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საქართველოს მეცნიერებათა აკადემიის

# მაცნე

ბიოლოგიის სერია
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# PROCEEDINGS

of the Georgian Academy of Sciences

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Biotechnology

# ANTIMICROBIAL ACTIVITY OF COMPOSITE FROM GREEN TEA, LEMON PEELS AND RED WINE LEES

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(Received March 9, 2010)

**Abstract**

Antimicrobial activity of the composite from green tea, lemon peels and red wine lees has been investigated. The composite showed good or moderate antimicrobial activity against some microorganisms. The composite inhibited the growth of gram positive bacteria *Staphylococcus aureus* and *Rhodococcus sp* (inhibition zones were 18.5 and 24.3 mm respectively), gram negative bacteria *Esherichia coli* and *Pseudomonas aeroginasa* (inhibition zones were 6.1 and 10.2 mm respectively), pathogenic fungi *Rhizoctonia sp.* and *Streptomyces glaucus 71MD* (inhibition zones were 8.5 and 20.3 mm respectively). The composite from green tea, lemon peels and red wine lees may be used in food industry as a natural antimicrobial food additive.

**Keywords**: antimicrobial composite, tea, wine lees, lemon



## Introduction

Food-borne illnesses and methicillin-resistant *Staphylococcus aureus (*MRSA) infections are still a major problem in the world today. To prevent and treat these illnesses many researchers have been studying the antimicrobial effects of various plant extracts [Burapadaja & Bunchoo, 1995; May et al, 2000]. Plant polyphenols have been demonstrated potential antibacterial activity [Garcia-Alonso et al, 2006; Balu et al, 2005, Baydar et al, 2006]. Recently, the antimicrobial activities of tea (*Camellia sinensis*) extracts and/or tea catechins have attracted special interest. [Yam et al, 1997; Kolodzeij et al, 1999]. Among tea catechins, epigallocatechin gallate (EGCG) has been shown to have the strongest antimicrobial activity [Amarowicz et al, 2000].

Other particular rich sources of the polyphenols are grapes [Kader and Barret, 2005]. The extracts of alcohol-free red and white wine exhibited antimicrobial activity to some pathogens such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* [Papadopoulou et al, 2005]. The results suggested that polyphenolic compounds contained in red wines were responsible for the antimicrobial effects. Some studies reported that phenolic compounds inhibited other food-borne species such as *Salmonella typhimurium* [Sivarooban et al, 2008] and *Listerial monocytogenes* [Luther et al, 2007]. Bioflavonoids from citrus also have antimicrobial properties and help milk preserve freshness if added before pasteurization [Braddock, 1999].

The aim of the presented work was to investigate antimicrobial activity of the plant composite from tea, lemon peels and red wine lees in order to use it as an antimicrobial food additive.

## Materials and Methods

80% green tea polyphenols (Kokusai Koeki Co., Ltd, Japan) and composite from green tea, lemon peels and red wine lees served as materials for investigation.

Preparation of the composite

The composite was prepared by mixing liquid red wine lees and dry powder of green tea (instant tea) with the subsequent spray-drying up to the powder form. Wine lees was preliminarily left over night in cylindrical glass vessel at 4 $^0$C temperature in order to separate insoluble sediment. After sedimentation the clear supernatant was pumped over by decantation and filtered through a dense filter under vacuum and then was poured into the vessel with pressed peels of lemon (ratio was 6:1 m/m), the mixture was heated up to 80 $^0$C, kept for 15 min and then filtered through a dense filter under vacuum. The filtered extract was added by green tea extract (1:2 respectively, based on dry matter), and spray-dried up to powder form.

The following microorganisms were used as test organisms: Gram positive bacteria – *Staphylococcus aureus, Rhodococcus* sp., Gram negative bacteria – *Esherichia* coli, *Pseudomonas aeroginasa*; Yeasts – *Candida utilis*, *Sacharomyces cerevisiae*, Pathogenic fungi – *Fusarium solani, Rhizoctonia* sp.

Nutrient media for incubation of microorganisms were Nutrient agar for bacteria; Sabouraud agar – for yeasts and pathogenic fungi.

### Antimicrobial tests

Agar well diffusion method was used to investigate antimicrobial properties of plant extracts [Aseeva et al., 1966]. The strains of test microorganisms were inoculated in Petri dishes containing agar media considered to be favourable for the growth of the microorganisms. 10% concentration solutions of the plant extracts were prepared in sterile water. 50 µl aliquots of the solutions were placed into agar wells (12 mm, diameter). The plates were incubated at 37 $^0$C for 24 h. Antimicrobial potential of the plant extracts was estimated by the diameter (mm) of the inhibition zone around the wells. All the plates were replicated twice and the results were averaged.

A subsequent dilution method was used to determine so called titre numbers of the plant extracts [Egorov, 1965]. 1 ml nutrient medium with the test microorganisms was placed in separate sterile tubes. 10 g of the plant dry extract was dissolved in 90 ml sterile water in a sterile flask. 1 ml of the original solution was diluted with 2 ml sterile water and placed in the first tube. 1 ml of the first tube solution was placed in the second tube and diluted with 2 ml sterile water and so on. Finally, 1:3, 1:9, 1:27, 1:81, 1:243, 1: 729 and 1: 2187 diluted samples series were obtained. One drop of the test microorganisms was added to each tube. The tubes were incubated at 30 $^0$C for 48 h and growth of the test microorganisms was monitored. Mean value of the dilution numbers in the tube wherein the microorganism was found grown and in the tube just before this one was applied as the titre number of the extract.

### Statistical analysis

Presented data are the mean of three or two replicates ± standard deviation. Data were subjected to one way analysis of variance and/or t-test. When necessary, the "least squares" method was used to calculate a straight line that best fitted experimental data, and returned an array that

described the line. All calculations were performed with Microsoft Excel (Version 4, statistical functions, Microsoft Corp., Redmond, WA, USA).

## Results and Discussion

As shown in the Table 1, the composite from green tea, lemon peels and red wine lees was active against gram positive and negative bacteria, and against pathogenic fungi *Rhizoctonia sp.* and *Streptomyces glaucus* 71 MD, but did not show any activity against yeasts and pathogenic fungus *Fusarium solani.* The composite expressed the highest activities against gram positive bacteria *Staphylococcus aureus* and *Rhodococcus sp.* as well as pathogenic fungus *Streptomyces glaucus 71MD.*

**Table 1.** Antimicrobial activity of the composite of green tea, lemon peels and red wine lees.

| **Microorganisms:** | **Inhibition zones (mm)** |
|---|---|
| **Gram positive bacteria** | |
| *Staphylococcus aureus* | 18.5 ± 1.2 |
| *Rhodococcus sp.* | 24.3 ± 1.5 |
| **Gram negative bacteria** | |
| *Esherichia coli* | 6.1 ± 0.7 |
| *Pseudomonas aeroginasa* | 10.2 ± 1.5 |
| **Yeasts** | |
| *Candida utilis,* | No effect |
| *Sacharomyces cerevisiae* | No effect |
| **Pathogenic fungi** | |
| *Fusarium solani,* | No effect |
| *Rhizoctonia sp* | 8.5 ± 0.4 |
| *Streptomyces glaucus 71MD* | 20.3 ± 1.2 |

Presented data are the mean of two replicates ± standard deviation. Differences between data within the same column are statistically significant at $\alpha = 0.05$ level.

**Table 2.** Antimicrobial titre numbers of plant products

| **Microorganisms** | **Titre numbers of plant products** | |
|---|---|---|
| | Composite of green tea, lemon peels and red wine lees | 80% green tea polyphenols |
| **Gram positive bacteria** | | |
| *Staphylococcus aureus* | $54^{a} \pm 0$ | $486^{b} \pm 0$ |
| *Rhodococcus sp.* | $18^{c} \pm 0$ | $486^{b} \pm 0$ |
| **Gram negative bacteria** | | |
| *Esherichia coli* | $54^{a} \pm 0$ | $54^{a} \pm 0$ |
| *Pseudomonas aeroginasa* | $18^{c} \pm 0$ | $54^{a} \pm 0$ |
| **Yeasts** | | |
| *Candida utilis,* | No effect | $162^{e} \pm 0$ |
| *Sacharomyces cerevisiae* | No effect | $18^{c} \pm 0$ |
| **Pathogenic fungi** | | |
| *Fusarium solani,* | No effect | $162^{e}$ |
| *Rhizoctonia sp* | $54^{a} \pm 0$ | $54^{a} \pm 0$ |
| *Streptomyces glaucus 71 MD* | $486^{b} \pm 0$ | $1458^{f} \pm 0$ |

Presented data are the mean of two replicates ± standard deviation.

$^{a, b, c, d, e, f}$ - means, without the same superscript are significantly different ($P < 0.01$)

Antimicrobial activities, expressed in titre-numbers of the composite and 80% green tea polyphenols against the pathogenic microorganisms were determined. In most cases the titre-numbers of the 80% green tea polyphenols were about by one order higher, but for *Escherichia coli* and *Rhizoctonia sp.* the titre-numbers were the same and equal to 54 (Table 2). The highest titre-numbers were found for pathogenic fungus *Streptomyces glaucus 71 MD* – 486 and 1458 for the composite of green tea and lemon peels extracts and red wine lees and 80% green tea polyphenols respectively.

**Conclusions.** The composite show good or moderate antimicrobial activity against some microorganisms and may be recommended as a natural antimicrobial food additive.

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# მწვანე ჩაიდან, ლიმონის ქერქიდან და წითელი ღვინის ლექიდან მიღებული კომპოზიტის ანტიმიკრობული აქტივობა

გულუა ლ., პატარაია დ., გურიელიძე მ., მჭედლიშვილი ნ.., ომიაძე ნ., აბუთიძე მ.

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(მიღებულია 09.03.2010)

## რეზიუმე

შესწავლილია მწვანე ჩაიდან, ლიმონის ქერქიდან და წითელი ღვინის ლექიდან მიღებული კომპოზიტის ანტიმიკრობული აქტივობა. აღნიშნულმა კომპოზიტმა გამოავლინა ანტიმიკრობული აქტივობა ზოგიერთი მიკროორგანიზმების მიმართ. კომპოზიტი აინჰიბირებს გრამ-დადებით ბაქტერიებს ***Staphylococcus aureus***-სა და ***Rhodococcus sp***-ს (ინჰიბირების ზონებია 18.5 და 24.3 მმ შესაბამისად), გრამ-უარყოფით ბაქტერიებს - ***Esherichia coli***-სა და ***Pseudomonas aeroginasa***-ს (ინჰიბირების ზონებია 6.1 და 10.2 მმ შესაბამისად), პათოგენურ სოკოებს - ***Rhizoctonia sp.***-სა და ***Streptomyces glaucus*** *71* -ს (ინჰიბირების ზონებია 8.3 და 20.3 მმ შესაბამისად). მწვანე ჩაიდან, ლიმონის ქერქიდან და წითელი ღვინის ლექიდან მიღებული კომპოზიტი შეიძლება გამოყენებულ იქნეს კვების მრეწველობაში როგორც ბუნებრივი ანტიმიკრობული საკვები დანამატი.

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Biotechnology

# BIOTECHNOLOGICALLY IMPORTANT CELLULASES OF MICROSCOPHIC FUNGI

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(Received December 10, 2009)

**Abstract**

Strains actively producing high technological, most appropriate for application cellulases have been selected from the collection of micromycetes (2300 strains) of Durmishidze Institute of Biochemistry and Biotechnology isolated from different ecological niches of South Caucasus. The industrial properties of selected enzyme preparations - heat stability, temperature optimum of action, mechanism of cellulose inhibition by the reaction products, enzymes ability to deep hydrolysis of microcrystalline cellulose, forming low molecular reducing sugars have been studied.

**Key words:** microscopic fungi, cellulase, heat stability, inhibition, conversion

## Introduction

One of the promising ways of sweeteners receiving from non-edible raw material is enzymatic hydrolysis of cellulose. Selection of cellulases with suitable biotechnological indices is essential for elaboration of industrial technologies of the process. Together with high enzymatic activity it means inhesion of characteristics like heat-resistance, activity under the high temperature regimen, less inhibition with terminal products, deep and effective hydrolysis of different ligno-cellulose substrates.

Among taxonomic groups of soil microorganisms, mycelia fungi posses the broadest genetic spectrum of cellulose-, hemicellulose- and lignin degrading enzymes [Chang, Holtzapple, 2000; Jacobsen, Wyman, 2000]. Cellulases used in laboratory conditions (obtained from genera: *Aspergillus, Trichoderma, Sporotrichum, Penicilium*) [Bhat, 2000; Klyosov, 1990] do not possess high technological properties and can't be responsible for fast and deep hydrolysis of cellulose. Accordingly, screening of cellulase producers following above mentioned indices and not by total activity is of great importance. The purpose of the given study was to select cellulose-degrading, biotechnologically promising enzyme-producer strains isolated from different ecological niches of Georgia.

## Materials and Methods

To reveal cellulase-producing strains tested material was cultivated in 750 ml volume Erlenmeyer flasks on a shaker (180-200 rot/min), containing the nutrient medium (in %): microcrystalline cellulose-1.0; corn extract-0.05; $NaNO_3$-0.36; $KH_2PO_4$-0.2; $MgSO_4 \cdot 7H_2O$-0.05,

$CaCl_2$-0.2, in a 30 l fermenter (New Brunswick, USA), at 40°C, for 5 days. After termination of cultivation the obtained liquid was filtered and cellulase activity of the filtrate was determined according to its effectiveness both on soluble (1.5% microcrystalline cellulose – carboxymethilcellulase) and insoluble (filter paper Whatman N1, 1x6cm) substrates. Amount of reducing sugars was estimated by Dinitrosalicylic acid reagent [Chang, Holtzapple, 2000]. To obtain technical cellulose preparation the cultural liquid was sedimented by ethanol (1/4).

## Results and Discussion

Fifty producers of cellulases with different extent of activity were revealed. As their cultivation took place at 40°C, they may be considered as thermophiles [Bilai, Zakharchenko, 1981]. Extracellulase activity of the mostly active producers is given in Table 1.

**Table 1.** Extracellular activities of mycelia fungi strains (units/ml)

| Strain | CMC | FP |
|---|---|---|
| *Aspergillus sp.* Z 1-7 | 12.5 | 0.82 |
| *Aspergillus wentii* Z 9-7 | 13.2 | 0.82 |
| *Aspergillus* sp. Z 9-2 | 8.0 | 0.46 |
| *Aspergillus wentii* Z6-3 | 8.4 | 0.45 |
| *Aspergillus terreus* K 61 | 14.0 | 0.85 |
| *Aspergillus* sp. Z 6-0 | 5.7 | 0.35 |
| *Aspergillus* sp. Ts 1-2 | 12.6 | 0.84 |
| *Aspergillus terreus* X 4-7 | 12.5 | 0.86 |
| *Aspergillus* sp. T1-6 | 9.2 | 0.56 |
| *Aspergillus versicolor* | 9.0 | 0,58 |
| *Sporotrichum pulv.* S-7 | 8.4 | 0.45 |
| *Sporotrichum pulv.* E 4-5 | 9.0 | 0.4 |
| *Sporotrichum pulv.* M 2-9 | 10.8 | 0.56 |
| *Chaetomium* sp. Ts 3-3 | 12.6 | 0.64 |
| *Chaetomium thermophile* | 8.6 | 0.50 |
| *Chaetomium* sp. Ts 4-4 | 8.8 | 0.50 |
| *Rhizopus* sp. V 4-6 | 7.5 | 0.33 |
| *Mucor* sp. S 10-1 | 7.2 | 0.30 |
| *Penicillium* sp Z 5-3 | 14.0 | 0.86 |
| *Penicillium* sp G 1-7 | 10.4 | 0.80 |
| *Penicillium* sp M 5-0 | 8.0 | 0.46 |
| *Allesheria terrestris* | 10.2 | 0.6 |

CMC (carboxymethilcellulase) activity (U/ml), FP-Filter paper activity (U/ml)

In bio-and enzymatic technologies running of a process at pasteurization (65°C) temperature is important, as it minimizes possibility of pollution of the incubation medium. Accordingly the temperature optimums of the selected strains were determined. For this purpose the activity of enzymes was measured in temperature intervals of 50-65°C and estimated by standard methods. The results were expressed as retained percentage of activities (Table2).

**Table 2.** Extra cellular cellulase activity at different temperatures

| Strain | 50°C | 55 °C | 60°C | 60°C Retained activity in % | 65°C | 65°C Retained activity in % |
|---|---|---|---|---|---|---|
| *Aspergillus* sp. Z 1-7 | 0.60 | 0.82 | 0.79 | 96.8 | 0.29 | 35.4 |
| *Aspergillus wentii* Z 9-7 | 0.63 | 0.82 (93.2%) | 0.88 | 100 | 0.52 | 59 |
| *Aspergillus* sp. Z 9-2 | 0.35 | 0.46 | 0.44 | 96.0 | 0.31 | 68.0 |
| *Aspergillus wentii* Z 6-3 | 0.30 | 0.45 | 0.24 | 53.3 | 0.06 | 13.0 |
| *Aspergillus terreus* K61 | 0.60 | 0.85 | 0.41 | 47.6 | 0.12 | 14.3 |
| *Aspergillus* sp. Z 6-0 | 0.30 | 0.35 | 0.22 | 63.6 | 0.06 | 18.0 |
| *Aspergillus* sp.Ts 1-2 | 0.70 | 0.84 | 0.47 | 56.0 | 0.10 | 12.3 |
| *Aspergillus terreus* X 4-7 | 0.60 | 0.86 | 0.71 | 89.0 | 0.43 | 50.0 |
| *Aspergillus* sp. T 1-6 | 0.43 | 0.56 | 0.46 | 83.0 | 0.18 | 33.0 |
| *Aspergillus versicolor* | 0.45 | 0.58 (89.2%) | 0.65 | 100 | 0.41 | 63.1 |
| *Sporotrichum pulv.* S-7 | 0.30 | 0.45 | 0.39 | 86.0 | 0.12 | 27.0 |
| *Sporotrichum pulv.* E 4-5 | 0.30 | 0.40 | 0.28 | 70.0 | 0.06 | 15.0 |
| *Sporotrichum pulv.* M 2-9 | 0.35 | 0.56 | 0.52 | 92.0 | 0.17 | 31.0 |
| *Chaetomium* sp. Ts 3-3 | 0.52 | 0.64 | 0.61 | 95.0 | 0.33 | 52.0 |
| *Chaetomium term.* | 0.4 | 0.50 | 0.55 | 88.7 | 0.62 | 100 |
| *Chaetomium* sp. Ts 4-4 | 0.40 | 0.50 (95%) | 0.53 | 100 | 0.26 | 49.0 |
| *Rhizopus* sp. V 4-6 | 0.25 | 0.33 | 0.30 | 91.0 | 0.09 | 27.0 |
| *Mucor* sp. S 10-1 | 0.22 | 0.30 | 0.30 | 100 | 0.09 | 30.0 |
| *Penicillium* sp. Z 5-3 | 0.66 | 0.86 | 0.81 | 94.0 | 0.43 | 50.0 |
| *Penicillium* sp. G 1-7 | 0.60 | 0.80 | 0.79 | 98.8 | 0.38 | 47.8 |
| *Penicillium* sp. M 5-0 | 0.30 | 0.46 | 0.32 | 69.0 | 0.07 | 15.4 |
| *Allesheria terrestris* | 0.47 | 0.6 | 0.72 | 88.9 | 0.81 | 100 |

Obtained results demonstrate that temperature optimums of action for cellulases from *A. versicolor* and *A. wentii* Z 9-7 were equal at 60°C, while for strains *Chaetomium termophile* and *Allesheria terrestris* under the same conditions it was 65°C. In others the temperature optimum of action did not exceed 55°C.

Estimation of thermostability of selected cellulases was done on further stage via the kinetic curve of thermo inactivation. Incubation of enzyme solution was carried out without a substrate at 65°C, within 300 minutes, in order to study the kinetics of thermo inactivation. Enzyme activity was estimated by the standard methods at particular intervals of time, and was calculated by the formula $A/A_0 \cdot 100\%$, were $A_0$ is an initial activity, and A – residual activity in t-period of time. Kinetics of thermo inactivation of one and the same enzyme was compared in thermophils and mesophylls, in particular, with the enzyme of *Trichoderma reesei* commercial preparation – Onozuka R. Kinetics of thermo inactivation of enzyme preparations according to filter paper activity is shown on (Fig.1).

The same results, have been received while analyzing heat inactivation of cellulose preparations according to CMC activity

Inhibition by the final products of hydrolysis is another limiting factor for cellulase application. Thus, on the next step of investigation the mechanism of cellulase inhibition by reaction product – cellobiose has been studied using method [Gusakov, Sinitsyn, 1992]. Dyed cellulase "OC-31" was used as a substrate, which in various quantities (2.5-12.5g/l) was added to 4 ml of 0.05% acetate buffer, pH 4.5, containing 0.1M cellobiose. The samples were centrifuged and optical density of the supernatant (at 450 nm) was measured. Colored products are formed as a result of hydrolysis of colored substrate, and are easily registered spectrometrically. Other methods

for studying inhibition are not effective in this case, because adding of cellobiose as inhibitor complicates its determination as of final product. While evaluating inhibition it must be taken into account that kinetics of products formation is influenced not only by the inhibition with final products, but also by structural changes of cellulose and inactivation of enzymes in the process of hydrolysis. These circumstances significantly change the constant of inhibition.

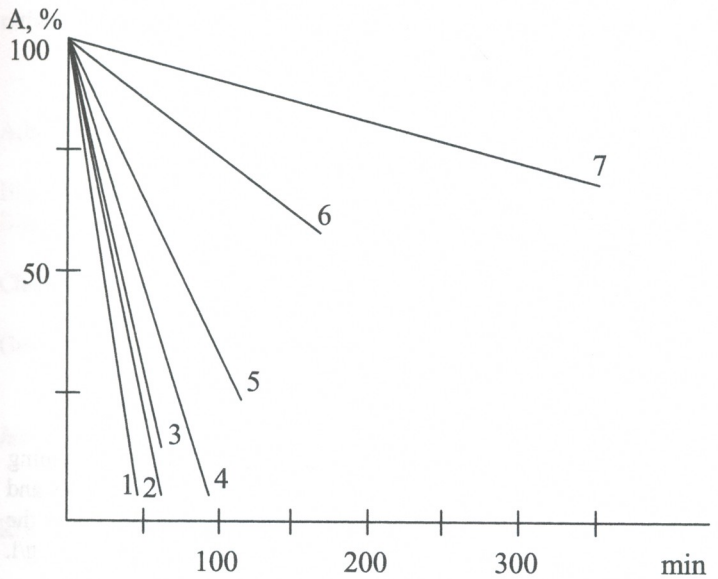


**Fig.1** Curves of thermal inactivation of cellulase preparations from mesophyll strains, assayed according to filter paper activity at 65°C (Schematized for clarity). 1 - "Onozuka R-10" *(Trichoderma reesei)*; 2 - *Spootrichum pulverulentum* M2-9; 3 - *Aspergillus terreus* K 61; 4 - *Aspergillus wentii* Z9-7; 5 - *Aspergillus versicolor*; 6 - *Chaetomium thermophile*; 7 - *Allesheria terrestris.*

Inhibitory analysis is not limited only by evaluation of the extent of influence of a particular substance on enzyme activity. Important index of analysis is quantitative evaluation of reactivity of the inhibitor, which may be obtained only by testing the kinetic parameters. Example of establishment of inhibitory type and coefficient calculation of the cellulase preparation of *A.wentii* Z 9-7 is given on Fig. 2.

Type of inhibition was determined on the base of analysis of 1/Vo, 1/[S] graphical relationship. From the figure it is clear that both curves transect in one point. This indicates that we have noncompetitive inhibition, and Ki is calculated by formula:

$$Ki = \frac{[In]}{\frac{V_0}{V_1} - 1}$$

The constant of inhibition – Ki was calculated according to kinetic analysis. For the cellulase preparation of *A. wentii* it was 814 g/l. With this principle we have investigated the inhibition types of 22 preparations of cellulase and calculated the constant of inhibition. In all cases inhibition was weak and of noncompetitive type. As it is known, linking of the enzyme with substrate occurs in two ways: productively (by means of the active center) and non-productively (by the peripheral parts of enzyme molecule). In spite of added amount of soluble inhibitor, in the second case, the total amount of enzyme can't be transferred from the substrate into the solution. In

the same manner, joining of enzyme molecules to cellulose won't be able to substitute a soluble inhibitor in the active center. It may be supposed that substrate joins with the peripheral parts of enzyme and not with its active center. Hence, it is easy to explain the non-competitive type of inhibition with cellobiose of the studied cellulases.

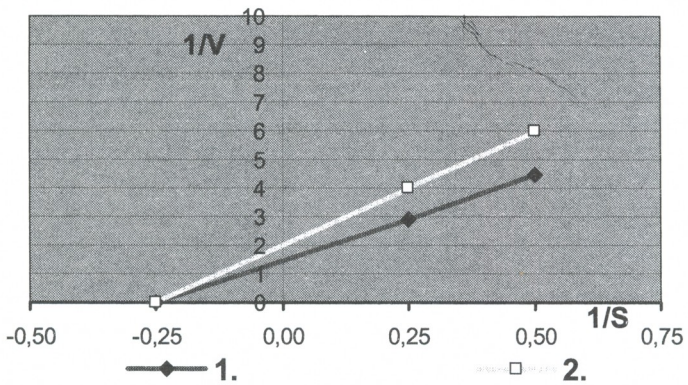


**Fig.2.** 1 - without inhibitor; 2 - with inhibitor

For determining the effectiveness of hydrolysis of the insoluble cellulose-containing substrates by the selected cellulase preparations its influence on microcrystalline cellulose and sawdust was investigated. Hydrolysis was performed in permanent type reactor, for 24h, under the optimal temperature regimen for each enzyme preparation. Concentration of the substrate was 1 g/l. Obtained results are demonstrated in table 3.

**Table 3.** Degree of conversion of microcrystaline cellulose and sawdust under the action of cellulase preparations

| Strain | MCC,% | Sawdust, % |
|---|---|---|
| *Aspergillus* sp. Z 1-7 | 10 | 20 |
| *Aspergillus wentii*. Z 9-7 | 22 | 28 |
| *Aspergillus* sp. Z9-2 | 15 | 34 |
| *Penicillium* Z 5-3 | 20 | 62.5 |
| *Penicillium* sp. G1-7 | 16 | 57 |
| *Aspergillus terreus*K-61 | 8.5 | 18 |
| *Aspergillus* sp.Ts1-2 | 6 | 16.5 |
| *Chaetomium* sp. Ts 4-4 | 13 | 24.5 |
| *Aspergillus terreus* X4-7 | 10 | 21 |
| *Aspergillus wentii* Ts1-1 | 20.5 | 42 |
| *Sporotrichum pulv.* M 29 | 11.2 | 20.5 |
| *Sporotrichum pulv.* S-7 | 16.5 | 32.5 |
| *Penicillium* sp.M 5-0 | 30 | 44.2 |
| *Aspergillus* sp. T 1-6 | 27.5 | 41.5 |

Conditions of hydrolysis: Microcrystalline cellulose -20 mg, 0.05M acetate buffer pH 4.5 - 5ml, E (culture solution) – 0.5 units, temperature of incubation - 58°C, time of incubation - 24hour.

Obtained results demonstrate that studied cellulases quite intensively hydrolyzed the insoluble substrate. Especially effective were producers from the genus *Penicillium*.

Performed experiments revealed that selected preparations of cellulase are practically rather feasible and meet the demands of biotechnology of cellulose enzymatic hydrolysis.

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## მიკროსკოპული სოკოებიდან მიღებული ბიოტექნოლოგიურად მნიშვნელოვანი ცელულაზები


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(მიღებულია 10.12.2009)


### რეზიუმე


დურმიშიძის ბიოქიმიისა და ბიოტექნოლოგიის ინსტიტუტში არსებული, სამხრეთ კავკასიის სხვადასხვა ეკოლოგიური ნიშებიდან გამოყოფილი მიკრომოცეტების კოლექციიდან შეირჩა ტექნოლოგიური ცელულაზების პროდუცენტი შტამები. მიღებულია შერჩეული ცელულაზების ტექნიკური პრეპარატები. შესწავლილია მათი ინდუსტრიული მახასიათებლები – თერმომედეგობა, ფერმენტული მოქმედების ტემპერატურული ოპტიმუმი, რეაქციის საბოლოო პროდუქტებით ინჰიბირება, მიკროკრისტალური ცელულოზის ჰიდროლიზის ხარისხი.

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# IN VITRO MICROCLONAL PROPAGATION OF *HIBICUS ESCULENTUS* L.

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**Abstract**

Plant cellular technology, as an untraditional way of propagation, along with its high efficiency enables us to model, realize and control morphogenetic processes. It provides to create a "gene pool" of elite, valuable plant bank. This method is applied in genetic-selection performances, such as obtaining the therapeutic agents and remedies. Okra - *Hibicus esculentus* L., which is practically not studied *in vitro,* belongs to such plants. So, production of an aseptic culture is the basic challenge in the study of plant morphogenesis.

**Key words:** okra, explant, microclonal propagation

## Introduction

The tropical regions of Northern Africa are considered as a homeland of *Hibicus esculentus* L. synonym: *Abelmoschus esculentus* (common names: okra, lady fingers, gambo, gombo, okura, ocra, bamia ochro, bhindi, bombo, chimbombo, Bhindee, Bhindi, Bindi, Cantarela, Gombaut, Gombo, Bumbo, Mesta, Ochro, Okro, Quiabo, Quimbambo, Quingombo, Rosenapfel, Vendakai, qiu kui, okura, Gumbo). Wild forms are still remained in Antilles. It is distributed in some countries of Southern Europe, America and Asia. Small plantations are spread in Krasnodar region, Kuban, Georgia, Armenia, Central Asia [The Compleat Botanica].

Okra is an herbaceous annual plant from Malvaceae family (Mallow family), bisexual, vegetable culture with main root growing through the soil at 1.5 m depth. A stem is somewhat woody attaining a height of 40-50 cm for low-growing forms and up to 2 m for tall ones, bearing alternate, serrate leaves of 3 varieties - angular, palmate, and subdigitate. The leaves are bisexual, setulose, thick, with long petiole, light or dark coloration. The flowers are solitary, large, hibiscus-like and showy; of a pale yellow, tinged at the base a dark crimson. The herbaceous portions of the plant are clothed with sharp bristles, and often bear purplish spots. Flowers are situated in the boot with short petiole. The gombo fruit, the edible part, is a pentagonal, narrow, cylindrical capsule, tapering at the base. It is often curved, and is covered with hairs, especially along the ridges. Length of the seed pod is 20-25 cm length (10-18 cm for some species). It contains smooth seeds in the cells of this capsule and is harvested while still tender and immature. The pods contain several roundish or kidney-shaped smooth gray or dark green seeds in each of the several cells [Phillips R.&Rix M., 1993].

Fruit of okra is rich in easy-assimilated proteins (1.5-2%), carbohydrates (2.2-6.6%), potassium salts, amino acids, B-group vitamins, carotenes, C-vitamin. Seeds consist of up to 20% of olive oil type oil.

Leaves of okra consist of flavonoids, glycosides, anthocyanins. Fruit of okra is tasted as aubergine and asparagus and thus they are widely used in cookery. Okra is demulcent, mucilaginous and is used as a thickener in soups and stews.

The mucilage binds cholesterol and bile acid carrying toxins dumped into it by the liver. It helps to lubricate the large intestines due to its laxative qualities. The fiber in okra helps to stabilize blood sugar (it regulates the rate at which sugar is absorbed from the intestinal tract). Okra recovers organism viability after some hard diseases. Fruit broth is used against cold, cough and bronchitis, stomach ulcer, gastritis [*Hibiscus esculentus*, 1999].

Mostly distributed cultivars of okra in Georgia are: Sona, White cylindrical 127, Lady fingers, White velvet, Green velvet, Green dwarf, Okra 1, Okra 4.

The aim of our work was to receive *in vitro* culture of okra, to work out the method of *in vitro* microclonal propagation.

The novelty of our work consists in getting the data on morphogenesis of *Hibicus esculentus* in tissue culture and working out methods of introduction of explant *in vitro* medium.

## Materials and Methods

Primary explant was isolated from donor-plant of okra of both mature and juvenile stages. Explants were obtained from: 1) boots and apical buds, young shoots, leaves and stems of mature plants; 2) dormant and apical buds, stem tissue and leaves of juvenile plants (3-4 month old saplings).

Primary material for explant isolation was thoroughly washed under tap water. The rest manipulations were conducted in aseptic conditions – in laminar boxes. Material was put in 96% ethanol for 1-2 min and then in sterilizing solution. As a sterilizing agents were tested the following solutions: a) sodium hypocloride : water of 50:50 and 25:75 ratio; b) 0.1, 0.2 and 0.5% water solutions of preparation "diocide" with composition ethanolmercurchloride : acetylpyridinchloride of 1:2 ratio; c) 10-15% water solution of chloramine-B with added detergent "Twin-80" for better wetting of plant material.

## Results and Discussion

The basic tasks of our work were: 1) to choose conditions for obtaining of *in vitro* culture of various okra explants; 2) to work out conditions for microclonal propagation of okra and their optimization; 3) to study the effect of physical conditions of cultivation just on the stage of microclonal propagation.

Concentration of sterilizing agent as well as exposition time of explants in sterilizing solution, explant type and plant development stage have effect on the yield of viable explants [Nanda, 2003; Kataeva&Avetisov, 1980].

Tested concentrations and expositions of Chloramine-B appeared less efficient and inexpedient for surface sterilization of lignified shoots with dormant buds (Table 1). The yield of uninfected material was low (15-30%). Sodium hypocloride solution has better effect. Amount of uninfected explants was 35-50%.

The high effect of surface sterilization was achieved at the usage of preparation "diocide". While using the solution of 0.5 % concentration the degree of infection was low and number of uninfected explants achieved 80%, but viability of aseptic cultures appeared lower as compared to

the two other sterilizing agents, which was caused by high toxicity of "diocide" and at high concentrations it aroused tissue intoxication. The 0.2% solution appeared the optimal from tested concentrations.

**Table 1.** Effect of sterilizing agents on the yield of viable, uninfected explants (boot and apical buds isolated from shoot vegetans)

| N | sterilizing solution | concentration | exposition (min) | % of uninfected explants | % of viable explants |
|---|---|---|---|---|---|
| 1 | chloramine-B | 10 | 20 | 15 | 85 |
| | | 15 | 20 | 30 | 80 |
| | | 15 | 35 | 70 | 10 |
| 2 | sodium hypocloride | 25 | 20 | 35 | 100 |
| | | 50 | 20 | 50 | 100 |
| | | 50 | 25 | 85 | 100 |
| 3 | "diocide" | 0.1 | 15 | 34 | 93 |
| | | 0.2 | 15 | 58 | 92 |
| | | 0.5 | 15 | 80 | 69 |
| | | 0.2 | 20 | 100 | 97 |

Usage of sodium hypocloride gave low degree of uninfected material, but as is seen from Table 1 received aseptic material appeared viable (100%). 50% solution of sodium hypocloride is characterized with slight effect.

Exposition time of explants in the sterilizing solutions affected the yield of uninfected viable material (Table 1). Variations of exposition of sterilization at the usage of all studied sterilizing agents changed slightly surface sterilizing effect, sometimes it caused decrease of the yield of viable explants; decrease of the concentration was conducted with increase of infection of primary material, and increase – with obtaining of unviable explants. This result is common for all sterilizing solutions.

Isolation of the primary material was carried out from field-growing plants of both, juvenile and surface generative phases and from intact plants grown in hothouse conditions of juvenile and generative phases. Apical and boot buds and seeds were applied as explants.

**Table 2.** Some parameters of growth and development of the primary explants in zero passage (juvenile phase)

| N | explant type | explant number | duration of zero passage (days) | growth rate of explant | induction time of callus | growth rate of callus | morpho-genetic potential | type of morpho-genesis |
|---|---|---|---|---|---|---|---|---|
| 1 | boot buds | 50 | 25-30 | high | 14-16 | medium | good | buds |
| 2 | apical buds | 50 | 20-25 | high | 12-15 | medium | good | buds |
| 3 | seed sprouts | 50 | 22-27 | high | 12-17 | medium | good | buds |

**Table 3.** Some parameters of growth and development of the primary explants in zero passage (mature phase)

| N | explant type | explant number | duration of zero passage (days) | growth rate of explant | induction time of callus | growth rate of callus | morpho-genetic potential | type of morphoge-nesis |
|---|---|---|---|---|---|---|---|---|
| 1 | boot buds | 50 | 40-45 | medium | 18-22 | weak | medium | histogenesis |
| 2 | apical buds | 50 | 35-45 | medium | 16-20 | medium | low | Buds histogenesis |
| 3 | seed sprouts | 50 | - | - | - | - | - | - |

According to the data of experiments the explants, which were isolated from donor plants being in juvenile phase of development, were characterized with higher rate of growth as compared to the explants isolated from donor plants being in generative phase. Explants isolated from mature donor plant rather hamper processes of growth and development in I stage of development. It should be also mentioned that callus formation occurred in explant basal part at the end of zero-passage, which development rate and potential differed from those of explants isolated from donor plants being in juvenile and generative phases.

Juvenile state of seed sprouts entirely repeated growth and development process of apical sprouts isolated from juvenile donor plant. To study this regularity plantlets were removed from the primary roots just at the beginning of sprouting. Comparison of the explants of I type showed that boot and apical buds differed from each other by potential of regeneration and growth rate (Tables 2, 3). Boot buds come from dormancy later and develop as a sprout slower than apical buds. The last ones begin primary leaf formation 6-8 days earlier. Consequently, the number of internodes in apical meristem was higher, which indicates the numerical growth of secondary explants in the following passages.

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## ბამიას (*Hibiscus esculentus* L.) in vitro პირობებში მიკროკლონური გამრავლება


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(მიღებულია 18.01.2010)


### რეზიუმე


მცენარეთა უჯრედული ტექნოლოგია, როგორც გამრავლების არატრადიციული გზა მაღალ კოეფიციენტთან ერთად, გვაძლევს მორფოგენეტიკური პროცესების მოდელირების, რეალიზაციისა და კონტროლის საშუალებას. უზრუნველყოფს ძვირფასი, ელიტურ მცენარეთა ბანკის "გენოფონდის" შექმნას. აღნიშნული მეთოდი გამოიყენება ისეთ გენეტიკურ-სელექციურ სამუშაობებში, როგორიცაა სამკურნალო და სამკურნალწამლო ფორმების მიღება. ასეთ მცენარეთა რიცხვს განეკუთვნება ბამია - (*Hibiscus esculentus* L.). in vitro სისტემაში ეს მცენარე პრაქტიკულად არ არის შესწავლილი. ამიტომ ყველაზე რთულ პრობლემას მცენარის მორფოგენეზის შესწავლაში წარმოადგენს ასეპტიკური კულტურის მიღება.

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# SOME RARE SPECIES OF CALCAREOUC COLCHIC FLORA IN TBILISI BOTANICAL GARDEN

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**Abstract**

The paper deals with the results of ***in situ*** and ***ex situ*** conservation of some rare endemic and relict species of calcareous Colchic flora. According to IUCN categories the investigated taxa are considered to be threatened by extinction in natural habitats.

**Key words**: endemic plants, endangered species, relict, refugium, calcareous, *ex situ, in situ.*

## Introduction

Colchis is a floristic refugium and botanical-geographical province of the northern hemisphere which is characterized by high endemism and florogenesis of plants that is conditioned by distinguished physical orography and eco-geographical conditions of Colchis.

The province of Colchis is rich in Colchic and Caucasian endemic species, where some Mediterranean, Palaeartic, East Asian and Lazetian species are also presented [Gagnidze, Davitadze, 2000].

The vertical zones and the composition of forest zones in Colchis are similar to the other regions of Georgia, but there are also many specific, typical calcareous plants.

The work gives information on protection measures of some rare, endemic and relict species of Colchis calcareous flora by means of ***ex situ*** conservation. The results of the investigation are published for the first time.

## Materials and Methods

With the purpose to define viability and fructification of the investigated species, Braun-Blanquet's method [Braun-Blanquet, 1951] was used. Phenological observations are carried out on all parameters of a plant. With this purpose the method of phenological observation in botanical gardens [Method of Phenological Observations in Botanical Gardens, 1975] was used. Vital form of a plant was studied according to Raunkier method [Raunkier, 1934]. Criteria of classification of the critically endangered species were estimated according to IUCN categories. The nomenclature of the species is ascertained according to S. Czerepanov (1995) and R. Gagnidze (2005).

## Results and Discussions

***Epimedium colchicum*** (Boiss.) Trautv.
**Family: Berberidaceae**

Relict species of the Tertiary flora. Mainly spread in Colchic refugium.

In Tbilisi Botanical Garden (subarid zone) up to 20-25 cm tall shrub in thin cinnamonic soil of medium depth is grown. Its leaves are ovate with serrate margins, growing red in winter. The plant develops well if it is properly watered and in the middle of March begins flowering abundantly with yellow flowers that lasts about 30-35 days. It usually bears fruit but produces seeds with poor germinability. Fruit capsule looks like a box.

With the purpose to enrich the collection the method of vegetative propagation is used. Each 4-5 year old plant is divided into 4-5 parts and each part should have more than 3 buds and well-developed root system.

***Leptopus colchicus*** **(Fisch.&C.A.Mey. ex Bois) Pojark.**
***(Andrachne colchica*** **Fisch.&C.A.Mey. ex Bois) Pojark.**
**Family: Euphorbiaceae**

80 cm tall shrub with thin, densely leaved branches. It is the Georgian endemic species of the Tertiary flora.

According to the indices of the growth and adaptation of the introduced Colchic trees and shrubs in Tbilisi Botanical Garden when they are regularly watered, the vegetation state of ***L.colchicus*** is considered to be satisfactory. The plant begins its vegetation in the first decade of March and keeps blooming from April till the end of September with axillary whitish green small flowers that become yellow to the end of its vegetation. Capsule bears fruit and it is flat, spherical. Seeds are triangular having poor germination ability (15-18%), and medium frost resistant. Its leaves and one year old shoots are damaged when the air temperature falls down to $-12^0$ $-13^0$. It is propagated by means of vegetative propagation.



***Scabiosa olgae*** Albov.
**Family: Dipsacaceae**

Mediterranean, north Colchic forest and alpine litophylous, Caucasian endemic species and relict of the Tertiary flora.

Under the cultural conditions the plant grows up to 40-60 cm. In autumn it develops root crown and remains in this phase the whole winter. The plant begins active vegetation in March, flowering begins from the second decade of June and goes on till November. Abundant flowering of the plant takes place in July. Each flower keeps blooming 7-10 days. The color of the flower varies from dark to light blue. Each plant produces 10-50 stalks. Intensity of flowering is conditioned by the specificity of the hydrothermic regime of the vegetation period, duration and succession of droughty and rainy periods and biological peculiarities of the plant itself. Seed ripeness begins 20-25 days after blooming.

*Scabiosa olgae* is a high light plants, frost and drought resistant. It is adequate to the nutritive order, organic and mineral fertilizers. Propagates by seeds and self-seeding.

***Centaurea barbeyi*** Albov.
**Family Asteraceae**

Spread in Mediterranean, north Colchic forest, rocky slopes and calcareous ecotopes. Rare endemic plant of Caucasus.

Under cultural conditions *C.barbeyi* begins vegetation in March. Despite the late frosts, characteristic of this period, the plant doesn't get damaged. Process of budding begins in the first decade of April and lasts till the end of May. Flowers twice a year. The plant begins its first flowering in the second half of April and lasts till the end of May. In this period the length of its stalk is 40-60 cm, flower diameter is 4-6 cm. The plant flowers abundantly with white or bluish-violet flowers during 6-8 days. A compact shrub produces 30-35 stalks, which are covered with silver velvet leaves.

The second flowering of the plant is particularly interesting under the cultural conditions. The observations showed that the plant has its second flowering when autumn is warm and sunny after heavy rainfalls. It has also been estimated that alongside some other factors, intensity of flowering is conditioned by the specificity of the hydrothermic regime of the vegetation period, duration and succession of droughty and rainy periods and biological peculiarities of the plant itself.

The plant propagates both by seeds and vegetative way. Both methods give positive results. Its seeds are fertile (approximately 70-80%). The offspring begins flowering 11-13 months after germination and the individuals obtained by division of a shrub, begins flowering the same year.

***Dioscorea caucasica*** Lipsky
**Family Dioscoreaceae**

The ancient Colchic relict and local endemic species of the West Caucasus.

Te plant occurs in the middle montane belt up to 1600 m, mainly in the open areas of oak and fir forests, on limy and carbonate ecotopes. It grows either single or in small groups.

Its stalk is creeping, 3 m long, which develops male and female flowers. The plant, under cultural conditions begins vegetation in the second decade of April, flowers in May that lasts till the end of June. Bears fruit from September and develops seeds with 10-15% germination ability. Fast propagation of the plant is accessible by means of vegetative method. It is worth mentioning that the plant gives the best medicinal raw material.

All the investigated species are considered to be stable as they are characterized by fast reaction and adaptability to the changeable environmental conditions. They aren't damaged as a result of frosts in winter, late spring or early autumn. In case of slight damages they have quick regeneration ability, flower yearly, propagate by means of seed and vegetative ways, show high plasticity and xerophilous ecological signs.

All these species have been cultivated in Tbilisi Botanical Garden for many years. They are being protected under the cultural conditions, the method of **ex situ** conservation is also in practice and thus they are not threatened to be disappeared. Though, the situation in Colchis, the area of their distribution, is quite different. According to IUCN categories they are considered to be disappearing taxa.

Proceeding from the above mentioned, it is essential to put **in situ** and **ex situ** conservation in practice with the purpose to survive and protect these unique species.

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## კოლხეთის ფლორის კირქვიანების ზოგიერთი იშვიათი სახეობები თბილისის ბოტანიკურ ბაღში

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(მიღებულია 15.03.2010)

### რეზიუმე

შრომაში განხილულია კოლხეთის ფლორის კირქვიანების ზოგიერთი იშვიათი ვიწროლოკალური გავრცელების ენდემური და რელიქტური სახეობების ბიოეკოლოგიური თავისებურებები, მათი გადარჩენის მეთოდები ***ex-situ*** კონსერვაციის საშუალებით.

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Botany

# EFFECTS OF GRAZING ON SOME COMMUNITIES OF BOTHRIOCHLOETA ISCHAEMUM IN SHIDA KARTLI (EAST GEORGIA)

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**Abstract**

Effects of overgrazing on some communities of the Bothriochloeta ischaemum formation was studied in Shida Kartli. Recovery of disturbed coenoses was observed in a short period after grazing was ceased: general projectional coverage of the coenoses and the edificator (*Bothriochloa ischaemum*) sharply increased; the above-ground vertical (layer) structure of the coenosis and productivity of the above-ground phytomass intensely recovered; coenotic role and frequency of occurrence of weeds and semi-weeds were minimized. At the same time, permanent absence of grazing caused negative effects, such as intensification of litter formation and accumulation, depletion of floristic diversity, reduction of species richness; significant decline in the number of annuals in contrast to the other life forms.

**Key words:** grazing, projectional coverage, species richness, frequency of occurrence, litter, floristic composition.

## Introduction

Formation and development of herbaceous vegetation (steppe, meadow, etc.) in time and space was closely connected to animals and its structure and normal functioning significantly depended on the factor of grazing. At present major part of herbaceous ecosystems is used under pastures and human impact has become an important factor of herbaceous plant community structure development. Misallocation of pastures has caused degradation of plant cover. A large number of studies are focused on the influence on grazing on vegetation [Kakulia, 1961; Nakhutsrishvili, 1962; Ketskhoveli, Nakhutsrishvili, 1963; Callaway, et al., 2005, etc.].

The aim of our study was to investigate effects of overgrazing on the structure of Bothriochloeta ischaemum communities, and in particular, determine changes in principal geobotanical parameters (general projectional coverage of the coenoses, projectional coverage of the edificator, sodding, litter formation, species richness, layer structure, phytomass productivity, life form composition) and floristic composition in connection with grazing.

## Materials and Methods

Communities of Bothriochloeta ischaemum formation on slopes in vicinity of the village Sakadagiano (51°53'20"N 44°33'14"E, 650 m asl), Kaspi district, the gorge of the river Ksani, were selected as the study objects. An area of 700 $m^2$ was fenced to investigate effects of grazing. 15 plots of 1 $m^2$ were randomly selected on the fenced area and geobotanical surveys were conducted in spring (May) and summer (July), i.e. in phases of maximum growth of ephemerals / ephemeroids and *Bothriochloa ischaemum*, annually during 4 years (before fencing in 2006 and after fencing in 2007-2009). The results of the 3-year fencing were compared with initial (2006) relevés as well as relevés compiled on grazed area (out of the fenced territory) in 2009. The relevés of 2009 were also compiled on randomly selected 15 plots of 1 $m^2$. Complete floristic inventory was made on the whole fenced area as well as adjacent pasture.

In order to assess phytomass productivity the above-ground mass was collected 5 times on 1 $m^2$ area in the period of maximum development of the herbaceous cover (July). The collected mass was divided into green (living) and dry (dead) parts and dried in thermostat at 80°C to determine absolute dry weight.

Geobotanical and ecological data were obtained via semi-stationary and itinerary method in 2006-2009. Geobotanical surveys were carried out using conventional geobotanical techniques [Shennikov, 1964; Vasilevich, 1985]. Species richness and frequency of occurrence were studied using methods by Korchagin (1964), Braun-Blanquet (1964) and Poniatovskaya (1964).

Species nomenclature follows R. Gagnidze (2005).

## Results and Discussion

Comparison between and analysis of geobotanical-ecological parameters and floristic data obtained on the grazed and ungrazed plots during 4-year study are given below.

**Projectional coverage.** Already in the first year of fencing general projectional coverage of the coenoses significantly increased. In the period of maximum growth of *Bothriochloa ischaemum* (July) this index (litter excluded) reached 93.5-94.3% (Table 1) and tended to slight decrease during the following years. The same pattern was observed for the edificator (*Bothriochloa ischaemum*) (Table 1). At the same time the respective initial data (2006) as well as the data collected on the overgrazed plots in 2009 were almost identical.

**Table 1.** General projectional coverage of the coenoses (litter excluded) (%) and projectional coverage of *Bothriochloa ischaemum* (%) on the grazed and ungrazed plots (July)

| Plot type and date | Projectional coverage (%) | |
|---|---|---|
| | General | *Bothriochloa ischaemum* |
| Grazed: | | |
| 2006 | 57.5-59 | 24-25 |
| 2009 | 57-58.5 | 23,5-25 |
| Ungrazed: | | |
| 2007 | 93.5-94 | 81-82.5 |
| 2008 | 92-93 | 67-68.5 |
| 2009 | 89.5-90 | 80-80.5 |

**Litter projectional coverage.** When grazing was ceased, litter accumulation process started, which was reflected in litter projectional coverage. After the first year the difference between initial (grazed, 2006) and ungrazed (2007) plot data was relatively small, while in the following years litter accumulation intensity remarkably increased and reached its highest value in

2009 (Table 2). At the same time, according to 2009 data, litter projectional coverage tended to decrease on the overgrazed plots.

**Table 2.** Litter projectional coverage (%) on the grazed and ungrazed plots (July)

| Date | Plot No. and type | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 |
| Grazed | | | | | | | | | | | | | | | |
| 2006 | + | 3-4 | + | 1 | 1-2 | 2-3 | 1-2 | 2-3 | 1 | 1 | + | + | 1-2 | 1-2 | 1-2 |
| 2009 | + | 0 | 0 | 2-3 | + | 0.5 | + | + | + | + | + | + | 0.5 | 0.5 | + |
| Ungrazed | | | | | | | | | | | | | | | |
| 2007 | 2-3 | 8-10 | 5-6 | 3-5 | 6-7 | 25-26 | 7-8 | 15-16 | 6-7 | 4-5 | 3-4 | 2-3 | 6-7 | 5-6 | 17-18 |
| 2008 | 2-14 | 40 | 20-22 | 20 | 30 | 32-33 | 22-25 | 20 | 5-6 | 6-8 | 5-6 | 5-6 | 22-23 | 20-22 | 12-13 |
| 2009 | 2-45 | 50 | 60-62 | 35-37 | 50 | 80-87 | 45-47 | 50 | 40 | 22-23 | 10 | 21-22 | 25-26 | 20-22 | 40 |

**Species richness.** Species richness (per 1 $m^2$) determined annually was as follows:

2006 (grazed) – 19.9 species
2009 "_____" – 19.7 species
2007 (ungrazed) – 13.4 species
2008 "_____" – 11.2 species
2009 "_____" – 10.6 species

According to the above data, fencing results in species richness decrease. The decrease must be connected with significant increase of projectional coverage of *Bothriochloa ischaemum* and especially litter. The thick layers of *Bothriochloa ischaemum* and litter hamper other plant growth and development, which leads to decline in the number of their individuals. Annuals and, in particular, ephemeral species are primarily affected by increase of *Bothriochloa ischaemum* and litter projectional coverage. Annual species richness was 4 times decreased on the ungrazed plots compared to the grazed ones in 2009 (Table 3). Hemicryptophyte and chamaephyte richness also decreased but at a relatively small extent. Consequently, perennials retain ability to renewal under litter and manage to grow and develop more or less normally, while annuals (especially ephemerals) either do not germinate, or their seedlings die at early stages of their development.

**Table 3.** Number of species of different life forms per 1 $m^2$

| Life form | Plot type and year | | | | |
|---|---|---|---|---|---|
| | Grazed | | Ungrazed | | |
| | 2006 | 2009 | 2007 | 2008 | 2009 |
| Chamaephytes | 2 | 2 | 1.7 | 1.8 | 1.7 |
| Hemicryptophytes | 7.9 | 8 | 6.1 | 6.8 | 6.5 |
| Geophytes | 0.1 | 0.1 | - | - | - |
| Therophytes: | 9.9 | 9.6 | 5.6 | 2.6 | 2.4 |
| Biennials | 0.4 | 0.5 | 0.3 | 0.1 | 0.1 |
| Annuals | 9.5 | 9.1 | 5.3 | 2.5 | 2.3 |

**Frequency of occurrence and coenotic role of some weeds and semi-weeds.** Prevention of grazing led to significant decrease of weed frequency of occurrence. The process proceeds gradually and proportionally to the length of the period since grazing was stopped. It is noteworthy that in 2009 some weeds completely dropped out of the coenosis composition (Table 4).

Along with decline in frequency of occurrence, coenotic role (projectional coverage) of the weeds and semi-weeds also decreases. By 2009 their majority was represented by single individuals in the coenoses and their projectional coverage was recorded as "+". It is also noteworthy that some weeds (*Aegilops tauschii*, *Adonis parviflora*, *Capsella bursa-pastoris*, *Setaria viridis*) with low frequency of occurrence (< 6.7%) on the grazed plots completely drop of the ungrazed coenoses. The exception was *Daucus carota*, frequency of occurrence and coenotic role of which increases on the ungrazed plots.

**Table 4.** Frequency of occurrence (%) of some weeds and semi-weeds on the grazed and ungrazed plots

| Species | Frequency of occurrence (%) | | | |
|---|---|---|---|---|
| | Ungrazed | | | Grazed |
| | 2007 | 2008 | 2009 | 2009 |
| *Carthamus lanatus* | 73.3 | 33.3 | 46.7 | 93.3 |
| *Salvia viridis* | 40 | 6.7 | 0 | 80 |
| *Filago arvensis* | 80 | 13.3 | 0 | 93.3 |
| *Euphorbia falcata* | 33.3 | 0 | 0 | 73.3 |
| *Althaea hirsuta* | 33.3 | 6.6 | 0 | 86.7 |
| *Xeranthemum squarrosum* | 6.6 | 6.7 | 6.7 | 20 |
| *Achillea biebersteinii* | 20 | 20 | 20 | 73.3 |
| *Daucus carota* | 6.6 | 53.3 | 73.3 | 6.7 |

**Layer structure.** Prevention of grazing also affected coenosis layer structure. In spring (April / May) the difference was slight, while in summer, i.e. period of maximum development of *Bothriochloa ischaemum* it was significant. During this period coenoses of the grazed plots are 1-layered and the height of the layer is only 1-5 cm. Despite the fact that this was the maximum development phase of *Bothriochloa ischaemum,* height of its vegetative and generative organs did not exceed 5 cm. Besides, generative organs were found only on a small number of individuals. In contrast, 2 (rarely 3) layers were clearly distinguished on the ungrazed plots. The first layer was mainly formed by *Bothriochloa ischaemum.* Besides, the first layer contained some perennial and annual fobrs (*Daucus carota*, *Scorzonera biebersteinii*, *Sonchus oleraceus*, *Linum corymbulosum*, *Plantago lanceolata*, *Onobrychis cyri*, *Medicago coerulea*, *Poterium polygamum,* etc.). During this period the first layer was (40)50-70(80) cm tall. The second layer was 5-20 cm tall. It was mainly made up of *Thymus marschallianus*, *Teucrium polium*, *Eryngium campestre* (vegetative organs), *Trachynia distachya* and leaves of various perennial forbs.

**Phytomass.** As shown on the chart (Fig. 1), overgrazing caused strong degradation of herbaceous cover (living and dead mass was minimized). When grazing is ceased, productivity of above-ground phytomass (both living and dead) significantly increases. Therefore, rapid increase of general projectional coverage of the coenoses and projectional coverage of litter (Tables 1, 2) is reflected in phytomass productivity.

**Sodding.** Grazing prevention did not affect sodding level. It must be related to sharp intensification of above-ground vegetative and generative organ development of *Bothriochloa ischaemum*, which leads to increase of its projectional coverage and above-ground phytomass rather than sodding process. However, sodding pattern may change in the following years.

**Floristic composition.** 3-year fencing resulted not only in changes of geobotanical parameters but also those of floristic composition. Coenoses were floristically pauperized. 77 species were recorded on the grazed plots in 2009, while only 54 species were recorded on the ungrazed plots. Shared species number on both plot types was 45. Jaccard similarity coefficient [Mirkin et al., 1989] calculated according to these data slightly exceeds 0.5, which indicates significant difference in floristic composition of the coenoses on the different plot types.

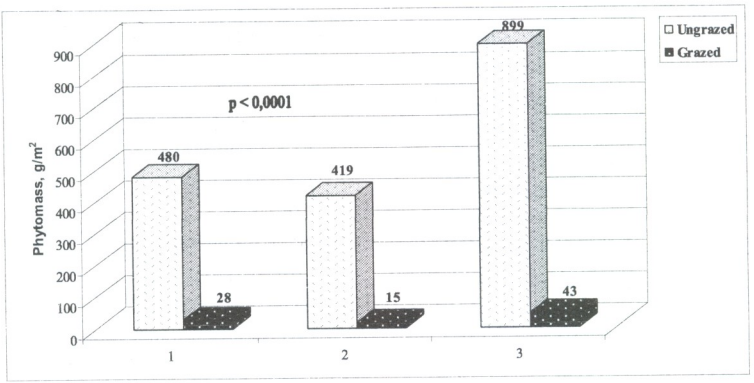


**Fig. 1.** Above-ground phytomass productivity (g/m$^2$ absolute dry weight) on the ungrazed and grazed plots (july, 2009). 1. Living mass, 2. Dead mass, 3. General mass.

**Life form composition.** Comparison of life form spectra of the fenced and overgrazed plots revealed interesting tendencies. On the ungrazed plots proportion of hemicryptophytes increased, while that of therophytes decreased (Table 5). However, it is noteworthy that the number of hemicryptophyte species decreased by 1 on the ungrazed plots, i.e. increase of their proportion is connected to decline in the annual species number. The same reason underlies some increase of other life form percentage. These changes per area unit (1 m$^2$) correlate with separate life form species richness.

**Table 5.** Life form (Raunkiaer, 1934) proportion on the ungrazed and grazed plots (2009)

| Life form | Plot type and year | | | |
|---|---|---|---|---|
| | Grazed | | Ungrazed | |
| | Number of species | % | Number of species | % |
| Chamaephytes | 3 | 3.9 | 3 | 5.6 |
| Hemicryptophytes | 24 | 31.2 | 23 | 42.6 |
| Geophytes | 1 | 1.3 | 1 | 1.8 |
| Therophytes | 49 | 63.6 | 27 | 50 |
| Biennials | 3 | 3.9 | 3 | 5.6 |
| Annuals | 46 | 59.7 | 24 | 44.4 |
| Total | 77 | 100 | 54 | 100 |

Comparison of the geobotanical-ecological and floristic parameters given above clearly show that ceasing of grazing results in both positive and negative changes in the structure of *Botriochloa ischaemum* coenoses. The positive changes are:

1. Sharp increase of general projectional coverage of the coenoses as well as projectional coverage of *Botriochloa ischaemum*;
2. Recovery of the above-ground vertical (layer) structure of the coenoses;
3. Sharp decrease of weed and semi-weed coenotic role and frequency of occurrence;
4. Intense increase of phytomass productivity.

The following should be considered as negative changes:

1. Pauperization of floristic diversity;
2. Decline in species richness;
3. Decrease of annual species proportion and consequently, possible drop-out of ephemeral synusia of the coenoses structure;
4. Intense process of litter formation and accumulation, which hampers natural renewal of the coenoses and causes their structure disturbance.

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## ძოვების ზეგავლენა შიდა ქართლის უროიანი ფორმაციის (BOTHRIOCHLOETA ISCHAEMUM) ზოგიერთ თანასაზოგადოებაზე

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(მიღებულია 15.12.2009)

### რეზიუმე

შესწავლილია ინტენსიური ძოვების ზეგავლენა უროიანი ფორმაციის ზოგიერთ თანასაზოგადოებაზე. დადგენილია, რომ ძოვების შეწყვეტისთანავე იწყება სტრუქტურადარღვეული ცენოზების აღდგენა: მკვეთრად იზრდება ცენოზის საერთო და ედიფიკატორის (*Bothriochloa ischaemum*) პროექციული დაფარულობა; ინტენსიურად მიმდინარეობს ცენოზების მიწისზედა ვერტიკალური (იარუსებრივი) სტრუქტურის აღდგენა და მიწისზედა ფიტომასის პროდუქტიულობის ზრდა; მინიმუმამდე მცირდება სარეველა და ნახევრად სარეველა მცენარეების ცენოზური როლი და შეხვედრილობა. ამასთავე, ძოვების მუდმივი შეწყვეტა უარყოფით შედეგებსაც იწვევს: ინტენსიურად მიმდინარეობს მკვდარი საფრის წარმოქმნისა და დაგროვების პროცესი. ადგილი აქვს ფლორისტული შემადგენლობის გაღარიბებას, მცირდება სახეობათა დატვირთვა; სხვა სასიცოცხლო ფორმებისგან განსხვავებით, მნიშვნელოვნად კლებულობს ერთწლოვანი მცენარეების მონაწილეობა.

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# FOR STUDIES OF DIVERSITY OF KVEMO KARTLI DENDROFLORA

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**Abstract**

The paper deals with diversity of woody plants of Kvemo Kartli floristic region. 86 species were revealed. Taxonomic structure of respective families, genera and species, and their phytogeography is presented. 5 species of woody plants not indicated for Kvemo Kartli in the "Flora of Georgia" are highlighted.

**Key words:** woody plants, systematics, phytogeography, topoecology

## Introduction

Kvemo Kartli is a historical region of Georgia remarkable for natural diversity and beauty. Scenic landscapes of volcanic mountains and uplands of Southern Georgia ranging from semi-deserts and steppes to broadleaved forests and mountains are represented here. Well-preserved historical monuments of V-VI cc and later periods are numerous in the region, which also supports city remnants founded in I millennium BC and other landmarks.

The oldest sample of the Georgian epigraphy survives at Bolnisi Sioni Church, which dates from V c. Up to 650 historical monuments are located in Kvemo Kartli; out of these, some 300 monuments are included in various tourist routes.

Discovery of primitive man site and remains at Dmanisi Settlement has attracted worldwide attention and is regarded as an archaeological find of utmost significance. Specialists have concluded that 1.8 m years ago Dmanisi hominids inhabited this area from where they migrated to Europe [Kvemo Kartli Passport 2007. http://www. moe.gov.ge].

**Natural Environment**

***Location, Area and Borders.*** Kvemo Kartli is located south of East Georgia. Kvemo Kartli floristic area (hereinafter Kvemo Kartli) is enclosed by the sources of r. Shavtskhala and r. Khrami as far as Arukhlo from the north, r. Mtkvari - from the east, Armenian state border – from the south and Javakheti range - from the west [Maruashvili, 1968].

The region occupies 2,238.2 km$^2$, which comprises 3.21% of the country area. Kvemo Kartli is divided into three administrative districts, which are as follows: Marneuli, Bolnisi and Dmanisi.

***Geology.*** Geologist Nikoloz Astakhov [Dolukhanov, 1989] divides Kvemo Kartli into two geomorphological parts: (1) Shua (middle) Khrami mountain group, and (2) Loki (Somkhiti) ridge. The first part is further subdivided into four areas while Loki ridge represents a single orographic unit, which extends along the Georgia-Armenia state border.

Khram-Loki mountainous region is mainly comprised of the Upper Tertiary, Middle Jurassic and Middle Eocene volcanogenic and partially calcareous strata, which overlie the Palaeozoic granitoids exposed at two places (Khrami and Loki mountains). The Upper Cretaceous formations are most widespread. Relief formation was strongly affected in some places by lava flowed from Javakheti range. The terrain is diverse ranging from flat and step-like forms to severely eroded areas and deep canyons.

***Soils.*** Soil cover is represented by a combination of chestnut, grey-cinnamonic, black and alluvial soil types [Maruashvili, 1968]. As a rule, alluvial soils occur in river gorges and valleys. Black soils are found at higher altitudes than alluvial soil, towards mountainous meadows (in our case – Zurtaketi). Cinnamonic and grey-cinnamonic soils occur in grasslands [Urushadze, 1977].

***Climate.*** Mean annual temperature ranges from 5.9 to 12.0°C in the main part of Kvemo Kartli. Mean temperature of the coldest month varies from -5.9 to -0.3°C; annual range of temperature is 23-24°C. Mean annual precipitation comprises 790-652 mm. It should be noted that steppes border onto the hypsometrically elevated part of Kvemo Kartli from the west and low-lying part to the east; steppes are characterized by the continental climate (cold continental in the higher and dry continental in the lower parts). Due to this, humidity increases up to low altitudes, namely – 1,300-1,400 m asl. Precipitation neither increases nor decreases at higher altitudes. Because of this and intensive anthropogenic pressure, the upper limit of the timberline runs at lower altitudes while lower limit is located higher narrowing the forest belt [Dolukhanov, 1989].

***Hydrological Regime.*** There are numerous rivers and lakes in the region, which are mostly located in the basins of r. Ktsia-Khrami and r. Algeti. The following watercourses are noteworthy: Khrami, Algeti, Mashavera, Debeda, Karabulakhi, Agrichai, Bolnisi.

The following lakes are situated in Kvemo Kartli: Jandara, Tsurbliani, Arkhiani, Bareti and Grdzeli [Kvemo Kartli Passport 2007. http://www. moe.gov.ge].

***Study Goals***: Compilation of an inventory of Kvemo Kartli woody plants; systematic, phytogeographical and topoecological analysis of woody plants; determination of their role in the landscapes.

## Materials and Methods

Field surveys were conducted using traditional methods [Grossheim, 1936; Tolmachev, 1947, 1986; Gagnidze, Ivanishvili, 1975].

Botanical-geographical analysis of Kvemo Kartli was undertaken using an Arealogical method. Determination of geographical elements is based on the recent geographical distribution of taxa [Gagnidze, Ivanishvili, 1975a, b; Gagnidze, 1974, 2005].

In addition to field surveys undertaken by us, botanical-geographical analysis is based on the first (1941-1952) and second editions (vol. I-XV, 1971-2007) of the "Flora of Georgia" and herbarium materials of Tbilisi Botanical Garden and Institute of Botany (TBI) and Tbilisi State University (TB).

The species nomenclature is in accordance with S. Cherepanov (1995) and R. Gagnidze (2005).

This study does not cover numerous introduced and cultivated woody plants found in this region.

## Discussion

### *1. History of Botanical studies of Kvemo Kartli*

"Description of Georgian Kingdom" by Vakhushti Bagrationi (1742) may be regarded as beginning of botanical studies of Kvemo Kartli. This source details vegetation cover and localities of entire Georgia including Kvemo Kartli.

Detailed studies of vegetation of the Caucasus and Georgia were initiated in XIX c. by such renowned scientists as Karl Kokh and Later – Rade. Since then, description of Kvemo Kartli vegetation cover is included in the works of various scientists, both well-known and relatively obscure. Among them, the following should be noted: I. Medvedev, 1870; A. Grossheim, D. Sosnovski, N. Troitski, 1928; N. Ketskhoveli, 1960; A. Dolukhanov, 1989; R. Gagnidze, 2000.

### *2. Characteristics of Flora*

Total number of higher plants of Kvemo Kartli is not determined accurately as yet (it is one of ultimate goals of this study); number of woody plants is 86 including six endemics of the Caucasus and one – of Georgia.

86 species of woody plants of Kvemo Kartli belong to 52 genera and 28 families.

The genera distribution by families is as follows: Rosaceae – 14 genera, Anacardiaceae – 3 genera, Caprifoliaceae – 3 genera; Betulaceae, Corylaceae, Fagaceae, Leguminosae, Moraceae, Oleaceae, Rhamnaceae, Salicaceae – 2 genera each; other families (Epedraceae, Aceraceae, Araliaceae, Berberidaceae, Celastraceae,Celtidaceae, Cornaceae, Elaeagnaceae, Ericaceae, Grossulariaceae, Viscaceae, Pinaceae, Punicaceae, Tiliaceae, Ulmaceae and Vitaceae) are represented only by one genus.

Rosaceae has the highest number of genera; other families include substantially fewer genera (Table 1).

Six leading genera with highest number of species are given below:

1. Acer – 6 species
2. Salix – 5 species
3. Rosa – 4 species
4. Rubus – 4 species
5. Quercus – 3 species
6. Populus – 3 species

The above six leading families include 25 species, which comprises 28.73% of the total number of species.

The six leading families with highest number of species are detailed below:

1. Rosaceae – 25 species
2. Salicaceae - 8 species
3. Aceraceae - 6 species
4. Rhamnaceae – 4 species
5. Fagaceae - 4 species
6. Corylaceae - 4 species.

The six leading families include a total of 51 species, which amounts to 58.62% of the total number of species.

### *3. Brief Overview of Vegetation*

Vertical zoning of the vegetation in Kvemo Kartli, as well as generally in East Georgia (excluding the nival zone above 3,700 m asl) is as follows: semideserts, steppes and light forests - 150-600 m asl; forests - 600-1,900 m asl, subalpine belt - 1,900-2,500 m asl, alpine belt - 2,500-3,000 m asl, subnival belt - 3,000-3,700 m asl [Nakhutsrishvili, 2000].

**The belt of semideserts, steppes and arid light forests (150-600 m asl)** covers Marneuli plain - right-hand part of Kvemo Kartli plain, which is irrigated by lower reaches of rivers Algeti and Khrami. The riverine terraces are comprised of gypsum-containing clays. The vegetation is mainly represented by bluestem, bluestem-common wormwood and saltwort dominated semideserts, communities of Christ's thorn, oriental hornbeam, spirea, Caucasian milkvetch, prickly thrift, etc. As regards rare vegetation types and plant species, Georgian iris occurs in Marneuli plain [Gagnidze, Davitadze, 2000]; fragments of arid light forests with mastic tree (*Pistacia mutica*), Caucasian hackberry (*Celtis caucasica*) and admixture of numerous hemixerophilous shrubs. Light forest fragments occur between Bolinisi and Kvemo Dmanisi, on the left bank of r. Mashavera, on hills on the bank of r. Poladauri in the vicinities of Shulaveri where they are found in the form of Caucasian hackberry and Christ's thorn communities [Ketskhoveli, 1960].

**The forest belt (600-1,900 m asl)** mainly includes Dmanisi environs enclosed by forestless Javakheti upland from the west and Marneuli and Gardabani plains from the east; it extends only to the north, towards Tetritskaro. Somkhiti ridge is also covered by forests. It is comprised of volcanogenic strata. Here the forests represented by beechwood, oakwood, hornbeam and pine forest fragments are developed.

**The subalpine belt (1,900-2,500 m asl)** is found in Loki and Lalvari mountain areas. Here the woody plants form the so called thin or park forest biome. Thin forests are mainly formed by *Acer trautvetteri* and *Quercus macranthera* as well as *Fagus orientalis* and *Sorbus caucasigena*. The foothills are occupied by bluestem-Christ's thorn communities [Nakhutsrishvili, 2000].

**The alpine (2,500-3,000 m asl) and subnival (3,000-3,700 m asl) belt** is found in Loki and Lalvari mountain areas as well as Somkhiti ridge. The subnival belt is represented only at peaks where altitudes reach 2,545 and 3,142 m asl.

The geographical structure of the endemic woody plants, conspectus of woody plants and total number of families and genera of Kvemo Kartli are given below.

**Table 1.** Woody plants of Kvemo Kartli

| FAMILY | NUMBER OF GENERA | GENUS | NUMBER OF SPECIES | NUMBER OF ENDEMIC SPECIES |
|---|---|---|---|---|
| **1. Ephedraceae** | **1** | **Ephedra** | **1** | |
| **2. Pinaceae** | **1** | **Pinus** | **1** | |
| **3. Aceraceae** | **1** | **Acer** | **6** | |
| **4. Anacardiaceae** | **3** | **Cotinus Pistacia Rhus** | **3** | |
| **5. Araliaceae** | **1** | **Hedera** | **1** | |
| **6. Berberidaceae** | **1** | **Berberis** | **2** | **1** |
| **7. Betulaceae** | **2** | **Alnus Betula** | **2** | |
| **8. Caprifoliaceae** | **3** | **Lonicera Sambucus Viburnum** | **3** | |
| **9. Celastraceae** | **1** | **Euonymus** | **2** | |
| **10. Celtidaceae** | **1** | **Celtis** | **1** | |
| **11. Cistaceae** | **1** | **Helianthemum** | **1** | |
| **12. Cornaceae** | **1** | **Swida** | **1** | |
| **13. Corylaceae** | **2** | **Carpinus Corylus** | **4** | **1** |
| **14. Elaeagnaceae** | **1** | **Elaeagnus** | **1** | |
| **15. Ericaceae** | **1** | **Rhododendron** | **1** | |
| **16. Fagaceae** | **2** | **Fagus Quercus** | **4** | |
| **17. Grossulariaceae** | **1** | **Ribes** | **1** | |
| **18. Leguminosae** | **1** | **Genista** | **2** | **2** |
| **19. Moraceae** | **2** | **Ficus Morus** | **2** | |
| **20. Oleaceae** | **2** | **Fraxinus Ligustrum** | **3** | |
| **21. Punicaceae** | **1** | **Punica** | **1** | |
| **22. Rhamnaceae** | **2** | **Paliurus Rhamnus** | **4** | |
| **23. Rosaceae** | **14** | **Amygdalus Cerasus Cotoneaster Crataegus Cydonia Malus Mespilus Padus Prunus Pyrus Rosa Rubus Sorbus Spiraea** | **25** | **3** |
| **24. Salicaceae** | **2** | **Populus Salix** | **8** | |
| **25. Tiliaceae** | **1** | **Tilia** | **1** | |
| **26. Ulmaceae** | **1** | **Ulmus** | **2** | |
| **27. Viscaceae** | **1** | **Viscum** | **1** | |
| **28. Vitaceae** | **1** | **Vitis** | **2** | |
| **28** | **52** | **Total** | **86** | **7** |

***Phytogeography.*** Kvemo Kartli belongs to ancient Mediterranean kingdom, namely Somkhiti or Loki region of Minor Caucasus province in phytogeographic terms [Gagnidze, 2004].

Kvemo Kartli flora is formed by the species of the Mediterranean, Caucasian, West Asian, Palaearctic and Holarctic range type species; their ratios are different in the floristical composition.

Composition of each geographical element is detailed below:

**1) Caucasian Species:**

◎ 1. *Corylus iberica* Wittm. ex Kem. – Nath.
This species is a tree, which is found from the middle to upper montane belts.
Georgia: 1 - Abkhasia; Racha-Lechkhumi; 9 - Kartli; 11 - Tusheti-Pshav-Khevsureti; 12 - Kakheti; 16 - Trialeti; 17 - Kvemo Kartli [Dolukhanov, 1989];
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.

◎ 2. *Genista transcaucasica* Schischk.
This species is a shrub found on dry stony slopes and shrubbery in the lower to middle montane belt forests.
Georgia: 8 - Shida Kartli; 9 - Kartli; 12 - Kakheti; 14 - Gare Kakheti; 17 - Kvemo Kartli.
Caucasus: South Caucasus: Azerbaijan, Armenia.

◎ 3. *Genista patula* Bieb.
This species is a shrub found in semideserts, grasslands and broadleaved forests from the lowlands to middle montane belt.
Georgia: 5 - Imereti; 8 - Shida Kartli; 9 - Kartli; 12 - Kakheti; 15 - Gardabani; 17 - Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.

4. *Pyrus caucasica* Fed.
This species is a tree, which occurs in the lower, middle and, sometimes, upper montane belt forests; it is more characteristic for broadleaved forests.
Georgia: All districts except Javakheti.
Caucasus: North Caucasus; South Caucasus: Black Sea coast, Azerbaijan.

◎ 5. *Sorbus caucasigena* Kom. ex Gatsch.
This species is a tree found in the middle, upper montane and subalpine belts, at the altitudes of 1,400-2,400 m asl, mostly in forests and subalpine habitats.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 8 - Shida Kartli; 9 - Kartli; 11 - Tusheti-Pshav-Khevsureti; 12 - Kakheti; 16 - Trialeti; 17 – Kvemo Kartli (R. Gagnidze); 18 – Javakheti; 19 – Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.

**2) East Caucasian:**

◎ 1. *Berberis iberica* Stev. & Fish. ex DC17 – Kvemo Kartli (R. Gagnidze).
This species is a shrub found in shrubbery and river terraces in dry places in the lower montane belt.
Georgia: 9 - Kartli; 13 - Kizikhi; 14 - Gare Kakheti; 17 – Kvemo Kartli (R. Gagnidze).
Caucasus: North Caucasus (Daghestan); South Caucasus: Azerbaijan, Armenia.

**3) South Caucasian – South-West Asian**

1. *Rubus woronowii* Lonacz.

This species is a shrub found at forest margins, thin birchwood and forest clearings in the upper montane and subalpine belts.
Georgia: 6 – Guria; 7 – Ajara; 9 - Kartli; 16 - Trialeti; 17 – Kvemo Kartli; 18 – Javakheti; 19 – Meskheti.
Caucasus: General distribution: South-West Asia (North-East Anatolia).

**4) East South Caucasian:**

◎ 1. *Rosa kozlowskii* Chrshan.
This shrub is found in open places, shrubbery and forest margins in the upper montane belt.
Georgia: 9 - Kartli; 17 –Kvemo Kartli.
Caucasus: South Caucasus: Azerbaijan (indicated in Lenkoran by Khrzhanovski).

**5) East Georgian:**

O 1. *Amygdalus georgica* Desf.
This shrub occurs in grasslands.
Georgia: 9 - Kartli; 14 - Gare Kakheti; 16 - Trialeti; 17 – Kvemo Kartli (R. Gagnidze).

**6) Caucasian-Mediterranean:**

1. *Berberis vulgaris* L.
This shrub grows at forest margins and gorges in the lower and middle montane belts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Central Europe; Mediterranean Region; Balkans; East Europe.

2. *Carpinus caucasica* Grossh.
This tree forms coenoses in the lower and middle montane belt forests; it is sometimes found even at higher altitudes.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Crimea; South-West Asia; North Iran.

3. *C. orientalis* Mill.
This species is a shrub or tree found in the lower and middle montane belt forests; it sometimes forms coenoses.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Mediterranean Region (eastern part); Europe (rare).

4. *Cerasus silvestris* (Kirschl.) Gars.
This tree grows in deciduous forests in the middle and upper belts.
Georgia: All districts.
Caucasus: All districts.
General distribution: Central Europe; Mediterranean Region; Balkans; South-West Asia; Iran; Crimea.

5. *Ulmus minor* Mill.
This shrub occurs at the altitudes up to 800 m asl in dry places, oakwoods, forest margins as well as floodplain terraces.
Georgia: 1 - Abkhasia; 5 – Imereti; 9 - Kartli; 10 - Mtiuleti; 12 - Kakheti; 14 – Gare Kakheti; 15 – Gardabani; 16 - Trialeti; 17 – Kvemo Kartli; 19 – Meskheti.

Caucasus: North Caucasus; South Caucasus: Black Sea coast; Azerbaijan, Armenia.
General distribution: Central Europe; Mediterranean Region; South-West Asia (East Anatolia).

**7) Caucasian, Mediterranean, South-West and West Asian:**

1. *Pistacia mutica* Fisch. et Mey.
This tree occurs in lowlands and foothills, mostly on southern slopes, dry stony areas; it forms arid thin forests.
Georgia: 9 - Kartli; 10 - Mtiuleti; 13 - Kizikhi; 14 – Gare Kakheti; 15 – Gardabani; 16 - Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Black Sea coast; Azerbaijan, Armenia.
General distribution: Mediterranean Region; Crimea; South-West Asia; Iran.

**8) Caucasian, Mediterranean, South-West, West and Central Asian:**

1. *Epehdra procera* Fish et. Mey.
This shrub is found on dry rocky and stony slopes as well as clayey-sandy, more or less saline, exposed places in the lower and upper montane belts, up to the altitudes of 500-1,600 (1,800) m asl.
Georgia: 9 - Kartli; 10 - Mtiuleti; 12 - Kakheti; 13 - Kizikhi; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Mediterranean Region; South-West Asia; Armenia; Kurtistan; Iran; East Asia.

2. *Euonymus europaea* L.
This shrub grows from lowlands up to the altitude of 2,000 m asl; it occurs in the understorey in the deciduous and dark coniferous forest belt.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 6 – Guria; 7 – Ajara; 9 - Kartli; 12 - Kakheti; 17 – Kvemo Kartli; 19 – Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Crimea; West Europe; Mediterranean Region; South-West Asia; North Iran.

3. *Fagus orientalis* Lipsky.
This tree ocuurs in moderately humid areas up to 1,000 – 2,300 m asl.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Black Sea coast; Azerbaijan, Armenia.
General distribution: Mediterranean Region (Eastern part); South-West Asia; Iran.

4. *Ficus carica* L.
This tree is found on rocky exposures in the lower montane belt, up to 1,000 m asl.
Georgia: 1 - Abkhasia; 4 – Samegrelo; 5 – Imereti; 7 – Ajara; 9 - Kartli; 12 - Kakheti; 13 – Kizikhi; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Black Sea coast; Azerbaijan, Armenia.
General distribution: Mediterranean Region; South-West Asia (East Anatolia); Iran.

5. *Fraxinus excelsior* L.
This tree is found in mixed deciduous forests from the lower to middle montane belts.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 6 – Guria; 7 – Ajara; 8 – Shida Kakheti; 9 - Kartli; 10 – Mtiuleti; 13 – Kizikhi; 14 – Gare Kakheti; 15 – Gardabani; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Black Sea coast; Azerbaijan, Armenia.
General distribution: Central Europe; Mediterranean Region; Balkans; South-West Asia; North Iran; Crimea.

6. *Lonicera caucasica* Pall.
This shrub is found in the middle and upper montane belt as well as subalpine forests; it is common in birchwood.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 6 – Guria; 7 – Ajara; 8 – Shida Kakheti; 9 - Kartli; 10 – Mtiuleti; 11 – Tusheti-Pshav-Khevsureti; 12 – Kakheti; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Balkans; South-West Asia (Lazistan; East Anatolia); North Iran.

7. *Paliurus spinachristi* Mill.
This shrub occurs on dry, loamy, gravelly and stony areas from the lowlands to the middle montane belt.
Georgia: 1 - Abkhasia; 3 – Racha-Lechkhumi; 7 – Ajara; 8 – Shida Kakheti; 9 - Kartli; 10 – Mtiuleti; 12 – Kakheti; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Black Sea coast; Azerbaijan, Armenia.
General distribution: Crimea; Central Europe; Mediterranean Region; South-West Asia (North-East Anatolia); Iran.

8. *Prunus divaricata* Ledeb.
This species is a shrub, which grows in the lower and middle as well as upper montane belts.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: East Mediterranean; South-West Asia (East Anatolia); Iran.

9. *Punica granatum* L.
This shrub is found on dry slopes and river terraces.
Georgia: 1 - Abkhasia; 5 – Imereti; 6 - Guria; 9 - Kartli; 12 – Kakheti; 13 – Kizikhi; 14 – Gare Kakheti; 15 – Gardabani; 16 – Trialeti; 17 – Kvemo Kartli.
Caucasus: South Caucasus: Azerbaijan, Armenia.
General distribution: Western part of Central Asia (Middle Asia); Mediterranean Region; Iran; Afghanistan.

10. *Rhus coriaria* L.
This shrub occurs in dry places, xerophilous shrubbery and arid thin forests in the lower and middle montane belts.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 5 – Imereti; 7 – Ajara; 9 - Kartli; 10 – Mtiuleti; 12 – Kakheti; 13 - Kizikhi; 16 – Trialeti; 17 – Kvemo Kartli; it can also be found in other districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Mediterranean Region; Crimea; South-West and Western Part of Central Asia (East Anatolia); Iran.

11. *Rosa corymbifera* Borkh.
This shrub occurs at forest margins, clearings and roadsides in the lowlands and lower and middle montane belts.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus.
General distribution: Europe; North Africa; South-West Asia; Iran; Afghanistan.

12. *Rosa foetida* Herrm.
This shrub is a cultivar.
Georgia: May be found in the wild.
Caucasus: South Caucasus (cultivated)
General distribution: South-West Asia; Western Part of Central Asia (Middle Asia); Iran; Afghanistan (cultivated); may be sometimes found in the wild.

13. *Sambucus nigra* L.
This shrub is found in forests, forest margins and ruderal areas in the lowlands of the lower montane belt.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 6 – Guria; 7 – Ajara; 8 – Shida Kartli; 9 - Kartli; 10 – Mtiuleti; 11 – Tusheti-Pshav-Khevsureti; 12 – Kakheti; 13 - Kizikhi; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Crimea; Central Europe; Mediterranean Region; South-West Asia; Syria; North Iraq; West Iran.

14. *Tilia begoniifolia* Stev.
This tree occurs in forests, forest margins and gorges up to the middle montane belt.
Georgia: Throughout the country.
Caucasus: North Caucasus; South Caucasus (all districts).
General distribution: Crimea; South-West Asia; North Iran.

**9) Caucasian, Mediterranean, South-West Asian:**
1. *Cotoneaster integerrimus* Medik.
This shrub grows mostly on rocky and stony slopes up to the subalpine belt.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: South (rarely East).
General distribution: Europe; South-West Asia; Crimea.

2. *Crataegus kyrtostyla* Fingerh.
This species is a shrub or tree, which occurs in the form of understorey in the lower, middle and upper montane belt forest; it sometimes forms thickets on sunny slopes.
Georgia: 2 – Svaneti; 3 – Racha-Lechkhumi; 7 – Ajara; 9 - Kartli; 12 – Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus.
General distribution: Central Europe; East Mediterranean; South-West Asia.

3. *Crataegus pentagyna* Waldst. & Kit.
This species is a shrub or tree, which occurs in the lower and middle montane belt forests, forest margins and shrubbery.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 7 – Ajara; 9 - Kartli; 10 – Mtiuleti; 12 – Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus.
General distribution: Central Europe; East Mediterranean; South-West Asia.

4. *Fraxinus oxycarpa* Willd.
This tree grows in dry and sunny places from the lower to the middle montane belts.
Georgia: 9 - Kartli; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus (Daghestan); South Caucasus: Azerbaijan.

General distribution: Mediterranean Region; South-West Asia; Iran; Crimea.

5. *Ligustrum vulgare* L.
This evergreen shrub occurs on open slopes in the lower forest belt.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 6 – Guria; 7 – Ajara; 9 - Kartli; 10 – Mtiuleti; 12 – Kakheti; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast, Azerbaijan, Armenia.
General distribution: Europe; Mediterranean Region; South-West Asia; Crimea.

6. *Pinus kochiana* Klotzsch ex C. Koch.
This tree is found from the lowlands up to the upper limit of the timberline; though it occurs mostly in the middle and upper montane and subalpine belts on steep rocky and stony slopes forming forests and isolated stands of only pine as well as together with beech, spruce, fir and rarely birch.
Georgia: Almost throughout the country (excluding Kizkhi and Gardabani floristic districts).
Caucasus: North Caucasus; South Caucasus: Black Sea Coast, Azerbaijan, Armenia.
General distribution: South-West Asia; Crimea.

7. *Populus canescens* (Ait.) Smith.
This tree occurs in terraces, floodplains and river banks in the lower montane belt.
Georgia: 1 - Abkhasia; 4 – Samegrelo; 5 – Imereti; 9 - Kartli; 12 – Kakheti; 13 – Kizikhi; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Crimea; South-West Asia.

8. *Quercus pedunculiflora* C. Koch.
This tree grows in the middle montane belt at the altitudes of 1,000-1,100 m asl.
Georgia: 8 – Shida Kartli; 9 - Kartli; 12 – Kakheti; 15 – Gardabani; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Mediterranean Region (eastern part); South-West Asia.

9. *Swida australis* (C. A. Mey.) Pojark. ex Grossh.
This shrub grows in the lower and middle montane belt forests, forest margins, river banks and shrubbery.
Georgia: All districts of West Georgia; 8 – Shida Kartli; 9 - Kartli; 12 – Kakheti; 13 – Kizikhi; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South and Central Europe; South-West Asia (North-West Anatolia).

**10) Caucasian – South-West Asian:**

1. *Acer trautvetteri* Medw.
This tree occurs in the subalpine belt forming park forests; it may be found at lower altitudes, namely – 1,400-2,500 m asl.
Georgia: Almost all districts except for Kizikhi, Gardabani and Javakheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia.

2. *Betula litwinowii* Doluch.
This tree occurs in the subalpine and upper montane belts at the altitudes of 1,900-2,600 m asl.
Georgia: Almost all districts except for Gardabani and Kizikhi.

Caucasus: North Caucasus; South Caucasus.
General distribution: South-West Asia (East Anatolia).

3. *Cotoneaster meyeri* Pojark.
This shrub grows in rocky places, thin forests and shrubbery in the middle montane belt up to 2,200 m asl.
Georgia: 9 - Kartli; 10 – Mtiuleti; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: Almost entire Caucasus except for West South Caucasus.
General distribution: South-West Asia (North Anatolia).

4. *Quercus iberica* Stev.
This tree occurs in the lower and middle montane belts forming forests at the altitudes of 400-1,000, sometimes, up to 1,500 m asl.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia (North Anatolia to Trabzon and southern slopes of Pontian ridge).

5. *Salix elbursensis* Boiss.
This tree occurs on river and gorge banks up to 2,000 m asl.
Georgia: 1 - Abkhasia; 5 – Imereti; 9 - Kartli; 14 – Gare Kakheti; 17 – Kvemo Kartli.
Caucasus: South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia.

**11) Caucasian, South-West and West Asian:**

1. *Ribes biebersteinii* Berl. Ex DC
This shrub occurs in the middle and upper montane belts sometimes reaching the subalpine belt.
Georgia: 1 - Abkhasia; 2 – Svaneti; 3 – Racha-Lechkhumi; 4 – Samegrelo; 5 – Imereti; 7 – Ajara; 8 – Shida Kartli; 9 - Kartli; 10 – Mtiuleti; 11 – Tusheti-Pshav-Khevsureti; 12 – Kakheti; 13 – Kizikhi; 17 – Kvemo Kartli (N. Ketskhoveli; Boghaskesanis Tkhe forest); 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast, Azerbaijan, Armenia.
General distribution: North Anatolia; North-West Iran.

**12) Caucasian, South-West, West and Central Asian:**

1. *Acer hyrcanum* Fisch. & C. A. Mey.
This tree occurs on sunny and rocky slopes in the middle montane, sometimes subalpine belt up to 2,400 m asl.
Georgia: 9 - Kartli; 19 – Meskheti; is indicated for Kvemo Kartli (17); it may also be found in other forested areas of East Georgia.
Caucasus: South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia; Iran.

2. *Acer ibericum* Bieb.
This shrub grows in arid thin broadleaved forests in the lower and middle montane belts.
Georgia: 9 - Kartli; 17 - Kvemo Kartli; 19 – Meskheti;; it may also be found in other forested areas of East Georgia.
Caucasus: South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia; Iran (North).

3. *Acer laetum* C. A. Mey.
This tree is found in groupings or as solitary individuals in the broadleaved forests of the lower, middle and upper montane belts up to 1,700 m asl.
Georgia: Almost all districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia; Iran.

4. *Alnus barbata* C. A. Mey.
This tree grows in humid places in the lowlands, lower and middle montane belts up to 1,500, rarely – 1,700 m asl.
Georgia: All districts except for Javakheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan.
General distribution: South-West Asia; North Iran.

5. *Celtis caucasica* Willd.
This tree occurs in stony and rocky areas, thin forests from the lower to middle montane belt (1,800 m asl).
Georgia: 9 - Kartli; 12 – Kakheti; 13 – Kizikhi; 14 – Gare Kakheti; 16 – Trialeti; 17 – Kvemo Kartli; 19 – Meskheti.
Caucasus: South Caucasus: Armenia.
General distribution: Western Part of Central Asia (Middle Asia); South-West Asia (East Anatolia); Iran.

6. *Cydonia oblonga* Mill.
This shrub occurs in forests, forest margins and slopes in the lower and middle montane belts up to 1,400 m asl.
Georgia: 7 - Ajara; 9 - Kartli; 12 – Kakheti; 13 – Kizikhi; 17 – Kvemo Kartli.
Caucasus: North Caucasus (Daghestan); South Caucasus: Azerbaijan.
General distribution: South-West Asia; Western Part of Central Asia (Middle Asia); Iran.

7. *Malus orientalis* Uglitzk.
This tree grows in forests and forest clearings in the lower and middle montane belts; it may also be found in the upper belt.
Georgia: All districts.
Caucasus: South Caucasus.
General distribution: South-West Asia; Iran.

8. *Pyrus salicifolia* Pall.
This species is a shrub or tree, which occurs in dry areas and participates in arid thin forests and semidesert communities.
Georgia: 8 - Shida Kartli; 9 - Kartli; 13 – Kizikhi; 14 - Gare Kakheti; 17 – Kvemo Kartli; 19 – Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia (East Anatolia); North Iran.

9. *Quercus macranthera* Fisch. & C. A. Mey.
This tree occurs in the middle, upper and subalpine montane belts at the altitudes of 800 - 2,400 m asl.

Georgia: 2 - Svaneti; 3 - Racha-Lechkhumi; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 11 - Tusheti-Pshav-Khevsureti; 12 - Kakheti; 16 - Trialeti; 17 – Kvemo Kartli (R. Gagnidze); 19 – Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West and West Asia (amended distribution is provided by Menitski, 1984).

10. *Rhamnus pallasii* Fish. & C. A. Mey.
This shrub occurs on dry rocky areas and arid thin forests in the lower and middle montane belts.
Georgia: 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 16 - Trialeti; 17 – Kvemo Kartli; 19 – Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia (North-East Anatolia); Iran.

11. *Rh. spathulifolia* Fish. & C. A. Mey.
This shrub occurs in dry grasslands and slopes, rocky areas, arid thin forests and gravelly, stony, rocky slopes.
Georgia: 9 - Kartli; 13 - Kizikhi; 14 - Gare Kakheti; 16 - Trialeti; 17 – Kvemo Kartli; 19 – Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia (North-East Anatolia); Iran.

12. *Rubus anatolicus* (Focke) Fccke ex Hausskn.
This shrub grows at forest margins, on river banks and sea coast in the lower and middle montane belts.
Georgia: 1 - Abkhasia; 2 - Svaneti; 4 - Samegrelo; 5 - Imereti; 6 - Guria; 7 - Ajara; 8 - Shida Kartli; 17 – Kvemo Kartli.
Caucasus: South Caucasus: Azerbaijan, Armenia.
General distribution: Part of Europe; East Mediterranean; South-West Asia; Western Part of Central Asia (Middle Asia); Iran.

13. *Salix excelsa* S.G. Gmel.
This shrub occurs on river and stream banks in the lower forest belt.
Georgia: 5 - Imereti; 9 - Kartli; 14 - Gare Kakheti; 15 - Gardabani; 16 - Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Western Part of Central Asia (Middle Asia); Iran.

**13) Palaearctic:**

1. *Acer platanoides* L.
This tree occurs in broadleaved and dark coniferous as well as mixed deciduous forests in the lower, middle and upper montane belts.
Georgia: Almost all forested districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: North and Central Europe; Mediterranean Region; South-West Asia; North Iran; West Siberia.

2. *Padus avium* Mill.
This species is a shrub or tree found on river banks and forest margins, mostly in the upper belt.

Georgia: 1 - Abkhasia; 2 - Svaneti; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 11 - Tusheti-Pshav-Khevsureti; 12 - Kakheti; 17 – Kvemo Kartli (N. Ketskhoveli); 18 - Javakheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: North and Central Europe; Mediterranean Region; South-West Asia; Central Asia; East Europe; West Siberia; West Part of Central Asia.

3. *Populus tremula* L.
This tree occurs at forest margins along river banks, forest clearings and exposed slopes in the lower and middle montane belts.
Georgia: All districts.
Caucasus: Entire Caucasus (except for Lenkoran).
General distribution: Central and North Europe; Mediterranean Region; South-West Asia (East Anatolia); Central Asia; Far East; Siberia.

4. *Rubus candicans* Weihe.
This shrub is found at forest margins, between shrubs, mountain slopes, river and stream banks.
Georgia: 1 - Abkhasia; 7 - Ajara; 9 - Kartli; 16 - Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Part of Europe (Crimea); South, West and Central Europe; Turkey.

5. *Salix alba* L.
This tree occurs at forest margins along river banks in the lower and middle montane belts at the altitudes of 1,500-1,600 m asl.
Georgia: 1 - Abkhasia; 4 - Samegrelo; 7 - Ajara; 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 15 - Gardabani; 16 - Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus.
General distribution: Entire Europe; Mediterranean Region; South-West, Central and East Asia; Iran; West Siberia; Himalaya.

6. *Salix triandra* L.
This species is a shrub or tree, which occurs on river, stream banks and lake shores at the altitudes of 1,300-1,800 m asl.
Georgia: 1 - Abkhasia; 4 - Samegrelo; 5 - Imereti; 8 - Shida Kartli; 9 - Kartli; 12 - Kakheti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: North and Central Europe; Mediterranean Region; South-West Asia; Iran; Central and East Asia; Siberia; Western Part of Central Asia (Middle Asia); Far East.

7. *Viscum album* L.
This species is a parasite shrub of deciduous woody plants and fruit-trees in the lower and middle montane belts.
Georgia: Almost all districts.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: North and Central Europe; Mediterranean Region; South-West Asia; Central Asia; East Asia.

**14) West Palaearctic:**
1. *Acer campestre* L.
This tree grows in broadleaved floodplain forests in the lower and middle montane belts.

Georgia: Almost all forested districts.
Caucasus: North Caucasus; South Caucasus.
General distribution: North, Central and East Europe; Mediterranean Region; South-West Asia (East Anatolia); Iran.

2. *Corylus avellana* L.
This shrub occurs in the lower, middle and upper montane belts.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: North and Central Europe; Mediterranean Region; South-West Asia.

3. *Euonymus verrucosa* Scop.
This shrub grows in river gorges and ravines in the broadleaved and dark coniferous forest belt.
Georgia: 1 - Abkhasia (rare); 9 - Kartli; 12 - Kakheti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: North and Central Europe; Mediterranean Region; South-West Asia.

4. *Helianthemum grandiflorum* (Scop.) DC.
This shrub occurs in broadleaved and arid forests, brichwood, stony meadows and steppefied areas in the alpine and subalpine montane belts.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Europe; Mediterranean Region; South-West Asia.

5. *Mespilus germanica* L.
This shrub mainly occurs in the understorey in the lower and middle montane belts up to 1,200 m asl; its main associate is Cornelian cherry.
Georgia: All districts.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Central and West Europe; South-West Asia; North Iran; Western Part of Central Asia; Crimea.

6. *Morus nigra* L.
This tree has become wild and is found in the floodplain forests in the lower montane belt.
Georgia: 9 - Kartli; 17 – Kvemo Kartli.
Caucasus: South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Mediterranean Region; South-West Asia; Western Parrt of Central Asia; Iran; Part of Europe.
7. *Sorbus torminalis* (L.) Crantz.
This shrub occurs in the lower, middle and upper montane belts up to 1,700 m asl.
Georgia: 1 - Abkhasia; 2 - Svaneti; 3 - Racha-Lechkhumi; 5 - Imereti; 6 - Guria; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 11 - Tusheti-Pshav-Khevsureti; 12 - Kakheti; 16 - Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Central and North Europe; Crimea; East Mediterranean; South-West Asia.

8. *Ulmus glabra* Huds.
This shrub is found in deciduous forests, moist areas, along floodplains and ravines in the middle montane belt up to 1,500 m asl.

Georgia: 1 - Abkhasia; 3 - Racha-Lechkhumi; 4 - Samegrelo; 6 - Guria; 7 - Ajara; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 11 - Tusheti-Pshav-Khevsureti; 12 - Kakheti; 16 - Trialeti; 17 – Kvemo Kartli.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Central and North Europe; Mediterranean Region; South-West Asia.

9. *Viburnum lantana* L.
This shrub is found on rocky and stony areas from the middle through alpine montane belts.
Georgia: 1 - Abkhasia; 2 - Svaneti; 3 - Racha-Lechkhumi; 4 - Samegrelo; 7 - Ajara; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 11 - Tusheti-Pshav-Khevsureti; 14 - Gare Kakheti; 16 - Trialeti; 17 – Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Central Europe; Mediterranean Region; South-West Asia (East Anatolia).

**15) South Palaearctic:**

1. *Cotinus coggygria* Scop.
This shrub grows in dry areas, shrubbery and forest margins in the lower montane belt.
Georgia: Almost all districts.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Central Europe; Crimea; Mediterranean Region; South-West Asia (East Anatolia); Western Part of Central (Middle Asia) and East Asia.

2. *Elaeagnus angustifolia* L.
This shrub or tree is found on river banks, floodplains, ravines and streams in the lower and middle montane belts; it is used in gardens and parks.
Georgia: 1 - Abkhasia; 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 15 - Gardabani; 19 - Meskheti; it may also be found in other districts of East Georgia.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Central Europe; Mediterranean Region; South-West Asia; Western Part of Central Asia (Middle Asia); West Siberia; Iran; East Asia.

3. *Hedera helix* L.
This liana is found on trees, rocks and old buildings in the lower and middle montane belts.
Georgia: All forested districts.
Caucasus: North Caucasus; South Caucasus.
General distribution: Central Europe; Part of East Europe; Mediterranean Region; South-West Asia; Iran.

4. *Populus nigra* L.
This tree occurs at forest margins along river banks in the lower and middle montane belts at the altitudes of 1,300-1,400 m asl.
Georgia: 1 - Abkhasia; 5 - Imereti; 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 15 - Gardabani; 16 - Trialeti; 17 - Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus.
General distribution: Central Europe; Mediterranean Region; South-West, Central and Western Parrt of Central Asia; Siberia.

5. *Rhamnus cathartica* L.
This shrub occurs in dry areas in the understorey and between shrubbery in the deciduous forest belt.
Georgia: 5 - Imereti; 6 - Guria; 7 - Ajara; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 13 - Kizikhi; 14 - Gare Kakheti; 16 - Trialeti; 17 - Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Western Part of Central Asia (Middle Asia); Siberia; North and Central Europe; Mediterranean Region; South-West Asia (North-East Anatolia); Central Asia.

6. *Rhododendron luteum* Sweet.
This shrub occurs in forest margins and slopes, forest clearings starting from the sea level up to the subalpine belt where it forms thickets in gorges.
Georgia: Throughout the country, especially in West Georgia.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Central Europe in the sources of r. Dnieper and middle reaches; South-West Asia; Balkans.

7. *Rubus idaeus* L.
This shrub occurs in forest and river margins, floodplain forests and shrubbery in the lower and middle montane belts.
Georgia: 1 - Abkhasia; 2 - Svaneti; 4 - Samegrelo; 5 - Imereti; 6 - Guria; 7 - Ajara; 8 - Shida Kartli; 9 - Kartli; 10 - Mtiuleti; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 16 - Trialeti; 17 - Kvemo Kartli; 18 - Javakheti; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: Part of Europe; West and East Siberia; Western Part of Central Asia (Middle Asia).

8. *Salix caprea* L.
This shrub or tree occurs in forest margins, along streams in the lower, middle, upper and subalpine belts up to 2,300 m asl.
Georgia: Widely distributed everywhere.
Caucasus: North Caucasus; South Caucasus.
General distribution: Europe; Central Asia; Iran; West Siberia; Korea; Japan; Western Part of Central Asia (Middle Asia).

9. *Spiraea hypericifolia* L.
This shrub occurs in shrubbery, forests and grasslands in the lower, middle and upper montane belts.
Georgia: 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 16 - Trialeti; 17 - Kvemo Kartli; 18 - Javakheti; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: South-West Asia (East Anatolia); Part of Europe.

10. *Vitis sylvestris* C.C.Gmel.
This climber is found in river gorges, flat areas and forests at the altitudes of 1,500-1,800 m asl.
Georgia: 1 - Abkhasia; 3 - Racha-Lechkhumi; 4 - Samegrelo; 5 - Imereti; 6 - Guria; 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 17 - Kvemo Kartli; 18 - Javakheti; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Mediterranean Region; South-West (East Anatolia) and Central Asia; Iran.

**16) Holarctic - Ancient mediterranean:**

1. *Prunus spinosa* L.
This shrub occurs in the lower and middle montane belt forests and forest margins, along gorges up to 1,200 m asl.
Georgia: 7 - Ajara; 8 - Shida Kartli; 9 - Kartli; 12 - Kakheti; 16 - Trialeti; 17 - Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Azerbaijan, Armenia.
General distribution: North and Central Europe; South-West Asia (East Anatolia); Mediterranean Region; Iran; North America (Introduced); West Siberia.

2. *Rosa canina* L.
This shrub occurs in forest margins, clearings, slopes from the lowlands to the upper montane belt.
Georgia: All districts.
Caucasus: Entire Caucasus.
General distribution: Europe; North America; South-West Asia; Syria; Iran.

3. *Vitis vinifera* L.
This climber shrub is a cultivar, which has frequently grown wild in forests (extends to South America via irradiation).
Georgia: 1 - Abkhasia; 3 - Racha-Lechkhumi; 4 - Samegrelo; 5 - Imereti; 6 - Guria; 7 - Ajara; 8 - Shida Kartli; 9 - Kartli; 12 - Kakheti; 13 - Kizikhi; 14 - Gare Kakheti; 16 - Trialeti; 17 - Kvemo Kartli; 19 - Meskheti.
Caucasus: North Caucasus; South Caucasus: Black Sea Coast; Azerbaijan, Armenia.
General distribution: Europe; Mediterranean Region; South-West (East Anatolia), Central and East Asia; Iran; North and South America.

***Geographical Structure of Kvemo Kartli Endemic Woody Plants***
In total, there are 6 Caucasian endemics and 1 endemic of Georgia among 87 woody plants occurring in Kvemo Kartli; these are as follows:

| | |
|---|---|
| ◎ 1. *Berberis iberica* | (Berberidaceae) |
| ◎ 2. *Corylus iberica* | (Corylaceae) |
| ◎ 3. *Genista patula* | (Leguminosae) |
| ◎ 4. *Genista transcaucasica* | (Leguminosae) |
| O 5. *Amygdalus georgica* | (Rosaceae) |
| ◎ 6. *Rosa kozlowskii* | (Rosaceae) |
| ◎ 7. *Sorbus caucasigena* | (Rosaceae) |

Kvemo Kartli endemic plants can be divided into four groups by distribution:
**Endemics of East Georgia:**
O ***Amygdalus georgica*** (Rosaceae) – is found in grasslands.

**Endemics of East South Caucasus:**
◎ ***Genista transcaucasica*** (Leguminosae) – is a forest element though it may also be found on dry slopes;
◎ ***Rosa kozlowskii*** (Rosaceae ) – grows in open areas, shrub thickets and forest margins in the montane belt.

**Endemics of East Caucasus:**

◎ ***Berberis iberica*** (Berberidaceae) – is a representative of steppe shrubbery, which grows on river terraces.

**Common Endemics of Caucasus:**

◎ ***Sorbus caucasigena*** (Rosaceae) – is characteristic for the subalpine forests, 1,600-2,400 m asl;

◎ ***Genista patula*** (Leguminosae) – grows in semi-deserts from the lowlands to the middle montane belt;

◎ ***Corylus iberica*** (Corylaceae) – occurs in forests from the middle to upper montane belts.

Thus, 7 endemic woody plants occur in Kvemo Kartli; among them, six are endemics of Caucasus and one is an endemic of Georgia.

***4. Changes in Landscape in Recent 50 Years***

Dmanisi hominid already inhabited Kvemo Kartli as long as 1.8 m years ago. Presence of man in this region since ancient times has affected development of our culture and civilization, on one hand and left indelible mark on the landscapes of Kvemo Kartli and, generally, East Georgia. Since then anthropogenic impact has been one of important factors altering natural conditions; however, this factor has assumed particularly negative role over the last century.

Anthropogenic impact (timber logging, intensive grazing) has resulted in development of xerophyte vegetation in many areas instead of oakwood cleared over the recent decades.

High mountain oak is a xerophyte and substitutes mesophilous mountain plants on slopes of southern exposure. High mountain oak – tree adapted to drier conditions colonizes areas where mesophilious beech or plants with similar ecology have been eradicated [Ketskhoveli, 1960].

Anthropogenic degradation of all biomes will be expressed more drastically under global warming. Many plants sensitive to high temperatures will change habitats and will be substituted by other species adapted to drier conditions. Due to climate change, semi-desert biome, which is under severe anthropogenic stress, may be gradually replaced by desert [Nakhutsrishvili, 2000].

***5. On Establishment of Protected Areas in Kvemo Kartli***

At present there are 27 protected areas (state reserves, national parks, managed reserves) in Georgia. None are located in Kvemo Kartli though there is sufficient basis for establishment of a protected area as 7 endemic plant species including 2 species included in the Georgian Red List occur in Kvemo Kartli. Establishment of a protected area (it can be allocated in Dmanisi vicinities, in r. Poladauri gorge at Bolnisi) will facilitate conservation of forests (which still survive), which are enclosed by steppes from the east and west. Coenoses of woody plants occurring in Kvemo Kartli form a so called 'link' connecting forests in Armenia with those in Georgia.

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# ქვემო ქართლის დენდროფლორის მრავალფეროვნების შესწავლისათვის

თედორაძე გ.

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(მიღებულია 10.03.2010)

## რეზიუმე

განხილულია ქვემო ქართლის ფლორისტული რაიონის მერქნიანი მცენარეების მრავალფეროვნება. გამოვლენილია 86 სახეობა. მოცემულია მათი ოჯახების, გვარებისა და სახეობების სისტემატიკური სტრუქტურა და გეოგრაფია. აღნიშნულია მერქნიანი მცენარეების ის 5 სახეობა, რომელიც "საქართველოს ფლორის" მიხედვით არ იყო ქვემო ქართლში მითითებული.

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Ecology

# SEASONAL DYNAMICS OF THE PHYTO- AND ZOOPLANKTON IN THE GEORGIAN COASTAL ZONE OF THE BLACK SEA

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**Abstract**

Monthly monitoring of four distinct sites in the Black Sea coastal zone of Georgia was carried out from June 2006 through October 2008. Abundance and species composition of phyto- and zooplankton were studied in relation to seasonal changes. In total, 178 phytoplankton species of six taxonomic groups were inventoried. Bacillariophyta (40%) and Dinophyta (32%) were most prevalent, while Cyanophyta comprised 13%, and Chromophyta and Xanthophyta made up 3% each of the total number of phytoplankton species. Over twenty-four months, the mature forms and larvae of thirty-one species of zooplankton representing eight classes of five phyla were observed. Among them Crustacea (sixteen species) and Rotatoria (nine species) were the most prevalent. High diversity was shown in the Cladocera and Copepoda groups, which made up 26% and 21%, respectively, of the total number of zooplankton species. The larvae of the benthic organism *Rapana tomasiana,* along with Rotatoria representative *Asplanchna priodonta* made up 32% and 20%, respectively, of total zooplankton numbers in samples collected in the autumn. *Centropages kroyeri* was the most frequently occurring zooplanktonic organism and was registered in 41% of the examined samples from every season.

A quantitative assessment revealed seasonal variations in the abundance and diversity of phyto- and zooplankton populations over the observation period. Regular increases in phytoplankton numbers were mainly observed in autumn (September-October) and spring (March-April), although abundance peaks varied slightly amongst sampling sites. In most cases, the zooplankton abundance peaks coincided with or followed the phytoplankton peaks, which reflects zooplankton's role as a consumer of phytoplankton. The ecological status of the coastal waters was determined based on the appearance and abundance of phyto- and zooplankton pollution indicator species. Local eutrophication was observed in the summer season at a majority of sampling sites, especially in the Supsa estuary from 2007 to 2008 where high numbers of Cyanophyta were observed. In the same period, an abundance of Cyanophyta and Xanthophyta was registered at the Batumi

* Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army or the US Department of Defense.

Boulevard and Green Cape sampling sites. The observed patterns in phytoplankton and zooplankton abundance and diversity in the Georgian coastal zone of the Black Sea reflect permanent environmental changes and continuing anthropogenic activity in the region.

## Introduction

The Black Sea is almost completely land-locked, only connected to the Mediterranean Sea through the narrow Bosporus Strait, and is the largest water body containing hydrogen sulfide. This unique ecosystem is burdened by excessive loads of nutrients and hazardous substances from surrounding countries and inflowing rivers, particularly near the northwestern shelf of the Black Sea, and the Danube, Dnestr, and Dnepr rivers [Black Sea Biological Diversity. Romania, 1997; International commission for the Protection of the Danube River, 2010]. However, the eastern Black Sea, including the Georgian coast, has faced similar but less drastic pollution problems including increased nutrient loads, influx of organic pesticide and heavy metals, oil spills, and invasion of exotic species.

Although there have been some improvements in the overall ecological status of the Black Sea over the last fifteen years, mainly due to a decrease in polluting activities and some progress in regional environmental management [Commission on the Protection of the Black Sea Against Pollution, 2009], environmental pollution remains one of the leading problems in the Georgian coastal zone of the Black Sea. This is in relation with regional activities such as extensive oil import/export infrastructure and tanker movement, construction, and traditional agricultural practices. These factors together with intensive tourism can have a significant and long-term negative impact on the ecological status of the area.

Large-scale transformations in aquatic ecosystems are believed to be accompanied by changes in all biotic components and reflected in the density of phytoplankton and zooplankton species and their subsequent biomass. Phytoplankton and zooplankton are the most sensitive components of marine ecosystems. Microalgae, in the form of phytoplankton, are the basis of the majority of trophic chains for plankton biocenosis, and have a major effect on the ecological status of a marine environment. Zooplankton, as an active consumer of phytoplankton, produces its own impact on local ecosystems. Thus by affecting plankton populations, anthropogenic pressure can have a significant negative influence on the ecological system of water reservoirs. For example, nitrates and phosphates have been observed to support the propagation of Centrophycae, Dinophyta, and Cyanophyta (e.g. *Oscillatoria* species), along with a decrease in the numbers of other phytoplankton species [Novikov & Naumovi, 1989; Tomas C.R.(Ed.), 1997]; such changes are often reflected in the deterioration of the ecological situation of a given body of water. Similarly, the propagation ratio of certain indicator species to one another (that is, those species indicative of water quality improvement to those indicative of an increase in pollution) is often a strong indicator of the water quality status of a particular site.

Since phyto- and zooplankton are biological indicators of the water quality, the study of their taxonomic diversity, numbers, biomass, and physiological parameters can provide background information on the ecological status of a given body of water and its particular region. A number of studies conducted over the last two decades in the eastern and northern Black Sea area have explored the relationship between qualitative and quantitative parameters of phyto- and zooplankton and the ecological status of the Georgian coast of the Black Sea, reflecting negative influence of anthropogenic activity [Gvarishvili, 1998a, b, c; 2007].

To study ongoing changes in the abundance and composition of phyto- and zooplankton populations, monthly monitoring was carried out from June 2006 through October 2008 at four different sites along the Georgian coastal zone of the Black Sea: the estuaries of the Supsa and Chorokhi rivers, and along the shores near Batumi Boulevard and the Green Cape. Through the

collection and analysis of samples from different sites along the Georgian coast, the presented study enriches and updates data from previous surveys.

## Materials and Methods

Samples of phytoplankton and zooplankton were collected monthly, or bi-weekly in the summer, from four sites on the Black Sea coast: Supsa estuary (N 42° 00.008', E 41° 41.401'); Green Cape (N 41° 41.791', E 41° 42.301'); Batumi Boulevard (N 41° 39.570', E 41° 38.006'); and Chorokhi estuary (N 41° 36.116, E 41° 34.021'). Samples were taken 50-60 meters from the shoreline at the Supsa and Green Cape sites and 100-150 meters at the Batumi Boulevard and Chorokhi sites. Samples were taken 20-50 cm below the sea surface.

Measurements of physical and chemical parameters such as water temperature, salinity, pH, conductivity, total dissolved solids (TDS), and dissolved oxygen (DO) were taken on-site using a portable multi-log meter (YSI 556 MPS, Yellow Springs Instruments, Co USA).

The plankton fractions were collected by filtering 100 liters of sea water through 64 μm and 200 μm-size plankton nets. The collected fractions were fixed with 3.7 % formalin (final concentration) and were stored at 4°C for three months. Planktonic cells in samples were counted manually in a Nageotte chamber (Azer Scientific, PA, USA) using a high-resolution light microscope (Leica DMLS or stereomicroscope M55, Germany; Kruss Biological inverse microscope MBL 3100, Germany) at 400x magnification.

Phytoplankton numbers were calculated as $n \times 10^4$ cells/per liter, and the quantity of zooplankton was given as individuals per cubic meter (ind/$m^3$). The taxonomic identification of the plankton was based on widely-accepted characteristics [Abrikosov et al., 1966; APHA, 1998; Dedusenko-Shegoleva & Gollerbakh, 1962; Gvarishvili, 1998a, b, c, 2007; Identification guide of Azov and Black Sea Fauna, 1968; 1969; 1972; Kiselev, 1950; Konivalova, 1998; Kosinskaya, 1948; Mitchell R. (ed), 1972; Proshkina-Lavrenko, 1955; Senichkina & Svirina, 1981; Tsiban, (ed), 1980].

## Results and Discussion

Water samples collected from June 2006 to October 2008 were investigated for seasonal fluctuations in the species abundance and composition of both marine phytoplankton and zooplankton with a special focus on detection and enumeration of pollution-indicator species, such as *Microcystis aeruginosa, Merismopedia punctata, Tribonema minus, Spirulina okensis,* and other organisms from the Cyanophyta and Xanthophyta phyla. In addition, data obtained during the 1990s and early 2000s was analyzed to determine if there were notable changes in the plankton abundance and species composition over the last decade.

In total, one hundred seventy-eight species and sub-species of phytoplankton were found in the aquatoria between Batumi and Poti. These species were attributed to six phyla (Fig. 1), among which seventy-two species belonged to Bacillariophyta, fifty-six to Dinophyta, twenty-three to Chlorophyta, sixteen to Cyanophyta, six to Chromophyta, and five to Xanthophyta.

From 2006 to 2008, zooplankton along the Georgian coast was represented by thirty-one species belonging to five phyla and eight classes dominated by Crustacea (sixteen species) and Rotatoria (nine species), with single species representing the other six classes. Among Rotatoria and Cladocera, nine species were identified per group, while seven species were found in the group Copepoda. Only one representative of the phylum Chaetognatha was registered (Fig. 2). The remaining groups consisted of the larvae of benthic organisms, including Bivalvia and Gastropoda, Polychaeta, Diptera, Cirripedia, and Ostracoda.

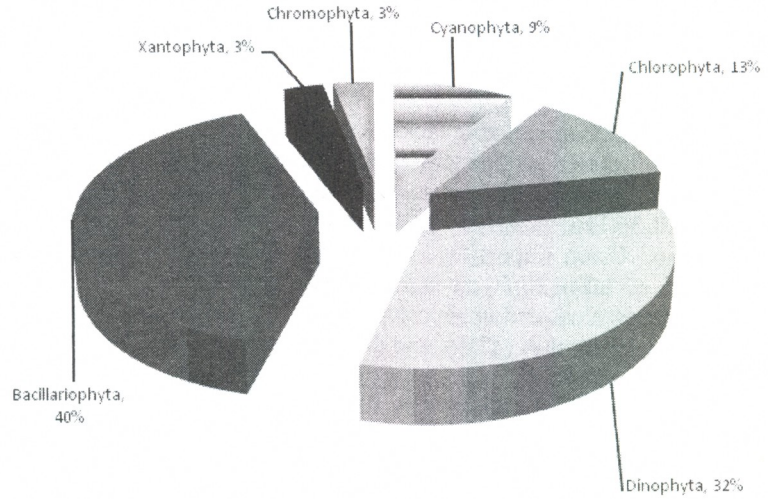


**Fig. 1.** Percent distribution of phytoplankton taxonomical groups in the Georgian coastal zone of the Black Sea from 2006 to 2008

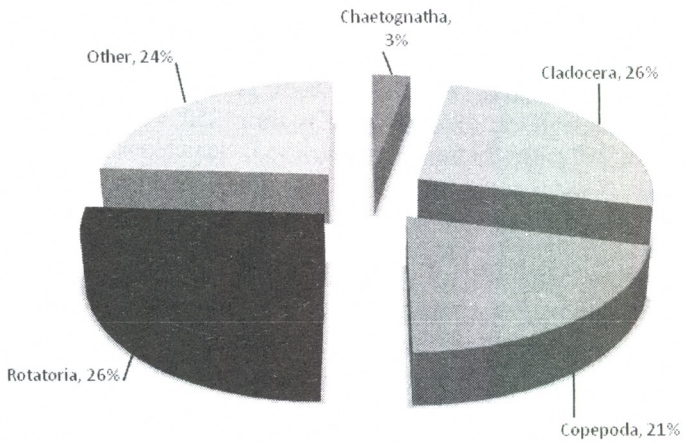


**Fig. 2.** Percent distribution of zooplankton species by taxonomical groups in the Georgia coastal zone of the Black Sea from 2006 to 2008

According to quantitative assessments conducted during this study, phytoplankton and zooplankton abundance fluctuate by season (Fig. 3, 4). Specifically, there was a consistent increase in phytoplankton numbers in the autumn (September-October) and spring (March-April) months, as well as in June and July of 2007 and 2008, with some differences observed in abundance peaks at different sites. For example, in September 2006, phytoplankton abundance peaks were observed simultaneously at the Batumi Boulevard, Chorokhi estuary, and Green Cape sites, while in spring 2007 the highest numbers were registered in the Batumi Boulevard area, and at the Chorokhi estuary in autumn 2007. The Supsa estuary expressed different abundance peaks, especially in October 2008, when total phytoplankton decreased at all sites but Supsa.

Similar profiles were observed for zooplankton quantitative data, with increases and decreases in zooplankton numbers in the same periods. In general, from 2006 to 2008, the

dynamics of zooplankton numbers varied significantly. In most cases, zooplankton abundance peaks coincided with or were followed by phytoplankton peaks, which is a reflection of the typical food chain as zooplankton feeds on phytoplankton.

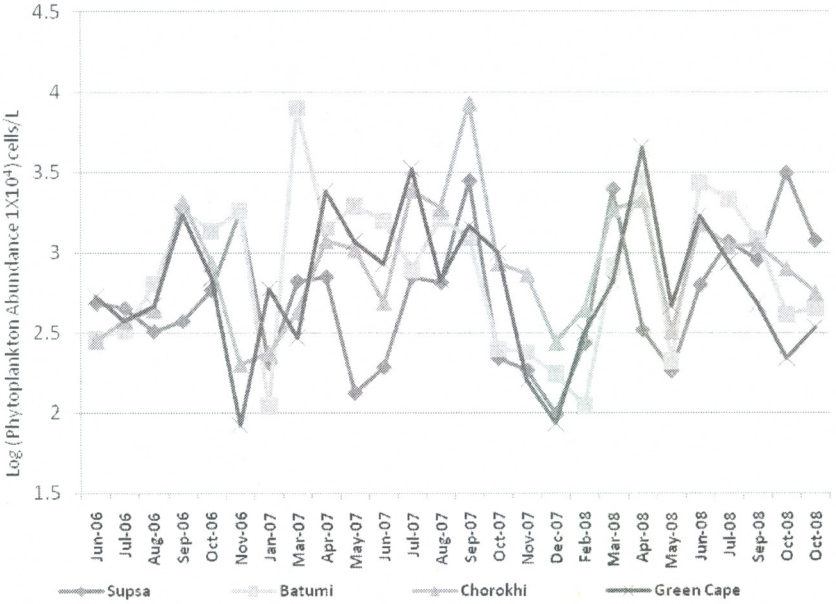


**Fig. 3.** Dynamics of phytoplankton abundance at four sampling sites in the Georgian Black Sea coastal zone from 2006 to 2008

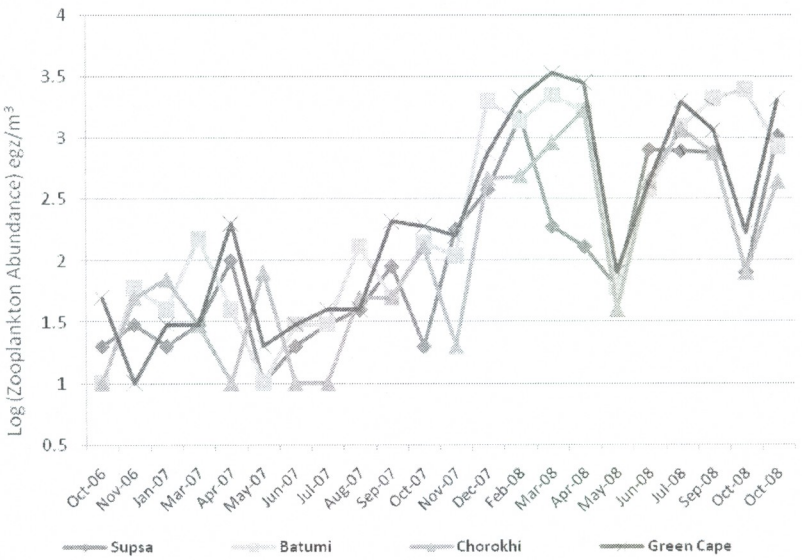


**Fig. 4.** Dynamics of zooplankton abundance at four sampling sites in the Georgian Black Sea coastal zone from 2006 to 2008

In 2007 and 2008, zooplankton numbers increased three times in comparison with 2006, due to an extension of the range of examined plankton fractions. Namely, 64 μm plankton fractions

were included in the counts starting in 2007, resulting in an increase in zooplankton numbers, mainly Rotatoria representatives.

The results obtained over thirty months of surveillance in the Georgian Coastal zone of the Black Sea are presented by season below, with an emphasis on the most abundant species of phyto- and zooplankton, and those indicative of water pollution.

SUMMER

Excess numbers of Cyanophyta and Dinophyta along the Georgian coast, especially in the summer months, may be indicative of increased water pollution, which may be due to an influx of biogenic elements often linked to seasonal burdens such as the tourist and holiday seasons, as well as malfunctioning of the sewage and drainage systems due to rainy seasons and increased usage. The propagation of Dinophyta, Cyanophyta and Xanthophyta at certain sites is indicative of this short term, local eutrophication.

Phytoplankton

Over the course of the study, the data obtained in the Chorokhi and Supsa estuaries in the summer months showed an increase in the abundance and diversity of diatoms in comparison with the data collected in the 1990s. In particular, in the 1990s, only sixty-two species of Bacillariophyta were reported in the Georgian coastal zone [Gvarishvili, 1998b] versus seventy-two species registered in our study.

According to monthly data, Bacillariophyta were detected in the greatest abundance in the Chorokhi estuary and the Batumi Boulevard areas (1.89 x $10^7$cells/L in July 2007, and 2.5 x $10^7$ cells/L in June-July 2008, respectively), while the lowest numbers in the region were detected to the north at the Green Cape and the Supsa estuary (1.5x$10^6$ cells/L in June 2006 and 1.7x$10^6$ cells/L in June 2007, respectively). Among the Bacillariophyta species detected, *Pseudosolenia calcar-avis* and *Proboscia alata* were dominant in the 1990s as well as the current study [Gvarishvili, 1998b], although in recent examinations these were accompanied by *Chaetoceros lorensianus, Chaetoceros insignis, Chaetoceros curvisetus,* and *Thalassionema nitzschioides,* as well as *Cylindrotheca closterium* and *Amphiprora paluosa.*(Fig.5 a,b)

According to previous data [Gvarishvili, 1998a,c; Tomas C.R.(Ed.), 1997] the prevalence of Dinophyta in the phytoplankton community in the summer months was characteristic for the Georgian coastal zone of the Black Sea. Our studies showed that Dinophyta numbers, in the summer from 2006 to 2008, were lower than those of Bacillariophyta. In comparison with levels from the 1990s [Gvarishvili, 1998b; 2007] current Dinophyta populations did not show significant differences in diversity by sampling site and were actually less abundant than in past decades. The greatest numbers of Dinophyta were observed at the Green Cape in June 2006 (2.28x$10^6$cells/L), which slightly exceeded the numbers at the Chorokhi and Batumi Boulevard sites in the same period (1.56x$10^6$cells/L and 1.82x$10^6$cells/L, respectively).

An abundance of *Cyanophyta* was observed at the Green Cape site during the summer of 2007, with a peak in July (1.47x$10^7$cells/L). Periodic increases of these phytoplankton groups in our studies were also registered in the Supsa estuary, reaching a maximum of 6.96 x $10^6$ cells/L in July 2008, and near the shore of Batumi Boulevard with a peak of 2.92 x $10^6$cells/L in August 2006. This increase of Cyanophyta counts can be explained by an elevated influx of biogenic elements from the surrounding areas after summer rainfalls. *Microcystis aeruginosa, Merismopedia punctata, Anabaena flos-aquae,* and *Oscillatoria planktonica* were dominant among the Cyanophyta species registered.

In August of 2008, increased numbers of Xanthophyta (5.54 x $10^7$cells/L) were observed in the Supsa estuary, with *Tribonema minus, Tribonema vulgare*, and *Spirulina okensis* registering as the predominant species. Despite this proliferation, which may be an indication of the elevated pollution at this site, Xanthophyta numbers did not exceed those of Bacillariophyta, which remained a leading taxonomical group by diversity at all sites.

Zooplankton

From 2006 to 2008, the total number of zooplanktonic organisms increased significantly: in 2006 the average abundance of zooplankton was 25 ind/$m^3$, increasing almost three-fold in 2007 (70 ind/ $m^3$), and finally reaching 1085 ind/$m^3$ in 2008. In addition to environmental factors that may have influenced the zooplankton fluctuation, this increase can be partially attributed to a change in the sampling protocol which expanded the screening fraction from organisms larger than 200 μm to those larger than 64 μm. This resulted in comparatively low zooplankton quantities and diversity recorded in the autumn of 2006 and spring of 2007 (before the screening fraction was increased) followed by an increase in zooplankton numbers in 2007 and 2008, mainly due to an increase in numbers of Rotatoria (sizes between 100 and 200 μm).

The greatest abundance of zooplankton in the summer season was observed at the Green Cape site in 2007, with dominance *of Bosmina* species among Cladocera, specifically *Bosmina longicornis* and *Bosmina longinostris* (Fig. 5d). Another Cladocera species, *Pleopis polyphemoides,* was detected at the Green Cape site and in the Supsa estuary throughout the year. This organism reproduces on a very large scale in the summer months. Interestingly, *Podura aquatica*, a river insect, was detected in samples from the Supsa estuary early in the summer of 2007. Although this is the first time this organism has been detected along the Georgian coast, due to a lack of data about the ecology and physiology of this organism, its significance in the ecosystem cannot be defined at this stage.

In the samples from all sites, especially in the summer, large numbers of nauplial copepods were detected, with the nauplii of *Centropages kroyeri* var. *pontica* observed in especially high numbers. The presence of these organisms throughout the year seems to be characteristic for this region of the Black Sea coast. Similarly, larvae of *Balanus improvisus* (Fig. 5c) occurred in plankton during much of the year, except for the few winter months.

At the Supsa estuary, the average zooplankton abundance in 2008 was 785 ind/L, compared to 1188-1260 ind/L as observed at other sites. This scarcity and lack of biodiversity amongst zooplankton observed in the summer months is particularly significant because, when coupled with the increase in Cyanophyta, is an indicator of elevated water pollution in this area.

AUTUMN

Phytoplankton

Bacillariophyta and Cyanophyta were detected in relatively high quantities at some sites in the autumn months. Examination of phytoplankton samples collected in the autumn from 2006 to 2008 revealed a periodic excess of Cyanophyta in the water of Batumi Boulevard and the Supsa estuary which may indicate elevated pollution at these sites. At the Supsa site, Cyanophyta were more prevalent than any other any group of phytoplankton, and although toxic species such as *Microcystis aeruginosa*, *Merismopedia punctata,* and *Oscillatoria limnetica* were observed, their quantities weren't high enough to pose a risk of water toxicity. This may be due to the seasonal water temperature, as this factor is believed to be an important one in controlling bloom development and the seasonal succession of *Microcystis* species [Tsiban (ed), 1980].

In general, phytoplankton populations of the Georgian coastal waters in the fall were characterized by a diversity of Bacillariophyta: *Chaetoceros* species were prevalent, as is typical for the autumn season [Gvarishvili, 1998a, c] and several other species were detected in considerable numbers. This included *Sceletonema costatum* (2.04 x $10^6$cells/L, Batumi Boulevard, October 2008), *Melosira moniliformi* (1.24 x $10^6$cells/L, Chorokhi, September 2006), *Dactyliosolen fragilissima* (8.8 x $10^5$cells/L, Green Cape, September, 2006), *Hemiaulus hauckii* (1.88 x $10^6$cells/L, Chorokhi, September 2006), *Thalassionema nitzschioides* (2.16 x $10^6$cells/L Batumi Boulevard, September 2007), and *Pseudo-nitzschia delicatissima* (2.64 x $10^4$cells/L, Chorokhi, September 2006).

Zooplankton

Samples of zooplankton collected in autumn months from 2006 to 2008 were represented mainly by the crustaceans Copepoda and Cladocera, along with Ostracoda and Rotatoria, and the benthic organisms *Diptera* and *Polychaeta.* The nauplii of Bivalvia and Gastropoda, along with *Sagitta setosa,* were also detected. The nauplii "bloom" typically began in the summer months and peaked in the autumn: the average monthly numbers were 584 ind/$m^3$ in the summer and 756 ind/$m^3$ in autumn. Among the zooplankton, the most common was *Oithona similis,* a representative of aquatic organisms that inhabit the surface layers and are characterized by tolerance to salinity gradients and vertical migration. In spite of this, no zooplankton or phytoplankton group was dominant in the autumn months.

WINTER

Phytoplankton

In the winter of 2007 and 2008, all phytoplankton species were low in abundance and diversity. Bacillariophyta and Dinophyta numbers were more or less equal, which differs from the summer, when Bacillariophyta tends to be the more prevalent of the two. Relatively high numbers of Cyanophyta were detected at the Green Cape and in the Chorokhi estuary (5.10 x $10^6$ cells/L and 1.72 x $10^6$ cells/L, respectively), among which *Microcystis aeruginosa, Merismopedia punctata, Lyngbia aestuaria,* and *L. lulae* (a freshwater form) were the most dominant.

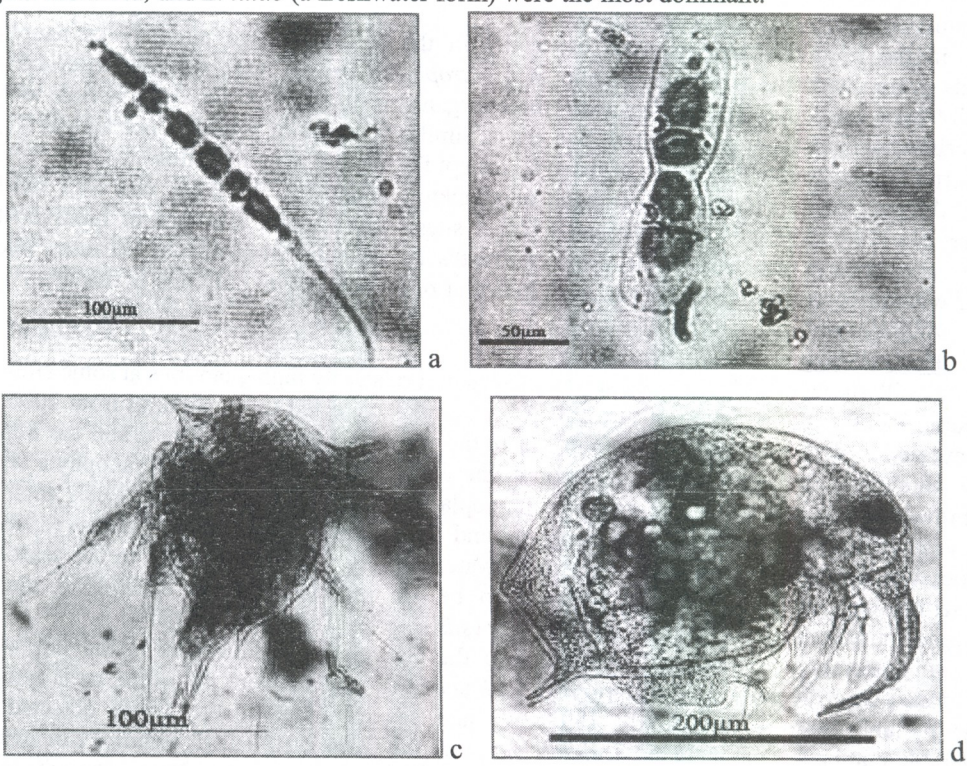


**Fig. 5.** Some of the Black Sea phytoplankton and zooplankton species registered during monitoring from 2006 to 2008 (Leica DMLS, Magnification x400). a) *Cylindrotheca closterium* b) *Amphiprora paluosa* c) *Balanus improvisus (larva)* d) *Bosmina longirostris*

In the winter of 2008, increased numbers of Xanthophyta were detected (1.98 x $10^4$ cells/L) at the mouth of the Chorokhi river, including the toxic species *Tribonema minus* and *T.*

*vulgare.*, although their presence was not interpreted as a threat because toxin production is believed to be insignificant at low water temperatures [APHA,1998; Hiroyuki et al., 2009].

Zooplankton

Zooplankton collected in winter samples mainly consisted of Rotatoria, Copepoda, Cladocera, and the larvae of an exotic hydrobiont from Japan, *Rapana thomaziana,* that was introduced into the Black Sea in the 1940s [Khurashvili, 1996]. A characteristically high abundance of zooplankton was observed in the Green Cape area, where their numbers reached 1.44 x $10^3$ ind/$m^3$, among which *Asplanchna priodonta* was most prevalent (4.70 x $10^2$ ind/$m^3$). At this site, the number of *Rapana thomaziana* larvae reached 4.65 x $10^2$ ind/$m^3$.

Winter samples from the Supsa estuary appeared relatively poor for zooplankton, but the chitin of *Centropages kroyeri* was detected in large numbers, indicating that these organisms were present in the water shortly before sampling and were consumed in large quantities by fish and third-level predators, such as marine mammals.

SPRING

The spring samples collected 2007 and 2008 were characterized by diversity amongst phyto- and zooplankton, as well as by the high numbers of organisms compared to other seasons.

Phytoplankton

An excess of diatoms was registered at a majority of the sites, with the most observed in the Batumi Boulevard area at a count of 1.87 x $10^6$ cells/L in May 2007, along with an abundance of Xanthophyta (7.17 x $10^6$ cells/L) in the same location in March 2007. On the other hand, in the Supsa estuary, numbers of both Bacillariophyta and Xanthophyta were relatively low.

Among the diatoms, Sceletonem costatum, Melosir moniliformis, Pseudosolenia calcar avis, Proboscia alata, Pseudo-nitzschia delicatissima and Leptocylindrus minimus were dominant, along with several representatives of Chaetoceros (at Chorokhi, Batumi Boulevard, and Green Cape) and Xanthophyta (Batumi Boulevard and Green Cape).

At the Supsa site, Cyanophyta peaked with a count of 1.27x$10^6$ cells/L in March 2008. The excess of Cyanophyta in the Supsa estuary in any season, particularly after rainfall, can be explained by the negative impact of the Supsa River, which is rich in biogenic elements and organic substances, and supports the propagation of competitive algae. The presence of the Xanthophyta group (namely, *Tribonema minus, T. crassum,* and *Heterothrix elegans*) in excess numbers in the spring samples from Batumi Boulevard and the Green Cape can be interpreted as an indicator of water pollution by organic substances. *T. minus,* detected in the Batumi Boulevard area, can be traced back to its known habitat in the Nurigeli Lake, which is close to the Batumi central beach and connected to the sea by a channel.

Zooplankton

During the spring months, forty-seven percent of the total zooplankton registered in water samples was attributed to the larvae of benthic species (558 ind/$m^3$). The dominant forms in the spring were *Asplanchna priodonta,* and in the summer, *Diadhanosoma brachyurum,* while *Centropades kroyer* var. *pontica* was present in all seasons. These benthic organisms usually spend late spring and summer amongst the plankton community before starting the benthic lifestyle in autumn, with their larvae appearing in zooplankton populations in the early spring.

## Conclusions

Our studies have shown that variations in the community structure and quantitative parameters of certain species of phyto- and zooplankton in the Georgian coast of the Black Sea can be influenced by multiple factors such as climatic conditions, water temperature, salinity, and the influx of organic matter from connecting bodies of water.

Estimation of the abundance of indicator species revealed local eutrophication at a majority of sampling points in the Black Sea during warm seasons. Fluctuations in phytoplankton and zooplankton abundance and diversity reflect seasonal changes in environmental conditions and periodic excesses of pollutants in the sea due to natural phenomena as well as anthropogenic activity in the region. The gradual introduction of ecological technologies improved the positive dynamics in the local ecosystem, which has subsequently been reflected in an increase in phytoplankton biodiversity.

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## შავი ზღვის საქართველოს სანაპირო ზოლის ფიტოპლანქტონისა და ზოოპლანქტონის სეზონური დინამიკა

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(მიღებულია 12.04.2010)

### რეზიუმე

2006-2008 წლებში შავი ზღვის საქართველოს სანაპირო ზოლის ცალკეულ მონაკვეთზე ჩატარებული რეგულარული ყოველთვიური კვლევითი სამუშაოების საფუძველზე შესწავლილი იქნა ფიტოპლანქტონისა და ზოოპლანქტონის სახეობრივი შედგენილობა, რიცხოვნება და სეზონური დინამიკა. სულ აღრიცხულ იქნა ფიტოპლანქტონის 6 ტაქსონომიური ჯგუფის 178 სახეობა, რომელთა შორის უმრავლესობას წამოადგენდა დიატომეები (40%) და დინოფიტა (32%). *Chlorophyta,*

Cyanophyta, *Chromophyta* და Xanthophyta-ს სახეობათა რაოდენობამ შეადგინა შესაბამისად 13%, 9%, 3% და 3%. კვლევის 24 თვიანი პერიოდის განმავლობაში დაფიქსირდა ზოოპლანკტონის 31 სახეობის ზრდასრული ინდივიდები და ლარვული ფორმები, რომლებიც მიეკუთვნებოდა 5 ტიპის 8 კლასს, მათ შორის დომინანტი კლასები იყო Crustacea (16 სახეობა) და Rotatoria (9 სახეობა). აღსანიშნავია Cladocera და Copepoda-ს მაღალი სახეობრივი მრავალფეროვნება (ზოოპლანქტონის სახეობათა საერთო რიცხვის 26% და 21% შესაბამისაად). წყლის სისუფთავისა და/ან დაბინძურების ხარისხის მაჩვენებელი ფიტოპლანქტონის და ზოოპლანქტონის ინდიკატორული სახეობების გამოვლენის საფუძველზე საკვლევი უბნების უმრავლესობაზე წლის ცალკეულ თბილ თვეებში დადგენილ იქნა ლოკალური ევტროფიკაცია, მათ შორის მდ. სუფსის აკვატორიულ წყლებში, სადაც 2007-2008 წლების შემოდგომაზე დაფიქსირდა Cyanophyta-ს ყველაზე მაღალი რიცხოვნობა. იგივე პერიოდში *Cyanophyt*ა-სა და Xanthophyta-ს მნიშვნელოვანი რაოდენობები აღინიშნა საკვლევი აკვატორიის სხვა სადგურებზეც (ბათუმის ბულვარი, მწვანე კონცხი). ზოოპლანკტონს შორის რიცხოვნობით დომინირებდა ბენთოსური ორგანიზმის *Rapana thomasiana* –ს ლარვები და ციბტუტელა ჭიების წარმომადგენელი *Asplanchna priodonta,* რომელთა 2007-08 წლის შემოდგომაზე ზოოპლანქტონის საერთო რაოდენობის 32% და 20% შეადგინა. ხვედრილობის სიხშირით გამოირჩეოდა *Centropages kroyeri,* რომელიც სინჯების 41%-ში დაფიქსირდა და მისი მაღალი შემცველობა აღინიშნებოდა მთელი 2008 წლის განმავლობაში. ჩატარებულმა კვლევამ გვიჩვენა, რომ შავი ზღვის ფიტო-და ზოოპლანქტონის რაოდენობრივ და თვისობრივ შემადგენლობას ახასიათებს სეზონური ცვლილებები, რაც ასახავს მუდმივად ცვლად ბიოტურ და აბიოტურ გარემო პირობებს და ასევე მზარდ ანთროპოგენულ დატვირთვას რეგიონში.

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# NATURE ENEMIES - PREDATORS, PATHOGENS AND PARASITIC NEMATODES OF BARK BEETLES IN HATILA VALLEY NATIONAL PARK OF TURKEY

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**Abstract**

The study of natural enemies of bark beetles *Ips typographus, Ips sexdentatus, Dendroctonus micans* from Hatila Valley National Park in Artvin, Turkey (1750-2200 m a.s.l) was carried out in different seasons (Summer and Autumn), 2008. *I.typograpus* population in spruce forest of HatilaVNP is monitored and controlled by 3-4 pheromone traps (*Ipstyp*®) per 1ha. 4 000 trap pieces were hung and a number of captured beetles was more than 3 334 860.

*I. typographus, I. sexdentatus* and *D. micans* are settled under bark in one biotope. Three species of predators *Thanasimus formicarius, Rhizophagu grandis* and *Rhizophagus depressus* were found in population of bark beetles. Approximately in 26% of *I.typographus*, 13% of *I. sexdentatus* and 8% of *D. micans* maternal galleries larvae and adults of *T. formicarius* were detected. 21% and 39% of *R. depressus* were found in the observed gallery of *I.typographus and I. sexdentatus* at the same regions of investigation. A high density (66%) of *Rhizophagu grandis,* predator of *D. micans* was observed. Various pathogen species and nematodes are observed in the population of bark beetles. Protozoa, *Gregarina typographi,* was revealed in *I. typographus* (41.1-49.5%) and *I. sexdentatus* (17.3%). *Chytridiopsis typographi* was found in *I. typographus* (9.1-15%) and *I. sexdentatus,* (4.1%). The first data on distribution and occurrence of *Ch. typographi* in Turkey was obtained. Fungal infection *Beauveria bassiana* (1.5%) and *Penicillium sp.* (7.6%) were found in adult *I.typographus.* Virus, like entomopoxvirus - EPV (1.5%) and nematodes (40.7-71.4%) *Contortylenchus diplogaster and Bursaphelenchus sp.* were observed in *I. typographus. Contortylenchus sp.* was found in *I.sexdentatus,* 26.1% of nematodes were associated with *D.micans.*

**Key words:** *Ips typographus, Ips sexdentatus, Dendroctonus micans, Thanasimus formicarius, Rhizophagu grandis, Rhizophagus depressus, Gregarina typographi, Chytridiopsis typographi, B.bassiana, Contortylenchus diplogaster, Contortylenchus sp.*

## Introduction

Artvin is located in Easter part of the Black Sea region of Turkey. It is bordered on Georgia and Ardahan in the east, Rize - in the west, Erzurum - in the south and the Black Sea in the

north. Hatila Valley National Park (HatilaVNP) is 10 km from Artvin. It consists of 16,988 hectare and 25 km of length of one of the main branches of Coruh River [Batan & Ozdemir, 2008].

Two different species Oriental spruce (*Picea orientalis* L.) and Scotch Pine (*Pinus silvestris* L.) are widespread in the Euro-Siberian floristic area of the Holarctic region of HatilaVNP [Eminağaoğlu & Anşin, 2003].

Among numerous bark beetles (Coleoptera: Scolytidae ) which damage spruce stands, the negative economic influence have spruce bark beetle - *Ips typographus* L, Great spruce bark beetle - *Dendroctonus micans* (Kugelann) and Six-toothed bark beetle (Six-spined engraver beetle) - *Ips sexdentatus* (Börner).

At present the bark beetles are very active and characterized by the massive increase and formation of focuses in the large tracts of coniferous forests of Artvin area (including HatilaVNP), they have infested 15,000 ha spruce forest and damaged 1.5 million $m^3$ wood [Aksu, 1987; Sunar Erbek et al., 2005; Akkuzu et al., 2009].

Proceeding from biological particularities it is difficult to develop ways of bark beetles control and protect environment from their invasion.

The question is to establish the natural factors of bark beetles number regulation. The role of natural enemies (parasitoids, predators, microorganisms) in the dynamics of insect pests is very important. The grate attention is paid to their complex study in Turkey.

Among insects the most abundant natural enemy of bark beetles are entomophagous (parasitoides and predators) which are important biological control agents. Extensive work has been carried out on the assemblages of entomphagous with bark beetles in Turkey [Eck, 1990]. Particularly, 27 species of entomophagous insects of this bark beetle were detected [Schopf et al., 1995; Weslien, et al., 1992; Weslien et al., 1999; Wermelinger, 2004].

*Thanasimus formicarius*, *Rhizophagus grandis, Rhizophagus depressus* are the most common entomphagous of bark beetles populations damaging *P.orientalis* in HatilaVNP [Batan & Ozdemir, 2008].

The occurrence and epizootiology of pathogens in bark beetles is one of the least studied aspects in their population dynamics. Recent studies brought evidence of several new pathogen species in bark beetles [Wegensteiner et al., 2000; Haidler et al., 2003; Händel et al., 2005; Händel & Wegensteiner, 2005; Wegensteiner & Weiser, 2004; Wegensteiner & Kleespies, 2005; Takov et al., 2007; Tonka et al., 2007; Burjanadze, 2006; Burjanadze et al., 2008; Yama, 2008]. About 20 pathogens of different bark beetles, that may be used for limiting the population density of bark beetles have been observed [Wegensteiner et al., 2000; Wegensteiner & Weiser, 2004].

In recent years concerted efforts have been made to reduce populations of the bark beetles *Ips typographus* and *Ips sexdentatus* in Turkey by means of numerous traps baited with synthetic pheromones *Ipstyp*® [Göktürk et al., 2005]. The interest in developing ways when the beetle's natural enemies can be used for control has been also grown [Burjanadze, 2007; Burjanadze et al., 2008; Wegensteiner et al., 2000; 2007].

The aim of the present investigation - identification of bioagents occurring in the populations of HatilaVNP, was focused on the natural enemies of bark beetles as biological control agents of great potential due to their ability to develop strong epizootics resulting in natural regulation of bark beetles population.

## Material and Methods

The survey of bark beetles investigation were carried in July and November of 2008 in HatilaVNP 1750-2200 m above sea level ( $\lambda = 41°03' - 41°13''N$ and long $\acute{\alpha} = 41°31' - 41°48'$ E).

From 1998 population of *I.typograpus* is monitored and controlled permanently in some spruce forest of the Artvin region by using 3-4 pheromone traps in 1ha (*Ipstyp*®) per year. The

traps were set up and baited at the end of April, checked weekly and all captured beetles were collected and counted.

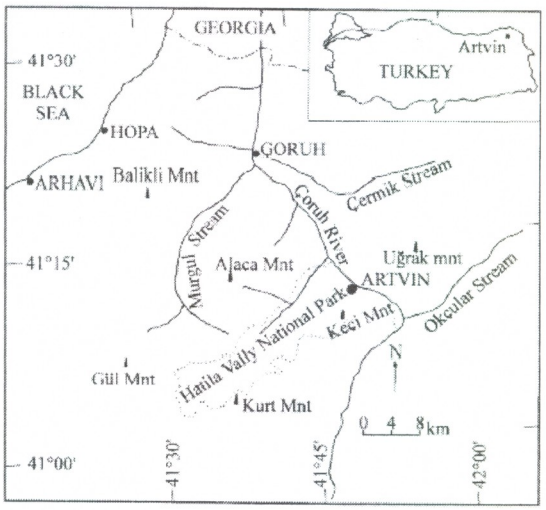



**Fig.1.** Map of the Hatila Valley National Park, Turkey (by Batan & Ozdemir,2008)

Adult bark beetles of *Dendroctanus micans, Ips typographus and Ips sexdentatus* were collected by hand as well, out of their galleries in the phloem of *Picea orientalis.* Log sections and bark with beetles were brought to the laboratory and kept at 15±1°C in a refrigerator. Beetles were dissected just after collection. Only living or slowly moving beetles were removed from their galleries every day and examined using a stereomicroscope (magnification 8-16-32-56x). Subsequently, beetles were dissected and a whole gut, gonads, fat body and other organs were removed for the examination. Diagnosis and search of pathogens was conducted on wet smears with light microscope (magnification 150-600x). After fixation with methanol smears were stained with Giemsa's dye [Weiser, 1977] and re-inspected in light microscope.

For the identification of entomopathogenic fungi, the barks were incubated in plastic vials at the atmosphere moisture. The conidia were stained with lactophenol-cottonblue and examined under light microscope. The material was studied by generally accepted methods in insect fungal pathology [Humber, 1997]. Isolated fungus cultivated on Potato Dextrose agar (PDA) for 10-14 days at $25^0$C, until they developed feature permitting their identification as species or genus [Evlakhova, 1961; Samson et al., 1978].

Isolated parasitic nematodes were studied by using of generally accepted methods in insect nematology [Kaya & Stok , 1997; Pavlovsky, 1957].

## Results

The study of natural enemies of bark beetles was conducted with beetles *I. typographus, I. sexdentatus, D. micans* from HatilaVNP in summer and autumn of 2008. Date and sites of collecting beetles, mature trees with ages, heights and diameters are given in Table 1 and 2.

For the monitoring and controlling of *I.typograhus* the pheromone traps were set up and baited at the end of April, and checked weekly. All captured beetles are collected and counted.

The flight activity of *I.typographus* is very extent and occurs over a period of about four month (May-August). During the monitoring it is possible to detect two main peaks of *I.typographus* capture, the first occurring in the middle of May and second at the beginning of July.

Spring captures include both overwintering and re-emerging adults, which fly around searching suitable spruce, where the emerging adults of both first and sister-generations are summer population. Spring captures may be higher than summer ones, but sometimes the opposite can be observed. Mean annual capture is very variable, indicating either epidemic or endemic *I.typographus* populations.

**Table 1**. Bark beetles collected in HatilaVNP (1850-2200 m a.s.l., λ = 41°03′- 41°13′′N and ά= 41°31′- 41°48′ E).

| # | Data of Collection | altitude a.s.l (m) | Name of Insects | Tree | H (m) | D (cm) | P (year) | Number of collected beetles |
|---|---|---|---|---|---|---|---|---|
| 1 | 18.07.08 | 1750 | *Ips typographus* | Picea orientalis | 20-25 | 40-55 | 75-100 | 197 |
| 2 | 19.07.08 | 1850 | *Dendroctanus micans* | Picea orientalis | 15-25 | 35-50 | 80-100 | 42 |
| 3 | 15.11.08 | 2100 | *Ips typographus*<br>*Ips sexdentatus* | Picea orientalis<br>Picea orientalis | 20-25 | 35-55 | 75-100 | 132<br>98 |
| | | | | | | Total | | ∑=469 |

H – Tree heights; D – Tree diameters; P – Age of trees

The number of hanging trap pieces and total captures of *I.typographus* adults per year are reported in Table 2. The comparative number of catching beetles in the last four years is given on Fig.2.

**Table 2.** Pheromone trap and catching degree of *Ips typographus* in 1 ha and the last 4 years in HatilaVNP.

| GPS | year | Trap piece (n) in 1 ha | Catching insect (n) in 1 ha | Total Trap piece (n) | Total Catching insect (n) |
|---|---|---|---|---|---|
| 1850-2200 m a.s.l, λ = 41°03′- 41°13′′N & ά= 41°31′- 41°48′ E) | 2005 | 4 | 3187 | 12 900 | 41 100 000 |
| | 2006 | 3-4 | 1863 | 5 100 | 9 500 000 |
| | 2007 | 3-4 | 794 | 4 790 | 3 802 500 |
| | 2008 | 3 | 835 | 4 000 | 3 334 860 |

It is seen that population density of spruce bark beetles was permanently reduced during the past years. In 2008, trap piece number was three times lower (4 000) than in 2005 (12 900) and captured beetles were about ten times lower (3 334 860) than in previous 2005 (41 100 000).

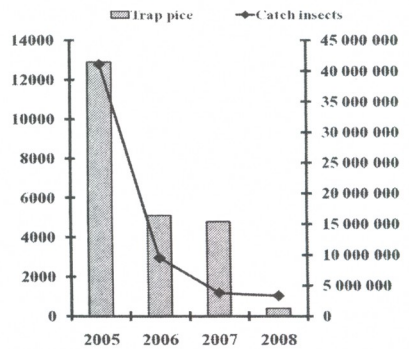



**Fig. 2.** Comparative rate of *Ips typographus* catching by using pheromone traps *Ipstyp*® in the past 4 years

In the study damaged trees of spruce forests in HatilaVNP were determined, where *I. typographus*, *I. sexdentatus* and *D. micans* settled under bark in one biotope. The two species *I. typographus* and *I. sexdentatus* have very similar way of living. Usually, the *I. sexdentatus* lives in low density populations than *I.typographus*.

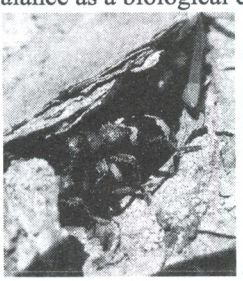
Among bark beetles the most abundant natural enemy is entomophagous. Three species of predators *Thanasimus formicarius* L (Cleridae), *Rhizophagu grandis* Gyllenha and *Rhizophagus depressus* F (Rhizophagidae), were found among population of bark beetles (Table 3).

**Table 3.** Predatores occurrence on bark beetles in Hatila Valley National Park in 2008

| Predatores (%) / Bark beetles | *Thanasimus formicarius* | *Rhizophagus grandis* | *Rhizophagus depressus* |
|---|---|---|---|
| *Ips typographus* | 26 | - | 21 |
| *Ips sexdentatus* | 13 | - | 39 |
| *Dendroctonus micans* | 8 | 66 | 14 |

Larvae and adults of these predators were observed in the galleries of bark beetles. Approximately, in 26% of the checked *I.typographus* maternal galleries, in 13% - *I. sexdentatus* and 8% - *D. micans,* larvae and adults of *T. formicarius* were detected. A bit different percentages (21% and 39%) of *R. depressus* were found in the observed gallery of *I.typographus and I. sexdentatus* in the same regions of investigation. A high density (66%) of the specific predator *Rhizophagu grandis* of *D. micans* was observed.

The predators, causing bark beetle mortality, able to regulate population density and make natural balance as a biological control means.



a

b

**Fig. 3.** Predatores occurrence on bark beetles in HatilaVNP in 2008. a - *Thanasimus formicarius,* b – larvae of *Rhizophagu grandis* and *Dendroctonus micans* under bark

For the identification of pathogens bark beetles were collected from pheromone trap of sampling plot. It is also taken from the inner and outer bark of *Picea orientalis*. Occurrences of pathogens and nematodes in populations of bark beetles are given in Table 3.

**Table 3.** Occurrences of pathogens and nematodes in bark beetles and infection rates (%) from different evaluations of HatilaVNP in 2008 (D.b= Dissected beetles; *G.t= Gregarina typographi*; *Ch.t=Chytridiopsis typographi*; Fun= fungus; EPV=entomopxvirus; Nem=Nematodes; n=number)

| Data | attutated (a.s.l m) | D.b (n) | Infected | | *G.t* | | *Ch.t* | | Fun. | | EPV | | Nem | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | n | % | n | % | n | % | n | % | n | % | n | % |
| *I. typographus* | 1750 | 197 | 124 | 62.9 | 51 | 41.1 | 18 | 9.1 | 15 | 7.6 | - | - | 40 | 71.4 |
| *D. micans* | 1850 | 42 | 11 | - | - | - | - | - | - | - | - | - | 11 | 26.1 |
| *I.typographus* | 2100 | 132 | 82 | 72.5 | 56 | 49.5 | 17 | 15 | 2 | 1.5 | 2 | 1.5 | 46 | 40.7 |
| *I. sexdentatus* | 2100 | 98 | 48 | 48.9 | 17 | 17.3 | 4 | 4.1 | - | - | - | - | 37 | 37.7 |

In total, 469 beetles were dissected individually. Different microorganisms and nematodes were found in bark beetles. The protozoan species, *Gregarina typographi* (Sporozoa, Gregarinidae) described by G.Fuchs (1915) as a parasite of *Ips typographus* was found in the mid-gut lumen (size of gamonts: 50-75μm x 70-130 μm) of adult beetles from different elevation of HatilaVNP (Fig.4,a). Frequency of that pathogen varied at different altitudes and was observed in different developmental stages. Infection rates in the beetles of *I.typogeraphus* varied within 41.1 - 49.5%. The pathogens were present in both genders. *G. typographi* was observed in population of *I. sexdentatus* as well, low infection rate among beetles was determinate (17.3%). During the study, several life stages of the gregarine pathogen, such as trophozoite gamont and cyst were observed in the intestinal lumen of the beetles.

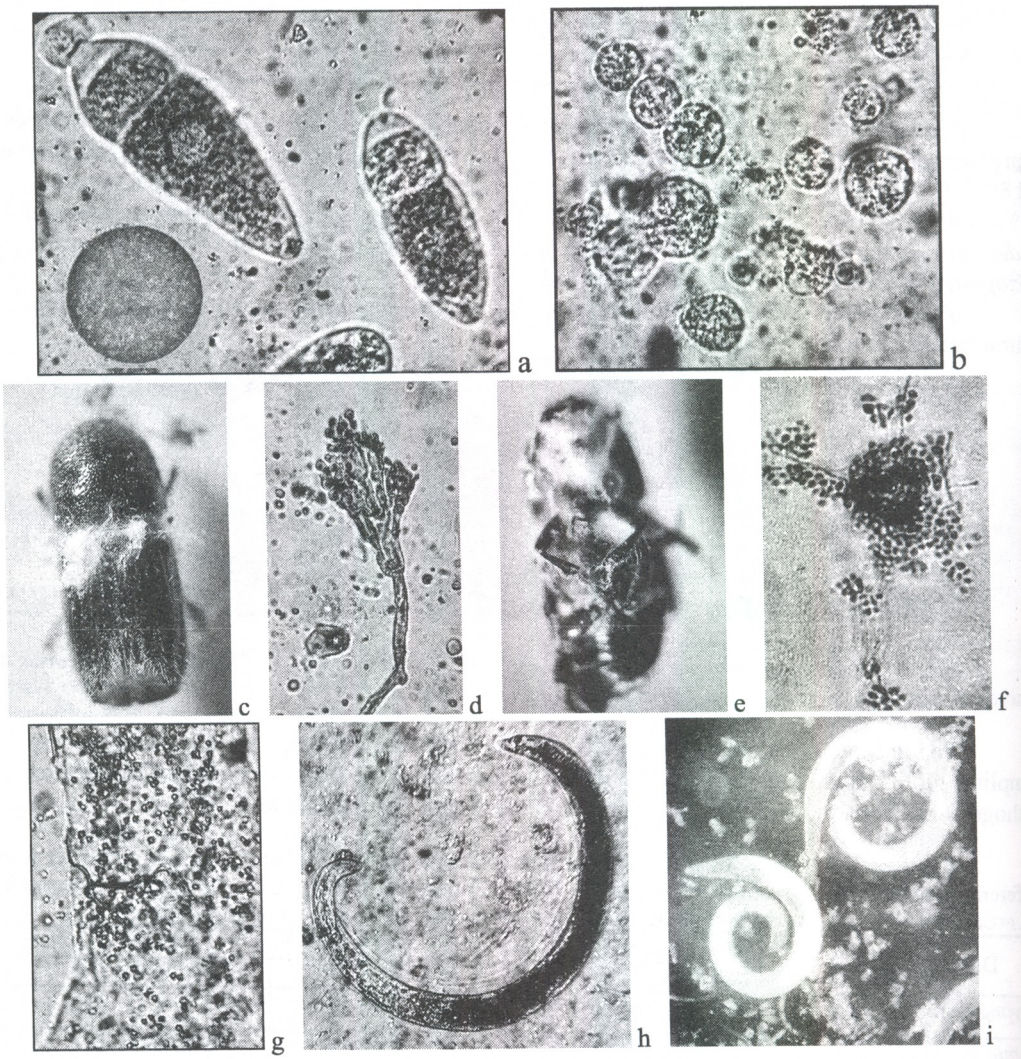



**Fig.4.** a - *Gregarina typographi* (600x); b - *Chytridiopsis typographi* (600x); c - *I.typographus* with micosys; d - *Penicillim sp.*, (250x); e - *I.typographus* infected by fungi; f - *Beauveria bassiana,* (600x); g - Entomopoxvirus (600x); h - *Contortylenchus diplogaster* (150x); i – *Contortylenchus sp.*(32x)

In light microscopical observations of fresh smears of *I. typographus*, the Microsporidium *Chytridiopsis typographi* J.Weiser [1954] was identified in the cells of the midgut epithelium (Spores 1.5 - 2.0 x 2.0 - 2.5 µm; Pansporoblasts (thick walled): 10 – 20 µm in diameter) (Fig.4,b). Infection rates were low (9.1% and 15%). This pathogen was also found in beetles of *I. sexdentatus* with low rate (4.1%). The distribution and occurrence of *Ch. typographi* in the populations of *I.typographus* and *I. sexdentatus* are first recorded in Turkey.

Adult beetles cadavers with mycosis symptoms, such as fungal growth were found in *I.typographus* population. *Penicillim sp.* (7.6%) (Fig.4, c,d) and single findings (1.5%) of the entomopathogenic fungus *Beauveria bassiana* (Fig.4, e,f) were observed of the barks from pheromone traps.

The virus infection (virus-like entomopoxvirus – EPV of *Ips typographus)* was observed only in two dead beetle specimens of *I.typographus*. The spheroid inclusion bodies were found in epithelial cells of the gut of the mature beetles (Fig.4,g). In this case infection rate was low (1.5%), but it is important that infection appeared.

The natural range of the *Chytridiopsis typographi* and virus-like entomopoxvirus and the number of locality was low.

Bark beetles were settled under bark in one biotope, assisted with various species of nematodes. Nematodes were observed in the hemolymph of the beetles at all investigated localities.

As a result of investigation different species of nematodes have been revealed: *Contortylenchus diplogaster* (Fig.h) occurred in the population of *I.typographus*, *Contortylenchus sp.* in *I. sexdentatus* (Fig. 4,i). They were found in gut, on the surface of body and under elytrum of *I.typographus,* also at the gallery of bark spruce. Nematodes were found in *D. micans* as well. Bark beetles number infected by nematodes was: 40.7 - 71.4% in population of *I. typograpus*, 37.7% in *I. sexdentatus* and 26.1% in *D. micans*.

Bacteria should be also occurred but were not identified because of specific difficulties of anatomical association with their host, isolation and taxonomy.

The Comparative numbers of pathogen occurrence in bark beetles population spreading in HatilaVNP at different evaluations are given on Fig. 5.

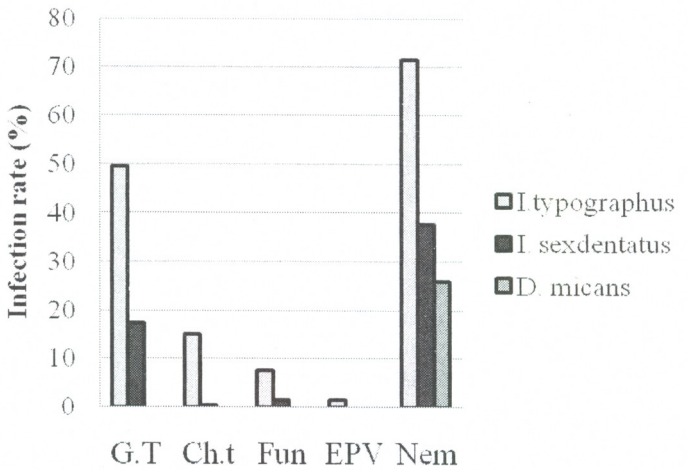



**Fig. 5.** Comparative number of pathogen occurrence of bark beetles population in HatilaVNP in 2008 (G.t= *Gregarina typographi*; Ch.t=*Chytridiopsi typographi*; Fun=fungi, Vir.=virus; Nem=Nematodes )

## Discussion

Modern strategies for *I.typographus* control include the use of traps baited with pheromones [Zumr, 1988; Faccoli & Stergulc, 2004]. Traps are useful both in control by mass-trapping and in population monitoring. It is often used to prevent attacks on living trees than to diminish its populations. This approach is often regarded as reasonable protection measure [Grodzki, 2004; Faccoli & Stergulc, 2006].

Beginning from 1998, populations of *I.typographus* are monitored permanently in some spruce forest of the Artvin region by using pheromone traps per year [Göktürk & Eldemir, 2005; Göktürk et al., 2005(a,b)]. The monitored stands in HatilaVNP are about 80-100 year old growings at altitudes between 1750-2200 m a.s.l. The number of bark beetles caught in pheromone traps very much depends on environmental and local conditions. A numerous investigations in Hatila VNP have shown that climate and topographic factors influence the population dynamics of bark beetles and its natural enemies [Akkuz et al., 2009]. Field experiments in HatilaVNP clearly showed that the complete control system with traps reduced *I.typographus* population by 80% in 2008 compared to its number in 2005.

The ant beetle *Thanasimus formicarius*, one of the most common predators in Turkey, feeds on 27 bark-beetle species belonging to 15 genera [Gauss 1954; Mills et al, 1991; Tommeras, 1988]. *T. formicarius* exerts a significant impact on the population dynamics of *I. typographus*, because of its high fecundity and high voracity at the adult stage as well as at the larval stage [Weslien & Regnander, 1992; Faccoli & Stergulc, 2006]. Adults of *T.formicarius* live 4 to 10 months [Mills, 1983] and respond to the pheromones of *I. ypographus* [Bakke&Kvamme, 1981].

*Rizophagus grandis* is the single most important and potentially useful natural enemy of *D. micans* throughout Eurasian range. The first notable artificial breeding and release of this predator began in 1963 in the Georgian Republic, and is still on going, being regarded as influential in reducing serious outbreaks of *D. micans* in the extensive oriental spruce forest [Kobakhidze et al., 1970]. A similar project began in Turkey in 1984 [Alkan & Aksu, 1990] and still on going.

The occurrences of Rhizophagus grandis, Rhizophagus depressus and Thanasimus formicarius in cut of Picea orientalis attacked by Dendroctonus micans were recorded in the field. The T. formicarius eggs, R. depressus adults were abundant in the I.typographus and I. sextendantus attacked trees. When both species were present the total number of larvae was reduced by 49%. The interaction between T. formicarius and R. depressus possible to be mutually antagonistic as well [Meydan et al.,2005; Meydan et al.,2005; Göktürk et al., 2005].

The new aspect of this examination is the occurrence on pathogens and nematodes in bark beetles population in HatilaVNP. The pathogen complex of the examined bark beetles was not very diverse, compared to the results of study in European country [Wegensteiner &Weiser, 1996; 2004].

One of the interesting results was to find numerous pathogen species distributed in *I.typographus* filed populations. The prevalence of *Gregarina typographi* was high but showed great variation in abundance. *G. typographi* was very common in *I. typographus* populations too and sometimes in high prevalence [Wegensteiner, 1994; 2004; Wegensteiner & Weiser, 1996; Kereselidze & Wegensteiner, 2007; Burjanadze & Goginashvili 2009]. M.Yaman (20) noted on *G.typography* as on a pathogens of *I. sexdentatus* in Turkey. According to our investigation the pathogens occurred as a population of *I.typographus* in population of *I.sexdentatus* in HatilaVNP. During the study several life stages of the *G.typography*, such as trophozoite, gamont and cyst in the internal lumen of both species of bark beetles were observed. Gregarines are presumed to be minor virulent pathogens, which do not evidently interrupt migration of beetles [Wegensteiner & Weiser, 2004.].

The occurrence of *Ch.typographi* in population of *I.typographus* is known from various investigations [Wegensteiner & Weiser, 2004; Wegensteiner & Kleespies, 2005; Burjanadze et al.,

2008]. J.Weiser [1954] was the first who described *Ch.typographi* (formerly *Haplosporidium typographi*) from the gut epithelium of *I.typographus* [Weiser, 1977]. The infection of beetles with this pathogen has a latent period and chronic disposition for a long period [Wegensteiner & Weiser, 1996].

The pathogens of *Chytridiopsis typographi* presented here are the first record of *I. typographus* and *I. sexdentatus* in Turkey.

The occurrence of ItEPV in *I.typographus* was reported by different authors [Haidler et al., 2003; Wegensteiner&Weiser, 1996; Wegensteiner, 1994]. Viruses should be very interesting pathogen for microbial control measure, especially from the point of selectivity and effectiveness. At this moment there is a lack of knowledge.

The entomopathogenic fungus *Beauveria bassiana* is a well known, natural occurring and widely distributed antagonist of *I. typographus* [Wegensteiner&Weiser, 1996; Burjanadze, 2006; Burjanadze et al., 2008]. Single findings of *Beauveria bassiana* occurred on the adult *I.typographus,* found under bark on the gallery with white mycelium on its surface. Later, on the adult *I.typographus* collected from pheromone traps, soft white mycelium discovered as well. After isolation *Penicillim sp.* was recorded by microscopical investigation. In general, this species likely appears in unfavourable conditions for pest development.

Nematodes were found free in the haemolymph and in the gut lumen of *I.typographus*. In case of presence of female nematodes it was possible to identify *Contortylenchus diplogaster* in the haemolymph. This species was already described for *I. typographus* from different parts of the world [Wegensteiner&Kleespies, 2005; Burjanadze et al., 2008, Burjanadze&Goginashvili, 2009 ]. *Contortylenchus diplogaster* (Tylenchida: Tylenchoidae) is endoparasitic nematode fed of fat, tissue and hemolymph. The parasite stays for survival in the host for long time. The gut of bark beetle is invised by J3 of this nematode [Fuchs, 1915]. Female of the species was found in hemolymphe and observed usually one or two, rarely 3-10 exemplars in one bark beetle. Number of infested larva in haemolymph and gut lumen of host bark varies between 50-600 exemplars per beetle. *Bursaphelenchus sp.*(Tylenchida: Aphelenchoididae) parasites under elytra. Under each elytra 30-50 samples occurred and 10-30% of bark beetles settled by this species. They are facultative ectoparasites [Kakulia, 1989].

*Contortylenchus sp.* was found both in the hemolymphe and gut lumen in population of *I.sexdentatus*. Its taxonomical belonging is the same as of *Contortylenchus diplogaster* (Tylenchida: Tylenchoidae). During the study several life stages of *Contortylenchus sp.,* such as egg, larvae, adult (mostly females) were observed. Usually 1-3, and maximum 7 exemplars per female bark beetle of the species were found in hemolymphe. Larvae were observed in hemolymphe and gut lumen, eggs in hemolymphe. The species *Contortylenchus pseudodiplogaster* as a endoparasitic nematode of *I.sexdentatus* from Georgia was described by G. Kakulia [1989]. M.Yaman [2007] reported on the nematodes associate with *I.sexdentatus* in Turkey, but did not describe it.

Low frequency of nematodes was observed in population of *D. micans*, but it was not possible to determinate its taxonomy, as all observed individuals were of juvenile stage.

It is significant that mixed infection in the bark beetles population was also observed. Usually two and rarely three infections are presented, such as Gregarins and nematodes, microsporidia, gregarines and nematodes.

The results of our study underlined the importance of investigations on bark beetles natural enemies of different geographical zones in order to get an accurate knowledge of their diversity and prevalence.

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# ჰატილას ხეობის ეროვნულ პარკში (თურქეთი) გავრცელებული ქერქიჭამიების ბუნებრივი მტრების – ენტომოფაგების, მტაცებლების, პათოგენების და პარაზიტული ნემატოდების გამოვლენა

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(მიღებულია 08. 02. 2010)

## რეზიუმე

ქერქიჭამია მავნე მწერების *Ips typographus, Ips sexdentatus, Dendroctonus micans* ბუნებრივი მტების შესწავლის მიზნით ჰატილას ხეობის ეროვნულ პარკში, ზღვის დონიდან 1750-2200 მ-ზე, 2008 წლის ზაფხულსა და შემოდგომაზე შეგროვებულია ზრდასრული ხოჭოები. *I.typographus* მონიტორინგი და კონტროლი ხორციელდებოდა ფერომონიანი სქესმჭერების (*Ipstyp*®) მეშვეობით. 1 ჰა-ზე გამოიყენება 3-4 ფერომონიანი საკიდი. სულ გამოყენებული იყო 4 000 საკიდი და მიზნდული ხოჭოების რაოდენობა აღემატებოდა 334 860 ცალს. გამოკვლევებმა აჩვენა, რომ ქერქქვეშ ერთდროულად თანაცხოვრებას ეწევა სამივე ქერქიჭამია მწერი *I.typographus I.sexdentatus* და *D. Micans* და სამი სახეობის მტაცებელი მწერი *Thanasimus formicarius, Rhizophagu grandis* და *Rhizophagus depressus. T. formicarius* მატლები და იმაგოების მიერ დაზიანება შედგენდა *I.typographus*-სათვის 26%-ს, *I. sexdentatus*-სათვის - 13% და *D. micans*-სათვის - 8%. *R.depressus* ზრდასრული ფორმების 21% და 39% გამოვლინდა შესაბამისად *I.typographus* და *I. sexdentatus* პოპულაციებში, ხოლო *R.grandis* (66%) აღინიშნა *D. Micans*-ის პოპულაციებში.

პროტოზოული ინფექცია *Gregarina typographi* გამოვლინდა *I. typographus* (41.1-49.5%.) და *I. sexdentatus* (17.3%) პოპულაციებში. ამავე პოპულაციებში ასევე გამოვლინდა *Chytridiopsis typographi* (9.1%-15% და 4.1%, შესაბამისად), რომელის გავრცელება თურქეთის ტერიტორიაზე პირველად არის აღნიშნული ჩვენს მიერ. *I.typographus*–ის ხოჭოებში აღინიშნა სოკოვანი ინფექცია *Beauveria bassiana* (1.5%) და *Penicillim sp.* (7.6%), ენტომოპოქსვირუსი – EPV (1.5%) და ნემატოდები (40.7-71.4%) *Contortylenchus diplogaster* და *Bursaphelenchus sp.*, ხოლო *I.sexdentatus* პოპულაციებში - *Contortylenchus sp. D.micans*-ის შემთხვევაში ნემატოდებით დაზიანება შეადგენდა 26.1%-ს.

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# INFLUENCE OF HEAVY METALS ON THE CONCENTRATION OF BIOGENIC ELEMENTS IN ORGANS OF COMMON CARP (*CYPRINUS CARPIO*)

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**Abstract**

The influence of heavy metals (copper, zinc) on the concentration of calcium, phosphorus and magnesium in the tissues (vertebra, gills, muscles and eye) of fish was studied. The investigation has revealed that concentrations of these elements before and after experiment are different. Due to the influence of heavy metals the level of calcium decreased by 5 and 11.5 times, in gills and muscles respectively, and increased 2 times in eyes. The concentration of magnesium decreased by 4.25 times in vertebra and by 2 times in eye, and increased in gills by 2 times. The level of phosphorus varies less than of the rest two elements.

**Key words:** heavy metals, biogenic elements, *Cyprinus carpio*

## Introduction

Contamination of aquatic ecosystems (e.g. lakes, rivers, streams, etc.) with metals has been receiving increased worldwide attention [Vinodhini & Narayanan, 2008; Vutukuru, 2005; Mansour & Sidky, 2002; Dirilgen, 2001]. Usually, many toxic compounds affect organisms in nature at the same time, each of them having a specific effect on physical and chemical processes that influence an organism's condition and reactions. Therefore, in order to maintain the quality of food it is important to regularly monitor and evaluate the pollution levels in fish as well as in water reservoirs [Staniskiene et al., 2006]. Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms [Farombi et al., 2007; Vosyliene & Jankaite, 2006; Ashraj, 2005]. Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants [Olaifa et al., 2004]. Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems [Farkas et al., 2002]. The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood [Canli, 1995].

Almost the entire store of calcium (99 %) and most of the phosphorus (80 %) in the fish's body are present in bones, teeth and scales. The one percent extra-skeletal calcium is widely distributed throughout the organs and tissues. In the muscle activity and osmoregulation, ionized calcium actively participates in the extra cellular fluids and in the circulatory system. Large amounts of extra-skeletal phosphorus are present mostly in combinations with proteins, lipids,

sugars, nucleic acids and other organic compounds. These phosphor compounds are vital exchange currencies in life processes and are distributed throughout the organs and tissues of the fish. The bulk of magnesium in fish (60 % in the carp) is stored in the skeleton. The remaining 40 % of the body's magnesium is distributed throughout the organs and muscle tissues (where it plays vital role as enzyme co-factors, and as an important structural component of cell membranes) and in extra cellular fluids [Chow & Schell, 1980].

The aim of the investigation was to determine heavy metals influence on the calcium, magnesium and phosphorus concentration in fish tissues: vertebra, gills, muscles and eyes. We chose these three elements for their importance for fish body. Common carp (*Cyprinus carpio*) was selected due to its adoption in polluted aquatic environment.

## Materials and Methods

Common carp (*Cyprinus carpio*), 62 individuals (15-30 cm length, 130-150 g weight), for the research were collected from the river Mtkvari (Gardabani region). The experiment was carried out in Lab of Hydrobiology and Ichthyology, Institute of Zoology. At the first stage of the experiment all collected fish were placed into 50 liter volume aquarium, filled by trap water, at 21°C temperature during 2 hours, aerated by the compressor. This stage was lethal for some part of experimental fish.

Survived fish were divided into 3 groups, by 12-12 samples (36 fish), first group served as control and other groups as experimental, second group of fish was placed in 0.5 mg/l $ZnSO_4$ solution and third group – in 1.0 mg/l $CuSO_4$ solution. These concentrations were choused by their lethal influence on fish. Organs from fish were removed after 24, 48, 72 hours. The vertebra, gills, muscles and eyes were removed from each fish and were analyzed separately [Dybem, 1983]. Afterwards P was assayed using spectrophotometer method and Ca, Mg - by titration method [Alekin, 1970].

## Results and Discussion

At the first stage 42% (26 individuals) of fish die. In all water samples before adding solution of heavy metals concentration of phosphorus, calcium and magnesium was lower. Afterwards concentration of P increased by 7-8 times, Ca - 1.5-2 times and Mg - 1.5 times. The concentration of these elements in most selected fish organs decreased respectively (Fig.1-3). Changes of biogenic elements in both parts of experimental groups were similar. Due to the influence of heavy metals the level of calcium decreased by 5 and 11.5 times, in gills and muscles respectively, and increased 2 times in eyes, concentration of Ca in vertebra changed insignificantly (Fig. 1).

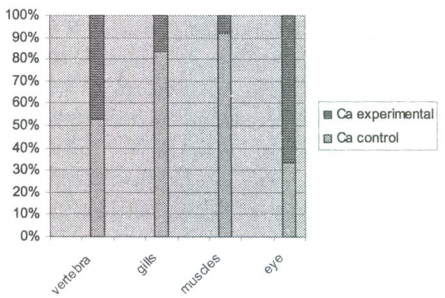


**Fig.1.** Concentration of Ca in fish organs in experimental and control samples.

The concentration of magnesium decreased by 4.25 times in vertebra and by 2 times in eye, and increased in gills by 2 times. In muscles changes of the level of Mg was not vital (Fig. 2).

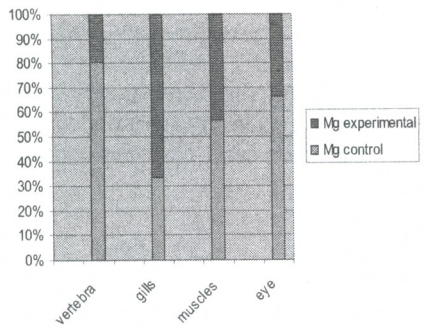


**Fig.2.** Concentration of Mg in fish organs in experimental and control samples.

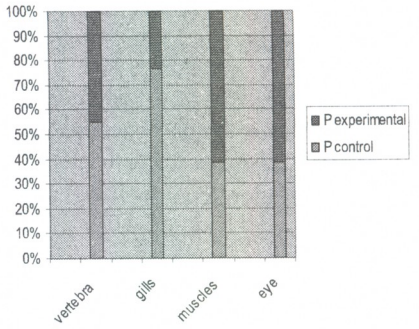


**Fig 3.** Concentration of P in fish organs in experimental and control samples.

Change of the concentration of phosphorus in gills was most significant – increased by 3 times, in other organs of fish changes were insignificant.

At the beginning of intoxication the oxygen demand increased, respiration was more frequent, afterwards breathe became labored, these symptoms were followed by arrhythmia and anoxic death. Skin and gills were covered by mucous.

It has raveled that influence of zinc sulfate mainly provoke decrease of calcium concentration in fish muscles than in gills. Concentration of magnesium decreased in vertebrae and concentration of phosphorus in – gills. In the eye quantity of calcium and phosphorus increased insignificantly and magnesium - decreased. 2-4 days after 80% of experimental fish die. In survived individuals concentration of biogenic elements vary insignificantly. The copper was adsorbed on the scales of fish which were placed in copper sulfate and in the all individuals provoked necrosis of hepatic cells.

The result indicates that the heavy metal contamination definitely affects the aquatic life of the fresh water fish.

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# მძიმე მეტალების გავლენა კობრის (*Cyprinus carpio*) ორგანოებში ბიოგენური ელემენტების შემცველობაზე

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(მიღებულია 10.03.2010)

## რეზიუმე

შესწავლილია მძიმე მეტალების (სპილენძი, თუთია) გავლენა კობრის ორგანოებში (ხერხემალი, ლაყუჩები, კუნთები და თვალი), კალციუმის, ფოსფორისა და მაგნიუმის შემცველობაზე. დადგენილია, რომ ამ ელემენტების კონცერნტრაციები ექსპერიმენტამდე და ექსპერიმენტის შემდეგ განსხვავებულია. მძიმე მეტალების ზეგავლენის შედეგად კალციუმის კონცენტრაცია მკვეთრად მცირდება ლაყუჩებში და კუნთებში, 5 და 11.5-ჯერ შესაბამისად, ხოლო თვალში იზარდება 2-ჯერ. მაგნიუმის შემცველობა ხერხემლაში შემცირდა 4.25-ჯერ, თვალში 2-ჯერ, ხოლო ლაყუჩებში გაიზარდა 2-ჯერ. ფოსფორის რაოდენობა დანარჩენ ელემენტებთან შედარებით ნაკლებად იცვლება.

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Microbiology

# EFFECT OF CHEMICAL PREPARATIONS ON VEGETABLE DISEASE INDUCING MICROFLORA DURING STORAGE

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**Abstract**

The inhibitory effect of fungicide preparations of 0.025% concentration on the sensitivity of beet and carrot phytopathogenic microorganisms was studied under conditions of storage, aimed at reduction of diseases. In case of both vegetables, Tylt and Thiabendazole appeared effective. These fungicides suppressed development of *Botrytis, Aspergillus and Fusarium.* Treatment with fungicides before storage proved effective.

**Key word:** fungicides, phytopathogenic, inhibition, vegetable

## Introduction

A low quality of products during storage is mainly associated with physiological, phytopathogenic and physical factors [Kudriaceva et al., 1986; Zhuchkov et al., 1986]. As the course of microbiological processes is inevitable during storage it is especially important to determine quality of products by the method of microbiological prognosis. Microorganisms find favorable conditions for growth and development in separate specimens of fruit and vegetables producing their disease that spreads very fast. Unless necessary measures are taken a great deal of stored products is rendered inedible [Zhuchkov et al., 1986].

It is also known that treatment of vegetables with various fungicides prior to harvest has a positive effect on diseases induced by microorganisms, including fungi, reducing their effect [Tlindsey et al., 2004]. The effect of fungicides on onion seeds infected with the fungus *B. aclada (B. allii)* was experimentally evaluated. The degree of fungi effect in fungicide-treated onion cultures (9 fungicides being tested) appeared reduced considerably compared to the untreated ones (control)

Spread of diseases induced by *Penicillium digitatum* on lemon fruit has been also studied using Thiobendazole and Benomil. The latter appeared to reduce infection more effectively than the former [Wild et al., 2008]. Though, the result was not always positive, it depends on the extent of the disease and microbe species inducing this disease.

Application of fungicides prior to storage reduces somewhat microbiological diseases. This is a very topical issue, as during prolonged storage fruits and vegetables are affected with a variety of microorganisms yielding a great loss (25-30%).

Therefore, we have studied the inhibitory action of chemicals at various concentrations on the sensitivity of beet and carrot phytopathogenic microorganisms.

Preliminary studies have shown that during storage beet is mainly affected by the phytopathogenic fungi – *Fusarium, Penicillium, Botrytis, Phoma*, while carrot by *Sclerotinia, Botrytis, Alternaria.*

## Materials and Methods

In our experiments we used beet and carrot and the following fungicides: *Azovite, Baitan, Tylt* and *Thiabenzole.* We have studied the inhibitory action of chemicals at various concentrations (0.01%, 0.02%, 0.025%) on the sensitivity of beet and carrot phytopathogenic microorganisms. 0.025% concentration appeared to be the most effective.

Vegetables were treated with fungicides shortly before storage. Effect of fungicides was recorded 90, 120 and 150 days after storage. The role of each fungicide in reducing the microbiological effect was studied.

## Result and Discussion

Wet beet was treated with fungicides prior to storage, which noticeably reduced microbial effect; in particular, Tylt and Thiabendazole yielded the best results. The diseases reduced 2 and more times compared to control (untreated) (Table 1).

**Table 1.** Effect of fungicides on beet diseases (concentration of fungicide was 0.025%)

| Variant | Treated before storage (affected) % | | |
|---|---|---|---|
| | 90 days | 120 days | 150 days |
| Control | 1.0 | 2.12 | 3.7 |
| Azovite | 0.22 | 1.73 | 2.5 |
| Baitan | 0.12 | 1.51 | 2.23 |
| Bolletin | 0.40 | 1.70 | 2.0 |
| Tylt | - | 0.3 | 0.71 |
| Thiabendazole | 0.15 | 0.5 | 0.85 |

A similar result was obtained when carrot was treated with fungicides; the best result was obtained also by Tylt and Thiabendazole, which decreased the microbial effect 1.2-2 times (Table 2). Especially, good result was obtained at 90-day storage, affection was reduced 1.5-2 times compared to the other fungicides. Microbiological assessments showed that Tylt and Thiabendazole suppressed particularly the development of *Botrytis, Aspergillus* and *Fusarium.*

**Table 2.** Effect of fungicides on diseases of carrot (concentration of fungicide was 0.025%)

| Variant | Treated before storage (affected) % | | |
|---|---|---|---|
| | 90 days | 120 days | 150 days |
| Control | 1.29 | 3.35 | 5.82 |
| Azovite | 1.05 | 2.75 | 4.0 |
| Baitan | 1.0 | 2.82 | 3.62 |
| Bolletin | 0.95 | 2.77 | 4.0 |
| Tylt | 0.32 | 1.31 | 2.17 |
| Thiabendazole | 0.15 | 1.51 | 2.15 |

Thus, treatment of vegetable cultures – beet and carrot with fungicides of 0.025% concentration, especially with *Tylt* and *Thiabendazole,* before storage reduced the diseases induced by microorganisms.

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## შენახვის დროს ქიმიური პრეპარატების მოქმედება ბოსტნეულის ავადმყოფობის გამომწვევ მიკროფლორაზე

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(მიღებულია 01. 02. 2010)

### რეზიუმე

შესწავლილი იქნა შენახვის პირობებში ფუნგიციდების 0,025%-იანი კონცენტრაციის პრეპარატების ინჰიბიტორული მოქმედება სუფრის ჭარხლის და სტაფილოს ფიტოპათოგენური მიკროორგანიზმების მგრძნობელობაზე დაავადების შემცირების მიზნით. ორივე ბოსტნეულის შემთხვევაში ეფექტური აღმოჩნდა ტილტი და თიაბენდაზოლი. აღნიშნულმა ფუნგიციდებმა დათრგუნა ***Botrytis, Aspergillus*** და ***Fusarium***-ის განვითარება. ფუნგიციდებით დამუშავება ეფექტური აღმოჩნდა შენახვის წინ.

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# KOTSAKHURI (EASTERN GEORGIA) PLIOCENE FAUNA OF VERTEBRATES AND TARIBANA ELEPHANT

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**Abstract**

Initially Kotsakhuri fossil vertebrate fauna was considered to be the Upper Akchagylian - Lower Apsheronian age. Detailed studies of new paleonotological discoveries, especially the one of the Southern elephant (*Archidiscodon meridionalis*) from Taribana allows precision of the geological age of Kotsakhuri vertebrate faunal assemblage. It is dated as the Upper Pliocene (Upper Akchagylian).

**Key words:** Kotsakhuri, Akchagyl, Taribana, Pliocene, *Archidiscodon meridionalis*.

## Introduction

Considering the lack of remains of fossilized vertebrate fauna on the territory of Georgia, the discovery of the Kotsakhuri locality (the Dedoplistskaro region) is undoubtedly a significant event in paleontology. Several years ago the scientific expedition of the Institute of Paleobiology found fossilized animals - a pile of mostly mammal bones on the southern slope of the Kotsakhuri Ridge in the Dedoplistskaro region. The material obtained after prospecting made up an interesting collection from stratigraphical point of view.

In the environs of the Kotsakhuri Ridge a rather thick series of the Akchagil-Apsheron deposits are cropped out. Its cross section has been described in details by V.Trubikhin and A.Chapaliga. The upper pert of the Kotsakhuri Ridge deposits are represented by alternation of conglomerates, sandstones and pelitic clays. In this part fresh water mollusk fauna (*Corbicula apscheronica, Valvata naticina, Unio apscheronicus*, etc.,) shells often occur, due to which the aforementioned deposits can be attributed to the Apsheron stage. In the lowermost part of the Apsheron deposits A.Chepaliga found remains of unionids and, accordingly, attributed the abovementioned deposits to the Damashkin horizon; whereas this horizon is generally characteristic to the lower part of the Southern Caucasian Apsheron deposits.

Geology and Fauna

The lower part of the Kotsakhuri Ridge deposits is characterized by marine Akchagil fauna (*Mactra subcaspica, Cardium pseudoedule, C.dombra, Potamides disjunctus*, etc.,). It should be noted that the transition of the Akchagil deposits into the Apsheron ones is gradual and no gap is observed between them; thus it gets difficult to draw a borderline between the Akchagil and Apsheron stages. The lower part of the Kotsakhuri Ridge deposits are positively magnetized (Gauss), while those of the upper one – negatively (Matuyame). Noteworthy is that V.Trubikhin

distinguishes positively magnetized thin horizon in the upper part of negatively magnetized Apsheron deposits and considers it to be an episode of Jaramilo.

Disputable is the geological age of the Kotsakhuri mammals fauna (the material found by the authors) underlying the Damashkin horizon deposits. V.Trubikhin attributed these deposits and, accordingly, the fauna of mammals to the Apsheron age, which is unacceptable to us. It is significant that in 1974 Mamedov carried out researches of the Kotsakhuri deposits from the paleontologic-magnetic point of view and defined positively magnetized layers in this horizon and attributed it to Olduvai episode. Consequently, the geological age of the Kotsakhuri deposits comprising bones is approximately 1.8 Ma. If this definition is true the Kotsakhuri fauna of mammals should be disposed in the 17th biological zone.


**Fig.1.** Kotsakhuri *Protorys sp.* Horn cores from the side views

At present in the Kotsakhuri fauna the following mammals have been established: Southern elephant (*Archidiskodon meridionalis*), Stenon equine (*Equus stenonis*), Etruscan rhinoceros (*Dicerorhinus etruscus*), Giant deer (*Croizetoceros aff.ramosus*), gazelle (*Gazella sp.*), fossilized antelope-protoryx (*Protoryx sp.*), Leptobos (*Leptobos sp.*), tortoise (*Testudo graeca*), and Giant ostrich (*Struthio transcaucasius*). When defining the geological age of the aforementioned fauna, due attention should be primordially paid to the representatives of *Proboscidea, Equidae* and *Bovidae.* In case of the Kostakhuri fauna, the Southern elephant, Stenon equine and Leptobose should be taken into consideration. In the Kotsakhuri Ridge fauna the Southern elephant belongs to the same subspecies (*Arch. meridionalis taribanensis*) which was defined in Taribana [Gabunia, Vekua, 1963]. The identity of the Taribana and Kotsakhuri elephant is undoubted: their remains have been found in the analogous deposits and their localities are nearly adjacent.

In the fifties of the last century master borer, Metreveli by the surname, noticed tusks sticking out of rock. Surely Metreveli could not establish to what animal they might belong; he broke off a piece and gave it to M.Dzvelaia, a collaborator of the Institute of Paleobiology. We immediately left for Taribana to check up the fact and it turned out that in the thick layer of sandstones an elephant's nearly complete fossilized skeleton occurred; the governing body of the Dedoplistskaro region detached a bulldozer; so, we excavated the skeleton and brought to Tbilisi. It

took about a year to carry out preparation and research works. At present the restored skeleton of the Taribana elephant is exposed in the exhibition hall of the Georgian National Museum. The Taribana elephant's skeleton is unique for its completeness. There have been found only three complete skeletons of Southern elephant in all over the world. One of them is exposed in Paris; the other – in St. Petersburg and the third one – in Tbilisi. Establishing the geological age of the Kotsakkhuri fauna we resorted to the fossilized Taribana elephant and the geological situation of its location.

**Fig.2.** Kotsakhuri. *Gazella sp.* Above – horn cores from the side views, below - astragalus

According to the data by D.Buleishvili (1960), the Akchagil deposits are represented by two facieses: in the lower one marine deposits with distinctly dated mollusk fauna occur while in the upper one – the Akchagil marine deposits gradually transit into continental deposits without any obvious gaps that comprise the upper part of the Akchagil stage and the whole of the Apsheron. It is impossible to draw a border between the Akchagil and Apsheron stages here. It is only known that the Akchagil marine deposits are positively magnetized but the Apsheron deposits – negatively. In a definite band changeability of inversion is observed that may coincide with the border between these stages.

The Taribana elephant's skeleton was found in deposits characterized by conditionally transitive inversion; they are represented by dense, fine-grained sandstones. While studying the Taribana elephant the authors [Gabunia, Vekua, 1963] considered that the geological age of the aforementioned deposits belong to the Apsheron stage but the subsequent researches convinced them in fallibility of their hesitant considerations and attributed the deposits, comprising the skeleton, to the Upper Agchagil stage.

It is known that taxonomy of elephants is mainly based on morphological characters of teeth; the most significant among them are the following: general number of dental lamellae, the quantity of lamellas within 10 cm and enamel thickness. According to these characters the taxonomy of Southern elephants is exactly defined. The less is the general quantity of teeth and that of lamella within 10cm and thicker is enamel the more archaic is the elephant from the geological point of view. The aforementioned regularity in morphology of teeth is well defined for elephants and it is especially trustworthy for Southern elephants. The geological age of Southern elephant and its subspecies is exactly established according to morphological characters of teeth and is successfully applied for nomination of Southern elephants and at comparison of the Upper Cenozoic continental rocks, comprising remains of mammals [Gabunia, Dubrovo, 1990].

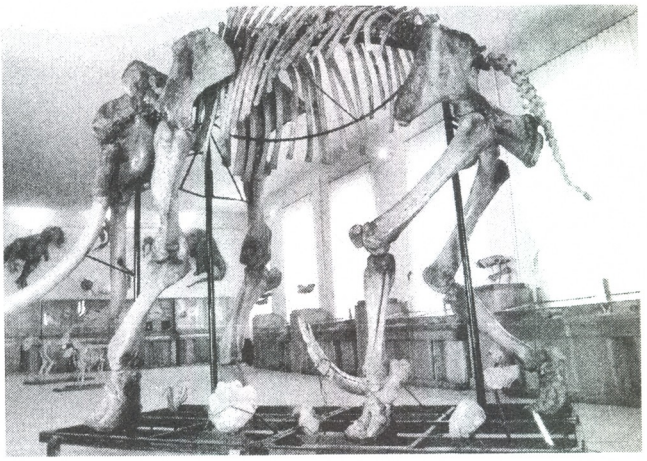

**Fig.3.** *Archidiskodon meridionalis taribanensis* Gabunia at Vekua. Mounted sceleton of Archidiscodon meridionalis in the Georgian National Museum

In Western Europe the remains of an elephant, found in the Upper Pliocene Valdarno location, in Italy, are regarded as archaic ones; they are nearly analogous to the Taribana elephant but there is obvious difference between them, especially in the morphological characters of teeth. The Valdarno elephant's last molar, in comparison with that of Taribana elephant, has more lamellas, higher frequency of lamellas and thinner enamel. According to those characters it is indisputable that Taribana elephant is not younger, if not more archaic, than the Pliocene Valdarno elephant.

We would like to interpret more loosely the geological age of the Southern elephant found in Khapri (the Azov seaside) sands, which is distinguished by Garutt and Alexseeva [Garut, Alexseeva, 1965] as a new species of Southern elephants *Archidiskodon gromovi* and consider it to be an archaic representative of this group. The deposits comprising Khapri bones are dated as the Upper Pliocene, while some researchers attribute them to the Upper Akchagil. The morphological parameters of the Khapri elephant's third molar are the following: 12–13 lamellas on the tooth, the lamella frequency – 3.6-5, enamel thickness – 3.0-5 mm that entirely correspond to the parameters of the Taribana elephant's molar and proves the Upper Akchagil age of the Taribana elephant.

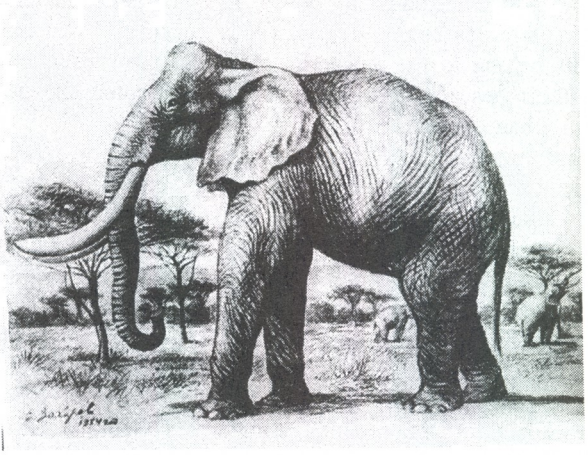

**Fig.4.** *Archidoskodon meridionalis* Nesti, drawing by E. Zakharov

## Conclusion

We'd like to return back to the geological age of the Kotsakhuri fauna. The ascertainment of the Taribana elefant's age facilitated the dating of the Kotsakhuri deposits that comprise bones. In the Kotsakhuri vertebrate fauna there appears an elephant, identical to the Taribana elephant and thus the Kotsakhuri deposits are considered to belong to the Upper Akchagil. Besides, in forming of the Kotsakhuri fauna there participate evidently Pliocene elements, such as: ostrich (*Struthio trascaucasicus*), antelope protorix (*Protorix sp.*), Stenon equine (*Equns stenonis*), etruscan rhinoceros (*Dicerorhinus etruscus*), the vertical development range of that slightly rejuvenates bone comprising layers but the complex, as a whole, should be attributed to the upper part of the Upper Pliocene.

According to E.Kvavadze, who has carried out the paleontological study of the Kotsachuri deposits, the spectrum represented here is characterized by the equal quantity of dust of trees and leaf-bearing plants; *Platanns, Alnus* and *Pinus* predominate especially. The latter reaches 45-55% of the spectrum. Besides *Pinus,* there occur *Picea.* The rock also comprises much dust of *Juglans; Fagus, Carpinus,* Quercus and *Tilia* occur comparatively rear. Dust of broad-leaf bearing plants is represented by several grains. In the spectrum of herbage *Chenopodiaceae* and *Artemisia* occur [Vekua, Kvavadze 1981].

As it turns out during the process of deposition the Kotsakhuri bone-bearing layers, on the Iori Plateau there should dominate a landscape of Savanna type with groves where Alnus was widely represented.

Palinological and paleontological data show that on the territory of Southern Caucasus at the end of the Akchagil the climate should be approximately the same as that of Mediterranean shore - mild with damp winter and dry summer [Vekua, Kvavadze 1981].

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# კოწახურის პლიოცენური ხერხემლიანთა ფაუნა და ტარიბანას სპილო

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(მიღებულია 05.02.2010)

## რეზიუმე

კოწახურის (დედოფლისწყაროს რაიონი) ხერხემლიანთა ფაუნის გეოლოგიური ასაკი თავიდანვე განისაზღვრა როგორც ზედა აღჩაგილ-ქვედა აფშერონი. ახალი პალეონტოლოგიური აღმოჩენებისა, განსაკუთრებით კი ტარიბანას სამხრეთის სპილოს დეტალური შესწავლის შედეგად კოწახურის ხერეხემლიანთა ფაუნა და მისი შემცველი ნალექები დღეისთვის ზედა პლიოცენურად (ზედა აღჩაგილი) თარიღდება.

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Phytopathology

# IMPORTANCE OF VIRUS FREE POTATO SEEDS FOR IMPROVED YIELDS

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**Abstract**

Our experiments show that after planting in the open soil potato varieties (Agria, Marfona, Impala) have been infected by viruses Y, L and M. Variety Nevski was not transplanted in the open soil and hence was not infected. Therefore, we conclude that for virus free potato seed production it is important to conduct laboratory checking of the virus status of potato plants at every stage of the vegetation and to follow all measures to avoid virus propagation.

**Key words**: virus, potato, resistant, diseases, seed

## Introduction

At present, in Georgia there is no virus free, high productive, resistant to diseases, potato seed (elite seed). Potato seed is propagated during one or two seasons, after which it is infected by viruses.

All over the world, to avoid virus infestation of seed potatoes, apical meristeme method is used which allows to receive test tube virus free plants. From later, minitubers are obtained, first generation of this minitubers is super-super elite seed. This material is used for further propagation and elite seed production.

In seed potato industry, main constrain is virus contamination of seed material. From generation to generation viruses are accumulated in potato tubers and each following generation yields less than previous.

Potato virus symptoms are not always revealed immediately, significant role in symptom manifestation play such factors as growth conditions, season, time of plant infestation, existence of virus vectors, etc. [Bagnall, 1981].

There are number of viruses which affect the plant productivity. For example, virus **Y** (**potato virus Y - PVY**) is most common virus which causes leaf and tuber necrosis, [De Bokx, 1981]. **$PVY^0$** strain of this virus is widely circulated and causes mosaic symptoms, strains **$PVY^C$**, **$PVY^N$**, **$PVY^{NTN}$** are less common, however their diagnosis is very complicated [McKinlay, 1992].

In Great Britain, ***Potato leafroll virus*** (**PLRV**) is widely spread. If plant is infected later in the season the symptoms might be not visible, however from the infected tubers weak, low yielding plants with abnormal leafs will be produced.

In warm climatic conditions, **virus S (PVS)** is common; this virus, in subtropical climatic conditions infects other crops as well. This virus was only identified in 1950, as it is characterized by nonspecific symptoms. Some strains of this virus infect plants only early in the season at planting time, later on, the plant becomes immune to this virus. Virus symptoms are hard to be visually seen, little damage of leaves sometimes occurs.

**Virus A (PVA)**, is also very common, the disease symptoms are visible on the leafs [Rich, 1983].

It is impossible to identify the virus based only on visual symptoms. Symptoms depend on plant age, infestation time, temperature and host plant genetics. Virus infections are serious problem as they cause significant decrease in yields, e.g. **virus M** decrease the yield by 10-15%, **virus L** by 75%, **virus Y** by 25-35%.

From said above it is clear what problems can be faced when infected potato seed is used. Therefore, laboratory control of potato plant status is very important.

## Materials and Methods

Potato varieties Agria, Marfona, Impala and Nevsky were investigated. Potatoes were tested on virus content using immunoenzymatic double antibodies method (ELISA test). For immonoenzymatic analysis, standard kit of LOEWE Biochemica GmbH for DAS-ELISA was used. Virus specific serum was brought into the micropad cells for their absorption on the surface. After four hour incubation they were washed by buffer solution. After incubation homogenized and centrifuged plant material extract (200 µl) was applied to the pad cells. Virus free and virus positive samples were applied simultaneously. After 12 hour incubation, pad cells were washed by buffer solution and specific antibody-enzyme (alkaline phosphatase) complex was applied to the pad cells. At the end of 4-hour incubation period and buffer solution washing, substrate – 4-nitrophenol-phosphate solution was applied to the cells and after one hour color change was determined at 405 nm length. Color changes were measured by Stat FaxR2100 micropad photometer (AWARENESS Technology inc).

The goal of the study was to determine virus content of different potato varieties (Agria, Marfona, Impala, Nevski) and detect the possibility of their infestation in the laboratory conditions and also after planting in the open soil.

## Results and Discussion

Sample tubers of mentioned varieties were imported form Nederland by humanitarian organization Mercy Corps. Sample tubers at the time of import were checked on viruses. As it is seen from Table 1 none of the tubers were virus infected.

**Table 1.** Virus determination in various potato varieties by immunoenzymatic analysis

| Potato virus | Optical density | | | | Positive control | Negative control |
|---|---|---|---|---|---|---|
| | Agria n=5 | Marfona n=5 | Nevski n=5 | Impala n=5 | | |
| A | 0.053 | 0.045 | 0.041 | 0.068 | 2.325 | 0.045 |
| X | 0.049 | 0.067 | 0.057 | 0.061 | 2.232 | 0.058 |
| Y | 0.052 | 0.068 | 0.072 | 0.069 | 3.306 | 0.079 |
| S | 0.056 | 0.074 | 0.054 | 0.058 | 3.106 | 0.056 |
| M | 0.085 | 0.070 | 0.075 | 0.053 | 3.282 | 0.073 |
| L | 0.054 | 0.058 | 0.069 | 0.070 | 2.228 | 0.060 |

In the laboratory mother plants were received from the tested tubers. After first reproduction and transplanting into the open soil some varieties such as Agria, Marfona and Impala were infected by different viruses. As is seen from Table 2 Agria was infected by virus L, Marfona - by virus M, and Impala - by virus Y. As for Nevski (Russian variety), it was not transplanted into the open soils and hence no virus was detected.

Based on the study results, we can conclude that potatoes were infected in the open soil, as for Nevski it was not transplanted into the open soil and hence was not infected. It should be mentioned that different varieties were infected by different viruses. This could be explained by different immunity of varieties to different viruses.

**Table 2.** Virus determination in different potato varieties by immunoenzymatic analysis.

| Potato viruses | Optical density | | | | Positive control | Negative control |
|---|---|---|---|---|---|---|
| | Agria n=5 | Marfona n=5 | Nevski n=5 | Impala n=5 | | |
| A | 0.045 | 0.070 | 0.044 | 0.061 | 2.325 | 0.045 |
| X | 0.057 | 0.068 | 0.060 | 0.058 | 2.232 | 0.058 |
| Y | 0.055 | 0.072 | 0.071 | **3.718** | 3.306 | 0.079 |
| S | 0.054 | 0.080 | 0.056 | 0.059 | 3.106 | 0.056 |
| M | 0.086 | 3.256 | 0.086 | 0.043 | 3.282 | 0.073 |
| L | 2.205 | 2.075 | 0.075 | 0.068 | 2.228 | 0.060 |

Climatic conditions in Georgia (1,500 - 2,000 m elevation) allow selecting plots for elite potato seed production where virus vectors are not observed. This will reduce the usage of insecticides and produce ecologically clean potato seeds.

It is evident that virus control in the open soil is very complicated; insecticides are effective only in the case of aphides population monitoring. Therefore, based on the results of our study it can be concluded that the only way to avoid reduced harvest yields is introduction of virus free potato seeds, which can only be achieved at the first stage in the laboratory conditions and thereon in field, controlling virus contamination.

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# უვირუსო კარტოფილის წარმოების მნიშვნელობა მისი მოსავლიანობისთვის

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(მიღებულია 15.03.2010)

## რეზიუმე

ჩვენს მიერ ჩატარებული კვლევის შედეგად გამოვლინდა, რომ ღია გრუნტში გადატანილი კარტოფილის ჯიშები (აგრია, მარფონა, იმპალა) დაინფიცირდნენ **Y, L** და **M** ვირუსებით. ხოლო ნევსკი არ ყოფილა გადატანილი ღია გრიუნტში და შესაბამისად არ მოხდა მისი დაინფიცირება. შეგვიძლია დავასკვნათ, რომ უვირუსო კარტოფილის თესლის საწარმოებლად აუცილებელია მათი ვირუსებზე კონტროლი ლაბორატორიულ პირობებში და ყველა შემდგომ ეტაპზე იმ პირობების დაცვა, რომელიც თავიდან აგვაცილებს ვირუსების გავრცელების შესაძლებლობას.

სამეცნიერო ნაშრომი გამოიცემა ინგლისურ ენაზე, მას უნდა დაერთოს რეზიუმე ინგლისურ და ქართულ ენაზე, სამეცნიერო მიმართულება, სათაური, ავტორთა გვარები და მათი სამუშაო დაწესებულების დასახელება, საკვანძო სიტყვათა მოკლე (4-6) სია.

წერილის მოცულობა არ უნდა იყოს 5 გვერდზე ნაკლები და 12 გვერდზე მეტი. წერილი უნდა გაფორმდეს შემდეგი რუბრიკაციით: შესავალი და მიზნები (Introduction), მასალა და მეთოდები (Materials and Methods), შედეგები და მათი განხილვა (Results and Discussion), დამოწმებული ლიტერატურა. უკანასკნელი უნდა იყოს დალაგებული ანბანის მიხედვით, ხოლო ტექსტში წყაროების მითითება უნდა ხდებოდეს ფრჩხილებში ჩასმული ავტორის გვარითა და წლით [Lernmark, Hagglof 1981].

მითითებული ლიტერატურა წარმოდგენილი უნდა იყოს შემდეგნაირად:

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Carvalho C., Pereira H., Pina C. *Chromosomal G-dark bands determine the spatial organization of centromeric heterochromatin in nucleus.* Mol. Biol. Cell, **12**, 5, 3563-3572, 2001.

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Kuhn T.S. *The structure of scientific revolutions.* Chicago, IL, Chicago Press, 2000.

Brush S. *Flowing waters or teeming crowds.* In: Mental Models. D. Gentner (Ed.), Chicago IL., Chicago Press, 865-900, 2001.

მასალა რედაქციაში წარმოდგენილი უნდა იყოს ქაღალდზე ამობეჭდილი და დისკეტით (ან CD-ით). წერილი ერთი ფაილით უნდა იყოს შენახული (ცალკე ფაილად შეიძლება ილუსტრაციების წარმოდგენა), ხოლო ფაილის სახელწოდება წერილის პირველი ავტორის გვარს უნდა ატარებდეს.

ქართული ტექსტისთვის ოპტიმალური ფონტებია **AcadNusx** და **AcadMtavr**, ინგლისური ტექსტებისთვის - **Times New Roman**. შრიფტის ზომა - 12 პუნქტი, ინტერვალი - 1,5. ცხრილებში დასაშვებია უფრო მცირე ზომის შრიფტები. წერილი უნდა დაიბეჭდოს A4 ფორმატით, ზევით და ქვევით - 2,5 სმ., მარცხნივ – 3 სმ. და მარჯვნივ – 2სმ. დაშორებით. ცხრილები, გრაფიკები და დიაგრამები (მხოლოდ შავ-თეთრი) შესაძლებელია დამზადდეს როგორც Microsoft Word-ში, ისე Excel-ში, ფოტოსურათები მიიღება აგრეთვე ორიგინალების (არაელექტრონული) სახითაც.

ჟურნალის გამოცემა ავტორთა ხარჯებით ხორციელდება. თანხა რედაქციაში უნდა შემოვიდეს ნაშრომზე დადებითი რეცენზიის მიღებისთანავე. ნაშრომის რეცენზირება ანონიმურია და ავტორს აქვს უფლება მიიღოს ან არ მიიღოს რეცენზენტის შენიშვნები. უკანასკნელ შემთხვევაში ნაშრომი, დამატებით გაეგზავნება სარედაქციო საბჭოს ერთ-ერთ წევრს. მეორე უარყოფითი დასკვნის შემთხვევაში, ნაშრომი არ გამოქვეყნდება.

ნაშრომის ჩაბარება შეიძლება სამუშაო დღეებში, 12-დან 16 საათამდე, შემდეგ მისამართზე: თბილისი, რუსთაველის გამზირი 52, საქართველოს მეცნიერებათა აკადემია, ბიოლოგიის განყოფილება, IV სართული, 429 ოთახი, ტელ: 93-58-92, პასუხისმგებელი მდივანი – მაია გრიგოლავა.