 67)	71.17 (on the territory of Georgia - 66.05)
Childiri Liva		
Kanarbeli	22 (on the territory of Georgia - 15)	19.64 (on the territory of Georgia - 11,98)
Total (within the Childiri Liva)	22 (on the territory of Georgia - 15)	19.64 (on the territory of Georgia - 11,98)
Fotskhovi Liva		
Chrdili	4 (on the territory of Georgia - 0)	3.19 (on the territory of Georgia - 0)
Total (within the Fotskhovi Liva)	4 (on the territory of Georgia - 0)	3.19 (on the territory of Georgia - 0)
Petre Liva		
Petre	0	0
Kashveti	0	0
Total (within the Petre Liva)	0	0
Total amount on the territory of the 'Gurjistan Villaiet' (in Georgia)	306 (on the territory of Georgia- 290)	311.75 (on the territory of Georgia - 295.78)

The average yield per one village was approximately one ton. For some villages the fewer yields were peculiar, while for others – far more, but this figure expresses the average indicator and indicates the importance of the mentioned crop. The 712 populated places are registered in the Defter, which are entirely or partly located at the territory of Georgia. The part of settlements of Kanarbeli, Ude and Chrdili (Potskhovi Liva) Nihies was located at the territory of Republic of Turkey. Thus, 683 of 712 registered villages were located at the territory of modern Georgia. Flax crop was cultivated in 290 populated places that equal to 42,5%. 242 of 683 settlements were already devastated by that time, therefore, for these populated places the total levied tax is given in 'Akhchis' and nothing is said about natural taxes. If we judge according to the rest 441 populated places, we can conclude that flax culture was cultivated approximately in 65.8% of settlements. Flax seed production remained the important branch even during the ensuing centuries that is confirmed by the results of the 1923 census (Table 2). In the Soviet period flax was gradually forgotten and its plantings were replaced with other crops. This caused in the end the disappearance of flax culture in the region.

Table 2. *Flax crop in the beginning of the XX century in the study region [12]*

Mazra	NN	Community	Flax crop	
			Flax field area	Flax seed yield
Akhalkalaki	1	Alastani	22.89	28.6
	2	Baraleti	167.969	209.96
	3	Gorelovo	1.744	2.18
	4	Dilska	148.24	185.3
	5	Eshtia	35.752	44.69
	6	Kondura	314.683	393.35
	7	Kulikami	128.075	160.1
	8	Okami	119.464	149.33
	9	Sathkhe	2.398	2.9975
	10	Khertvisi	0	0
		Total	941.215	1176.5
Akhaltsikhe	1	Adigeni	0	0
	2	Ats'kuri	0	0
	3	Vale	0	0
	4	Varkhani	0	0
	5	Idumala	0	0
	6	Klde	0	0
	7	Lepisi	0	0
	8	Ude	0	0
	9	Uraveli	3.052	3.815
	10	Akhaltsikhe town	0	0
		Total	3.052	3.815
Gori (Communities according of 'Gurjistan Villaiet' territory)	4	Akhaldaba	0	0
	7	Bakuriani	21.364	26.7
	8	Borjomi	0	0
	11	Gujareti	0	0
	12	Dviri	0	0
	32	Kvishkheti	0	0
	36	Tsagveri	0	0
		Total	21.364	26.7
Total (within the entire region)			965.631	1207.04

The difference between the 1574 and 1923 data shows that flax plantings have been increased. The total indicator is almost 4-times bigger as far as the population number was increased five and more times. In the Akhaltsikhe hollow and Mtkvari valley at Atskuri-Klde-Tsnisi-Zikilia section flax plantings were already disappeared by the mentioned period. There were no more flax plantings in the vicinity of Khertvisi, as well, but instead the mentioned crop was appeared in the surroundings of Bakuriani that solidifies the above-mentioned argument that was expressed regarding Petre and Kashveti Nihies – flax culture was not observed here due to depopulation of these places. But one can observe the concentration of flax crop at the Javakheti plateau and, as we can see, in the last century it played an important role in the local life. We still can face the stones and special warehouses, remained in particular villages that were used for gathering and treatment of flax seeds (Fig. 2).

Among flax seed products one can single out flax oil or 'Beziri' as it was called in Ottoman Empire, which was very popular product and it was even exported. On the topographic maps the titles 'Bezirkhana' are mentioned sometimes instead of village names, and this fact points at the mistake made by map compiler, who taken the term named by the resident to be an own name of the village and, correspondingly, plotted it on the map. However, in the Defter the particular settlements are levied by the tax exactly according to 'Bezirkhanas', which presented the flax oil producing enterprises. The 'Bezirkhanas'

Fig. 2. *„Gelazi" – oil-pressing stone, village Foka*

data shows that flax plantings have been increased. The total indicator is almost 4-times bigger as far as the population number was increased five and more times. In the Akhaltsikhe hollow and Mtkvari valley at Atskuri-Klde-Tsnisi-Zikilia section flax plantings were already disappeared by the mentioned period. There were no more flax plantings in the vicinity of Khertvisi, as well, but instead the mentioned crop was appeared in the surroundings of Bakuriani that solidifies the above-mentioned argument that was expressed regarding Petre and Kashveti Nihies – flax culture was not observed here due to depopulation of these places. But one can observe the concentration distribution map shows us that they were mainly concentrated in the eastern part, basically to the east of Akhaltsikhe and their abundance is observed at Javakheti plateau (Fig. 3) and at Niala field, as well. Presumably, the increase of their production became the reason that the role of the mentioned flax crop was raised here; however, the reason of its total disappearance at the territories of Ude, Chacharaki, Atskuri and Aspindza Nihies, as well as in the section of Vardzia-Tmogvi canyon is unclear.

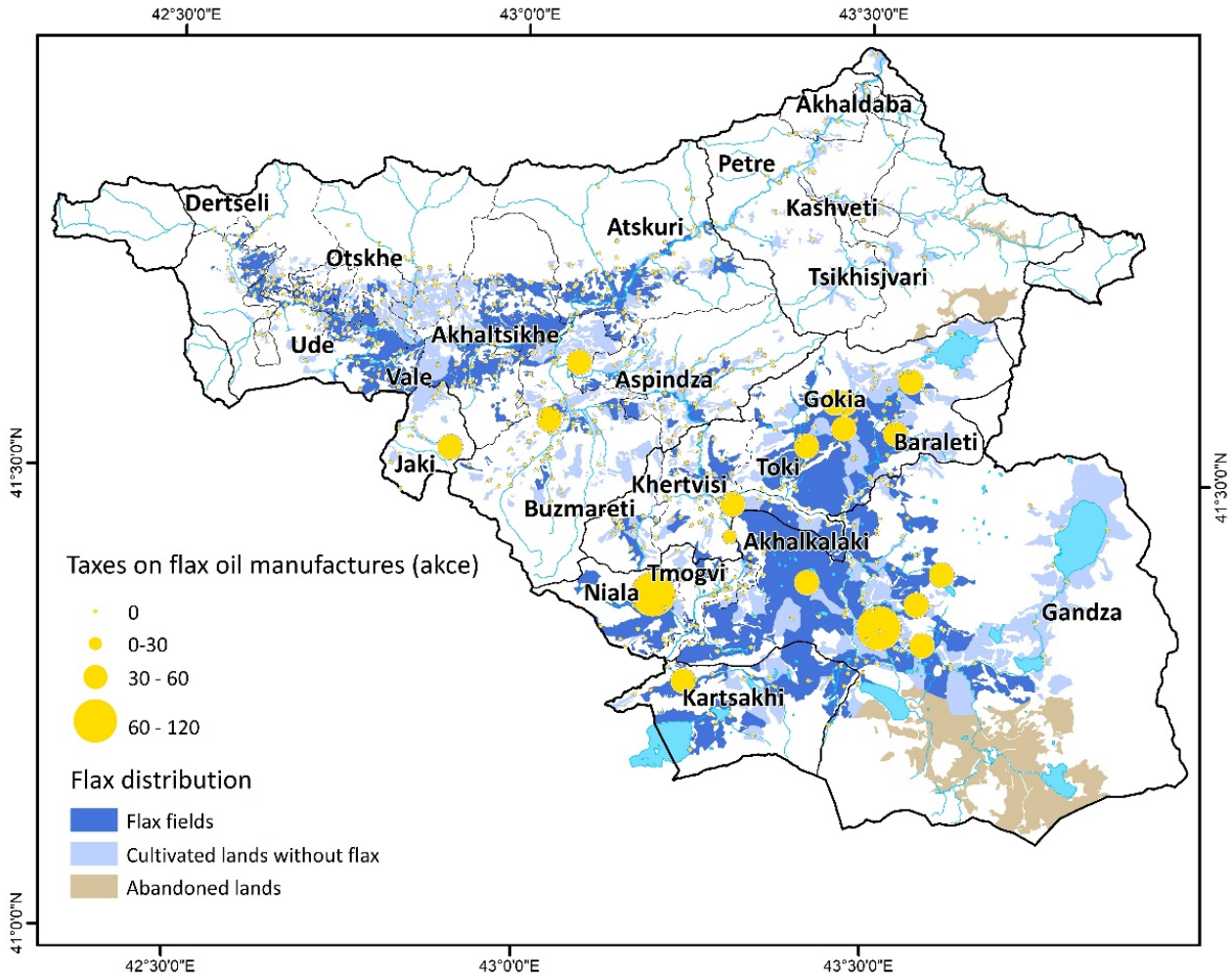

Fig. 3. *'Bezirkhanas' at the territory of Samtskhe-Javakheti according to 1574 data*

Conclusion

1. Flax was the important agricultural crop for Georgia in the middle ages. Oily flax was widely cultivated in the Samtskhe-Javakheti. Flax seeds and oil were used as a food and medication, for dyes and for other intended purposes, too.
2. Due to great demand for the flax oil-pressing centers were emerged, which also played important role in the trading as the enterprises manufacturing the exported goods.
3. Flax crop was propagated throughout the Samtskhe, while the most of 'Bezirkhanas' were concentrated at the Javakheti plateau and Niala field. The reason was the fact that the massive boulders necessary for manufacturing 'Gelazis' and construction of 'Bezirkhanas', were concentrated exactly in the volcanic landscapes of Javakheti.
4. In the ensuing centuries the flax culture was shifted mostly to the Javakheti plateau and in the beginning of the XX century its yield was far more than in the last centuries.
5. From the very beginning of the Soviet period this crop was oppressed and by the turn of the XXI century it was totally disappeared.
6. Disappearance of flax culture in Samtskhe-Javakheti is related to the series of agricultural reforms conducted in the Soviet period that had several times the impact on Georgian villages. The flax crop can be ranked among such traditional crops which became the victims of the Soviet state-planned economy in the middle of the XX century.

Acknowledgements

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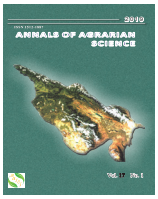

Bacteriophage preparation for Treatment and Prevention of Salmonella Infection in Poultry

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A B S T R A C T

Enteric Salmonella infection is a global problem both in man and animals, and has been attributed to be the most important bacterial etiology for enteric infections worldwide with massive outbreaks occurring in recent years. Food animals are the primary reservoir for human non-typhoid Salmonella infections. Poultry products are considered one of the major sources of Salmonella infections. The development of alternative anti- microbial remedies has become one of the highest priorities of modern medicine and biotechnology. One of such alternatives might be bacteriophages as a prospective biocontrol method against contaminations caused by antimicrobial resistant pathogens. The aim of this study was to develop bacteriophage-based product that can be used to control salmonella contamination on farm level. 31 *S. typhimurium* strains were isolated from 200 poultry meat and eggs. All of the isolates showed high resistance to antibiotics used in the test. For formulating polyvalent phage preparation 3 phages out of 8 with wide, complementary, not-fully-overlapping host ranges were selected. Lytic activity and host specificity of each individual phage was compared with that of the phage cocktail. It was observed that the cocktail was superior to the individual phages. .Salmonella phage cocktail was effective against 46 out of 45 (98%) tested *Salmonella* strains in in vitro experiments.

Keywords: Salmonella, Bacteriophage, Antibiotic resistance, Treatment, Prevention, Chikens

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Introduction

Food animals are the primary reservoir for human non-typhoid *Salmonella* infections [1]. According to the Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA) 75% of the annual cases of human salmonellosis are due to the consumption of contaminated poultry, beef, and egg product. In general, poultry products are considered one of the major sources of Salmonella infections [2, 3]. In fact, several Salmonella strains persistently colonize chickens but without causing any signs of illness [4]. The ceca of chickens are the major sites of Salmonella colonization, and chickens can become asymptomatic carriers of Salmonella. This lack of clinical symptoms facilitates the dissemination of Salmonella within flocks. *Salmonella* can be transmitted into foods at different stages of the food production chain. Reduction of pathogens colonization in animals during primary production is very important strategy to reduce the probability of cross-contamination with the animal feces during food processing. Controlling of food-born pathogens at the firm level will reduce their occurrence in the environment and food line and dissemination. Consequently, it could help prevent

the occurrence of food pathogens in animal, food, humans and environment [5].

Recent years show a dramatic rise both in terms of incidence and severity of human salmonellosis cases. The continuing emergence of Salmonella strains that are resistant to antimicrobials is also a cause of increasing concern [6].

Existence of such pathogens is problematic not only for animal health, but also because of possible transmission of antibiotic resistant bacteria from animals to humans through the food supply. Antibiotic-resistant bacteria from poultry houses contribute to the spread of antibiotic-resistant bacteria in the air, water, and soil so antibiotic-resistant bacteria from poultry production can contribute to rising rates of antibiotic resistance [7, 8].

The development of alternative anti- microbial remedies has become one of the highest priorities of modern medicine and biotechnology. One of such alternatives might be bacteriophages – bacterial viruses that infect and replicate within bacterial cells causing irreversible cell death [9].

Recently, there has been an increased interest in the application of bacteriophage as an alternative antimicrobial chemotherapy in various fields including human infections, food safety, agriculture, and veterinary applications [10, 11]. This interest is enhanced by a number of positive properties of bacteriophages. These include the possibility of high specificity towards the pathogenic agents while sparing the regular flora of the human, their lytic capability against antibiotic-resistant strains and, most important, their safety for the animals and environment. They have been identified as a prospective alternative biocontrol measure for infections and contaminations caused by antimicrobial resistant pathogens. The use of bacteriophages may be a safe and effective alternative to antibiotics for the treatment and prevention of Salmonella infection in poultry [12].

The main goal of this work is the development of bacteriophage-based commercial product that can be used to control salmonella contamination at the farm level.

Materials and Methods

Isolation and identification of Salmonella strains: For isolation of salmonella strains total 200 samples of poultry meat and eggs were purchased at the farmer markets and supermarkets in Tbilisi area. Fresh samples were placed to sterile sampling tubes and transported to the laboratory. For primary enrichment, 25g of each sample was incubated in 225 mL of buffered peptone water (BPW) during 24-48 hours at 37°C. For secondary enrichment, 1 mL of the first enriched broth was added to 10 mL of Hajna tetrathionate broth and incubated for 22-26 hours at 42°C. After incubation, broth was inoculated onto desoxycholate hydrogen sulphide lactose agar and brilliant green agar, both supplemented with 20 mg/L of novobiocin sodium, and incubated at 37°C for 18 h. Suspected colonies were isolated for biochemical and microbiological studies.

Bacterial strains

For investigation of bacteriophage host range, apart from new isolated salmonella strains 15 clinical S. typhimurium strains from laboratory collection were used. For assessment of lytic activity of the selective phages toward essential bacteria characteristic of GI tract 8 probiotic strains from institute collection were also enrolled in this study.

Antimicrobial susceptibility testing. All the S. typhimurium isolates were exposed to different antibiotics for its antimicrobial susceptibility and drug resistance pattern determination using disk diffusion assay following the guidelines of clinical and laboratory standard institute [13]. Pre-incubated 24 h cultures of S. typhimurium were swabbed over Brain Heart agar. After placing the antibiotic discs aseptically, the plates were incubated at 37oC for 18-24 h and zone of inhibition were measured subsequently. 8 different antimicrobial agents, most widely used in clinics and also used in this study were: Ampicillin (10 μg), Streptomycin (10 μg), Ciprofloxacin (5 μg), Trimethoprim (5 μg), Chloramphenicol (30 μg), Tetracycline (30 μg), Gentamicin (10 μg), Kanamycin (30 μg). Each strain was identified susceptible (S), intermediate (I), or resistant (R) in accordance with the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Characterization of selected bacteriophages

Spotting assay

The host bacteria were grown overnight and then 100 µl of cell suspension was added to 5 ml of soft LB agar (0.6% agar), which had been pre-heated to 42°C in a water bath. Resultant mixture was gently vortexed, poured over LB agar plates (1.5% agar), and allowed to solidify at room temperature during 30 min to produce bacterial lawns. Then, 10 µl of phage stock dilutions (10-fold serial dilutions in SM buffer) were spotted onto the upper agar layer, and the plates were dried at room temperature for 30 min. Then, the plates were incubated overnight at 37°C, and inspected for single plaques or bacterial growth inhibition zones after 24 hours.

Preparation of concentrated phage stocks (Bilayer agar method)

100µl of the host bacterium culture grown overnight and 1 ml of phage (103PFU/ml) were mixed; then 3 ml of molten soft-agar (0.7%) was added to each tube and the mixture was gently vortexed and poured over LB agar plates (1.5% agar). The Petri dishes were incubated at 37oC. After 18-20 hours incubation, 3 ml of broth was spread over the agar and left for 15-20 min. Using spreading rod or spatula, the soft-agar with broth were scraped I and transferred to a centrifuge tube, and centrifuged at 6000 g for 45 min. The supernatant was filtered through 0.22 nm filter, transferred into the sterile vial and titrated.

Bacteriophage host range and selection of the most efficient phage

The phages were investigated for host range specificity and lysis efficiency against Salmonella strains. Each strain was inoculated on Tryptic Soy Agar (TSA) and 10 µl of phages (1 × 104 - 1 × 107 PFU m^{l}-1) was dropped over the plate with inoculated culture. The plates then were incubated during18 hrs at 370C and the presence of plaques was observed.

Characterization of phage genomes Restriction analysis

For investigation of phages genome, phage DNA was isolated though standard Phenol–Chloroform extraction method from high-titer phage lysates. Phage nucleic acids were purified in a three-stage organic extraction using equal volumes of buffered phenol, then phenol-chloroform (1:1), and finally chloroform. At each stage the aqueous and organic phages were mixed by gentle inversion for 3 min, followed by centrifugation at 13,500 × g for 5 min at room temperature. DNA was precipitated by adding sodium acetate to a final concentration of 0.3 M and adding an equal volume of 100% ethanol, until the phage DNA had just precipitated. The precipitated DNA was collected by centrifugation at 15,000 × g for 15 min at 4°C, washed with 70% ethanol, and air dried before being resuspended in 10 mM Tris-HCl (pH 7.5) and stored at −20°C. The restriction patterns of phages DNA were determined with restriction endonucleases HindIII, EcoRI from Sigma-Aldrich Inc. according to the manufacturer's instructions. Incubation time was 2 h at 37oC. Fragments were separated on a 1% agarose gel at 70 V for 2 h and stained with ethidium bromide (1 µg/ml).

Results

Isolation and identification of S. *typhimurium* strains

A total of 200 samples of poultry and eggs were taken from several markets in Tbilisi area. The samples were analyzed for the presence of Salmonella using standard biochemical and serologic methods. A total of 31 S. *typhimurium* strains were isolated.

Antimicrobial susceptibility testing

The next step of our work was testing antibiotic susceptibility of isolated Salmonella strains. 7 different antibiotics widely used in clinics and veterinary were used in this study.

The study revealed that the majority of salmonella cultures were characterized by high levels of antibiotic resistance. Multiresistance was revealed when strains were resistant to four and more antibiotics (Table 1).

Table 1. *Antibiotic resistance of isolated S. typhimurium strains*

		Resistant strains[a]		Multiresistant strains[b]	
Serotypes	Number of strains	n	%	n	%
S.typhimurium	46	46	100	35	76

[a] Isolates resistant to one and more antibiotics
[b] Isolates resistant to four and more antibiotics

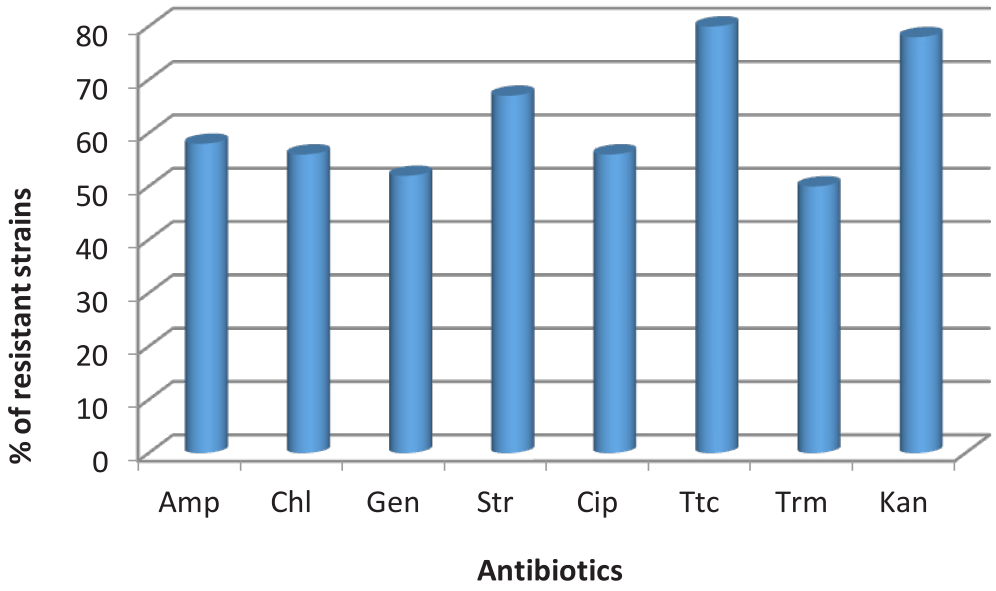

Fig. 1. *S. typhimurium antibiotic susceptibility test*

As follows from the Table 1, investigated *Salmonella* isolates were resistant to 4 from 7 examined antibiotics. S. *typhimurium* strains revealed high resistance to Tetracycline (80%), Kanamycin (78%), Streptomycin (67%). Actually, all newly isolated strains were characterized with high to moderate level of antibiotic resistance.

Selection and study of effective bacteriophages against Salmonella

The aim of our work was selection and study effective bacteriophages against salmonella strains. Our purpose was to create an effective, non-overlapping phage cocktail against salmonella. We considered that it would also be important to select bacteriophages with different genotypes.

A host specificity of Salmonella phages

In the test, we used 46 S. typhimurium strains to determine the range of host cells susceptible for previously selected 8 salmonella bacteriophages. Sal.phi13, Sal.phi18 and vB_stm 21 were characterized by the wide range of host cells. From the results obtained, Sal.phi13 phage lysed 36 out of 46 strains (82.6%). The activity of Sal.phi18 was high compared to the Sal.phi13 phage. The Sal. phi18 lysed 43 out of 46 strains (93.4%) and vB_ Stm phage lysed 35 out of 46 strains (76%) (Table 2).

Table 2. *A host specificity of Salmonella phages*

Tested *S.typhimurium* phages	A total *S.typhimurium* strains	Lysed *S. typhimurium* strains	Lysed *S. typhimurium* strains, %
vB_Stm 17	46	29	63
vB_Stm 18		30	64
vB_Stm 21		35	76
vB_Stm 29		24	52
Sal.phi 13		36	82,6
Sal.phi 14		32	69,5
Sal.phi 16		31	67,4
Sal.phi 18		43	93,4

Three lytic salmonella phages, *Sal.phi13, Sal. phi18,* and *vB_Stm 21,* which belong to two families, were selected to compose the phage cocktail. Each of these phages was shown lytic and highly specific toward *Salmonella typhimurium* strains. These three phages have been isolated from individual cell-phage lysates, purified, and stock suspensions of approximately equal concentration of the phages were prepared. The phages were mixed in the proportion 1:1:1 and lytic activity, and host specificity of each individual phage was compared with that of the phages cocktail (Fig 2). It was observed that the phage cocktail possessed broader host specificity within *S. typhimurium* serotype than each of three phages alone. The cocktail composed of these phages was shown lytic toward 65 out of 66 tested *S. typhimurium* strains (98%).

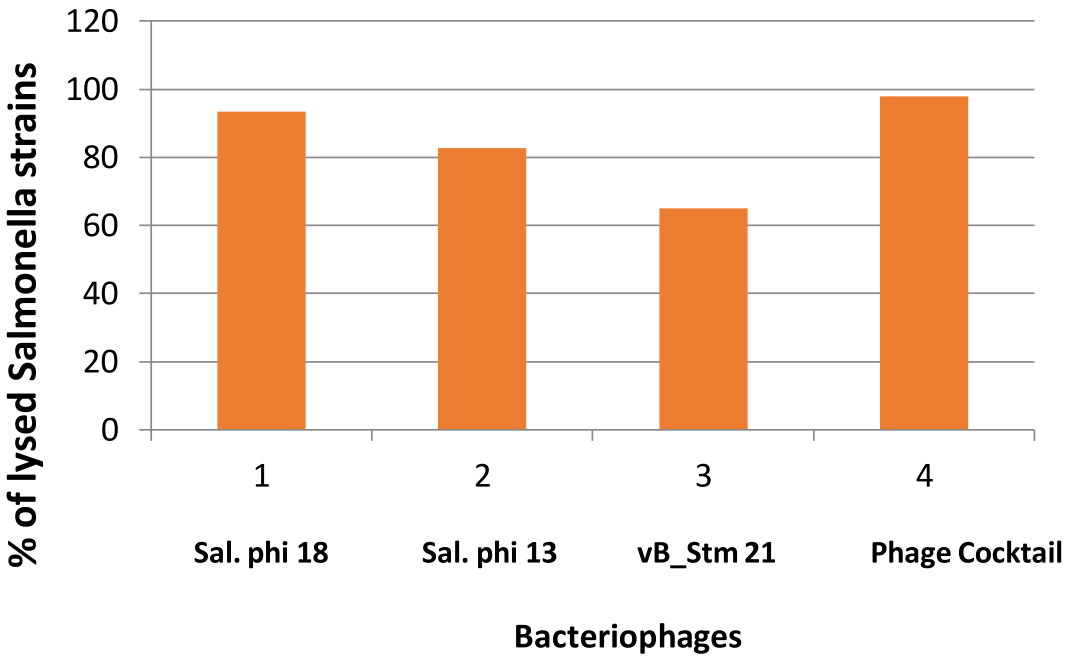

Fig. 2. *Comparison of host specificity of single Salmonella phages and their combination within S. typhimurium serotype*

Figure 2 demonstrates that a host specificity of *Salmonella* cocktail was noticeably wider than that of the individual *Salmonella* phages. It can be easily explained by the fact that host specificities of the individual phages were not completely overlapping, and thus, each phage in the cocktail contributed its activity toward species for which other phages were not lytic. We are going to study the efficacy of *Salmonella* phage cocktail preparation in elimination, reduction and prevention of colonization of poultry in the chicken infectious model.

Morphological characteristics of selected Salmonella phages

Electron microscopy study of pre-selected salmonella phages allowed classifying them (Fig. 3, Table 3). Phages Sal. phi 13 and vB_Stm 21 had a tubular tail with helical symmetry and the diameter of 10-20 nm. The tail length of phages was in the range from 80 to 90 nm, and the head diameter of 50-60nm. These phages were referred to *Myoviridae* family. Phage Sal. phi 18 had relatively thin, long, non-contractile, and flexible tail with an isometric head. The tail length of phage Sal. phi 18 was of 140 nm, and its head diameter was 45 nm. It was referred to *Siphoviridae* family.

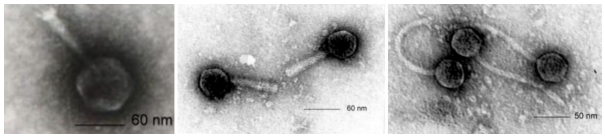

Fig. 3. *vB_Stm 21, Sal.phi13, Sal.phi18 phages electron micrograph*

Table 3. *Morphological characteristics of selected Salmonella phages*

Phage	Head diameter nm	Head length nm	Tail diameter nm	Tail length nm	Family
vB_Stm 21	50	50	15	90	*Myoviridae*
Sal.phi13	60	60	15	80	*Myoviridae*
Sal.phi18	45	45	10	140	*Siphoviridae*

Characterization of phage genomes

Restriction analysis

Bacteriophages vB_Stm 21, Sal.phi 13 and Sal. phi 18 showed distinct restriction endonuclease patterns after digestion with EcoRI and HindIII endonucleases (Fig. 4, 5, 6, 7).

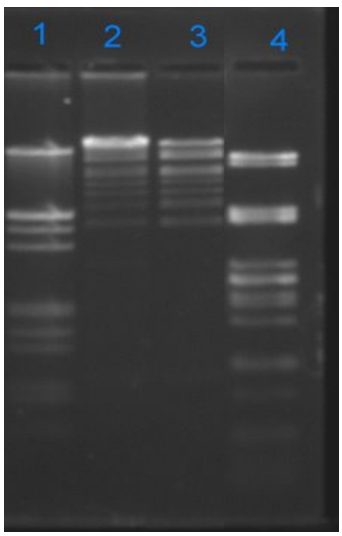

Fig. 4. *Digestion of the Sal. phi 13 and Sal. phi 18 phage DNA with HindIII endonuclease. 1. λ DNA (HindIII digest), 2. Sal. phi 13, 3. Sal. phi 18, 4. λ DNA*

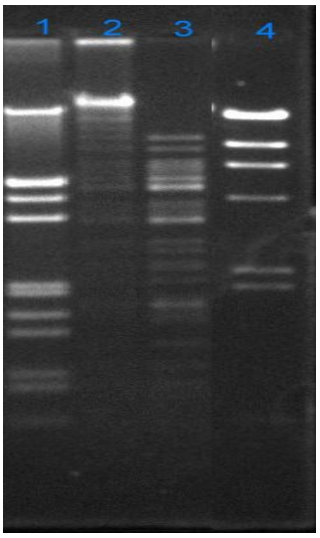

Fig. 5. *Digestion of the Sal. phi 13 and Sal. phi 18 phage DNA with EcoR1 endonuclease. 1. λ DNA (EcoR1 digest), 2. Sal. phi 13, 3. Sal. phi 18, 4. λ DNA*

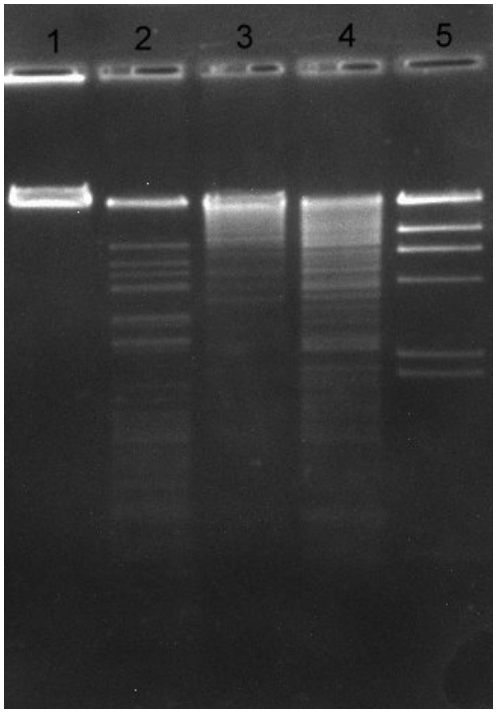

Fig. 6. *Digestion of the vB_Stm phage DNA with HindIII endonuclease. 2. vB_Stm 21, 5. λ DNA (HindIII digest)*

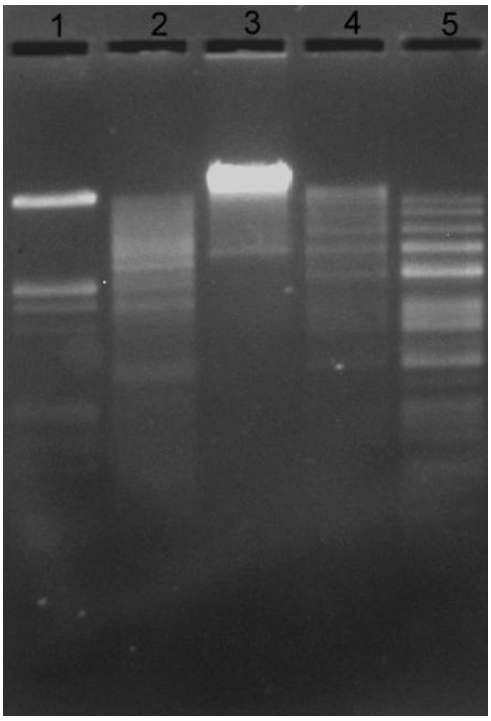

Fig. 7. *Digestion of the vB_stm 21 phage DNA with EcoR1 endonuclease. 1. λ DNA (EcoR1 digest), 4. vB_Stm 21*

Lytic activity of the selected Salmonella phages toward essential bacteria characteristic of GI tract

We examined lytic activity of the Sal. phi 13, Sal. phi 18 and vB_Stm 21 phages toward essential bacteria characteristic of GI tract. Results presented in the Table 4 demonstrates that no lytic activity of examined phages was observed toward essential microorganisms characteristic of GI tract.

Table 4. *Lytic activity of Sal.phi13, Sal.phi18, and vB_ Stm 21 phages toward essential bacteria characteristic of GI tract*

Phages	*vB_Stm 21*	*Sal phi 13*	*Sal phi 18*
Bacterial strains	Lysis with phages		
Bifidobacterium bifidum Ac-1246	-	-	-
Bifidobacterium longum Ac-1252	-	-	-
Bifidobacterium adolescentis Ac-	-	-	-
Lactobacillus acidophilus B-2585	-	-	-
Lactobacillus fermentum B 7580	-	-	-
Lactobacillus plantarum B 7583	-	-	-
Escherichia coli M17 B 8208	-	-	-
Enterococcus faecium B 4054	-	-	-

Discussion

Bacterial antibiotic resistance is an increasingly important problem in farm animal and human medicine. Although resistance in bacterial species responsible for human infections is mainly caused by indiscriminant use of antibiotic in humans, for a range of bacteria, farm-animal use of antibiotics is a significant contributor, and for some infections is the main source of resistance development. In food animal production antimicrobial resistance is increasing in both zoonotic and commensal bacteria. This has raised concerns about the risks of transmission of resistant zoonotic bacteria and resistance genes from food animals to humans and the consequences for health care and public health. Also, results obtained from our work showed high level of antibiotic resistance of the studied S. *typhimurium* strains.

Considering the above-mentioned problems, interest in the use of bacteriophage preparations as an alternative to antibiotic treatments has been steadily increasing worldwide. This interest is enhanced by a number of positive properties of bacteriophages. These include i) high specificity towards the pathogenic agents, which means that the regular human microflora is spared, ii) ability to lyse antibiotic-resistant strains and, iii) safety for the animals and environment, and iv) virtually unlimited number of phages, which should allow facile isolation of phages specific for any given bacteria, including isolation (or selection) of phages capable of lysing bacteria that acquired resistance to a phage(s) used in the initial round of therapy.

These properties give bacteriophages or phage-derived products wide potential to be used: a) as possible alternatives to antibiotics for the treatment of bacterial diseases in humans and animals b) to minimize the pathogen loads in food products of animal and plant origin -biocontrol of microorganisms in food c) an alternative to industrial disinfectants and sanitizers.

Using bacteriophage preparation as a phage cocktail composed of two or more individual bacteriophages enhances the efficiency of the preparation activity. Formulation of phages into cocktails increases their potential to be used presumptively, that is, prior to identification of pathogens (e.g., in terms of phage susceptibility), and the more phages are included, the greater the potential that there will be sustainable levels of medical as well as commercial demand for a given formulation. Perspective of using bacteriophage endolysins instead, or along with intact bacteriophages increases a potential value of bacteriophages as natural alternative to antibiotics.

Therapeutic use of phage cocktails, attracts more and more attention. According to authors [14, 15] the primary motivation for the use of cocktails is their broader spectra of activity in comparison to individual phage isolates: they can impact either more bacterial types or achieve effectiveness under a greater diversity of conditions. The combining of phages can also facilitate better targeting of multiple strains making up individual bacterial species or covering multiple species that might be responsible for similar disease states, in general providing, relative to individual phage isolates, a greater potential for presumptive or empirical treatment. Also, it is expected that cocktails consisted of several genetically distinct phages has lower chance to induce microbial resistance. Our study demonstrates the lytic activity of selected bacteriophages against multidrug-resistant S. Typhimurium strains, including antibiotic-induced resistant and clinically isolated strains. The three bacteriophages used in this study, were chosen from our collection of Salmonella bacteriophage based on their broad host range, different restriction patterns with EcoRI and HindIII and their morphological characteristics. It was observed that the phage cocktail possessed broader host specificity within S. typhimurium serotype than each of three phages alone (Fig. 2).

Advantages of phage therapy over the use of chemical antibiotics can be framed in terms of phage properties that can contribute substantially to phage therapy utility. These include: i) effective against multidrug-resistant pathogenic bacteria; (ii) substitution of the normal microbial flora does not occur because the phages kill only the targeted pathogenic bacteria; (iii) respond quickly to the appearance of phage-resistant bacterial mutants because the frequency of phage mutation is significantly higher than that of bacteria [16, 17]. Also, according to our results (Table 4) no lytic activity of examined phages was observed toward essential microorganisms characteristic of GI tract. Though more essential microorganisms must be tested, the data presented in the Table 4 are of a

great importance. The lack of lytic activity of pre-selected phages toward essential microorganisms means that no harm to human and animal health should be expected from application of these phages as anti-microbial remedies. We are going to study the efficacy of Salmonella phage cocktail preparation in elimination, reduction and prevention of colonization of poultry in the chicken infectious model.

Conclusion

The obtained results show that the selected salmonella bacteriophages were not only lytic to many S.typhimurium strains, but had also broad specificity to these strains. That indicate the use of phages as a biocontrol of foodborne pathogens. Control of pathogenic microorganisms will help reduce their frequency in the environment and food products, which in turn will reduce the risk of the spread of foodborne diseases caused by this pathogen in humans.

Acknowledgement

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Some aspects of agroecological assessment of soils on the development of economic conditions of the Lankaran lowland of Azerbaijan

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A B S T R A C T

Agroecologically evaluating of the soils under the perennial plantings (citrus and tea plantations) in the humid subtropic climate condition if the Lankaran Lowland has been performed. Unlike the traditional comparative evaluation, besides the soil parameters, the environment indices are also used. The main and open scales of the soils spread under the citrus and tea plantations have been built, the average calculation scores and comparative valuable coefficients have been found. A state of the perennial plantings (citrus and tea plantations) has been analyzed under the humid subtropic climate condition, decrease of the tea plantations area from 135000 hectares to 600 hectares has been indicated. Agroecologically evaluation of the soils under the perennial plantings was fulfilled. Unlike the traditional comparative assessment, The environment indices (rainfalls amount, Md, Σ T > 10 °C, BIP) beside the soil indications (humus, Ph, > 0.25 mm aggregates quantity, bulky weight) are used. At this time the correction coefficients of psevdopodzol which is character for the zone are used: weak psevdopodzolic-1.0; mean psevdopodzolic-0.80; strong psevdopodzolic-0.60; The agroecological scores of the soils under the perennial plantings (citrus and tea plantations) in the humid subtropical climate condition got the following values: weak psevdopodzalic yellow-forest-96 scores; mean psevdopodzalic yellow-forest -77 scores; weak psevdopodzalic yellow-95 scores, mean psevdopodzalic yellow-75 scores; strong psevdopodzalic yellow-58 scores; psevdopodzalic yellow-gleyey-93 scores; yellow-gleyey-91 scores; dark yellow meadow-100 scores; ordinary yellow-meadow-66 scores, bright yellow-meadow 56 scores.

Keywords: Soil, Parameters, Citrus and Tea plantation, Agroecological factors. Environment, Perennial planting.

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Introduction

Now are some important aspects of the ecological valuation of the soils under the perennial plantings (citrus and tea plantations) in the humid subtropics climate condition. Thereby a role of the natural and anthropogenic factors which participate in soil cover formation of the Lankaran Lowland differs from the other regions of the republic.

The climate condition possesses more influence and the climate factors actively participate in soils genesis.

A superiority of the teaching regime is observed in most cases in the center and south of the Lankaran Low Land.

The motley landscapes formation, soil cover variety, soil formation of the various facial zones are reflected [1]. A variety of the soil cover and climate condition shows itself in a character of the soil resources use and structural system of the faming lands.

A central and southern part of the Low Land is very good for citrus-growing and tea-growing development. Therefore agro ecologically evaluating of soils under the citrus and tea plantations in the humid subtropics condition assumes scientific-theoretical and practical importance.

On the basis of our investigations a total area of the soils concerning the humid subtropics soil formation is 32214,8 hectares in the plain part of the Lankaran Lowland.

Objectives and methods

The Lankaran Lowland is situated on the Caspian Sea shore of the east part of the Lankaran province. A geological structure in the research object is characterized by the deposit rocks collection in the zone. The Lankaran Low Land is entered the humid subtropics climate zone which is conditioned by little rainy summer and mild winter. An amount of the yearly rainfalls is 1200-1700mm in the research object. An annual average temperature changes by 14,6-15,0`C. all the rives which get a source from Talish, Peshtasar, Burovar mountains runs into the Caspian Sea. A composition of the perennial planting possess a special structure. This region is known with its tea plantations and subtropics fruit gardens. But it`s very pity, from the end of the 80th years of the XX century and in the first years of our independence some farming actions in the Low Land, inducing the tea-growing fell into decay. So, the tea plantations area descended from 13.4000 hectares in 1985 to 600 hectares in 2010. But last years there are some progresses in this area. Thereby the areas under the plantations reached 1000 hectares in 2016. This tendency is going on.

While the soils under citrus and tea are agro ecologically evaluated, the researches have been performed at three stages: cameral-preparation, field-laboratorial, summarizing-cameral. The methods and approaches have been used according to each stage.

Results and analysis

There is a need for an assessment in any area of the human`s activity. Ther soils assessment is a great conception. Some researches were devoted to the evaluation of the soils under the annual plantings, including citrus and tea plantations in the moisture subtropics from a different point of view [2-12].

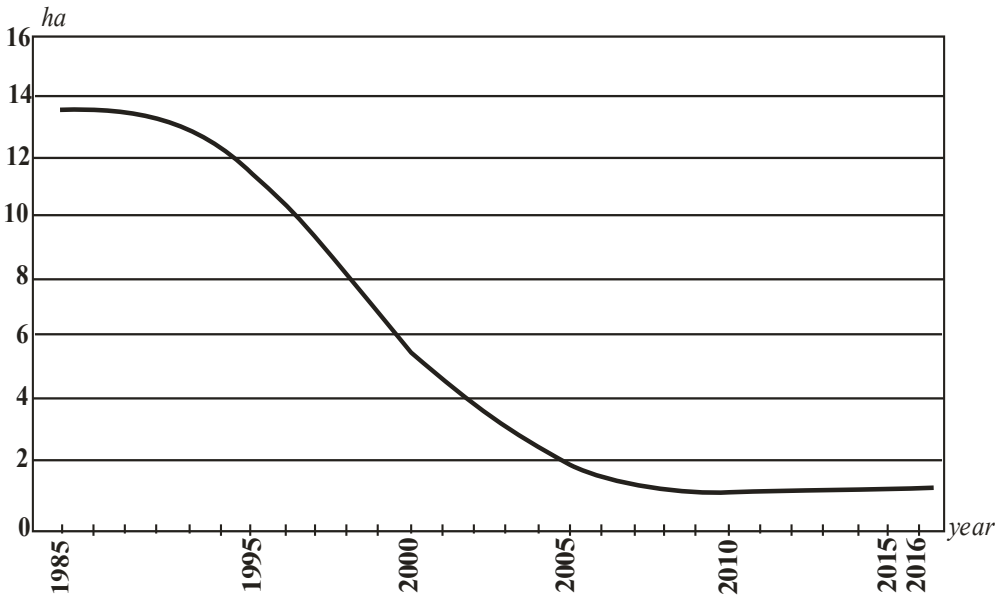

Fig. *Perennial dynamics of the farming lands under the tea-plantations in the Lankaran Lowland (1985-2016).*

But wholly different approach was suggested by S.Z. Mammadova [3] in the 90th years of the last century, while performing an ecological assessment of the soils under tea plant.

According to this approach not only the soil but also the environment factors (height of the zone, rainfalls, inclination, a sum of the positive temperatures and etc), are taken as a criterion while ecologically evaluating the soils under agroecosystems. [13-15]. It is known, that a

role of the climate and soil factors is great in subtropic plants development (citrus and tea) of the Lankaran Low Land. The agro ecological scores are found for the environment and soil parameters of the soils under the same plants considering this feature of the zone. At this time S. Z. Mammadova`s [3] method which was modificated by us has been used. There by the agro ecological assessment of the soils under the subtropics perennial plants in the moisture subtropics climate condition was realized by the following sequence:

At the first stage-the environment (rainfalls, Md, Σ>10`C, BİP) and soil (humus, pH, water-resistant aggregates, bulky weight) factors have been used, the agroecological scores of the soils are found) and the main agroecological scales are built, at the second stage-the total agroecological scores are found and an open agroecological scale was built by an application of the correction coefficients (Table).

As a result of the calculations it was known that a value index of the facial group of the psevdopodzolic yellow-forest and podzolic yellow-gleyey soil formation used under tea and citrus plant was 95 scores.

It unprovided with the heat and humidity to a high degree of zone where the same facial group spreads.

It was so, because the zone with the same facial groups arean`t provided withthe heat and humidity to a high degree. An agroecological score of the environmentparameters changed by 91-100 scores here. An average index was 88 scores on facial group. There are some factors besides eight environment and soil factors during formation of the agroecological scores in soil with facial group, they are taken into account by the correction coefficients as they show themselves in the local zones. The soil irrigation, granulometric structure, (psevdo) podzolic, erision and other characters include in here. But sometimes the psevdopodzolic rate participates as a factor which limits fertility under the moisture subtropics condition. This factor was considered by the correction coefficients: psevdopodzolic to a week degree – 1,0; psevdopodzolic to an average degree – 0,80; psevdopodzolic to a strong degree – 0,60. The following mathematic formula was used to build a scale of the total bonitet scores:

$$B = B_{я} \cdot Я_{sşn} \cdot Я_{şk}$$

B – a total bonitet score of soil:

B_2 – a bonitet score taken from the basic bonitet scale in soil.

So, an open scale of the agroecological scores of the soils under citrus and tea plant in the humid subtropics was compiled by using from the correction coefficients (Table).

It was connected with unproviding of the zone with the same facial group.

As is seen from (Table) an average calculation score of the soils under citrus and tea plant in the moisture subtropic condition of the Lankaran Lowland after the correction coefficients application was 81 scores. A map at a scle of 1:100000 in agroecological evaluation of Lankaran soils was composed on the basis of our consequences.

Table. *Open agroecological value scale of the soils under citrus and tea plant in the Lankaran Lowland*

Name of soils	Agroecological scores of the soils	Area, hectares	Comparative valuable coefficient
Weak psevdopodzolic yellow-forest	96	534,3	1,09
Average psevdopodzolic yellow-forest	77	2728,9	0,88
Weak psevdopodzolic- yellow	95	14466,0	1,09
Average psevdopodzolic yellow	75	6936,8	0,88
Strong psevdopodzolic yellow	58	1315,1	0,65
Psevdopodzolic yellow-gleyey	93	2093,0	1,07
Yellow-gleyey	91	485,4	1,03
Dark-yellow-meadow	10	1217,7	1,14
Ordinary yellow-meadow	66	1415,6	0,75
Bright yellow-meadow	56	1022,0	0,66
Average calculation score on moisture subtropics	81	32214,8	1,00

Conclusion

The following approach was used during an agro ecological assessment of the soils under citrus and tea plants in the Lankaran humid subtropics condition: at the first stage – the environment (rainfalls, Md, ΣT>10`C, BİP) and soil (humus, pH, >0,25mm, bulky weight) factors are used, basic agro ecological scores on soils facial groups are used and a main agro ecological scale is built, at the second stage- agro ecological scores of the soils in soil forming facial groups are found by the correction coefficients application and an open) agro ecological scale is built.

The agro ecological scores of the soils under citrus and tea plants after the correction coefficients got as the following values: weak psevdopodzolic yellow-forest – 96 scores; mean psevdopodzolic yellow-forest – 77 scores; weak psevdopodzolic yellow-forest – 95; average psevdopodzolic yellow- 75 scores; strong psevdopodzolic yellow - 58 scores; psevdopodzolic yellow - gleyey – 93 scores; yellow - gleyey – 91 scores; dark yellow – meadow – 100 scores; ordinary yellow meadow – 66 scores; bright yellow - meadow – 56 scores.

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A new generation of biocompatible nanoparticles made of resorbable poly(ester amide)s

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A B S T R A C T

A new generation of resorbable nanoparticles (NPs) were prepared on the basis of amino acid based biodegradable (AABB) poly(ester amide)s (PEAs) for drug delivery application. The NPs were fabricated by cost-effective polymer deposition/solvent displacement (nanoprecipitation) method on the basis of three different AABB PEAs recently developed by our group: (i) PEA composed of amino acid leucine as a basic component, (ii) cationic PEA composed of amino acid arginine for imparting positive charge, and (iii) functional PEA composed of amino acid leucine and lateral poly(ethylene glycol) groups acting as surfactant as well as PEGylating agent. The mean particle diameter (MPD), polydispersity index (PDI) and zeta-potential (ZP) were determined by Dynamic Light Scattering (DLS). Moreover, the stability (resuspendability) of the NPs over the time at low temperature was investigated. The NPs were studied for in vitro cell compatibility using four different stable cell lines: A549 (human), U937 (human), RAW264.7 (murine), Hepa 1-6 (murine). Prepared nanoparticles exhibit high stability and cell compatibility and have potential for the application as drug delivery devices.

Keywords: Biodegradable polymers; Nanoprecipitation; Nanoparticles; Biodegradable surfactant; PEGylation; in vitro cell compatibility

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Introduction

There is increasing interest to develop new nanoscale drug delivery vehicles for targeted therapy [1-3]. Targeted drug delivery compared to conventional one, has potential to increase delivery efficacy and reduce side effects. Nanoparticles (NPs) used as nanocontainers should meet following requirements to be suitable for drug delivery: high particle stability, tunable carrier capacity, feasibility of encapsulation of both hydrophilic and hydrophobic drugs, feasibility of variable routes of administration, including oral application and inhalation, and ability to allow controlled drug release from the matrix. Moreover, it is highly desirable to prepare nanoparticles without surfactants, because the surfactant residue is difficult to remove and can cause toxicity, affect drug delivery and cellular uptake efficiency [4-6].

Among various types of drug nanocarriers, biodegradable polymer based nanoparticles emerged as one of the most promising class, as they have ability safely to be cleared from the body after the fulfillment of their purpose [7]. Number of synthetic biodegradable polyesters of low immunogenicity such as poly(caprolactone), poly(lactic acid) and poly(lactic-co-glycolic acid) were reported as suitable candidates for design of nanocarriers [8].

However, degradation products of these polymers are glycolic and lactic acids with pKa 3.83 and 3.86, accordingly, that are considered to be toxic and induce undesired phenotype modulation in cells [9]. Besides, polyesters showed lower affinity to living tissues (due to the lack of hydrophilic CO–NH bonds in the backbone) [10] that can decrease the bioavailability of the NPs prepared from this type of polymers. Therefore, biodegradable poly(ester amide)s (PEAs) are considered as better candidates for biomedical applications, as they contain in the backbone CO–NH links along with ester bonds leading to an increased polymer-tissue affinity. The most promising are the PEAs composed of physiological building blocks − naturally occurring α-amino acids and other non-toxic building blocks such as fatty diols and dicarboxylic acids - amino-acid-based biodegradable (AABB) polymers [9,11–19]. The AABB polymers showed better biocompatibility compared to polyesters [9,15–17]. Besides, after the biodegradation of the AABB polymers very low or no local acidic environment causing inflammation is formed [20]. It has to be emphasized that the AABB PEAs were successfully used for constructing nanobiocomposites [21,22], drug eluting vascular stent coatings [19,23–25], microfibrils [26] and microspheres [27].

One of the main factors limiting the application of NPs is the problem of so-called protein "corona", that is conjugated with the immune system of the organism. When nanosystems are in a physiological environment, they rapidly adsorb biomolecules such as proteins and lipids on their surface forming a protein corona [28,29]. Therefore, in addition to size, shape, and other nanoscale parameters of the nanomaterial, the long-lived (hard) corona has an important impact on the behavior of NPs in biological media. The formation of protein corona changes the size, surface chemistry, solubility, aggregation, and surface charge of the NPs and hence can influence their biodistribution, cellular uptake, and capture by macrophages. In other words, the therapeutic potentialities of polymeric NPs may be compromised by particle recognition by the macrophages [28,29]. It has been established that the NPs functionalized with hydrophilic polymers (NPs having so called stealth coatings [28]) show more long-lasting circulation and decreased macrophage recognition of many types of nanoparticles. One of the efficient ways to render the NPs surface hydrophilic is their PEGylation which represent the process of pretreatment of polyethylene glycol (PEG) on the surface of NPs. PEG decreases the affinity of plasma proteins (opsonins) for adsorption on NPs - long chains of PEG form a random cloud around the NPs, thereby preventing absorption of opsonins and in that way suppressing phagocytosis. Along with the protection of the NPs from phagocytosis the PEGylation substantially increases the bioavailability of NPs [28−33].

Positive surface charge (positive zeta-potential) is favourable for penetration of biological barriers, including ophthalmic ones, 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 [30,31].

Recently we reported on a systematic study of the preparation of resorbable NPs by cost-effective nanoprecipitation method using AABB PEAs [34]. The present work deals with the preparation and study of the new generation of modified NPs having the PEGylated and positively charged surfaces. The study also includes the cell compatibility assessment of the new NPs with four established cell lines.

1. Materials and Methods

1.1. Materials

Surfactants − Tween 20 Sorbitanmonolaurate (MW 1,228), Tween 40 Sorbitanmonopalmitate (MW 1,277), Tween 80 Sorbitanmonooleate (MW 1,310), Kolliphor P188 PPO-PEO-PPO triblock copolymer (MW, 7,680-9,510), Brij 010 Polyoxyethylene(10) oleyl ether (MW 709), Poly(vinyl alcohol)s (PVAs) such as Mowiol 4-88 (MW 31,000 of 86.7%–88.7% hydrolyzed) and Mowiol 8-88 (MW 67,000 of 86.7–88.7% hydrolyzed), purchased from Sigma-Aldrich (St. Louis, MO, USA), and Ttriton X100 Poly(ethylene glycol)p-(1,1,3,3-tetramethyl-butyl)-phenyl ether (MW 647) purchased from Ferak Berlin GmbH (Berlin, Germany), were used as received. Methoxy-PEG-amine with average molecular weight 2,000 Da (mPEG-amine-2000) was purchased from Laysan Bio. Organic solvents − N,N-Dimethylformamide (DMF) and N,N-dimethylacetamide (DMA) were purchased from Sigma-Aldrich, and Dimethylsulfoxide (DMSO) from Carl Roth (Karlsruhe, Germany). All the solvents were used as received. The dialysis bag

(MWCO 25 kDa) was purchased from Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA. The AABB PEAs, selected for the proposed study (Fig. 1), were originally synthesized as reported previously – the leucine (L) based PEA 8L6 via the Interfacial Polycondensation (IP) [15,20], and the arginine (R) based biodegradable cationic PEA 8R6 - via Solution Active Polycondensation (SAP) [35].

Na2EDTA (Ethylenediaminetetraacetate disodium salt) solution (Santa Cruz Biotechnology, Dallas, USA) has been used. One of the used cell lines - U-937, grows in suspension. This particular cell line was maintained in RPMI1640 medium (Santa Cruz Biotechnology, Dallas, USA) supplemented with 10% FBS.

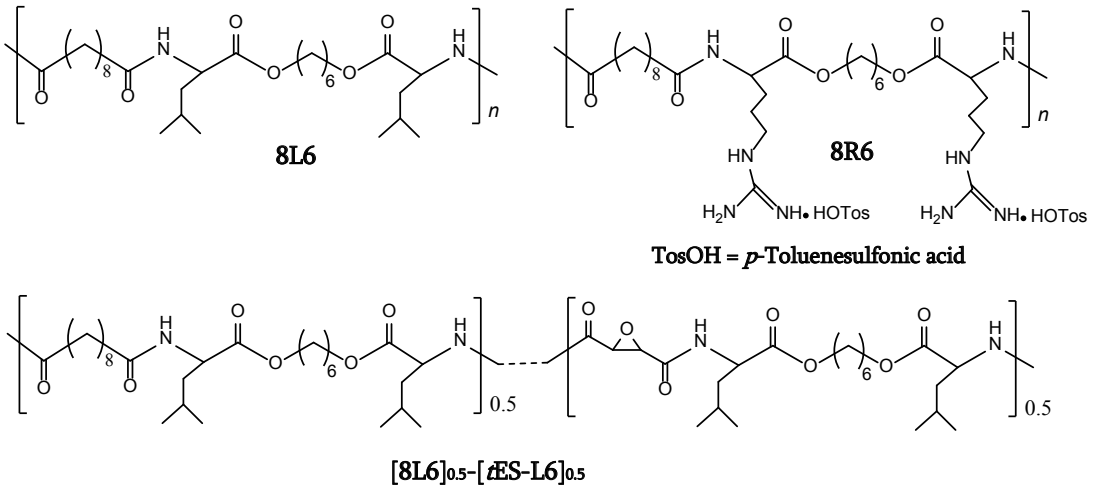

Fig. 1.*The chemical structures of the PEAs used for NPs fabrication: PEA 8L6, cationic PEA 8R6, and epoxy-co-PEA [8L6]$_{0.5}$-[tES-L6]$_{0.5}$ - a precursor of the new surfactant/ PEGylating agent PEG-co-PEA.*

The new surfactant/PEGylating agent composed of amino acid L and containing lateral PEG chains, PEG-co-PEA, was synthesized by interaction of epoxy-co-PEA [8L6]0.5-[tES-L6]0.5 with mPEG-amine-2000; the epoxy-precursor [8L6]0.5-[tES-L6]0.5 was synthesized via SAP as reported previously [36].

Four different established cell lines (two - human, two - murine) were used: A549 − human alveolar epithelial type II cells derived from lung carcinoma (ATCC® CCL-185TM), U-937 – human monocytic cell line from histiocytic lymphoma (U-937 ATCC CRL-1593.2TM , city, US state, USA), Hepa1-6 − mouse hepatoma-derived cells (ATCC®CRL-1830TM), and RAW264.7 − mouse leukemic monocyte macrophage cell line (ATCC®TIB-71TM). Three cell lines (A549, Hepa 1-6, and RAW264.7) are adherent and have been maintained in complete Dulbecco's Modified Eagle's Medium(DMEM) Santa Cruz Biotechnology, Dallas, USA) culture medium supplemented with 10% fetal bovine serum(FBS) Santa Cruz Biotechnology, Dallas, USA). For the harvesting trypsin (0.25%)/

1.2. Characterization of polymers

The number-average (Mn), and weight-average (Mw) molecular weights (MW), and molecular weight distribution (MWD) of the polymers were determined using the GPC. The MWs of the polymers 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, λ = 240 nm). A solution of LiBr (0.1 M) in DMF was used as an eluent. Injected volume was 100 μL, 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 μL, the sample concentration 2.0 mg/mL, and the flow rate 0.5 mL/min. The columns were calibrated with PMMA standards.

The [8L6]0.5-[tES-L6]0.5 and PEG-co-PEA co-polymers were also characterized by FT-IR and 1H and 13C NMR spectroscopy. Thermo Nicolet Avatar 370 FT-IR spectrophotometer (coupled with EZ OMNIC software) was used for IR analysis. To obtain the polymers spectra the thin films were cast from dichloromethane solution on KBr plates, solvent was evaporated at r.t., and films were dried in a vacuum at 40° C for 24 h. The 1H and 13C NMR spectra were recorded using JEOL ECA-400 MHz NMR spectrometer at r.t. in DMSO-d6 as a solvent and internal standard.

1.3. Preparation of PEG-co-PEA

For PEGylating NPs we have originally developed a new biodegradable PEGylating agent which at the same time represents a surfactant. The new surfactant/PEGylating agent - PEG-co-PEA was synthesized via polymeranalogues modification reaction: 200 mg (0.42 mmol) of $[8L6]_{0,5}$-$[t$ES-L6$]_{0,5}$ and 840 mg (0.42 mmol) of mPEG-amine-2000 was dissolved in 2 mL DMA and stirred for 24 h at 60°C. After completing the reaction, the resulting solution was poured in 50 mL hexane and the precipitated product was separated and dried under reduced pressure at 60°C for 48 h.

The structures of initial co-polymer $[8L6]_{0,5}$-$[t$ES-L6$]_{0,5}$ and the obtained PEG-co-PEA were confirmed by FTIR and NMR spectroscopy.

$[8L6]_{0.5}$-[tES-L6]$_{0.5}$. ^{1}H NMR (400 MHz, DMSO-d6, δ): 0.9 (24H, s, CH(C<u>H</u>$_3$)$_2$), 1.1-1.84 (40H, O-CH$_2$-(C<u>H</u>$_2$)$_n$-, C<u>H</u>(CH$_3$)$_2$ and –CH-C<u>H</u>$_2$-CH-), 2.05 (4H, t, -CO-C<u>H</u>$_2$), 3.60 (2H, s, C<u>H</u>-O epoxy), 4.04 (8H, -O-C<u>H</u>$_2$-), 4.27 (4H, -NH-C<u>H</u>-CO), 8.1 (2H, d, CH$_2$-CO-N<u>H</u>-CH-), 8.82 (2H, d, O-CO-N<u>H</u>-CH- and O-CH-CO-N<u>H</u>-CH-).

^{13}C NMR (100 MHz, DMSO-d6, δ): 23.0, 24.6, 25.2, 28.3, 29.03, 46.1, 50.8, 52.7, 64.7, 166.1, 172.0, 172.5, 172.8.

PEG-co-PEA. ^{1}H NMR (400 MHz, DMSO-d6, δ): 0.88 (24H, s, CH(C<u>H</u>$_3$)$_2$), 1.12-1.84 (40H, O-CH$_2$-(C<u>H</u>$_2$)$_n$-, C<u>H</u>(CH$_3$)$_2$ and –CH-C<u>H</u>$_2$-CH-), 2.09 (4H, t, -CO-C<u>H</u>$_2$), 3.51 (PEG -O-CH$_2$-CH$_2$-O-), 3.60 (1H, C(OH)-C<u>H</u>), 4.04 (8H, -O-C<u>H</u>$_2$-), 4.24 (4H, -NH-C<u>H</u>-CO), 4.62 (1H, C(OH)-C<u>H</u>), 7.90-8.35 (5H, N<u>H</u>). Together with ^{1}H NMR spectroscopy, successful modification of epoxy group with PEG-amine was proved by complete disappearance of the epoxide band at 895 and 3062 cm^{-1} combined with appearance of the broad band at ≈ 3500 cm^{-1} characteristic for OH group (signal is partially overlapped with absorption band of amide at 3302 cm^{-1}).

2.4. Preparation of the PEGylated NPs

The PEA NPs were prepared according to the polymer deposition/solvent displacement (nanoprecipitation) method under the optimal conditions previously established for amino acid based biodegradable ester polymers [34]: 6.0 mg of PEA 8L6 was dissolved in 1.0 mL of DMSO (organic phase) and dropwise added (dropping rate 12 drops/min) to 10.0 mL of water (inorganic phase) containing 50.0 mg of the initially synthesized biodegradable surfactant PEG-co-PEA (organic/water phases (O/W) ratio 1:10 v/v) at a stirring rate of 700 rpm using a magnetic stirrer. All manipulations were done at room temperature.

PEGylated NPs were also fabricated using the modified nanoprecipitation method as reported previously [34]: the half of the surfactant PEG-co-PEA (25.0 mg) was dissolved in 1.0 mL of DMSO together with 6.0 mg 8L6, or the 70/30 mixture of 8L6/8R6 (organic phase) and dropwise added to 10.0 mL of water phase containing the other half of the surfactant PEG-co-PEA (25.0 mg), i.e. in this method the surfactant is equally distributed in both 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 1 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 at 4-5°C.

2.5. NPs size, size distribution and zeta-potential

The obtained PEGylated NPs were characterized by size (Mean Particle Diameter - MPD), 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 MPD and PDI are presented as an average of five measurements ± standard deviation (SD). The PDI < 0.04 corresponds to a narrow distribution, 0.04 ≤ PDI ≤ 0.16 − to a mean distribution, and PDI > 0.16 − to a wide distribution.

2.6. Cytotoxicity (MTT) assay

For the cytotoxicity testing of A549, U-937, Hepa1-6, and RAW264.7, cells were cultured at a density 0.5 × 106 cell/mL in 96-well cell microplates. After 24–28 h of growth, after 80% confluence has been reached, the cell culture medium was changed to serum-free and nanoparticles were added at a concentration of 5.0 μg/mL. After 24 h, the cytotoxicity of NPs was assessed by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay [37] as described by us previously [28]. The absorbance at a wavelength of 570 nm was read on the ELx800 Absorbance Reader (Biotek). In control samples cells were cultured with medium only. Cell viability was calculated using the equation:

where [OD]test, [OD]control, and [OD]blank represented the absorbance values of the wells with cells and NPs, cells without NPs, and without NPs and cells, respectively. For each experiment the absorbance was the average value measured from 12 wells in parallel. Five independent experiments have been performed in case of each cell line used.

$$\text{Cell viability (\%)} = \frac{([OD]\text{test-}[OD]\text{blank})}{([OD]\text{control-}[OD]\text{blank})} \times 100\% \qquad (1)$$

2. Results and discussion

2.1. Selection of the PEAs

The PEA 8L6 composed of L-leucine (L), 1,6-hexanediol (6) and sebacic acid (8) was selected as a basic polymer for preparing the PEGylated NPs. We have found this PEA as an optimal for fabricating resorbable NPs considering such parametes as stability upon storage and cell compatibility [34]. For imparting a positive charge to the NPs enhancing both their stability and cellular uptake [30,31], arginine-based cationic PEA 8R6 was used. Among designed arginine-based PEAs [35] 8R6 showed a high hydrophobicity - it dissolves in water only upon heating to 60–70 °C and precipitates when cooled to r.t. We have assumed it will be retained by the NPs, i.e. will not easily be washed out from the NPs in the water phase. The third polymer we have selected in the present work was the functional epoxy-co-PEA [8L6]0.5-[tES-L6]0.5 containing reactive oxirane rings. It was demonstrated [36] that the oxirane rings are suitable active sites for covalent binding to primary amines under mild conditions. This approach was used for preparing the new amphiphilic polymer by reacting [8L6]0.5-[tES-L6]0.5 with mPEG-amine-2000. The new amphiphilic copolymer labeled as PEG-co-PEA combines the properties of both surfactant and PEGylating agent: the copolymer contains backbone 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-co-PEA with NPs made of the 8L6 or 8L6/8R6. The structures of the selected PEAs are depicted in Fig. 1, their MWs are given in Table 1.

Table 1. *MW characteristics of the PEAs*

Polymer	M_w	M_n	M_w/M_n
8L6	76,100	44,200	1.72
8R6	17,500	7,200	2.43
$[8L6]_{0.5}$-$[t$ES-L6$]_{0.5}$	27,200	14,700	1.85
PEG-co-PEA	36,800	28,400	2.58

3.2. Fabrication of the PEGylated NPs

As it was mentioned above, for PEGylation of NPs we have initially prepared the new biodegradable amphiphilic polymer PEG-co-PEA, which at the same time serves as a surfactant when preparing the NPs. In contrast to its precursor - epoxy-co-PEA [8L6]0.5-[tES-L6]0.5 the new amphiphilic polymer is soluble in water and similar to the known amphiphilic compounds (surfactants) forms micelles (Table 2). The mean particle diameter (MPD) of the PEG-co-PEA micelles is 16.2 nm that is close to MPD of the micelles formed by Brij 010 and Mowiols. As regards the zeta-potential (ZP), there is no significant difference between the PEG-co-PEA micelles (ZP=-13.1 mV) and micelles of known surfactants (Table 2).

A high affinity of the backbones of the PEG-co-PEA, 8L6 and 8R6 provided effective conjugation of PEG-co-PEA with the surface of the NPs and promoted the fabrication of the NPs (Table 3). The results given in Table 3 show that the MPD of the negatively charged 8L6 NPs is less than the MPD of the positively charged 8L6/8R6 NPs. Thus, the MPD of 8L6 NPs is 70.1 nm (in case of nanoprecipitation method, NM) and 97.6 nm (in case of modified nanoprecipitation method, MNM) vs. 125.7 nm and 130.2 nm obtained for 8L6/8R6 NPs.

Table 2. *Characteristics of micelles of standard surfactants and new biodegradable surfactant*

Surfactant	MPD (nm) ± SD	PDI ± SD	Z
Tween 20	11.3 ± 0.3	0.244 ± 0.029	
Tween 40	11.1 ± 0.8	0.231 ± 0.023	
Tween 80	10.8 ± 0.7	0.263 ± 0.018	
Brij010	19.2 ± 0.3	0.173 ± 0.016	
Kolliphor P188	9.2 ± 0.4	0.373 ± 0.031	
Triton X-100	10.4 ± 0.7	0.254 ± 0.021	
Mowiol 4-88	20.0 ± 1.3	0.463 ± 0.039	
Mowiol 8-88	23.4 ± 1.5	0.479 ± 0.041	
PEG-co-PEA	16.2 ±1.0	0.174 ± 0.012	

Table 3. *Characteristics of 8L6 and 8L6/8R6 PEGylated NPs prepared by nanoprecipitation (NM) and modified nanoprecipitation methods (MNM)*

Method	MPD (nm) ± SD	PDI ± SD	ZP (mV) ± SD
8L6 NPs			
NM	70.1 ± 2.3	0.188 ± 0.002	-14.5 ± 1.2
MNM	97.6 ± 2.6	0.112 ± 0.008	-14.7 ± 1.1
8L6/8R6 (70/30) NPs			
NM	125.7 ± 4.3	0.221 ± 0.014	+6.9 ± 1.2
MNM	130.2 ± 3.8	0.143 ± 0.011	+7.5 ± 0.4

As we can see from Table 3, obtained PEGylated NPs showed mean ($0.04 \leq PDI \leq 0.16$) to wide size distribution ($PDI > 0.16$). It has to be noted that in case of MNM for both 8L6 and 8L6/8R6 NPs the size distribution was mean − 0.112 and 0.143 and in case of NM it was wide - 0.188 and 0.221, accordingly. With regard to the surface charge two types of NPs were prepared: (i) negatively charged NPs based on PEA 8L6 and (ii) positively charged NPs based on the mixture 8L6/8R6 (70:30 w/w). The ZPs of the NPs, listed in Table 3, were determined right after the fabrication of the NPs. The results show that the PEGylated 8L6 NPs had moderate negative charge: -14.5 mV in case of NM and -14.7 mV in case of MNM. We suppose that the negative ZP of the NPs is caused by a partial hydrolysis of the ester links of the PEAs generating free carboxyl groups (carboxylate anions –COO−). In case of the NPs prepared from 8L6/8R6 mixture, the ZP values were positive for both applied methods; +6.9 mV (NM) and +7.5 mV (MNM). The positive charge of the 8L6/8R6 NPs is provided by guanidine groups of the cationic PEA - 8R6. In spite of relatively low surface charge value of the 8L6/8R6 NPs, it is sufficient to ensure the stability upon storage (see below).

3.3. Stability of the NPs

A firm anchoring of the PEG-co-PEA with NPs made of the 8L6 or 8L6/8R6 provides good stabilization of the NPs. Both types of the PEGylated NPs prepared by NM and MNM were studied for stability upon storage at low temperature. The NPs' MPD and PDI were measured right after the fabrication and then the NPs suspensions were stored refrigerated at 4–5 °C. After predetermined time (30, 60, and 90 days), the suspensions were thoroughly shaken and analyzed for the MPD and PDI. The results, listed in Table 4, show that the fabricated NPs were highly stable – no substantial change of the MPD and PDI, or aggregation is observed after 90 days of storage.

3.4. Cell compatibility study of the NPs

For cell compatibility studies we have used established cell lines of different origin: two murine cell lines (Hepa1-6 and RAW264.7) and two human cell lines (A549, U-937). In case of both species one cell line was monocytic (RAW264.7, U-937). We have chosen monocyte-macrophage cell lines for the cytotoxicity studies, as these particular cells are characterized by high phagocytic activity. Therefore, presumably, these cells will actively phagocytose added NPs, which will lead to the higher concentration of NPs inside the cells, compared to the cell types which aren't able to perform phagocytosis and will engulf NPs only by the process of endocytosis. Our aim was to investigate how the high concentration of intracellular NPs inside the phagocytes will affect cell viability. In parallel, we have taken a non-phagocytic cell line for each of the species: hepatocytes (Hepa1-6) - in case of mouse and alveolar epithelial type II cells (A549) - in case of human. For the viability assessment standard MTT test has been used, which is based on the ability of a mitochondrial dehydrogenase enzyme in viable cells to cleave the tetrazolium salt leading to coloured reaction [37]. As it can be seen from the Fig. 2, both types of NPs haven't affected cell viability in case of all four cell lines used: no statistically significant change of the cell viability is visible compared to subsequent control cultures. This means that both types of NPs are characterized by high biocompatibility. Considering the fact, that in cultured cell lines NPs might affect other physiological parameters besides viability, in our future experiments we plan to evaluate NPs effect on cells growth and functional characteristics.

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

Type of NPs	Method	Time			
		Freshly prepared	After 30 days	After 60 days	After 90 days
		MPD (nm) ± SD [PDI ± SD]			
8L6 NPs	NM	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]
	MNM	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]
8L6/8R6 NPs	NM	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]
	MNM	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]

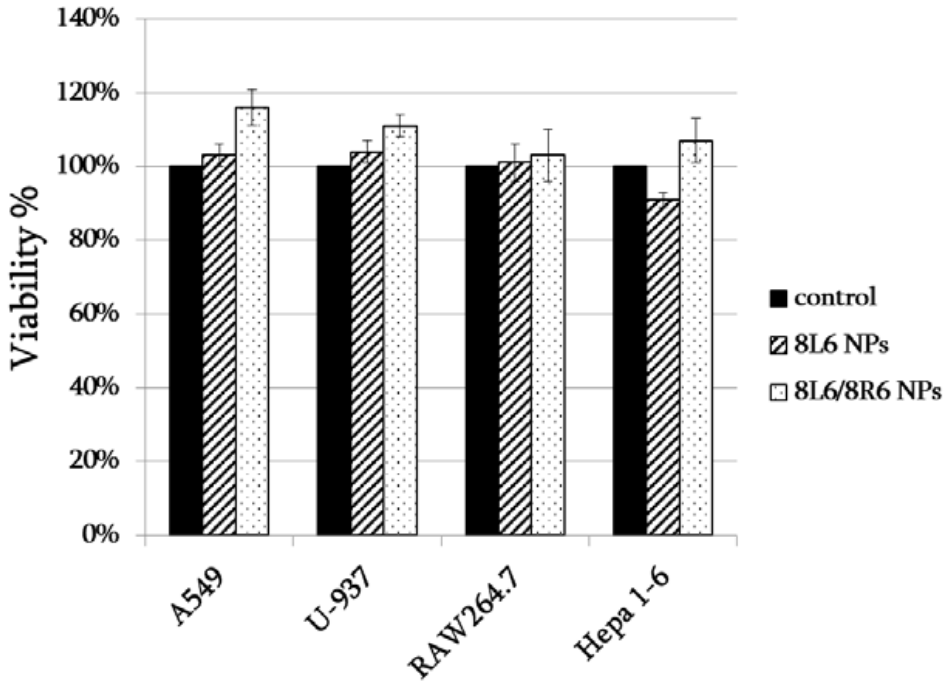

Fig. 2. *Percentage of viable cells after 24 h incubation with 8L6 and 8L6/8R6 NPs. Results for four cell lines shown: A549 − human alveolar epithelial type II cells derived from lung carcinoma, U-937 – human monocytic cell line from histiocytic lymphoma, Hepa1-6 – mouse hepatoma-derived cells, and RAW264.7* − mouse leukemic monocyte macrophage cell line.

3. Conclusion

Two types of PEGylated NPs (negatively and positively charged) on the basis of AABB PEAs were prepared using cost-effective nanoprecipitation and modified nanoprecipitation methods. For preparing NPs new biodegradable surfactant/PEGylating agent was specially designed via polymeranalogues modification reaction. The stability (resuspendability) of the PEGylated NPs upon storage was investigated using DLS method. *In vitro* biocompatibility study of the NPs with four different stable cell lines: A549 (human), U-937 (human), RAW264.7 (murine), Hepa 1-6 (murine) showed that they are biocompatible. Considering the high stability and biocompatibility, prepared NPs are considered as promising candidates for the application as drug delivery nanocarriers.

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Georgian Natural Resources (Brief Review)

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A B S T R A C T

In the paper the main natural resources of Georgia are considered. Among them are: soil (more than 20 types), land (the ways of efficient use of its resources), water (including 26060 rivers with total length of 60000 km; the resources of fresh water of distinctive wealth), forest (98% at the slopes of various incline with protective functions), mineral and energetic resource potential of raw material, variety of the reserves of mineral products, the results of their many years and intensive exploitation, the potential of reproducible resources of energy (hydro, solar, wind, geothermal water, biomass), recreational resources as the basic for development of health resort and touristic industry; protected territories with a large set of state reserve, national parks, wildlife preserves, landmarks and protected landscapes.

Keywords: Soil, Land, Water, Forest, Power resources, Recreation.

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Introduction

At the modern stage of the society development the determination – estimation of the potential of local natural resources is necessary, since, in spite of the wide promotion of newest resource – saving technologies, natural resources and their rational use is a main factor of social – economic development of the country.

Natural resources, existing in the country, are the basis of development of its economics and considerably determine the directions of sectoral and regional economics.

In the nearest years, in the conditions of intensive and uncontrolled use, the natural resources may be really found on the verge of pointed deficit and degradation. Therefore, this problem is in the focus of World society.

At globalization, when the solving of many problems requires the joint efforts of World countries, the realization of regular, transparent state policy is necessary in the problems of the use and protection of natural resources to avoid their non-rational and, in some cases, the predatory exploitation and to provide the conservation – sanitation of living space for population.

Natural resources and the peculiarities of their use determine the necessity of permanent research of mentioned problems since the resources of all kind and the demand for them is in constant dynamics, varies with space and time. On this basis the research of modern state of natural resources and of the prospects of their use is very topical [1].

Main part

Georgia is characterized by very distinctive natural conditions. In the country the all thermal groups of World climate (excluding tropical one), all types of relief (macro-, meso- and microreliefs), three types of organisms (green, plants-arboreal, arboreal-grass, grass, desert and lichen and moss; microorganisms and animals, magmatic, metamorphic and sedimentary rocks) are marked out.

Richness of natural conditions determines the uniqueness of soils and soil covering. "The museum of natural conditions of the soils outdoor – the soil covering" is explained by such manner. There is all types of the soils of the most countries of the world and of Europe in our country. Among them are the soils which are not found in Europe [2].

At present more than 20 types of the soils are marked out: Brown Forest soils (WRB: Humic Cambisols, Ferric Cambisols, Eutric Cambisols, Dystric Cambisols), Mountain Forest Meadow soils (WRB: Haplic Umbrisols), Mountain Meadow Soils (WRB: Hyperdistric Umbrisols), Cinnamonic soils (WRB Chromic, Calcaric, Humic, Eutric Cambisols, and Leptic, Haplic, Calcic Kastanozems); Meadow-cinnamonic soils (WRB: Chromic, Calcaric, Gleyic, Eutric Cambisols, and Gleyic, Vertic, Haplic Kastanozems), Yellow brown forest soils (WRB: Stagnic, Ferric, Luvisols, Skeletic Luvisols, and Dystric, Gleyic, Luvic Stagnosols), Terra Rossa (WRB: Rendzic Leptosols (Brunic), Mountain-meadow humus-illuvial soils (WRB: Humic Cryosols (Sombric), Brown forest black soils (WRB: Dystric Stagnosols) Andosols (WRB: Vitric Andosols), Red soils (WRB: Ferralic Nitisols, Haplic Nitisols), Yellow soils (WRB: Ferric Luvisols), Bog soils (WRB: Dystric Gleysols, Eutric Gleysols, Histosols), .Yellow Podzolic soils (WRB: Stagnic Acrisols, Ferric Acrisols), Raw Carbonate soils (WRB: Rendzic Leptosols), Grey Cinnamonic soils (WRB: Calcic Kastanozems, Vertic Kastanozems), Meadow Grey Cinnamonic soils (WRB: Haplic Kastanozems, Gleyic Kastanozems, Vertic Kastanozems), Black soils (WRB: Haplic Vertisols), Chernozems (WRB: Voronic Chernozems, Calcaric Chernozems), Mountain Meadow Chernozems (WRB: Phaeozems), Saline soils (WRB: Vetric Solonchaks, Mollic Solonetz), Alluvial soils (WRB: Gleyic Fluvols, Eutric Fluvisols, Dystric Fluvisols) [2]

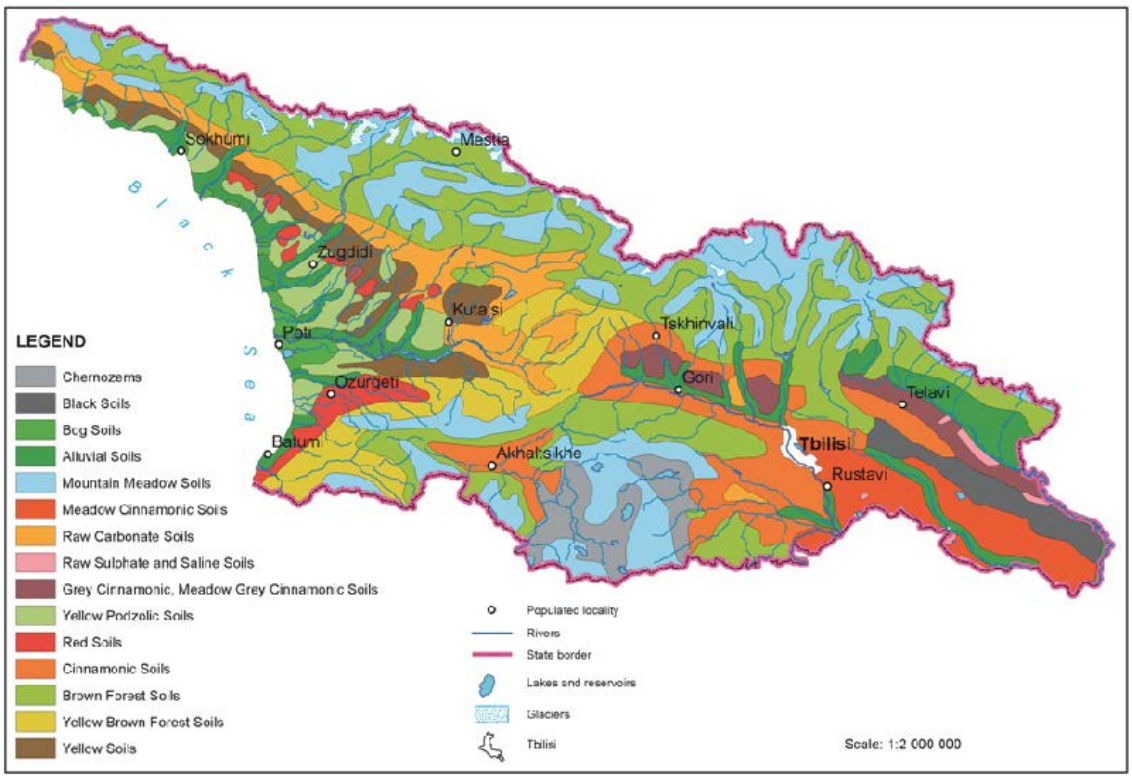

Fig.1. *Soil map of Georgia*

Land resources are very important for country development [3-6]. The use of the land is nonuniform and the land is more intensively used in plain and submountain zones, the soils up to 1000 m above sea level. They comprise 46.1% of country territory. The distribution of non-agricultural lands is very interesting by the property form.

The distribution of non-agricultural lands is the following: settlements – 88.4 thousand hectares, protected territories – 300.7 thousand hectares, forest resources – 2456.2 thousand hectares, industrial, transport, energetics and of other function – 171.9 thousand hectares, religious organisations 4.9 thousand hectares, water resources (including the flow of territorial waters) – 835.1 thousand hectares.

In the modern conditions of agriculture development the rational use of land resources attaches much importance. It must be based on objective estimation of land quality, on well considered, proper organization of the territory, on economic motivation of land protection, on continuous enhancement of the level of agricultural education and on the use of modern progressive methods.

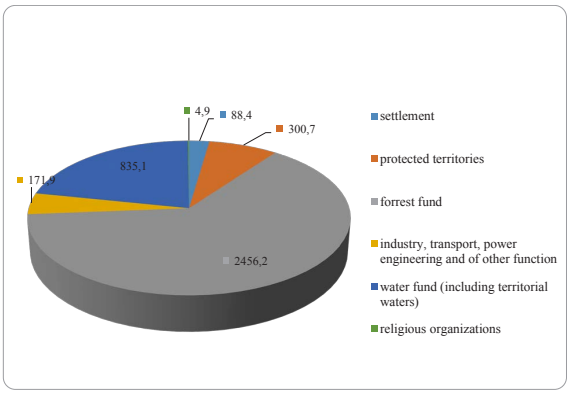

Fig.2. *Distribution of non-agricultural lands in accordance with property form (in thousand hectares)*

Soil protection is a daunting, global problem of modernity which must be directed to qualitative improvement and rational use of the land for conservation and increase of its fertility.

From the 90's of the last century the unstable social-economic events were revealed most sharply in agriculture. By this reason, conduction of the required measures for soil protection and fertility increase were considerably retarded.

Over last 20-25 years the soil degradation was intensively occurred. 30.5% (205.7 thousand hectares) of total area of the cropland is eroded by various degrees. 14.2% of total area of the cropland is highly and, in the mean, eroded. Moreover, over last years the natural calamities (landslide, torrent, flood, etc.) gained the scale effect, which impair considerable losses to agricultural lands. Humus horizon of the soil decreases, fertility and haying-pasture areas reduce, etc.

As a result of the difficulties in the country, caused by unprepared privatization of agricultural lands, careless relation to agrarian sector, hurried disintegration of old economic organization forms was favoured for deepening of crisis state in agriculture. The sphere wasn't provided by agricultural techniques, qualitative fertilizers, pesticides and chemicals. Seed and transplant farms, systems of testing and renewing of the species will cease to operate. Main indices of agrarian sector were sharply impaired. The areas of agricultural plants – arable-seeding, long-standing transplants (orchard, vineyard) were significantly reduced.

Crop production was sharply decreased. Provision of the country by the food was impaired. Existing state made deeper further the unemployment and aggravated the problem of the poverty. This was favoured for activation of the processes of mass migration, especially, from the mountain regions.

There are many unsolved and insufficiently studied problems in the country as yet, in particular, land cadastre and appraisement, soil-climate zoning, study of soil covering by aerospace methods, soil technological research, etc.

Elaboration of large-scale measures against land erosion, landslide and torrent, for recultivation, melioration, exsiccation, lime treatment and their execution by accelerated rate is necessary.

For efficient use of land resources, the agrochemical research of the soil is reasonable. It must be based on the principles and methods of

large-scale mapping of the soils. For this purpose the formation of a scientific center for complex research of land (soil) holdings is necessary which will totally provide scientific service in this direction.

The data bank of land resources must be organized and the control on its use must be established. Qualitative accounting of the land isn't ordered in the country. Determination of land quantitative and qualitative indices, its economic estimation isn't carried out. Land code and land cadastre must be elaborated which will be a basis for regulation of holding relations in modern conditions, for developing of land market. For rational use of land holdings and for organizational-legal regulation, in accordance with their state interests, the creation of integrated state system of land management is reasonable in the country.

After the land privatization the problem of land consolidation became acute. The solving of mentioned problem depends on a number of the factors. Therefore, it requires the system approach. Together with the further developing of the cooperation, the use of other, alternative forms is reasonable. For example, the state land fund(corporation) may be organized which will have the appropriate financial resources for land area purchase, for its ruggedness and hereafter, according to economic reasonability, to alienate it or to hand it on the basis of lease contracts.

The land must not be sold on foreign citizens and legal persons. The land must be transferred to them only on the basis of lease relations.

There are all possibilities for organization of highly developed agricultural production in Georgia, so that the country may become self-sufficient, primarily by ecologically pure food production in the nearest years which will exclude the problem of food safety in the country.

At the use of land resources the role of territorial administration and self-government is of importance. They must assist in improvement-stimulation of business sphere, in creation-developing of processing entities on the basis of local raw material and in stable increase of agricultural production, in creation of working places, in increase of standard of life in the villages and in self-employers welfare.

Development of agriculture and associated spheres, requires a complex approach. For this purpose the elaboration of the State goal program of sustain and safe development of the sector is reasonable, which determines, by stages, development tasks, financial sources and mechanisms for their realization [3-6].

Georgia is characterized by richness of the resources of fresh water [7-15]. Annually the flow comprises 56.5 km3, and including transit flow – 65.8 km3. 75.5% or 49.7 km3 of the latter flows in the Black Sea and 16.1 km3 in the Caspian Sea by passing of neighbour states.

Water resources are presented by river flow, lakes, glaciers, marshes, water reservoirs and underground waters. Water resource in Georgia attains 100 km3 totally (Fig.3)

Thickness of the layer of fresh water, formed locally, comprises 810 mm. By this value Georgia occupies a major place among former soviet republics.

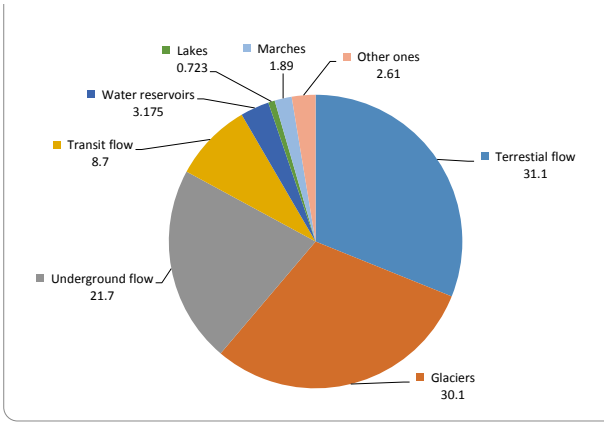

Fig.3. *Percent distribution of water resources*

In the country the following is accounted: 26060 rivers, the total length of which is 60 thousand km; Mean compactness of river set is 0.85 km/km2, in West Georgia – 1.07 and in East Georgia – 0.68 km/km2. Hydrographic set consists of mainly (99.4%) little (<25 km) and very little (<10 km) rivers. From adjacent countries, in average, 9.3 km3of water is entered. Among them are: river Mtkvari – 0.9125 km3, river Potskhovi – 0.252

km3, river Debeda – 0.883 km3, river Chorokhi – 7.25 km3. The river head of the major river of Georgia – Mtkvari is located in Turkey and in the territory of our country flows its middle part (351 km). The most part of the rivers of East Georgia compose an integrated system of Mtkvari basin. Among the rivers of West Georgia river Rioni is the most big and full – flowing (length – 333 km, flow - 12.7 km3), the river head of which is located at south slope of Caucasian mountain ridge and it flows in the Black Sea.

The biggest rivers of Georgia are the following ones (by length and by the area of water collection basin): Alazani (390 km, 11.8 thousand km2), Mtkvari (351 km, 21.1 thousand km2), Enguri (231 km, 4.06 thousand km2), Ktsia-Khrami (201 km, 8.34 thousand km2), etc. The rivers are characterized by mixed feeding: rains, snow, molten glaciers and underground waters.

The most part of Georgian rivers are characterized by high volume of solid flow. Big rivers inject a considerable amount of the floated alluvion in the Black Sea: from 2 to 11 mln tons.

There are 856 lakes in Georgia; the total area of their water surface comprises 170 km2 and water reserve – 0.72 km3. More than one third of the lakes are located in volcanic upland of South Georgia. The biggest lake is Tabatskuri which contains 221 mln m3 of water. Lake Paravani has highest water surface – 37.5 km2. The area of water surface of more than half of the lakes is less than 0.1 km2. In the five big lakes (Ritsa, Paravani, Paliastomi, Sagamo, Tabatskuri), the total area of water surface of which comprises 72.6 km2 , water amount is 535 mln m3. 97.3% of the total volume of Georgian lakes is fresh water and it may be used for economic purposes.

Glaciers occupy nearly 0.7% of Georgian territory. The total number of them comprises 786 with total area 555.9 km2 in which nearly 30130 mln m3 of water mass is accumulated. But there are other data about the amount of glaciers, which is associated with global climate warming. The biggest glaciers are: Lekhziri, Chalaati, Tsaneri, Adishi, Khalde, Boko, Gergeti, Devdoraki. Glaciers are mainly located in the basins of Enguri, Rioni, Kodori and Tergi, 85.2% of total amount and 94.9 % of the surface of glaciers falls on mentioned ones. World global warming caused the decrease of glacier area on Caucasian mountain ridge. Over 1890-1990, the surface of glaciation reduced meanly by 29%.

Only 4% of river flow is regulated in Georgia. Hence there are considerable reserves for increase of sustain flow. At present 44 water basins are in exploitation, total volume of which comprises 3.32 km3 and annual renewable useful volume – 2.27 km3. Total area of their surface comprises 163 km2 which consists of 0.23% of Georgian territory. Water basins of West Georgia are of energetic function. Their total useful volume attains 0.85 km3. Most part of water basins in East Georgia is used for irrigation. Jvari water basin on river Enguri is the biggest and deepest one in South Caucasua. Its volume comprises 1093 mln m3. 37 water basins are located in East Georgia. Total volume of each of them exceeds 1 mln m3. Their total volume comprises 1.45 km3. Water basins are mainly located in Mtkvari basin were 34 water basins are located with total volume of 1.002 km3. 2 of them are of energetic function and one – Zhinvali is of complex purpose. 5 water basins are located in the basin of river Iori. Total volume of three ones – Tbilisi, Sioni and mountain Dali comprises 773 mln m3. To fill the seasonal deficit in the basin of river Aragvi, Zhinvali complex hydrosystem and Narekvavi water reservoir are constructed, the total volume of which comprises 526.8 mln m3.

Marches and highly humid lands (area – 225 thousand hectares) are mainly located on Kolkheti plain, in coastal zone. Their water reserve is 1.9 km3. Kolkheti marches are important hydro-ecosystems which participate in the formation of climatic, hydrological and hydrogeological processes. In 1997, Georgia joined up to Ramsari International Convention which provides the protection of highly humid lands of important marches of the World. Pichora – Paliastomi (1328 mln m3), Chaladidi-Poti (194 mln m3) and Eris Tskali (93.6 mln m3) marshy massifs are characterized by large volume of water reserve.

The part of their water reserve is evaporated, another part flows in the Black Sea by infiltration in water set.

Natural resource of underground fresh waters comprises 18 km3 or 572 m3/sec in Georgia. 63.4% of their total amount falls on West Georgia, 24.1% - on East Georgia and 12.5% - on South Georgia. Approved amount of underground fresh drinking water comprises 145.5 m3/sec. 48% of them falls on West Georgia, 45% - on East Georgia and 7% - on South Georgia. There are many powerful appearances of fresh underground waters in the form of the springs which are characterized by high output, by hydrocarbonate calcium-magnium composition and by low mineralization which determines the possibility of industrial bottling and export of drinking water on their basis.

From numerous springs the output of 135 ones exceeds 5 l/sec. Among powerful springs the group in South Georgia must be marked. Their output comprises 1100-3500 l/sec. Big springs of karst waters are located in Abkhazian Autonomous Republic and their output comprises 1500-26000 l/sec. Output of the springs on Kolkheti plain consists of 300-3500 l/sec. Georgian volcanic upland is characterized by numerous and powerful springs. Among them Samkhari spring in the basin of river Paravani, Ablari spring in Khrami basin must be remarked. Their output comprises 20 m3/sec. High output springs (100 l/sec) are located in upper parts of the rivers – Khvabliani, Uraveli, Injasu. The springs Zilbukhari and Paska-Pufi in the basin of river Khvabliani are among them. Bulachauri, Choporti, Natakhtari and Saguramo springs are located in Aragvi basin. They provide the water supply of Tbilisi. Numerous springs are located in left side of Alazani. Among them the biggest ones are Apeni (220-250 m3/sec) and Patmasuri (188 m3/sec) springs. There are more than 2000 boreholes by highly output in the country for population water supply. Most part of them is located in East Georgia, mainly on Alazani plain.

By data of water balance on country territory 96.9 km3 of precipitations fall on, among them 65.3 km3(2009 mm) in West Georgia and 31.6 km3(859 mm) in East Georgia. 40 km3 (580 mm) is evaporated, among them 22.8 km3 (701 mm) in West Georgia and 17.2 km3 (406 mm) in East Georgia; 56.9 km3 (810 mm) flows, among them 42.5 km3 (1300 mm) in West Georgia and 14.4 km3 (393 mm) in East Georgia. The share of underground flow consists of 30% in total flow: 40% in West Georgia and 27% in East Georgia.

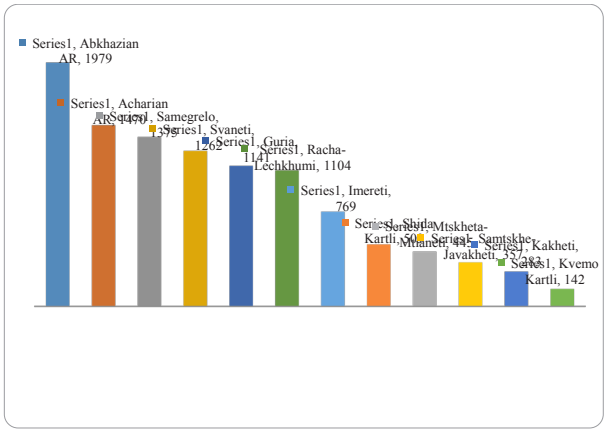

Fig.4. *Distribution of water resources by Georgian regions*

Forest has a very important function among Georgian natural resources. It has a pronounced effect on the environment which is expressed in stabilization of natural processes, Forest regulates surface flow, intensity of snow melting, air temperature and relative humidity, purifies water and air from mechanical and other impurities, reduces wind speed, absorbs carbon dioxide and other harmful gases and liberates oxygen, suppresses or annihilates harmful microorganisms, reduces noise. Forest hinders significantly soil erosion, improves its structure, provides soil high water and air conductivity [16-20].

The forest role is particularly strained in mountain countries such as Georgia where 98% of the forests are located at the slopes of various inclination, among them 78% at steep and highly steep slopes (36o and more). This fact restricts considerably the industrial exploitation of the forest from technical as well as, what is more important, from ecological viewpoint.

The forests serve essential social-protective

functions: soil protection, water saving, water regulation, recreation, etc. Georgian forests protect the population of high-mountain villages from avalanches, mud streams and torrents. Forest richness is mainly expressed by their social-protective, recreative and aesthetic importance instead of pure economic one.

According to the researches of the scientists of various countries, economic profit, obtained from forest ecological functions, exceeds by the factor 20-30 the profit, obtained by wood sale, in direct or indirect forms [21-23].

By the data of 2015, 33.9% of Georgian territory is covered by the forest. In comparison with the indices of 2012 (40.5%), percentage of forest land reduced by 6.6% which is a very anxious fact and is a problem of isolated consideration, since the same was a percentage of forest land in the country after Second World War. And in the conditions of proper management this value increased up to 40%. At present the regress is presented.

Georgian forests are characterized by their biodiversity. Here up to 400 types of trees and bushes raise in wild conditions, among them a number of endemic and relict species. From main forest-creating types the following must be remarked: Beech (occupies 45% of all forests), Oak (10%), Horn beam (9%), Fir (7%), Alder (7%), Spruce (5%), Pine (4%), Chestnut (4%), Birch (3%) [24-26].

By the data of 2016, total area of Georgian State Forest Fund comprises 2.36 mln hectares, 33.9% is covered by the forest. Over last 5 years area of mentioned fund reduced by 460000 hectares.

By relief conditions Georgian forests are divided in mountain and plain ones. Mountain forests occupy 98% of all territory and plain ones - 2%, which are mainly located on Kolkheti plain as well as in near low parts of the rivers Mtkvari, Alazani, Iori, Khrami and other ones.

The most part of Georgian State Forest Fund is degraded and has need for corresponding restorative forest-economic measures. Although, at present, because of low financing, restorative - constructive works are reduced to scanty level. For example, by the data of 2016, the works of forest restoration and cultivation is performed on 142 hectares on total territory of above-mentioned fund, forest seeding and planting - on 21 hectares and works, favourable for natural renewing of the forest - on 121 hectares [27, 28].

At present in the sphere of forest exploitation a number of the problems are accumulated which have need for timely solving, on the basis of the principles of International forest exploitation; in particular: forest exploitation must be based on standard principles of sustain development. The annual amount of required wood must be reasonably estimated, so that the ecological state mustn't be impaired by single economic profit; forest certification must be carried out since certified production is more costly in several times at international market; the entities of wood-working industry, for exportation of only assortmented bucked wood production, must be restored; total account and processing of forest nonarboreal products (wild fruits and berries, medicinal plants, walnuts, etc.) is necessary which will give economic profit to the country as well as will partially fill the basket of goods for local population; accounting of wood secondary resources (remained after tree felling) and their use in various fields of the economic (building, furniture production, combined feed production in the form of biomass, etc.). Timely solving of the problems will be favourable for increase of the level of forest bioresources rational and complex use [29, 30].

Industrial development is considerably determined by existing resource potential of mineral raw material and its valuable and rational use. Georgia has diverse, but non large-scale reserves of mineral products: fuel-energy (oil, gas, coal, geothermal waters, peat); ferrous, non-ferrous and noble metals (manganese, copper, lead-zinc, gold, silver); non-ore raw material for metallurgy (flux limestone, dolomite); mine-chemical raw material (barite, diatomite, talc, serpentinite, mirabilite, lithographic stone, bentonite clay, zeolite, mineral pigments);

building materials (facing and sawing stones, gypsum, cement raw material, quartz - feldspar sand, ceramic raw material, brick and tile clay, sand - crushed stone material, light fillers for concrete, etc.), mineral waters, etc.[31-41].

Over last years (especially from 90-th of last century) the rates of mining-processing of mineral products considerably reduced which, in a certain degree, is determined by less demand for them on local market. Mentioned fact had an adverse effect on export-investment possibilities of Georgian mineral raw material and, respectively, on further development of the sphere. In our country there are real possibilities for expansion of mineral - raw material base, which will allow the increase of the facilities for mining, processing and production of various products.

Geological structure, the results of scientific-research, field-geological, survey and drilling works, performed in the country, allow to conclude that in Georgian interior there is a certain reserve of oil and gas (by some data - solid reserve). Unfortunately, at present real results from viewpoint of indices of their extraction are unfavourable. But it should be noted that in this field were the optimistic believes when over 70-80 years of last century the oil annual extraction exceeded 3 mln tons.

In eastern part of Georgia, especially, in South Kakheti, in deposit suites of various age there is a real reserve, development of which will allow the increase the prospects of the extraction of hydrocarbon containing energy carriers.

In Georgian territory five and, probably, two regions with oil and gas may be divided. Along with it nowadays Georgian territory is divided in license blocks.

Within recent years in Georgia an attention is given on reveal of shale gas. The corresponding works are conducted.

By deposit and mine-geological conditions Tkibuli-Shaori coal deposit is dedicated to complex one. Working of steep inclined and steep layers is accepted reasonable by the method of lower lift failure and by the use of mechanical lining.

In Tkvarcheli all infrastructure is really degraded. Preparation of the deposit for exploitation must be begin by restoration of terrestrial deposit economy and by making it serviceable. Special attention must be placed on Tkvarcheli deposit №8, where in due time the mine-hydropower, mounted in the mine, was constructed. This was unique engineering decision in former USSR.

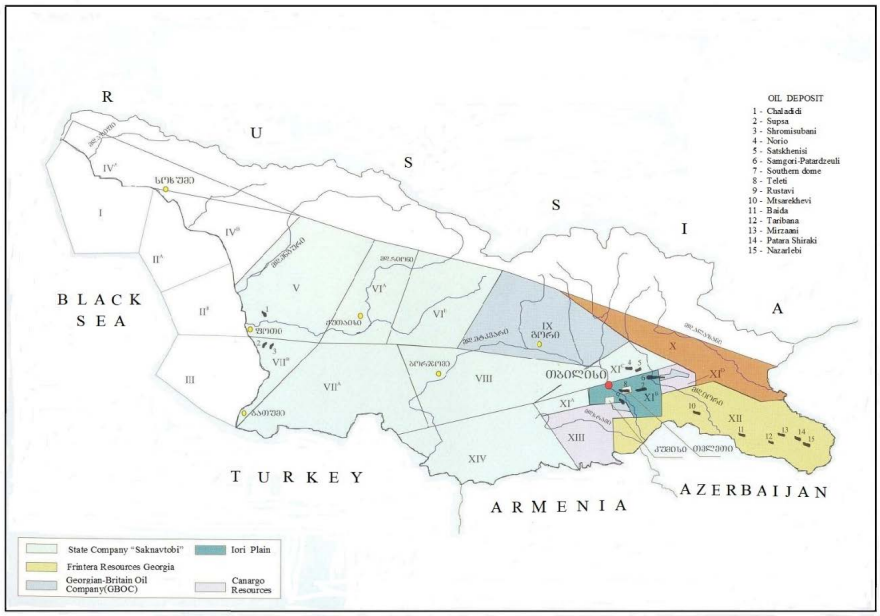

Fig.5. *Map of Georgian License Blocks*

At Akhaltsikhe deposit of brown coal the cover of coal dust and bed are presented by montmorilinite clays. In natural state the rock is characterized by high humidity which is favorable for adsorption of the most heat-conducting compounds - resins, humus acids, etc. from the coal by coal-containing bentonite clay. Hereafter coal depletion and bentonite enrichment takes place and hence, in natural conditions, organic-mineral fertilizer is formed. Coal selective mining by special holing mechanisms is reasonable which will allow the mining of the coal and hereafter – of bentonite clays ("black bentonites") enriched by organic compounds.

There is a serious reserve of the peat in Georgia which must be rationally used as the fertilizer component and as a fuel for home use.

Metallic mineral products occupy the important place in Georgian mineral-resource potential. From ferrous metals manganese must be noted. It is presented by several industrial deposits among which Chiatura deposit should be primarily noted. Its ore resource comprises 201 mln tons. Other marked deposits are Chkhari-Ajameti, Kvirila depression and Shkhmeri.

At present Chiatura manganese deposit occupies the most important place from viewpoint of mining, enrichment and processing. But it should be noted that over last period the reserve of high-grade ore is significantly reduced. Existing conditions at the deposit must be reconsidered which will give the possibility to expanse the contours of manganese-containing blocks.

Copper, lead, zinc, aluminum must be noted from non-ferrous metals. Explored copper reserve is mainly presented by Bolnisi and Achara ore regions. Predicted resources of Kvemo Svaneti and other regions are not estimated. Prospective territories (Abkhazian and Acharian Autonomous Republics, Kakheti, Svaneti and Bolnisi regions) are marked out.

Among prospective objects Chotio ore block, Zheskho and Devdorak deposits deserve attention and further study.

Lead and zinc were mined only at Kvaisi deposit and ore was sent in North Osetia. Unfortunately there are no data about mentioned deposit.

Reduction of boxite resources all over the World caused the need for use of non-traditional minerals for aluminum production. From this viewpoint alunites are prospective compounds. Technology of their processing was elaborated. Apart from aluminum the preparation of a number of useful products such as sulphuric acid, aluminum sulfate, potassium fertilizer, caustic potassium, etc. may be obtained from alunites.

In Achara reasonably large areas of alunite and alunitized ricks are know in several places of Achara. Alunite content varies from 40% to 70%, Al2O3 – from 22% to 36%. Mentioned objects are not studied in detail but, by preliminary data, it may be said that predicted resources of alunite is impressive.

Argilites of Tkibuli-Shaori coal deposit are also prospective for production of limestone and aluminum in which the content of aluminum oxide varies from 27% to 32%.

Study-development of raw material (Achara alunites, Tkibuli-Shaori argilites) for production of limestone and aluminum allow the production of costly ferro-silikoaluminum in the conditions of Zestafoni ferroalloy factory.

From noble metals gold-containing deposits are of importance for Georgia. At present Bolnisi ore region is considered as the most prospective region for gold where existing ores are characterized by gold significant content (Madneuli, Sakdrisi, Tsiteli Sopeli, etc.). It should be noted that region gold potential isn't limited by mentioned deposits (also for copper and other mineral priducts). From this viewpoint the prediction of some researchers about the prospects of Bolnisi ore region deserves attention. They, on the basis of the data obtained by remote methods, divide up to ten cyclic volcanic structures, similar to well-known Zhurab-Nabakrebi (Madneuli). This also must be studied in detail.

The wide spectrum of mine-chemical raw material is presented in Georgia. Among them barite, diatomite, lithographic stone, mineral pigments must be pointed out. Efficiency of their industrial development depends on a number of economic factors. Unfortunately, at present the

objects are in very heavy conditions.

Georgia is rich by various building materials. Among them are: facing and sawing stones, gypsum, cement raw material, quartz- feldspar sand, ceramic raw material, brick and tile clay, sand-crushed stone material, rocks for production of basalt fiber, covering shales, concrete light fillers (volcanic slag, perlite, pumice stone, etc.) It should be noted that the methods of mining and processing of building materials and technological facilities are, in many cases, outdated and require renewing and re-equipment.

As result of long-standing and intensive exploitation of mineral products the share of rich and easily enriched ores decreases in their reserves, and the share of low-grade and hardly enriched ores increases. By the use of traditional mechanical methods of enrichment the preparation of selective conditional concentrates is difficult. For intensive development of the sphere the promotion of innovative technologies is necessary – remote methods for deposit search, automated and robotized systems for drilling, etc. Since the share of hardly enriched ores increases in mining of mineral products, the wide promotion of combined technological schemes of enrichment by the use of bio-hydrometallurgical processes is reasonable in the practice of primary processing of the ores together with traditional methods.

On Georgian territory there are numerous mineral waters of diverse composition (up to 1500 springs and boreholes). From this viewpoint the mountain region of the country is the peculiar national laboratory were the analogues of all types of mineral water, known all over the World, are formed: started from cold and low-mineralized (0.5 g/l) carbon acid waters ("Narzan" type), hot (30-112oC) and finished by highly mineralized (340 g/l) chlorine-calcium brine.

Primarily Borjomi deposit water, well-known all over the World, should be noted. Such mineral waters as Sairme, Nabeglavi, Utsera. Java, Tskhaltubo, Menji, Zvare, Nunisi, etc. are well-known.

From 80's of the last century the industrial extraction and familiarization of natural carbon dioxide (CO2) for food industry has started by its separation from mineral waters.

In 2007, by drilling works in the head of river Tergi, in Truso gorge, the powerful stream of carbon dioxide was obtained. Its industrial bottling is provided which will be favourable for employing of the population of Kazbegi region and for improvement of social conditions.

Within all south slope of Caucasian mountain ridge, where young volcanic centers are existed and up to 800 springs, containing CO2, are accounted, carbon dioxide is intensively emitted. A number of the springs contain pure CO2 (99.9%) as accompanying gas. This is a very important condition for its use in food industry. In mentioned places the production of carbon dioxide in large quantities is possible by inexpensive drilling works.

Hence, inexhaustible resources of mineral waters in Georgia, their diversity and excellent landscape conditions of appearance, on the basis of foreign experience of their use, allows to conclude that Georgia has the wide prospects for large-scale familiarization and for increase of industrial capacities for the resources of mineral waters.

Power engineering is a basic sphere of country economics which provides all sectors and population by energy content.

There are nearly all types of fuel-energy resources in Georgia: coal, oil, natural gas, thermal waters, peat, brown coal, hydro-, wind- and solar energy resources. Their reserves are not important with the exception of hydropower resources and coal [42-49].

Over last two decades in the development of fuel-energy complex of the country, certain quantitative and qualitative variations took place. On the basis of statistical data, the adverse tendencies may be revealed in the sphere of power engineering in future. In particular, preferential increase of national produce is expected in comparison with power generation. This fact will have an adverse effect on country's economic development. This state is caused by low rate of electric facilities which is due to the absence of own investments. Hence the way out is only in

foreign investments.

Performed investigations have shown that, in perspective, the index of the use of local energy resources will be low in fuel-energy balance. By 2020-2025, the hydro resources, coal and biofuel (including firewood) will be mainly used from local energy resources, the total share of their production comprises nearly 36-37% of consumed energy resources. By 2025, 31-37% of technical reserve of country's hydro resources will be familiarized.

The rates of familiarization of sun, wind and geothermal waters will be low in future. Therefore an increase of the scales of the use of mentioned non-traditional, renewable energy resources is necessary which, respectively, will reduce the expected risks of adverse ecological effect. They may be used in agriculture, housing and communal sector, food and light industries, etc. 60% of theoretical potential and more than 50% of technical potential of Georgian hydro energy resources is accounted for 5 rivers and their tributaries among which, 4 rivers-Rioni, Enguri, Kodori and Bzipi are located in West Georgia. More than 70% of technical hydro energy potential of the whole country is accounted for West Georgia.

On the basis of above-mentioned, in perspective, the familiarization of hydro energy resources will be mainly carried out in western part of the country where the construction of hydropower stations will be continued.

Recommencement of the construction of Khudoni hydropower station in Samegrelo-Zemo Svaneti and starting of the process of investments search for construction of prospective hydropower stations of Enguri cascade in upper part of river Enguri is extremely important.

In perspective, the construction of powerful hydropower stations on the rivers Rioni and Tskhenistskali (in Imereti and Racha-Lechkhumi regions) (Namakhvani cascade, Utsera, Tsageri, Orbeli, etc) are also important.

Construction of complex hydrosystems is reasonable on the rivers of West Georgia which will be favorable for regulation of river flow and for rational use of water resources for energetic as well as for water feeding and irrigation purposes.

Familirization of the rivers of West Georgia for energetic purposes will cause the reduction of solid alluvium in coastal zone of the Black Sea. Because of this fact, performance of protective and restorative works in the sea coastal zone is the immediate task.

For increase of the share of solid fuel in fuel-energy balance and to enhance its competitive ability in comparison with other types of the fuel, elaboration and promotion of modern technologies will provide radical restructurization of power engineering. In perspective, the organization of power entity of newest type is possible where, apart from the obtaining of main products: electric and thermal energies, the production of building and insulating materials, marketable nitrogen, oxygen, argon and sulfur, as well as of various metals and secondary products will be carried out.

At gasification in the slag melt from one ton of Tkibuli-Shaori coal secondary product by the cost of 20-30 US dollars may be obtained, which will reduce the cost price of electric power to 0.9-2.5 cent/kW.hour, generated in multiprofile thermal power station.

Production of ferroalloy – ferro-silikoaluminum from the ash, formed after burning of Tkibuli coal in power plant, will also give a great economic effect.

Because intense variation of cloudiness in Abkhazian Autonomous Republic, Samegrelo-Zemo Svaneti, Imereti and Racha-Lechkhumi, the use of solar energy is mainly prospective in the systems of individual heat supply (for heating of buildings and for realization of the processes of hot water feeding).

Regions of Guria and Achara, where heliopower potential is high, Samtske-Javakheti (especially its central and south parts), Kvemo Kartli, plain of Shida Kartli and piedmont territories are prospective for large-scale use of solar energy.

In Mtskheta-Mtianeti region, the municipalities: Mtskheta, Akhalgori and Dusheti have good prospects for generation of thermal energy by the use of thermal collectors of the sun.

Tbilisi and Kakheti region are characterized

by large heliopower potential. In perspective, the large-scale use of solar energy is possible here for generation of thermal and electric power. For this purpose the construction of powerful thermal and photoelectric stations is necessary.

There are good prospects for the use of wind power resource in Georgia. From this viewpoint the perspective locations are: in Racha-Lechkhumi: Mamisoni pass and adjacent territory of Oni municipality; in Samegrelo: vicitity of settlement Jvari, Kolkheti plain, city Poti and adjacent territory; in Imereti: Kutaisi-Ajameti zone and central part of Likhi mountain ridge (mountain Sabueti); in Shida Kartli: Khashuri-Gori section of the gorge of river Mtkvari; in Kvemo Kartli, Mtskheta-Mtianeti and Tbilisi: Mtskheta-Rustavi section of river Mtkvari, Samgori municipality and airport zone, high-mountain zone of Kazbegi municipality and Dusheti municipality. Wind regime in Samtskhe–Javakheti, in Tskhratskaro and Achara, on Kakhaberi plain allows the use of the plants of slow as well rapid wind.

Together with the construction of wind power plants the corresponding measures must be conducted for liquidation or for minimization of such adverse results as losses of land areas, landscape damage, formation of low-frequency harmful noises and of electromagnetic disturbances for communication.

Among the deposits of geothermal waters, West Georgia is characterized by great number of operational and reinjective boreholes as well as by the amount of revealed resources, especially, Samegrelo Region (Zugdidi – Tsaishi deposit) and in East Georgia-Kartli region (Tbilisi deposit). From other deposits Samtskhe-Javaklheti region and Abkhazian Autonomous Republic are of interest in country scale.

The use of the heat of geothermal water for domestic-communal purposes, in agriculture (in greenhouses for supply of technological processes by heat and cold) and in the case of geothermal power stations will be highly favorable for improvement of energy supply of individual regions and for solving of ecological problems.

For correct and efficient functioning of fuel-energy complex, the performance of some measures are necessary. Among them major ones are:

- Adjustment of the potential of resources and creation of integrated data base;
- Rational use of the whole complex of fuel-energy resources of Georgia – hydropower, coal, oil, natural gas and non-traditional sources of energy taking into account ecological requirements;
- Formation of optimal structure of fuel-energy complex appropriate for Georgian conditions;
- Establishment of reliable and favorable energetic contacts with the world's advanced and neighboring countries.

Recreation resources are the base for the development of resort and tourist business for Georgia [50-54]. Those are resources which may be used for recreation and tourism: natural complexes and their components (relief, climate, water reservoirs, vegetation, animal world, etc.); cultural and historical-architectural sights. Although the objects of anthropogenic nature also belong to recreation resources (primarily historical-architectural monuments), yet natural-recreation resources are their basis and have an important function in the sphere of population health protection. They not only have medical-preventive-sanitary and cultural-educational function but also are of great social-economic importance since they are favorable for economic and social activity in health resort - recreation zones and totally in the country.

Climate is the important component of recreation resources. Hereunder by this the totality of weather conditions favorable for various recreation activities (recreation, treatment, travel, etc.) is meant.

Georgian climate is rather comfortable for recreation goals, giving good possibility for the development of health resort - tourist sphere. Country territory is a classical example of vertical zonality of geographical landscapes where all spectrum of climatic zones is presented: from wet subtropics to eternal snow zone.

Georgia is rich in diverse health resort - recreation resources: the Black Sea coast, mountain-climatic, balneological, balneoclimatic.

The most important resources are located in mountain and sea coast zones. The most favorable climatic conditions are in low-mountain (500-1000 m above sea level) and middle-mountain (1000-2000 m above sea level) zones were the part of mountain-climatic and balneological health resorts are located. Balneological health resorts: Nabeglavi, Tkvarcheli, Zvare, Nunisi, Borjomi, Sairme, Surami and others are mainly located at a height of 1000 m. And at a height of 1000-1500 m above sea level - mountain-climatic health resorts: Abastumani, Tsagveri, Tsemi, Kojori, Kiketi, Manglisi and others are located. The upper part of middle-mountain zone (1500-2000 m above sea level) is characterized by soft winter, long-term and high snow cover, moderate cool summer, long duration of sun shining. From the health resorts of the mentioned zone the following ones are well-known: in West Georgia: Shovi, Bakhmaro, Lebarde, Avadkhara (Abkhazian Autonomous Republic), in East Georgia: Bakuriani, high-mountain zone (higher than 2000 m above sea level) is not used at present as resorts. It is mainly used for mountain and downhill skiing.

The Black Sea coast (more than 300 km long) is rich in recreation resources. It is characterized by unusual beaches and climatic conditions. On the Black Sea coast there are 130-135 days annually favorable for recreation, whereas in Baltic countries this index comprises only 60-90 days and in Crimea – 129 days. Rich and diverse vegetation, efficient combination of mountain and sea coast landscapes as well as large possibilities for the use of hydromineral resources enhances considerably aesthetic and recreational importance of the Black Sea beach. In the mentioned zone the following health resorts and resort places are located: Gagra, Bichvinta, Akhali Atoni, Sokhumi, Gudauta (Abkhazian Autonomous Republic), Batumi, Kobuleti, Makhinjauri, Mtsvane Kontskhi, Tsikhisdziri, Gonio, Kvariati, Sarpi (Acharian Autonomous Republic).

The sands containing magnetic iron which are located in the central part of Georgian Black Sea zone, are important natural recreation resources. The sands contain up to 4% of magnetite and create constant magnetic field which is especially useful for children organism as well as for the treatment of cardiovascular, peripheral and locomotor systems (health resort Ureki).

Georgian mineral waters effect on the specialization of the country's territorial-recreational system, determine the high relative share of medicinal-recreational service. There are nearly all kinds of mineral waters in accordance with modern classification.

At present, the mineral springs are used more intensively which are located in plain and low-mountain zones of intermountain areas where transport infrastructure is well developed. Exactly in mentioned places the balneological and balneoclimatic health resorts were organized: Borjomi, Utsera, Java, Tskaltubo, Menji, Surami, etc.

Therapeutic muds are one of the types of natural-recreational resources. Nowadays, two mud treatment health resorts – Akhtala and Kumisi are functioning. Sulfide ooze mud of Kumisi lake is also used in Tbilisi Balneological Health Resort.

The forests rich in vegetation are important recreation resources of Georgia. Their total area comprises 2.7 mln hectares. Georgian forests are mainly broad-leaved, coniferous ones occupy only 35%. 57% of the forests are in West Georgia where they vary from Kolkhurian to subalpine type forests.

In the course of consideration of natural-recreational resources the protected territories of double function: ecological and recreation must be singled out. By the data of the Agency of Protected Territories, at present the total area of mentioned territories comprises 597556 hectares, that is to say, nearly 8.57% of the country territory. In accordance with the criterion of IUCN, there are 88 protected territories in the country by 2018: 14 state wildlife areas with a total space of 139048 hectares, 11 national parks (349327 hectares), 19 wildlife preserves (59857 hectares) 42 natural sanctuaries (2941.43 hectares) and 2 protected landscapes (34708 hectares).

Karstic caves present an important recreation

resource. Georgia occupies one of the first places in the world by the amount of mentioned caves. The total length of more than 1300 revealed underground natural sanctuaries attains 240 km. It should be noted that several caves located in Georgia are among ten deepest caves of the world. Those are: Kruberi (2197 m) and Sarma (more than 1500 m) caves in Abkhazeti, on Arabic massif (Gagra mountain ridge) and Pantyukhin cave (1500 m) on Bzipi mountain ridge.

The functions of caves are diverse: scientific-research, excursion-educational, sporting (speleotourism) and medical (speleotherapy).

Two karstic caves may be used for medical purpose: Anakopia in Abkhazeti, near health resort Akhali Atoni and "White cave" in Tskhaltubo. Microclimate of mentioned karstic caves is characterized by excellent medical properties for the treatment of respiratory tract, cardiovascular, neurological and other diseases.

The part of Georgian caves is characterized by their attractivity: large halls, underground rivers, lakes, stalactites and stalagmites, etc. which opens good prospects for ther development of speleoturism.

Remarkable is "The Promete cave" (former "Kumistavi cave") near Tskaltubo where the simultaneous development of several kinds of speleotourism is possible: educational, extreme and medical ones.

The well-known Ritsa lake (in Abkhazeti) is tourist-recreational object of special importance. Millions of guests visited this object annually. Nowadays, because of the well-known events, the information about this place is restricted. There are interesting lakes within the mountain system of the Great Caucasian ridge: Keli lake, Bazaleti lake (Mtsketa-Mtianeti region), lakes Ertso and Tsiteli Khati (Shida Kartli), Kedi lake (Racha region).

South Georgia highland is rich in lakes: Kakhisi, Tabatskuri, Tsero, Paravani and Sagamo (Samtskhe-Javakheti region).

Artificial water reservoirs may be successfully used for recreation purposes: Tbilisi, Sioni, Gali, Jvari, Lajanuri, Shaori, Tsalka, Algeti and Zhinvali.

At present, there is a great demand on winter sports for the development of which Georgia has unique natural conditions. There are many places in the country with corresponding relief, zones with high and long-term snow cover which may be used for mountain tourism and recreation. From this viewpoint Svaneti, Tusheti, environs of Abastumani, Bakuriani, Gudauri and Bakmaro are the most prospective.

Georgian natural-recreational resources provide unique possibilities for mountaineering, the best conditions for which are in Zemo Svaneti with the highest peaks such as Ushba, Shkhara, Tetnuldi, etc. Prospective for mountaineering development are mountainous Abkhazeti and municipalities of Oni, Java, Kazbegi, Dusheti, Akhmeta, Lentekhi.

The important recreation resource of the country are objects of cultural-educational tourism: historical and architectural monuments, theatres, concert halls, galleries of art, exhibition halls, ethnographic museums, house-museums of well-known persons, pantheons, etc.

Centuries-old history of Georgian people is materially expressed in historical monuments which are located in large quantities in all regions of the country. These are spiritual, defensive buildings and secular ones of last centuries.

All these man-made monuments present anthropogenic tourist-recreational resources and mainly serve for to increase human intellect, to deep knowledge, to provide entertainment, recreation and attract foreign tourists.

The diversity of natural and economic conditions of Georgia effected significantly on the total picture of population settling and determined the originality of the view of populated places. Mountain villages of the following municipalities: Mestia, Lentekhi, Oni, Dusheti, Kazbegi, Akhmeta and others are of great interest to tourists by peculiar architecture and specific style of living. One of them - Svaneti settlement Ushguli (in village Chazhashi) is included in the list of the World Heritage.

The analysis of modern state of natural-recreational resources shows that there are many difficulties and problems to be solved on rational

use and protection of these resources.

In the first place the measures must be carried out for minimization of catastrophic results of natural disasters (landslides, torrents, snow avalanches, erosion events, river washouts, etc.) For this reason planning and realization of large-scale preventive measures are necessary.

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Modern attributes of the Republic of Armenia financial system stability

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A B S T R A C T

The financial and economic crisis in global and regional economies over the past decade has shown that, in parallel with the price stability goal, central banks should focus on financial sustainability in macroeconomic stability assurance process. In order to ensure financial stability, the Central Bank of Armenia implements a macro-prudential policy, which implies the use of appropriate tools. The use of tools is aimed at minimizing systemic risks, or in other words, minimizing risks that hinder the normal functioning of the entire financial system. In the article are discussed the main indicators characterizing the stability of the financial system of RA and based on recent studies and analyzes made in 2005-2017, prove that the Central Bank's financial risks are within the scope of governance, maintaining the stability of the financial system.

Keywords: Financial stability, Central Bank of Armenia, Financial soundness indicators, Macro-Prudential Policy, Financial Stability Report, World Economic Growth.

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Introduction

For the last several decades the central banks of the developed countries have united the price stability problem with financial stability, considering that the last is a preliminary condition of effective monetary policy. Almost all the governments of the world beside the study of the development of separate components of the financial system carried out the study of the country's financial stability as a whole. Ensuring of macroeconomic, monetary and credit stability is a direct comparison to the decrease of risks of the financial system stability. The Central Bank of Armenia is responsible for ensuring financial stability in the Republic of Armenia what is also enshrined in the Law of the Republic of Armenia "On Central Bank"[1].This is due to the fact that high economic growth, low inflation and unemployment levels ensuring, and effectiveness of monetary policy measures are impossible without stable and developed financial system intermediation, without development the parts forming the financial system - financial institutions, financial markets and financial infrastructures.

Since 2005 the Central Bank of Armenia regularly implements financial stability vulnerability monitoring including results in the annual report of the Central bank of Armenia and according to the amendments to the Law on the Central Bank of Armenia, the latter has undertaken to publish an Annual Financial Stability Report since 2007.The purpose of the Financial Stability Report is to inform the financial market participants and the public about the risks of the RA financial stability, the reasons for their occurrence or transfer, to assess the elasticity of financial stability in RA, the ability not only to neutralize, but also to absorb the potential risks. The Bank of England [2] is one of the first, which not only adopted financial sustainability as the second main objective, but also since 1996 began to publish Financial Sustainability Report with a semiannual period (Table 1).

Table. 1. *Financial Stability Report Publication Guide by States [3]*

State	Publishing Institution	Frequency	Starting Year	Website Address (short)
Great Britain	Bank of England	semi-annual	1996	https://www.bankofengland.co.uk /
Norway	Norges Bank	semi-annual	1997	www.norges-bank.no
Sweden	Sveriges Riksbank	semi-annual	1997	https://www.riksbank.se
Philippines	Bangko Sentral ng Pilipinas	semi-annual	1999	http://www.bsp.gov.ph/
Australia	Reserve Bank of Australia	semi-annual	1999	http://www.rba.gov.au/
Hungary	National Bank of Hungary	semi-annual	2000	http://www.mnb.hu/
Iceland	Central Bank of Iceland	semi-annual	2000	http://www.sedlabanki.is/
Ireland	Central Bank and Financial Services Authority of Ireland	semi-annual	2000	www.centralbank.ie
Austria	Österreichische Nationalbank	semi-annual	2001	www.oenb.at
The Netherlands	National Bank of Poland	semi-annual	2001	www.nbp.pl
Russia	Bank of Russia	annual	2001	www.cbr.ru
Belgium	National Bank of Belgium	annual	2002	www.nbb.be
Canada	BANK OF CANADA	semi-annual, annual	2002	https://www.bankofcanada.ca/
Finland	Bank of Finland	annual	2003	suomenpankki.fi
Switzerland	SWISS NATIONAL BANK	annual	2003	snb.ch
Slovakia	National Bank of Slovakia	annual	2003	nbs.sk
Poland	National Bank of Poland	semi-annual, annual	2003	nbp.pl
Australia	Commonwealth Bank of Australia	semi-annual, annual	2004	commbank.com.au
Czech	Czech National Bank	annual	2004	cnb.cz/en/
Japan	Bank of Japan	semi-annual, annual	2005	boj.or.jp
Korea	Bank of Korea	semi-annual, annual	2005	bok.or.kr
RA[4]	Central Bank of Armenia	annual	2007	www.cba.am

The monitoring of the financial risks of the financial system has always been one of the priorities included in the CBA strategy [5]. Financial risks can be external and internal but the Central Bank of Armenia does not exclude the risks of the financial system itself. If the Central Bank develops, approves and implements monetary policy to ensure price stability but for financial stability it implements macro-prudential policy (Fig.1) [6].Moreover, the impact of monetary policy on financial stability is regarded as an indirect effect.

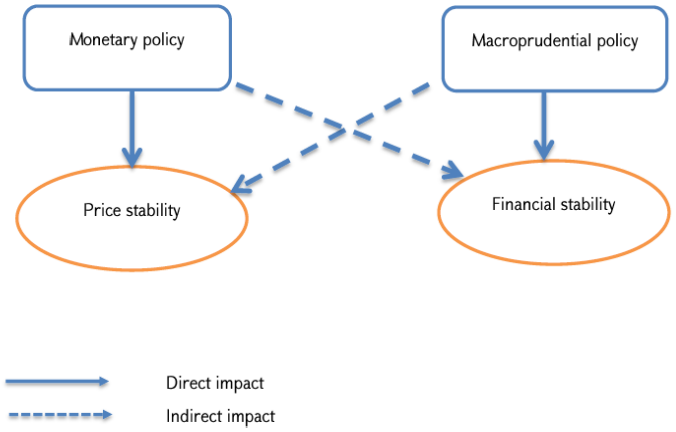

Fig.1. . *Interaction of Monetary and Macro-Prudential Policy [7]*

Based on the above mentioned, the potential risks to the RA financial stability are presented in the report in 5 main directions [8]:

1. Risks deriving from global economic developments,
2. Risks derived from the macroeconomic development of RA,
3. Risks derived from developments in RA financial market,
4. Risks derived from financial institutions of RA,
5. Risks derived from financial infrastructures of RA

Getting back to the risks inherent in the global economy, according to the IMF data globally was observed a decline in economic growth. The main reasons for the decline of the latter are conditioned by the slow pace of economic growth in developing and developed countries, with global trade instability, reduction of prices for raw materials and the reduction of capital flows (Table 2).

Table. 2. *Developed and Developing States Economic Growth and Inflation in 2005-2017*

Indicators	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018 projections	2019 projections
Economic Growth															
World Economic Growth	4,4	5	4,9	3,2	-0,8	5	3,9	3,1	3	3,4	3,2	3,1	3,7	3,9	3,9
Developed States	2,5	3	2,7	0,9	-3,2	3	1,6	1,3	1,4	1,8	2,1	1,7	2,3	2,3	2,2
Developing States	7	7,7	7,8	6,1	2,1	7,1	6,3	5,1	5	4,6	4,1	4,4	4,7	4,9	5
Inflation															
Developed States	2,3	2,3	2,2	1,9	0,7	0,9	1,3	1,2	1,2	1,4	0,3	0,8	2	1,9	2,1
Developing States	5,3	5,1	6,5	9,2	5	5,6	7,1	5,8	5,5	4,7	4,7	4,4	4,7	4,4	4,1

Global economic growth of the International Monetary Fund (IMF) in 2016 was 3.1% [9]. External and internal variations in the first half of 2017 have a favorable impact on the stability of the financial system. RA economic growth in the first half of 2017 was 6.0 % (2016 in the first half of the year was 0.2%) capital outflow leading to fluctuations in the national currency. Revival of activity in the world economy and increase in prices in the stock markets was led to formation of rather high inflationary environment. According to the IMF data in developed countries in 2017, inflation was 2%, and in developing countries - 4.7%.In developing countries notices the tendency of toughening monetary and credit conditions on the international scale due to the yearly decline in economic growth [10]. In the first half of 2017 there has been an increase in prices for commodity markets according to predictions of IMF in 2018 the inflationary pressures will not be significant.

Risks derived from macroeconomic developments in RA

RA trade partner countries' low economic growth rates, geopolitical tensions and maintaining of low levels of raw materials prices have negative impact on RA economic stability.

As a result of the monetary policy pursued by the RA CB and the stimulating fiscal policy developed by the RA Government partially neutralized the risks that threaten financial stability. In the first half of 2017, in RA was recorded high economic growth (higher than expected) largely due to industry (value-added increase 7.6%) and services (value-added increase 8.4%) sectors [11]. In the first half of 2017 to the recovery in the gross demand also contributed continuous recovery of external demand in particular 8.7% of private consumption and 6.8% increase in investment. 1.6% increase in lending is remarkable. It has been accompanied by 0.4 percentage point decrease in non-performing loans and account receivable specific weight, making it 5.8%.

Risks derived from developments in RA financial market

In the first half of 2017 weakening of monetary conditions had a significant impact on the rates of interest of financial market. The Central Bank, implementing an expansive monetary policy, reduced the refinancing rate to 6.0 percentage points (a decrease of 4% compared to the end of the previous year).

Reduction of the mandatory reserve rate by the Central Bank to 10% as well as the surplus cash generated by the replenishment of the commercial banks' capital, has resulted in a decrease in the amount of contraction in the financial market at the same time raising the economic activity in the country which indicates a significant increase in the level of financial intermediation.

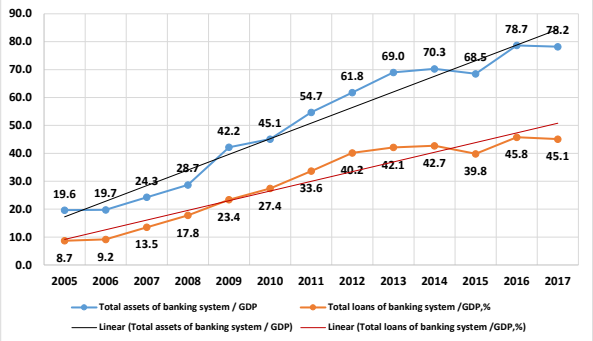

Fig.2. *Dynamics and trends of indicators Total assets of banking system/GDP and Total loans of banking system/GDP*

In particular, the assets / GDP and credits / GDP indicators decreased by 0.5 and 0.7 percentage points and made respectively 78.2% and 45.1%.According to the trends presented in Figure 2, although assets / GDP and credits / GDP indicators have declined in the last year, nevertheless, their stable growth is predicted in subsequent years. The volatile trends in the RA currency market have also been preserved in 2017 (Fig. 2).

In the first half of 2017 the exchange rate of AMD against the USDvalued by 0.72%, against the RUR devalued by 2.72% and against the euro devalued by 6.55% (in 2016, the exchange rate of the Armenian dram almost did not change against the USD, registering 0.04% devaluation in comparison with the same period of the previous year, AMD against RUR devalued by 15.99%, and against EUR valued by 3.22%).

Risks derived from financial institutions in RA

In 2016 financial intermediation has increased due to the growth of economy lending volumes by the commercial banks, which was accompanied by the

increase of the quality of the loan portfolio. Capital adequacy and cash liquidity ratio of the banking system have been improved due to the inflow of financial resources as a result of replenishment of the statutory capital by banks contributing to the strengthening of financial stability.

Risks derived from financial infrastructures of RA

The risks of this section include the risks associated with the RA CB Electronic Payment System (EPS) interbank and valuable security of settlements system.

It should be noted that from the point of view of financial stability more important are the banking system risks and sector development trends because of the fact that the banking system's assets in the financial system of RA by the end of 2017 make up around 88.1% of the entire financial system's assets (see Fig. 3).

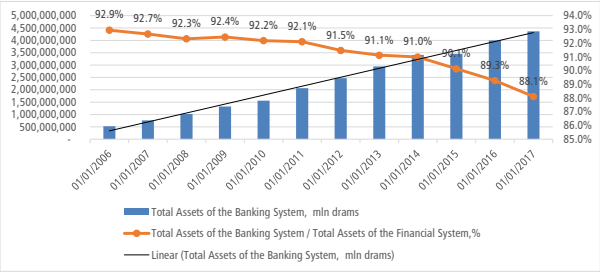

Fig.3. *Dynamics and composition of total assets of the banking system in total assets of the financial system*

Financial stability in the country is largely conditioned by the level of stability and development of the banking system, and the identification and evaluation of the banking system's risks is an important object of analysis. The following indicators of financial stability are highlighted for RA banking system[12] (see Tabel 3) .

1. Total regulatory capital/risk weighted assets - for enhancing the financial intermediation and strengthening financial sustainability, from January 1, 2010 the minimum amount of the normative total capital for commercial banks has been set at 30 billion drams. The growth of normative total capital, of course, contributes to improving the ability and flexibility of the banking system to withstand shocks in various economic situations at the same time raising the efficiency of the banking system.The minimal correlation of this standard since 2005 has been declining, which is largely conditioned by an immense increase in the normative total capital.

2. Normative sunk capital (Tier1)/risk weighted assets - As for ability to absorb risks, the general normative capital is characterized by high quality structure: the share of normative sunk capital in the total capital over the past years exceeds 80% , while the correlation between normative sunk capital and risk-weighted assets was continuously decreasing by 2015, and since 2016 has a tendency to grow.

3. Non-performing Loans / Total Loans – In terms of financial stability, for ensuring solvency of banking system management of credit risk is considered important. The loans extended to the economy by the banking system in comparison with the previous year increased by 13.2% (growth in 2016 - 15.4%). In recent years, there has also been a tendency towards gradual improvement of non-performing loans and receivables The share of non-performing loans and receivables (supervised, non-standard and doubtful risk classes) in total loans and receivables decreased by 1.2 percentage points and amounted to 5.5%.

4. Profitability by assets – According to the RA CB presented audited statements net profit of the banking system in 2017 has made 38.2 billion AMD(for 2016 31,7 billion AMD) [13], moreover 15 banks worked with profits and with loss 2. Profitability of assets in 2017 was 1.2% (In 2016 1.1%).

5. Profitability by capital – By capital in 2017 profitability increased by 0.7 percentage points compared to the previous year. It should be noted that in the structure of income and expenses decreased the share of interest and non-interest income, interest expenses of the banking system and the share of returns from the reserve for potential losses of assets has increased. The specified amendment is explained mainly by improvement of quality of the loan portfolio (Fig. 4).

6. Liquidated assets / total assets - During the year the liquidity level of the banking system has increased mainly due to the total capital replenishment of banks. The overall liquidity ratio has dropped by 0.4% making 32,1%, whereas the minimum limit set by the Central Bank is 15%[14].

7. Liquid assets / demand liabilities – The current liquidity level has also decreased in RA banking system, during 2016 the index grew by 28.4% reaching up to 170.8%, and in 2017 decreased by 29.1% and recorded at 141.7%, with a minimum limit of 60% [15] .

Table. 2. *Developed and Developing States Economic Growth and Inflation in 2005-2017*

Basic Financial Stability Indicators for the Banking System (in percentage terms)	Dec - 2005	Dec - 2006	Dec - 2007	Dec - 2008	Dec - 2009	Dec - 2010	Dec - 2011	Dec - 2012	Dec - 2013	Dec - 2014	Dec- 2015	Dec - 2016	Dec- 2017
Regulatory capital to risk-weighted assets	33,7	34,9	30,1	27,5	28,4	22,2	18,3	16,8	16,7	14,5	16,2	20,0	18,6
Regulatory Tier 1 capital to risk-weighted assets	31,7	32,7	29,0	26,9	26,7	20,2	16,7	15,2	14,5	12,7	14,0	16,4	15,2
Nonperforming loans to total gross loans	2,0	2,4	2,4	4,3	4,9	3,0	3,4	3,7	4,5	7,0	8,0	6,7	5,5
Return on assets	2,9	3,9	3,5	3,5	1,0	2,8	2,4	2,5	1,9	1,0	-0,5	1,1	1,2
Return on equity	13,7	17,2	16,0	15,2	4,8	12,3	12,7	14,7	12,0	6,4	-3,5	7,0	7,7
Liquid assets to total assets	41,8	41,2	33,7	23,8	34,4	29,5	27,9	25,6	29,1	25,1	28,0	32,5	32,1
Liquid assets to demand deposits	110,5	106,1	98,2	103,1	140,8	131,5	120,8	126,1	142,3	129,4	142,4	170,8	141,7

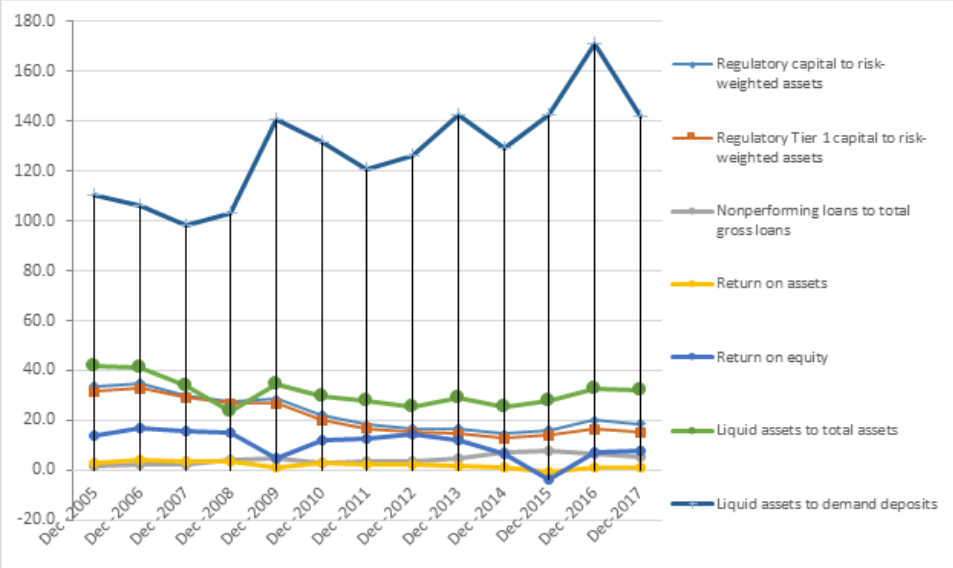

Fig.4. *Dynamics of main indicators of financial stability of the banking system*

Conclusion

It should be noted that, parallel to the main goal of price stability starting from April 9, 2018, financial stability also becomes the main goal of the Central Bank of Armenia caused by various financial crises and their consequences at the global level in recent years [16]. Nevertheless, the policy aimed at ensuring financial stability in the Republic of Armenia still does not have a regulated and complex character compared to countries with developed financial system. There is no single financial sustainability index that will identify the level of financial stability in the country. Taking into account the large share of assets of the banking system in the financial assets of Armenia, the Central Bank of Armenia (CBA) emphasize the stability of the banking system in terms of ensuring financial stability. In the work were discussed main indicators of financial stability established for bank system of RA.

It should be noted that, according to the observations there was noted the tendency to decrease financial system risks, the established norms are considered to be manageable, without risks for the maintenance of financial stability.

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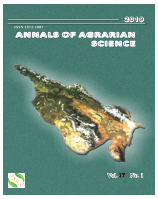

Spatial-Territorial Development Prospects of Borjomi Resort

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A B S T R A C T

Georgia is notable for the abundance of tourism and recreational sites. Between them the southern Samtskhe-Javakheti region is outstanding for its peculiarities like: diverse geographic conditions, several protected areas, recreational places, natural monuments, balneology resorts, cultural landscape, a lot of monuments of cultural heritage and the borderline location that is interesting from the cross-boundary aspect. The article discusses issues of the spatial-territorial development of the resort city Borjomi and hydrogeological research of Borjomi mineral water, as well as some ecological problems of Borjomi, the recent natural disasters and their relation to the project of sanitary protection zones of the deposit. The role and importance of the mineral water deposit as city-forming factor for the development history and spatial-territorial planning prospects of the resort-city are considered. Based on the Project of Borjomi mineral water deposit sanitary protection zones and the General Land Use Plan of Borjomi city, envisaging the environmental problems and natural hazards occurred in recent years, some spatial development directions of the resort Borjomi were identified, as well as recommendations for the implementation of preventive planning activities are suggested.

Keywords: Spatial-territorial development, Resort ecology, Hydrogeological resources, Natural hazards, Recreational tourism, Borjomi resort.

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Introduction

Regions of Georgia are characterized by unequal economic development. According to the data of Geostat's (National Statistics Office of Georgia) GIS analysis, by the economic characteristics Samtskhe-Javakheti ranks the eighth among 10 regions [1]. Although, regardless of agricultural, tourism and resort potential, it may be considered as a less developed region. At the same time, there is a group of recreational resorts in Samtskhe-Javakheti, which economic, social and ecological development would have a significant impact on the region and the country.

There are 14 health resorts and the same number of resort destinations in Samtskhe-Javakheti [2]. This resort potential integrity is called the Samtskhe-Javakheti tourism and recreational cluster. The Borjomi-Bakuriani Group is particularly well known in Samtskhe-Javakheti tourism and recreation cluster. Noteworthy is that according to the indicator of the implemented investments, Samtskhe-Javakheti has a better position between the regions that is mainly due to the Borjomi and Bakuriani factor [1]. Among the municipalities of the region, Borjomi leads by all features. Generally, more attention is paid to the arrangement of the ski resort infrastructure while the scale of spatial-territorial development of the health resorts is very limited. Beginning from the second half of the 19th century to the 90es of the 20th century, health-care tourism was the leading industry of the region's economy, and its weakening has led to economic stagnation; the negative impact on the ecosystem has increased

because of the improper economic activities of the local population and the illegal logging. A large part of ecological problems is related to the economic, planning and logistical problems. Below we will discuss the case of Borjomi, the most important health resort of Samtskhe-Javakheti. Main urban and city-forming factor of Borjomi is its famous mineral water deposit.

The face of use of mineral water for treatment from the ancient times has been confirmed by archaeological finds. The medieval wars of conquest forced the population to leave the Tori Gorge (the historical name of the Borjomi Gorge) [3]. Formation of Borjomi as a resort is related to the re-discovering history of the mineral water healing properties by the Russian Empire's military servants in 1825 [3],[4]. "In the XIX century, Borjomi development undergone three major stages: the establishment - 1825-1842, revival - 1844-1854 and the development of 1862-1900 periods, which were characterized by the study of the chemical composition and medical properties of Borjomi mineral waters"[5]. Geological and hydrogeological surveys of the gorge started from 1872 were carried out episodically, in repeated stages [5]. By the beginning of XX century, Borjomi was a well-equipped and arranged resort like the well-known European spas.

During the Soviet period, development of the resort, as well as mineral water production, achieved the extensive scale. In the 80es of the 20th century, there were 14 sanatoriums, rest houses and boarding houses in Borjomi, while the number of visitors of medical institutions reached 80 thousand there. In addition, 40-50 thousand unofficial holidaymakers visited the resort every year [3]. In the crisis period of the 90es, the sanatoriums were used for the resettlement of the Internally Displaced Persons that actually stopped functioning of Borjomi as a health resort.

Nowadays, the growing tendency of tourism in the country is recorded in both urban and rural areas [6-8]. The number of spa-hotels in Borjomi is small and the prices are not affordable for the middle class, though the growing dynamics of the number of tourists using healthcare centers in the spa-hotels is positive. The spatial-territorial and functional-planning development of tourism attraction centers is relevant to the "spatial arrangement" component of the 4-point plan of the "Freedom, Rapid development, Prosperity, the Government Platform 2016-2020" [9].

The geological and hydrogeological study of the Borjomi mineral water deposit and its district has a history of up to 200 years. Numerous published works, scientific researches, monographs, articles and geological reports devoted to the studies of it, are kept in the local geological library holdings. Some of them are listed in the References hereunder. "A large body of literature on the physical and chemical properties of the Borjomi mineral waters and their physiological action is available in Georgia, Russia and the European countries. Many doctors have written scientific works and defended theses on their research. In this respect the last decade of the 19th century is worth noting" [5].

Among the books of the recent period, noteworthy is the work "Resort Borjomi", prepared by the authors group, which details the state of hydro-mineral base and useful resources of the resort, the possibilities of use of mineral waters and climatic factors for medical and prophylactic purposes [10].

The scientific novelty of the article is considering the urban planning prospects of the resort, namely, in terms of its city-forming factors, in particular in view of the importance of the mineral water deposit.

Objectives and methods

The aim of this study is to determine the planning activities with regard to the Borjomi resort potential and mineral water deposit, considering most recent ecological problems and natural hazards and to develop recommendations in this direction. The planning activities intend to strengthen the road system using historical roads, renewed zoning and planning of protective landscaping.

The main objectives are: the use of city planning approaches to mitigate the adverse impact of ecological problems at the resort - analyzing the spatial zoning defined by the General Land Use Plan of resort and mineral water sanitary protection zones; outlining the preliminary planning measures to mitigate the consequents of natural hazards.

The following methods of research have been used in the article: analysis of the materials of mineral water deposit sanitary protection zones project, legislative framework, GIS data, mapping data, development strategy and spatial-territorial planning documents.

The study examines the Geostat's GIS analysis data [1]; materials of the Borjomi deposit sanitary protection zones project are used as the relevant legislative framework supporting the protection of mineral water deposit [11]; Borjomi General Land Use Plan maps, developed in 2007, where the factor of mineral water deposit sanitary zones is outlined [12]; Borjomi Local Development Strategy 2016-2019 [13]; materials of Environment Agency on the development of natural geological processes [14]; the generated profile of hazards and risks for Borjomi Municipality and Borjomi city [15].

A brief analysis of the sanitary protection zones project

The history of establishing and approving the sanitary protection zones of Borjomi mineral water deposit begins in 1914. On the initiative of the Society for Promotion of the Caucasian Healing Places, the project of sanitary protection zones was developed for Borjomi and Abastumani, which was approved by the Russian Provisional Government in 1917. It was the first official document on the Borjomi mineral water sanitary protection zones [5].

Under the Law of Georgia on "Sanitary Protection Zones of Resorts and Vacation Destinations" (1998) [16], due to natural conditions, three sanitary protection zones will be allocated in the Borjomi mineral water deposit area and in the resort Borjomi considering the location of mineral water wells, resort facilities and forest-parks: the first – the exclusion zone, the second - the restricted zone and the third - the supervisory zone.

According to the above-mentioned law, the boundaries of the first (exclusion) zone of sanitary protection for the mineral water outcrops and boreholes are determined in consideration of their natural protection quality, but at least 15 meters from their outlines. Total 21 sites of the first (exclusion) sanitary protection zone are designed round the exploitation and surveillance boreholes. Their total area is 3.39 ha and the total length of the boundaries is 2761.3 m. The objective of the first zone arrangement is to provide reliable sanitary protection of boreholes, springs and hydro mineral facilities (captivities, reservoirs, pumping stations, measuring equipment etc.).

Due to the hydrogeological conditions of the Borjomi deposit, the depth of the mineral water aquifer, its impermeable cover and high-pressure mineral water, ensure the high quality of natural protection of mineral water in the exploitation and surveillance boreholes. The many years' experience of exploitation of these boreholes has shown no microbiological or other chemical contamination of water as well as no change of its chemical and physical properties.

The second (restricted) zone is allocated in consideration of the structural, hydrogeological and geomorphological peculiarities of the territory, the nature and flow of surface and ground waters to provide the effective protection of mineral water from pollution, spoiling and exhaustion. Its total area is 15390 ha, total length of the boundaries - 46800 m.

The zone covers the area where the surface and underground waters flow to Borjomi and all three exploitation sites of the deposit. All the exploitation and surveillance boreholes, sanitary-resort and recreational facilities, gardens, forest-parks and other greenery, a part of the Borjomi- Kharagauli National Park are located here. The second zone also includes the areas where the general plan envisages the expansion and development of the resort.

The outer boundaries of the sanitary protection zone of the Borjomi deposit and resort Borjomi coincide with the outer boundaries of the third zone. The third (supervisory) zone includes the area of feeding, formation and distribution of Borjomi mineral water, as well as the area of Borjomi climate formation [11]. The main woodland is located in this zone, which plays an important role in the formation and protection of climate and mineral water. The area of the third zone is 133,885 ha, the total length of the boundaries – 155,800 m. Almost all Borjomi mineral water feeding sites which are connected to the outcrops of the aquifer system of the Upper Cretaceous –Lower Paleocene age are located in the supervisory zone. Such outcrops are known on the Borjomi deposit: 1)5-20 km northwest, at the headstreams of the rivers Vakhanistskali, Shavitskali, Chincharauli and on the Lomi mountain; 2)15-25 km northeast, in the valleys of the rivers Satibe, Saterdze and Tkemlovani, and 3)25 km southwards – in the area of Chobareti mountain. These outcrops are spread at the absolute elevation of 1300-2000 m. These are unpopulated areas with healthy sanitary state. The

areas within which the underground water transit takes place to Borjomi deposit, is distinguished by good sanitary conditions.

It should be noted that the company "IDS Borjomi Georgia" that holds the state license for Borjomi water extraction pays great attention to the protection of Borjomi mineral water deposit. According to the current legislation, the company developed the first zones of mineral water sanitary protection of Borjomi mineral water deposit, which were approved under the decree N 367 (06.06.2014) of the Government of Georgia [17]. The project is attached with the list of prohibiting and curative measures, which are strictly controlled by the company's relevant department.

The Borjomi General Land Use plan (2007) envisages the factor of sanitary protection zones of the mineral water deposit [12]. However, when updating the urban planning documents it is necessary to specify the sanitary protection zones by their actual status, as in the general plan of 2007 the sanitary protection zones of the Borjomi mineral water deposit were simply outlined, while in the project of sanitary protection zones of 2011 their borders are updated and adjusted.

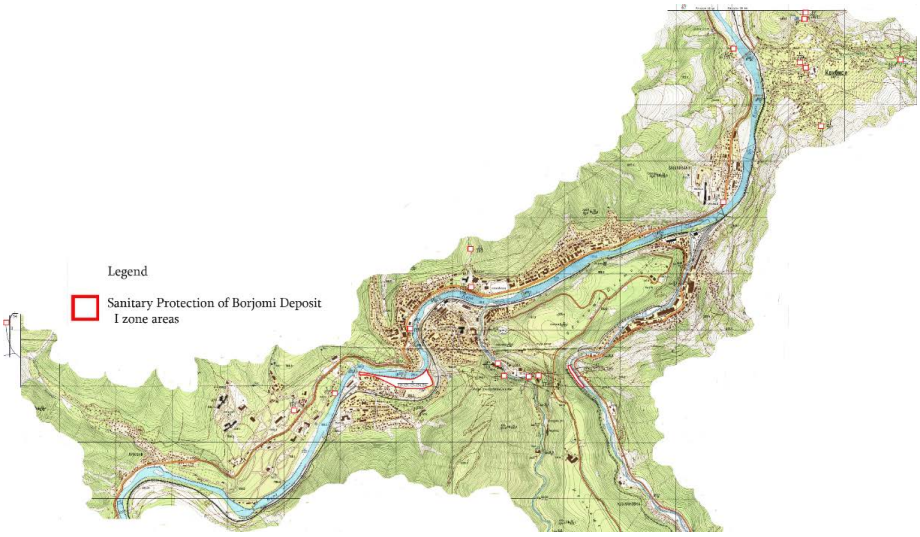

Fig.1. *Map of sanitary protection zones of Borjomi mineral water deposit, 1 zone [11]*

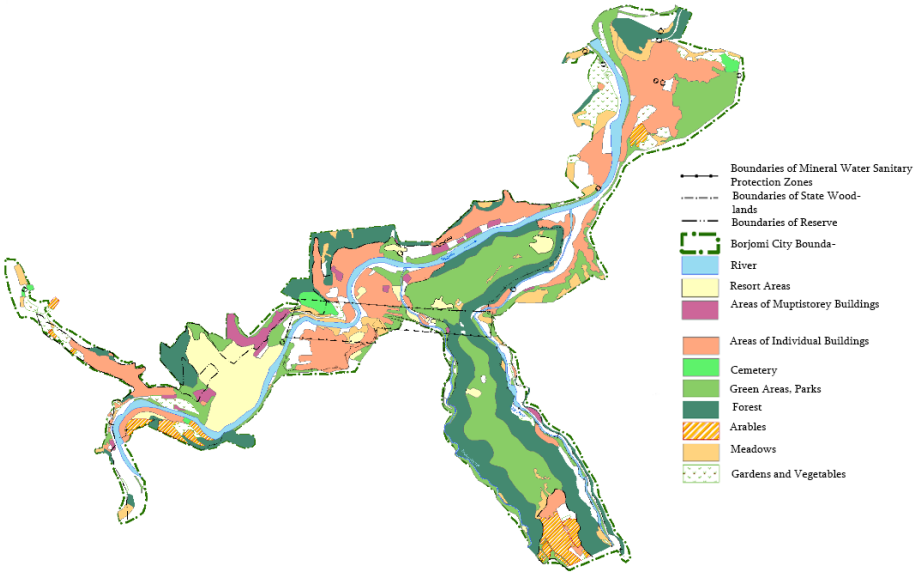

Fig.2. *Borjomi Land Use General Plan, Basic Plan [12]*

We consider the mineral water deposit as the urban forming and tourism-promoting factor, while the impairment of ecological conditions and activation of natural hazards is the factor impeding the city development as a resort. Due to the Environmental Agency data, the landslide processes are activated in the areas damaged by wildfires [14]. According to the recent generated statistical data of natural disasters available on the Geoportal of natural hazards and risks (CENN), the hazards of flash flood and wildfires have been identified for Borjomi.

Results

The survey results are the determination of the Borjomi mineral water deposit role and its sanitary protection zones for the spatial-territorial planning of the resort city; comparison of the sanitary protection zones project and the materials specified by Land Use General Plan of Borjomi city. It offers the planning solutions for prevention and mitigation of environmental problems and consequences of natural disasters.

Discussion

The world-famous resort Borjomi, with its mineral water deposits and climatic resort factors, represents the most important center for wellness tourism development in Georgia. With its rich resort history and modern potential, the strengthening of wellness, medical and spa tourism should be a strategic direction for resort and spatial-territorial development of Borjomi. However, Local Development Strategy document focuses on the sustainable tourism prospects [13]. The resort is overloaded seasonally, during the warm seasons of the year.

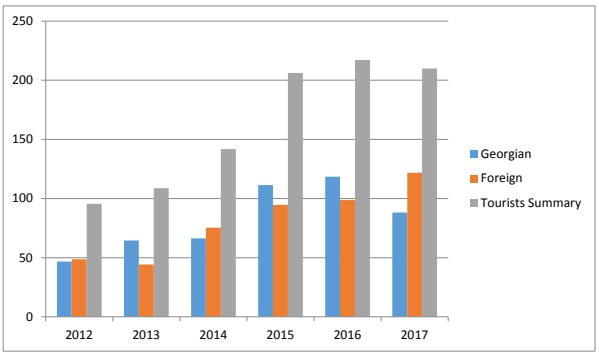

Fig.3. *Number of tourists in Borjomi Municipality during last 5 years (thousand persons). Source: Borjomi Municipality*

Geographical and climate data of Borjomi

Geographical location – among the slopes of Meskheti and Trialeti ridges, in the valley of river Mtkvari and its tributaries Borjomula and Gujaretiwater. The height above the sea level - 800-1000 m. The Borjomi climate characteristics derive from the location of the resort in the low mountain forest zone with moderate climate. The average temperature in January is -2.8°C. The average temperature in July and August is 19°C. The average annual temperature is 8.3°C. The average annual relative humidity is 77%. The average annual precipitation is 658.6 mm. The amount of precipitation is greater in warm seasons (April-September - 356.6 mm), and less in cold seasons (October-March - 302 mm). The sunshine duration is 1350-1400 hours per year." [10]. According to the 2014 general census, the population of Borjomi municipality is 25214 inhabitants, where 15162 live in Borjomi and 10052 - in the rural area [18].

The main reason for the establishment and development of the resort is its carbon dioxide, hydro carbonate-sodium type mineral water and climatic healing factors. The Borjomi mineral water deposit is located in the central part of the Ajara-Trialeti folded zone, between the Ajara-Trialeti and Meskheti ranges, in the valley of the river Mtkvari (Borjomi Gorge). It occupies a length of 8 km in the valley between the villages of Likani and Rveli. The area of the deposit is about 20 sq. km. [19]. Today, the licensed useful resource of Borjomi mineral water deposit totals 682.9 cubic meters per day. The license holder has delivered free of charge daily consumption of 20,000 liters mineral water from the three exploitation wells located in the Mineral Water Park to the Borjomi Municipality for the tourism potential development of the resort and the region.

As mentioned above, the existence of the Borjomi water deposit and its reserves contribute to the development of wellness tourism, as well as to the spatial-territorial development of the city. While the adverse factors, such as environmental problems and resulting natural disasters: green areas reduction because of the improper economic activities, illegal logging and wildfires [20]; river pollution as a reason that water-surfaces are less used for recreational purposes, are impeding this process.

The growth of tourism flow intensifies the impact on environment. The especially crowded places are exposed to risk. The recent statistical data of Borjomi and the municipality shows that the cases of flash flood and wildfires as well as the hazard of inundation and landslides are relatively high.

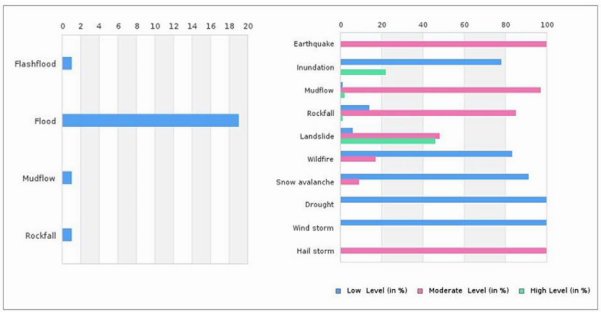

Fig.4. *Generated data of natural disasters for the town of Borjomi: historical disasters; exposition of facilities to risks [15].*

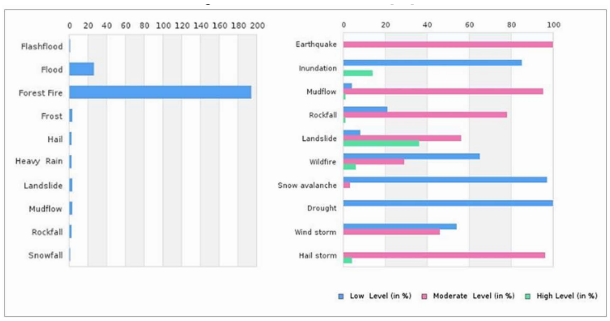

Fig.5. *Generated data of natural disasters for the Borjomi Municipality: historical disasters; exposition of facilities to risks [15].*

The analysis of the natural geological processes map of Samtskhe-Javakheti shows that almost all the Samtskhe-Javakheti resorts are located close to the natural geological hazard zones, and the surroundings of the Borjomi city outline a particular hazard zone. [21]

Therefore, it is necessary to consider the preliminary planning solutions in the process of updating the spatial planning documents of the region, municipality and settlements, in our case, of the Borjomi city.

The planning solutions include: updating the functional zoning in the spatial-territorial documents of the municipality; applying the emergency management plan on the map for the cases of natural disasters; proper planning of roads as possible evacuation corridors (the paving of forestry roads shall antecede but not be made during the occurred natural disaster) [22], [23]; targeted protective, especially terraced planting to enforce the landslide slopes and improve the ecological conditions of the settlements [24].

According to the European experience of forestry roads, the purpose of these roads is different: recreational, touristic, economic, and other [23]. During natural disasters, it is possible to use historical roads and hiking trails for safety purpose.

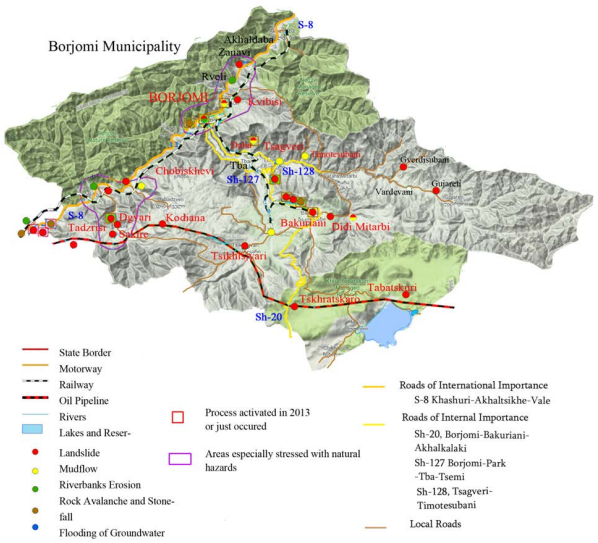

Fig.6. *Borjomi municipality map, settlements falling in the zone of hazard of natural geological processes, main roads and communications*

From the historical sources it is known that the road to Khashuri-Akhaltsikhe (now the highway S-8), which passes through Borjomi, was reconstructed in the middle of the 19th century. The old route traced from the Ateni Gorge along the river Tana to the Borjomi Gorge - the same historic Tori, passing the Gujareti Gorge and Mitarbi, from where it went in the Tadzrisi-Sakire direction to Atskuri. These roads are now hiking trails that can be used for movement during natural disasters.

An important place in the planning solutions should be given to the planning of green areas for the tourism and recreational purpose as well for solving ecological problems [22]. The terraced protective planting of slopes on the landslide areas, through the careful correction of the terrain using the plants with rapidly growing, strong root system can reduce the threat of soil erosion in the troubled areas of Borjomi Gorge. For example in the village Kvabiskhevi there is arranged a demonstration plot planted with Paulownia. This plant

is characterized by rapid growth, good soil protection ability and is used in beekeeping. It is possible to use this variety to protect the landslide areas [25].

Spatial-territorial development of Borjomi

According to the municipal council data, free state-owned land plots in the town of Borjomi are limited (basically they exist in kind of parks, public gardens and small green areas), so one of the promising direction of the urban development may be considered the revitalization of the so called brownfields, i.e. the functionless industrial-technical areas. In this regard, the influence of the mineral water deposit protection zones factor must be considered in the design solution and spatial development strategy. Revitalizing of brownfields is especially relevant in terms of protective green strips reduction. Transformation of former industrial areas into public green spaces is a global trend [24]. These areas are mostly found in the periphery. Sanitary protection zones of Borjomi water deposit are located adjacent to these areas. The planning of intensive green public spaces and protective greenery along the roads will improve the Borjomi deposit surroundings area, the ecological conditions and the aesthetic visual aspect of the entire resort town of Borjomi in view of the tourist traffic in the direction of Bakuriani.

Conclusion and recommendation

The study of Borjomi mineral water deposit and its assessment as the crucial urban and town-forming factor for the resort Borjomi shows that the spatial-territorial planning of the resort should take into account the actual condition of the sanitary protection zones of the deposit. The growing tourist flows in the resort city, positive trend of revival of the recreational tourism and increased interest in the use of Borjomi mineral water makes this issue particularly actual.

Considering the environmental problems and natural hazards as the impeding factors, the spatial-territorial documentation of the resort city can provide for the following preliminary planning measures:

- To respect that the sanitary protection zones of mineral water deposit are highly important during the planning of bypass roads and spatial territorial zoning;
- To apply the damaged and restorable areas of the green cover on the municipality map; to consider the protective greenery, particularly the protection of slopes from erosion in order to minimize the risk of development of natural geological processes;
- To plan and construct/pave the forestry roads, taking into account the best practices [23];
- In order to improve logistics, including communication during the natural disasters: to organize the extended urban communications, as well as to use the historical roads and hiking/forestry paths for the emergency evacuation corridors.

Acknowledgements

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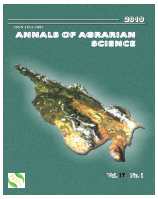

Genotype diversity and pathogenicity of Beauveria spp. Isolates from different ecoregions of Georgia

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A B S T R A C T

In this study entomopathogenic fungi *Beauveria* spp. from various ecoregions of Georgia have been studied morphological and on the genome level. After morphological analysis of monocultures, 6 strains were selected for further investigations. Several DNA fragments of isolated strains were amplified and sequenced, ITS region (the rRNA gene cluster), EF1 (the Elongation Factor 1-alpha) and (the intergenic) Bloc region. A BLAST analysis of sequences strains and their morphological studies has shown that the strains belong to two species: (1) *Beuveria bassiana* (strain- Bb001; Bb006) and (2) *Beauveria pseudobassiana* (strain - Bb007; Bb009, Bb010; Bb011). The regional differences between strains were identified through phylogenic tree. Base on this research, it was confirmed that the samples taken from the same region were located at the same species on phylogenic tree. This is a first report where most virulent strains identified and their virulent against the fall webworm, *Hyphantria cunea* have been studied. Two entomopathogenic fungal strains: Bb001 and Bb011 evaluated the pathogenicity on the larvae (L5-L7) *H. cunea.* Both fungal isolate were pathogenic. The mortality 68% - 71 % for *B.bassiana* (Bb001) and 76.9% -87% for *B.pseudobassiana* (Bb010) were observed. Mortality caused by Bb001 was significantly different between the concentration ($p<0,05$). One-way Anova, Single factor, $\alpha=0.01$: significance difference were found between the pairs of treatments: for Bb001 $1x10^8$ and $1x10^7$: $p=0,0025$, $F=18.7$; for Bb010 $1x10^8$ and $x10^7$: $p=0,000156$, $F=44.6$; for Bb001 $1x10^8$ and Bb010 $1x10^8$: $p=0,0001$, $F=46$. *B. pseudobassiana* proved highly pathogenic. Maximum of larvae mortality were observed in 4-9 day after treatment, were as in variance Bb010 mortality indicate later 5 -13 day.

Keywords: *Beauveria bassiana, Beauveria pseudobassiana*, morphological and molecular study, ITS, EF1, BLOC region.

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Introduction

Entomopathogenic fungi (EPF) are distributed in a wide range of habitats, including aquatic, forest agricultural, pasture, desert, and urban habitats [1-3]. Such fungi have the ability to regulate invertebrate pest populations and play an important role in biological control of various harmful insects and mites [4-7]. EPF, which naturally reside in soil, comprise an environmental reservoir of insect pest antagonists and are readily isolated from soils in various ecosystems [8].

Beauveria (Hypocreales: Cordycipitacea) is an anamorphic genus of soil borne facultative necrotrophic arthropod-pathogenic fungi [1, 9-11]. Many species of *Beauveria* grow naturally in all continents, except Antarctica, Arctic and have a cosmopolitan distribution [12]. The fungus has been isolated from more than 700 insect species belonging to nine orders [13-16].

There are several benefits of using *Beauveria* spp. to control invertebrate pest populations: it does not harm non-target organisms or the environment, it is easy to mass-produce and disseminate, it causes rapid host mortality, it has the potential to recycle in the environment, it is compatible with agricultural chemicals, and it is susceptible to genetic selection and DNA transformation [17-22]. Traditional identification of *Beauveria* spp. is principally based on conidial morphology. However, molecular phylogenetic analyses have revealed that the *Beauveria* genus includes cryptic species [23,24].

Beauveria spp. are widely used entomopathogenic fungi in agriculture. These fungi naturally produce biologically active toxins during their life cycle, which cause sustained, significant death of harmful insects of various species without human intervention. Importantly, this fungus is highly effective in controlling vectored human pathogens, specifically adult mosquitoes [25,26]. Also, pathogens that occur as saprotrophs and plant endophytes [27]. Therefore, it is crucial to undertake molecular characterization of *Beauveria* spp. for developing an effective strategy of biocontrol.

The South Caucasus region, particularly Georgia, is distinguished by its diversity of climate zones and biological species. Thus, some level of genetic diversity of entomophatogenic fungi in this region is expected due to the variability and uniqueness of local climates and host organisms. Local strains of such fungi may be adapted to their environment, which renders them of particular interest for use in biological control. Georgia has a large diversity of such fungi, due to its varying altitudes, eco-and cropping systems, which offers a unique opportunity to study local insect pathogens.

The objective of this study was to explore diverse habitats as potential sources of local strains of the entomopathogenic fungus *Beauveria,* their relative distribution in various geographical areas in Georgia, and their potential application as biological control agents against the target insect.

Materials and methods

Study site and collections of samples

Soil samples were obtained in 2007-2009, from four different geographical areas at different altitudes (600-1700 m a.s.l), representing different agro-and forest ecosystems of Georgia.

Isolation of entomopathogenic fungi

Single insects with the symptoms characteristic of entomopathogenic fungus have been detected in the population of Spruce bark beetles - *Ips typographus* L. adults and Colorado Potato beetles – *Leptinotarsa decemlineata* Say.

Fungi isolated from *I.typographus* and *L.decemlineata* were cultivated on artificial media and incubated at 23 ± 2° C for 12-15 days.

Colony growth and morphology

Colony descriptions and measurements were determined from cultures grown on full strength potato dextrose agar (PDA) (DifcoTM) at 23 ± 2° C in darkness at 14 day from inoculation. Terms and notations used to describe colony coloration, hyphae and conidia [28-30]. Microscopic measurements of conidiogenous cells and conidia were taken from PDA cultures at 5–15 days and images were acquired with a light microscope. Terminology for conidial shape follows [31], hyphae and conidia's measurements were taken.

Mycroscopial analyses

Infected insects, at first were examined for under stereomicroscope (MBC-9, USSR, magnification 40x). For the identification of entomopathogenic fungi, symptomatic insects and pathological material were incubated for five days in plastic vials for the moisture atmosphere.

Isolates of fungi cultivated on the artificial media: Potato Dextract Agar (PDA), and *Beauveria* selective media (BSM), for 10-14 days at 23±2^{0}C, until they developed feature permitting their identification as to species or genus [30,32].

The conidia were stained with lactophenol-cottonblue and examined with light and phase- contrast microcopy, to accurately detect morphological peculiarities (Zuzi,S120; magnification 400x, 1300x) for entomopathogenic microorganisms [24,28].

DNA Extraction and PCR amplification

Genomic DNA was extracted using the DNeasy Plant Mini kit (Qiagen, USA) [33]. Extracted DNA was quantified by gel electrophoresis [34]. PCR amplification of *Beauveria* spp. DNA was performed in 25 µl reaction volumes using primer pairs indicated in Table 1. ITS region (ITS4-ITS5)

Table. 1. *PCR Primers used in the study*

Name	Sequence [5´-3`]	PCR Fragment Size (bp)
ITS4 Fwd	5 ' -TCCTCCGCTTATTGATATGC-3 '	600
ITS5 Rev	5 '-GGAAGTAAAAGTCGTAACAAGG-3 '	600
EF2F Fwd	5 '-GGAGGACAAGACTCACATCAACG-3 '	700
1567R Rev	5 '-ACHGTRCCRATACCACCSATCTT-3	700
B22U Fwd	5 ' -GTCGCAGCCAGAGCAACT-3 '	1500
B822L Rev	5 ' -AGATTCGCAACGTCAACTT-3 '	1500

of the rRNA gene cluster [14, 33,35]. Elongation Factor 1-alpha [36,37] and the intergenic Bloc region [7,38].

For the ITS region, the PCR conditions included 3 min initial denaturation at 94 °C, 35 cycles of 1 min at 94 °C,1 min at 50 °C, and 1.5 min at 72 °C followed by final extension of 7 min at 72 °C. In the case of EF1-a and Bloc regions, 3 min initial denaturation at 94 °C, 35 cycles of 1 min at 94 °C,1 min at 55 °C, and 1 min at 72 °C followed by final extension of 10 min at 72 °C.

Bands of expected size were run on a 1% agarose gel containing ethidium bromide in 1X TAE buffer and were visualized by UV illumination using a digital imaging system. Nucleic acid concentration and purity ratios were obtained using NanoDrop.

Purified PCR products were sequenced from both ends using the following primers (Table 1).

Sequencing

Approximately 3-20ngof DNA were used in sequencing reactions with the ABI Prism® BigDye® Terminator Cycle Sequencing Ready Reaction kit v3.1 (Applied Biosystems, Foster City, CA). Cycle sequencing was performed on a GeneAmp® PCR System 9700 or 2720 Thermal Cycler (Applied Biosystems) according to the manufacturer's protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit Protocol, Rev A, Applied Biosystems). Dye terminators were removed from the cycle sequencing reactions using Multiscreen-HV plates (Millipore, Mississauga, ON) loaded with Sephadex G-50 superfine (Sigma, Oakville, ON). Clean reactions were analyzed on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems). A minimum read length of 700bp was generated for each of the reactions. Chromatograms were analyzed using ABI Prism® DNA Sequencing Analysis Software Version 4 (Applied Biosystems) to generate quality target sequences within the software's clear confidence range. Single nucleotide polymorphism (*SNP*) analysis, Indels (insertion/deletion) and mutation *detection,* and phylogenetic tree construction were performed using the computer programs Mafft and Blast **[39-41**] http://www.ncbi.nlm.nih.gov)

Target Insect – The fall webworm (FWW)

The fall webworm (FWW), Hyphantria cunea Drury (Lepidoptera: Arctiidae) is a one of most dangerous polyphagous pests in Georgia. Different instars of living larvae of H. cunea were collected manually from orchards and forest trees in West Georgia.

Inoculums preparation

Fungal suspensions of the isolates were prepared from 4 week-old cultures grown on PDA at 23±2°C, with good sporulation, using distilled water containing 0.01% (w/v) Twin 80. The obtained suspension was filtered through two layers of sterile muslin into a sterile 50-mL plastic tube, to remove the medium and fungal debris and was then shaken for 5 min using a vortex for homogenization. The concentration of spores in the suspensions from each fungus was determined using a haemocytometer and adjusted to two concentrations of $1x10^7$ and $1x10^8$conidia ml^{-1} for bioassay [30].

Bioassay

The 5th and 7th instars of larvae (L5-L7) target insects - H. cunea performed for the bioassay was treated with fresh culture suspension of Bb 001 and Bps010 (1.0 X 107, 1.0 X 108), placed in a glass jar with mulberry tree leaves and kept at room temperature ~23 ºC (day) /18 ~ ºC (night), RH with

14/10 light/dark regime. Dead or infected larvae with fungal symptoms were removed and placed in moister environment for development of conidia. Mortality of larvae was recorded on 3-18 days after treatment [32].

Data Analysis

All mortality data were corrected for control mortality Abbott's formula (1925) [42]. The percentage of larvae mortality for each concentration was analyzed using one way ANOVA; means were separated by Turkey's mean separation test. Mortality was considered statistically significant ($P<0.01$).

Results and analysis

Fungal distribution and isolation

Adult beetles of *Ips typographus*, infected with entomopathogenic fungi were found under tree bark in the maternal gallery of *Picea orientalis* in Borjomi-Bakuriani gorgeous. The fungi were identified using microscopic preparations made directly from mycelia developing on beetles in dead bark. After morphological analysis of monocultures, individual isolates of *Beauveria* spp. were collected. Conidia dimensions were between (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0) µm.

Samples were obtained from 2 different geographical sites at different altitudes (600-1700 meters above sea level (m a.s.l.) and climate zones, each representing a unique agricultural and forest ecosystem in Georgia. First investigation site is the Borjomi-Bakuriani region, located on the South Caucasian Mountain, second is the south slope of the Great Caucasus Mountain.

Adults of *Leptinotarsa decemineata* with mycosis symptoms were collected in the potato filed. Six isolates of *Beauveria* spp. were obtained from two regions of Georgia, as described in Table 2.

Morphological study of fungal isolation

Colony growth characteristics and appearance are similar among the majority of *Beauveria* species. All six isolates were cultured on full-strength Potato dextrose agar (Ø 90mm) at 23 ± 2° C at 12-15 days grows average 10–40 mm diam. The culture appears as white to light yellow colonies having a cottony, powdery, velutinous and woolly texture that frequently becomes farinaceous as conidia accumulate on the surface of aging cultures.

Microscopic observation of colony growth and conidia arrangement showed general and typical characteristics of *Beauveria.* After morphological analysis of monocultures, two species of *Beauveria* were identified: (1) *Beuveria bassiana* (Bb001; Bb006) and (2) *Beauveria pseudobassiana* (Bb007; Bb009, Bb010; Bb011). Morphological characterization of two *Beauveria* species are given in Table 3.

Table. 2. *Sites of investigation*

Name of isolates	Host	Region	Geographical location (lat.N,long.E)	Altitude	Habitat		year
					Habitat	Sub-habitate	
Bb001	*Ips typographus*	Borjomi-Bakuriani, LCM*	41⁰ 52' 40'' 43⁰ 22' 35''	950	Natural	Forest	2007
Bb006	*Ips typographus*	Borjomi-Bakuriani, LCM	41⁰ 45' 45'' 43⁰ 30' 58''	1000	Natural	Forest	2008
Bb007	*Leptinotarsa decemlineata*	Shovi, GCM**	42⁰ 35' 36'' 43⁰ 00' 88''	1800	Cultivated	Field crop	2009
Bb009	*Leptinotarsa decemlineata*	Shovi, GCM	42⁰ 23' 76'' 43⁰ 10' 13''	1800	Cultivated	Field crop	2009
Bb010	*Leptinotarsa decemlineata*	Shovi, Glola, GCM	42⁰ 33' 36'' 43⁰ 00' 58''	1730	Cultivated	Field crop	2009
Bb011	*Leptinotarsade cemlineata*	Shovi, Glola, GCM	42⁰ 44' 07'' 43⁰ 77' 01''	1780	Cultivated	Field crop	2009

*LCM- Lesser Caucasian Mountain; GCM**- GrateCaucasian Range

Table. 3. *Morphological characterization of Beauveria spp. isolates from different ecoregion of Georgia*

Species	Colony description	Colony size (Ø mm)	Size of conidia (µm)	Form of conidia
Beauveria bassiana (Bb001; Bb006)	cottony, powdery, velutinous, white or yellow white appressed to agar surface	13–30	(1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0)	globose, subglobose , broadly ellipsoid forming short chains
Beauveria pseudobassiana (Bb007; Bb009, Bb010; Bb011)	subvelutinous, velutinous to cottony, closely appressed to agar surface white or yellowish white	14–34	2–3 X 1.5–2.5 (1.5) 2.0 – 3.0 (3,5) x (1.2) 1.5 – 2.0 (- 2.5)	subglobose, ellipsoid

Colony of *Beauveria bassiana* on full strength PDAat 12 days (23°C), 13–30 mm diam., cottony, powdery, velutinous, white or yellow white, appressed to agar surface, at first white and becoming creamyyellowish white. Conidia aggregated in ball-shaped, spherical clusters among hyphae and white in mass. Colony reverseun colored to yellowish white. Vegetative hyphae septate, branched. Conidiogenous cells solitary, usually dense clusters, denticulate rachis, produced hyphae and conidia. Conidia (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0)µm, globose, subglobose, broadly ellipsoid forming short chains.

Colony of *Beauveria pseudobassiana* growth on full-strength PDA 14–34 mm diam. at 12 day at 23°C. Surface mycelium subvelutinous, velutinous to cottony, closely appressed to agar surface, white or to yellowish white. Conidia aggregated as, 0.1 mm spherical clusters, white in mass, conidia occasionally. Forming a farinaceous surface layer in older cultures. Colony reverse either uncolored or yellowish white. Vegetative hyphae septate, branched, hyaline. Conidia 2–3 X 1.5–2.5 (1.5) 2.0 – 3.0 (3,5) x (1.2) 1.5 – 2.0 (- 2.5) µm, primarily subgloboseor broadly ellipsoid, rarely ellipsoid, occasionally with inconspicuous hilum at base, hyaline, aseptate walls thin and smooth (Fig. 1).

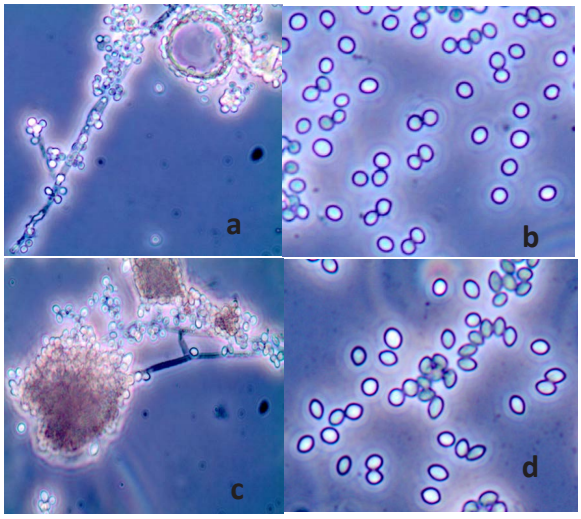

Fig.1. *Conidiogenous cells and conidia of Beauveria species. a-b B.bassiana; c-d B.pseudobassiana Molecular characterization of Beauveria spp.*

In samples that were obtained from two different regions of Georgia, the following sequence differences were identified: two samples from the Borjomi region and three samples from Shovi were identical to one another, respectively (Table 4).

The following genetic markers for characterization of *Beauveria* were used: the ITS region of the rRNA gene cluster, a region of EF1-α and the intergenic Bloc region. Mega6, Mafft, Blast, and DNASTAR were used to analyze nucleic acid sequence data [43].

Table. 4. *Strains isolated from two different regions of Georgia*

Strain designator	Short name of isolates*	Origin, species	Region of Isolates
Bb001	1B	*Beauveria bassiana*	Borjomi-Bakuriani
Bb006	2B	*Beauveria bassiana*	Borjomi-Bakuriani
Bb007	3S	*Beauveria pseudobassiana*	Shovi, CM*
Bb009	4S	*Beauveria pseudobassiana*	Shovi, CM
Bb010	5S	*Beauveria pseudobassiana*	Shovi, Glola, CM
Bb011	6S	*Beauveria pseudobassiana*	Shovi, Glola, CM

*Short name of isolates were given according for region

In this study we characterized genotypes of the entomopathogenic fungi of *Beauveria spp.* from different geographical and ecological regions of Georgia.To the best of our knowledge, this report describes the first time that *Beauveria* spp. have been characterized in Georgia using molecular genetics. Strains from different geographical regions were compared using phylogenetic analysis of sequences of multiple gene fragments. Samples obtained from the same region were grouped together as clades within the phylogenetic tree (Fig. 2, 3).

Fig.2. *EF1- alpha region of samples 5S, 4S, 3S, 2S (Shovi) and 6b, 1b (Borjomi)*

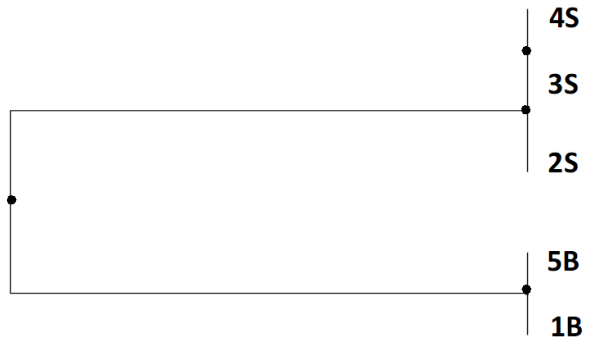

Fig.3. *ITS region 4S, 3S, 2S (Shovi) and 5B, 1B (Borjomi)*

Sequences of Georgian species were compared to other strains from database. Georgian strains were found to belong to multiple clades, with the greatest homology in samples belonging to the A (*Beauveria bassiana*) or C (*Beauveria pseudobassiana*) clade [12]. Samples collected in this study were identified as *Beauveria bassiana* (99-100% coincidence) through comparison of sequences obtained from the ITS region of the ribosomal gene with those published in GenBank.

ITS5 and ITS4 primers are verified reference sequences for PCR amplification of unknown species. Partial sequences of two nuclear loci, including EF1-α and the intergenic Bloc region, were also used in analysis. EF1-α was selected for analysis because of its widespread use in phylogenetic studies of *Beauveria* and other fungi [23, 44]. Bloc is a nuclear intergenic region initially developed for investigation of cryptic speciation in *B. bassiana* [38].

Georgian DNA samples, which were compared with database samples, have shown that selected strains of *Beauveria* spp. are located in diferent clades. The highest level of homology was detected between samples located in A and C clade (Fig. 4).

The EF1-alpha and the intergenic Bloc region were also compared (in contrast to ITS region - the rRNA gene cluster, EF1-alpha, Bloc region are more variable and contains more information) of Georgian samples with the samples taken from various country, located in the different clades.

Strains from Borjomi belong to clade A (*B.bassiana*), whereas strains from Shovi belong to clade C (*B. pseudobassiana)* (Fig. 4).

Only two samples were obtained for the Bloc region (Borjomi region). Both samples are identical and differ by one SNP from GenBank isolates. Comparison of KVL 03_76 [7] with Georgian samples is described in Table 5.

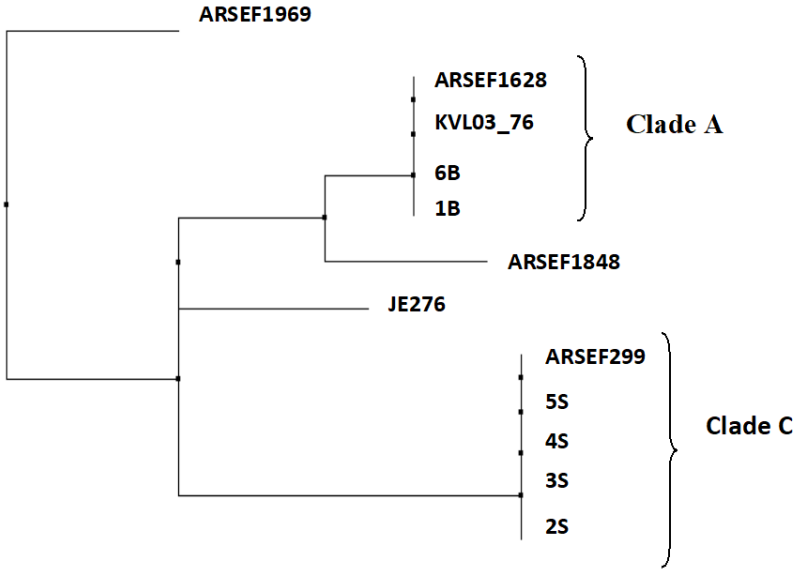

Fig.4. *Elongation factor-1a (EF1-alfa) phylogeny of Beauveria bassiana (B1, B6, S2, S3, S4, S5) Constructed Neighbour Joining tree using PID(Waterhouse et al 2009).The GenBank accession used for the analyses are Beauveria bassiana samples: AY531896(ARSEF 1628), EF193188 (KVL 03-76), AY531904(ARSEF 1848), EF193181(KVL 03-114), DQ376244(JE276), EF193192(KVL 03-91), AY531921, (ARSEF 292)AY531923(ARSEF 299), AY531907(ARSEF1969).*

Table. 5. *SNPs in Bloc region of Beauveria Bassiana*

Samples	Nucleotide position	Nucleotide
KVL 03 76	1118	G
B1, B6	549	A

After identification, we selected two entomopathogenic fungal strains: Bb001 and Bb011 and evaluated the pathogenicity on the larvae (L5-L7) *Hyphantrya cunea*. Both fungal strains were pathogenic. The mortality 68% - 71 % for *B.bassiana* (Bb001) and 76.9% -87% for *B.pseudobassiana* (Bb010) were observed (Fig. 5).

Mortality caused by Bb001was significantly different between the concentrations ($p<0$, 05). One-way Anova, Single factor, $\alpha=0.01$: significance difference was found between the pairs of treatments: for Bb001 $1x10^8$ and $1x10^7$: p=0,0025, F=18.7; for Bb010 $1x10^8$ and $x10^7$: p=0,000156, F=44.6; for Bb001 $1x10^8$ and Bb010 $1x10^8$: p=0,0001, F=46.

B. pseudobassiana proved highly pathogenic against *H. cunea* causing 76.9 % and 87,6 % mortality in both cases and the highest rates of fungal outgrowth (Fig. 5).

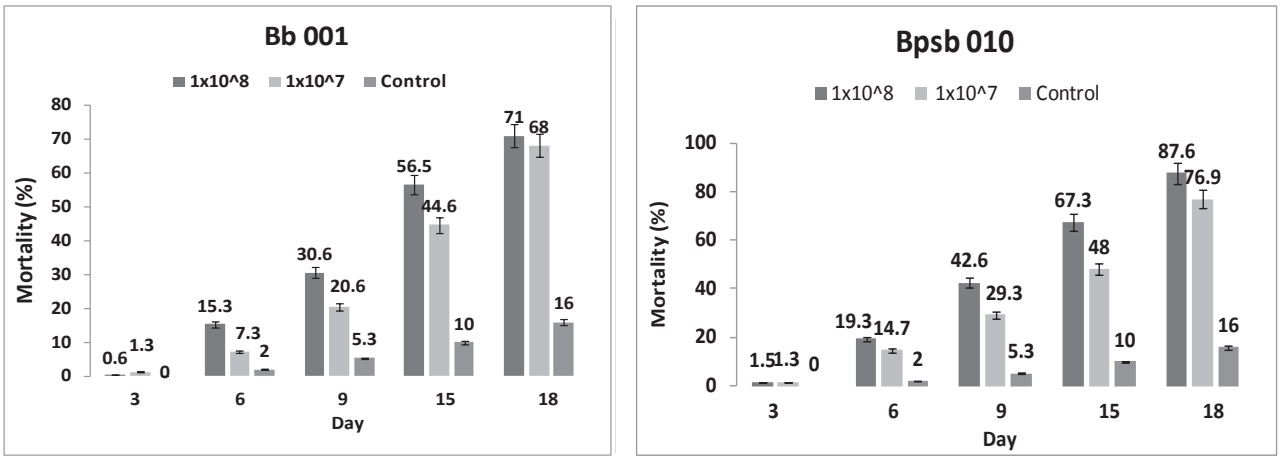

Fig.5. *Mortality (%) of larvae H. cunea after treatment with different concentration of EPF in laboratory per replicate (Mean %±SD), significant level α=0.01*

At high concentration ($1x10^8$) of promising isolates Bb001 showed significantly different results compared to Bb010. Maximum of larvae mortality were observed in 4-9 day after treatment, were as in variance Bb010 mortality indicate later at 5 -13 day (Fig. 6). However, virulence varied considerably. Development of mycosis in variance Bb001 were observed in the L5-L6 larvae and in cocoon, in variance Bb010 mycosis symptoms mostly were observed in L6-L7 instars larvae. A quick development of mycosis was observed in variance of Bb010.

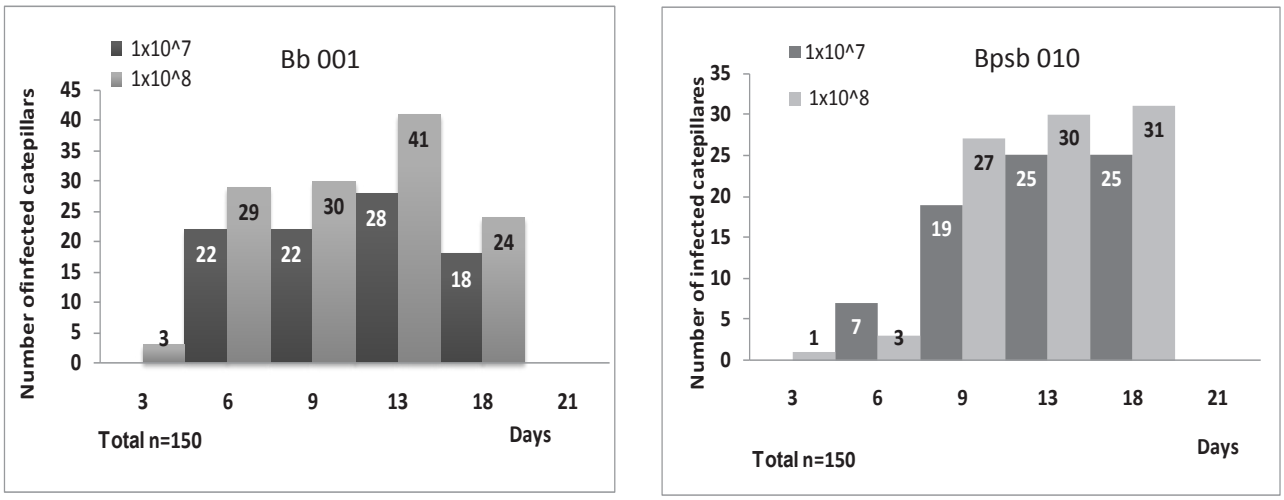

Fig.6. *More susceptible time (in days) for the appearance of mycosis infection in larvae of H. cunea by Bb001 and Bb011.*

Vertical transmission of Beauveria spp.

The surviving larvae hidden under the leaves and cordon, made cocoons and were transformed into pupae. They have been left overwinter until spring at 8±5 º C.

In the spring adult moths started to appear from the pupae, followed by mass emergence in 4-5 days with subsequent mating. The mass laying eggs lasted for 10-12 days. The numbers of oviposition groups and eggs were significantly larger in variance of Bb010 (2 ovipositions group) then in Bb001 (Fig. 7).

The emerged adults in Bb001was 68.4-15% and inBb010 - 48.4-42.18 60%. The larvae hatched seven to ten days later (Bb001 - 76.3%, Bb010-70%, Control - 89.5%) and began feeding on fresh leaves intensively in-group.

It should be noted, that in the instance of Bb010 a dimorphic male with incompletely formed right wings was observed.

These results suggest that theBb001and Bb010 isolates may be used to control *H. cunea*.

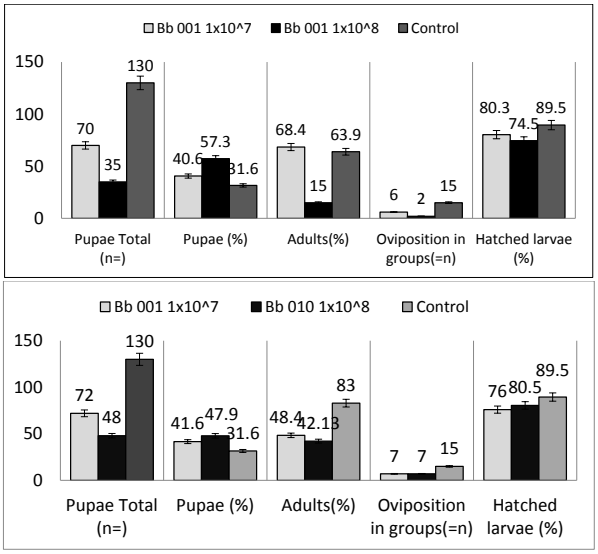

Fig.7. *The number of emergences adult of H. cunea from the overwinter pupae and they reproductive rate (=n - number)*

According to the results, the number of larvae that transformed into pupae was considerably less in the experimental group than in the control group.

Conclusion

Beauveria is a cosmopolitan anamorphic, a facultative soil-borne pathogen that has well-established characterization of the genus [45]. The genus *Beauveria* is characterized morphologically by globose to flask-shaped conidiogenous cells from which one-celled, terminal holoblastic conidia are produced in sympodial succession on an indeterminate, denticulate rachis. *Beauveria* species are distinguished principally according to characteristics of their conidia, which are typically smooth-walled, hyaline, 1.5–5.5 mm and globose to cylindrical or vermiform [46,47].

For the study of the genetic diversity and molecular ecology of *Beauveria, B. bassiana* and *B. brongniartii* are redescribed, and typifications are proposed for these species. Genealogical criteria were used to delimit the six novel clades orphy logenetic terminals that are described here as new species, including: *B. asiatica, B. australis, B. kipukae, B.pseudobassiana, B. sungii B.varroae*. and *B. amorpha*has been published and an epitype specimen has been proposed [10,24], which occur as multispecies assemblages in both natural and agricultural habitats [7,38].

Fall webworm (FWW) - *Hyphantria cunea* Drury (Lepidoptera: Arctiidae) is the most polyphagous insects all over the world's, has invaded in Georgia and well adapted to local climate condition. There are found rare Georgian endemic plant in Caucasus within its host range (some 600 plant species). Population of *H. cunea* increased last two decade, so the Georgian Government has declared as a national problems, because our purpose was to evaluate efficiencies of local isolates of *Beauveria* spp. on the *H. cunea* [48]. Within the framework of the assessment of the biodiversity of *Beauveria* spp. isolated from different ecoregion of Georgia, has been evaluated using both morphological criteria and the molecular taxonomic characterization. Previous classification of isolates to presents *Beauverri* spp.

Morphologically and the molecularly different single-spore-derived cultures here referred: (i) *Beauveria bassiana*, (Bb001; Bb006) isolated from *I.typographus* population in the Lesser Caucasus Mountain and (ii) *Beauveria pseudobassiana* (Bb007; Bb009; Bb010; Bb011) isolated from the population *Lepthinotarsa decemlineata* and from South slope of the great Caucasus Mountain soil.

In this study, we characterized genotypes of the entomopathogenic fungi *Beauveria* from various regions of Georgia. To the best of our knowledge, this report describes the first time that *Beauveria*

spp. The goal of our research was to test the isolated virulent *Beauveria* sp. against a target insect species. The fall webworm – *H. cunea* appeared to bean excellent candidate because it is one of the most dangerous polyphagous insects in Georgia that affects more than 600 species of plants. Two isolates from the mountanious regions of both East and West Georgia were tested. In both cases, that the virulence of these strains towards the *H. cunea* was high. There are preliminary data where used to ascertain the entomopathogenic activity of the isolates.

Sequencing data of the IF1 a, ITS and and the BLOC DNA regions of the *Beauveria* isolates confirmed the presumed species: sample 6, 1 was confirmed as *Bauvaria bassiana*, while samples 2,3,4,5 were confirmed as *Beauveria pseudobassiana*. The initial evaluation of these species was done on the lowest taxonomic level. At the same time, the picturesclearly show the difference between the conidia of *B. bassiana* and those of *B. psedudobassiana*. About distribution of *B. pseudobassiana,* it is found both in Europe and North America, and likely elsewhere [24, 45, 49].

GenBank database material of *B.bassiana* with respect to other *Beauveria* species and the fact that above all older entries are referred to as *B. bassiana* [45]. Compared the sequencing results with the genebank's outdated data, all samples were determined to belong to *B. pseudobassiana*, despite the fact that, according to the modern classification, part of these amples can now be classified as *B. pseudobassiana*. Molecular testing, therefore, turned out to be more effective in species-specific identification of the highly virulent fungi isolates from the distinct ecological areas of Georgia.

Acknowledgments

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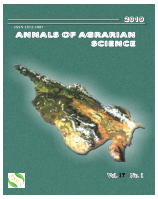

Effect of the concentrate "Rumifos" on the mass and the degree of reproduction of rain worms

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A B S T R A C T

The article deals with study of effect of a new, innovative, plant product concentrate of local origin "Rumifos" on the mass and the degree of reproduction of the rain worms and, establishes its efficient and optimal doses. The maximal (Max.), nominal (Nom.) and minimal (Min.) doses in milliliters of the plant substance of the concentrate "Rumifos" to be added to the substrate of the trial group are calculated.

Weighting and counting the cocons were conducted in three stages, on 21st, 31st and 41st days after beginning the experiment. Average change of masses by grams and percent in the control and trial groups and average number of cocoons in pieces and percent, were studied in each stage, while. An average change of the mass of trial groups in comparison with the control one during the total period of the experiment and an average degree of increase of the reproduction of cocons, as well as the efficient and optimal doses of the concentrate "Rumifos" were established also. It is found that when balancing the substrates of the rain worms trial in comparison with the control group, no significant changes take place. As to the degree of reproduction of the cocons, on the first stage, this indicator in comparison with the degree of reproduction of the control group cocons (100%), it varies within 1411.0–1633.67% ranges, while the optimal and efficient dose of the plant concentrate "Rumifos" is 0.31ml., calculated for 600g substrate. Thus, balancing the rain worms substrate by "Rumifos", has a positive effect on the mass growth of the worms and increases considerably the degree of reproduction of the cocons, that, as we suppose, is preconditioned by diversity of various biologically active chemical class compounds existing in the composition of the concentrate "Rumifos". The obtained results will have importance for that direction of vermiculivation, where the protein mass of the rain worms is used for balancing the combined feed for farm animals and poultry, as a vitamin-protein, high quality concentrative additive.

Keywords: Vermiculture, Rain worm, Cocon, Concentrate, Rumifos, Substrate.

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Introduction

Improvement of the ecological state of the environment and the health of population is a global problem of nowadays. This problem is as actual for the contemporary world as never [1-12]. To resolve them, production of the ecologically friendly agricultural products is of an essentially important, where the use of bio-humus - the ecologically clean, natural, biologically active organic fertilizer received by processing the organic waste (wastes of the farm animal manure and various plants waste) instead of the chemical fertilizers, herbicides and pesticides, would play a decisive role, were the rain worms have a central role. So, bio-humus is an unique microbiological mineral fertilizer received from the worms. It is approved that the humates contained in the bio-humus, are not toxic,

cancerogenic and mutagenic. The most important difference between the bio-humus and the simple organic fertilizer is that bio-humus contains a large quantity of of the water soluble necessary elements-nitrogen, phosphorus, potassium. The microelements are also transiting into the movable form. Content of the water soluble fractions in the bio-humus is too high. On the other hand, the protein mass of the rain worms is a high-quality vitamin-protein concentrative addition for balancing the combined food of the farm animals and poultry, that, in its turn, is a guarantee of production of the agricultural bioproducts: bio-meat, bio-milk, bio-egg. For this purpose, one of the obstructing factors is its expensiveness. That is why, study of impact of various factors on increase of the protein mass and the degree of reproduction of the rain worms seems to be very important.

As earlier as in the ancient Egypt period, the farmers considered the warms as a precondition of future harvest and, used successfully the river Nile sludge processed by the rain worms. To receive the agricultural cultures, the old Egyptians worshiped the rain worms, since considered them the sacred animal and, restricted their export from the country. Aristotle named them the "earth intestines and, it is true: while passing the earth and the plant waste through their intestines, the worms make the soil rich. They are the unique representatives of the alive nature. All the species of the rain worms differ from each other by their body structure, place and rule of living. However, together with the distinguished features, all the species have the following common features:

- They do not die since they never become ill;
- They are not carriers of human, animal, bird, fish diseases;
- They do not need expensive foods, because they process the organic waste;
- They do not need light and ventilation;
- They can stay in a building that has no windows;
- They do need big energy consumption. A necessary temperature in a building is 18-240C;
- They need the food just once in 2-3 days. They process during a day the bio-humus equal to their body weight.

Today, nobody makes it questionable that the rain worms can play an important role in resolving the food product-related worldwide problem and ensure a sustainable and ecologically balanced development of the agriculture. The rain worms are the source of growth of stimulators of proteins, vitamins, and other biologically active substances not only for the plants but for the animals as well. There are more than 40 proteins just in the celomic fluid of the rain worms, which demonstrate number of the biological effects: cytolytic, proteolytic, hemoglutterial, anti-tumor, mitogenic antibacterial, antioxidative, immunogenic effects, etc. The researchers pay attention also on the giant molecule of hemoglobin, neuropepdide, growth factors. By a set of the biologically active components, exclusivity of the effects of some species, and their availability, the rain worms have no equal analogues in the world [13-17]. Together with a variety of these properties, a rapid growth of biomass of the rain worms and a high degree of reproduction, not pretentious attitude to the food composition and the feeding conditions and a high percentage content of the protein in the organism, as well as high proficiency and ecologically friendly nature–all this determine (ensure) a possibility of a massive industrial production of the rain worms and necessity of use of this hew bio-resource for receiving the high-quality vitamin-protein containing food addition in the fields of poultry, livestock and fishery, that enables us to move to the production of organic meat, egg, and milk [18-23]. For studying the effect of the concentrate "Rumifos" on the mass and degree of reproduction of the rain worms, a new specie of the rain worm "Georgian New" was selected, which is produced in the bio-farm of the company "Macro-Prim LLC." by a breeder Guram Gejadze [24].

Computational Method

On the basis of the sources of literature available to us, we could not find other researches similar to the one conducted by us. Therefore, we have implemented the study using our own methodology, where we used the method of weighting for determining the alive mass of the worms and the method of counting for determining a quantity of cocons. The object of research is: Rain worm, substrate, cocons and concentrate "Rumifos".

In the coursed of the experiment we have studied:

- The dynamics of change of the rain worms mass; Weighing was conducted in three stages-on 21st, 31st and 41st days after beginning the experiment (average change of the mass in

grams, in each stage and each group);
- Quantity of the cocoons produced by the rain worms. Average number of cocoons in the same time ranges;
- Change of the mass in comparison with the control group and number of the cocons by percent, in each stage;
- Average quantity of total masses and cocons in percent, for all three stages during the total period of experiment;
- Efficient and optimal doses of the testing sample of the concentrate "Rumifos" for the substrate of the rain worms.

Results and Analysis

Joint researches targeted at studying the effect of various additives on the protein mass and the degree of reproduction of the rain worms are continued by participation of the Iv. Javakhishvili Tbilisi State University, Laboratory for the Agricultural Chemistry Problems at Petre Melikishvili Institute of Physical and Organic Chemistry, and Bio-rational Technologies Research Center ("BrTRC") with involving the effect of the concentrate "Rumifos" on the mass and the degree of reproduction of the rain worms. The product - concentrate "Rumifos" is the intellectual property of the company "Lark LLC.", the trade mark and the technology of receipt are protected (an unique technology of receipt of the product is developed on the base of the Bio-rational Technologies Research Center). The concentrate "Rumifos" is received through extraction of the endemic raw grain crops spread in the mountainous areas of Georgia. The biologically active substances contained in the concentrate are the total of the compounds of various chemical classes [ferments, co-ferments, amino-acids, poly-phenolic substances, flavonoids, phyto-biotics) [25, 26].

Its positive effect on the intestinal tract of the cattle (restoration of a natural microflora of intestines), and high rate of food consumption are established.Besides, its stimulating effect on the immune system and metabolic processes in the animals, on growth of their live mass, preservation of steadiness, etc., are studied [27].

Based on the important sciebntific sources, the derivatives of imadizole and pyrazole, which are identified in the "Rumifos" presumably have the anti-tumor properties [28-30].

The purpose of our work was to balance the substrate by the concentrate "Rumifos" and establish its effect on the changes and degree of reproduction of the protein mass of rain worms. We calculated the defined quantities (200-200g.) of the dry substrate for both the control and trial groups, and 3-3 doses of defined quantity of the "Rumifos" for adding to the substrate of the trial groups (Max=0.62ml.; Nom=0.31ml.; Min=0.16ml.), in milliliters.

In the course of the experiments on the rain worms, our goal was a. To determine changes of the protein mass and the degree of reproduction in the works, caused by effect of the "Rumifos" being added to the substrate; b. To define the efficient and optimal doses of "Rumifos". For this purpose, we have conducted test for four groups, each with three repetitions: in all three repetitions, the substrate of the control group was soaked by an ordinary water, the I trial group–with a water solution of maximal (0.62ml.) doze of "Rumifos", II trial group - with a water solution of nominal (0.31ml.) doze of "Rumifos", and the III trial group–with a water solution of minimal (0.16ml.) doze of "Rumifos". For repetitions in each group, we selected 5-5 worms of comparably equal masses. The first weighting and counting of the cocoons were conducted on the 21sty day after beginning the experiment, while the second and the third procedures were conducted on the 31st and 41st days., respectively. In cases of all three weighting, we calculated average change of the mass in each group, mass changes by, Average and total number of cocons by percent. After the end of the experiment, average masses and average number of cocoons were counted in percent, for all stages and for the control group and 3 trial groups (Table).

Table. Results of The Test on the Rain Worms

Groups (Dose, ml)	Container #	Stages of testing																											
		initial stage			I							II							III							Result	
		Number of worms	Mass (g)	Mass (average)	Mass (g)	Mass (average)	Mass change, (g)	Mass change (%)	Number of coconuts	Number of coconuts (average)	Number of coconuts (%)	Mass (g)	Mass (average)	Mass change, (g)	Mass change (%)	Number of coconuts	Number of coconuts (average)	Number of coconuts (%)	Mass (g)	Mass (average)	Mass change, (g)	Mass change (%)	Number of coconuts	Number of coconuts (average)	Number of coconuts (%)	Mass change, (%)	Number of coconuts (%)
Control. (0)	1	5	1.22	1.27	3,05	2.99	1.72	100	1	1	100	3.25	3.20	0.21	12.02	4	3.33	333.0	3.31	3.25	0.05	2.91	2	1.67	167.0	38.31	200
	2		1.40		3.03				2			3.15				6			3.25				3				
	3		1.18		2.89				0			3.19				0			3.18				0				
Max. (0.62)	4	5	1.40	1.18	3.85	3.40	2.22	129.65	3	3.67	367.0	3.24	3.23	1.01	-10.06	21	21.33	2133.0	3.22	3.20	2.19	-0.52	17	17.33	1733.0	39.69	1411.0
	5		1.12		3.21				4			3.25				21			3.24				18				
	6		1.01		3.15				4			3.01				22			3.15				17				
Norm. (0.31)	7	5	1.22	1.23	3.29	3.23	1.99	116.09	8	6.67	667.0	3.45	3.34	0.11	6.39	21	21.67	2167.0	3.34	3.27	-0.07	-3.89	20	20.67	2067.0	39.53	1633.67
	8		1.19		3.15				6			3.25				22			3.22				21				
	9		1.28		3.24				6			3.31				22			3.25				21				
Min.	10	5	1.18	1.22	3.79	3.41	2.19	127.32	6	6.33	633.0	3.80	3.52	0.11	6.39	21	20.67	2067.0	3.82	3.50	-0.02	-1.16	21	20.33	2033.0	44.18	1577.67
	11		1.34		3.15				7			3.40				21			3.35				20				

As the Table shows, at the beginning of the experiment, a maximal growth of the mass by 1.72g. (100%) is observed in the control group on the first stage, while on th second and the third stages, a rate of mass growth is reduced and makes 0.2g. (12.02%) and 0.05g. (2.9%), respectively. In parallel to such mass change in the control group, an average number of the cocoons on the second stage is at first increased from 1 (10%) to 3.33 (333.0%) while afterwards on the third stage is reduced to 1.67 (167%).

In the I trial group where "Rumifos" was added with the maximal dose (0.62ml.), on the first stage an increase of the mass by 129.65% in comparison to the control group was observed, while on the second and the third stages the masses were reduced by 10.06% and 0.52%, respectively. As to the degree of reproduction of the cocons, in comparison to the control group, their number on the first stage was increased by 367.0%, on the second stage – by 2133.0%, and on the third stage – by 1733.0%.

In the II trial group where the nominal dose (0.31ml.) of "Rumifos" was added the mass increase rate on the first stage was by 116.09%, on the second stage – by 3.34%, while on the third stage the mass reduction was reported by 3.89%. In parallel to such changes of masses, a degree of reproduction of the cocons on the first stage was increased by 667.0%, on the second stage–by 2167.0%, and on the third stage –by 2067.0%.

In the II trial group with adding the minimal doze (0.16ml.) of the "Rumifos", changes of masses of the worms are of a following nature: On the first stage, the mass is increased by 127.32%, on the second stage–by 6.39%, while on the third stage the mass is reduced by 1.16%. A degree of reproduction of the cocons se in the same trial group is increased by 633.0% on the first stage, by 2067.0% on the second stage, and by 2033.0% on the third stage.

During the whole period of the experiment, (in total for all three stages) an average mass increase is reported and, in the control group it is equal to 38.31%, in the I trial group – 38.69%, in the II trial group – 39.53%, and in the III trial group – 44.18%. as to the total change of the cocoons reproduction degree, it has the following nature: In the control group – 200%, in the I trial group 1411.0%m in the II trial group – 1633.67%, and in the III trial group – 1577.67%.

Conclusion

Based on the analysis of the obtained results of the experiment, we can conclude the following: In the whole period of the experimental process, the mass change of the rain worms is characterized by one and the same nature. Namely, on the first stage, in both the control and trial groups, a maximal increase of masses of the rain worms is reported, while on the second and the third stages, a rate of the mass increase is reduced and, such a reduction is as lower as the degree of reproduction of the cocons is higher.

Increase of the degree of reproduction of the cocons is of the following nature: In all the trial groups in comparison with the control one increase of the degree of reproduction of the cocons is reported and, such the increase reaches at the maximal value on the second stage (2167%), to which a maximal reduction of the masses corresponds (-3.89%).

By total results of all the three stages, we conclude: A total change if the mass for all groups are positive and, reaches at the maximal value in the III trial group (44.18%), while total number of cocoons reaches at the maximum in the II trial group (1633.67%). Based on the obtained results, effective and optimal dose of substrate concentrate "Rumifos" 0.31 ml for 600mg. substate is selected.

Balancing the warms substrate by "Rumifos" has a positive weffect on the mass growth of the warms and increases considerably the degree of reproduction of the cocoons that, as we suppose, is preconditioned by diversity of various biologically active chemical class compounds existing in the composition of the concentrate "Rumifos". The obtained results will have importance for that direction of vermiculivation, where the protein mass of the rain worms is used for balancing the combined feed for farm animals and poultry, as a vitamin-protein, high quality concentrative additive.

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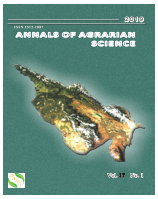

Genetic and Farming Features of the Kakhetian Pig Gene Pool and Epizootic Characteristics of Helminthiases of This Breed

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A B S T R A C T

The work reflects the genetic and economic potential of Georgian aboriginal Kakhetian pig. Based on the genetic systems of erythrocytical antigens, blood serum proteins, a number and morphology of chromosomes, the parameters of the skull structure (craniology) and the phenotypic features, it can be strongly suggested that the Kakhetian pig descended from the Caucasian population of European wild boar- Sus chrofa attila - through selective breeding in a process of the direct domestication. The results of our research including an amazing phenotypic similarity of the Kakhetian pig with its wild ancestor highly support the above suggestion. The Kakhetian pig farm was established with the purpose to reproduce this breed and provide both small family household farms and relatively large farms of the villages adjacent to fruitful forests located on the southern slopes of the Caucasus ridge, with piglets of the above breed. The first results of the Kakhetian pig breed recovery activities are presented in our research. In 2015-2017, the first slaughtering of Kakhetian pigs was carried out, which was followed by the drying of raw meat. The products of Georgian Hamon are already available on some Georgian markets. The conducted research has revealed four most prevalent pig helminthoses in Georgia. The study has shown that the Kakhetian pigs were less infected by helminthes when compared to the hybrid pigs, which can be suggestive of their relatively high resistance potential to these intestinal parasites.

Keywords: Pig, Immuno-genetics, Chromosome, Selection, Craniology, Helminthiasis.

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Introduction

The Kakhetian pig is unique, because during an entire year they can be kept in fruitful forests on the southern slopes of the Caucasus ridge, where they feed on forest fruits (rue, chestnut, nuts, wild pear, wild apple, medler, cornel, blackberry, hawthorn, barberry, currant, bilberry, etc.), resulting in the low cost and high quality delicious meat.

Over the years, a little attention has been paid to Kakhetian pigs. Due to this fact they have faced the threat of extinction. At present, investigations using selective breeding are underway in order to recover this breed, increase its reproduction and productivity, and to provide both small family household farms and large farms with its off-springs (piglets of the above breed).

The collaborative research of the SRCAG with the Spanish company "DERAZI iberico" is aimed at the promotion of the research on the Kakhetian pig, and at the production of the world famous brand Hamon and other meat products based on this breed in Georgia for local and international markets. The products of Georgian Hamon are already available on some Georgian markets.

The Kakhetian pig is almost identical to the wild pig. Hence, its breeding and rearing will facilitate the development of hunting farms and subsequently, sustainable hunting tourism in Georgia.

It should be indicated that one of the prerequisites, for the successful accomplishment of the targeted tasks, and for the production of the high

quality meat, is the rearing of healthy livestock. Therefore, alongside the implementation of disease control measures, it is necessary to determine the current epidemiological status of pig helminthiasis, without which the prevention of helminthosis will be impossible.

Materials and methods

The research object was a nomadic Kakhetian pig population. Its genetic and craniological studies were conducted with the commonly accepted methods (taking the measures of a skull and calculating the craniological indexes, determining antigenic and genetic frequencies in the genetic systems of blood groups, studying the number of chromosomes and morphological structure according to leukocyte analysis) [1-5] in the laboratory of Animal Immuno-genetics and Hybridization of the Institute of Cytology and Genetics (Novosibirsk) of the Siberian Division of the former Soviet Union Academy of Sciences and at the department of Animal Husbandry and Genetics of the Zootechnical-Veterinary Research Institute of Georgia.

For the experiments, the Kakhetian pigs were selected in their classical distribution zones only in the villages of Akhmeta and Dusheti municipalities (East Georgia). Presently, they are farmed in the experimental base of the SRCAG, where scientific research works are underway to increase their body mass and productivity. The consolidation of the breed and determination of the breed standards will provide the basis for determining of the gene pool of the Kakhetian pig.

For determining the epidemiological status of helminthiasis in pigs in Georgia, in 2014-2017, the scatological studies were conducted on pigs aged 4-10 months in both the small family household farms and the large farms. In these studies, the flotation method [6] was applied for the diagnosis of helminthiasis in the Kakhetian and hybrid pigs.

Results and discussion

As suggested above, the Kakhetian pig descended from its wild ancestor via the selective breeding in the process of direct domestication, and which is exhibited in its phenotypic similarities-including transversal stripes of newborn piglets,- with the wild ancestor and in the results of our studies on genetic and craniological patterns/indicators. In particular, , our experiments showed that erythrocyte antigen Ga from the genetic system G of blood groups was predominantly characteristic to the Kakhetian pig and the Caucasian wild pig, while in cultural breeds they occur less frequently [7-10].

Radically different data have been received regarding to a double-allele genetic system F of the blood groups. In particular, neither the Kakhetian pigs nor European and Caucasian wild pigs had an antigen - Fa, which indicates their great phylogenetic similarity. However, almost all individuals of the Asian pigs have an antigen – Fa on the erythrocyte membrane. The vast antigenic diversity and significant differences in gene frequencies were revealed with the genetic system E in some of the previous studies [11-15,].

It is known that a diploid number of the chromosomes of domestic pigs is 38, while the European wild boar has 36 or 38 chromosomes. According to our research, the Kakhetian pigs had 38 chromosomes, and the north and south Caucasian wild populations has 38 chromosomes – a karyotype identical to the one of the Kakhetian pig (out of 18 pairs of autosomic chromosomes, 9 pairs are submetacentric, 6 pairs – telocentric, 3 pairs – metacentric, and also 1 pair – sex chromosomes, 19 pairs in total). This indicator, along with the phenotypic similarity and transversal stripes in newborn piglets, is one of the proofs that the Kakhetian pig is descended by direct domestication of its wild ancestor [3-5]. The above assumption is supported by the results of the craniological study of different breeds of pigs (the skull structure, the shape and size of the lachrymal bone, a general shape, height, width and length of the skull) (Table 1).

Table. 1. *The measurements of skulls of South Caucasian wild, Kakhetian and Landrace pig breeds*

Skull measurements (mm)	Pig breeds					
	South Caucasian		Kakhetian		Landrace	
	♀ 4- year-old	♂ 6- year –old	♀ 5-year-old	♂ 4,5 – year-old	♀ 4-year-old	♂ 4-year-old
Total length	305	420	337	355	310	310
Basal length	269	363	299	316	287	294
Length of the brain part	78	108	82	89	84	83
Length of the facial part	227	312	255	266	226	227
Width between zygomas	127	152	154	161	167	191
Width between eye sockets	63	84	78	73	89	108
Length of the lower part of lachrymal bone	27	39	24,5	33	25	27
Height of lachrymal bone	21	27	20	22	23	25
Maximum length of a skull	184	220	192	195	238	266

As shown in Table 1, it is clear that a total length of the skulls of the Caucasian wild and Kakhetian pigs (420 and 355 mm respectively) significantly exceeds the one (310 mm) determined for the Landrace pig. Based on the analysis of the length and height of a lachrymal bone, we identified a form of the oblong parallelepiped on the skulls of the South Caucasian wild and Kakhetian pigs, while in Landrace pigs the skull shape appeared to be square. It was found that the height of the Landrace breed skulls (266 mm) exceeds the ones (220 and 195 mm respectively) that were determined for the South Caucasian wild and Kakhetian breeds. There are also significant differences between the craniological patterns of the skulls of the South Caucasian wild and the Kakhetian breeds and the ones of the skull of the landrace breed, that exhibit the phenotypic similarity between the South Caucasian wild and Kakhetian breeds. These similarities are further shown in the photo material as follows.

The Kakhetian pig is a late-maturing and low-productive animal. Its late-maturing and low productivity have been due to the direct domestication of this breed under extreme conditions. Its live weight is 100-110 kg, fertility - 5-8 piglets, body length - 100 cm, hearth girth - 100-105 cm, withers height - 65 cm, milk production - 25-30 kg, carcass yield - 63%. The above features allow the Kakhetian pig to be at the same level with cultural breeds; moreover, in some cases, it exceeds some other breeds [4].

In 2014-2017, in the villages of Kakheti and Mtskheta-Mtianeti regions, we explored the areas of Kakhetian pig distribution and condacted initial zoo-technical studies. Based on the obtained results, the typical individuals of the Kakhetian pig were selected, which were then transferred to the experimental farm, where currently the selective-research works have been underway. Currently, the experimental farm maintains 140 pigs of different ages.

In 2015-2017, the control slaughter of Kakhetian

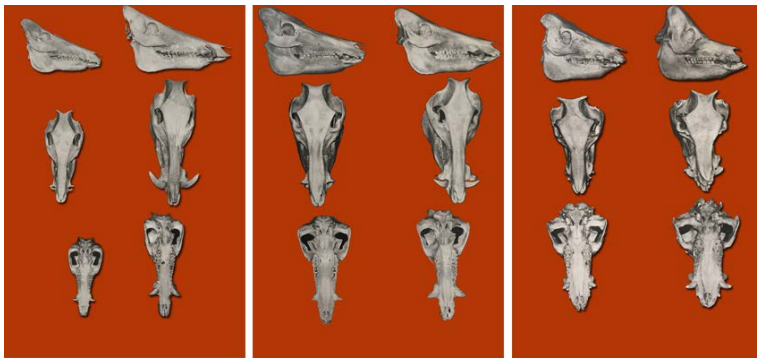

Fig.1. *Conidiogenous cells and conidia of Beauveria species. a-b B.bassiana; c-d B.pseudobassiana Molecular characterization of Beauveria spp.*

The skulls of the Kakhetian pigs are low, the profile - long and straight, while the skull of the Landrace breed is relatively high, the profile - short and bent.

It must be also indicated that newborn South Caucasian wild and Kakhetian piglets carry transversal stripes on their body, which disappear at the age of 3-4 months. The Kakhetian pigs can be characterized by a herd instinct, due to which they stay together on the pastures or in the forest. They have a well-developed ability to navigate and memorize their location. Occasionally, a few days before farrowing, a pregnant pig leaves the herd, choosing a dry place for farrowing on a hill that is usually covered with shrubs. After a certain period of time, together with piglets it returns to the herd, but sometimes, it chooses to stay in the woods and becomes wild.

pigs was held, which was followed by drying of raw meat. The process has been completed, and the first Georgian Hamon made from the Kakhetian pig breed is already available. At present, the third series of pig slaughter is planned and the production of other meat products alongside Hamon is intended.

In Georgia, pigs are kept in nomadic or semi-stationary conditions. In homestead farms, locals keep predominantly 2-3 pigs, which move around the settlement during the day. Such a practice bears a risk for acquiring and transmission of helminthiasis.

In the second half of the XX century, incidence and prevalence of helminthiasis in pig populations were studied in Georgia [6,17]. The results of our study, which was conducted to determine the epidemiological status of pig helminthiasis in the country, are shown in Table 2.

Table. 2. *Helminthiasis cases in populations of the Kakhetian and complex hybrid pigs in Georgia in 2014-2017*

Breed	District, country	Invest. (No.)	Infect .(No.)	% of Infect.	Among them							
					Asc	%	Oes	%	Tr	%	M et	%
Kakhetian	Telavi	13	7	-	0	-	7	-	0	-	0	-
	Akhmeta	135	54	40,0	3	2,2	50	37,1	2	1,5	2	1,5
	Tianeti	45	21	46,7	0	-	21	46,7	0	-	0	-
	Dusheti	21	13	61,9	2	9,5	13	61,9	0	-	0	-
	Gardabani	95	29	30,5	0	-	28	29,5	3	3,1	0	-
	Kvareli	10	7	-	0	-	7	-	0	-	0	-
	Total	319	131	41,1	5	1,6	126	39,6	5	1,6	2	0,6
Hybrid	Georgia	728	347	47,7	117	16,1	237	32,6	34	4,7	37	5,1
Total		1047	478	45,6	122	11,6	363	34,7	39	3,7	39	3,7

Invest. – investigated; infect. - infected
Asc – Ascarida; Oes – Oesophagostomum; Tr – trichocephalus; Met – Metastrongylus;
The percent of infection was not calculated when a number of the examined pigs was less than 15.

Our study revealed that in Georgia, the following four helminthiases predominate in the pig populations: ascaridosis, oesophagostomosis, trichocephalosis and metastrongylosis, - the agents of which had infected 45.6% of the animals investigated. It is noteworthy that mixed helminths predominate more in the hybrid pigs (47.7%) than in the Kakhetian pigs (41.1%). The most common helminthiasis was oesophagostomosis, which was observed in 34.7% of the pigs tested. The extensiveness indicators of infection of the hybrid and Kakhetian pigs with oesophagostomosis made 32.6% and 39.6% respectively. It was found that the Kakhetian pigs were less infected with ascaridas, trichocephalus and metastrongylus (1,6%, 1,6%, and 0,6% respectively) than hybrid pigs (16,1%, 4,7%, and 5,1% respectively).

After the control slaughter of the Kakhetian pig, the carcass of five pigs were examined by the trichinelloscopy method. No trichinellas were observed during the examination.

Conclusion

1. Double-allele genetic systems G and F of blood groups, these immuno-genetic indicators of North and South Caucasian wild and Kakhetian breeds are identical. The above two breeds have identical karyotypes - 38 chromosomes as well. In the European wild pig, karyotypes reflect either 36 or 38 chromosomes. These indicators, along with transversal stripes in newborn piglets, strongly suggest that the Kakhetian pig descended from its wild ancestor in a process of the direct domestication.

2. The shapes of skulls of the South Caucasian and Kakhetian pigs are similar. However, they are dramatically different from skulls of the Landrace pig. In particular, the skulls of South Caucasian wild and Kakhetian pigs are low with the long and straight profile, while the skull of the Landrace breed is relatively high with theshort and bent profile.

3. Our studies on the selection of pig populations allowed us to form a herd consisting of 140 typical Kakhetian pigs of different ages. First slaughters were carried and first Georgian hamon was produced.

4. In Georgia, the following four helminthiases predominate in the pig populations: ascaridosis, oesophagostomosis, trichocephalosis and meta-strongylosis.

It is suggested that the Kakhetian pigs are more resistant to these diseases than the hybrid pigs. This can be partially explained by the fact that the Kakhetian pig is more resistant, which, as we suggest, is due to the lifestyle and feeding habits of the above pig; the vegetation (rue, chestnut, nuts, wild pear, wild apple, medlar, cornel, blackberry, hawthorn, barberry, currant, bilberry, etc.) that they feed on in the woods must have some helminthicide properties establishing special environment negatively affecting helminths in the digestive tract

of a pig. However, more in-depth studies are needed to strongly support the above suggestion.

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Parasitic mites of animals and fighting them

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A B S T R A C T

The dissemination of ixodid mites has been studied throughout in four regions (Marneuli, Bolnisi, Gardabani, Dmanisi) of Kvemo Kartli (Georgia). 9 species of parasitic mites were identified from 5 families of Ixodidae.. Periods of attack of parasites have been identified on agricultural animals. The highest number of pupa was observed in May-July, nymph - August-September (Marneuli, Gardabani, Bolnisi), and in the highlands (Dmanisi) imago is active from the second half of March until the end of May, pupa-from the end of July to the end of August, nymph-from the second half of March until the second half of May.

For the increase of fighting efficiency against the parasitic mites, on the basis of synthetic pyrethroids there was developed environmentally little dangerousy preparation (conditional name "Giometrin") with the prolonged acaricidal effect. Prolongation of acaricidal effect of "Giometrin" became possible in the result of combination of water-oil emulsion and synthetic piretroides. The simultaneous use of hydrophilic and organophilic surfactants in the formulation led to an increase in oil up to 80%.

By diluting the obtained preparation in the water, milk-like liquid is formed, that has longer acaricidal action (25-28 days), than the imported preparations (12-15 days) presented on the local market. It was confirmed, that after 96 hours of the treatment of hungry imago by the 0.015-0,02% solution of the preparation in lab conditions 100% mortality was identified, and in case of 0,0001-0,0075% of solution the mortality rate varried within 78.9-95.9%. In real conditions, in four farms, cattle were treated with of working solutions with concentration 0.0075-0.001-0.015-0.02%. Totally a 4-5 adult and adolescent cattle was treated with "Giometrin" in each farm. In daily monitoring, the viability of ticks and the number of dead ticks fallen from the skin of cattle were monitored. It has been confirmed, that imago forms totally die during 3-4 days, while the satiated mites die 4-5 days later after the treatment. The clinical condition of observed cattle was in norm, milk yield was not decreased. Conducted macro and micro morphological studies have shown that the spraying of 0,02% solution of the oil-emulsion preparation "Giometrin" does not cause changes in skin and internal organs. The reaction of the organism is within the norm. 1 hour later after spraying, weakly expressed capillary hyperemia and perivascular infiltration is detected in skin and regional lymph nodes. 6 hrs later after the spraying no deviation from the norm is indicated, it means that the change is not the result of the toxicity of the preparation or the cumulative effect of the skin. During the season for the guaranteed protection against the ticks 15 procedures are required in case of insecticide-acaricide preparations presented on veterinary market, while in case of treatment with the developed oil-emulsion preparation "Giometrin" with prolonged action only 7-8 times is enough. If we take into account the self-price of "Giometrin", that is, 10-15% less compared to imported, preparations, overalls developed preparation can reduce almost 2 times the expenses for the protection of animals from ectoparasites. Georgian Patent (GEP2011.5346B) has been adopted for the development of a concentrated oil-water emulsion concentrate with acaricide properties.

Keywords: Parasitic mites, Animals, Veterinary preparation, Ectoparasites, Ixodidae, Species.

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Introduction

From many of the existing species of ectoparasites, mites from the family Ixodidae are the most harmful for the agricultural farming, which spread all Diseases caused by endogenous parasites. [1-4]. The dissemination of ixodid mites has been studied throughout four locations of Georgia (Marneuli, Bolnisi, Gardabani, Dmanisi) in Kvemo Kartli region, lowland, foothill and mountainous zones (fieldy, bushy, hilly meadows, riverside and forest-field areas).

From ticks of family Ixodidae there are identified 9 species of parasitic ticks (*Haem. punctata, Haem. sulcata, Haem. otophila, H. marginatum, H. anatolicum. B. calcaratus, Rh. sanguinalis, Rh. Bursa and I. ricinus)* from 5 genus *(Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus, Boophilus)*.

On pastures these species are crawling on the fur of cattle and stick to the surface of their skin. In host organism ticks inoculate saliva with anticoagulant action, which leads to both general and local toxicity. In general, allergic reactions occur when a metabolic disorder occurs in the body. In this case, the general reaction of the organism changes significantly, which is mainly due to the inhibition of the animal, decrease its productivity and the number of red blood cells in the blood. According to studies, mites for one feed can suck out from 0.5 to 3 ml. of blood from the cattle. Thus, the estimated total loss of blood can be 166 mg / per day on one adult animal [5]. Mites are transmitters of viral, bacterial, fungal and rickettsiose pathogens in animals [6,7,8]. In addition, ticks of this family are also spreaders of various human diseases (Encephalitis, Barrelos-Lyme disease, Fever, Tularemia, Brucellosis, etc.), [9-12].

Significant change in climatic conditions on the earth is gradually becoming noticeable due to global warming. Radiation activity of the sun increased. In some regions there is a decrease in the amount of precipitation, and in some cases, an increase. In comparison with previous years, the air temperature has increased by 1 - 2.1^{0}C [13]. These climatic changes significantly affect the development of ticks. As well as other countries, Georgia is also focused on determining the area of distribution of various types of parasitic ticks in accordance with the geographical zones and seasons. Data of studies conducted in this direction are not available since 1958 (14).

Parasitic mites are widespread in Georgia. Consequently, cases of agricultural animal diseases caused by tick attacks are regular, which is a difficult task for farmers. The fight against them will not be a desirable result if the country does not take into consideration the bio-ecological, climatic and geographical factors of ticks in different zones of the countries. Treatment of diseases caused by attack of mites is associated with many difficulties. It is easier to carry out complex preventive measures against mites, which are implemented by regular treatment of cattle with effective and safe acaricidal preparations.

Measures against parasitic mites in Georgia are carried out by imported means. In recent years, insecticie-acaricidal preparations prepared on the basis of synthetic pyrethroids are widely used against parasitic mites [15]. Unlike previously used preparations, made from organochloric, organophosphoric and carbamic acids, they are less toxic to worm-blooded animals. They have poorly expressed allergic properties; do not have teratogenic, mutagenic and carcinogenic effects, as well as photosensibiling properties.

The aim of the study was to develop an environmentally low-hazard preparation, with long-lasting acaricidal effect based on synthetic pyrethroides using natural compounds and the evaluation of its effectiveness. The low self-cost of the developed preparation for competition with other similar imported means was desirable.

Computational Methods

Four raions - Marneuli, Bolnisi, Gardabani and Dmanisi were selected for the research in Kvemo Kartli region. 1628 cattle, 841 sheep and goats, and 27 dogs were clinically examined in these areas. 4032 units parasitic mites were totally collected, including: imago – 2916, nymph - 624, larva - 492. Parasites were collected from the animal skin, from animal farms, as from adjacent territories and pastures. The morphological examination of the collected ticks has been done through Galuzo and Zakhvatkin's tables [16] and using MBC-9 microscope. The temperature of the environment was determined by the thermograph, measuring soil temperature at 4-5 cm depth - using a thermo probe, and determination of moisture – using a psychrometer.

Systemic observation was carried out on cattle to determine the quantity of mites. Research

works were conducted both in laboratory and field conditions, in areas of the region. To determine the activity of ticks on livestock and pastures in different seasons of the year, tests were conducted for individual parazites (imago, nymph, and pupa) at different temperatures in warmth (at a temperature of 7-10-20-25°C) and in the cold (1-3-5°C using dry ice).

In order to determine the influence of the preparation "Gimetrin" on the reactivity of the animal organism, totally 9 clinically healthy rabbits were examined. After treatment of the animal's skin with 0,02% solution of "Giometrin", the reaction of the animal and clinical signs were observed within 5 days after application of the preparation. In parallel, pathomorphological changes in the body were studied.

After 1, 6, 24 hours and 3 days of treatment the animals slaughter and autopsies were carried out. Were cut off in order materials from subcutaneous tissue, parenchymic organs and lymph nodes for pathomorphological examination. The primary fixation of pathomorphological material was carried out in a 10% solution of formalin, which was then transferred to a 15% solution. The material was embedded in paraffin, in the case of pathomorphological changes in organs and tissues, the coloring method with Sudan-3 was used to study fatty dystrophy and lipids.

Results and Discussion

There are identified 9 species of parasitic mites (*Haem. punctata, Haem. sulcata, Haem. otophila, H. marginatum, H. anatolicum. B. calcaratus, Rh. sanguinalis, Rh. Bursa* and *I. ricinus*) from 5 genus (*Haemaphysalis, Hyalomma, Ixodes, Rhipicephalus, Boophilus*) of family-*Ixodoidae.*

In the lowland territories of selected regions of Kvemo Kartli there are spread mites of species *B. Calcaratus, H. marginatum, Rh. Bursa, H.anatolicum, Haem. punctata* and *haem. sulcata*, the dominants are *B. calcaratus, H. Marginatum* and *Rh. Bursa.*

B.Calcaaratus - species are widely spread throughout the country, especially in western Georgia. In the Kvemo Kartli zone, these species have been collected in large quantities in lowland areas that are abundantly covered with vegetation. High quality of tick activity was observed on the territory of Mtkvari, Iori and Khrami.

Duration of life-cycle of these parasitic ticks in 4 regions of Kvemo Kartli lowlands is 56 days, in foothill - 80 days, in mountainous areas 92 days.

In foothill territories there are spread *Rh. Bursa, H.anatolicum, I. ricinus, Haem. punctata, Haem. sulcata, B.Calaratus* and *Haem. otophila* species, dominants are *Rh. Bursa* and *H.anatolicum*.

Ryipicephalus bursa – Parasit mainly of large and small cattle. It is distributed on non-irrigated pastures, shrubs and hills of all four regions. Even in case of high quantity of ticks on large cattle, in Gardabani region, only few individuals of imago parasites were found on sheep.

Hyalomma anatolicum – This parasite is of a large size. In addition to the foothill zones, it is widespread in the lowland areas, where the territories are abundantly covered with a vegetation and more are observed in the bushy areas.

H. Marginatum, I.ricinus, H. punctata and *Rh. Bursa* are widespread in the mid-mountain zone, dominant is *H. Marginatum*.

Hyalomma marginatum - Adult parasite is large. Its distribution is observed throughout the whole territory of all four regions.

In mountainous areas genus of mites «*H.Otoophila*» and «*Haem. Punctata*» are common out. Of these, the most common is «Haem. Punctata».

Haem. Punctata –is spread in lowlands, foothills and mountainous areas of all four regions . They are tiny mites. The main owner of adult mites is the large cattle.

A high price for high-quality imported preparations, represented on the Georgian veterinary market, is too expensive for most farmers engaged in cattle breeding. Accordingly, prophylactic processing of the animal's body is not performed at all or partially carried out. As a result, there are often cases of various infectious and invasive diseases and the fall of livestock. Therefore, it was important to develop a low-cost and effective acaricidal preparation of local production, the use of which would be economically acceptable for farmers, engaged in cattle breeding, in order to protect livestock from parasitic mites.

It is not possible to produce cheaper local analogues of imported preparations, since there is no production of the necessary components in Georgia. Therefore, it was decided that the studies

were aimed at prolonging the acaricidal activity, in particular, on the development of the preparation, which will have a longer period of action than the imported (12-15 days). This became possible by the controlled isolation of molecules of the main active substances (evaporation). At the same time, the modern requirements for insecticide-acaricidal preparations necessary for veterinary practice were considered [17].

Prolongation of the acaricidal action of the developed preparation made possible by the combination of water-oil emulsion concentrate with the synthetic pyrethroides.

In case of increase of the oil (vaseline) content up to 80% in the composite system, two types of hydrophilic and organophilic surfactants were introduced into the system to avoid the stability decrease of the emulsion concentrate and phase separation during long-term storage. By diluting the resulting preparation in water (ratio 1: 100), a milky-like working solution is formed which has an acaricidal effect for 25-28 days. High oil content in the working solution ensures reliable fixation of the product on the treated surface and maintenance of acaricidal properties for a long time even in case of contact with water.

The effectiveness of the acaricidal preparation (conditional name «Giometrin») prepared on the basis of a microemulsion concentrate was performed in laboratory conditions and in real environment.

Impact on the hungry imago B. calcaratus with different concentrations of preparations was carried out for 1 minute. Dead and paralyzed ticks were recorded through 12, 48, 72, 96, 120 and 144 hours. The results are presented in Table 1.

In real environment, the study of acaricidal properties of the preparations was continued in 4 farmings in Dmanisi region on large cattle with mites (20-25 mites were recorded on animals). On average 4-5 adult and young animals were treated by the preparation "Giometrin" in each farm. The processing of animals was carried out with 0,0075-0,001-0,015-0,02% of working solution containing cypermethryn.

Three periods of attack of the species B.Calcaaratus were registered on livestock in the pastures:

I. In early spring - from the second half of February to the first half of April (Gardabani, Marneuli and Bolnisi lowlands); From the beginning of March to the first half of May (Dmanisi),

II. In summer - from the beginning of June to the end of August (Gardabani, Marneuli and Bolnisi); From the second half of June to the end of August (Dmanisi).

III. In autumn - from the beginning of the september to the second half of October (Marneuli, Gardabani) in the foothills of Bolnisi. autumn attack period was not observed in Dmanisi.

Treatment

P - Paralyzed D - Dead

Table. 1. *The results of the impact of «Giometrin» on hungry imagoes of Boophylus calcaratus*

Concentration of Cypermethrin in the preparation	Quantity of ticks	Tick mortality %													
		12 hr		24 hr		48 hr		72 hr		96 hr		120 hr		144 hr	
		P	D	P	D	P	D	P	D	P	D	P	D	P	D
0,0001	20	100	-	85.7	14,3	65,5	34,5	43,9	56,1	21,1	78,9	4,3	95,7	-	100
0,001	20	63,1	26,9	41,9	58,1	29,1	80,9	16,3	83,7	9,4	90,6	1,2	98,8	-	100
0,0075	20	52,2	47,6	36,7	67,3	18,2	81,6	11,1	88,9	4,1	95,9	-	100	-	-
0,015	20	40,4	59,6	20,1	79,9	12,3	87,7	6,5	93,5	-	100	-	-	-	-
0,02	20	10,2	89,8	7,1	92,9	4,5	95,5	1,2	98,8	-	100	-	-	-	-
Control water	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Experiments in the laboratory and environmental conditions showed that the major host of parasitic ticks B.Colcaaratus is 95% of the livestock.

The massive attack of adult ticks of Hyalomma anatolicum on livestock throughout Gardabani and Marneuli districts, and in the lowlands of the Bolnisi region, was observed from mid-March to the end of May, nymph and pupa from the beginning of June to mid-July. Some mites are observed on the skin cover of bovine livestock in late period (late September), parasite was fixed on the skin cover of the animal in Bolnisi region from the end of March to the first half of April.

Imago «Hyalomma marginatum» are attacking cattle in early spring - at the end of February and reaches the maximum in the first half of May. Nymph and pupa is observed later from the second half of March. The major host of parasites are cattle and other animals.

In the laboratory conditions not hungry imago and nymp dies at -10 C (using dry ice) in 33-35 days.

When the mites (imago, nymph and pupa) are placed in water for 3 hours, death occurs in 18 days, and with a 2-day placement - the mites die on day 6.

First attack of adult mites of *Haem. Punctata* on animals is observed from early spring, at the end February to the end of April. Second attack in autumn, in August-September. The largest number of ticks is observed in May-July, nymph-August-September (Marneuli, Gardabani, Bolnisi). In the Dmanisi region imago is active from the second half of March until the end of May, pupa - from the end of July to the end of August, nymph - from August to September.

The quantity of mites on cattle reaches its maximum in May. From the end of May, the number of animal mites decreases and only one parasites is found on the surface of the host skin. In Dmanisi district the attack of ticks is observed later.

A study of the relationship between the environmental climate and the activity of parasite mites was confirmed that *Haem. Punctata, I.ricius, H.anatolicum, H. marginatum* attack agricultural animals after wintering, when the air temperature reaches 6-80 C and the soil temperature is - 70C. Species-B.Colaratus and Rh. Bursa attack animals when air temperature is 11-120C and the soil 90 C. These indicators make it easier to predict attack period.

According to the results of efficacy testing of preparation "Giometrin" in laboratory conditions presented in Table 1. it is confirmed, that 100% mortality was observed after 96 hours after the contamination of hungry imago with 0.015-0,02% of solution containing cypermethrin and in case of treatment with 0,0001-0,0075% solution after 96 hours the mortality varied within 78.9-95, 9%.

In real environement the preparation efficiency was evaluated according to the the status of mites after the processing of skin cover of cattle. In the daily monitoring mode, the viability of the ticks was observed, the number of dead and number of dropped from the cattle skin cover was recorded.

It has been established that imago forms of hungry mites completely die for 3-4 days, and non hungry full parazites - 4-5 days later. The clinical condition of the cattle that was in the scope of norm, milk yield was not reduced.

Visual Observation was performed on the skin of treated animals for 20 days to determine the residual acaricidal activity of the preparation. Within 15-18 days after processing, there were no ticks on skin cover of animal, whereas on on the skin of untreated animals, being together on the herd an average number of mites was 10-15 pcs/ day.

Skin vessels, especially reticular layers are enlarged and filled with blood. This circulatory reaction was primarily concerned to capillaries, around which in a single cases weak infiltration of serous fluid and leucocytes (eosinophilic) was detected in a small areas. Histological structure of vessel wall was maintained. Fatty glands and ducts were well expressed. Totally was maintained the connective tissue of animal fur.

The nearest (regional) lymph nodes adjacent to the site of spraying the preparation were not enlarged in volume (with characteristic grayish-white color and consistency). According to histological research, the histological structure of the lymph node has been preserved; the primary and secondary follicles were well expressed (with well-maintained light zones in the secondary follicles). The follicles were filled with lymphocytes and reticular cells.

Hyperemia in lymphaden and medullary layer is kept, that mainly was expressed in venous capillaries. Anatomic parameters of the liver were

preserved and macro-morphological changes were not observed. Pathomorphological studies have shown weakly expressed blood reaction in the form of hyperemia in venous capillaries.

Micro- and macromorphological changes in the heart, lungs and kidneys were within the norm and no deviation from them was observed.

After 6 hours of spraying with the preparation previously expressed hyperemia and weak peripheral infiltration were not marked.

The clinical signs of animals were within the norm. Weakly expressed hyperemia in regional lymph nodes has returned to norm. Deviations from the norm of histological changes were not recorded in the investigated organs. A similar picture is expressed 24 hours after the use of the preparation.

According to the studies carried out on the preparation - «Giometrin», conducted in G. Natadze Sanitation, Hygiene and Medical Ecology Scientific-Research Institute of the Ministry of Labour, Health and Social Affairs of Georgia issued a hygienic and toxicological decision on its safety / harmlessness for humans and animals, which makes it possible to use a preparation for parasitic tick control.

After receiving such a conclusion, a large-scale test of the preparation was conducted in field conditions. Agricultural animals were treated in the mountainous and lowland regions of Kakheti, Samegrelo, Imereti, Shida Kartli and Kvemo Kartli. The original design of the mixer-stirrer was developed to provide large-scale field trials, and the finished product/preparation was packaged in 100 ml bottles.

Throughout the field trials, 585 souls of cattle was treated, including milker cows, adolescents and calves. Continuous monitoring of the cattle was carried out within 30 days. The result was satisfactory, the contraindication had no place.

According to the results of the field exams, the use of «Giometrin»significantly decreases need processing repetition of cattle. During the season only 7-8 fold treatment is enough for the guranteed protection of animals from mites, with developed emulsion preparation -»Giometrin» with prolonged insecticide-acaricidal action, instead of 15 fold. As a result, the quantity of used preparation is reduced by 40%. If we take into account the self-cost of the emulsion preparation, which is 10-15% less than the cost of imported preparations, the costs for protection of animals from ectoparasites, with preparation «Giometrin» (for which a Georgian patent GEP2011.5346B was obtained), will be reduced almost 2-fold, as shown in Table 2.

Conclusion

A study of the bioecological, climatic and geographic features of ticks in the Kvemo Kartli zone established that in the phenology of ticks there are certain pecularities considering species. The highest number of pupa was observed in May-July, nymph - August-September (Marneuli, Gardabani, Bolnisi), and in the highlands (Dmanisi) imago is active from the second half of March until the end of May, pupa-from the end of July to the end of August, nymph-from the second half of March until the second half of May.

By G. Natadze Sanitation, Hygiene and Medical Ecology Scientific-Research Institute of the Ministry of Labour, Health and Social Affairs of Georgia issued hygienic and toxicological decision on the safety /harmlessness for humans and animals

Preparation	Acaricidal activity, days	Repetition of processing in season	Preparation expense /per cattle ml	Price for expenced Preparation GEL
Pyrethroid	12-15	15	30	1,5
Giometrin	22-25	8-9	17	0,76

Table. 2. *The effectiveness of «Giometrin» in comparison to imported acaricidal preparations*

of the developed preparation "Giometrin" with prolonged acaricidal effect.

Conducted macro and micromorphological studies have shown that the spraying of 0,02% solution of the oil-emulsion preparation "Giometrin" does not cause changes in skin and internal organs. The reaction of the organism is within the norm. 1 hour later after spraying, weakly expressed capillary hyperemia and perivascular infiltration are detected in skin and regional lymph nodes. 6 hrs later after the spraying no deviation from the norm is indicated, it means that the change is not the result of the toxicity of the preparation or the cumulative effect of the skin. Probably, it is associated with manipulations on rabbits, as a sign of a stressful reaction that does not go beyond the physiological limits of the organism.

"Giometrin" fully meets the modern requirements for veterinary preparations:

- Easy to use
- Safe for human and animal health;
- Does not have cumulative feature;
- It is characterized by a wide spectrum of action and the effect of prolonged acaricidal action;
- During storaging there is no change in physical-chemical characteristics;
- Suitable for use during 3 years.

The compositional preparation "Giometrin" is an innovation for the Georgian veterinary market, therefore, an introductory work was carried out for farmers and the rural population, which showed the advantage of using "Giometrin" in comparison with foreign analogues.

Based on the above, the widespread use of the preparation "Giometrin" will significantly facilitate the improvement of cattle care in Georgia and increase the production of livestock products.

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Invasive pest - box tree moth Cydalimaperspectalis Walker. (1859) - and main biological aspects in Georgia

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A B S T R A C T

The paper concerns box tree (*Buxus colchica*), the relic of Georgian flora and endemic of the Caucasian dendroflora and the very dangerous invasive pest, that spread on these trees - *Cydalima perspectalis,* that in 2012together with the planting material of evergreen box tree (*Buxus sempervirens*), imported from Italy for greening of the Olympic village entered in Sochi; the pest invaded the territory of Sochi, and since 2013 from there the outbreak of the best began, firstly on the territory of the town and then on plantings of the National park, that caused almost 100% defoliation of box tree. From Sochi the pest spread on box trees of the Black Sea coastland of Georgia and began their aggressive destruction. After that the pest has spread in parks and gardens of the whole Georgia, where box tree grew. The pest feeds on box tree leaves, and when leaves exhaust, they may additionally feed on peel of young shoots. Now the Colchic box tree is in very bad condition. Before the box tree moth, in 2009-2010 the invasive disease box blight *Cylindrocladium buxicola* has spread in Georgia, that destroyed more than a half box trees, and further box tree moth *C. perspectalis* added to it. The paper describes the results of study of the main aspects of biology of box tree moth, promotion of reproduction of resistant species by artificial grafting and pest control measures.

Keywords: *Box-trees*, *Cydalima perspectalis*, Biology, Flight of imago, laying of eggs, larvae

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Introduction

There are up to 49 species of box tree; from them a relic species *Buxus colchica Pojark.* grows in Georgia. It is mainly spread in the subtropical zone of Western Georgia. Sometimes its distribution is mentioned in this zone on the altitude of 1700 m above sea level.

It is an evergreen bush or a tree, grows very slowly, in favorable environmental conditions its height attains up to 15-18 m and diameter - 40-50 cm. It has a very excellent wood. Sprouts are covered with green bark, and a stem is grayish yellow with very thin bark [1]. Its flower is rich in nectar, seed contains up to 37% of oil. Boiled wood and leaf give a brown paint; it is also used in medicine.

Wood is yellowish, very heavy (it sinks in water, even dry), dense and hard, when machined and polished it resembles an ivory. It is used in building and lathework (for figure handiworks), for manufacturing of music instruments etc. [2]. 1 cu.m of box tree weighs 1000 kg.

Trees of the Colchic box tree were cut down in large numbers in the XIX and the beginning of the XX century, because it represented the export

material. Its area in the forests of Georgia is 8,443 ha. There are many decorative forms of box tree. It well endures shearing. It is a very valuable species for decorative gardening. Box tree lives 500-600 years, is introduced in the Red Book.

In the end of the XX and in the beginning of the XXI century in Georgia and the neighboring countries (Black Sea region), due to weakening of a quarantine new invasive dangerous pests broke through, in their number - box tree moth *Cydalima perspectalis*.

The native land of the box tree moth is Eastern Asia: China, Japan, Korea, Russian Far East and India. Supposedly the pest occurred in Western Europe from China with sapling material; the first data about finding of box tree moth have been recorded in Germany in 2006, after that the pest has been introduced in the list of the most dangerous pests of Europe. After that up to today, besides Germany, it is spread in: Hungary, Romania, Switzerland and in other countries of Europe, and also in Turkey [3, 4].

In 2012 the pest box tree moth *Cydalima perspectalis* entered to the territory of Sochi with sapling material of the evergreen box tree *(Buxus sempervirens)*, used for gardening of the Olympic village in Sochi. Since 2013 the pest outbreak began from there: firstly - on the territory of the town and then on the plantings of the National Park. It caused almost 100% defoliation of box tree and its drying [4]. The scientists S.A. Belokobylskij & Yu.I.Gninenko in the Northern Caucasus developed from pests of the North-Caucasian box tree an endoparasitoid - parasitic wasp (insect that lives in tissues of other organisms) and have demonstrated the parasitic wasp [5], which is an endoparasitoid of the dangerous pest - box tree moth.

From Sochi the pest has spread on Colchic box trees of the Black Sea coast of Georgia and aggressively destructed it. After that the pest has spread in parks and gardens of the whole Georgia, where box tree grew. The pest feeds on box tree leaves, and when leaves exhaust, they may additionally feed on peel of young shoots [6]. Now the Colchic box tree is in very bad condition. Before the box tree moth, in 2009-2010 the invasive disease box blight *Cylindrocladium buxicola* spread in Georgia, that destroyed more than a half of box trees [7], and further (since 2013) box tree moth *C. perspectalis* added to it.

It should be noted, that at present the Colchic box tree is in very bad situation, because 2-3 years ago box blight *Cylindrocladium buxicola* spread, that began destroying of box tree and, unfortunately, in Georgia and in other countries a control of this fungal disease is hampered; there is no point in spraying the 15-20 m length trees by above ground equipment (that is - by bio preparation). It is impossible to spray all leaves diseased by fungi (they must get wet). As we know from various scientists in literature [8], a spore of this fungus lives in soil 5 years; imagine how difficult a complete elimination of this disease is. In addition, as we know a majority of box tree copses grow under the crowns of other trees and contact of the bio preparation with these trees is a problem.

To the spread of this fungal disease and strong damage, or defoliation of box tree a very dangerous and aggressive pest - box tree moth *C. perspectalis*- was added; this became a reason of study of its main biological aspects (mode of life) and research.

Objectives and methods

We identificated Box tree moth for the first time in Georgia on 6th of August 2014 in the resort Grigoleti of Lanchkhuti municipality, in the private yard of an inhabitant, on the bushes of the Colchic box tree *Buxus colchica Pojark.* After that we carried out observations in the forest massifs of box tree in Adjara: in Kintrishi protected area, Mtirala national park, Machakhela national park, Khelvachauri, in general in the whole Georgia, where box tree is distributed. We carried out all kinds of observations: on distribution, biology and a mode of life, in general.

Actively feeding larvae of the final age of the pest box tree moth *Cydalima (=Diaphania) perspectalis* (Lepidoptera: Pyralidae*)* were placed in small bags of black fabric and delivered to the laboratory of Vasil Gulisashvili Forest Institute of Agricultural University of Georgia, there were placed in big insect rearing chamber and a feed - box tree shoots with leaves - has been given to them. Larvae pupated on 14.08.2014 and after 12 days, on 26.08.2014 moths (imago) flied out, they were straightened on the insect straightening board, moths were identified and material was placed in the entomological box that is kept in the laboratory.

Since 2014 Box tree moth that broke through the

Black Sea region, spread in the whole Georgia and also in Turkey.

The intensity of pest spread was determined according to a percentage of damage (with destruction) of tree crown - leaves - in this way:

- if tree leaves were not damaged or eaten - 0 number;
- if tree leaf damage was one tenth of a crown, or 10% - weak damage;
- if tree leaf damage was one fourth of a crown, or 25% - moderate damage;
- if tree leaf damage was a half, or 50% - strong damage;
- if tree leaf damage was from 50% up to 75% - very strong damage;
- if tree leaf damage was 75% or more - the tree already dies (defoliation occurred).

We researched and studied various aspects of biology of the pest: different phases of development of the pest, quantity, density of settling, degree of damage etc., we also measured phases by 8x ocular microscope МБС-1 with diopter scale, sizes of various phases of box tree moth (50 specimens from each phase).

Results and discussion

We have found box tree moth for the first time in Georgia in the town let Grigoleti, Lanchkhuti district on 6th of August 2014; it migrated from Sochi to Abkhazia [3], and from there it very quickly spread in big numbers in Adjara box tree plantations, and in 3 years it spread basically in Western Georgia and then in Kartli and Eastern Georgia.

Intensive spread of the pest in 2-3 years has severely damaged and defoliated box trees [4], drying of trees began, mainly in Western Georgia and Adjara, where there was the most box trees; a period of their destruction began; the 400- 500 years old box trees dried in Tkibuli, Martvili, on Kolkheti lowland, in Kintrishi, Mtirala, Machakhela, Khelvachauri etc. (Fig. 1, 2).

Fig.1. *Old dried box trees in Kintrishi*

Fig.2. *Small dried box tree in Mtirala*

#	Location	damage of box tree leaves (eating) in %					
		healthy 0 -%	weak 10%	moderate 25%	strong 50%	very strong 50-75%	defoliated 75% and >
1	Tkibuli (Mukhuri)	1-2	+	+	+	+	-
2	Martvili (Gachedili - Salkhino)	-	-	-	+	+	+
3	Kolkheti lowland	-	+	+	+	+	+
4	Kintrishi	-	+	+	+	+	+
5	Mtirala	-	+	+	+	+	+
6	Machakhela	-	+	-	+	_	-
7	Khelvachauri (Kirnati)	-	+	+	+	+	+

Table. *Box tree moth spread in Western Georgia and coastland of Black Sea and damage caused by it in % in 2016-2017*

We recorded a damage of tree by box tree moths - Table (% of damage - defoliation of leaves). As we see from the Table, box trees are almost destroyed, due to high density of the pest and its aggressiveness that caused almost total devastation of box trees in the entire Georgia.

We have delivered young larvae of box tree moth, found in the field conditions (in Western Georgia and also in Eastern Georgia) to Tbilisi and began in the laboratory of the Forestry Institute of the Agricultural University of Georgia their study and observation on their biological aspects - mode of life etc.

Egg. The female moth lays egg clusters on the underside of a leaf, winters in the egg phase. Egg is yellowish in color, 1 mm on average. On Spring 2017 larvae hatched from wintered eggs (Fig.3)

Fig.3. *larvae of I age, hatched from egg clusters laid by moth*

Fig.4. *Mesophyll of underside of leaf eaten by larvae of moth of I-II age*

Larva. Larvae of moth of the first age hatched, began active eating of mesophyll of underside of box tree leaf (Fig.4). We have measured the newly hatched I age larvae: they were 1.1-1.2 mm; the II age larvae were 2.3-2.4 mm; these newly hatched larvae are white or yellowish-green in color, heads are black. The larvae of 3-4 ages became green, again with black head. Larvae have black stripes on the both sides. The size of larvae of the last age is up to 2.5 cm (Fig.5). They actively feed and are insatiable - they eat leaves completely (they change skin 4 times). When feed is in short supply, they also eat skin of young shoots; at last on the 25th-27th day of their development larvae make a gossamer, wrap up in it and pupate.

Fig.5. *larva of box tree moth of the last age*

Pupae. The larvae of the last age of the I generation pupated on 14-15 May, when the temperature was 20-23^{o}. The first moth of the I generation flied out on 28-29 May, i.e. the phase of chrysalis lasted for 14 days (Fig. 6, 7).

Fig.6. *The largest chrysalis 1.95 cm*

Fig.7. *The smallest chrysalis 1.65 cm*

Imago. Moths that flied out from one and the same population of box tree moth - 12-15% are brown, and the remaining 85-88% - of ordinary white color, with brown edging around wings (Fig.8, 9).

Fig.8. *Moths of box tree moth, whiteandbrown*

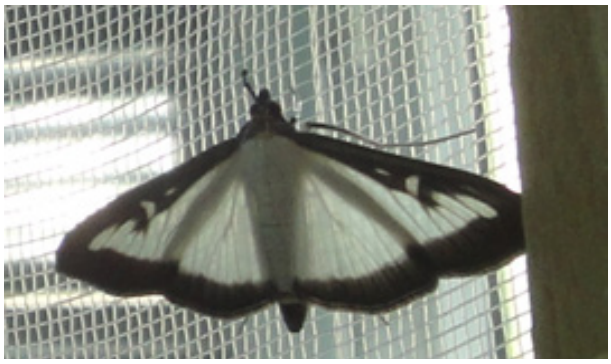

Fig.9. *Moth of box tree moth with open wings*

Fig.10. *Imago of box tree moth*

The moth (imago) of the pest with open wings is 4.0-4.5 cm (Fig.10). On a head it has big embossed eyes of dark color and thread-like whiskers (Fig.6). Males have white wings with dark brown edging, females - light brown with white spot and with bluish violet nacreous shine. In the ends of wings - short thin hairs in a form of fringe. It gives yearly 3 or 4 generations.

Ovaries of moths. We cut the newly flied out female moths; eggs of yellowish color were discovered in ovaries - on aver. 1mm, 59-61 pcs (Fig. 11, 12).

Fig.11. *Cross section of ovary of female moth*

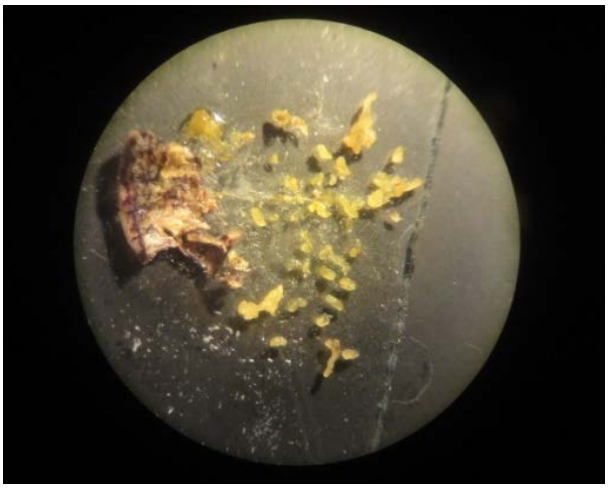

Fig.12. *Average number of eggs in ovary 59-61 pcs*

1. **Mechanical** control measures may be carried out against box tree moths, when larvae and eggs of box tree moths are collected by hand and burnt.

2. **Biologic**al by means of useful insects and **insect traps with pheromones.** From bio preparations there recommended the bacterial preparation - lepidocide, that is produced on the basis of the bacteria *Bacillus thuringiensis var. kurstaki,* only by the Russian company "Sibbiopharm" from Berdsk, which has wide experience in producing of bio preparations, is characterized by good results on the larvae of first - second- third ages, or on young larvae [9, 10], when a larva eats leaves processed with this bacteria.

3. **In a deadlock the pyrethroids are used from the chemical pesticides** against box tree

moth: the preparations "Decis", "Fastac", "Karate", as well as drastic systematic preparations - "Bi-58" or Carbophos; however at use of chemical preparations we must be very careful, avoid from children and animals. In general use of chemicals in forest in Georgia is prohibited.

A network of nurseries should be established, where the box tree moth-resistant forms will be grown; then they will be artificially reproduced in box tree lost massifs, when box tree moth will begin reducing its outbreak. Rescuing of unique plants from total destruction must be helped by the organization of special reserves, where selectionists will can to select the box tree forms.

Conclusion

1. In 2009- 2010 in Georgia the invasive disease - box blight *Cylindrocladium buxicola* spread in Georgia, that destroyed more than half of Colchic box tree, and then (since 2013) the pest box tree moth *Cydalima perspectalis* added to it.
2. In 2012 the pest box tree moth *Cydalima perspectalis* entered to the territory of Sochi with sapling material of the evergreen box tree *(Buxus sempervirens)*, used for gardening of Olympic village in Sochi. Since 2013 the pest outbreak began from there: in Abkhazia and in the entire Georgia, also in Turkey. It caused almost 100% defoliation of box tree and its drying.
3. The pest turned out to be very aggressive; in a season it gives 3 or 4 generations, that is destructive for trees and plants.
4. In 2013 – 2014 the pest has spread in the entire Adjara, and then in the whole Georgia.
5. By route walking we have researched and determined a damage caused by pests and diseases, massive spread of the pest. Before the means for their control have been determined, the pest has brought box trees to the brink of extinction.
6. After spread of a box tree moth, a part of box trees turned out to be resistant in respect of the pest disease, so we should take scions from these box trees and to plant box tree nurseries by grafting and then replant them in the areas with lost box tree, where the climate was favorable for box trees.Acknowledgments
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Spirea formation (Spiraeta hypericifoliae) in Tbilisi environs (East Georgia, South Caucasus)

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A B S T R A C T

Spirae formation (Spiraeta hypricifoliae) of Tbilisi environs is studied for the first time. This formation is one of the typical representatives of hemixerophilous shrubberies of shibliak type for vegetation cover of Tbilisi surroundings. Plant communities of Spirae formation with different plots area are fragmentary spread almost all over the territory of Tbilisi environs from 600 to 1000(1100) m above s.l.. Plant communities are developed on slopes with various exposure and inclination, mainly on the cinnamonic and grey-cinnamonic soils. In Tbilisi environs the Spirae's plant communities are either primary or secondary origin. Formation is characterised by rich typological and floristic composition. We identified 5 plant communities: (1) Spiraetum gramino-mixtoherbosum, (2) Spiraetum muscosum, (3) Paliuroso-Spiraetum gramino-mixtoherbosum, (4) Spiraetum festucoso-bothriochloosum, (5) Spiraetum festucoso-bothriochloosum. From them first plant community is widespread and others are rare. For each separated plant communities the basic structural characteristics (general projective coverage, projective coverage, distribution and height of layers, sodding degree, dominant-edificator plants, characteristic species, number of species, moss cover, litter, species richness, spectrum of life forms), distribution area in the Tbilisi environs and main physical-geographical conditions (topography, altitude, exposure, inclination, soil type) are given. 189 species of vascular plants, which belong to 43 families and 133 genera, were recorded. In the floristic spectrum leading families are: 1. Poaceae – 26 species (13,7%), 2. Asteraceae – 20 species (10,6%), 3. Fabaceae – 13 (6,9%), 4-5. Lamiaceae, Rosaceae – 12-12 (6,3-6,3%), 6. Brassicaceae – 10 (5,3%), 7. Apiaceae – 9 (4,8%), 8. Caryophyllaeae – 7 (3,7%), 9-10. Asparagaceae, Rubiaceae – 6 (3,2-3,2%). The life form spectrum is as follows: hemicryptophytes (including biennials) – 92 species (49,2%), therophytes – 50 (26,4%), phanerophytes – 18 (9,5%), chamaephytes – 6 (3,2%), geophytes – 23 (12,2%).

Keywords: Tbilisi environs, East Georgia, Spiraeta hypericifolia, plant communities, structural characteristics, floristic composition.

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Introduction

The part of basin of the Mtkvari River, which is spread from the village Dzegvi to the section between Ponichala and Rustavi, is considered in the environs of Tbilisi. Endings of the thick geographic units of various origins and geographic age are gathered in the vicinities of Tbilisi that make its topography considerably complicated. Privately, they are involved in the environs of Tbilisi: (1) extreme southern branching of Caucasian range – Saguramo-Ialno ridge. (2) Eastern part of Kvernaki low range (Skhaltba low range), (3) Eastern endings of Trialeti ridge (the ranges of Satskepela and Armazi, Mskhaldidi and Lisi, Mtatsminda, Narikhala, Tabori, Telet-sakharaulo), (4) Western and extremee north-western part of Iori upland (Samgori, Vaziani, Tbilisi Sea and its adjacent

territory, etc.), (5) Extreme north-western ending of Mtkvari-Arax lowland (plains of Ponichala and Kumisi-Tsalaskuri). Hypsometrical amplitude of the Tbilisi vicinity is from 350 m to 1875 m above s.l.. Types of low and middle-height mountain-gorge and stepped plain relief are highlighted in the topography [1-5].

In the environs of Tbilisi, two types of climate with the appropriate two zones of the climate are apportioned [2, 3]: (1) The climate with insufficient humidity, dry and hot summer and mild but well expressed winter, (2) moderately humid climate with moderately warm summer and moderately cold snowy winter. The first zone of the climate contains the whole left side of the river Mtkvari, plains of Ponichala and Kumisi-Tsalaskuri and the river floodplain as well. The secondary climate zone contains the mountainous part of the right side and the main part of Saguramo-Ialno range.

Mainly grey-cinnamonic soil is developed on the western ending of the Iori upland and on the slopes of middle-height low range, which is skeletal in most cases. There are the same types, but slightly salinity soils are on the plains of Ponichala and Kumisi-Tsalaskuri, where the solonets and solonchak are speckled. Mainly different modifications of cinnamonic soil (cinnamonic leached, cinnamonic calcareous, cinnamonic light) are spread on the slopes of eastern endings of Trialeti range, on the slopes of southern exposure of Saguramo-Ialno ridge and partly on the Skhaltba low range. And, brown forest soil of various modifications is met in the upper parts of these ridges. Alluvial soils remained on the terraces of the rivers. Thin primitive skeletal soil and scree-stony are widely spread in the grey cinnamonic soil areal. Rocky bareness of groundless soil covering is also met. The section of clay and clay-sand badlands is met on the southern slope of Skhaltba low range [2, 3, 5-9].

Tbilisi environs are characterized by large biodiversity. Both ecosystem and floristic biodiversity are expressed [10-16]. From the point of landscapes creation and taken space, shrubberies of shibliak type are one of the important and are represented by diverse modifications. From them Spirea formation (Spiraeta hypericifoliae) is one of the characteristic for vegetation cover of Tbilisi surroundings [12, 16]. According to our research it is spread in the other regions of East Georgia too (Shida Kartli and Iori plateau).

Though, the literature data about this formation is scanty. There is not presented distribution area, structural and physical-geographic characteristics of plant communities of Spirea formation in the above-mentioned scientific works; floristic composition and life forms spectrum of formation is not given.

The literature data about distribution of this formation in other regions of Caucasus cannot be found.

Objectives and methods

The object of research is Spirea formation (Spiraeta hypricifolia) of Tbilisi environs. The main aim of the research was to establish area, typological, floristic composition and spectrum of life forms of Spirea formation in the Tbilisi environs; determination of area and main physical-geographical conditions of identified syntaxa (plant communities); study their geo-botanical structure and evaluation of ecological situation.

Geo-botanical data was obtained by the route method. 50 geo-botanical surveys (releves) 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 [17-23].

On the each plots were registration general projective cover (in %) of plant communities, projective cover (in %), distribution, height and floristic composition of each layers, sodding degree (in %), general floristic composition, coenotic role of each species (projective cover in %), as well physical-geographical characteristics (topography, exposure, inclination, soil type, altitude). In the process of cameral work for each plant community were established frequency of occurrence of each species and were determined constant (typical) species, were calculated species richness on 25 m² and spectra of life forms. Based on the geo-botanical surveys floristic composition of Spirea formation was established.

Results and analysis

I. Area and distribution regularities

This formation is one of the characteristic for vegetation cover of Tbilisi surroundings. Plant communities of this formation with different plots area are fragmentary spread on the both sides of the

river Mtkvari. Plant communities of Spirea formation mainly are spread in second climate type zone and in first climate type zone are comparatively rare. Their altitudinal range is from 600 to 1000 (1100) m above s.l.. Plant communities are developed on slopes with various exposure and inclination, mainly on the cinnamonic and grey-cinnamonic soils. Often soils are skeletal. Mainly, soils are thin or middle depth. Spirea plant communities are rare on plane places.

In the Tbilisi environs the Spirea's plant communities are either primary or secondary origin. Nowadays, to draw the line between the primary and the secondary coenoses is impossible in most cases. Secondary plant communities were formed by digressive succession processes of post-forest vegetation. Accordingly, secondary plant communities of Spirea formation are included in the area of forests and xeromezophilous shrubberies of lower mountain belt [12, 24].

II. Typological composition and geo-botanical characteristic

Within the Spirea formation we identified 5 plant communities: (1) Spiraetum gramino-mixtoherbosum, (2) Spiraetum muscosum, (3) Paliuroso-Spiraetum gramino-mixtoherbosum, (4) Spiraetum festucoso-bothriochloosum, (5) Spiraetum festucoso-bothriochloosum.

From them first plant community (Spiraetum gramino-mixtoherbosum) is widespread. In some cases area of this plant community is large and comprises almost all slopes. Others plant communities are rare and presented by comparatively small plots. Their plant communities are fragmentary spread in the distribution range of first plant community.

(1) Spiraetum gramino-mixtoherbosum

Physical-geographical characteristics:

Area in the Tbilisi environs: fragmentarily is spread almost all over of the range of Spirea formation; **Altitude (m)**: 600-1100; **Topography:** slope; **Exposure (macro)**: N (rare S, W); Exposure (micro): N, N-E, N-W, W, S-W, S-E; **Inclination:** 20°-35° (rare 10°-18°); **Soil:** cinnamonic or grey-cinnamonic, often skeleton, middle or thin depth;

Geo-botanical characteristics:

General projective coverage: 90-95% (100%); **I layer (shrubs):** ***Projective coverage*** **-** from 50-60% to 95-98%, ***Distribution*** - more or less evenly or evenly (rare, uneven), ***Average height (cm)*** **-** 100-120; **II layer (herbs, semi-shrubs & dwarf semi-shrubs):** ***Projective coverage*** **-** from 40-50% to 70-75% (rare 25-30%), ***Distribution*** **-** uneven; **III layer (moss cover):** ***Projective coverage*** **-** 60-90% (rare 3-5%), ***Distribution*** **-** more or less evenly; **Sodding degree:** (rare, 1-6%); **Litter:** from + to 50-60%; 0,1-1,5(2) cm depth (mostly are developed on the moss cover);

Dominant-edificator: *Spiraea hypericifolia* (projective coverage from 40-45% to 90-95%);

Characteric species:

Shrubs: *Paliurus spina-christi*, *Prunus incana* (frequency of occurrence 40-40%), *Jasminum fruticans* (32%), *Rhamnus pallasii* (28%), *Ephedra procera* (24%), *Cotoneaster morulus* (20%); **Semi-shrubs & dwarf semi-shrubs:** *Teucrium nuchense* (frequency of occurrence 41.7%); **Perennial plants:** *Galium verum* (frequency of occurrence 100%), *Phleum phleoides* (96%), *Potentilla recta* (92%), *Falcaria vulgaris*, *Rumex tuberosus* (84-84%), *Filipendula vulgaris*, *Thalictrum collinum* (80-80%), *Dactylis glomerata*, *Euphorbia boiisieriana*, *Melica transsilvanica* (68-68%), *Elymus repens*, *Medicago caerulea* (60-60%), *Salvia nemorosa*, *Stachys atherocalyx* (56-56%), *Dictamnus albus*, *Hypericum perforatum*, *Stipa pennata* (48-48%), *Inula oculus-christi*, *Asparagus verticillatus* (40-40%); **Annual plants:** Despite the diversity of species, the constant species were not identified;

Number of species: 178;

Species richness on 25 m²: 33,3;

Spectrum of life forms:

Phanerophytes – 18 species (10,1%), Chamaephytes – 4 (2,3%), Hemicryptophytes (including biennials) – 87 (48,9%), Geophytes – 23 (12,9%), Therophytes – 46 (25,8%).

(2) Paliuroso-Spiraetum gramino-mixtoherbosum

Physical-geographical characteristics:

Area in the Tbilisi environs: lower part of Digmistskali River gorge (between of Lisi Lake and settlement Vashlijvari); **Altitude (m):** 650-700; **Topography:** relief is uneven with slightly expressed terraces and paths; **Exposure (macro):** N; **Exposure (micro):** N, N-E; **Inclination:** 18-20

to 25-30; **Soil:** cinnamonic or grey-cinnamonic, skeleton, middle or thin depth;

Geo-botanical characteristics:

General projective coverage: 95-100%; **I layer (shrubs):** ***Projective coverage:*** 55-60 to 80%, ***Distribution*** **-** uneven or more or less evenly, ***Average height (cm)*** **-** 120-140, ***Maximum height (cm)*** **-** 180-200 (*Paliurus spina-cristi*); **II layer (herbs, semi-shrubs & dwarf semi-shrubs):** ***Projective coverage*** **-** as an average 50-80%, ***Distribution*** **-** uneven (rare, evenly); **III layer (moss cover):** ***Projective coverage*** **-** 80-100%, ***Distribution*** **–** evenly; **Sodding degree:** 2-8%; **Litter:** from 5-10% to 35-45%; 0,1-1 cm depth (mostly are developed on the moss cover);

Dominant-edificator: *Spirea hypericifolia* (projective coverage 40-55%);

Subdominant-edificator: *Paliurus spina-christi* (projective coverage 18-25%);

Characteric species:

Shrubs: *Rhamnus pallasii* (frequency of occurrence 100%), *Prunus incana* (60%), *Jasminum fruticans* (40%); **Semi-shrubs & dwarf semi-shrubs**: *Teucrium nuchense* (frequency of occurrence 40%); **Perennial plants**: *Asparagus verticillatus*, *Galium verum*, *Falcaria vulgaris*, *Melica transsilvanica*, *Thalictrum collinum*, *Phleum phleoides*, *Potentilla recta*, *Rumex tuberoum* (frequency of occurrence 100-100%), *Allium rotundum*, *Dianthus subulosus*, *Achillea nobilis*, *Alyssum murale*, *Stachys atherocalyx* (80-80%), *Eryngium campestre*, *Inula germanica*, *I. oculus-christi* (60-60%); **Annual plants:** the constant species were not identified;

Number of species: 68;

Species richness on 25 m²: 41;

Spectrum of life forms:

Phanerophytes – 7 (10,3%), Chamaephytes – 2 (2,9%), Hemicryptophytes (including biennials) – 42 species (61,8%), Geophytes – 3 (4,4%), Therophytes – 13 (20,6%).

(3) Spiraetum muscosum

Physical-geographical characteristics:

Area in the Tbilisi environs: fragmentarily is spread almost all over of the range of Spirea formation; **Altitude (m):** 600-1100; **Topography:** Slope; **Exposure (macro):** N (rare S, W); **Exposure (micro):** N, N-E, N-W, W, S-W, S-E; **Inclination:** 20°-35° (rare 10°-18°); **Soil:** cinnamonic; middle or thin depth;

Geo-botanical characteristics:

General projective coverage: 95-100%; **I layer (shrubs):** ***Projective coverage*** **-** from 80-85 to 95-98%, ***Distribution*** **-** evenly, ***Average height (cm)*** **-** 100-120; **II layer (herbs, semi-shrubs & dwarf semi-shrubs):** ***Projective coverage*** **-** 2-10%, ***Distribution*** **-** uneven; **III layer (moss cover):** ***Projective coverage*** **-** 60-90%, ***Distribution*** **-** evenly; **Sodding degree:** –; **Litter:** +; 0,1-0,5 cm depth (mostly are developed on the moss cover); **Dominant-edificator:** *Spiraea hypericifolia* (projective coverage from 60-70% to 95-98%);

Characteric species:

Shrubs: *Prunus incana* (frequency of occurrence 90%), *Paliurus spina-christi*, *Rhamnus pallasii*(40-40%), *Jasminum fruticans* (30%); **Semi-shrubs & dwarf semi-shrubs:** the constant species were not identified; **Perennial plants:** *Galium verum* (frequency of occurrence 70-70%), *Thalictrum collinum* (60-60%), *Filipendula vulgaris* (50%), *Phleum phleoides*, *Silene latifoia* (40-40%), *Festuca valesiaca*, *Melica transsilvanica* (30-30%); **Annual plants:** *Bromus squarrosus*, *Lolium rigidum* (frequency of occurrence 30-30%);

Number of species: 68;

Species richness on 25 m²: 16;

Spectrum of life forms:

Phanerophytes – 9 (13,2%), Chamaephytes – 2 (3,0%), Hemicryptophytes (including biennials) – 33 species (48,5%), Geophytes – 8 (11,8%), Therophytes – 16 (23,5%).

(4) Spiraetum stiposum pennatae

Physical-geographical characteristics:

Area in the Tbilisi environs: western foothills of Saguramo range, Mamadaviti Range (vicinities of Kustba Lake), Lisi range (vicinities of Lisi Lake), lower part of Digmistskali River gorge (between of Lisi Lake and settlement Vashlijvari); Altitude (m): 650-850; Topography: Slope; Exposure: N, N-W, S-W, S-E, E; Inclination: 25°-35°; Soil: cinnamonic or grey-cinnamonic, very skeletal, thin depth;

Geo-botanical characteristics:

General projective coverage: 80-90%; I layer (shrubs): Projective coverage - from 35-45% to 65-70%, Distribution - in many cases, uneven, Average height (cm) - 90-100; II layer (herbs, semi-shrubs &

dwarf semi-shrubs): Projective coverage - 40-70%, Distribution - more or less evenly; III layer (moss cover): Projective coverage - from 15-20% to 50-60%, Distribution - more or less evenly; Sodding degree: 8-10%; Litter: from 8-10% to 50-60%; 0,5-3 cm depth;

Dominant-edificator: Spiraea hypericifolia (projective coverage from 35-45% to 65-70%);

Subdominant-edificator: Stipa pennata (projective coverage 15-25%);

Characteric species:

Shrubs: Paliurus spina-christi (frequency of occurrence 50%), Prunus incana (37,5%); Semi-shrubs & dwarf semi-shrubs: Teucrium nuchense, Teucrium polium (frequency of occurrence 50-50%), Thymus coriifolius (37,5%); Perennial plants: Galium verum (frequency of occurrence 100%), Phleum phleoides, Potentilla recta, Rumex tuberosus (87,5-87,5%), Filipendula vulgaris, Melica transsilvanica, (75-75%), Dactylis glomerata, Salvia nemorosa, (62,5-62,5%), Euphorbia boiisieriana, Stachys atherocalyx, Thalictrum collinum (50-50%), Hypericum perforatum, Inula oculus-christi (37,5-37,5%); Annual plants: the constant species were not identified;

Number of species: 90;

Species richness on 25 m²: 34;

Spectrum of life forms:

Phanerophytes – 9 (10,0%), Chamaephytes – 5 (5,6%), Hemicryptophytes (including biennials) – 43 species (47,8%), Geophytes – 11 (12,2%), Therophytes – 22 (24,4%).

(5) Spiraetum festucoso-bothriochloosum

Physical-geographical characteristics:

Area in the Tbilisi environs: foothills of Saguramo range, vicinities of Jvari Monastry; Mamadaviti range, vicinities of Kustba Lake; **Altitude (m):** 650-800; **Topography:** slope and upper plane places of slopes; **Exposure (macro):** S, N; **Inclination:** from 12°-15° to 30°-35°; **Soil:** grey-cinnamonic, very skeleton, middle or thin depth; denuded bedrocks are observed also;

Geo-botanical characteristics:

General projective coverage: 90-100%; **I layer (shrubs):** ***Projective coverage*** **-** from 30-50% to 70-80%, ***Distribution*** **-** uneven, **Average height (cm) -** 90-120; **II layer (herbs, semi-shrubs & dwarf semi-shrubs):** ***Projective coverage*** **-** from 20-30% to 50%, ***Distribution*** **-** uneven; **III layer (moss cover):** ***Projective coverage*** **–** is not or + (S Exposure), 40-50% (N Exposure), ***Distribution*** **-** uneven; **Sodding degree:** 12-14%; **Litter:** +; 0,1-0,5 cm depth;

Dominant-edificator: *Spiraea hypericifolia* (projective coverage from 30-50% to 70-80%);

Subdominant-edificators: *Festuca valesiaca* (projective coverage 20-25%), *Bothriochloa ischaemum* (projective coverage 5-10%);

Characteric species:

Shrubs: *Paliurus spina-christi*, *Prunus incana, Rhamnus pallasii* (frequency of occurrence 40-40%); **Semi-shrubs & dwarf semi-shrubs:** *Teucrium nuchense*, *Teucrium polium* (60-60%); *Helianthemum nummularium* (frequency of occurence 40%); **Perennial plants:** *Galium verum*, *Melica transsilvanica* (frequency of occurrence 100-100%), *Rumex tuberosus*, *Stachys atherocalyx* (80-80%), *Dactylis glomerata*, *Eryngium campestre*, *Medicago caerulea*, *Potentilla recta* (60-60%), *Allium rotumdum*, *Astragalus brachycarpus*, *Dictamnus albus*, *Filipendula vulgaris*, *Hypericum perforatum*, *Phleum phleoides*, *Polygalla transcaucasica* (40-40%); **Annual plants:** *Arenaria serpyllifolia* (frequency of occurence 100%), *Alyssum hirsutum*, *Medicago minima* (80-80%), *Bromus squarrosus*, *Helianthemum salicifolium*, *Lolium rigidum* (60-60%), *Petrorhagia prolifera*, *Alyssum linifolium*, *Trifolium arvense* (40-40%);

Number of species: 55;

Species richness on 25 m²: 28;

Spectrum of life forms:

Phanerophytes – 5 (9,1%), Chamaephytes – 3 (5,4%), Hemicryptophytes (including biennials) – 21 species (38,2%), Geophytes – 5 (9,1%), Therophytes – 21 (38,2%).

III. Floristic composition

189 species of vascular plants, which belong to 43 families and 133 genera, were recorded. In the floristic spectrum leading families are: 1. Poaceae – 26 species (13,7%), 2. Asteraceae – 20 species (10,6%), 3. Fabaceae – 13 (6,9%), 4-5. Lamiaceae, Rosaceae – 12-12 (6,3-6,3%), 6. Brassicaceae – 10 (5,3%), 7. Apiaceae – 9 (4,8%), 8. Caryophyllaeae – 7 (3,7%), 9-10. Asparagaceae, Rubiaceae – 6 (3,2-3,2%).

First plant community is outstanding by rich floristic composition – 179 species of vascular plants. From 55 to 90 species of vascular plants were recorded in others plant communities.The life form spectrum is as follows: hemicryptophytes (including biennials) – 92 species (49,2%), therophytes – 50 (26,4%), geophytes – 23 (12,2%), phanerophytes – 18 (9,5%), chamaephytes – 6 (3,2%).

Most of constant species are perennial herbs (in many cases hemicryptophytes) and shrubs. In spite of species diversity (50 species), there are no constant species for Spirea formation among therophytes.

Full list of recorded plants is given bellow.

GYMNOSPERMAE

Cupressaceae

Juniperus communis L. subsp. *oblonga* (M.Bieb.) Galushko (*Juniperus oblonga* M.Bieb.; *Juniperus communis* var. *saxatilis* Pall.

Ephedraceae

Ephedra procera C.A.Mey. [*Ephedra major* subsp. *procera* (C.A.Mey.) Bornm.]

ANGIOSPERMAE

DYCOTYLEDONEAE

Sapindaceae (Aceraceae)

Acer ibericum M.Bieb. [*Acer monspessulanum* subsp. *ibericum* (M.Bieb. ex Willd.) Yalt.]

Anacardiaceae

Cotynus coggygria Scop.

Rhus coriaria L.

Apiaceae

Bupleurum marschallianum C.A.Mey.

Bupleurum rotundifolium L.

Daucus carota L.

Eryngium campestre L.

Eryngium caeruleum M.Bieb. (*E. caucasicum* Trautv.)

Falcaria vulgaris Bernh.

Heracleum antasiaticum Manden.

Malabaila dasyantha (K.Koch) Grossh.

Seseli grandivittatum (Sommier & Levier) Schischk.

Apocynaceae

Vinca herbacea Waldst. & Kit.

Asteraceae (Compositae)

Achillea biebersteinii Afan.

Achillea nobilis L.

Carduus hamulosus Ehrh.

Centaurea ovina Pall. ex Willd.

Centaurea reflexa Lam.

Crepis rhoeadifolia M.Bieb. [*C. foetida* L. subsp. *rhoeadifolia* (M.Bieb.) Čelak.]

Crepis sancta (L.) Babc.

Galatella linosyris (L.) Rchnb.fil. [*Crinitaria linosyris* (L.) Less.]

Galatella villosa (L.) Rchb.f. [*Crinitaria villosa* (L.) Cass.]

Crupina vulgaris Pres. ex Carss.

Filago eriocephala Guss.

Inula aspera Poir.

Inula oculus-christi L.

Inula germanica L.

Jurinea blanda (M.Bieb.) C.A.Mey.

Psephellus carthalinicus Sosn.

Klasea radiata (Waldst. & Kit.) Á.Löve & D.Löve [*Serratula radiata* (Waldst. & Kit.) M.Bieb.]

Tragopogon graminifolium DC.

Tragopogon tuberosus K.Koch

Xeranthemum squarrosum Boiss.

Boraginaceae

Aegonychon purpurea-coeruleum Holub.

Echium rubrum Jacq.

Lappula barbata (M.Bieb.) Gürke

Lycopsis orientalis L. (*Anchusa arvensis* subsp. *orientalis* (L.) Nordh.

Brassicaceae

Alyssum alyssoides (L.) L.

Alyssum desertorum Stapf

Alyssum hirsutum M.Bieb.

Alyssum linifolium Stephan ex Willd.

[*Meniocus linifolius* (Stephan ex Willd.) DC.]
Alyssum murale Waldst. & Kit.
Erophila verna (L.) DC.
Draba nemorosa L.
Hirschfeldia incana (L.) Lagr.-Foss.
Thlaspi orbiculatum steven
Turritis glabra L.

Caprifoliaceae (Dipsacaceae)

Cephalaria media Litv.
Lonicera iberica M.Bieb.
Scabiosa columbaria L.
Scabiosa micrantha Desf.

Caryophyllaceae

Arenaria serpyllifolia L.
Dianthus subulosus Freyn & Conrath
Gypsophila bicolor (Freyn. & Sint.) Grossh.
Petrorhagia prolifera (L.) P.W.Ball. & Heywood [*Kohlrauschia prolifera* (L.) Kunth]
Silene latifoia Poiar. [*Melandrium latifolium* (L.) Maire; *M. boissieri* Schishk.]
Silene italica (L.) Pers.
Silene cyri Schischk.

Cistaceae

Helianthemum lasiocarpum Janques & Herincq [H. ledifoliyum (L.) Mill.]
Helianthemum nummularium (L.) Mill.
Helianthemum salicifolium (L.) Mill.

Convolvculaceae

Convolvulus arvensis L.
Convolvulus cantabrica L.

Crassulaceae

Sedum pallidum M.Bieb.
Sedum caucasicum (Grossh.) Boriss. [*S. maximum* subsp. *ruprechtii* (Jalas) Soó]
Sempervivum transcaucasicum Muirhead

Euphorbiaceae

Euphorbia boissieriana (Woronow) Prokh.
Euphorbia helioscopia L.
Euphorbia iberica Boiss.
Euphorbia seguieriana Neck.

Fabaceae (Leguminosae)

Astragalus brachycarpus M.Bieb.
Securigera varia (L.) Lassen (*Coronilla varia* L.)
Cytisus caucasicus Grossh.
Dorycnium herbaceum Vill. [*D. pentaphyllum* Scop. subsp. *herbaceum* (Vill.) Rouy]
Genista sp.
Lotus corniculatus L.
Medicago caerulea Less. ex Ledeb.
Medicago minima (L.) Bartal.
Mellilotus neapolitanus Ten.
Onobrychis cyri Grossh.
Onobrychis radiata (Desf.) M.Bieb.
Trifolium arvense L.
Trifolium campestre Schreb.

Geraniaceae

Geranium lucidum L.
Geranium robertianum L.
Erodium cicutarium (L.) L'Her

Hypericaceae

Hypericum perforatum L.

Lamiaceae

Nepeta mussinii Spreng. ex Henckel
Origanum vulgare L.
Phlomis pungens Willd. [*Phlomis herba-venti* subsp. *pungens* (Willd.) Maire ex DeFilipps]
Phlomoides tuberosa (L.) Moench (*Phlomis tuberosa* L.)
Salvia nemorosa L.
Salvia verticillata L.
Scutellaria orientalis L.
Sideritis montana L.
Stachys atherocalyx K.Koch
Teucrium nuchense K.Koch [*Teucrium chamaedris* subsp. *nuchense* (K.koch) Rech.f.]
Teucrium polium L.
Thymus coriifolius Ronniger

Linaceae

Linum austriacum L.

Linum corymbulosum Rchb.

Oleaceae

Fraxinus excelsior L.

Jasminum fruticans L.

Orobanchaceae

Melampyrum arvense L.

Papaveraceae

Papaver arenarium M.Bieb.

Papaver dubium L.

Plantaginaceae

Plantago lanceolata L.

Veronica multifida L.

Polygallaceae

Polygala transcaucasica Tamamsch.

Polygonaceae

Rumex tuberosus L.

Primulaceae

Cyclamen vernum Sweet [*Cyclamen coum* subsp. *caucasicum* (K.Koch) O.Schwarz]

Primula macrocalyx Bunge [*Primula veris* subsp. *macrocalyx* (Bunge) Lüdi]

Primula woronowii Losinsk.

Ranuncilaceae

Adonis flammea Jacq.

Thalictrum collinum Wallr.

Resedaceae

Reseda lutea L.

Rhamnaceae

Paliurus spina-christi Mill.

Rhamnus pallasii Fisch. & C.A. Mey.

Rosaceae

Cotoneaster morulus Pojark.

Cotoneaster racemiflorus (Desf.) Booth ex Bosse

Filipendula vulgaris Moench

Fragaria vesca L.

Fragaria viridis Weston

Potentilla recta L.

Sanguisorba minor subsp. *balearica* (Bourg. ex Nyman) Muñoz Garm. & C.Navarro (*Poterium polygamum* WAldst. & Kit.)

Prunus incana (Pall.) Batsch [*Cerasus incana* (Pall.) Spach]

Prunus spinosa L.

Pyrus salicifolia Pall.

Rosa spinosissima L.

Spiraea hypericifolia L.

Rubiaceae

Asperula arvensis L.

Crucianella angustifolia L.

Galium album Mill.

Galium tenuissimum M.Bieb

Galium spurium L. (*Galium vaillantii* DC.)

Galium verum L.

Rutaceae

Dictamnus albus L.

Scrophulariaceae

Verbascum formosum Fisch. ex Schrank

Thymeleaceae

Thymelaea passerina (L.) Coss. & Germ.

Violaceae

Viola alba Besser

Viola arvensis Murray

Viola kitaibeliana Schult.

MONOCOTYLEDONEAE

Amaryllidaceae (Alliaceae)

Allium pseudoflavum Vved.

Allium rotundum L.

Allium sp.

Asparagaceae (Hyacinthaceae)

Asparagus officinalis L.

Asparagus verticillatus L.

Bellevalia montana (K.Koch) Boiss.

Muscari armeniacum Leichtlin ex Baker (*Muscari szovitsianum* Baker)

Ornithogalum ponticum Zahar.

Scilla siberica Haw.

Colchicaceae

Colchicum trigynum (Steven ex Adams) Stearn [*Merendera trigyna* (Steven ex Adams) Stapf]

Cyperaceae

Carex liparocarpos Gaudin subsp. *bordzilowskii* (V.I.Krecz.) T.V.Egorova

Carex humilis Leyss.

Iridaceae

Crocus biflorus Mill. subsp. *adamii* (J.Gay) B.Mathew.

Crocus speciosus M.Bieb.

Iris pumila L.

Iris reticulata M.Bieb.

Lilaceae

Fritillaria caucasica Adams

Gagea chlorantha (M.Bieb.) Schult. & Schult.f.

Gagea commutata K.Koch

Poaceae

Aegilops ovata L. (*Ae. neglecta* Req. ex Bertol.)

Aegilops tauschii Coss.

Agropyron cristatum subsp. *pectinatum* (M.Bieb.) Tzvelev

Avena sterilis L. subsp. *ludoviciana* (Durieu) J.M.Gillet & Magne

Bothriochloa ischaemum (L.) Keng

Brachypodium distachyon (L.) P.Beauv. [*Trachynia distachya* (L.) Link.]

Brachypodium sylvaticum (Huds.) P.Beauv.

Bromus biebersteinii Roem. & Schult. [*Bromopsis biebersteinii* (Roem. & Schult.) Holub]

Bromus japonicus Thunb.

Bromus squarrosus L.

Cleistogenes serotina (L.) Keng

Cynosurus echinatus L.

Dactylis glomerata L.

Elymus repens (L.) Gould [*Agropyron repens* (L.) P.Beauv.; *Elytrigia repens* (L.) Nevski]

Elymus hispidus (Opiz) Melderis [*Agropyron intermedium* (Host) P. Beauv.; *Elytrigia intermedia* (Host.) Nevski]

Festuca valesiaca Schleich. ex Gaudin

Koeleria cristata (L.) Pers.

Lolium rigidum Gaudin

Melica transsilvanica Schur

Phleum paniculatum Huds.

Phleum phleoides (L.) H.Karst.

Poa angustifolia L.

Poa bulbosa L. subsp. *vivipara* (Koeler) Arcang.

Poa nemoralis L.

Stipa capillata L.

Stipa pennata L.

Conclusion

Area of Spirea formation (Spiraeta hypericifolia) in Tbilisi environs is in foothills and lower mountain belt, approximately 600-1000 (1100) m above s.l.. Plant communities of formation are developed on slopes and plane places with various exposure and inclination, mainly on the cinnamonic and grey-cinnamonic soils. Often soils are skeletal. Mainly, soils are thin or middle depth. In Tbilisi environs the Spuirea's plant communities are either primary or secondary origin.

Typological composition of Spirea formation (Spiraeta hypericifolia) of Tbilisi environs is such: (1) Spiraetum gramino-mixtoherbosum, (2) Spiraetum muscosum, (3) Paliuroso-Spiraetum gramino-mixtoherbosum, (4) Spiraetum festucoso-bothriochloosum, (5) Spiraetum festucoso-bothriochloosum.

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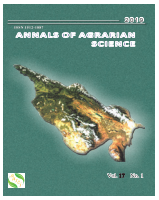

Forecasting Seasonal Rainfall Patterns for Crop Production in Juba County, South Sudan Using the Artificial Neural Networks

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A B S T R A C T

A simple Multi-Layered Feed Forward (MLF) training process of the Artificial Neural Networks model with a learning back-propagation algorithm was applied to forecast rainfall data of Juba County, Central Equatoria State in South Sudan from 1997-2016. Annual rainfall data were aggregated into three seasons MAMJ, JAS and OND and later trained for best predictions for the period 2017-2034 using the Alyuda Forecaster XL software. Best training was attained once the *minimum error or cost function* of the weight $\Delta\omega$ was attained during gradient descent and expressed as Mean Square Error (MSE) and AE of the input variable. The results showed that for MAMJ and JAS months, the number good forecasts were over 97% whereas this was between 60-80% for OND months. The Seasonal Kendal (SK) test on future rainfall forecasts as well as the Theil-Sen slope showed a declining monotonic trend in the mean amounts in all three seasons with MAMJ, JAS at OND at 100, 150 and 80 mm respectively towards the end of 2034. Forecast of the Standardized Precipitation Index (SPI) showed that the MAMJ months for the years 2019 to 2027 will be moderately wet with near to normal drought except in April 2021 which will experience some severe wetness. Interdecadal severe drought is expected between 2028 to 2033 after almost two decades. The SPI of JAS and OND seasons will remain near normal to moderate drought during the same period. Declining onset of MAMJ rains is expected to significantly affect the timing for land preparation and crop planting. The forecast accuracy of the MLFFNN can be used as a vital tool for decision makers in projecting future rainfall and drought events.

Keywords: Artificial Neural Networks, Standardized Precipitation Index,Cumulative Distribution Function, Theil-Sen Slope Estimator Rainfall Forecasting, Precipitation Index, Drought Events.

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Introduction

Agriculture and more specifically crop production is the mainstay of much of the rural population of Juba County of Central Equatoria State (CES), South Sudan. It is predominantly rain-fed and therefore, sustainable crop production is not only contingent upon the frequency, intensity and magnitude of rainfall but also on the spatial-temporal variations. These factors all make rainfall predictions more challenging especially when coupled with the effects of climate change. Although no prior studies on the seasonality of rainfall distribution in CES have been conducted, experiential evidences from farming communities in the region over the last two decades report of clear deviations and decrease below average values and intensity especially during the onset of the rainy season (*Ja´be*). Generally, the annual onset of rain starts during the second to third dekad of April and continues till June punctuated with a dry spell around July. This then continues from August till October, significantly decreasing toward November and December dry season (*Méling*). The rainfall

pattern may be described as more or less bimodal in nature. Such belated occurrences of onset rains during this period over the last two decades could be attributed to the prolonged impacts of *El Niño*. The effects of such temporal shift from the traditional farming calendar caused by *El Niño* resulting to untimely availability of soil moisture [1] are often poor harvests or crop failure of crops like cowpea, maize or peanuts. Understanding such erratic rainfall events and assessing seasonal rainfall trends would require a better understanding of the effects of "*meteorological drought*" on the "*agricultural drought*". The former is expressed entirely based of the degree of dryness (usually related to rainfall anomaly from the long-term mean) whereas the latter is based on temporal soil moisture deficit during crop phenology coupled with intensive actual evapotranspiration.

Much of the rainfall predictions for South Sudan encompassing the study area of CES have in the last decade been issued by diverse regional and international institutions like the IGAD Climate Prediction and Applications Centre, (ICPAC); UN Food and Agriculture Organization (FAO); United Nations Office for the Coordination of Humanitarian Affairs (UNOCHA). These predictions are however, monthly with short decadal time-scales and often in the form of probabilities relative to monthly or seasonal rainfall averages. Spatial and temporal rainfall patterns do not often correlate with soil moisture contents and dynamics. [2] showed that surface soil moisture dynamics generally follow rainfall patterns at the two gravel plain sites, whereas this was not the case soil moisture dynamics in the sand dune site. Therefore, depending on intensity of rainfall, soil structure, surface sealing and infiltration, clear distinctions between meteorological and agricultural droughts should be made and how both are interlinked.

Developing drought risk insurance models for any country in sub-Sahara Africa where drought accounts for about 40% of the economic losses to smallholder farmers [3], would be of paramount significance especially, if the ANN training of historical data and subsequent prediction were based on shorter hourly or daily time scales. Other indices e.g. Soil Moisture Deficit Index (SMDI) [4] or the Normalized Soil Moisture Index (NSMI) [5] may be more appropriate indices than the SPI in developing drought risk insurance models as they directly capture the moisture status and available volumes of water in the soil. The Global Climate Observation System (GCOS) program has acknowledged soil moisture as one of the Essential Climate Variable (ESV SM) that will have to be routinely measured and monitored in space and time. In the last three decades, temporal and spatial in-situ soil moisture contents have continuously been measured through satellite-based soil moisture products obtained from active and passive microwave sensors like the Advanced Microwave Scanning Radiometer on Earth Observing System (AMSR-E) by [6] or the AQUA AMSR-E by [7]. Such geo-referenced soil moisture data would be used as input in ANN and in making spatial-temporal projections in drought risk insurance models.

In the last two decades, much research on rainfall prediction using the ANNs have been conducted in different parts of the world from monsoon summer rainfall using time series [8]; seasonal [9]; daily [10]; hourly [11]; dekadal [12]; monthly [13]. A comprehensive overview of ANNs use in temporal rainfall prediction has been reported by [14]. However, only a few similar studies have been conducted in Africa, in Ethiopia [15]; in Algeria [16] and in West Africa [17]. Other studies on predicting monthly tropical rainfall used hybrid linear stochastic with non-linear extreme machine learning method [18, 19]**.** Other researchers have applied linear stochastic methods e.g. autoregressive moving average ARMA for rainfall prediction [20]; Box-Jenkins SARIMA model [21] or predicting solid weekly waste generation using ANN and ARMA [22]; monthly river inflow using the seasonal autoregressive integrated moving average-adaptive neuro fuzzy inference systems (SARIMA-ANFIS) [23]; oil-gas production engineering [24,25]; in healthcare engineering [26]; image-based plant disease detection [27]; soil clay content mapping using hyperspectral image [28]; limited medical datasets [29]. Basically, the ANN is a type of Machine Learning (ML), whereby a computer-based model fed with historical data in a time series is trained to identify specific patterns and the derived "*intelligence*" later used to predict future events. Some major advantages of the ANN are its ability to: i) model nonlinear and complex relationships between input and output variables; ii) it can learn and make *generalization* from this input-output relationship. This learned processes can be used to

make inferences on previously unseen data, iii) it is not affected by *heteroscedasticity* and therefore does not impose any restrictions on the distribution of the input variable. This means, it can still model an input-output relationship irrespective of the non-constant nature of the variance in the time series.

to produce their respective outputs $f(\Sigma h_n)w_{hn}$. The total output ($\Sigma\Sigma\sigma_1$) is then the output of the entire ANN for that specific input (x_1) and is compared to the target value (σ_T). The difference is expressed as the measure of error (E) between the computed and expected values. The main

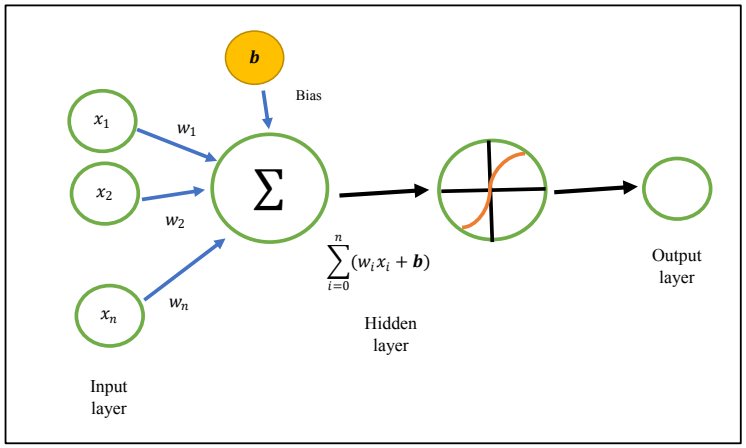

Fig. 1. *Simplified architecture of a feedforward two-layer ANN model with an input, hidden and output layer.*

However, one major drawback of the ANN is, its proneness to overfitting.

ANN Architecture

The neural network is based on a simplified and popularly used architecture called the Multi-Layer Feed Forward Neural Network (MLFFNN) as in Fig.1. Hereby, the product of the values of neurons or impulses $(x_1, x_2, x_3 \ldots . x_n)$ from the input layer and their respective weighted values $(wx_1, wx_2, w_{x3} \ldots . wx_n)$ are passed on to the middle neurons $(\Sigma h_1, \Sigma h_2, \Sigma h_3 \ldots . \Sigma h_n)$ in the hidden layer(s). Further, the products from the individual neurons in the hidden layer(s) $(\Sigma h_1, \Sigma h_2, \Sigma h_3 \ldots . \Sigma h_n)$ and the weighted sum of their respective inputs are passed on upon activation through a *transfer or logistic function* advantage of this MLFFNN model is that, the process of back propagation from the output to the hidden layer ensues and continues iteratively depending on margin of error till a minimum error value is attained.

Standard Precipitation Index, SPI

The Standardized Precipitation Index (SPI) is a popularly used index to characterize meteorological drought [30], on varying timescales. SPI is a probability index based entirely on precipitation as input variable. Formulated by [31] it can better represent rainfall anomalies in terms of wetness or drought than for example the Palmer Drought Severity Index (PSDI).

Table1. *Classification scale for the SPI*

Drought		**Wetness**	
Description	**SPI**	**Description**	**SPI**
Extreme drought	< -2.0	Extreme wetness	>2.0
Severe drought	-1.5 to-2.0	Severely wet	1.5 to 2.0
Moderate drought	-1.0 to -1.5	Moderately wet	1.0 to 1.5
Near normal	-1.0 to 0.0	Near normal	0.0 to 1.0

The first step in calculating the SPI is to determine a Cumulative Distribution Function (CDF) that describes the long-term time series of precipitation observations in either 1, 3, 6-month, etc. intervals. The CDF with mean zero (*corresponds to the median precipitation*), is then applied to the cumulative probability and the SPI estimated. For a given time-scale, SPI values are positive for greater than and negative for less than median precipitation. The magnitude of the SPI departure from zero is a probabilistic measure of the severity of a wet or dry event that can be used for risk assessment. The time series of the SPI can be used for drought monitoring by setting application-specific thresholds of the SPI for defining drought beginning and ending times

Basically, the SPI was envisaged to express the spatial-temporal drought events and variability as influenced by rainfall deficit. It is defined as the number of standard deviations from which normally distributed random variable deviates from its long-term mean. Generally, precipitation whether monthly, seasonally or otherwise are not normally distributed and therefore, an equiprobability transformation is performed such that the derived SPI values follow a normal distribution with mean = 0 and standard deviation = 1. This is obtained by fitting a gamma function $\Gamma(\alpha)$ of the cumulative distribution to precipitation values in the time series (*as in our study a 3-month period*). For most part, the SPI estimates were let to vary between -2.0 and +2.0 which contained approximately 95% of the SPIs values with close to 68% within the range -1 to +1.

In our study therefore, we attempted to use a simple Multi-Layer Perceptron model of the artificial neural network in predicting rainfall patterns for crop production for the period 2016-2033. We evaluate its relevance in forecasting "*lumped*" seasonal precipitation derived from historical data after training and testing.

Methodology

This study evaluated the significance of the ANNs in the forecasting of seasonal rainfall patterns in Juba County of Central Equatoria State (CES), South Sudan. In general, there are five basics steps: (1) collecting data, (2) preprocessing data, (3) building the network, (4) training and (5) test performance of model. The basic flow in designing ANNs model is given in Fig. 1. The daily rainfall data for Juba weather station as from the years 1983 to 2015 were downloaded from the US National Oceanic and Atmospheric Administration (NOAA). However, daily and consistent rainfall data were only recorded as from 1997 to 2016 and were used for this study. Data preprocessing involved aggregating the daily rainfall amounts to monthly means of March-April-May-June (MAMJ), July-August-September (JAS) and October-November-December (OND). Due to the unpredictable onset of rains especially between mid to end of March of each season, the MAMJ was "*lumped*" together. Rainfall around mid-March prior to the onset of the rainfall season in April was characterized by drizzles and light rainfall showers. With these monthly rainfall data sets, neural networks were then created and later proceeded by training and forecasting.

The chosen rainfall data for each season were divided into two random groups, the training and test sets corresponding to 82% and 18% respectively. Networks were trained for a fixed number of epochs or iterations till a minimum error function was reached. The optimal number of neurons in the hidden layer was obtained experimentally running the training process several times until a good performance was obtained or when no other changes were observed.

The Seasonal Kendall (SK) Test

The rank-based nonparametric Seasonal-Kendall method was applied to the long-term rainfall to detect any statistically significant trends. In this SK test and for the null hypothesis (H_0), assumed that there was no monotonic trend in precipitation amounts over time; and for the alternate hypothesis (H_1), it assumed that there was either an increasing or decreasing monotonic trend over time.

Once the seasonal rainfall data from 1997-2015 for MAMJ, JAS and OND months were trained and future forecasts made using neural network, test for the presence of any monotonic trend in the seasonal rainfall during the entire period between 1997 to 2034 was conducted using the Seasonal Kendall (SK) test [32,33,34]. The SK statistic for the *i-th* season S_i may be computed as:

$$\boldsymbol{S_i = \sum_{k=1}^{n-1} \sum_{j=k+1}^{n} sgn(x_{ij} - x_{ik}), \quad i = 1, 2, 3, \ldots n} \qquad (1)$$

$$sgn(x_{ij} - x_{ik}) \begin{cases} 1 \; if \; x > 0 \\ 0 \; if \; x = 0 \\ -1 \; if \; x < 0 \end{cases} \quad (2)$$

Where $sgn(x_{ij} - x_{ik})$ is the indicator function for the month (i) for the two respective years j and k. The SK statistic $\vec{S}$ for the entire series may be computed as:

$$\vec{S} = \sum_{i=1}^{n} S_i \quad (3)$$

A positive value of $\vec{S}$ would indicate that there is a positive monotonic trend and vice versa. This is the alternative hypothesis (H_α) whereas the null hypothesis (H_0) would suggest that there is no monotonic trend in the time series at $p < 0.05$. The value of $\vec{S}$ and Var ($\vec{S}$) may be used to compute the test statistic Z as:

$$Z = \begin{cases} \dfrac{\vec{S} - 1}{\sqrt{\text{Var}(\vec{S})}} & if \; \vec{S} > 0 \\ 0 & if \; \vec{S} = 0 \\ \dfrac{\vec{S} + 1}{\sqrt{\text{Var}(\vec{S})}} & if \; \vec{S} < 0 \end{cases} \quad (4)$$

The presence of a statistically significant trend is evaluated using the Z statistic. A positive value of Z indicates an upward trend and a negative value indicates a downward trend. Incorporating the Theil-Sen slope estimator to the SK test gives a better understanding of the magnitude (*change of unit per time*) of the slope. Generally, the slope Q between two successive values in a time series is expressed as:

$$Q = \frac{x_j - x_k}{j - k}, j \neq k \; and \; j > k \quad \text{for } i = 1,2 \ldots N \quad (5)$$

Where x is the value at the j and k-th interval for n observations and N = n(n-1)/2. The overall Theil-Sen estimator is the median of these N values of Q can then be expressed. Significant trends at either the 95% or 99% confidence intervals can then be computed with the confidence limits defined by M_1 and M_2. Derivation of these indices is referred to [35].

Cumulative Distribution Function (CDF) of gamma distribution

For some chosen rainfall season and time scale, the CDF $G(x)$ of a gamma distribution (Γ_α) is defined as:

$$G(x) = \frac{1}{\beta^\alpha \Gamma_\alpha} x^{\alpha - 1} e^{-\frac{x}{\beta}} \quad (6)$$

Where;

$$\Gamma_\alpha = \int_0^\infty e^{-t} t^{\alpha - 1} dt \quad (7)$$

Where $x > 0$ is the rainfall amount and the gamma distribution parameters $\alpha > 0$ as the shape and $\beta > 0$ the scale parameters and can be estimated through the Maximum Likelihood Estimation (MLE). First, a measure of the skewness (A) with median $m = 0.5$ of all (x_i) non-zero values in the rainfall time series and ($\overline{x_i}$) the arithmetic mean is estimated as:

$$\text{A} = \ln(\overline{x_i}) - \left(\sum_{i=1}^{n} \ln(x_i) \right) n \quad (8)$$

The values for the gamma distribution parameters can then be estimated as:

$$\tilde{\alpha} = \frac{1}{4A} \left(1 + \sqrt{1 + \frac{4A}{3}} \right) \quad (9)$$

$$\tilde{\beta} = \frac{\overline{x_i}}{\tilde{\alpha}} \quad (10)$$

Data pre-processing and training of the network

In order to enhance a faster convergence, the monthly input variables were normalized relative to the seasonal averages. The normalized values consistent with the sigmoid activation function were between 0 and 1. Since probability is between 0 and 1, the normalized values would give better predictions during training. The Alyuda ForecasterXL basically splits the data into two sets (1) training and validation sets (2) training set. During training, the weights of the neural network were adjusted whereas the validation increases the

accuracy by minimizing the error function (**E**) during iteration. The training stopped once the error function reached a global minimum. Finally, the performance of the network was evaluated on the test data set which had not been involved in the training process. In this study, the neural network was trained with 76, 56 and 55 datasets for the MAMJ, JAS and OND months respectively.

Model performance

The performance of the neural network was best done by using the linear regression coefficients (r^2) of the actual and forecasted data during training. Hereby, the regression coefficients for each season for the test period 1997-2015 were calculated for the entire dataset, as well as the best model predictive performance in terms of good and bad forecasts (*expressed relative to 100% highest accuracy*) for the training (P_{train}) and test (P_{test}) data respectively. As in our case, $r^2 \geq 0.9$, $P_{train} \geq 90\%$, $P_{test} \geq 70\%$ were considered as good model performance indicators within the error of tolerance as in (Table 2)

Similarly, prediction accuracy of the ANN was tested by using both the Mean Standard Error (MSE) and Absolute Error (AE) during training. AE is the absolute difference between the predicted and observed values.

Training using a single input variable (x_{ij} measured rainfall amount for the *i*-th training case at the *j*-th network output) for (n) observations in a time series was conducted and the best forecast or prediction ($\hat{x}_{ij}$) after each iteration was estimated by minimum *error function* denoted by the AE or MSE as:

$$\mathrm{AE} = x_{ij} - \hat{x}_{ij} \tag{11}$$

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}\left(x_{ij} - \hat{x}_{ij}\right)^2 \tag{12}$$

In both cases, the error function (**E**) is directly dependent on the weight component (**W**) which in turn influences the learning rate (η). This is updated or changes iteratively during gradient descent as:

$$\Delta\mathbf{W} = \eta\frac{\partial\mathbf{E}(\mathbf{W})}{\partial\mathbf{W}} \tag{13}$$

The smaller the error function the better the prediction during the training process. A minimum of five training runs were done on the same data set to obtain the best MSE. Thereafter, the neural network was perceived to have learned and could then be used for making predictions for unknown data. Training parameters like number of hidden layers, stopping condition, iterations number, learning rate and generalization loss were estimated on trial-and-error basis for each dataset.

On a similar note, monthly SPI values were estimated from respective CDF curves of historical rainfall data from 1997-2015 These SPI were trained and predictions for the period from 2016 to 2034 subsequently made.

Results and discussion

Neural Network Performance

The trained JAS with smaller dataset (n=56) and single hidden layer appeared to outperform the MAMJ dataset with larger dataset (n=76) and two hidden layers demonstrating the difference in performance as influenced by data size. The variances for MAMJ (μ=0.252), JAS (μ=0.332) and OND (μ=0.345) were 0.01, 0.006 and 0.07 respectively. Comparing the variance effects on all datasets, there was a notable difference on learning especially of JAS and OND datasets with similar data size. The MAMJ and OND were characterized by high standard deviation (σ=0.266) and (σ=0.1) respectively, whereas for JAS, this was σ=0.076). However, all training sets achieved statistically significant performances ($r^2 \geq 0.99$) with number of good forecasts over 60%. Figure 2 shows a plot of MSE and AE vs iterations during training for the MAMJ, JAS and OND datasets for the years 1997-2015. The accuracy of both error function estimates showed steep gradients prior to 1000-th iteration till to convergence at global minima. During training of the MAMJ dataset for example, there was a sharp decrease of the MSE from about 0.016 to as low as 0.0007 while for the AE this was between 0.1 to 0.016. It is seen that both error functions were large at low iteration values decreasing till convergence and subsequently increasing with further iterations. The AE and MSE during training for OND was ten-fold larger that of either MAMJ and JAS. The learning rate (η) as measured by number of iterations to reach global minimum is fastest for OND at 1008 than for

JAS and MAMJ at 1625 and 1363 respectively. Low iteration number for OND would suggest, that the stochastic gradient descent algorithm effected larger step size parameter with large errors. This accounted for faster and poor learning rates and therefore, poor generalization. Conversely, smaller step sizes with smaller gradients resulted into larger number of iterations and comparatively lesser errors and better generalization. Better generalization was manifested by the comparatively higher percentage of good forecasts in the training sets of both MAMJ and JAS. Further, the rate of change of the error function ΔW as in Eq. (13) had a significant effect on the performance and accuracy of the ANN.

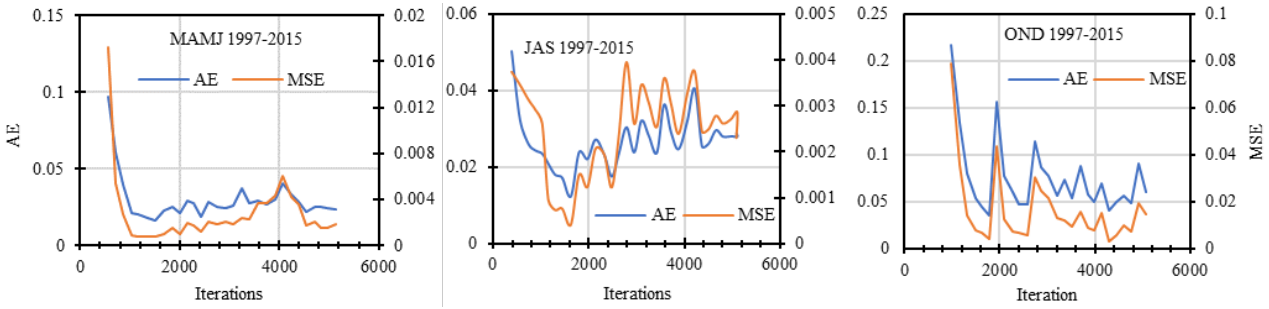

Fig. 2. *Error development and convergence to global minimum during training of MAMJ, JAS and OND of time series data between 1997 to 2015.*

Table 2 shows the training parameters and accuracy according to the r^2, number of good forecasts, hidden layer(s), MSE, and AE. The network demonstrated better performance rate for MAMJ and JAS when using two and one hidden layers respectively. The results here suggest, that model performance in terms of the number of good forecasts (98%) and approximation during validation for both seasons was independent of the number of hidden layers. In effect, one hidden layer performed just as good as two layers. Similar results on neural performance with a single hidden layer were reported by [36,37,38,39,40]. Although both JAS and OND trained datasets had each one hidden layer and almost equal data size, the latter gave a low number of good forecasts at 61% and high number of bad forecasts (39%).

Generally, better accuracy was shown by both MAMJ (2 hidden layers) and JAS (one hidden layer) trained data with a ten-fold less error than that of OND. However, training in terms of number of iterations needed for convergence, percentage of good and bad forecasts was observed in JAS dataset with one hidden layer, while the second best was MAMJ with two hidden layers. On the other hand, OND showed the highest inaccuracy with one hidden layer. This could be due to the inability to learn from a small dataset, although the learning logistic regression algorithm for JAS with similar data size seemed to work well. Similar observations were reported by [29,41]. Such conflicting generalizations in terms of the number of hidden layers for MAMJ-JAS as well as for JAS-OND on model performance and accuracy indicate striking instability especially for smaller datasets. For instance, using one hidden layer, the OND dataset had a learning rate of 0.0062 and reached the global minimum at lower iterations than JAS at 0.0021. After that, the error functions AE and MSE started to increase indicating that the model was getting over-fitted. Moreover, the MAMJ dataset with two hidden layers had a learning rate at 0.004 (Table 2) and was comparatively lower than that of OND but greater than that of JAS dataset with one hidden layer.

Conclusively, one can say, that the learning rate during gradient descent is inversely related to the number of iterations in reaching a global minimum. Judging by the rule-of-thumb in estimating the number of neurons in the hidden layer(s), our study showed that this was between 105 and 210 neurons for one and two hidden layers respectively for MAMJ dataset, whereas these were 99 and 100 neurons for OND and JAS datasets respectively. Despite such striking inconsistency between the JAS and OND datasets with the single hidden layer, the accuracy and generalization performance of the two-layer feedforward neural network model was satisfactory. With the error tolerance (%) as indicator for overall performance, the results demonstrate that, this model was able to achieve remarkable performances on predictive tasks with limited data size as in MAMJ and JAS datasets, but unable to perform well on smaller datasets as in OND.

Table 2. *Training parameters and network structures showing the goodness of error estimation between the training and test sets.*

	MAMJ		JAS		OND	
	Training set	**Test set**	**Training set**	**Test set**	**Training set**	**Test set**
Nr. of data set	63	13	47	9	46	9
AE	0.005	0.005	0.003	0.006	0.015	0.02
MSE	4.91E-05	2.35E-05	2.71E-05	7.42E-05	0.0003	0.0004
Error tolerance (%)	10	30	10	30	10	30
Nr. of good forecasts (%)	61(98%)	13(100%)	46(98%)	9(100%)	28(61%)	7(78%)
Nr. of bad forecasts (%)	2(3%)	0(0%)	1(2%)	0(0%)	18(39%)	2(22%)
r^2	0.994		0.996		0.997	
No. of hidden layer (s)	2		1		1	
No. of input layer (s)	1		1		1	
No. of output layer(s)	1		1		1	
Best at iteration number	1363		1625		1008	
Learning rate (η) till global minimum	0.0040		0.0021		0.0062	

Fig. 3 compares the performance of the linear-nonlinear method in predicting rainfall during training. The results show that both linear and nonlinear estimations had excellent abilities to forecast the seasonal rainfall amounts in the time series between 1997-2015. The average regression coefficient in the linear method was about (r^2=0.99). Although the actual and forecasted data during training seemed to give a high correlation and (r^2), it still showed some amount of error or noise as in (Fig. 2) for most part of the seasonal dataset trained. These errors were within the tolerance range put forth by the software. Hereby, the ANN model for rainfall forecasting may be assumed to be probabilistic and containing both deterministic as well as random error components. Therefore, both linear-nonlinear methods presented here during training of datasets can be regarded as good tools to be use for forecasting seasonal rainfall patterns

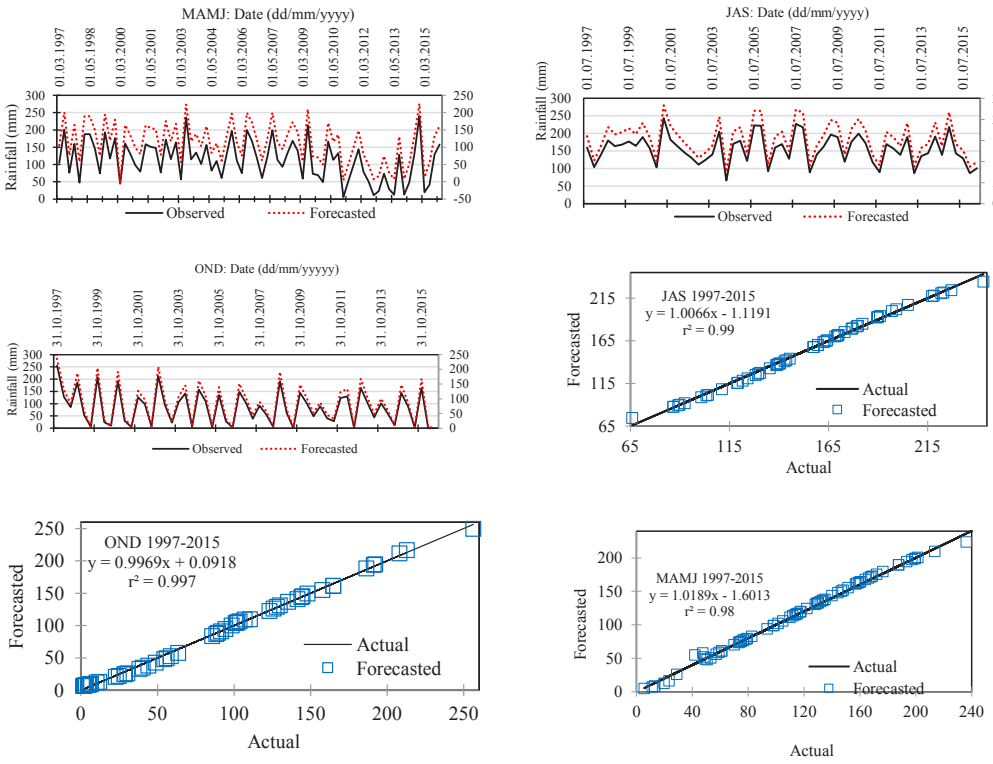

Fig. 3. *Scatter plots showing the correlation between the actual and forecasted data during training of neural network.*

Trends in mean seasonal rainfall and SK

Trained rainfall data for the MAMJ. JAS and OND months from 1997-2015 were used to forecast the mean rainfall over the period 2016-2034. Obtained results are shown in Fig. 4. The SK test *(S=-317; Z=0.774 and p=0.029*) showed a negative monotonic trend and statistically significant at 99% confidence level. The mean rainfall at the start of measurement for the MAMJ in 1997 was about 125 mm with about 5-10 mm reduction in 2015. This was a mean rainfall reduction of approximately 0.278 to 0.556 mm/year. Model projections from 2016-2034 forecasted a near 18% decrease in mean rainfall to about 100 mm. The total MAMJ rainfall reduction for Juba county between 1997 to 2034 is projected to be close to 32 mm. The JAS months also showed a decrease in the mean rainfall amount towards the end of 2034 forecasting period. The SK test (*S= -1171; Z= 1.901; p=0.234*) also showed a negative monotonic trend and statistically significant at the 95% confidence level. This was a slight 6-7% decrease from about 160 mm in 1997 to about 100 mm in 2034. The Theil-Sen slope Q* forecasted an annual drop of 0.2 mm/year which on average would be close to 7.64 mm as from 1997 to 2034. The OND also showed similar negative monotonic trend with SK test (*S= -317; Z=0.774; p=0.220*). There was a 21% decrease from about 95 mm in 1997 to about 75 mm forecast in 2034. The estimated Theil-Sen slope Q* value of 0.100 mm/year would be about 3.8 mm in 2034. Trend analysis in of all the seasons revealed a general decrease of rainfall in Juba County with highest decrease during the MAMJ and lowest in OND.

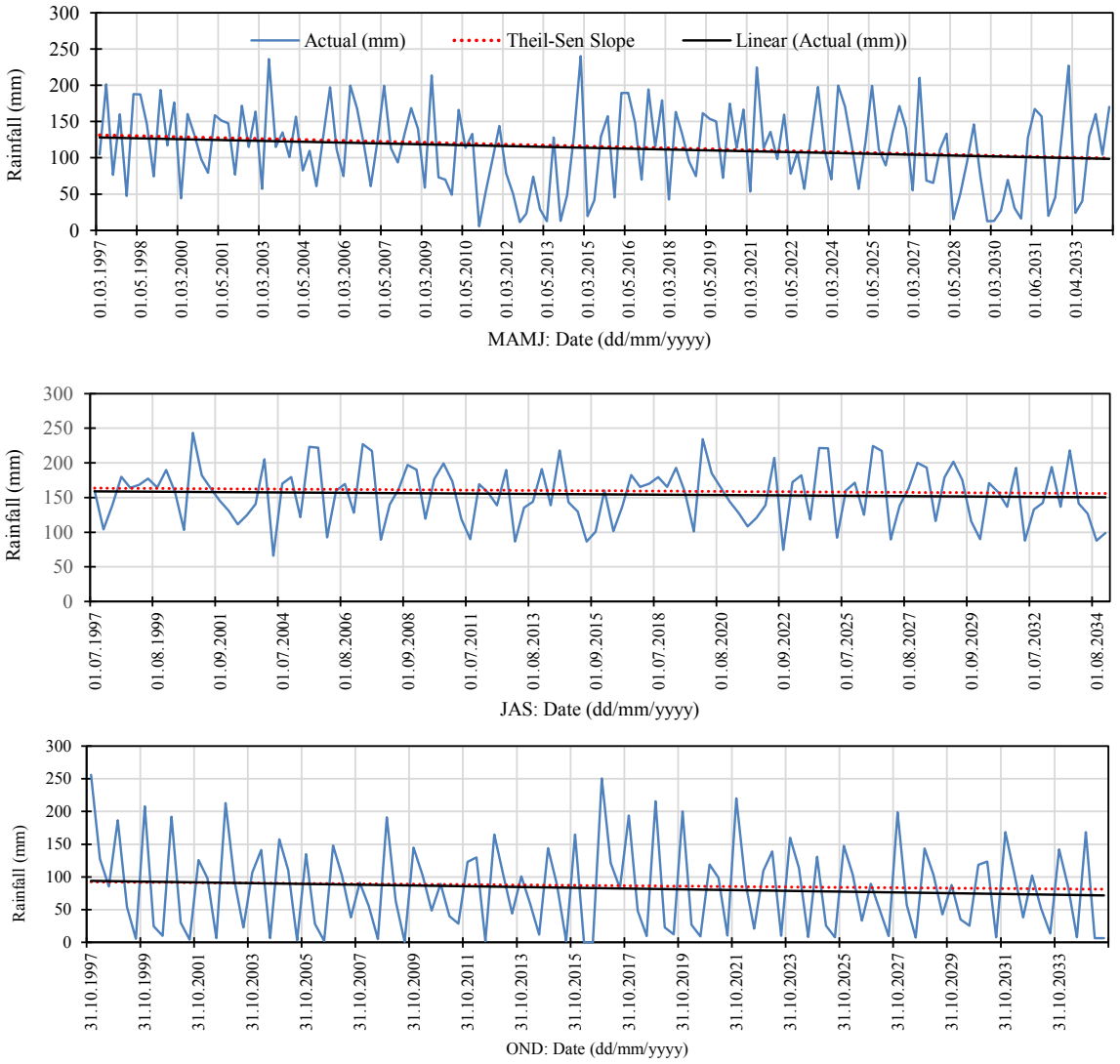

Fig. 4. *Ordinary Least Squares (OLS) Trendline (black line) of actual and the Theil-Sen slope (red-dotted line) between 1997-2034 for the MAMJ, JAS and OND months.*

Inter-seasonal SPI changes and drought

The histograms of the SPI during the MAMJ months is shown in Fig. 5. It was found that most of the MAMJ months of the preceding years from 1997 to 2016 showed near to normal to moderate wetness with similar drought SPI values. Recurrent severe drought for MAMJ months was witnessed during the years 2010-2013 with unusual extreme wetness in July 2014. However, the years 2015 till 2017 show normal to moderately wet MAMJ months. During 2018, the SPI for the AMJ months are expected to be near normal with moderate drought in March Table 3. It is forecasted, that the MAMJ months for the years 2019 to 2027 will be moderately wet with near to normal drought except in April 2021 which will experience some severe wetness (due to intensive rainfall). Interdecadal severe drought at SPI -1.5 to -2.0 with rainfall ranges less than 10th to 5th percentile is expected between 2028 to 2033 after almost two decades and is anticipated to have a long duration.

The SPI in July 2019 is expected to be about 0.6 with average rainfall of about 170 mm, while July 2019 with SPI 0.1 and about 150 mm. Occasional heavy rainfall downpour is expected between November/December 2019 despite the seasonal dryness during this period.

For the JAS months, 2018 is expected to be near normal Fig. 6. Sporadic and heavy rainfall is also expected during this period with 7-10 days of dry spells with absolutely no rainfall. In 2019, moderate drought in August with SPI -1.3 with rainfall range between 20th and 10th percentile is forecasted followed by severe wetness in September due to intensive rainfall that may cause serious flooding. However, inter-seasonal drought in September 2022 at SPI -1.9 is expected with rainfall range less than the 10th to 5th percentile. In our study, we found out that recurrent droughts in August followed by severe wetness in September is forecasted to continue till 2034. For the JAS months, no extreme drought is anticipated.

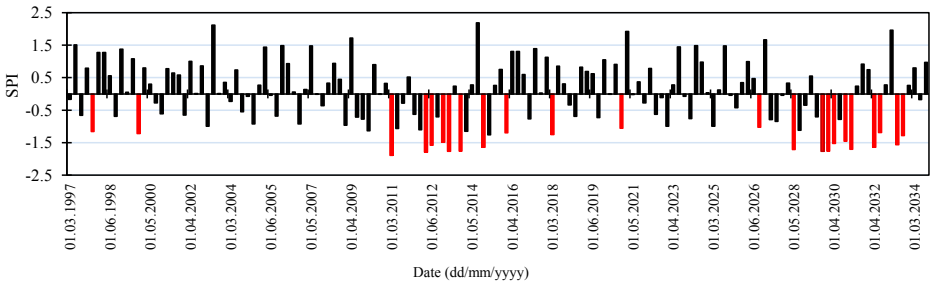

Fig. 5. *Calculated and projected SPI for the MAMJ months during the period 1997-2034*

Description	Month/Year
Moderate drought	03/2018, 03/2021, 03/2027, 04/2031, 05/2033
Severe drought	05/2028, 03/2030, 04/2030, 04/2032, 04/2033
Extreme drought	

Table 3. *Projected drought periods using SPI values from 2018 to 2034 for the MAMJ months*

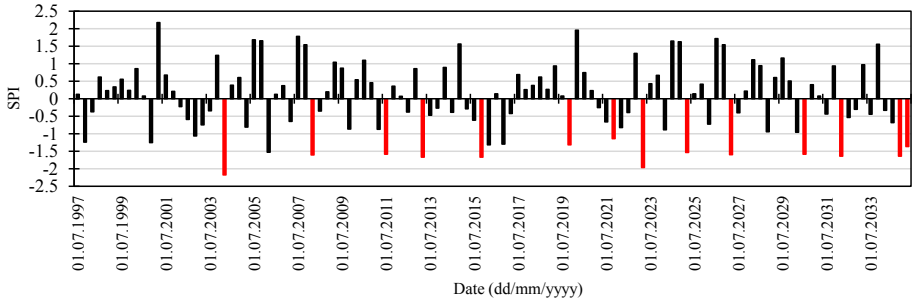

Fig. 6. *Calculated and projected SPI for the JAS months during the period 1997-2034*

Description	Month/Year
Moderate drought	08/2021
Severe drought	09/2022, 09/2024, 09/2026, 07/2030, 09/2031, 08/2034
Extreme drought	

Table 4. *Projected drought periods using the SPI values from 2018 to 2034 for the JAS months.*

For OND months, 2018 is expected to be remain near to normal with moderate drought at SPI -1.0 to -1.5 (rainfall range less than the 20th to 10th percentile) for much of the period except in October which is forecasted to be severely wet (Fig. 7). The cumulative effect of moderate drought especially during November and December months with SPI values between -0.93 to -1.1 respectively and rainfall probability between 10-20% or 20th to 10th percentile is expected to continue into the following new MAMJ season and significantly delay the onset of rainfall in March-April. This recurrent phenomenon will ostensibly interrupt the traditional farming-calendar especially during land preparation and sowing.

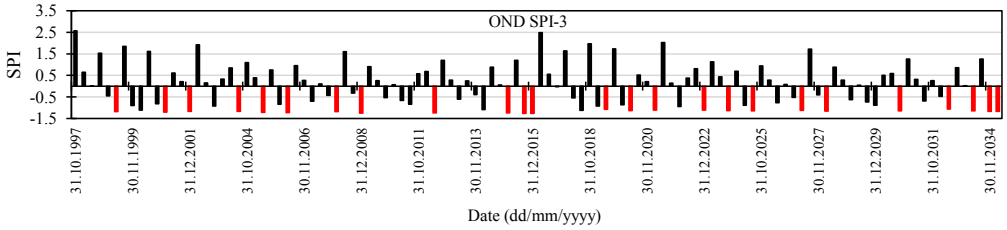

Fig. 7. *SPI histograms for the OND months in Juba County*

Changing rainfall patterns and impacts on crop production

Fig. 8 shows the anticipated decline in the amount of mean rainfall at the onset of rain during the MAMJ. The onset rains varied between the 4th dekad of February and 1st dekad of March with daily rainfall values generally below 4.0 mm level. The March rainfall amounts locally termed as *´doko kulunyit* (*that which carries away grass cinders after burning*) are barely enough for any effective land preparation and planting. Thus, most farmers tend to shift their land preparation and planting dates toward the 3rd and 4th dekad of April. Most farmers plant cowpeas (*ngete*), amaranth (*kwedekwede*), jute mallow (*mulukhiya/khudra*), okra (*bamia*) whose short growing and maturity periods (from 21 to70 days) often offers best food security options prior to the onset of the longer rainy JAS season. Increasing inter-seasonal rainfall variability with declining mean rainfall amounts during MAMJ is forecasted to continue, thus much crop production will have to be shifted toward the 4th dekad of April or 1st dekad of May while for maize, sorghum, sesame will have to be grown during the JAS to OND season. Mean onset rainfall amounts in 2018 is expected to be around 65 mm with a 25% probability. With declining amounts of the onset rains, there is need to intensify inter-cropping of fast and slow growing crops during the MAMJ-JAS seasons as much time, energy and water resources can effectively be utilized. These findings corroborate similar studies by [42] on declining rainfall trend in the March-May rains within the East African region.

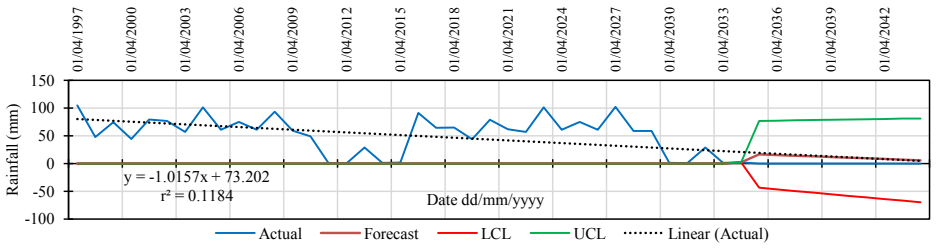

Fig. 8. *Observed and projected mean rainfall amounts during onset at first dekad of March (70 days from start of each new year where LCL=Lower Confidence Level and UCL= Upper Confidence Level).*

Interpretation of the gamma parameters (α) and (β)

Unlike the normal distribution curve that requires mean, median and mode to define the skewness, the gamma distribution requires that both the shape (α) and scale parameter (β) that are interlinked by Equation (10) be interpreted concurrently. For our study, we varied a *priori* the scale parameter (β) with different values around the mean values to observe the resultant effect on the shape parameter (α). The scale parameters for MAMJ, JAS and OND months with mean values of 113.32 mm, 154. 6 mm and 84.54 mm were 36.3, 30.6 and 77.3 respectively. We observed that a greater scale parameter *(with correspondingly low shape parameter*) had low ean and variance. Hereby, large scale parameter depicted large variability in rainfall patterns resulting in irregular rainfall typical for the comparatively drier OND months. Conversely, with increasing rainfall during the rainy season (Ja´be), the wetter JAS months with high mean and lowest scale parameter (*with correspondingly higher shape parameter*) showed low rainfall variability. The MAMJ months transitioning from the relatively drier OND months showed more, or less similar patterns to the JAS months with intermediate scale parameter and mean *(compared to JAS but higher than OND months*) that was often characterized by occasional rainfall showers in March prior to the onset of the rainy season in April. Hereby, the scale parameter was low with comparatively higher mean suggesting less rainfall variability and therefore wetness. With such interpretation of gamma parameters, it is likely to describe periods of relative wetness or dryness as well as drought-prone times.

For example, for the months JAS, the probability for normal wet conditions at SPI=0.5 increased with each scale parameter β as shown in Figs. 9 (a, c, e). The rainfall probability here at β=60.594 was about 0.56, at β=0.594 about 0.67 and at β=20.594 it was about 0.97. To illustrate the inter-linkage between scale parameter and rainfall amount at SPI=0.5 constant, a straight line was drawn from Fig. 9(a) that cut the cumulative rainfall in Fig. 9(b). The estimated rainfall amounts at β=60.594, β=30.594 and β=20.594 were about 140, 168 and 227 mm respectively and showed increasing rainfall amounts with decreasing scale parameter resulting into a more negatively skewed CDF. In general, this implies that wetter rainfall conditions have smaller β values than drier ones. This inverse relationship between scale parameter and rainfall amount may give insight into the rainfall patterns during anytime of the season as in the MAMJ and OND months

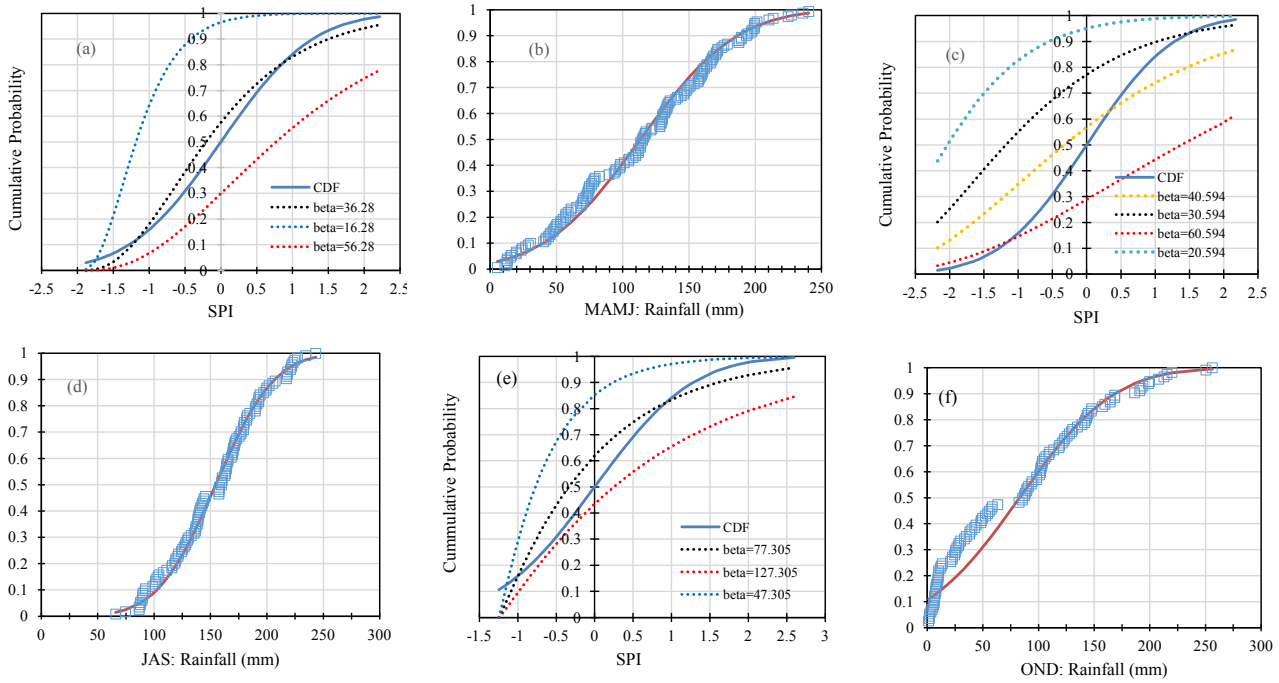

Fig. 9. *CDF plot of different shapes of the gamma distribution curves for the different seasons as influenced by different scale parameters β.*

SPI future projections

Fig. 10 shows the observed (1997-2016) and forecasted (2017-2034) SPI trends for the MMAJ, JAS and OND months. The results showed that the SPI decreasing trend of −0.15, -0.05 and -0.04 per decade for MAMJ, JAS and OND respectively was significant at p= 0.05. For JAS and OND this decrease in the next 100 years is forecasted to remain within the near to normal range while for MAMJ, this is forecasted to have moderate drought for the same period. In the short term, August 2019 is expected to be dry with SPI at about -1.3 (< 50 mm) and increase in September to about 2.0 (200 mm) and October at about 1.7 (<30 mm). November and December are expected to be dry with SPI at -0.8 (<30 mm) and -1.1 (< 25 mm) respectively. The reason is due to the low mean rainfall during OND coupled with high daily temperatures around this period that often continues into the MAMJ months prior to the onset of the first rainfall. Manufacturing industries and large-scale agricultural farming are practically non-existent in South Sudan and therefore, $C0_2$ or methane emissions due to "*anthropogenic compulsions*" are unlikely to be the causes for climate change and so changing rainfall patterns. However, the increased burning of fossil fuel, indiscriminate cutting down of forest trees as cheap energy source [1] over the last 50 years suggest a possible anthropogenic cause for the increase of dry events and thus reduced rainfall mean over Juba County. The results of our study on the negative trend of the SPI also suggest an overall shift of the SPI from near to normal towards more moderate drought events. This is coupled with occasional severe wetness characterized by flooding events especially during September-October months as anthropogenic compulsions continues to increase.

No conclusive reasons are accountable to the decreasing SPI trend with corresponding reduction in the mean rainfall amounts. However, the effects of global warming exacerbated by El Niño on rainfall patterns at the regional level may have occurred, but this could not be statistically identified and verified within the available historical rainfall data and time series.

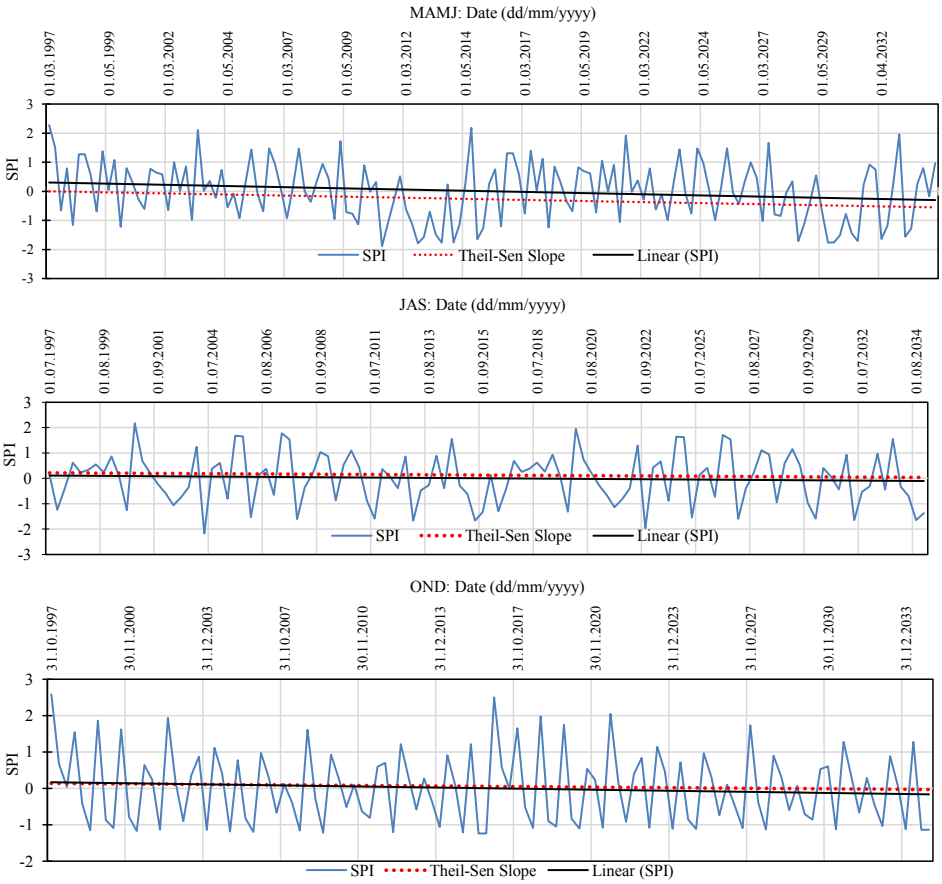

Fig. 10. *Measures SPI values (1997-2016) and projected SPI values (2017-2034) showing decreasing trend using the Theil-Sen Slope estimator.*

Conclusion

Time series rainfall data from 1997 to 2015 were trained, tested and used to make 3-months ahead forecast. Our study has shown, that the MLFFNN model with single input, hidden and output layers is a versatile tool in modelling and detecting complex nonlinear relationships and features between the input and output variables in a time series. After training of the respective datasets, the model was able to learn and generalize this relationship and thereby make predictions on future rainfall patterns for each season irrespective of the data size.

Rainfall and their subsequent projections to year 2034 using the ANNs showed that there was negative monotonic trend significant at the 99% confidence level for the MAMJ, JAS and OND months with rainfall amounts varying between 5-12% below seasonal averages. There was also decreasing trend in the average amounts during rainfall onset (*March-April*) with much rainfall events occurring towards the end 3rd and 4th dekad of April and in other instances in the 1st dekad of May of each year significantly affecting the timing for land preparation and subsequently planting.

Future SPI projections using the ANNs also showed that there was a decreasing trend in all the seasons or months with values forecasted to remain within the near normal range for JAS and OND months while for MAMJ forecasted to have moderate drought in the next 100 years. Rainfall amounts during these seasons are expected to be slightly below the seasonal averages with SPI values between 0 and -1.0. Hereby, national and state governments as well as development partners will be urged to prepare contingency and intervention plans that could quickly and timely be implemented to avert any disruptions to crop production.

However, challenges by the application of ANNs models in projecting spatial and temporal rainfall patterns especially on shorter hourly and daily time scales persist. Similarly, questions on the amount of historical data needed for training from which predictions can be made still pose huge challenges, especially in regions where such data are scanty. Understanding rainfall variability and intensity on hourly and daily basis within Juba County would increase the capacities and readiness of all stakeholders to timely and adequately respond to uncertainties arising from erratic rainfall patterns due to climate change. This paper recommends further studies to investigate whether such seasonal projections of rainfall can be corroborated with empirically measured rainfall amounts from several spatially placed stations within the county. However, one major disadvantage of this model is that in all datasets, the unusually high regression coefficients (r^2=0.99) between the observed and simulated was no panacea for model fitness alone, but simply highlighted the proneness to overfitting during training irrespective of error differences.

Conflicts of interest

None

Acknowledgment

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