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### Diversity and local use of wheat in Georgia

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### ABSTRACT

Georgia is characterized by remarkable diversity of wheat and an important tradition of wheat production, which have left significant mark on the local language, production practices, culture and everyday life of the local population. The Racha-Lechkhumi province in western Georgia was a refuge of hulled wheat species of makha and zanduri. Naked tetraploid dika and hexaploid bread wheat were major wheat species cultivated in the east and south of Georgia. The harvest practices differed between hulled and naked grain wheats. Some of the hulled wheat spikes are brittle and must be harvested first to avoid shattering. After cutting off heads, the spikes were thrashed and hulled grain was de-husked before milling. Stems were harvested separately. Naked-grain wheats were harvested in bundles at once and thrashed with special sleds on flat yards. The major types of ovens in Georgia were tóné and pourne. In tóné, the pieces of dough are stuck on the hot walls, while in pourne, dough is baked on flat stones. Both naked and hulled wheat grain produced flour of sufficient gluten quality to stick on the walls. The major types of bread are Georgian lavashi and shoti. The Georgian lavashi is very different from the Asian lavahs. The Georgian lavashi is round, relatively thick and is baked in tóné, while the Asian lavash is very thin and baked on flat stones. Other type of local breads like somini and Meskhuri lavashi were spread in South Georgia. Various meals are produced from wheat flour and dough in Georgia. Wheat flour was widely used in folk medicine to treat wounds, diarrhea, stomatitis, while sprouted wheat was used to support people with broken health. The chaff of wheat is fed to livestock. Wheat bundles were used to cover the roofs of houses and barns in rural areas until the middle of the  $20^{th}$  century.

Keywords: Wheat, Hulled, Naked-gain, Use, Bread, Production practice.

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### Introduction

To this day, the South Caucasus region, especially Georgia, is renowned for its delicious ancient wheat species that are almost unknown to the rest of the world. It is largely overlooked by wheat researchers that Georgia is one of the main centers of wheat origin and the most important center of wheat diversity in the world. The archeological excavations of Neolithic sites suggest that popula-

tion of Ancient Georgia began cultivation of wheat 8000 years ago [1, 2]. Cultivation of wheat by Georgians is mentioned in the works of Greek historians Herodotus and Xenophon [3, 4]. The names of the wheats 'Ipkli' 'Dika' and 'Asli' were first mentioned in Georgian written sources as early as the 5th century AD.

Wheat production and use have left significant impact on the local language, production practices and everyday life of the local population. The role of bread, wheat grain and flour in local diets is very

high. Georgia is among the top ten countries in terms of consumption of wheat per capita. Although wheat is not a big crop in modern Georgia, its histori- cal role for local agriculture and impact on the culture of the local dwellers cannot be overestimated.

### The diversity of wheat in Georgia

Georgia is the only country in the world, where 15 out of 20 wheat species recognized worldwide of wheat are present: Triticum boeoticum Boiss., T. monococcum L., T. dicoccum (Shrank) Schübl., T. palaeocolchicum Menabde, T. timopheevii (Zhuk.) Zhuk., T. durum Desf., T. turgidum L., T. carthlicum Nevski, T. macha Dekapr. & Menabde, T. zhukovskyi Menabde & Ericzjan, T. turanicum Jacubz., T. polonicum L., T. spelta L., T. compactum Host, and T. aestivum L. Five species (T. timopheevii, T. zhukovskyi, T. macha, T. palaeocolchicum, T. carthlicum) are endemics to Georgia [5]. Out of the 15 wheat species represented in Georgia, 8 are hulled, and 7 are naked (free-trashing). All endemic species except T. carthlicum are hulled. Georgia is distinguished by the highest number of tetraploid wheat species.

Wheat species: *T. macha, T. palaeocolchicum, T. compactum* and *T. spelta* as well as some forms of *T. dicoccum* and varieties of *T. aestivum* have a vernalization requirement and belong to winter wheats (are sown in the fall). *T. monococcum, T. timopheevii, T. zhukovskyi, T. durum, T. turgidum, T. carthlicum* and some varieties of *T. aestivum* do not have a vernalization requirement and are characterized by spring growth habit. Winter is mild in Georgia and most of the spring wheats often produce reasonable yields when sown in the fall.

### **Hulled wheats**

The tough glumes of hulled wheats give excellent protection to the crop in the field and in storage. These wheats are also resistant to poor soil conditions and a range of fungal diseases [6]. Although the share of the hulled wheats decreased even more over the centuries in Georgia, the West of the country (predominantly Province of Lechkhumi) still represented a repository of hulled wheats by the first half of the 20<sup>th</sup> century as evidenced by many researchers. Two major hulled wheat landraces, Zanduri and Makha were widely produced there.



**Fig. 1.** T. timopheevii (chelta zanduri) in the living collections of the Institute of Botany and Georgian National Botanical Garden (Photo of I. Maisaia)

Zanduri landrace consists of three species: *T. monococcum* var. *hornemanii* (gvatsa [narrow] zanduri), *T. timopheevii* (chelta [wide] zanduri) and *T. zhukovskyi* (zanduri). Gvatsa zanduri is not endemic to Georgia, as it was widely spread in other wheat producing regions as well and it is not considered in the present paper. However, chelta zanduri and hexaploid zanduri are endemics and found only in Georgia. Zanduri landrace was sown in Lechkhumi and Racha until 1960-ies.

The makha landrace is composed of *T. macha*, a hulled hexaploid (AABBDD) and *T. palaeocolchicum* (Colchis emmer) a hulled tetraploid (AABB) wheat, both are endemics to Georgia. *T. palaeocolchicum* was first described in Lechkhumi by V. Supatashvili in 1929 [7].

In Makha fields, *T. macha* itself was presented in great variation for spike color (white and red) awnedness (awned, semi-awned and awnless) and hairness of glumes. The most widespread form was white spike with short awns and without hairs. Fourteen varieties of Makha have been identified by Georgian researchers Dekaprelevich and Menabde in 1932 [8, 9].

### Naked (free-thrashing) wheats

Among the naked wheats, Kartlian (east Georgian), not "Persian" [9] wheat - *T. carthlicum* (tetraploid) and bread wheat - *T. aestivum* (hexaploid) had the most remarkable economic significance in

Georgia, which grew over the centuries. Both *T. carthlicum* (local name Dika) and *T. aestivum* (under the old name Ipkli) were mentioned in the written sources of the 5th century AD together with the hulled wheats [4]. They occupied much larger areas than hulled wheats and were cultivated in various environments, mostly in the Samtske-Javakheti Region (South Georgia) and Kakheti (East Georgia). Dika was produced predominantly in the mountains, while bread wheat production was confined with the lowland areas.

Triticum carthlicum - Dika ranks second to T. aestivum according to its distribution and significance for the Georgian agriculture. Typically, Dika (Fig. 1) was produced in the highlands of Georgia, within 1000-2000 m asl. However, some its fields were spread as low as 750 asl and as high as 2300 m asl. Dika is characterized by great intraspecific diversity, which allowed to identify eleven varieties. However only three of them covered significant areas. Black dika, with black glumes (T. carthlicum var. fulliginosum Zhuk.) was distributed across the Great Caucasus Range forest belt (900-1400 m asl) [10]. Red dika (T. carthlicum var. rubiginosum Zhuk.) dominated in more drought-prone areas of Samtskhe-Javakheti, Trialeti, Kartli, Pshav-Khevsureti, Mtiuleti, Tusheti, Imereti, Racha-Lechkhumi, and Svaneti. White dika (var. stramineum Zhuk.) is found as a mixture in bread wheat fields of Trialeti, within the Rioni, Liakhvi, Enguri, and Kvirila river valleys (Fig. 2).

Although dika and bread wheat production areas did not overlap, dika was often mixed with bread wheat. Farmers were not able to maintain purity of the fields because dika is strikingly morphologically similar to bread wheat (*T. aestivum*). However, mixture of bread wheat grain provides for better bread baking quality of the flour and is beneficial for farmers. E.g. the most widely spread wheat landrace in Samtskhe-Javakheti Region was called Javakhetian dika, which was a combination of Red dika with a spring soft [11-13].

Dika-barley mixtures were also widespread in the cold areas. In the mixed plantings two layers are formed in the grass stand, as barley is much shorter than dika. Such stands can be sown with higher density as competition between plants of the different species is alleviated because of the layer differentiation. The bread baked from the flour of this mixed crop outperformed in quality the barley bread.



**Fig. 2.** Dika field near village Tsnisi, Meskheti (Photo of M. Mosulishvili)

Dika mixed with rye was also widespread in the mountain areas of Georgia. It is not surprising as wild rye is a widely spread weed in the wheat fields in Georgia. In this case, two-layer stands were also formed as rye is significantly higher than dika. The bread produced from the mixture of dika and rye had a specific flavor, which was praised for good taste by the local population of the western Georgian provinces of Racha and Lechkhumi.

Bread wheat - *T. aestivum* (local name Doli, Dolis-puri) was cultivated in every locality of Georgia, which had at least minimal environmental conditions for wheat cultivation. This was possible due to the large diversity of the species including a large number of botanical forms demonstrating different growth habits (winter, spring and facultative varieties) and adaptation to different abiotic and biotic stresses.



**Fig. 3.** Meskhuri doli field near village Tchobareti, Meskheti (Photo of M. Mosulishvili)

However, the most widespread bread wheat variety was tsiteli [red] doli (*T. aestivum* var. *ferrugineum*). The diverse ecological conditions of Georgia made it possible to develop several ecotypes within doli, which formed the following three major local landraces: Meskhuri Tsiteli [red] Doli, Kakhuri Doli, and Svanuri Doli. They developed under completely different ecological conditions of the provinces of Meskheti, Kakheti and Svaneti, respectively (Fig. 4). These landraces are noted for their prolific tillering ability, yield stability, resistance to shattering and some diseases. Georgian Red Doli is produced as a heritage wheat in southern France and is known there as 'the Caucasus Rouge' [13].

### Wheat harvest

Naked grain wheat plants were harvested in bundles and threshed on a flat yard called Kalo (Fig. 4) using a special tool - threshing sled (local name Kevri, Fig. 5), a flat wooden board with stone teeth on the lower side. When the naked species are threshed, the glumes and chaff break and the grains are immediately released. Kalóóba, the day of wheat thrashing was an important event celebrated by local people. This celebration is revived in modern Georgia.



**Fig. 4.** Kakhuri doli field near town Dedophlis-Tskaro (Photo of M. Mosulishvili)

Wheat grain was milled on water mills. Large round stone, which was used for grinding of wheat grain was called 'dolabi'. The used-up surface of the dolabi was regularly up-graded using a cavil to improve cohesion with grain, which provided for well-grinded flour. This work was called 'mokhod-

va'. When a new or up-graded dolabi was installed for the first time, the initial portion of flour was discarded as it could contain small stones or mild rocks removed by cavil.



Fig. 5. Kalóóba



**Fig. 6.** Kevri thrashing sled (Photo of G. Chkhutiashvili)

In case of the hulled wheats, because their spikes were brittle, the local population harvested spikes on the first place to avoid shattering using a special tool Shnakvi. It consisted of two sticks, which were tied together [4]. The wheat spikes were gathered in baskets and taken from the field separately from stems. As thrashing was not sufficient to liberate grain from chaff, the grain was further dehusked by pounding before milling. However hulled seed was used for seeding. After the harvest of the spikes was finished, wheat stems were cut with sickles and bundled. A similar approach was used in areas where spelt wheat was produced in Spine [14].

### Uses in food

Georgian bread was mostly baked in two kinds of the oven: tóné and pourne (Fig. 7). Tóné is a bottomless cylindrical clay construction, embedded in



soil, of about 1 m height. Bread is stuck on the hot walls in tóné, which is heated by burning firewood on the soil (Fig. 7a). Pourne is constructed from stones and looks more similar to house ovens (Fig. 7b), where bread is arranged on flat stones, which are heated with burning wood.

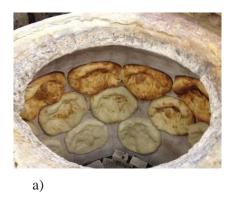


**Fig. 7.** Traditional ovens in Georgia: a) tóné (Photo of Nino Ustiahsvili) and b) pourne (Photo of M. Mosulishvili)

It is important that dough easily to sticks to the hot walls, doesn't not fall off and burn while baking for baking bread in tóné. The local varieties of bread wheat, as well as other wheat species of dika, makha and zanduri, which are discussed in this paper, are suitable for baking of bread in tóné as have a sufficient gluten content and gluten viscosity. Hulled wheat Makha's bread was considered as of high quality among the local population of Racha-Lechkhumi. Makha bread is white, tasty and flavorful, not to mention its ability to remain soft for several days. It was honor to treat guests to 'makha' bread at feasts.

Bread with different forms are baked in Georgia (Fig 8). The major local wheat types include Geor-

gian lavashi and Kakhuri [Kakhetian, east Georgian] shoti. Georgian lavashi is very different from the Asian lavash. The Asian lavash is widely spread in Iran, Armenia, Azerbaijan, Central Asia. It looks like thin sheet. It is produced from very flat, well-rolled unleavened dough, which is baked on 'saj' – a flat round or square stone or metal pans heated by firewood inside the traditional ovens or outside them. The Georgian lavashi is much thicker and it has a round shape. Unlike to the Asian lavash, the Georgian lavashi can be baked stuck to the walls of tóné. The major difference of shoti from Georgian lavashi is in its form. Kaketian shoti has a prolonged, sometimes arced shape with sharped ends (see Fig. 8b).







**Fig. 8.** Examples of Georgian bread: a) Georgian lavashi and b) Kakhetian shoti and c) somini (Photos of N. Ustiashvili and L. Meskhi)

a)

Yeast is not applied directly in the traditional breads, but some amount of the old sour dough is retained and mixed in the new dough for leavening. Mixed dough is left for a night before baking to achieve good level of leavening.

According to Jalabadze *et al.* bread was a staple food in Meskheti and Javakheti (South Georgia)

[15]. It was baked in pourne. Meskhuri [Meskhetian] bread produced from doli wheat was widely spread in the region. However, the local population also differentiated bread produced from dika, which was called Makhnia bread. This bread was mainly produced in the villages located in the uplands of the river Mtkvari basin [16].





**Fig. 9.** *Meskhuri [Meskhetian] breads: a) podola and b) somini (Courtesy of L. Meskhi)* 

Local bread in Meskheti is often baked in the form of 'podola' (round flat bread with a whole in the center; named also Meskhetian bread) and 'somini' (big spherical) breads (Fig. 9), 'kakala' (small spherical) bread were also widespread.

Various meals are produced from wheat flour and dough in Georgia [17]:

**Tatarbega** - dough is cut in small pieces of the form of butterfly, which is boiled in salted water and is seasoned with matsoni (Georgian sour yoghurt - fermented milk of local production), garlic and panfried onion.

**Katmari** – five layers of dough filled with ghee between the layers and spliced edges baked in an oven.

**Lukhumi** – walnut-size balls produced through mixing flour with milk and frying these balls in ghee on a pan.

**Sironi** – thin dough is rolled over a wooden stick (called 'ukhlavi'), cut in circles, baked in an oven, fried on a pan and dipped with ghee and matsoni. After boiling it is kept in warm temperature for a short time before serving (Fig. 10).



**Fig. 10.** *South Georgian [Meskhetian] meal sironi (Authors: Sh. Vanadze and E. Poladishvili Source:* https://mtianiachara.wordpress.com/2015/11/ in Georgian)

**Erishta**—long and narrow pieces of dough, dried in pourne, are rinsed in boiling water and dipped in ghee and matsoni.

**Makarlama** – boiled pieces of dough are seasoned with pan-fried onion and shredded cheese.

**Khavitsi** – mixture of flour with water, milk and ghee is boiled on light fire until it gets consistency of thick creamy soup.

**Korkoti** (**kolio**) – boiled grain mixed with milk, honey and walnuts. Meal, which is used in rituals organized for remembrance of someone who passed away.

**Kumeli** – roasted flour, sometimes mixture of wheat and barley.

### Uses in folk medicine and other uses

Wheat has been widely used in folk medicine in Georgia. Wheat flour was a component of many ointments. Khavitsi (mentioned as meal in the previous section) was used to drain pus from wounds. Wheat grain broth was mixed with alum and used mouth rinse to treat stomatitis. Wheat starch was used to control diarrhea. Meals cooked with sprouted wheat seed was used to strengthen people with broken health or pregnant women. Local people also used to drink a decoction of Makha grains to improve eyesight [12].

Chaff of wheat is fed to livestock. Wheat bundles were used to cover the roofs of houses and barns [18]. In the province of Lechkhumi, the small houses and farm buildings were covered with "Koroli" the clustered stalks of Zanduri [4].

### Conclusion

Georgia is a country of ancient agriculture, characterized with outstanding diversity of wheat species. These species represent different directions and stages of wheat evolution and suggest that the population of Georgia was involved in wheat domestication. These involvement is reflected in diverse production practices, heavy use in local cuisine and folk medicine. Wheat has become an integral part of the local culture.

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# Study of honey Andromedotoxin in Western Georgia by UPLC-MS method

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### ABSTRACT

Cases of honey poisoning have been reported in Western Georgia (Adjara), meaning there is a need for methods that detect "mad honey" or honey contaminated with plant-derived toxins to protect human health. The purpose of this study was to determine the levels of Grayanotoxin-III, Total phenolic compounds (TPC) and antioxidant activity of honey produced in mountainous Adjara (West Georgia), using ultra-high-performance liquid chromatography mass spectrometry (UPLC-MS) and by spectrophotometric methods. Honeys were provided by beekeepers and collected from different locations in mountainous Adjara (an altitudinal range of 200-3007 m above sea level). The harvest was taken in the autumn of 2019. In this study, we compared whole of a Flower of Rhododendron extracts, mad honey and 12 samples of autumn honey. Liquid chromatography with mass spectrometry (Waters, UPLC Acquity, QDa Detectore) was used for the identification of andromedotoxin (grayanotoxin-III). The analytical column was a Acquity UPLC BEN C18. GTX-III was eluted using a mobile phase consisting 50:50 water/methanol solution containing 1% acetic acid at flow rate of 0.3 mL/min in 8 min. A Mettler Toledo UV-5 model UV-VIS spectrophotometer was used for Determination of phenolic compounds and Antioxidant activity Results and discussion - Using the UPLC-MS method grayanotoxin-III were identified in the honey samples. MS data were acquired by running electrospray ionization (ESI) in negative ion mode using selected reaction monitoring (SRM) after describing the real molecular weight of GTX-III by full scan in the range of 200-500 m/z. For comparing chromatography analyses was used rhododendron's flower and mad honey samples, where consistency of toxin was much higher than in other honey's Samples. The toxin content in honey increases with increasing height (Mean sea level). No correlation is observed between toxin content and antioxidant activity, while there is a direct correlation between phenolic compounds content and antioxidant activity. Honey grown in highlands of Adjara, in parallel with the toxin content (4.2 – 24.0 mg/kg), is characterized by a high content of phenolic compounds (407.54 – 1004.5 g/kg) and it also has a strong antioxidant activity (61.5 j- 175.0 50% inhibition mg of samples).

Keywords: Honey, Grayanotoxin-III, Phenolic compounds, Antioxidant activity, Chromatography, Mass spectrometry.

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### Introduction

Honey is one of the most widely sought products due to its unique properties, that are attributed to the influence of the different groups of substances it contains [1]. In addition to being used as food, honey has been used as an alternative medicine for thousands of years. Honey has a great potential to be used as a medicine because it is not suitable for microorganisms, it is very acidic and has a very high sugar content [2], which causes an osmotic effect that prevents the growth of some microorganisms, moreover, in some honey, hydrogen peroxide is

found, which has a strong antibacterial effect [3. 4].

One of the most common and persistent therapeutic uses of honey has been as a wound dressing [5, 6], almost certainly due to its antimicrobial properties [7]. With the advent of highly active antibiotics in the 1960s, honey was dismissed as a "worthless but harmless substance". However, the current and growing crisis of antibiotic resistance has revived interest in the use of honey, both as an effective agent in its own right and as a therapeutic lead to develop new methods of treatment [8, 9].

Properties and appearances of honey vary greatly according to the floral source in which the bee

collects the nectar [3, 7]. Honey made with poisonous nectars contain toxic chemicals that pose a direct risk to consumers [10, 11]. These toxic honeys, commonly mentioned as "mad honey" (also referred to as "bitter honey"), have usually reddish-brown color with unusual sharp and biting taste that is irritating to throat [12-15]. Mad Honey is Different from the Common Commercial Honey. It is contaminated with grayanotoxin, which causes intoxication [16-18]. Mad honey poisoning was first described in 401 BC by Xenophon, an Athenian author and military commander [19, 20]. Mad honey was also used in Northern Anatolia against the armies of Pompey by King Mithridates Eupator of Pontus in 97 BC [15].

A typical course of poisoning consists of the gastrointestinal system irritation, cardiac arrhythmias, and neurological symptoms [10,21]. In untreated cases of severe intoxication, the worst signs and symptoms last about 24 hours. By the end of that time, the patient is alert and vital signs are normal. Complete recovery may take several more days [15]. Mad honey poisoning is frequently reported in the Eastern Black Sea region of Turkey. It was also well described in North America and Europe 100 years ago [15, 22-24].

Since toxins taken up from rhododendron flowers by bees are not detoxified in their organisms, they cause poisoning by becoming directly mixed in with honey (mad honey poisoning) [25-27]. The grayanotoxins bind to the sodium channels in cell membranes. These compounds cause activation or inactivation of the channels; excitable cells are thus maintained in a state of depolarisation, during which entry of calcium into the cells may be facilitated [26, 28-30].

The flowers of Rhododendron species are widely spread over countries such as Spain, Portugal, Japan, Brazil, the United States, Nepal, Great Britain, and especially Turkey [12,16].

The Rhododendron family contains more than 750 plant species, most of which, although not all, contain toxins (e.g., grayanotoxins [GTXs]) [12, 31, 32]. Purple-flowered (Rhododendron ponticum) and yellow-flowered (Rhododendron luteum) rhododendrons are widespread along the Black Sea coast (Georgia, Turkey) [29, 13, 20]. The former is also known as "black poison" and the latter as "yellow poison" [29]. Since these plant species grow in forested areas at specific altitudes and in specific valleys the local inhabitants know which honey produced in which region is mad honey [29]. Intoxication is caused by the grayanatoxin content of the flowers which honey is produced, also called andromedotoxin, acetylandromedol or rhodotoxin [14, 33]. Grayanotoxins are toxic polyhydroxylated diterpenes [31,34]. More than 25 GTX isoforms have been isolated from the Rhododendron family. While GTX-I and GTX-II are found in smaller amounts, the GTX-III isoform is the principal toxin in mad honey (Figure 1) [12,36].

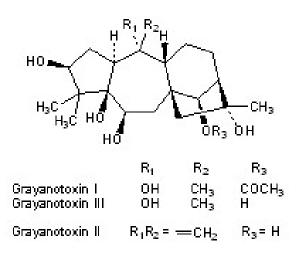


Fig 1. Chemical formula of grayanotoxin

Mad honey produced in spring is more toxic and contains more grayanotoxin than that produced in other seasons [16, 35]. These plants usually bloom in late May and early June. The rate of GTX in these plants varies depending on whether the weather is rainy or not when the plants are blooming. During periods when rain is low, the GTX ratio is high and this increases the likelihood of honey poisoning [28].

The average amount of honey taken from GTX poisonings is generally between 5 and 30 g. Symptoms start within 1.5 to 3 hours after eating honey [28]. Despite its toxic effects, Rhododendron honey is consumed as an alternative medicine against various diseases [37,12] (used for hypertension, diabetes, gastrointestinal disorders, abdominal/gastric pain, arthritis, stimulating sex, various viral infections, skin ailments, pain, and cold) [38], This honey contains thousands of different phenolic compounds which possess antioxidant properties that defend cells against attacks by free radicals [39, 37, 12].

Commercial honey packers receive honey from thousands of hives; therefore, toxin coming from any one hive is diluted. Also, the honey sold by an individual bee keeper is often unprocessed [20]. Honey can be potentially therapeutic or toxic depending on its dose. Lower doses of honey could have potentially therapeutic short-term antiarrhythmic and long-term cardiovascular benefits. As the old adage goes, "One man's poison is another man's cure" [40]. Mad honey can be also found in Western Georgia. The Georgians call it Shkeri Honey. At present, around 3-4 thousand tonnes of honey are produced annually in Georgia. Georgia mainly produces five types of honey in large amounts. These

are acacia honey, blossom honey, alpine honey, linden honey and chestnut honey [41]. Beside these types, there are special honeys in Georgia such as Jara honey (wild honey), rhododendron honey, honey from laurel, willow, solidago and others. However, their output is low (up to 3 tonnes) mainly due to low demand from the market [13].

Cases of honey poisoning have been reported widely in Western Georgia (Adjara), meaning there is a need for methods that detect "mad honey" or honey contaminated with plant-derived toxins to protect human health [42]. For example, in cold spring conditions, when yale and sage precede the flowering of the main melliferous plants, bees feed on the nectar of yale and sage.

The purpose of this study was to determine the levels of Grayanotoxin-III, Total phenolic compounds (TPC) and antioxidant activity of honey produced in mountainous Adjara (West Georgia), using ultra-high-performance liquid chromatography mass spectrometry (UPLC-MS) and by spectrophotometric methods.

### 2. The purpose of this study

The purpose of this study was to determine the levels of Grayanotoxin-III, Total phenolic compounds (TPC) and antioxidant activity of honey produced in mountainous Adjara (West Georgia), using ultra-high-performance liquid chromatography mass spectrometry (UPLC-MS) and by spectrophotometric methods.

### 2.1 Samples

Honeys were provided by beekeepers and collected from different locations in mountainous Adjara (an altitudinal range of 200–3007 m above sea level). The harvest was taken in the autumn of 2019. In this study, we compared whole of a Flower of Rhododendron extracts, mad honey and 12 samples of autumn honey (Table 1).

The honey specimens were kept in containers made of sealed polyethylene at 4-5 ° C. Liquid or crystallized honey without impurities was homogenized for 3 minutes. The honey with impurities was filtered in a stainless steel grating with a diameter of 0.5 mm before its homogenization.

Compiler	Canadanasada	Hamisak daka	Mean sea level							
Samples	Samplers code	Harvest date	(MSL)							
Autumn Honey 1	AH - 1	2019	450 m							
Autumn Honey 2	AH - 2	2019	600 m							
Autumn Honey 3	AH - 3	2019	780 m							
Autumn Honey 4	AH - 4	2019								
	MSL – 420 – 2662 m									
Autumn Honey 5	AH - 5	2019	720							
Autumn Honey 6	AH - 6	2019	760							
Autumn Honey 7	AH - 7	2019	1400							
Autumn Honey 8	AH - 8	2019	1600							
	MSL – 400 –	3007 m								
Autumn Honey 9	AH - 9	2019	923 m							
Autumn Honey 10	AH - 10	2019	1000 m							
Autumn Honey 11	AH - 11	2019	1750 m							
Autumn Honey 12	AH - 12	2019	2040 m							

**Table 1.** Samples of Autumn honey taken for analysis

### 2.2 Chemicals and instruments

All reagents and chemicals used were analytical grade from Sigma and Merck Chemical Company, A Mettler Toledo UV-5 model UV-VIS spectrophotometer was used for absorbance measurements, A Waters Acquity UPLC/H Class (PDA Detector) was used for identification of Grayanotoxin.

### 3. Materials and methods

Liquid chromatography with mass spectrometry (Waters, UPLC Acquity, QDa Detectore) was used for the identification of andromedotoxin (grayanotoxin-III). The analytical column was a Acquity UPLC BEN C18. GTX-III was eluted using a mobile phase consisting 50:50 water/methanol solution

containing 1% acetic acid at flow rate of 0.3 mL/min in 8 min. A Mettler Toledo UV-5 model UV-VIS spectrophotometer was used for Determination of phenolic compounds and Antioxidant activity

### 3.1 Research methods

### 3.1.1 Determination of Grayanotoxin-III

Approximately 5 g of honey sample was extracted with 30 mL methanol in a flask attached to a condenser at 60°C in 6 h. Extract was subsequently filtered to remove particles, and the final volume was determined, with 5 mL methanol extract being set aside for antioxidant activity analyses. The remaining extract was evaporated until dry using a rotary evaporator (INGOS RVO 400) at 40°C. The residues were dissolved in 10 mL distilled water and transferred to a C18 solid phase extraction (SPE) cartridge (Waters Sep-Pak, Vac 6cc, C18 – 500mg) which was initially conditioned with 5 mL methanol followed by 5 mL water. The cartridge was washed with 5 mL water to remove unbound materials. GTX-III was eluted from C18 SPE using 5 mL methanol. Finally, the organic solvents were evaporated in a rotary evaporator under reduced pressure at 40°C. The residue was weighed and dissolved in methanol for LC/MS-MS analysis [16].

Liquid chromatography-tandem mass spectrometry (Waters, UPLC Acquity, QDa Detectore) was used for the identification of grayanotoxin-III. The analytical column was a Acquity UPLC BEN C18. GTX-III was eluted using a mobile phase consisting 50:50 water/methanol solution containing 1% acetic acid at flow rate of 0.3 mL/min in 8 min [16].

# **3.1.2 Total content of phenolic compounds** (TCPC)

Honey solution (100 mg/mL-1) was previously homogenized and filtered through quantitative filter, 500 mL of honey solution was added to 2,5 mL of Folin Ciocalteu (0.2 N), After 5 min, 2 mL of sodium carbonate solution (Na2CO3-75 g L1) was added and incubated for 2 h in the dark, The absorbance was measured at 760 nm in a spectrophotometer [24.29]. Standard curve was defined by known concentrations of gallic acid, ranging between 0 and 200 mg L1 and results expressed in milligrams of gallic acid equivalents (mgGAE·100 g1).

### **3.1.3 Antioxidant activity** (assay with DPPH)

The DPPH assay was done by using 750 mL of honey solution that was mixed with 1,5 mL of

DPPH solution in methanol (0,02 mg/ mL), The mixture was homogenized for 30 min at room temperature and then the absorbance was determined at 517 nm [44, 43, 14]. Antioxidant activity DPPH - 50% inhibition mg of samples was calculated using the following formula 1 and 2:

AA % inhibition = [A(DPPH) - A(sample)\*100]/A(DPPH) (1)

Where:

A (DPPH) - A(sample) - Absorbance of DPPH at 517 nm

A(sample) - Absorbance of sample at 517 nm AA of mg sample = m\*50\*1000/V\*AA% (2), where

m – weight of sample,

V – volume of sample,

50 - % of inhibition,

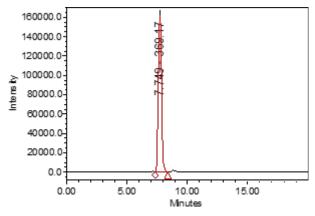
1000 – unit conversion from gram to mg

AA% - % inhibition.

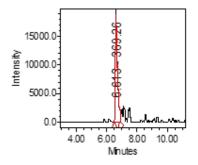
### 4. Results

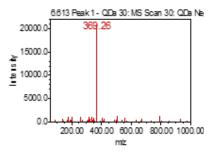
The identification of grayanotoxin-III - Using the UPLC-MS method grayanotoxin-III were identified in the honey samples. MS data were acquired by running electrospray ionization (ESI) in negative ion mode using selected reaction monitoring (SRM) after describing the real molecular weight of GTX-III by full scan in the range of 200–500 m/z. The molecular weight of GTX-III is 370 g/mol, appearing at m/z 369 in negative ion mode.

A substance 1 (Fig.2) is retention time 7.749 min, m/z 369 [M-H]+, λ max 289 nm; according to the obtained results and compounds mass database METLIN (https://metlin.scripps.edu) the substance 1 is grayanotoxin-III. - C20H36O6 Negative FABMS: m/z= 369.26 [M-H+], Molecular Weight: 370 g/mol.



**Fig. 2.** Chromatograms of a Flower of Rhododendron, scan ESI-MS m/z: 369 [M-H+]





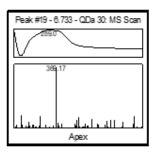


Fig. 3. Chromatograms of a Mad honey, scan ESI-MS m/z: 369 [M-H+]

For comparing chromatography analyses was used rhododendron's flower and mad honey samples (Figures 2 and 3), where consistency of toxin was much higher than in other honey's Samples (Figures 4 - 8).

Among the 12 samples of honey taken for analysis, the toxin was identified in 5 samples of honey - AH-4, AH-7, AH-8, AH-11 and AH-12. In particular, in samples taken at 1566, 1400, 1600, 1750 and 2040 meters above sea level (Figure 4 - 8) (Table 2). The toxin content in honey increases with increasing height (4.2 – 24.0 mg/kg) (Table 2). It is likely that the blossoming of honey plants in cold and late spring conditions precedes the flowering of rhododendrons, which is consequently reflected in the composition of honey [13,20].

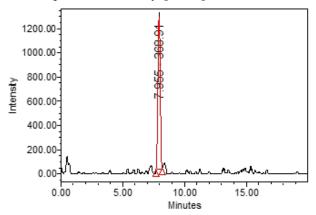
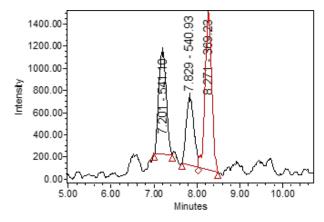
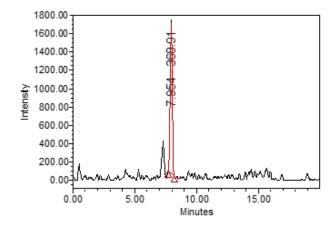


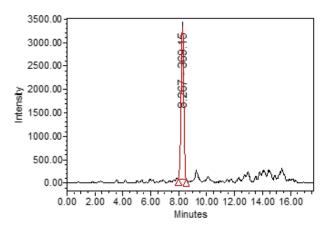
Fig. 4. Chromatograms of Honey produced in autumn; MSL – 1566 m; (AH 4), scan ESI-MS m/z: 369 [M-H+]



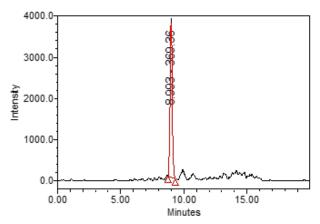
**Fig. 5**. Chromatograms of Honey produced in autumn; MSL – 1400 m; (AH 7), scan ESI-MS m/z: 369 [M-H<sup>+</sup>]



**Fig. 6.** Chromatograms of Honey produced in autumn; MSL – 1600 m; (AH 8), scan ESI-MS m/z: 369 [M-H<sup>+</sup>]



**Fig. 7.** Chromatograms of Honey produced in autumn; MSL – 1750 m; (AH 11), scan ESI-MS m/z: 369 [M-H<sup>+</sup>]



**Fig. 8.** Chromatograms of Honey produced in autumn; MSL – 2040 m; (AH 12), scan ESI-MS m/z: 369 [M-H<sup>+</sup>]

**Table 2**. The grayanotoxin-III content of autumn honey

Samples	Grayanotoxin-III mg/kg
Mad honey	500
AH - 4	4.2
AH - 7	6.3
AH - 8	8.4
AH - 11	17.0
AH - 12	24.0

In mountainous Adjara, the alpine and subalpine forest zone is distinguished by the diversity of honey plants [43, 44], therefore, most of the honey harvested in this area is polyfloral and rich in its chemical composition. In addition to the toxin, the quantitative content of phenolic compounds and antioxidant activity were also determined in the honey samples taken for analysis.

The total content of phenolics was between 407.54 and 1004.5 mg/kg (Table 3). The highest phenolic content of samples was found in honey samples: AH -4 (907.37 mg/kg), AH -4 (932.2 mg/kg) and AH -11 (1004.5 mg/kg) (Table 3).

**Table 3**. *Total phenols and antioxidant activity of autumn honey* 

	·	
		Antioxidant activity
Camandaa	Total phenols	DPPH - 50% inhibition
Samples	mg/kg	mg of samples
AH - 1	407.54	145.2
AH - 2	468.98	130.0
AH - 3	642.82	110.0
AH - 4	907.37	66.5
AH - 5	776.8	85.2
AH - 6	503.11	107.0
AH - 7	658.2	95.5
AH - 8	932.2	65.0
AH - 9	562.61	175.0
AH - 10	895.0	70.0
AH - 11	1004.5	61.5
AH - 12	808.12	80.11

Among honey samples, those with a total phenol content of 808.12 to 1004.5 mg / kg have a relatively high antioxidant activity (50% inhibition 80.11-61.5 mg of samples). In the presented samples by the relatively high activity stands out honey samples - AH -4 (66.5mg), AH -8 (65.0 mg) and AH -11 (61.5 mg) (Table 3).

### 5. Conclusion

For comparing chromatography analyses was used rhododendron's flower and mad honey samples, where consistency of toxin was much higher than in other honey's Samples. The toxin content in honey increases with increasing height. No correlation is observed between toxin content and antioxidant activity, while there is a direct correlation between phenolic compounds content and antioxidant activity. Honey grown in highlands of Adjara, in parallel with the toxin content, is characterized by a high content of phenolic compounds and it also has a strong antioxidant activity.

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# Antibacterial activity screening of some endemic plants of Adjara floristic regionand secondary metabolites - essential oils

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### ABSTRACT

In order to find alternative remedies for replacement of so-called food antibiotics in livestock and poultry, antibacterial activity screening was carried out on the methanolic extract of some endemic plants of Adjara and Adjara-Lazeti, as well as some essential oils and compositions based on them. Based on conducted expiremental researches, it was established that Hypericum nordmanni Khokhr., Hypericum ptarmicifolium var. adzharicum and Linaria adzharica Kem. methanolic extracts have pronounced and wide spectrum antibacterial action. Also, it should be noted that 0.1% aqueous solutions of Thymus vulgaris L., Salvia sclarea L. and Monarda didyma L. essential oils and compositions made of them was found to have high antibacterial activity. The obtained results testify to the necessity of further research of the identified antibacterial plants and essential oils for their use in medical practice.

Keywords: Endemic plants, Secondary metabolites, Essential oils, Antibacterial activity, Methanolic extracts, Spot test.

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### Introduction

Modern technologies for farming animals and poultry involve wide use of antibiotics. In the 1950s, took start usage of antibiotics to eradicate the pathogenic microflora in the food, to prevent disease, as well to stimulate growth and productivity in animals and poultry. Most of the antibiotics used for these purposes are synthetic. However, the constant and unsystematic use of antibiotics in poultry has dramatically reduced their effectiveness. Inappropriate or excessive use of antibiotics causes their accumulation over than permissible in food products, which endangers human health, causes dysbiosis, allergic reactions, weakens immunity, etc. The first signal regarding antibiotics enter the human body

through the food chain appeared in scientific literature as early as the '60s of past century. Along with destroying the intestinal microflora they produce resistant forms of bacteria. Resistant strains of pathogenic microorganisms have become a huge problem worldwide. That is why in 2006 the EU banned the use of antibiotics in the territories of its countries, in stock farming and poultry as a stimulant for growth, for neutralizing food from pathogen microorganisms and for prophylactic goals [1]. The growth of antibiotic-resistant strains and the reduction of their efficacy have paved the way for research of new ways to combat pathogenic microorganisms. Recent researches have led scientists to conclude that antibiotics for the above mentioned purposes in stock rising and poultry can be replaced with herbal remedies. The abundance of infections caused by

multiresistant microbes and the complexity of combating them has brought widespread recognition to biologically active substances of plant origin. They do not cause addiction, have no side effects and most importantly, do not develop resistance in bacteria and fungus [2]. From plant origin biologically active substances of antibacterial activity, essential oils have special place with high antibacterial, antioxidant and immune modulating activity [3-5]. The use of essential oils prevents the development of various intestinal infections, which significantly affects the productivity and maintenance of stock and fowls. In addition, essential oils provide a pleasant aroma and make food attractive, also they have anti-stress activity, increase production of digestive enzymes, even improve their mood [6]. Essential oils are products of secondary metabolism that contain numerous easily evaporated substances: terpenes, terpenoids, phenolic products, aliphatic and aromatic components [7]. It is known that essential oils have a wide range of biological activity, above all with bactericidal and fungicidal properties [8]. The mechanism of their action on microorganisms is following: different organic compounds within it change speed of biochemical reactions, resulting in their destructive effects on microorganism's mesosomes and cytoplasmic membranes, thus reducing oxidative phosphorylisation activity, also inhibit cellular respiration [7,9].

In recent years, in the scientific community interest in medicinal plants and herbal extracts has increased significantly in terms of antimicrobial activity. Researchers [2] found that essential oils of bergamot, carnation, cypress, big fennel, eucalyptus, lavender, rosemary, peppermint, clary, thyme, show pronounced antibacterial activity against various pathogens. That is why for today there is no doubt regarding use of essential oils as new antibacterial chemical modifiers, on the basis of which it is possible to create different compositions with therapeutic, prophylactic effects [10]. Studies in poultry, particularly in chickens, have shown that watery extract of Salvia sclarea L. flowers has anti-inflammatory, antiseptic properties, also has a positive effect on the musculoskeletal system [11]. Studies have also established the antioxidant and antibacterial activity of Salvia sclarea L. [12]. Besides, the essential oils of Monarda didyma L. posses interesting antibacterial action for poultry [13]. Among well-known essential oils one with high antibacterial activity is the essential oil of Thyme (Thymus vulgaris) containing 50% thymol [14]. Chemistry,

Antioxidant, Antibacterial and Antiviral Activity of essential oils of Thymus Transcaucasicus Ronniger, widespread in Georgia was studied in details [15]. We must also take into account the fact that usage of only one type of food supplement cannot completely replace synthetic food antibiotics. In this case, it is necessary to create alternatives with complex composition and action. In the modern stage One of the most effective ways to combat resistant bacteria is to use antibacterial ingredients that act with different mechanisms at the same time. In this case, it is possible to inhibit different processes of metabolism in the microbial cell at the same time, leading to its rapid death and significantly inhibits the development of resistance in microorganisms.

Based on experimental studies carried out by the authors of the presented article [16-19], was obtained and studied antibacterial activity of Clary sage, Eucalyptus, Perilla essential oils [20]. The antioxidant and anti-inflammatory effects of Perilla was also explored [21-23].

The aim of the study is to determine the antibacterial spectrum of some endemic plants of Adjara and Adjara-Lazeti, essential oils and bio-composition.

To achieve the goal following task is set: Research antibacterial activity of some endemic plants of Adjara and Adjara-Lazeti, essential oils and biocomposition.

### Objectives and methods

Research objects are:

Endemic plant species of Adjara:

- 1. Angelica adzharica M.Pop. Umbellifereae Juss., Apiaceae Lindl.
- 2. Centaurea adzharica Sosn. Asteraceae Dumort. (Compositae Giseke)
- 3. Erysimum contractum Somm. et Levier. Crucifereae Juss.(=Brassicaceae Burnett.)
- Ranunculus ampellophylus var.adzharica Ranunculaceae Juss.
- 5. Rubus adzharicus Sanadze Rosaceae Juss. Endemic plant species of Adjara-Lazeti:
- Amaracus rotundifolius(Boiss.) Briq. (Origanum rotundifolium) - Lamiaceae Juss.(=Labiaceae)
- 7. Astragalus sommieri Freyn. Fabaceae Lindl.
- 8. Hypericum nordmanni Khokhr. Hypericaceae

Juss.

- 9. Hypericum ptarmicifolium var.adzharicum Hypericaceae Juss.
- 10. Linaria adzharica Kem.-Nath.(=L.syspirensis C. Koch.) Scrophulariaceae Juss.
- 11. Primula megasaefolia boiss. Et Bal. Primulaceae Vent.
- 12. Quercus petra var. dshorochensis c. Koch. Fabaceae Lindl.
- 13. Rhododendron smirnovii Trautv.- Ericaceae DC.
- 14. Rhododendron ungernii Trautv. Ericaceae DC.
- 15. Rhynchospora caucasica Vahl. Cyperaceae Juss.

### Essential oils:

- 16. Thymus vulgaris L. essential oil 0.1% aqueous solution
- 17. Salvia sclarea L. essential oil 0.1% aqueous solution
- 18. Monarda didyma L. essential oil 0.1% aqueous solution
- 19. Perilla nankinensis Decne. essential oil 0.1% aqueous solution
- 20. Composition of Thymus vulgaris L., Salvia sclarea L. and Monarda didyma L. essential oils 0.1% aqueous solutions in the ratio of 2:1:1
- 21. Control object

The research objects were prepared in Adjara region in the period of May-August of 2019. Preparation of methanolic extracts from dry and finely fragmented raw herbal materials was carried out by the maceration method, with the raw material and menstruum in a ratio of 1: 5 and left to stand by for 24 hours at room temperature.

Extraction of essential oils from the study objects was carried out by the method of hydrodistillation.

### **Experimental Section**

Antibacterial activity research of study objects was conducted using Spot test (screening). 5ml of LB broth was added to each LB agar slant containing overnight bacterial culture and eluted using vortexes. Tenfold dilution (using LB broth) of eluted bacterial suspension was prepared: 0,5ml of bacterial suspension was added to reaction tubes with 4,5ml LB broth and vortexed gently on low speed;

The lawn of the diluted bacterial suspension was made on 1.5% LB agar plate. Plates were allowed to set on bench top or in biosafety cabinet for at least 10-15 min and then sequentially spotted 10µl of each research substance on the bacterial lawn. To avoid mixing of spotted drops max 4 different

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	K
Streptococcus		R		±	R	R															
pyogenes	R		R				R	2+	3+	±	±	R	R	R	R		1+		1+	4+	
Escherichia		R		R	2	R															
coli	R		R				R	R	±	2+	R	±	R	R	R		4+	4+		4+	
Enterobacter		土		R	R	R															
cloacae	R		R				R	4+	土	3+	R	R	R	R	土	4+				4+	
Salmonella		R		±	2	R															
typhimurium	R		R				R	2+	R	2+	R	土	R	R	R	4+	2+			4+	
Klebsiella		土		2+	2	±															+
pneumoniae	2+		R				R	4+	2+	2+	±	R	R	R	2+	4+	4+			4+	1
Proteus		R		R	+	R															
vulgaris	R		R				R	3+	3+	土	2+	R	R	R	土	4+	4+	3+	1+	4+	
Shigella		R		R	+	3															
flexneri	土		R				R	2+	4+	3+	3+	R	R	R	土	4+	4+	4+		4+	
Enterococcus		2+		2+	2	3															
faecalis	土		R				R	2+	2+	R	±	R	R	R	土	4+	4+			4+	
Staphylococcus		R		R	+	R															
aureus	2+		R				R	R	2+	2+	2+	R	R	R	R	4+	4+			4+ 1+	
Pseudomonas		R		2+	+	2														1+	
aeruginosa	R		R				R	R	2+	R	R	R	R	R	R	4+					

**Table.** Results of determination of antibacterial action of research objects

research substances were spotted on one bacterial lawn. After drying the drops, the Petri dishes were placed upside down into the incubator at appropriate temperature; Spot test results were examined 18-24 hours after incubation. The presence of clear zones in the research substance spot area indicated a positive result.

### Results and analysis

The investigation antibacterial activity of methanolic extracts obtained from 15 endemic herbs, 0.1% aqueous solutions of 4 essential oils and essential oils composition took place. The results are presented on Table.

Given data shows that against listed strains no antibacterial effect was found for Erysimum contractum Somm. et Levier., Astragalus sommieri Freyn., Rhododendron smirnovii Trautv. and Rhododendron ungernii Trautv. Week antibacterial effect was determined for Angelica adzharica M.Pop., Centaurea adzharica Sosn., Ranunculus ampellophylus var.adzharica, Rubus adzharicus Sanadze, Quercus petra var. dshorochensis c. Koch. and Rhynchospora caucasica Vahl. With pronounced antibacterial effect and narrow spectrum of action is characterized Amaracus rotundifolius (Boiss.) Briq. and Primula megasaefolia boiss. Et Bal., while Hypericum nordmanni Khokhr., Hypericum ptarmicifolium var.adzharicum and Linaria adzharica Kem. Methanolic extracts along with pronounced antibacterial effect have comparably wide spectrum of action.

From secondary metabolites, aqueous solution of thyme and clary essential oils is standing out with pronounced and wide antibacterial activity. Monarda didyma L. essential oil 0.1% aqueous solution has pronounced antibacterial activity but with narrow spectrum. Perilla nankinensis Decne essential oil 0.1% aqueous solution shown week antibacterial activity. Essential oils composition, with constitution of thyme, clary and monarda in the ration of 2:1:1, had effect of each bacterial strain on each bacterial strain.

### Conclusion

Based on conducted expiremental researches, it was established that *Hypericum nordmanni* Khokhr., *Hypericum ptarmicifolium var. adzharicum* and *Linaria adzharica Kem*. methanolic extracts have pronounced and wide spectrum antibacterial action . 0.1% aqueous solutions of *Thymus vulgaris* L., *Salvia sclarea* L. and *Monarda didyma* L. essential oils and of their compositions with 2:1:1 ratio possess high antibacterial activity.

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### Soil-melioration peculiarities in valley of Kish river

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### ABSTRACT

The presented study explains the scientific approach to the creation of mathematical models based on the concept of a systematic approach to solving the existing problems of the impact of floods on soil ecosystems in the Kish river valley, increasing their adequacy and applying research results to practical problems. The article presents some results of studies carried out on the Kish River and its floodplains over 3 years. Soil sections were placed at different depths, soil samples were taken and examined for their morphogenetic properties. At the same time, samples of groundwater and river irrigation water were taken for laboratory analysis at different times of the year (spring, summer, autumn). In accordance with the hydrographic regime and relief of the Kish River, the qualitative composition of the water flowing in different parts of the river, dependent particles, impurities, and the degree of clay content were studied. It has been clarified that the use of river water for irrigation of agrocenoses is expedient. Mathematical models have been developed to calculate floods.

Keywords: Valley of the river, Agroecology, Soil formation, Agrochemistry, Soil fertility, Floods.

### Introduction

The hydrographic network also plays an important role in soil formation. In the river valleys of Azerbaijan, there are widespread alluvial-hydromorphic soils with high potential fertility, different landscape characteristics and different stages of development, formed in a complex geomorphological structure, microrelief and bioclimatic conditions. However, in comparison with zonal soils, morphogenetic diagnostics and classification of alluvial-hydromorphic soils of river valley with a complex soil cover structure have been insufficiently studied. The Kish River has a well-developed river network. The use of ICT (information and communication technologies) in solving many practical problems,

such as assessing soil fertility and forecasting productivity, product quality management, etc is very important. The results are based on the rational use of natural resources and environmental protection.

### Objects and methods of investigations

Systems of coordinates of investigate section were determined with GPS - GPS. N 41 15 22 " E 47 12 30"; N 41 15 24" E 47 12 37"; N 41 14 51" E 47 11 49"; N 41 15 25" E 47 11 50"; N 41 14 36" E 47 11 11"; N 41 15 25" E 47 12 37"; N 41 15 31" E 47 12 23".

Investigations were done in valley of the Kish river, which is a tributary of the Ayrichay river; its

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length is 33 km<sup>2</sup>, and its area is 265 km<sup>2</sup> [1]. The Kish river, one of the most flooded rivers in the Caucasus, begins at an altitude of 2900 meters from the southern slope of the Greater Caucasus Range. The valley of the Kish River, whose channel is located near the western part of Sheki, is considered one of the strongest floodplains [2, 3].

To study the soils of the studied river valleys, we used geographical comparisons, cartographic, stationary and statistical calculation methods. Field work was carried out to determine of alluvial soils in the cones of the Kish River tributary.

Field work was carried out on the basis of soil cartographic material. In each individual contour, in characteristic places for them, ditches were made, where the structure of the soil, the depth of occurrence of carbonates (boiling from 10% HCl) and groundwater, particle size distribution, degree of erosion, as well as climatic conditions of the territory in the form of bioclimatic potential were determined, the degree of culture, the contrast of the relief, rockiness, bushiness and others. Soil and groundwater samples were taken from soil plots at a depth of 1.5 and 2 meters for laboratory analysis. At the same time, water samples were taken from certain places in the Kish river valley, pH was measured immediately, and the samples were delivered to the laboratory for analysis.

Modern methods of laboratory analysis of soil and water samples were used. Analysis of readily soluble salts in soil and groundwater E.V.Arinushkina [4], hygroscopic humidity -105 °C, drying in a drying oven 6 hours by weight, particle size distribution - N.A.Kachinsky [5] - Na<sub>2</sub>P<sub>2</sub>O<sub>7</sub>, soil density - with a cylinder in the field according to the method N.A. Kachinsky [5], the degree of rockiness of the soil was determines at a depth of 0-30 and 30-50 cm

Soil genesis wee investigate according international soil classification system [6].

Determination of the surface area of plants (5 times repeat) of soil on an area of 1 m<sup>2</sup> 2 cm above the surface of the grass were cut, after drying in air, the dry weight per hectare was determined and calculated. The amount of root mass was investigated in 3 replicates at a depth of 0-20, 20-50 cm using a monolithic method (25x25 cm<sup>2</sup>).

Taking into account the geomorphological structure, relief, alluvial sediments, soil and vegetation, the level of groundwater, the seasonal nature of river floods in the Sheki region, key study areas were selected.

In the valley of the Kish river, a large number of land plots have been laid along the routes (from bedrocks to ancient terraces in the direction of the width of the floodplain) and the stages of evolution of alluvial hydromorphic soils have been studied. In the last 60-70 years, anthropogenic impacts have replaced forests with arable land.

# Physical-geographical conditions of Kish river valley

It is known that the role of parent rocks in the process of soil formation is very great. Thus, the rock transfers its physical, chemical and biological properties to the soil. Soil-forming rocks in the Sheki region, located on the southern slope of the Greater Caucasus, developed mainly on eluvial-diluvial deposits [1].

The mountainous part of the area is composed of limestone, dolomite, andesite, shale, sandstone. In addition, diluvial-proluvial deposits are widespread in the foothills, valleys and depressions of gentle slopes [2].

The initial development of alluvial-hydromorphic soils with high natural potential fertility at the research site began with deforestation by humans. Currently, due to the protection of forests, the area of land for agriculture is limited [3].

The annual number of hours of sunshine in Sheki region is 2350 hours. 40 % of hours of sunshine are in the summer months. During the year, there are 122 kcal of solar radiation per 1 square centimeter of the earth's surface. The climate of the Sheki region is influenced by cyclones and anticyclones, various air masses and local winds. In winter, the influx of arctic and moderate air masses forces the weather to pass at relatively moderate temperatures. In summer, local winds are formed in the mountains and valleys.

This is due to the pressure drop between mountains and valleys. The mountains of the Greater Caucasus block the cold masses coming from the north. Thus, the average January temperature in Sheki is  $0.5 \, \mathbb{C}$ . The average annual temperature is  $11 \, \mathbb{C}$ . The average temperature in June-August ranges from  $20\text{-}25 \, \mathbb{C}$ .

Strong winds (with a speed of more than 15 m/s) are rarely recorded, only 1012 days a year.

In Sheki, winds are often observed at a speed of less than 1 meter per second. The amount of precipitation is 730 mm. Half of them are in May, June,

July, September and October. The smallest amount of precipitation is in August (35 mm), January (29 mm) and February (36 mm).

The amount of precipitation is 775 mm in the village of Kish, Sheki region and more than 1000 mm on the Khan plateau. Natural phenomena such as floods, storms and heavy hail are typical of Sheki and the surrounding mountains and plains.

Here atmospheric phenomena such as storms and hail are closely related. The mountainous terrain causes hail and heavy evaporation during the summer months. The average annual number of hail days is 1.4. 14 hail days in 10 years.

At an altitude of 500-850 meters above sea level, the surrounding mountain forests protect the city from overheating. At an altitude of 500-850 meters above sea level, the surrounding mountain forests protect the city from overheating. The hydrographic network also plays an important role in soil formation. The main rivers are fed by snow, small rivers and canals, groundwater and partial rainwater. The amount of precipitation varies from year to year and from month to month. A drought has been observed here for several years.

In the summer months, the relative humidity sometimes drops to 6-7 %, and many hot and dry winds blow. All this plays an important role in the abundant flow of the river. Due to the large slope of the Kish river, the water dries up and flows quickly in the spring and summer months.

Kish river is very destructive and it plays a large role in the topography and soil fragmentation. The role of artificial canals and groundwater in the natural hydrographic network of the Sheki region is also great.

The nature of the soil formation process in the river valleys of the republic and the morphogenetic characteristics of soils depend on the density of the river network, the annual flooding regime, the degree of turbidity, the lithological and chemical composition of alluvial deposits, the degree of groundwater mineralization, and other processes have a significant impact.

These soils are mainly used as summer pastures and hayfields, partly for sowing tilled crops. The ecological significance of these soils is not limited to agricultural aspects and play an important role in the hydrological mountain areas.

### **Results of investigation**

The problems of land reclamation in the South Caucasus are very urgent and great scientific and practical importance is considered to their solution [7-14].

According to preliminary estimates, in the foothills of Azerbaijan, especially in the tributaries of mountain rivers, there are about 80,000-100,000 hectares of alluvial-stony soils. However, most of this land is not used for agriculture. This is explained by insufficient knowledge of the physicochemical properties of these soils and the degree of rockiness. Stony soil is one of the main properties that reduce its low economic value.

Thus, stones of different sizes and quantities in the surface and profile of the soil, reducing the fertility of the soil, make it difficult to carry out the necessary agrotechnical and reclamation measures of technology on these soils.

For the restoration of stony soils for the needs of agriculture, first of all, the study of the degree of rockiness and physical and chemical properties, as well as the collection and cleaning of stones from these territories is one of the most important agrotechnical measures and is one of the most important measures of agricultural technology and has valuable scientific and practical significance. Mineralization of groundwater increases to 25-70 g/l, and in some cases up to 100 g/l. Depth of placement ranges from 2 meters to 10-15 meters. Although the main source of irrigation in this area is river water, other sources of water are also used - artesian wells and kakhriz shafts. Mineralization of artesian wells is 0.4-1.1 g/l, and in the waters of Kakhriz shaft -0.3-0.6 g/l.

There are freshwater deposits in the Sheki region. There are 2 deposits of fresh water with reserves of 128.8 thousand m³/day. And one deposit of mineral waters with reserves of 0.284 thousand m³/day. In the course of our study, it was found that the average diameter of water-resistant aggregates in the first 25-30 years on newly irrigated alluvial meadow soils is 0.28-0.30 mm. However, on irrigated lands (up to 100 years of irrigation), this indicator is 0.31-0.33 mm, which must be taken into account when adapting the degree of erosion of ancient irrigated accumulative soils to the modern classification.

Dependent small particles that enter with irrigation water deteriorate the particle size distribution, and the particles move inside the field under the action of the water flow.

On smooth areas, these changes are equal, but on sloping areas they are concentrated below. Since ancient times, irrigated lands are mainly heavy clay and clayey. Changes affect soil density, porosity, structural composition and water regime. Excavation and embankment of leveled soil reached 30-40 cm. Although this process facilitated irrigation, drilling and spilling destroyed the structure, displacing 1000-3000 m³ of fertile soil per hectare.

In the process of alluvial-hydromorphic soil erosion, and especially for studying the important role of vegetation in the formation of humus, the surface phytomass and the mass of plant roots were determined over 3 years. The amount of phytochemicals is determined once a year, with the maximum growth of the growing season (end of May).

The Kish River has a well-developed river network.

The amount of nitrogen and ash elements in the composition of plant species prevailing in the herbariums was determined.

Most of the foothills and plains in the Sheki region are irrigated. Erosion resistance in these soils is significantly improved.

Indicators are a source of information on the quantity and quality of the final state of land. Anthropogenic impacts play an important role in the depth of groundwater, the dynamics of mineralization, and water-salt balance.

A diagram of the distribution of humus at different depths in anthropogenically altered soils of natural cenoses and agrocenoses of river basins has been established, showing that the amount of humus in the upper layers is large and decreases with depth.

Dependent river tributaries provide a sufficient amount of humus (1.19-1.59%) and nitrogen (0.09-0.17%), a slightly alkaline medium (pH = 7.3-7.5). Low mineralization of groundwater is characteristic (0.85–1.98 g/l). The total chemical composition of the alluvial deposits forming the soil is  $SiO_2$  (59.4-64.7%) and  $R_2O_3$  ( $Al_2O_3$ =20.0-25.6%;  $Fe_2O_3$ =8.4-10.7%) prevails in the composition. The amount of dry residue in the middle reaches of the river is 0.47 g/l, and in the lower reaches of 0.92 g/l.

The salinity of groundwater in the river beds increased to 1.53–3.46 g / l, and in microdepressions to 4.28–9.35 g/l, which led to varying degrees of soil salinity. The amount of physical clay (particles <0.01 mm) in irrigated meadow-gray soils is 81.24-88.0 % in wet soils and 45.72-81.40 % under cereals. According to agrochemical indicators, the amount of humus in meadow gray soils is 0.41-2.92 % and 0.53-2.45 %, respectively, and the highest carbonization rates are 12.32% and 13.90 %. This variation is considered to be an indicator that the soil samples are suitable for cultivation.

$$S_{or} = k \cdot \frac{v^m}{H^n \cdot u^k}$$

The following formula is most often used as a result of processing field research results:

$$S_{or} = 11 \cdot v_{or} \sqrt{\frac{RI \cdot v_{or}}{u}}$$

R is the hydraulic radius of the flow,

$$S_{or} = 0.0022(v_{or} / u)^{1.5} \cdot \sqrt{RI}$$

the following dependencies are suggested for small items.

Main physical-chemical indexes in naturally chenos and agrochenos are shown on the two characterization soil profile (Table ).

Main soils in basin of Kish river are Fluvisols, Cambisols, Leptosols [6].

Fluvisols accommodate genetically young soils in fluvial, lacustrine or marine deposits. This soils are characterized by regular flooding and the sedimentation of new alluvial layers. Latin fluvius - river. This soil characterized by various hydrological regimes, conctructions and properties. Its properties are mainly determined by the nature of the basin, where these soils are developed. The soil profile usually has the following structure: A-BC-C-CD. Predominantly recent, fluvial, lacustrine and marine deposits. River plains and fans, valleys, lake depressions and tidal marshes on all continents and in all climate zones; no groundwater and no high salt contents in the topsoil; many Fluvisols under natural conditions are flooded periodically. Profiles with evidence of stratification; weak horizon differentiation but a district topsoil horizon may be present. The good natural fertility of most Fluvisols and attractive dwelling sites on river levees and on higher parts in marine landscapes were recognized in prehistoric times. Later, great civilizations developed in river landscapes and on marine plains.

Cambisols combine soils with at least an incipient surface soil formation. Transformation of parent material is evident from structure formation and mostly brownish discoloration, increasing clay percentage, and/or carbonate removal. Brown forest soils profile has the following horizons: Ao-A-Bm-C; Cinnamonic soils has next structure:

A-B(Ca)-BC(BCCa)-Cca. Soils with at least the beginnings of horizon differentiation in the subsoil,

evident from changes in structure, colour, clay content or carbonate content. Medium and finetectured materials derived from a wide range of rocks. This soils are characterized by slight or moderate weathering of parent material and by absence of appreciable quantities of illuviated clay, organic matter, Al and/or Fe compounds.

Leptosols comprise very thin soils over continuous rock and soils that are extremely rich in coarse fragments. Leptools are particularly common in mountain regions. Leptosols are thin soils; from Greek leptos, thin. Various kinds of continuous rock or unconsolidated materials with less than 20 percent (by volume) fine earth. Leptosols have continuous rock at or very close to the surface or are extremely gravelly.

### **Conclusion**

- 1. In the channel of the Kish river marks increase of the bed water and in microrelief cause different degree of soil salinization.
- Main soils in basin of Kish river are Fluvisols, Cambisols, Leptosols, The ecological significance of these soils is not limited to agricultural aspects and play an important role in the hydrological mountain areas.

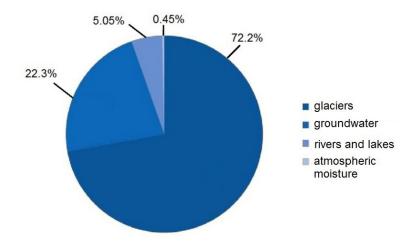


Fig. 1. Water content of Kish river valley

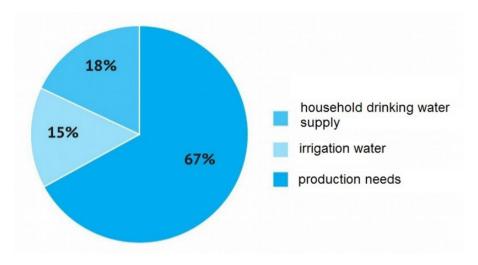


Fig. 2. Kish river water use scheme



Fig. 3. Laboratory analysis of soils taken from the riverbed



Fig. 4. Some of the land plots and water samples

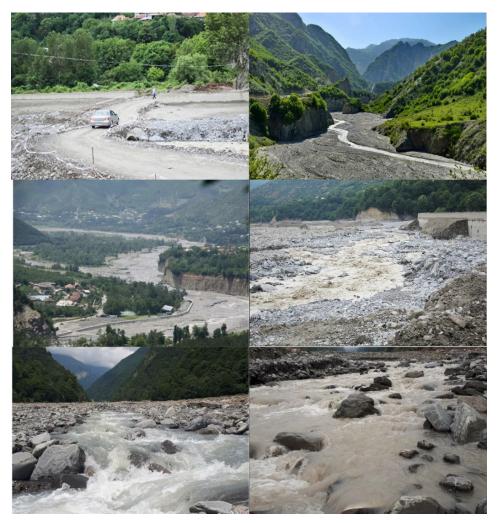


Fig. 5. Kish river in Sheki region

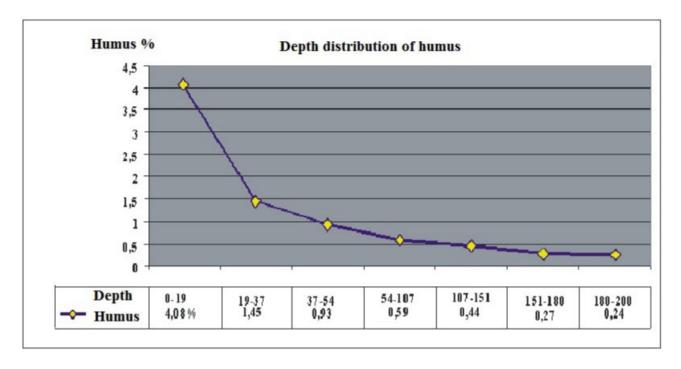


Fig. 6. Distribution of humus at different depths in the soils of natural thenoses in river valley

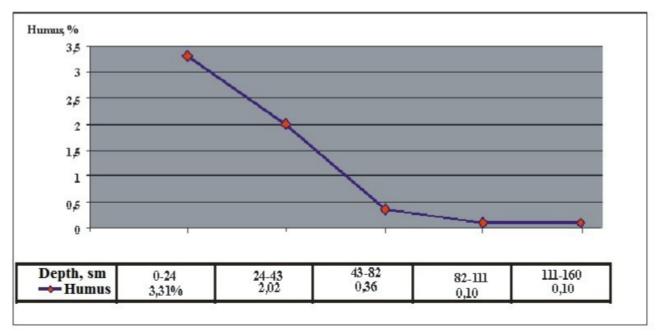


Fig. 7. Distribution of humus at different depths in the soils of cultivated thenoses in river valley

Table. Basic physicochemical indicators in the soils of natural thenosis and agrothenosis

Cenoses	Depth,	Humus	Azot	CaCO <sub>3</sub>	Dry	Dry Absorbed bas mg/ekv		pН	Hygros- copic	Ca/Mg	
	<b>344</b>	%	%	%	%	Ca	Mg	in solution	humidity %	Carrig	
Soils of	0-19	4.08	0.29	47.64	0.200	0.77	1.26	7.1	3.09	0.61	
natural cenosis	19-37	1.45	0.12	52.18	0.120	0.63	0.49	7.2	2.33	1.28	
cenosis	37.54	0.93	0.09	50.52	0.154	0.49	0.49	7.0	2.36	1.00	
	54.107	0.59	0.07	47.46	0.162	0.49	0.56	7.4	2.38	0.87	
	107-151	0.44	0.06	48.43	0.216	0.56	0.63	7.3	2.45	0.89	
	151-180	0.27	0.05	48.45	0.220	0.58	0.65	7.3	2.47	0.90	
	180-200	0.24	0.03	47.40	0.200	0.57	0.64	7.2	2.46	0.90	
Soils of	0-29	3.58	0.26	18.04	0.165	0.91	0.42	7.2	4.90	2.17	
agrosen oces	29-57	3.12	0.23	16.33	0.184	0.91	0.35	7.1	5.23	2.60	
	57-102	2.24	0.17	36.32	0.155	0.84	0.42	7.4	4.23	2.00	
	102-150	1.87	0.15	45.12	0.167	0.56	0.63	7.4	3.62	0.89	
	150-170	0.27	0.05	42.78	0.165	0.70	0.42	7.3	8.95	1.67	

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# The reasons for the low effectiveness of agro-ameliorative measures on heavy soils of Kolkhida

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#### ABSTRACT

The article analyzes the reasons for the low efficiency of ameliorative measures on heavy clay soils of Kolkhida. It is shown that: the low efficiency of the investigated ameliorative measures can be explained by the unfavorable conditions of the formation of the structure in clay soils of wetlands Colchis:

- Mechanical and mineralogical composition of soils contains 50% or more silt, which includes minerals characterized by intracrystalline absorption and a large force that prevents the formation of structures.
- Drainage and high standing of soil-groundwater cause a waterlogging of the biologically active layer.
- Reversibility of colloids prevents their coagulability, and thus the structuring of the soil during draining.

In conclusion, all of the above factors prevent the formation of a solid agronomically valuable structure of the clay swelling soils of Colchis and determine the low effectiveness of ameliorative measures.

Keywords: Heavy clays, Swelling, Shrinkage, Porosity, Humidity, Aeration.

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## Introduction

The main purpose of reclamation of heavy waterlogged soils of Kolkhida is to bring them to a state that allows them to be used for high-performance agricultural development. However, in spite of huge capital investments, no agro-ameliorative measure produced any significant effect.

The effectiveness of land-reclamation measures and with it the degree of return on investment depends on how scientifically proved are the recommended measures. This, in turn, is determined how correctly we understand the reasons, causing unfavorable soil properties of the ameliorative object.

The design of rational land reclamation measures requires a clear picture of the water-air regime of the soil of the territory both before and after their

implementation, which requires preliminary study of a number of issues:

- What is the impact of swelling shrinkage process on water-air regime?
- What amount of water should be removed from the root zone to ensure optimal water-air regime;
- What is the ratio of water and air at different volume masses of arable and sub-plow layers?
- How can agromeliorative measures affect on unfavorable properties of heavy structure less soil?

## The main part

In the clay soils of Kolkhida, the most active silt fraction of the soil is contained in a large amount - up to 50% or more to the weight of the soil. It contains clay minerals of montmorillonite type capable

of intracrystalline soaking, which causes a strong swelling of these soils. In the swollen state, the clay soils of Kolkhida are found most of the year. Only in spring and early summer they usually dry out and shrink.

Data obtained during the study of the process of swelling – shrinkage of the initial field humidity (during the period of water-logging) to the humidity of the air-dry state, allowed us to identify two very important features of heavy clay soils of Kolkhida. The first is the specific character of the shrinkage of these soils during drying, the second - in a very large amount of water swelling [1-3]. A clear idea of the nature of the shrinkage of podzolic gley soil is shown in Fig.

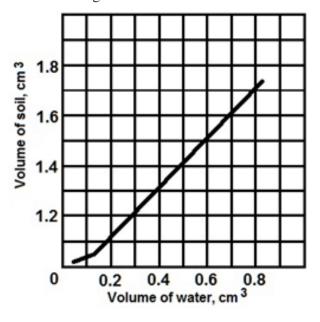


Fig.1. Typical shrinkage schedule

The coordinates for plotting the curves are the ratios  $-\frac{V}{V_0}$  on the ordinate axis,  $\frac{W}{V_0}$  on the abscissa axis, where - V the soil volume in cm3,  $V_0$  - the soil volume after shrinkage, W - the volume of water (equal to the weight) in the sample.

The graph shows that, for heavy genetic horizons in the mechanical composition, shrinkage occurs almost in the entire range of humidity change. The linear nature of the dependence means normal shrinkage. This is the kind of shrinkage when the decrease of the volume of the soil is equal to the volume of evaporated water, and remains in a wide range.

In connection with this type of shrinkage, drying does not cause the expected improvement in the air regime, which is a very important feature in practice of heavy clay soils of Kolkhida (4-6).

While determining the shrinkage, data was obtained, allowing the studied soils to calculate the water content of swelling for the first time, by considering the water that caused an increase in the volume of soil equal to the volume of this water (7,8).

Table 1 provides data from which shows the swelling water is 31 to 47% of the weight of the soil, or 55-76% of the initial humidity. If hygroscopic water is added to this amount (also in % of the weight), then we get the amount of water that cannot be removed by any hydro technical measure.

Depth,	Humidi	ty in % at	Volume o	of 100 g	Differ	rence	Shrinka-	Water
cm							ge factor	swelling
			of soil in	cm³ for			for	in %
	field	air-dry	field	air-dry	water	volume	France-	from the
	humidity	condition	humidity	condi-	content	in cm³	son	start
				tion	in cm³	(water		
						swelling)		
2.12	F0 0C	F 70	104 5	60.0	F4 2	26.5	0.67	60.0
3-13	59, 96	5, 79	104, 5	68, 0	54, 2	36, 5	0, 67	60, 8
15-25	55, 57	6, 10	95, 7	58, 5	49, 5	37, 2	0, 75	66, 9
37- 47	55, 88	8, 79	94, 2	55, 7	47, 1	38, 5	0, 82	68, 9
68-78	51, 40	7, 07	88, 9	53, 1	44, 3	35 <i>,</i> 8	0, 81	69, 6
5-15	66, 87	6, 88	108, 3	68, 7	60, 0	39, 6	0, 66	59, 2
33-43	53, 24	6, 82	94, 3	59, 9	46 4	84, 4	0, 74	64, 7
63-73	51,15	10, 34	90, 6	55, 7	40,9	34, 9	0, 85	68, 2

**Table 1.** Water content of swelling in clay soils of Kolkhida (Settlement, podzolic-gley)

Studies carried out to determine the effect of drying the soil on swelling, showed that even a three-time drying of the soil at 1050 could not eliminate it. The final swelling value after all the drying was 38% versus 47% in the original soil. In some cases, 46% versus 49% in the original (Table 2), which in-

dicates the reversibility of the colloids of these soils.

Thus, swelling cannot be eliminated by drying, and, consequently, the idea that under the influence of drying, the colloids of the soil coagulate and as a result of which the soil is structured, applies only to soils in which the colloids are irreversible (7).

		ement I	Measur	ement II	Measure	ement III	Measure	ement IV
Depth,	Swelling,	Water	Swelling,	Water	Swelling,	Water	Swelling,	Water
cm	%	swelling,	%	swelling,	%	swelling,	%	swelling,
		%		%		%		%
3-13	47,0	82,6	40,6	67,0	38,9	62,2	37,9	64,3
"	44,3	83,8	37,1	67,4	35,9	65,6	34,3	64,9
25-35	48,3	86,0	44,3	69,1	41,9	67,9	39,2	68,2
"	47,5	81,6	42,8	69,3	41,2	65,5	39,2	66,1
60-70	49,2	85,4	49,0	75,6	46,7	67,8	46,2	67,5
"	48,4	81,4	47,8	73,4	47,2	67,2	42,5	64,3
90-100	43,8	75,9	40,7	67,0	36,3	61,4	36,4	62,8
u	43.1	78.5	37.8	:65.2	36.6	61.9	35.8	61.2

Table 2. Effect of drying on soil swelling

According to the table 2, due to swelling, that heavy clay soils of Kolkhida have the ability to keep from 31 to 47% of water swelling, which cannot be removed by any hydro technical measures.

Determination of the optimum moisture content in percentage from MFD (Maximum field discharge) and evaluation of the water-air regime by humidity regime is based on the opinion that the difference between the MFD and the lower limit of available moisture for normal soils gives a range of field moisture (6,7). If this position is acceptable for irrigated and non-irrigated soils, it cannot be accepted for excessively moistened, swampy, swelling soils of Kolkhida, where MFD often coincides or is very close to full saturation, since the air capacity is zero. This means that air is a limiting factor for the use of water by the plant. Without the necessary minimum (determined for each plant), the plant cannot develop.

Consequently, the entire range from MFD to wilting moisture cannot be considered as useful water, and its upper limit is the moisture content at which the soil contains the minimum air required for a given plant.

What is the minimum volume of aeration pores that should be in the soil? Generally, it is considered that if pore volume occupied by air, when MFD is less than 10-15% of the soil volume, agro technical techniques will be required to improve aeration (2).

It can be explained by the fact that the flow of water into the soil causes a disproportionately large disconnection of pores compared with the volume of water that has arrived, through which soil aeration could be carried out. Studies have shown that the deactivation of pores and the associated decrease in the diffusion coefficient is particularly revealed in structure less soils and in soils of strongly swelling (8).

As it is shown above, in the clay soils of the central part of the Kolkhida lowland contains minerals of the montmorillonite group. In montmorillonite clays, swelling is associated with an increase in both inter-packet distances and distances between primary particles.

While drying these soils begins the process of volumetric shrinkage, which occurs mainly in 2 phases: first - when the decrease in the volume of the soil is equal to the volume of evaporated water - normal shrinkage, and the second - when the volume reduction is less than the volume of evaporated water - residual shrinkage. In this regard, the decrease in the moisture content of individual genetic horizons differently changes their aeration. The humidity range in which the soil aeration improvement depends on its structural state. In more structured soils it is wider than in less structured soils. In the upper humus horizon, some improvements in aeration occur at high humidity, evidently in connection with the release of a small pore volume from the gravitational water. Later, some stability is observed and, from about 40-42% humidity, the aeration curve begins to rise (1, 3).

The influence of agro-ameliorative measures on the water-air regime affects mainly on the upper 0-10 cm layer of the arable layer, where the air content is higher than on the control. In the lower layers, the difference was not found (Table 3).

Depth in	Option	Humidity of	Volumetric	in% to the v	olume of
cm		soil in% to	weight in g /	soi	
		weight	cm³	Water	Air
0-10	already	48,5	0,99	48,04	14,3
10-20	plowed	65,7	0,93	61,1	3,4
0-10	Row on the	53,2	0,84	44,7	23,2
10-20	drainage	58,5	1,01	59,1	2,6
0-10	The deposit	43,7	1,20	52,4	2,4
10-20		56,4	1,03	58,1	2.2

*Table 3.* The ratio of water and air in different versions

To determine the effect of agro-ameliorative measures on the water-air regime of heavy soils in Kolkhida, studies were carried out at the Khorga experimental base of the Kolkhida experimental and ameliorative station, where these options were chosen: old-fall (10 years), skills with drainage (3 years) and a reservoir for comparison.

Table 4 provides data on the water and air content and their ratio in the soil on options with agro-reclamation measures and on controls. Thus, neighboring areas without activities (virgin land or fallow land).

Analysis of the data in the table shows that in the Khorga, in the experience with tea, in the soil (podzolic-gley) in the row with drainage, the ratio of water and air in the upper layer 0-10 cm varies in both terms from 2: 1 to 2.4: 1. Deeper, this ratio increases, reaching 15.7: 1.

Based on the data given, it appears that the apparent effectiveness of agro-ameliorative measures has not been determined by the air content in the soil and by the ratio of water and air in row with drainage.

The data show that even in the dry period, when there should be a maximum air in the soil, its content is sufficient only in the 0-10 cm layer and often less in the lower layers.

The above mentioned is confirmed by the results of studies obtained at the Khorga experimental base for soils with different degrees of compaction (Table 5) (1, 6, 7).

		I term - May										
Depth in cm		ture of soil	in%	in% to v	volume '	Ratio	of	Unavailable				
		to weight			water a	and	water in% to					
					Λ:	air	-	volume				
				water Tea o	│ <u>Air</u> n row with	n a drain						
0-10		34,9		38,7	19,3	2,0 :						
10-20		33,9		43,7	7,8	5,6 :		18,9				
				48,0	'	12,0 :		21,9				
20-30		37,5		,	4,0	-		21,5				
40-50		32,3		45,2	3,3	13,7 :	1	21.4				
0-10		25,7		31,5	22,1	1,4:1		22,2				
10-20		29,8		39,3	11,1	3,5 : 1		23,5				
20-30		31,9		40,8	11,3	3,6 : 1		21,9				
40-50		32.1		42,7	7,7	5,5 : 1		21,5				
				II teri	m - Octob		21,3					
			Topio	n row with								
0-10	34,7	40,2	1600	16,5	2,4:1			10.7				
10-20		47,0		9,3	5,1:			19,7				
20-30	40,2	53,1		3,4	15,6			19,9				
	45,8	55,1	l	3,5		15,7:1		19,4				
40-50	49,2	33,1		3,3	13,7			17,1				
		44.5		12.4	2.4							
0-10	34,6	48.4 47,9		13,4	3,1:			21,6				
10-20	40,0			6,1	7,9 :			21,6				
20-30	39,9			7,0	6,8 :			20,5				
40-50	38,7	49,5		2,9	17,1	: 1		20,7				
	55,7											

Table 4. The content of water and air in the soil at low degrees of moisture

Bulky weight, g/cm³	Porosity, %	maximum moisture capacity, %	minimum moisture capacity, %	Optimal air content, %	Aeration,%	Non- productive humidity, %	productive humidity, %
1,3	51,3	39,6	40,0	15,4	0,0	33,0	7,0
1,2	55,0	45,8	42,0	16,6	3,5	33,0	9,0
1,15	56,9	49,5	43,0	17,4	6,5	33,0	10,0
1,1	58,8	53,5	46,0	18,2	7,5	33,0	13,0
1,0	62,5	62,5	48,0	20,0	14,5	33,0	15,0
0,9	66,3	73,6	48,0	22,0	25,6	33,0	15,0

**Table 5.** The ratio of water and air at different bulky masses

The data given clearly indicate that, even against of systematic drainage with a backfill, acceptable water-air ratios are possible only with a bulky weight of less than  $1.0~{\rm g}$  / cm<sup>3</sup>.

Naturally, grinding the soil to such a state (especially in the Kolkhida conditions) requires the use of special equipment and huge energy inputs, which is unprofitable. However, even in this case, the effect obtained is very short-lived, as the aggregates obtained in this way, when in contact with water, are dispersed and compacted to the original state. The radical improvement of the aggregate composition of the Kolkhidian soils by cultivation with agro technical and other methods is practically unreal (8).

#### Conclusion

The mechanical and mineralogical composition of soils in the central part of Kolkhida contains more than 50% of silt, consisting of minerals of montmorillonite, which are characterized by intracrystalline absorption, and irreversible soil colloids, which prevent the formation of structures under periodic swelling and shrinkage.

Very large (up to 2000 mm) and long (dry period not more than 1.5 months) precipitation, as well as big amount of groundwater standing against the lack of structure determines the maximum water saturation of soil and the lack of air content practically throughout the year. In the dry period, there is no productive moisture.

Agromeliorative measures affect only superficial 0-10 cm of soil.

The above prevents the formation of a solid, agronomical valuable structure and the provision of an optimal water and air regime, which makes it impossible to conduct productive agricultural development of this part of Kolkhida.

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# Georgian Autochthonous red grape and wine Antioxidant activity

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### ABSTRACT

Phenolic compounds, anthocyanins and their antioxidant activity of 7 autochthonous grape varieties of Western Georgia and wines produced from them (Aladasturi, Mujuretuli, Chkhaveri, Kabistoni Shavi, Mtevanddi, Mtredispekh and Ojaleshi) were studied. The largest amount of phenolic compounds in the studied grape varieties was found in Ojaleshi , Kabistoni Shavi, Mujuretuli, Otskhanuri Sapere ((5691.75 - 4091.05 mg / kg), kg), relatively less - in Usakhelauri , Kachichi, Aladasturi and significantly less in Chkhaveri, Mtredispekha, Mtevandidi (1340.1 - 3535.25 mg / kg). The content of anthocyanins in each variety has similar patterns: Otskhanuri Sapere (631.16 mg / kg) and Ojaleshi (576.29 mg / kg) are also dominant here; The total content of phenolic compounds in wine varies in different ways: Otskhanuri Sapere (3674.0 mg / kg), Kabistoni Shavi (3619.0 mg / kg), Ojaleshi (3566.32 mg / kg) have the highest content in this respect. During the production of wine, a certain amount of anthocyanins is transferred into it. Their transition from grape to wine is somewhat correlated. The largest amount of anthocyanins is observed in wines made from such varieties as Ojaleshi (485.0 mg / kg), Otskhanuri Sapere (400.9 mg / kg), Usakhelauri (397.1 mg / kg), Kachichi (390.8 mg / kg ). There is a direct correlation between the content of phenolic compounds in grapes and its wines and their antioxidant activity. Ojaleshi grapes and wine have the highest antioxidant activity among these varieties. \*Keywords\*: Autochthonous Grape, Wine, Phenolic compounds, Anthocyanin, Antioxidant activity, Red Grape.

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### Introduction

Grapes and natural wine, made from it, are a source of antioxidants [1]. The main representatives of antioxidants are polyphenols. Grapes are one of the fruits with a high content of phenolic compounds. Some of them, naturally, get into wine, prepared from these grapes [2]. Phenolic compounds are also found in grape skin, pulp and seeds [3]. The content of polyphenols in grapes and wine, made from it, is associated with color, aroma, taste and antioxidant activity, which is considered a good means of preventing ischemic diseases and arteriosclerosis [4]. The content of polyphenols in grapes and, accordingly, in wine depends on the type [2] and variety [3] of grape, as well as on the rules for the production and storage of wine [5]. Autochthonous varieties of grapes and, correspondingly, wine are of great interest [6, 7]. Polyphenols are one of the most common compounds found in fruits, vegetables and herbs. They are divided into several groups, although they are all biologically active and have antioxidant activity [8, 9]. In recent years, most attention has been paid to the antioxidant activity of grapes and wine, produced from it [10,11]. Obviously, they are involved in the prevention of many diseases caused by stress [12-14], and they also have anti-cold, anti-viral, anti-mutagenic and other actions [15]. The high content of phenolic compounds in red wine is associated with the peculiarity of production, when during fermentation the skin and grape seeds contact with each other for a long time in grape juice, and then they are extracted with alcohol [16].

According to some recommendations [17], moderate consumption of wine (150 ml per day for women and 300 ml for men) has a positive effect on human health. The positive effect is associated with the antioxidant activity of polyphenolic compounds

in wine [18]. Usually, the red wine contains more of them than the white one. Drinking wine reduces the risk of developing vascular disease [19, 20]. Many components of wine have antioxidant activity, so it is difficult to determine the activity of any one component taken separately. There are often deep internal connections between them [21]. Currently, antioxidant parameters for wines produced in Georgia are not defined (with a few exceptions, for example, our article on white wine [22], so commercial wines cannot use this important component for advertising.

## 1. The purpose of this study

Currently antioxidant parameters for wines produced in Georgia are not defined (with a few exceptions, for example, our article on white wine, so commercial wines cannot use this important component for advertising.

## 1.1 Samples

The object of the research was the widespread and popular autochthonous red and black grape of various varieties (Kabistoni Shavi, Otskhanuri Sapere, Aladasturi, Kachichi, Mujuretuli, Ojaleshi, Chkhaveri, Mtredispekha, Mtevandidi) and wine samples, prepared from them, grown in different regions. The grapes were harvested during the period of technical ripeness (period of mass harvest). The wine was prepared in the laboratory of the Batumi Shota Rustaveli State University using Kakhetian technologies. The grape mass was boiling on the "chacha" until the completion of wine fermentation.

#### 2. Methods

A Mettler Toledo UV-5 model UV-VIS spectrophotometer was used for Determination of phenolic compounds, anthocyanins and Antioxidant activity.

The object of the research was the widespread and popular autochthonous red and black grape of various varieties (Kabistoni Shavi, Otskhanuri Sapere, Aladasturi, Kachichi, Mujuretuli, Ojaleshi, Chkhaveri, Mtredispekha, Mtevandidi) and wine samples, prepared from them, grown in different regions. The grapes were harvested during the period of technical ripeness (period of mass harvest). The wine was prepared in the laboratory of the university using Kakhetian technologies. The grape mass was boiling on the "chacha" until the completion of wine fermentation.

## 3.1 Research methods

## 3.1.1 Total phenolics assay

The Folin–Ciocalteu method was used for the determination of the total phenolics. In brief, an aliquot (1 mL) of the appropriate diluted extracts was added to a 25 mL volumetric flask, containing 5 mL of distilled water. Then, 1.0 mL of Folin-Ciocalteu reagent was added and the contents mixed. After 3 min, 10 mL Na2CO3 solution of concentration 7% was added and made up to a total volume of 25 mL distilled water. Their absorbances were read at 765 nm against distilled water as the blank. A calibration curve was constructed using gallic acid standard solutions (0–100 mg/L). The concentration of total phenolics is expressed as the gallic acid equivalent (GAE) per 1 g of fresh sample. All samples were prepared in triplicate [23].

## 3.1.2 The total monomeric anthocyanin

The total monomeric anthocyanin content of the red wine was determined using the pH-differential method. The red grape extract and wine was dissolved in buffers of KCl (0.025 M, ph 1.0) and CH3COONa (0.4 M, pH 4.5) with a predetermined dilution factor. Absorbance (A) was measured using UV-Vis spectrophotometer at 520 and 700 nm, and the results were calculated as follows: The monomeric anthocyanin (MA) pigment concentration was calculated as: where represents the molar mass of malvidin-3-glucoside (493.5 g/mol), is the dilution factor, is molar extinction coefficient (28,000 L/mol cm), and is the cuvette optical path length (10 mm). The final anthocyanin concentration is expressed as milligram per 1000 mL or kg of red grape and wine of malvidin-3-glucoside. All analyses were done in triplicate [24, 25].

## 3.1.3 Antioxidant activity

Antioxidant activity (assay with DPPH) [25], The DPPH assay was done by using 1 mL of red grape extract and wine solution that was mixed with 1,5 mL of DPPH solution in methanol (0,02 mg mL-1), The mixture was homogenized for 30 min at room temperature and then the absorbance was determined at 517 nm. Antioxidant activity DPPH 50% inhibition mg of samples was calculated using the following formula 1 and 2:

AA % inhibition = [A(DPPH) - A(sample)\*100]/A(DPPH) (1),

Where A (DPPH) - Absorbance of  $0.01\ mM$  DPPH at  $517\ nm$ 

A(sample) - Absorbance of sample at 517 nm AA of mg sample = m\*50\*1000/V\*AA% (2), Where m – weight of sample, V – volume of sample, 50 - % of inhibition, 1000 – unit conversion from gram to mg

## 4. Results

The largest amount of phenolic compounds in the studied grape varieties was found in Ojaleshi (5691.75 mg / kg), Kabistoni Shavi (4756.36 mg / kg), Mujuretuli (4350.65 mg / kg), Otskhanuri Sapere (4091.05 mg / kg), relatively less - in Usakhelauri (3535.25 mg / kg), Kachichi (3456.52 mg / kg), Aladasturi (3269.27 mg / kg) and significantly less in Chkhaveri (2250.23 mg / kg), Mtredispekha (1411.9 mg/kg), Mtevandidi (1340.1 mg/ kg). The content of anthocyanins in each variety has similar patterns: Otskhanuri Sapere (631.16 mg / kg) and Ojaleshi (576.29 mg/kg) are also dominant here; the amount of anthocyanins is comparatively less in Usakhelauri (481.96) and Kabistoni Shavi (480.79 mg/kg), a bit less, almost 2 times, - in Chkhaveri (399.5 mg / kg), Aladasturi (392.83 mg / kg) and Mujuretuli (391.69 mg / kg), and much less - in Mtevanddi (230.15 mg / kg) and Mtredispekha (191, 21 mg / kg).

The autochthonous grape varieties have been processing with natural yeast (to preserve varietal characteristics), until the chacha was completely settled (about 20 days). The analysis of wines was carried out on wines of one year old aging. The total content of phenolic compounds in wine varies in different ways: Otskhanuri Sapere (3674.0 mg / kg), Kabistoni Shavi (3619.0 mg / kg), Ojaleshi (3566.32 mg / kg) have the highest content in this respect. The transition of phenolic compounds in these wines is almost 60% of the original. Although the proportion of transitions is about the same, they are relatively less in Kachichi (3012.0 mg / kg), Mujuretuli (2914.48 mg / kg), Aladasturi (2613.60 mg/kg), Usakhelauri (2374.36 mg/kg). Chkhaveri (1899.7 mg / kg), Mtredispekha (1121.04 mg / kg) and Mtevandidi (1032.53 mg/kg) have significantly less phenolic compounds.

During the production of wine, a certain amount of anthocyanins is transferred into it (most of the anthocyanins in grapes are soluble in alcohol). Their transition from grape to wine is somewhat correlated. The largest amount of anthocyanins is observed in wines made from such varieties as Ojaleshi (485.0 mg / kg), Otskhanuri Sapere (400.9 mg / kg), Usakhelauri (397.1 mg / kg), Kachichi (390.8 mg / kg). Accordingly, the content of anthocyanins in wines, produced from other varieties, is relatively lower: Aladasturi (368.45 mg / kg), Mujuretuli (344.8 mg / kg), Chkhaveri (335.3 mg / kg), Kabistoni Shavi (315,7 mg / kg). It is almost 3 times less in Mtevandidi (161.4 mg / kg) and Mtredispekha (141.8 mg / kg) than in Ojaleshi, although more than 60% of anthocyanins are transferred from these grapes to wine.

There is a direct correlation between the content of phenolic compounds in grapes and wines, prepared from it, and their antioxidant activity. Ojaleshi grapes have the highest rate among varieties (3.17 mg of a sample can inhibit 50% 0.01 mM DPPH, i.e. the lower this characteristic is, the more active the product is). The same picture is observed among varieties and in wines as well. Wine, prepared from Ojaleshi variety, compared to grapes, has lower characteristics, however, compared to other wines, it has higher characteristics (8.7 mg). Kabistoni Shavi (4.1 mg), Otskhanuri Sapere (4.1 mg), Mujuretuli (4.18 mg / kg), as well as Kachichi (4.7 mg) and, therefore, the wines produced from them: Kabistoni Shavi (10.2 mg), Usakhelauri (11.93 mg), Kachichi (13.4 mg), Otskhanuri Sapere (13.47 mg) and Mujuretuli (17.1 mg) have high antioxidant activity. The rest of the wines (Chkhaveri (20.3 mg), Aladasturi (20.81 mg), Mtevandidi (25.74 mg) and Mtredispekha (37.26 mg) and, therefore, their raw materials have less AA (Chkhaveri (8.2 mg), Aladasturi (10.39 mg)), Mtevandidi (14.56 mg) and Mtredispekha (21.85 mg).

	Grape			Wine	Wine				
Samples	Total phenols mg/kg	Anthocyanins mg/Kg	Antioxidant activity  DPPH - 50% inhibition mg of samples	Total phenols mg/l	Anthocyanins mg/l	Antioxidant activity DPPH - 50% inhibition mg of samples			
Kabistoni Shavi Imereti, Municipality of Tsageri, Village Okhureshi	4756,36	480,79	4,1	3619,0	315,7	10,20			
Otskhanuri Sapere Imereti, Municipality of Terjola, Village Zorvethi	4091,05	631,16	4,1	3674,0	400,9	13,47			
Aladasturi - Imereti, Municipality of Bagdadi, Village Phersathi	3269,27	392.83	10,39	2613.60	368.45	20.81			
Kachichi - Adjara, Municipality of Qeda, Village Kharaula	3456,52	441,08	4,7	3012,0	390,8	13.4			
Mujuretuli - Racha, Ambrolauri municipality, Village Tola	4350,65	391,69	4,18	2914.48	344.8	17.10			
Usaxelauri - Racha, Ambrolauri municipality, Village Tola	3535,25	481,96	4,12	2374.36	397.1	11.93			
Chkhaveri - Guria, Municilality of Chokhatauri, Village Erkethi	2250,23	399,5	8,2	1899.7	335.3	20.3			
Mtredispekha - Guria, Municilality of Chokhatauri, Village Erkethi	1411,9	191,21	21,85	1032.53	141.8	37.26			
Mtevandidi - Guria, Municilality of Chokhatauri, Village Erkethi	1340,1	230,15	14,56	1121.04	161.4	25.74			
Ojaleshi - Samegrelo, Municilality of Martvili, Village Salkhino	5691,75	576,29	3,17	3566.32	485.0	8.7			

Table 1. Grape and Wine Phenolic Compounds, Anthocyanin and AA.

## 4. Conclusion

Phenolic compounds, anthocyanins and their antioxidant activity of 7 autochthonous grape varieties of Western Georgia and wines produced from them (Aladasturi, Mujuretuli, Chkhaveri, Kabistoni Shavi, Mtevanddi, Mtredispekh and Ojaleshi) were studied. There is a direct correlation between the content of phenolic compounds in grapes and its wines and their antioxidant activity. Ojaleshi grapes and wine have the highest antioxidant activity among these varieties.

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# Assessment of ecological condition of Kosh stone pit adjacent agrocenoses and the improvement measures

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### ABSTRACT

The article analyzes and presents the results of the impact studies of the Kosh stone pit on the productivity of the agro-ecological systems of the Kosh, Ujan and Avan communities in the Ashtarak district of the Republic of Armenia. Studies have shown that under the influence of tuff quarry dust, scrap, among other anthropogenic factors, decreased the productivity of natural agro-cenoses in the adjacent communities of the stone pit (Kosh, Ujan), deteriorated the photosynthetic capacity of plants, decreased air permeability reduced humus and macro-food-elements amounts, while no significant negative impact was observed on the productivity of agro-cenosis in the Avan community, which is essentially away from the stone pit. At the same time, it was substantiated that in order to mitigate the negative impact of the stone pit on the agro-cenosis of the adjacent surrounding communities and to increase the fertility of the soils, it is necessary to carry out fertilization works with mineral-organic fertilizers, and perennial grasses in grasslands sowing works according to the degree of land degradation and prescribed improvement measures.

Keywords: Stone pit, Agricultural systems, Productivity, Organic fertilizers, Improvement measures, Communities.

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## **Background**

Human economic activity, especially misuse of natural resources, causes many negative phenomena in the environment, as a result of which the balance of nature is disturbed, the state of the biota and its bio-genic habitats is weakened, the current and future processes in natural social systems become unpredictable.

In the territory of the Republic of Armenia, 7.0 - 7.5 thousand ha of land is occupied only under the waste of stone materials (tuff, basalt, etc.). These wastes, as a result of winds and tornadoes, as well as heavy rains and floods, constantly pollute the air, water and land areas of the settlements, causing significant damage to the natural and agricultural systems in the surrounding areas, reducing their productivity, significantly affecting biodiversity,

particularly on conservation of individual endemic animal and plant species [1].

There are many stone pits with different colors of tuff in Armenia, where more than 92 million m3 of tuff has been extracted since 1928 [2,3].

The Kosh tuff stone pit, where ore mining was carried out in the 55s and 60s of the last century, has been mechanized since 1969. Over the past 50 years, about 15.3 million m3 of tuff has been extracted, 37% of which has been used as masonry, and the rest - 63% or 9.6 million m3 as waste, dumped into the environment in the form of scrap and dust [2, 4]: The mentioned wastes, as a result of unwise exploitation of the quarry, not only caused the formation of industrial deserts, where only certain species of weeds (lice, thorns, etc.) grow scarcely, but also caused formation of floods and sediments due to mixture of dust from the quarry and heavy rains. As

a result, the productivity of natural agricultural systems decreases, the yield of horticulture fields and the productivity of livestock decreases. Therefore, any study aimed at identifying the negative effects of the stone pit to identify measures to improve them is relevant to the requirements of the regional development strategy plans.

### Materials and methods

A task was set to study and assess the land degradation in the Kosh quarry adjacent Kosh, Ujan and Avan communities productivity of the agricultural systems due to waste generation from mine exploitation. In particular the following tasks were fulfilled:

- a) The level of degradation of agricultural lands (arable lands, natural pastures) in Kosh, Ujan and Avan communities, adjacent to the quarry, over the last three years.
- b) Develop a system of measures to mitigate the land degradation and increase their productivity.
- c) Develop recommendations to the farmers of the communities on the possible application ways of the developed measures.

To study the condition of arable lands, pastures and grasslands of the mentioned rural communities, to make comparative analyses with the data of the non-degraded areas of those systems, as well as to determine the degree of their degradation due to the quarry activity, we performed visual, cartographic [5,6] and laboratory study, as well as the results of agricultural activities for the last three years to determine the impact of the quarry, conditioned by the distance, on the economic efficiency of the agricultural activities of the communities through the comparison of crop yields.

Soil samples were taken from community arable lands and natural grasslands. The content of humus, environment reaction (pH), available nutrients (NPK) were determined in the samples. These indicators were compared with similar indicators of non-degraded or considered agricultural land types. As well as the production data of 3 years were taken from the three communities located at different distances from the stone pit (Kosh, Ujan, Avan) through the comparisons revealed the impact of the quarry activity on the results of agricultural production received by those communities.

Laboratory analyzes of soils were performed by

universal methods, which are given in manual of methods of agrochemical analysis, edited by B.A. Yagodin [7].

The hummus was determined by Turin method, with the help of phenyl andranyl acid, vie a titration method, The reaction of the soil solution pH was determined by the electropotentiometric method, the total nitrogen was determined by Kjeldal, and the easily hydrolyzable nitrogen was determined by I.V. Turin and M.M. Kononova methods. The content of mobile phosphorus was determined according to Arenius and Machigin, and the exchange potassium was determined by Maslova's methods.

#### Research results

Analyzing the agrochemical parameters of the lands of Kosh, Ujan and Avan communities located at different distances from Kosh quarry, it became clear that in the arable lands and natural grasslands of communities immediately adjacent to the quarry are more severely degraded (Kosh, Ujan) by mine waste (dust, rubble), than similar land areas of Avan community, located 21-25 km from the quarry (Table 1). Thus, if the humus content in the pastures and arable lands of Kosh community adjacent to the quarry was only 0.8 and 1.6%, the content of available nutrients (NPK) in pastures were only 2.2, 2.8 and 26 mg per 100 grams of soil, in arable lands -3.2, 5.2 and 27 mg, than at a distance of 10 km from the Kosh stone pit mine, east of Kosh community, in Ujan community the same land type pastures and arable lands, the mentioned indicators have better values than in Kosh community. However, the same could not be said about Avan community, which is located 21 km north-west of Kosh stone pit mine: despite the lands of mentioned communities are less degraded, both the humus content and the nitrogen, phosphorus-potassium levels are higher than the similar indicators in the pastures and arable lands of Kosh and Ujan communities, they are almost close to the agrochemical indicators of the non-degraded soils of the region (Table 1). Due to the activity of the mine, studying of the average yields of agricultural crops grown in Kosh, Ujan and Avan communities over the last three years (2017-2019), comparing the distances of communities from the Kosh stone pit mine, reavealed that near the mine or in the nearest communities, the yield of agricultural crops and orchards is less than the yield of similar crops grown in the community lands farther away from the mine [8].

Thus, if in the Kosh community, which is near to Kosh stone pit area, the average yield of grain (autumn wheat) was 24.6 c / ha, plum - 34.2 c / ha, green bean - 57.5 c / ha, apple - 128.5 c / ha, in the Ujan community, which is 11 km away from the mine, the average yield of the same crops were-

26.2 c / ha of autumn wheat, 62.5 c / ha of green beans, 63 c / ha of stone-fruits, 62.4 c / ha of grapes, 156.8% of apples. c / ha, while farmers and individual peasants of Avan community, located 21-25 km away from Kosh mine, provided higher yields from the same crops in 2017-2019 than in Ujan community - almost 26-50% higher than near the mine located Kosh community.

**Table 1.** Structure of the land fund, agrochemical indicators of arable lands and pastures of the communities adjacent to the Koshi quarry\*

			, yes			S	ım	%	ract		ilable nu ent, mg p g of soi	er 100
Community name and distance from the mine	Total land. ha	Arable land, ha	Perennial planting, yes	Grasslands, ha	Pastures, ha	Land-use types	Sample depth, sm	Humus content,%	pH in aqueous extract	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
Kosh	690	152	32	_	194	arable land		1.6	8.0	3.2	5.2	27.0
1.5- 2,5 km						pasture		0.8	8.2	2.2	2.8	26.0
Ujan	1948	328	82	6	6 918	arable land		1.8	7.7	3.6	5.0	29
9.5- 11 km	1740	320	02	0	710	pasture	20	1.1	7.6	3.7	3.0	30
Avan	1300	448	162	2	245	arable land	- 0	2.2	7.4	4.8	6.3	32
21- 25 km	1300	110	102	.62   2		pasture		1.5	7.2	3.9	4.8	33
Non-degraded	_	_	_	_	_	arable land		2.4	7.3	5.6	6.4	34.0
1 ton degraded						pasture		1.8	7.1	5.4	6.9	33.0

<sup>\*</sup> Considering that there are no or almost insignificant grasslands in the studied communities, no studies were conducted in such areas.

The average yield of autumn wheat in Avan community was 39.5 c / ha, green beans - 87.0 c / ha, cherry - 124.6 c / ha, apple - 197.0 c / ha (Table 2).

The results of agricultural activities are different in the communities at different distances from the mine; the farther from the mine, the higher yield of crops are observed and on the contrary, if this or that community is closer to Kosh quarry, than the yield of crops grown in that community is much lower. By the way, such a pattern was observed when determining the level of degradation and soil fertility, especially the content of humus and mobile nutri-

ents (NPK) in the pastures of those communities, especially in rural-side areas.

Thus we can definitely conclude that under the influence of Kosh quarry dust and scraps, among other anthropogenic factors, decreases in natural (in this case pastures, because in these communities there is almost no natural grassland) and agricultural systems' productivity is observed. Also water and air permeability of arable lands is worsened, humus content and generally field yield, as well as pasture use efficiency is decreasing.

**Table 2.** Sown areas of main agricultural crops and yield data in Kosh, Ujan and Avan communities of Ashtarak

Communities	Crops	Occupied areas, ha				The actual collected harvest, c				
	Autumn Wheat	20	17	20	492	418.2	492		24.6	
	Green bean	5	4	6	287.5	230.0	345.0		57.5	
Kosh	Plum	5	5	5	170	173	170		31.2	
	Apple	4	4	4	518	514	510		128.5	
	Cucumber	5	3	6	564	582	576		123.0	
	Autumn wheat	50	57	55	1310	1493.4	1441		26.2	
	Green bean	6	8	8	372	504	500		62.5	
Ujan	Plum	141	141	143	8883	8980	8786		63.0	
U.j.	Apple	6	6	6	950	930	942		156.8	
	Grapes	62	62	62	3731	4030	4028		63.4	
	Potato	18	12	18	2340	1660	2240		130.0	
	Autumn what	29	35	40	1146	1462	1501		39.5	
_	Green bean	7	8	8	696	690	615		87.0	
Avan	Cherries, plums	41	41	42	5230	5208	5010		124.6	
	Apple	53	54	54	10608	10508	10144		197.0	
	Walnut	4	4	5	247	196	198		49.3	

Therefore, taking into account the low level of fertility of the arable lands of the communities, as well as the negative impact of Kosh quarry functioning on the agronomic productivity (agro-cenosis productivity) of neighboring communities (Kosh, Ujan), especially on the agricultural crops, we propose to apply the following mixtures of organic and mineral fertilizer in Kosh community.

- Combining the norm of 5-8 t / ha of biohumus or organomix with 60 (N60P60K60) kg / ha norm of nitrogen, phosphorus and potassium active substance.
- 2. For the fertilization works in arable lands in Ujan community use 70-75% of the fertilization norm proposed for the Kosh community, and in Avan community -up to 50% of the proposed norms for the Kosh community.
- 3. In order to increase the content of humus and available nutrients, the density and composition of vegetation per unit area, in the degraded pastures of Kosh, Ujan and Avan communities- it is necessary to carry out the following surface improvement works,
- Fertilize with the rotten manure at the rate of 10-15t / ha and 30 kg / ha of NPK measure and collect stones.
- Carried out sowing with the seeds of perennial herbs with the folloing norms in the communities: in Kosh- 20 kg/ha, in Ujan - 15 kg / ha and in Avan of 9-10 kg/ha

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# Synthetic analysis of energy tariffs and conceptual bases for its change (on example of Georgia)

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#### ABSTRACT

The article describes the factors affecting energy tariffs that lead to its change. These include globalization, the existence and utilization of natural energy resources in the country, energy balance, energy efficiency, the role of society in energy development, etc. The peculiarities and principles of the field are mentioned in this regard, first of all, the need to utilize increasingly expensive energy resources and the need to take into account environmental requirements. According to the author, in order not to increase tariffs, we must achieve the introduction of scientific-technological and managerial progress in the field at a faster pace than the deterioration of natural conditions and other negative challenges.

Keywords: Georgia, Tariff, Energy, Technical Progress, Management Electrical Balance.

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### Introduction

Georgia has long linked its destiny to a market economy. The transition to a new way of life has created many problems in the country. Among them were energy challenges and, above all, tariffs. Added to these and other negative developments was the occupation of 20% of the territory. Despite international outcry, the country found itself in dire straits. This can be clearly seen from the data below [1].

General information about Georgia:

- 1. Georgia is located in the South Caucasus, on the crossroad of eastern Europe and western Asia
- 2. Total area is 69 700 km2. 20% out of total area is occupied by Russia
  - 3. Population: 3 716 900
  - 4. Capital: Tbilisi
  - 5. Currency: Lari (1 US Dollar=3,30 GEL)
  - 6. GDP: 44599,3
- 7. Fuel and other energy sources are found in slight amount, though not very rich in this aspect.
- 8. Rivers hold huge amounts of hydropower, largely compensating fuel shortage of the country.

Currently, resources of technical hydropower is utilized at only 12%

9. Georgia is rich with alternative sources of energy (solar, geothermal, wind, etc.).

This article will play a positive role not only for Georgia, but also for countries in a similar situation.

## Basic Part

## 1. Factors affecting the tariff

Among the many problems facing Georgia today is the issue of secure and sustainable energy supply [2]. We all know that energy is the lifeblood of the economy. The socio-economic development and strength of the country, the well-being of the population, etc. depend on it. We can not achieve economic security without achieving energy security; Moreover, the independence and statehood of the country will be threatened. Particularly noteworthy is the fact that the increase or decrease in energy prices by chain reaction is immediately reflected in

the consumer market. It is therefore not at all surprising that every increase in the consumer tariff on electricity has a severe impact on society. This was also the case at the end of 2020, when a significant increase in tariffs was observed, although for some time it was subsidized for a large part of consumers. This is obviously a heavy burden for our economy [3]

Now there is a question if this decision of the National Energy Regulatory Commission (GNERC) was objective or not. However, I will say from the beginning that both companies and consumers are dissatisfied with this decision. This is the main recognized indicator of the objectivity of the regulatory body in the world. Practically neither side of the regulatory body should be satisfied. After all, he is the defender of justice and universal balance in the field [4].

First of all, about the methodological correctness of the tariff determination. This process is fully based on a methodology recognized by international standards, adapted to local conditions; And statistics on operating and investment costs are audited by relevant experts. Here falsity or human error is virtually ruled out. The calculation is done using modern means. The process will go from a regular employee of the regulatory body to an employee of all levels including senior management. They have a high responsibility before the law and the country. The final decision will be made publicly with the participation of all stakeholders. The tariff is published in the press, and in case of dissatisfaction the party is entitled to apply to the court at all levels, including the Constitutional Court. GNERC had such a precedent in the past [5].

What is the objective environment and operating factors related to energy tariffs in Georgia, which undoubtedly have an impact on determining the level of tariffs.

Globalization. Our country has long been connected to its future market economy and has become a full member of the international community. Here, too, the principle of value is the basis for setting tariffs. It does not take into account whether the country is rich or what the incomes of the population are. Georgia has so far maintained relatively low tariffs. Only in energy-rich countries is it lower than us. In most states, however, it is much higher. According to the results of 2019, our The electricity tariff in the neighborhood was as follows: in Georgia (at white kWh) 18.89; In Azerbaijan - 13.88; In Armenia - 24.69; In Turkey - 27.09; In Russia - 16.34.

Leading European countries: France - 56.44; In Germany - 98.75; In Belgium - 90.79; In Denmark - 95.43. In post-socialist countries: Lithuania - 40.13; In Latvia - 52.09; In Moldova - 29.23; In Estonia - 43.40.

Table. Household electricity tariffs operating in different countries, 2019 [6].

Country	Household tariff (white / kWh)
Azerbajan	13.88
Ukraine	14.13
Russia	16.34
Georgia	18.89
Serbia	22.58
Armenia	24.69
North Macedonia	25.04
Turkey	27.09
Bosnia and herzegovina	27.92
Moldova	29.93
Bulgary	31.88
Montenegro	33.00
Hungary	35.82
Lietuva	40.13
Malta	41.73
Croatia	42.24
Poland	42.95
Estonia	43.40
Romania	43.43
Island	44.96
Slovakia	50.43
Latvia	52.09
Slovenia	52.25
Greece	52.77
Finland	55.45
Czech Republic	55.90
France	56.44
Luxembourg	57.50
Norway	59.70
Sweden	64.44
Austria	65.05
Netherlands	65.62
UK	67.86
Portugal	68.75
Cyprus	70.45
_ Italy	73.58
Spain	76.85
	77.49
Belgium	90.79
Denmark	95.43
Germany	98.75

Such are the consequences of globalization. The situation was exacerbated by the events of 2020, primarily the widespread and prolonged spread of the Corona virus. As a result, energy has also become a hostage of medicine. During the first months of the pandemic, companies suffered huge losses. If before the pandemic electricity consumption in Georgia was significantly ahead of production, now the picture has changed, consumption is lagging behind production. This happened both in the capital and in Georgia as a whole [7].

Power generation structure. The impact of this factor on tariffs is well known. The existence of hydro and thermal capacities in the electrical system has its necessity, but also its pros and cons. Construction of hydroelectric power plants requires relatively more time and investment compared to thermal capacities. But due to the need for expensive fuel, the tariff in thermal power plants is 5-6 times higher. Their tariff also includes the so-called "guaranteed capacity" factor in summer. In Georgia, in the conditions of increased electricity consumption during the last 5 years (2015-2019), it became necessary to increase electricity production in thermal power plants from 2378.7 to 2420.2 million. KW. Per hour, ie its share in total output increased from 21.4 to 22.2%. The comparison is especially noticeable compared to 2010. Then the thermal power plants will produce 655.9 million kWh, or 3.7 times less than in 2019. If we add to the above the fact that the increase in electricity imports increased the fact that in 2015-2009 it became necessary to increase imports of imported electricity from 699.3 to 1626.5 million kWh, or 2.3 times, the picture becomes clearer [8].

Deficient energy balance. The energy balance of our country is traditionally deficient. Georgia currently imports about 70% of the energy resources it needs, only 30% of which is locally produced. Natural gas and petroleum products are almost completely imported in the energy balance. In recent years, especially in the autumn-winter period, the electricity balance is also deficient. In 2019, the country imported 1,762.7 million kWh from abroad - about as much as the incomplete Khudoni HPP would generate [9].

Existence and utilization of natural energy resources. Almost all of them are more or less energetic on the territory of Georgia. We are especially rich in hydropower and non-traditional (solar, wind, geothermal) resources. We have coal and oil fields. The first of them is in the region only in Georgia.

As for their assimilation it is unsatisfactory. We use only 10-12% of the technical capacity of hydropower resources and 20-25% of the economically advantageous potential. Oil and coal absorption levels are low. Only at the initial stage is the use of solar and wind yet expensive energy [10].

The role of society. The conscious participation of the community in the development of energy is special. Under the influence of this factor, Khudoni HPP and Namokhvani cascade could not be built in Georgia in recent years. The same picture continues now. If in the past large hydropower plants were targeted, now a certain part of the society is against the construction of hydropower plants of any size and in parallel they are also protesting against the increase of electricity tariffs. We unfortunately have many examples of this [11].

Energy efficiency. The ancient truth is that without an economy, society cannot successfully manage its limited resources. An intensive form of extended reproduction is an organic characteristic of the economic system of a society. It creates objective guidelines for the rational and efficient use of resources to achieve better end results [12].

Although the energy and electricity capacity of products in Georgia has significantly decreased in recent years, this figure is still much higher than in developed countries. According to the latest data, the energy capacity of GDP in Georgia was equal to 0.87 kg. USD, while this parameter was in Germany - 0.03; In Japan - 0.005; In Turkey - 0.029, etc. It turns out that we consume more energy per unit of production than countries rich in energy. Special mention should be made of energy losses In 2019, 155.7 million kWh of energy was lost in the distribution network of "Telasi". In the network of "Energo Pro Georgia" 526.7 million kWh. Therefore, 682.7 million kWh were lost on electricity transportation in both companies. That is almost as much as it was generated in 2019 by such a large HPP as Vartsikhe HPP. (727.3 million kWh) [13].

### 2. Peculiarities of field development

Tariff change is significantly influenced by the fact that the characteristics of energy are practically taken into account in the development of the sector. In addition to the fact that the production and consumption of electricity coincide in time, this sector is characterized by high intellectual labor capacity and capital capacity among the sectors of the economy. Second, the industry requires intensive and

continuous funding to maintain its ability to function while achieving progress in meeting the needs of the macroeconomic environment. Third, unlike other sectors, energy has a high socio-economic responsibility. Fourth, it as a typical representative of a natural monopoly needs independent regulation. Fifth, it is necessary to attract a significant amount of investment. This is due to the combined influence of other objective factors (ecology, the need to use increasingly expensive energy resources, etc.) to further increase costs, which leads to tariff increases [14].

The role of management in the practical consideration of these features is special. It has been proven that the issue of management is uniquely relevant for energy, which is characterized by a number of features. First, it is characterized by high intellectual labor capacity and capital capacity among the sectors of the economy; Second, the sector requires intensive and continuous funding in order to maintain its ability to function while achieving progress in line with the requirements of the macroeconomic environment; Third, unlike other sectors, energy has a high socio-economic responsibility; Fourth, it, as a typical representative of a natural monopoly, requires state regulation; Fifth, it is necessary to attract a significant amount of investment. This, as a result of the combined influence of other objective factors (ecology, the need for increasingly expensive energy resources, etc.), further increases the capital capacity of the sector [15].

During the last five years (2015-2019), a total of GEL 1,140 million was invested in Georgia's electricity, including 22.1% in electricity generation, 33.6% in transmission-dispatching and 44.3% in distribution. Annually 228 million GEL on average. It was desirable that the share of investment in production be higher.

In large companies such as Telasi and Enguri HPP, the total number of employees is solidly represented by engineering and technical staff and managers. According to the results of the last three years (2017-2019), the share of managers (administration) in Enguri HPP varied from 13.3 to 17.3%, and from 21.7 to 26.7% in engineering and technical staff, and in Telasi respectively. Within the optimal range of 4 - 7,6% and 26,8-28% [16].

### 3. The tariff change paradigm

Independent regulation of the sector is the big-

gest obstacle to tariff changes. It makes tariffs more stable and acceptable to the public. The regulatory body has been functioning in Georgia for almost twenty-five years and its results are already well known. Research has shown that prices in a regulated market rise much more slowly than in a free market, including for energy products that are not regulated. Assuming that the Consumer Price Growth Index for Ten Years is 1; Then a similar rate of increase in the price of gasoline is 3.39; Kerosene 3.23; Diesel 3; Liquefied gas 3.69 and so on [17].

Particularly important in terms of tariff changes is the factor that has a cumulative impact on tariff growth (ecology, exploitation and investment costs). If we add to this the need to take into account environmental requirements, it becomes clear that obtaining the same amount of energy - production is becoming more and more expensive. Consequently, the technical-economic indicators of the sector, including the tariff, are objectively deteriorating. Then where is the solution?

Independent regulation of the sector is the biggest obstacle to tariff changes. It makes tariffs more stable and acceptable to the public. The regulatory body has been functioning in Georgia for almost twenty-five years and its results are already well known. Research has shown that prices in a regulated market rise much more slowly than in a free market, including for energy products that are not regulated. Assuming that the Consumer Price Growth Index for Ten Years is 1; Then a similar rate of increase in the price of gasoline is 3.39; Kerosene 3.23; Diesel 3; Liquefied gas 3.69 and so on.

We think there is such a solution. We need to achieve the pace of scientific-technological and managerial progress in the field at the pace of deteriorating natural conditions and other negative challenges. Otherwise, the technical and economic indicators of the sector will deteriorate, prices for energy sources will increase.

I believe that humanity will achieve the protection of such a ratio it has such potential. Signs of this are already being observed in the world. For example, Japan, which is arguably isolated from the rest of the world and is not really rich in natural energy resources, has been able to achieve reliable and sustainable electricity supply through scientific, technological and managerial progress, while making the country economically rich and powerful. It now generates \$ 48,472 per capita GDP while consuming 8,816 kWh of electricity, about as much as large, energy-rich and developed countries. As for

the tariff, it is 29.1 cents, which is almost the same as in these countries. It is true that so far there is also a trend of tariff queues, but at a relatively moderate pace [18].

The process of tariff increase is a characteristic feature of market economy countries. The main thing is to be provided with electricity. Sadly, millions of people around the world are now deprived of this goodness.

## 4. Development concept

For the normal functioning of the Georgian economy, it is especially necessary to take effective measures for the development of the energy complex. For this, it is necessary: 1. Considering the requirements of nature protection, to rationally use the whole complex of fuel and energy resources in the area - hydropower, coal, oil and non-traditional sources; 2. To establish the optimal structure of the fuel-energy complex suitable for the conditions of Georgia. In the fuel-energy complex, taking into account the energy, ecological and socio-economic requirements, their place should be given to both hydro and thermal power and other alternative energy sources; 3. It is necessary for the economic and rational use of these resources to play a great role in the energy supply of the Republic; 4. Georgia must establish reliable and favorable external energy ties with the world's leading countries, including neighboring countries [19].

The interests of the sustainable development of the sector require constant improvement of regulation, improvement of its standards using international recommendations and accumulated experience, including the development of a comprehensive tariff methodology, ensuring the reliability of supply and thus promoting a sustainable investment environment in the energy sector. Increase efficiency.

An important precondition for the transition to the European energy model was Georgia's accession to the energy community in 2017. With this fact, our country was forced to comply with the EU.

Approximation with legislation that is being successfully implemented. At the end of 2019, the Parliament of Georgia has already adopted a new law "On Energy and Water Supply". There is a lot of work to be done in this regard in the future. The energy saving factor is especially important. According to expert calculations, the untapped technological potential of energy efficiency is equal to

about 20% of the total energy consumption of the country. Thus it can be boldly considered as a new energy resource. Electricity capacity in Georgia has been declining in recent years, but its level is still unfavorable [20].

The key directions of energy saving for our country are: acceleration of scientific and technical progress in the field of production and consumption of energy resources; Improving the sectoral, technological and territorial structure of the economy; Expanding the use of non-traditional energy (solar and wind energy; thermal waters, biomass, secondary energy resources, etc.) and small rivers, improving agricultural management and mechanism in energy saving [21].

Energy development obviously requires significant funds. This is often the reason for the underdevelopment of the field. However, a special study showed that if we compare the amount of investment needed for energy development and the possible loss of national income caused by the loss of electricity, we will see that the latter is 5 times more than the former. Therefore, the capital investments required for the development of electricity in Georgia are 5 times less than the losses that will be caused by the underdevelopment of this sector [22].

#### Conclusion

The increase in energy tariffs is due to many objective factors, including natural conditions and environmental requirements. In order not to increase tariffs, it is necessary to achieve the advancement of scientific-technological and managerial progress in the field at a faster pace compared to the rates of deterioration of natural conditions and other negative challenges.

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# Polysaccharide-hydrolysing secretome of Schizophyllum commune during growth on different carbon sources

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#### ABSTRACT

In the present work, the polysaccharide-hydrolysing secretome of Schizophyllum commune BCC 632 was analysed in submerged fermentation conditions to elucidate the effect of chemically and structurally different carbon sources on the expression of cellulases and xylanase. Among polymeric substrates, crystalline cellulose appeared to be the best carbon source providing the highest endoglucanase (53.5 U/mL), total cellulase (9.2 U/mL), and xylanase (636.1 U/mL) activities. The use of mandarin pomace as a growth substrate also allowed to achieve high volumetric activities of all target enzymes whereas wheat straw and xylan turned out to be the weakest inducers of enzyme production. The supplementation of the Avicel or wheat straw-based medium with a low concentration of easily metabolizable carbon source (mandarin pomace or glycerol) favored enzyme secretion. The addition of 0.5% glucose to the Avicel-containing medium caused short-term catabolite repression of the synthesis of both cellulase and xylanase while the addition of  $\alpha$ -deoxy-D-glucose prevented enzyme secretion. It was shown that the presence of compounds inducing the formation of cellulase and xylanase by S. commune BCC 632 depends on the age of the fungal culture.

Keywords: Schizophyllum commune, Submerged fermentation, Cellulase, Xylanase, Carbon sources, Regulation.

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### Introduction

Agro-industrial plant residues accumulating worldwide in huge quantities as wastes/by-products of crop cultivation and food processing are renewable, abundant, and cheap resources for bioconversion to various value-added products including biofuels, chemicals, animal feeds, and human nutrients [1,2]. Hydrolysis of biomass polysaccharides into fermentable sugars by cellulases and hemicellulases is the key step for enzymatic conversion of lignocellulose. Cellulases comprise endoglucanases (EC 3.2.1.4) which cleave internal  $\beta$ -1,4-glucosidic bonds of cellulose chains, exoglucanases (EC 3.2.1.91) which processively act on the reducing and non-reducing ends of cellulose to release shortchain cello-oligosaccharides, and β-glucosidases (EC 3.2.1.21) which hydrolyze soluble cello-oligosaccharides to glucose. As far as hemicellulases are concerned, endo- $\beta$ -1,4-xylanases (EC 3.2.1.8) and  $\beta$ -xylosidases (EC 3.2.1.37) and auxiliary enzymes are required for the complete hydrolysis of xylan.

The main challenges for wide and large-scale application of cellulases and xylanases are reducing their cost and developing more efficient enzyme cocktails with high specific activity and stability [3,4]. Therefore, considerable research efforts were devoted to the bioprospecting of enzyme producers from less studied environments and exploitation of alternative sources of cost-effective enzymes. Wood- and litter-degrading basidiomycetes produce a variety of extracellular enzymes including glycoside hydrolases. Moreover, some of them have shown exceptional potential for the production of individual groups of hydrolytic enzymes under appropriate cultivation conditions. Thus, Coprinellus disseminatus produced 469 U/mL of alkali-ther-

mo-tolerant xylanase along with negligible cellulase activity [5] while Armillaria gemina secreted up to 146 U endoglucanase/mL, 15 U  $\beta$ -glucosidase/mL, and 1.72 U FPA/mL [6]. Moreover, Jagtap et al. [7] achieved very high  $\beta$ -glucosidase activity (45.2 U/mL) in the submerged cultivation of Pholiota adiposa in a medium containing rice straw and corn steep powder.

Recently, during the extensive screening of wood and litter-deconstructing basidiomycetes for lignocellulolytic enzyme production, Schizophyllum commune BCC 632 has been revealed as a potent enzyme producer [8]. Several studies already reported on the ability of S. commune to secrete cellulase and xylanase and optimization of these enzyme productions [9-12]. It is worth noting that the crude enzyme cocktail derived from S. commune demonstrated superior performance over a commercial enzyme preparation from Trichoderma longibrachiatum in the hydrolysis of pre-treated lignocellulosic biomass at low enzyme loadings [11]. Moreover, S. commune was exploited for ethanol production from wood chips by consolidated bioprocessing [13]. However, studies on physiological mechanisms of cellulase and xylanase production by S. commune have been limited and more indepth studies are required to understand how specific environmental factors modulate the secretion of individual cellulases and xylanase isoenzyme and develop the fungus enzyme system for biotechnological application. Therefore, in the present work, the polysaccharide-hydrolysing secretome of S. commune BCC 632 was analysed in submerged fermentation conditions to elucidate the effect of chemically and structurally different carbon sources on the expression of cellulases and xylanase.

## Materials and methods

Organisms and inoculum preparation

Schizophyllum commune BCC 632 isolated from a tree branch in Georgia and deposited in the Institute of Microbial Biotechnology basidiomycetes culture collection has been used in this study. The fungal inoculum was prepared by growing the mycelium on a rotary shaker at 150 rpm and 27 oC in 250 mL flasks containing 100 mL of standard medium (g/L): glucose – 15.0, KH2PO4 – 0.8, K2HPO4 – 0.6, MgSO4 x H2O – 0.5, peptone – 3.0, yeast extract – 3.0, pH 6.0. After 7 days of fungal cultivation mycelial pellets were harvested and homogenized using a Waring laboratory blender.

Cultivation conditions for hydrolases production The submerged cultivation was carried out using rotary shakers Innova 44 (New Brunswick, USA) at 160 rpm and 27 °C in 250-mL flasks containing 100 mL of the medium of following composition (g/L): KH2PO4 – 0.8, K2HPO4 – 0.6, MgSO4 x H2O - 0.5, (NH4)2SO4 – 5.0, yeast extract – 5.0, pH 5.8. Crystalline cellulose (Avicel), soluble carboxymethyl cellulose (CMC, low viscosity, Sigma, USA), xylan from birchwood (Sigma, USA) at a concentration of 15 g/L and milled to powder wheat straw and mandarin peels at a concentration of 40 g/L were used as the fungus carbon sources. Moreover, the effect of adding glucose, α-deoxy-D-glucose, methyl-α-D-glucose, and cycloheximide to the 1.5% Avicel-containing medium on enzyme synthesis was assessed in short-term experiments. Also, in separate short-term experiments, the fungus was grown in a medium with cellulose for 5 and 10 days, then the solids (biomass and cellulose) were separated by filtration. The resulting filtrate was boiled 5 min to inactivate available enzyme activity, added with 1.5% Avicel, then inoculated with homogenized biomass of the fungus grown both in a medium with glucose and in a medium with cellulose.

During the fungus cultivation, at predetermined time intervals, 1-2-ml samples were taken from the flasks, the solids were separated by centrifugation at 10,000 g for 10 min at 4 °C and the supernatants were analysed for pH, reducing sugars, protein content, and enzyme activities.

### Analytical methods

Protein concentration in culture liquids was determined using the Bradford Reagent (Serva, Heidelberg, Germany) according to the manufacturer's instructions. The total cellulase activity (filter paper activity, FPA) was measured with Whatman filter paper No. 1 according to IUPAC recommendations [14]. Endoglucanase (CMCase) activity was assayed using 1% low-viscosity carboxymethyl cellulose in 50 mM citrate buffer (pH 5.0) at 50 oC for five minutes [14]. Xylanase activity was determined using 1% birchwood xylan (Roth 7500) in 50 mM citrate buffer (pH 5.0) at 50 oC for 10 min [15]. Glucose and xylose standard curves were used to calculate the cellulase and xylanase activities. In all assays, the release of reducing sugars was measured using the dinitrosalicylic acid reagent method [16]. One unit of enzyme activity was defined as the amount of enzyme, releasing 1  $\mu$ mol of reducing sugars per minute. To measure  $\beta$ -glucosidase and  $\beta$ -xylosidase activities, the reaction mixture containing 1.8 mL of 2 mM solutions of p-nitrophenyl- $\beta$ -D-glucopyranoside or p-nitrophenyl- $\beta$ -D-xylopyranoside in 0.05 M acetate buffer, pH 4.8, and 0.2 mL of the enzyme solution was incubated at 50 °C for 10 min [17]. One unit of enzyme activity was defined as the amount of enzyme releasing 1  $\mu$ mol of p-nitrophenol per minute.

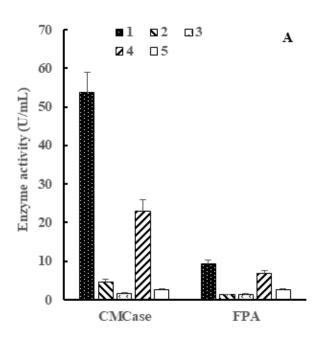
## Zymogram analyses of CMCase activity

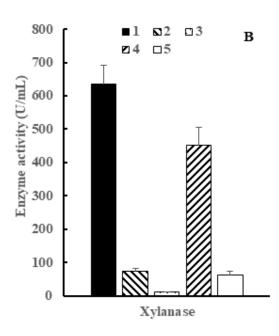
The native-PAGE was performed using 10% separating and 5% stacking gels. Separating gels were incorporated with 1% carboxymethyl cellulose. A sample containing 10  $\mu g$  protein was loaded in a well. Electrophoresis was performed at a constant 100 V for 3 h using a Vertical Gel Electrophoresis System (MRC, Israel). After electrophoresis, the

native gel was removed from the glass plates, rinsed twice with distilled water, and then incubated in so-dium citrate buffer (0.1M, pH 5.0). CMCase bands were developed after staining with 0.1% Congo red (w/v) for 30 min followed by de-staining with 1 mM NaCl until the clear zone was observed. Staining and de-staining procedures were performed on Rocker Shaker MR-1 (Biosan). The reaction was fixed with 5% acetic acid solution for 5 min.

## Statistical analysis

All experiments were performed twice using three replicates each time. The results are expressed as the mean  $\pm$  SD. The mean values, as well as standard deviations, were calculated by the Excel program (Microsoft Office 2010 package) and only values of p  $\leq$  0.05 were considered as statistically significant.





**Fig. 1**. Effect of the polymeric carbon sources on the S. commune BCC632 cellulases (A) and xylanase (B) activities. Legend: 1-1.5% Avicel, 2-1.5% CMC, 3-1.5% xylan, 4-4% mandarin pomace, 5-4% wheat straw.

#### Results

Effect of the polymeric carbon sources on the S. commune BCC632 enzyme activity

Initially, *S. commune* BCC 632 was cultivated in media containing crystalline cellulose, carboxymethyl cellulose, xylan, wheat straw, and mandarin pomace as carbon sources and the potential stimulators of cellulase and xylanase activities pro-

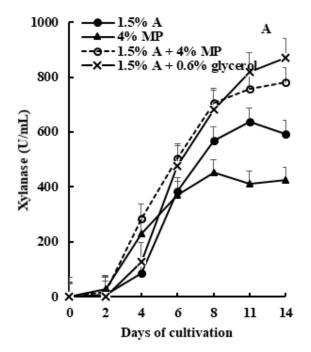
duction. Growth on mandarin pomace followed by Avicel occurred at a faster rate while that on wheat straw being lowest. S. commune BCC 632 secreted cellulases and xylanase activities regardless of the material tested; however, the enzyme yield differed significantly. Crystalline cellulose appeared to be the best carbon source providing the highest endoglucanase (53.5 U/mL), total cellulase (9.2 U/mL)

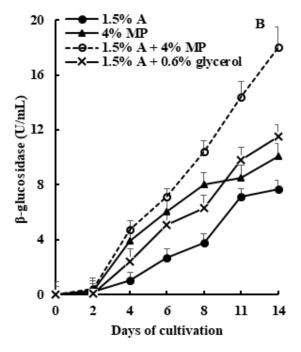
mL), and xylanase (636.1 U/mL) activities (Fig. 1). The use of mandarin pomace as a growth substrate also allowed to achieve high volumetric activities of all target enzymes. These results were far superior to those achieved with other materials used as substrates for fungal growth and enzyme production. Among them, xylan turned out to be the weakest

inducer of enzyme production, including xylanase activity - 59 times lower than that in the microcrystalline cellulose-containing medium. It is worth noting that CMC and wheat straw induced comparatively significant xylanase activity secretion by S. commune BCC632, although the fungus cellulase activity was low.

**Table 1.** Effect of easily metabolizable additional carbon sources on the S. commune BCC632 enzyme activity

Substrate	CMCase	Xylanase	FPA	β-glucosidase	β-xylosidase
(carbon source)	(U/mL)	(U/mL)	(U/mL)	(U/mL)	(U/mL)
1.5% Avicel	52.5 ± 6.1	673.1 ± 70.2	9.0 ± 1.3	7.7 ± 0.6	0.14 ± 0.02
4% Mandarin pomace (MP)	22.4 ± 2.8	460.7 ± 57.3	$7.1 \pm 0.8$	10.1 ± 0.9	$0.18 \pm 0.03$
4% Wheat straw (WS)	$3.8 \pm 0.5$	63.8 ± 8.6	$2.2 \pm 0.3$	$3.4 \pm 0.4$	$0.14 \pm 0.03$
1.5% Avicel+4% MP	32.5 ± 3.8	780.1 ± 52.7	13.5 ± 1.5	18.0 ± 1.5	$0.37 \pm 0.07$
1.5% Avicel+0.6% glycerol	50.8 ± 4.7	869.4 ± 86.4	14.0 ± 1.6	11.5 ± 0.9	$0.41 \pm 0.06$
4% WS+4% MP	11.6 ± 1.4	364.8 ± 41.0	$5.2 \pm 0.5$	5.9 ± 0.6	0.07 ± 0.01
4% WS+0.7% glycerol	16.4 ± 1.9	617.0 ± 51.8	$6.8 \pm 0.8$	2.5 ± 0.4	0.17 ± 0.03



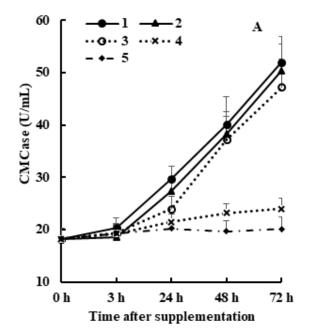


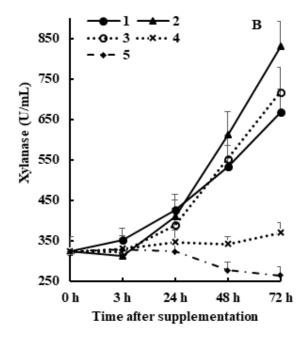
**Fig. 2.** Effect of easily metabolizable additional carbon sources on the S. commune BCC632 xylanase (A) and  $\beta$ -glucosidase (B) activities.

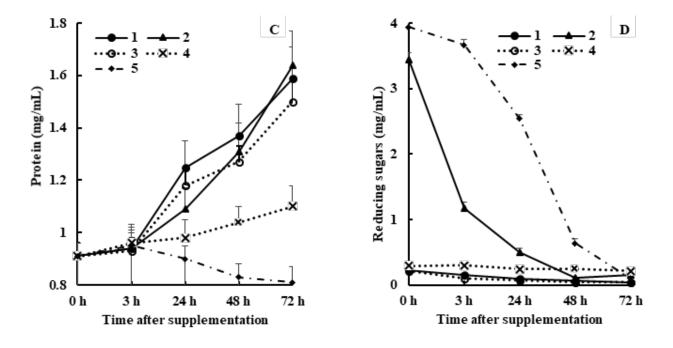
Effect of easily metabolizable carbon sources on the S. commune BCC632 enzyme activity

Subsequently, the effect of easily metabolizable carbon sources on hydrolases production by S. commune during submerged fermentation of Avicel and wheat straw was studied. We hypothesized that microcrystalline cellulose and lignified straw are recalcitrant growth substrates that retard the initial development of mushroom culture. Therefore, the use of mandarin pomace or glycerol as additional sources of easily metabolized carbon can accelerate fungal growth and rapid biomass accumulation, thus favoring enzyme accumulation. Indeed, supplementation of the Avicel-based medium with mandarin pomace caused a significant increase in both xylanase and FPA as well as a more than 2-fold increase of β-glucosidase and β-xylosidase activities of S. commune BCC632, although a 65% decrease of CMCase activity was observed (Table 1). Moreover, when mandarin pomace was added to the wheat straw-based medium, the fungus CM-Case activity increased 3-fold along with the raised activity of other enzymes with the exclusion of β-xylosidase activity. The supplementation of the

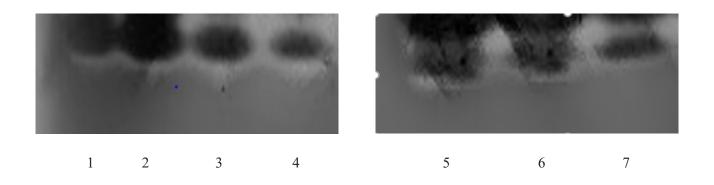
wheat straw-based medium with glycerol was even more beneficial for cellulase, xylanase, and β-xylosidase production, although the β-glucosidase activity was decreased. The addition of glycerol to the Avicel-containing medium did not change the fungus endoglucanase activity but gave the highest xylanase, FPA, and β-xylosidase yields. It is worth noting that the presence of glycerol in the Avicel-based medium caused a short-term delay in cellulases and xylanase accumulation, obviously due to catabolite repression of enzyme synthesis (Fig. 2). When this easily metabolizable carbon source was depleted, the fungal culture switched to cellulose metabolism and cellulase and xylanase induction took place with a gradual increase of the enzyme activity until the end of the experiment. In general, the addition of glycerol to the Avicel medium resulted in a 29% and 56% increase of xylanase and FP activities of S. commune BCC632, respectively. Interestingly, the supplementation of a cellulose-based medium with mandarin pomace especially promoted an increase in β-glucosidase secretion during the entire cultivation period of the fungus, ensuring the accumulation of as high as 18 U/mL of enzyme activity (Fig. 2B).







**Fig. 3.** Effect of glucose analogues and cycloheximide on the S. commune BCC632 xylanase activity (A) and reducing sugars content. Legend: 1-1.5% Avicel, 2-1.5% Avicel +0.5% glucose, 3-1.5%+0.25% methyl- $\alpha$ -D-glucose, 4-1.5% Avicel +0.25%  $\alpha$ -Deoxy D-glucose, 5-1.5% Avicel +1 µg/mL cycloheximide.



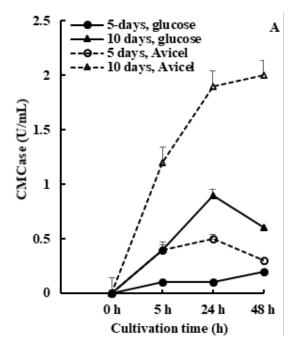
**Fig. 4.** CMCase activity intensity in native electrophoresis of culture liquids after short-term cultivation of S. commune BCC632 with different carbon sources. Legend: 1 – 1.5% Avicel, 0 h; 2 – 1.5% Avicel, 24 h; 3 – 1.%5 Avicel + 0.5% glucose, 24 h; 4 – 1.5% Avicel + 0.5% glucose + 1 μg/mL cycloheximide, 24 h; 5 – 1.5% Avicel, 72 h; 6 – 1.5% Avicel + 0.5% glucose, 72 h; 7 – 1.5% Avicel + 0.5% glucose + 1 μg/mL cycloheximide, 72 h. The arrow shows bands of CMCase activity.

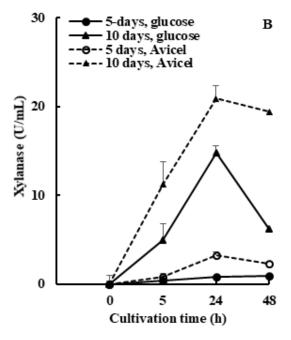
Effect of glucose, glucose analogues, and cycloheximide on the S. commune BCC632 enzyme activity

In the next step, cellulolytic secretome was assessed in short-term experiments after the addition of glucose,  $\alpha$ -deoxy-D-glucose, methyl- $\alpha$ -D-glucose, and cycloheximide to the *S. commune* BCC632 culture grew during 6 days in the 1.5% Avicel-containing medium. Monitoring of the pH values of the media did not reveal significant differences between cultures containing Avicel and cultures with added sugars.

The addition of 0.5% glucose to the Avicel medium caused catabolite repression of the synthesis of both cellulase (Fig. 3A) and xylanase (Fig. 3B) during at least 3 hours of cultivation. Subsequently, the production of cellulase resumed at the same rate as in the control variant, while the accumulation of extracellular xylanase was even more intense. This phenomenon is confirmed by the data on the concentration of reducing sugars in the fungal culture (Fig. 3C), which decreased after 3 hours to less than 0.1%, apparently sufficient for the derepression of enzyme synthesis. Supplementation of growing culture with methyl-α-D-glucose, a non-metabolizable glucose analogue, slightly reduced the secretion of both enzymes only within 24 h, especially in the first 3 hours of cultivation. On the contrary,

the addition of α-deoxy-D-glucose to the S. commune BCC632 culture actively synthesizing target enzymes prevented the active secretion of cellulase and especially that of xylanase. As was expected, no CMCase production occurred in the presence of cycloheximide whereas xylanase activity gradually decreased obviously due to partial inactivation of the available enzyme. It is important to note that the protein content in the culture liquids obtained after cultivation of the fungus in media with Avicel and additives largely correlated with the dynamics of enzymatic activity (Fig. 3D). Finally, zymogram analyses showed that proteins with hydrolytic activity on CMC were detected in all gels. Native electrophoresis confirmed that crystalline cellulose acted as an inducing substrate for endoglucanase production by S. commune BCC632. In parallel with volumetric CMCase activity, the size and intensity of bands with CMCase activity after 24 h and 72 h cultivation increased (Fig. 4, lanes 2 and 5) compared with that inoculation time (Fig. 4, lane 1). The intensity of the band in lane 3 correlated with the CMCase activity measured after 24 h after the addition of 0.5% glucose but it was increased after 72 h of the fungus cultivation (Fig. 4, lane 6). When cycloheximide was used as an inhibitor of protein synthesis, significantly decreased size and intensity of bands with CMCase activity were observed after 24 h and 72 h cultivation (Fig. 4, lanes 4 and 7).





**Fig. 5.** Secretion of cellulase (A) and xylanase (B) activity of S. commune BCC632 depending on the "age" of the filtrate and the carbon source in the inoculum culture.

Testing the culture filtrate as a source of enzyme synthesis inducers

The results obtained in this study show that the secretion of cellulases and xylanases in the Avicel-containing medium takes place throughout the entire cultivation period, even when the growth of the fungus has practically ended. It can be assumed that the culture constantly contains substances that initiate the expression of genes responsible for the synthesis of these enzymes. To test this hypothesis, the filtrate of S. commune BCC632 grown in a medium with cellulose for 5 (logarithmic phase of growth) and 10 (stationary phase of growth) days was inoculated with homogenized biomass of the fungus grown both in a medium with glucose and in a medium with cellulose. The results shown in Fig. 5 indicate that the activity of both enzymes was significantly higher when the filtrate from the stationary growth phase was used for the cultivation of the fungus. Moreover, the kinetics of endoglucanase and xylanase secretion and the magnitude of the enzyme activity were preferable if the inoculated biomass was grown in a cellulose-containing medium.

#### **Discussion**

The secretome of S. commune BCC632 grown on crystalline cellulose showed a combination of all tested enzymes: endoglucanase, xylanase, β-glucosidase, and  $\beta$ -xylosidase. This fungus is a promising producer of cellulases and xylanase that play a crucial role in biomass polysaccharide hydrolysis and supplying the mushroom culture with a carbon and energy source. It is worth noting that this strain is an excellent producer of  $\beta$ -glucosidase. Another S. commune KUC9397 secreted as high as 43.51 U/ mL β-glucosidase in the medium containing 2.96% cellulose, 2.3% soy peptone, and 0.11% thiamine [18]. Besides, Villavicencio et al. [19] showed remarkably high levels of xylanase and β-glucosidase activities of S. commune in solid-state fermentation of birch wood.

Like in Fomes fomentarius BCC 38, Panus lecometei BCC 903, Pseudotrametes gibbosa BCC 17, and Trametes versicolor BCC 13 [20], production of cellulase and xylanase by *S. commune* BCC 632 requires the presence of inducing substrate in the culture medium. However, similar to the listed mushrooms, xylan and carboxymethyl cellulose appeared to be poor substrates for *S. commune* BCC 632 growth and enzyme secretion whereas cellulose was the most appropriate growth substrate. Similar-

ly, wild and mutant strains of S. commune produced cellulolytic and hemicellulolytic enzyme activities and as high as 1145 U xylanase/mL in the fermentation of microcrystalline cellulose Avicel-PH101 [12]. Likewise, cellulose caused the highest inductive effect on Ganoderma applanatum LPB MR-56 cellulase and xylanase production [21]. However, unlike *S. commune* BCC632, xylan and CMC appeared to be potent inducers of G. applanatum hydrolases synthesis.

Literature data indicate that cellulolytic and xylanolytic activities of basidiomycetes depend on lignocellulosic substrate chemical composition. Rice straw was the best growth substrate for endoglucanase production by Armillaria gemina [6]. This enzyme activity reached 146 U/mL when the fungus was cultivated in the presence of yeast extract at 10 g/l as a nitrogen source. Among lignocellulosic materials tested, rice straw provided the highest CM-Case (44.4 U/mL) and β-glucosidase (6.5 U/mL) activities of S. commune, while alkaline pre-treated sugarcane bagasse promoted accumulation of 1326 U/mL xylanase [12]. Unlike these studies, in our work, wheat straw appeared to be a poor substrate for both cellulases and xylanase secretion by S. commune BCC632 while mandarin pomace provided high enzyme activity and contained some compounds favoring enhanced xylanase production. We assumed that one of the reasons for the low enzymatic activity of the fungus in the presence of wheat straw may be the lack of easily metabolizable sugars necessary to accelerate the initial fungal growth. In fact, the supplementation of wheat straw-based medium with mandarin pomace or glycerol resulted in a significant secretion of cellulase and xylanase by the fungus.

As seen, the production of xylanase by *S. commune* BCC632 is strictly linked to the presence of cellulose. Hence, this fungus released low molecular weight soluble catabolites from the cellulose polymer, which served as signaling molecules promoting the simultaneous formation of both cellulase and xylanase. Based on the results, it can be concluded that the formation of cellulase and xylanase is inducible in *S. commune* BCC632 and it is under common control. At the same time, one should pay attention to significant differences in the regulatory control of these enzyme activities. The most obvious distinction concerns the predominant production of xylanase by the fungus regardless of the growth substrate composition.

The results obtained in this study show that in the

microcrystalline cellulose-based medium the fungal culture contains compounds initiating/maintaining the secretion of cellulase and xylanase. Obviously, a metabolically less active 10-days culture of S. commune BCC632 that has reached the stationary growth phase contains a higher concentration of inducing compounds (cellooligosaccharides and xylooligosaccharides) than an intensively developing 5-days culture. Upregulation of cellobiohydrolase and endoglucanase gene expression in culture medium containing cellotriose and cellotetraose derived from cellulose was shown for Polyporus arcularius [22]. Likewise, the addition of cellotriose and cellotetraose increased transcript levels of several cellulolytic genes in Phanerochaete chrysosporium [23]. It is worth noting that in our short-term experiments, intensive secretion of CMCase and xylanase was observed during 24 h of cultivation. Subsequently, the production of enzymes sharply decreased, as we assume, due to the hydrolysis of oligosaccharides by  $\beta$ -glucosidase and  $\beta$ -xylosidase. Further research is needed to understand the mechanisms of regulation of cellulases and hemicelluloses by S. commune BCC632, which may have potential in large-scale applications of lignocellulose degradation.

## Acknowledgement

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# Studying some characteristics of cyanide destructors for the purpose of decontamination of cyanide-containing waste

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## ABSTRACT

The subject of article is about isolation and study of endemic cyanide-resistant strain on the Madneuli mine, for further microbiological decontamination of cyanide-containing waste of the gold mining company. In the result of performed studies, for the first time in Georgia, two active cyanide-destructor stains were isolated from the liquid and solid objects contaminated with industrial cyanides: The strain #5 Bac.cyanooxidans and #12 Bac.subtilis. In the result of performed works, in laboratory conditions, in the mode of serial passage, on model solutions, some characteristics of development of cyanide-destructor microorganisms in the process of cyanide-destruction were determined. Nutrient is an impact of content, impact of the bacterial mass on the process of destruction, cyanide destruction characteristics of specific strains and their association.

Keywords: Cyanide-resistance, Microorganism, Strain, Waste, Cyanide-destruction, Performed works.

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## Introduction

Development of mining and processing industry is dependent on increase of anthropogenic impact on the environment. These phenomena are especially visible during operation of gold mines of various formations, where cyanides are used for extracting gold from mostly gold-containing ores. During a heap leaching of these ores, which is performed on open pits, potentially dangerous cyanide-containing waste is produced. They are negative factors of environment impact, because there might be their leakage into soil, water and atmosphere after evaporation. The gold-copper-polymetallic mine of Madneuli is located in the ore-containing zone of Bolnisi, which is the forming part of the Artvin-Bolnisi belt and is situated in the central part of the Alpine-Himalayan belt. The mine is the northern-western part of the Armenia-Karabagh structural metallogenic zone.

Secondary quartzites are widespread in the mine. The secondary quartzites containing gold and silver are located in the superficial part of the mine, above the gold and copper. Mineralization is associated with the late blueish-gray quartzes, which are in the form of stockworks, separate rich veins and vein zones [1]. During mining of chalcopyrite, gold-containing quartzites were stored on dumps, with the perspective of their further processing. Later the technology of gold extraction from these quartzites were implemented. This is the heap leaching method using cyanides, which is carried out in open pits. A thick polyethylene film covers the bottom of the pit. The ore is arranged in the form of 5-meter layers. Extracted gold is collected in the heap cover and is pumped to the collecting tank, from where it is transferred to adsorption columns. Depending on ore type and company's capacity, an annual consumption of cyanides reaches tens of even hundreds of tons. Therefore, there is a problem of cyanide contamination, and it is necessary to neutralize the cyanides in the waste. Destruction of cyanides may be carried in three ways: chemical, biological and complex chemical-biological methods.

Among the biological methods, most promising, economical and environment-friendly is the micro-

biological method of cyanide destruction. Therefore, researchers focus on obtaining cyanide-destructor bacterial strains and their use in bio-decontamination of cyanide-containing waste.

Currently, various types of heterotroph microorganisms are obtained, which carry out destruction of cyanide compounds. These are the representatives of the following genus: Pseudomonas, Bacillus, Achromobacter, Rhodococus, Serratia and other [2-5].

## Study objects and methods

The objects of isolation of cyanide-destructor microorganisms were solid and liquid waste of heap leaching of gold-containing secondary quartzites carried out by cyanidation technique. Isolation of strains and their further study was conducted by means of techniques approved in microbiology, which are given in the textbook of practical works [6].

For the purpose of microflora revival and activation, the solid probes were moisturized by sterile water, and all probes were placed into a thermostat at the temperature of 28 0C for 24 hours. After incubation, probe of 10 g was diluted with sterile water (1:10), the mix was placed on shaker for 10 minutes, after which the probes, according to the approved technique, by tenfold dilution, were inoculated 3 times repeatedly in Petri dishes on beef peptone agar, to which 10 mg/l of sodium cyanide was added to suppress otherassociated microflora. Cultivation of microorganisms were conducted in stationary conditions in the thermostat at 28 0C for 4 days. Microorganisms were recorded by visual examination of the inoculations and also by their microscopic study. The number of microorganisms were calculated for 1 g of absolutely dry ore or 1 ml of water.

After cultivation for 4 days, isolated colonies were produced on the nutrient medium. In the result of conducted study, 18 cyanide-resistant strains were isolated and obtained. Those strains are considered cyanide-resistant, which grow well or weakly on cyanide-containing nutrient medium.

From 18 strains, isolated for obtaining working strains, were selected the strains with clearly expressed ability for destruction of cyanides.

The selection was conducted on Petri dishes on Podolskaya nutrient agar #1 [7] with the following content: K2HPO4•3H2O - 3,0; MgSO4•7H2O -

0,5; FeCl3 – 0,01; CaCl2•6H2O – 0,01; saccharose - 5,0; agar - 2%. The prepared source of nitrogen was not added to the nutrient medium, to give a possibility to microorganisms to use nitrogen from NaCN as a source of nitrogen. Cyanide was added to the nutrient medium after sterilization. Cyanide concentration intervals of 10-20 mg/l; 30-40 mg/l and 50-60 mg/l were taken. Cultivation was conducted in stationary conditions in the thermostat at temperature of 28 OC Resistance of the strains against cyanides were assessed according to growth on nutrient medium.

It should be noted that all strains from 18 expressed resistance to cyanide to some extent, but two active strains were distinguished among them: The strains #5 and #12.

These strains were examined on purity in accordance with inoculation technique on various nutrient media. In parallel, they were microscopically studied using a digital microscope Omax.

For strengthening and improving cyanide-destruction features of the isolated strains, they were adapted to the increased concentrations of cyanides by means of their serial high passage.

Adaptation of strains was conducted on Petri dishes in #1 solid synthetic nutrient medium, to which sodium cyanide were gradually added with increased concentrations (50-135 mg/l) according to the results of tests. 3-5 passages were needed for adaptation to each increased concentration of cyanide (I passage - 50 mg/l, II passage - 70 mg/l, III passage - 100 mg/l, IV passage - 115 mg/l, V passage - 135 mg/l).Cultivation was conducted in stationary conditions in the thermostat at temperature of 28 0C.

After obtaining active working strains, their morphological-cultural and physiological-biochemical characteristics were studied. Based on the obtained data, the strain # 5 was identified as Bacillus cyanooxidans, and the strain #12 - Bacillus subtilis. Identification of bacteria was conducted according to the Bergeys bacteria determinant technique.

As it is known, for growth and development of live organisms, including microorganisms, availability of two elements - nitrogen and carbon is critically important. Synthetic nutrient medium used by us, was providing the microorganisms with critically important elements. For clarifying the issue - whether the isolated cyanide-resistant microorganisms had the ability to absorb these elements or not, we conducted tests, in which the solid and liquid nutrient media of similar content were used.

For determining nitrogen absorption ability of microorganisms from cyanide compound, the tests were conducted in Petri dishes, agar synthetic medium #1, which contained NaCN (20 mg/l) as a source of nitrogen in one variant, and NH4Cl (0,5 g/l) in another.

For determining the source of carbon, the same cyanide-containing synthetic medium #1 in one variant contained carbohydrates (saccharose - 5 g/l, Na lactate - 1.5 g/l), and in the second variant it did

not contain an organic compound. The 24 hours cultures of strain #5 Bac.cyanooxidans and the strain #12 Bac.subtilis were used as inoculants. The tests were conducted at the temperature of 28 0C in the thermostat in stationary conditions for 5 days. We assessed the results of tests according to growth of bacteria on the solid nutrient medium, and also by analyzing cyanide in the solutions. The results of the test are given in the Table 1.

**Table 1.** The nutrient is an impact of growth of cyanide-destruction microorganisms

Strain #	Source of	f nitrogen	Source of carbon			
	7 an a # 1	Zone # 1 N		Zone # 1 NaCN		V
	Zone # 1 NaCN	Zone # 1 NH <sub>4</sub> Cl	Saccharose Na lactate		Without carbohydrate	
5, Bac.cyanooxidans	+	+	+	+	-	
12, Bac.subtilis	+	+	+	+	-	

- + Growth of bacterium
- absence of growth

The amount of bacterial mass is very important for studying the cyanide destruction process.

The tests for studying this issue were conducted in Erlenmeyer flasks of 250 ml volume, in which 100 ml of synthetic nutrient #1 were introduced as well as increased concentrations of cyanide (10-50 mg/l). Cyanide-destruction microorganisms - 24 h cultures of the strain #5 Bac.cyanooxidans and the

strain #12 Bac.subtilis were used as inoculants. The tests were conducted in stationary conditions in the thermostat at temperature of 28 0C. We assessed the results of the tests according to the number of microorganisms using the direct counting method in microscope, and also by determining a dry bacterial mass and analyzing cyanide compounds in the solution. The results of the test are given in the Table 2.

**Table 2.** *Impact of the bacterial mass on the cyanide destruction* 

Value, №	1	2	3	4	5
Cyanide concentration, mg/l	10	20	30	40	50
Bacterial titer	109	109	107	$10^{6}$	$10^{3}$

The issue was studied concerning the impact of certain strains (types) of isolated heterotroph bacteria and their association on the activity of cyanide-destruction.

The tests for studying this issue were conducted in Erlenmeyer flasks of 250 ml volume, in which 100 ml of synthetic nutrient #1 with saccharose were introduced and CN was added in the amount

of 20 mg/l and 30 mg/l. The 24 h cultures of strain #5 Bac.cyanooxidans and the strain #12 Bac.subtilis and their united bacterial suspension were taken as inoculants. The amount of inoculant was 10% of the initial liquid (each strain in the association in equal amount of 5%). Tests were conducted in condition of shaking, by thermostating, at 28 0C. The results of the test are given in the Table 3.

Strain #	Concentration	Incubation time, h		
Strain //	Initial	Final		
5,	20	15	24	
Bac.cyanooxidans	30	20	48	
12,	20	4	24	
Bac.subtilis	30	08	48	
5, 12	20	0	24	
Bac.cyanooxidans; Bac.	30	0	48	

**Table 3.** Cyanide compounds destruction by isolated heterotroph bacteria and their association

#### Results and discussion

For the first time in Georgia, works were conducted on the Madneuli mine for isolation and study of endemic cyanide-destructor microorganisms, for using them later for microbiological decontamination of gold mining company's waste.

During the study process, 18 strains of cyanide-resistant microorganisms were screened from solid and liquid waste of the mine, contaminated with cyanides, from which two active strains #5 and #12 were selected. Based on the study of morphological-cultural and physiological-biochemical characteristics of the strains, the strain #5 was identified as Bac.cyanooxidans, and the strain #12 as Bac.subtilis.

The results of conducted adaptation showed that after each passage the resistance ability of microorganisms against cyanide was increasing (which we assessed according to the developed bacterial mass). The strain #5 was adapted to the concentration of cyanide of 100 mg/l, and the strain #12 - to 135 mg/l concentration. Further increase of cyanide was not performed because further increase of NaCN concentration significantly suppressed the growth of bacteria.

In the study process of ability of isolated strains to use cyanide as a nitrogen and carbon source, good growth of bacteria was detected in the nutrient medium when NH4Cl, and also NaCN were present. During visual examination of microorganisms on the solid medium, larger growth of strains was detected in the variant with NH4Cl compared to

the variant with NaCN, however, the latter variant also produced a good growth. Therefore, it became evident that the strain #5 and the strain #12 Bac. cyanooxidans have the ability to absorb nitrogen from cyanide, but, at the same time, they do not use cyanide as a carbon source, because, without organic compounds, autotrophic growth of strains on NaCN-containing medium was not detected. While in case of use of saccharose and lactate as carbon and energy source, the strains produced a good growth.

These data match the characteristics of cyanide-destruction. In stationary conditions, after incubation for 5 days, the content of CN was determined in the final solutions (initial solution CN - 20 m). In case of source of nitrogen being NaCN and NH4Cl, the destruction of CN was 99.2% and 99.5%, respectively. In case of source of carbon being saccharose and lactate, the destruction of CN was 99.2% and 99.5%, respectively, and the obtained results are close to each other, and in case of carbohydrate presence in the nutrient medium, CN destruction did not occur. It became clear that the presence of organic compound in the medium is necessary for development of cyanide-destructor microorganisms and the process of cyanide destruction.

Limitation occurs during the serial passage of microorganisms (gradual decrease of nutrient components in the medium and accumulation of metabolism substances), as well as inhibition (toxic impact of cyanide compounds' concentration). After introducing in the bacteria nutrient medium, they continue reproduction until a content of some component needed for them reaches a minimum value after which the reproduction stops.

The test results showed that concentration of CN impacts the number of microorganisms (mass). Total destruction of cyanides depends on the initial number of microorganisms in the solution. As the test results showed, 10-20 mg/l cyanide concentration in the medium had weak impact on the growth of microorganisms, no limitation and cyanide inhibition effect on the microbic mass occurred.

With cyanide concentrations above 30 mg/l, the inhibition effect appears and the specific growth rate of bacteria decreases. With cyanide concentrations of 50 mg/l, the inhibition effect even more increased and biochemical activity of the microorganisms decreased.

The test results show that in the serial passage, the more is CN concentration, the less is bacterial mass. Highest value of cyanide destruction is obtained in case of bacterial titer of 109 CFU/ml.

In case of combination of isolated strains in an association, they complement each other, because of which the destruction ability of the association increases.

During conducting the test, the change of ration between various bacteria was detected in the population. At the same time, the continuity of type content was retained, which was proven microscopically, and by inoculation on an agar synthetic nutrient medium. Also, the strains in the pure culture showed different ability of CN destruction. The strain #12 Bac.subtilis showed more active ability of CN destruction compared to the strain #5 Bac.cyanooxidans. The strain #5 Bac.cyanooxidans destructs 10 mg/l cyanide for 48 hours (initial - 30 mg/l), and the strain #12 12 Bac.subtilis in the same time destructs 22 mg/l cyanide. Their combined association carries out the total destruction of cyanide within the same time period.

#### Conclusion

For the first time in Georgia, works were conducted on the Madneuli mine for isolation and study of endemic cyanide-destructor microorganisms, for using them later for microbiological decontamination of gold mining company's waste.

18 strains of cyanide-resistant microorganisms were screened from solid and liquid waste of the mine, contaminated with cyanides, from which two active strains #5 and #12 were selected. Based on

the study of morphological-cultural and physiological-biochemical characteristics of the strains, the strain #5 was identified as Bac.cyanooxidans, and the strain #12 as Bac.subtilis.

For strengthening and improving cyanide-destruction features of the isolated strains, they were adapted to the increased concentrations of cyanides, after which the strain #5 Bac.cyanooxidans was adapted to the cyanide concentration of 100 mg/l in the nutrient, which the strain #12 Bac.subtilis - 135 mg/l of cyanide concentration.

The isolated strains can use cyanide as a source of nitrogen, but they cannot use it as a source of carbon. For the source of carbon, they need existence of organic compound in the medium. In these conditions, the strains may carry out 99% destruction of cyanide with its initial concentration of 20 mg/l.

The cyanide concentration impacts the number of microorganisms (mass); the more is cyanide concentration, the less is bacterial mass. Highest value of cyanide destruction is obtained in case of bacterial titer of 109 CFU/ml. In case of combination of isolated strains in an association, they complement each other, because of which the destruction ability of the association increases. The strain #5 Bac.cyanooxidans destructs 10 mg/l cyanide for 48 hours (initial - 30 mg/l), and the strain #12 12 Bac.subtilis in the same time destructs 22 mg/l cyanide. Their combined association carries out the total destruction of cyanide within the same time period.

The concentration of cyanide in the medium has an impact on the cyanide destruction process. Bacterial titer, nutrient is content.

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# Terrain morphometry and soil erosion topographic factor (LS) in upper Alazani basin (Georgia)

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#### ABSTRACT

Terrain morphometry, landforms, and the resulting LS component are generally acknowledged as important factors in soil erosion studies. The aim of this research was to identify and analyze terrain morphometric elements in the upper Alazani basin, as well as determine their impact on soil erosion. We examine terrain morphometry, identify landforms, and calculate the LS factor for the upper Alazani valley in this analysis. Arc map 10.8 was used to perform all calculations. For the measurements, an SRTM 1 arc-second DEM (resolution 30m) was used. The slope angle and slope aspect were calculated using the D8 algorithm. MFD analysis was used in order to calculate the flow path. As a result of it The flow accumulation was computed. Stahler's method was chosen to calculate stream order, which allows drainage density to be calculated. The slope position and the topographic position index (TPI) were computed. TPI values were obtained in order to obtain landforms. The MFD algorithm was used to compute the LS factor. In general, the LS factor is higher in Alazani's left tributaries than in its right tributaries. The maximum values were found in the Alazani headwaters, in the Samkuristskali channel, which is a tributary of the Alazani, and in the Stori channel. These results demonstrate that the upper Alazani valley has a high erosion potential. Future work should concentrate on the DEM resolution, which also has an impact on overall soil loss.

Keywords: Soil erosion, Landforms, LS, RUSLE, Alazani, Georgia

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#### Introduction

The fluvial geomorphological processes in the river basin are generally accepted to be complex and multifaceted. Many factors can affect the volume of runoff in a river basin, including glacial sediment, rockfall, sheet, rill, and gully erosion, weathering, etc. Soil erosion in river basins and the resulting sediment supply are inextricably linked [1], and they are the dominant geomorphic processes in many regions of the world [2]. Numerous experiments have already shown that these processes manifest differently in different geographical environments; for example, water erosion is a frequent and serious concern that impacts all European countries, although at different degrees [3]. In this case, a major current focus is on how topography plays a key role. Soil erosion models were the first to use topography factor modeling.

There are numerous methods for modeling the 174

factors affecting soil erosion. The (Revised) Universal Soil Loss Equation - (R)USLE - is one of the most widely used models. Because of their simple, robust forms, USLE [4] and RUSLE [5] are still the most commonly used equations for estimating soil erosion [6]. The (R)USLE equation calculates average annual soil erosion by multiplying several factors together, including: rainfall (R) factor (MJ mm ha 1 h 1 y 1); soil erodibility (K) factor (Mg h 1 MJ 1 mm 1); slope length and steepness (LS) factor (dimensionless); cover management (C) factor (dimensionless), and support practice (P) factor (dimensionless).

Geomorphological research has been critical in the development and application of soil erosion assessment tools [3]. All terrain factors include terrain curvature, slope aspect, steepness, length, and direction [6]. Once runoff begins to flow across surface areas and into streams, the quantity and size of material transported increases with its velocity [3]. For soil erosion modeling, LS is the most important topographic factor.

The original equation for LS calculation is below:

```
LS=L*S (1)

L= (\lambda/22.13)m (2)

m=\beta(1+\beta) (3)

\beta=(\sin\theta)/[3*(\sin\theta)0.8+0.56] (4)
```

Where  $\lambda$  is the slope length, m is a variable exponent calculated from the ratio of rill-to-interrill erosion and  $\beta$  is the factor that varies with slope gradient.

```
S=10.8 \sin\theta+0.03, slope gradient\leq 9\%
S=16.8 \sin\theta-0.50, slope gradient\geq 9\% (5)
```

Where, S is the slope factor, and  $\theta$  is the slope angle.

USLE and RUSLE were originally designed for gently sloping cropland with a one-dimension topography factor (LS) [7]. In newer research for catchment-scale studies, the one-dimensional slope length factor of individual slopes in the USLE was replaced by the upslope contributing area to respect the topography of complex watersheds or vast two- or three-dimensional areas [8]. As a result, new methods and modifications to existing ones emerged. The most commonly used are the unit stream power method [9,10], the multiple flow direction method [11,12], and the upslope contribution area method and its improvement [13]. The authors of the paper [12] compared the values of the LS factor calculated by various methods with field data and concluded that MFD performed better than other methods in calculating the slope length and LS factor.

Because of the spatial nature of (R)USLE factors, they can be integrated with geographic information systems (GIS) [2]. The reliability of the calculated slope length and slope gradient (LS) factor is determined by the availability and precision of topographic data [14]. The LS factor is calculated in several steps, including depression filling, flow-direction and slope-steepness calculations, obtaining the slope length, and calculating the LS factor [6]. The combination of geographical information systems (GIS) and computer processing power allows better resolution input data to be used for modeling studies and projects [8]. GIS-based methods are one of the few ways to investigate the role of spatial variability in soil properties, rock types, and a variety of other geologic and climatic properties in

landscape evolution [3]. The resolution and quality of the digital elevation models used in the study are a separate topic of discussion. It is well known that producing topographical map-based DEMs takes some time. In contrast, 2m resolution DEM yields lower mean LS values than 25m resolution DEM. As a result, the soil loss would be overestimated [8]. As a result, as grid sizes would be increased, the relative computation errors of the LS factors increased [14]. So far, it has not been systematically investigated whether different DEM resolutions produce different LS-factor values and whether the use of high-resolution DEMs produces higher L-, S-, and LS-factors [8]. According to studies, SRTM has a slight advantage over ASTER when using publicly available DEMs [15].

The goal of this research is to describe and analyze morphometric elements of terrain in the upper Alazani basin and assess their influence on soil erosion.

#### Study area

The upper reaches of the Alazani River are included in the study area (fig.2.A). The study area is 5309 square kilometers. The study area is distinguished by its mountainous terrain. The hydrographic network is quite frequent, as shown in fig. 1. The study area has a moderately dry subtropical climate. According to the meteorological stations here, there is 2-3 months of drought per year, with heavy showers following the dry period [16], which contributes to the intensification of floods and mudflows. According to historical records, a mudslide in Kvareli on May 23, 1899, destroyed 25 houses and destroyed 665 desetina (724.85 ha) of arable land and vineyards, killing 50 people [17]. A natural disaster struck Telavi on the night of June 14, 1977. This was due to the wet winter and spring. Previously, the river Telavi had a wide (30-40 m) ravine, a 1.5 km wide debris cone, and a length of up to 6 km. At the time of the disaster, the flow height was 1.6 m, the width was up to 50 m, and the flow rate was approximately 280 m3/s [18. The Tsivistskali flood on June 14, 1977, cost the Soviet farm in Tsinandali 60,000 manats (\$ 44,400 at the time) and destroyed the growing vineyards [18]. There are numerous cases that are similar. This is why topography and morphometric analysis of topography are critical in assessing these fluvial geomorphological processes in the study area.

#### **Methods and Materials**

The primary source of information for this study was elevation data. The DEM used in the study, SRTM 1 Arc-Second (resolution 30 m), was obtained from www.earthexplorer.gov.com. It was projected WGS 84, UTM, 38N projection. All calculations were carried out in the following order in the software Arc map 10.8:

- 1. The Fill Sink tool was used to eliminate DEM anomalies:
- 2. The D8 algorithm was used in order to calculate slope angle and slope aspect (fig. 2.G and H);
- 3. MFD analysis was used to compute flow direction, the most basic geomorphometric attribute. The number of upstream cells that flow into each cell according to their flow directions was used to calculate flow accumulation. [2]:
- 4. Stahler method was chosen to calculate stream order (fig. 2.I), which provides a way of calculation of drainage density (fig.2.J);
- 5. On the basis of slope angle raster, slope position (fig. 2.E) and topographic position index (TPI) (fig. 2.F) were calculated [19]
- 6. TPI values were used for the purpose of obtaining landforms [19]. To make landforms smoother focal statistics (5X5) was used;
- 7. LS factor was calculated with the MFD algorithm [12].
- 8. Based on the filled DEM hill shade of the study area was created and it was used only for visualization of the results.

#### **Results**

As outlined in the introduction, the main purpose of this work was to analyze the morphometric features of the study area that directly or indirectly affect erosion and denudation, based on this analysis we performed a landform classification and determined the LS factor in this section.

**Table 1.** Hypsometry of Stydy area, covered area and percent

Elevation (m)	Area (sq. km)	Percent
100-200	72.0751	1.356
200-500	1843.371	34.72
500-1000	1457.1252	27.45
1000-1500	963.2614	18.14
1500-2000	466.627	8.79
2000-2500	302.001	5.69
2500-3000	190.7485	3.59
3000	14.5491	0.27

Table 2. Slope (degree) of study area

Slope	Area (sq. km)	Percent
0-5	1544.341	29.09
515	1478.729	27.85
15-30	1352.307	25.47
30-45	815.9882	15.37
45-	118.1547	2.22

Table 3. Slope Apect of study area

Aspect	Percent
Flat	3.8
N	10.73
NE	12.28
Е	13.09
SE	13.19
S	13.07
SW	11.52
W	11.92
NW	10.40

According to Table 1, a large portion of the study area (34.7%) is located between 200 and 500 meters above sea level. This hypsometric step is best suited for resettlement and agricultural activities. In the 500–1000-meter hypsometric step, it lags slightly (27.4%). The area above 2500 meters is very small, accounting for only about 4% of the study area.

According to the data reported in table 2, which illustrated the slope angle value distribution the area is almost evenly distributed as a percentage, however comparing the results, we will see that the steep slopes cover a large part.

The slope aspect is another factor that does not directly affect soil erosion but is affected by the amount of heat and light received from the sun as well as the characteristics of the vegetation, which in turn affects depletion and runoff. Table 3 shows that slopes with an east, southeast, or south aspect predominate in the study area. This means that the area receives a lot of heat and light, and weathering processes will be active due to a lack of vegetation and relevant geological conditions.

The next step, the flow direction gives us a very good idea of the alluvial cones, allowing us to make a visual interpretation of the evidence for this is in fig.2.D. Automatic delineation of alluvial cones requires additional data and field surveys. In our case, we used flow direction raster to calculate flow accumulation, which in turn we used in further calculations, for example, we used it to automatically draw a stream network, after which we calculated the drainage density displayed in fig.2.I and J respectively. It is evident that the maximum values (1 km/km2) are on the alluvial cones near the Alazani channel. This figure is also important because it can affect the shape of the river hydrograph during storm events. A high drainage density indicates a high risk of flooding and a high bifurcation ratio, which means that the higher it is, the higher the risk of flooding. We used a flow accumulation raster to show the profile graph of some tributaries (in this case Duruji (fig.1.A) and Shavkaba (fig.1.B)). It shows the change in elevation of the surface along a line. In both cases, especially in the case of Duruji, the channel gradient is quite high.

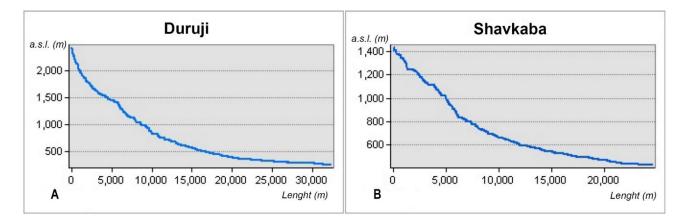


Fig. 1. A. Profile Graph-Duruji; B-Profile Graph-Shavkaba

The next step was landform classification. From fig.2.B it can be seen that we have identified 10 landforms. It helps us to identify various geomorphological features, including the visual interpretation of alluvial cones.

Finally, there is the topographic (LS) factor for the study area. It stands to reason that the high LS values corresponded to the highland valleys. Figure 2.C shows that the highest values are found in the Alazani headwaters, in the Samkuristskali channel, which is a tributary of the Alazani, and in the Stori channel. According to fig. 2.C, the LS factor is relatively high in the case of Alazani's left tributaries compared to right tributaries.

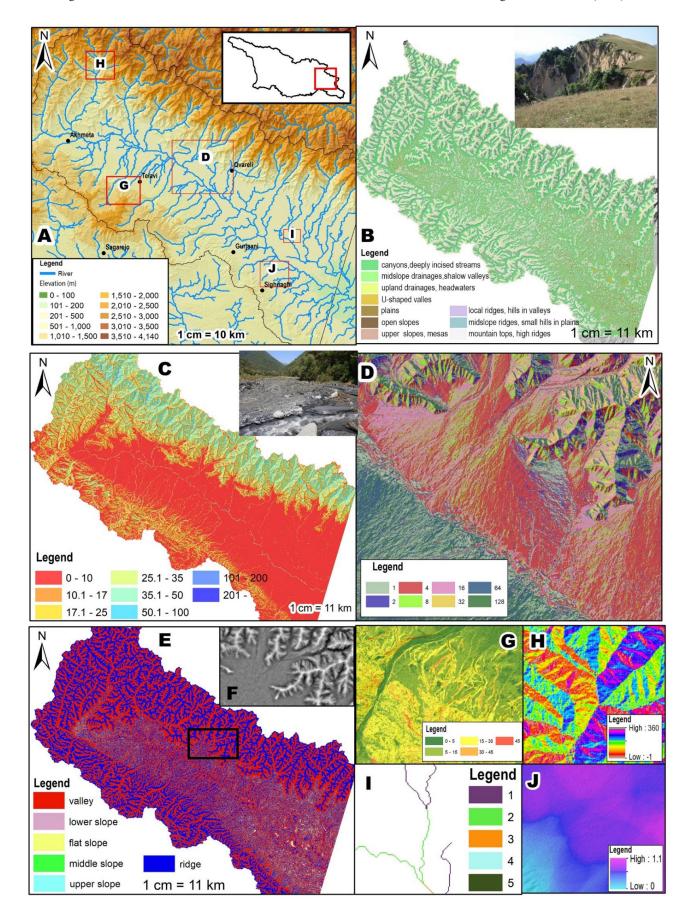
Our findings strongly support previous predictions. It should be noted, however, that in this case, the LS values describe the overall picture and indicate the spatial distribution of the min and max LS values.

#### **Conclusion**

Prior works have documented the importance of the LS factor in soil erosion studies. In this paper, we used the Weiss and MFD algorithms to delineate landforms and assess the LS factor in the upper Alazani valley's terrain morphometry. Our results provide compelling evidence that the study area is characterized by high erosion potential. Our results are in general agreement with previous studies in the landform classification. Our current findings expand prior works with the assessment of LS factor for the study area. Our results mean that the upper Alazni valley is very sensitive to the factors affecting erosion. In our case, we analyzed only the topography factor. An important question for future studies is to analyze each factor to see and/or calculate overall soil loss in the study area. Future work should focus on the DEM resolution because it has a huge influence on the maximum values of LS factor, which on the other hand affects the results of soil loss. However, our calculations give the general overview of the spatial distribution of minimum and maximum LS values but further detailed calculations need better DEM resolution.

#### Acknowledgement

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**Fig. 2.** A-Study Area; B-Landform Classification; C-LS factor; D-Flow Direction; E-Slope Position; F-TPI; G-Slope angle; H-Slope Aspect; I-Stream Order; J-Drainage Density

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# The outcomes of field survey of sensitive areas at Kobi - Gudauri section of the Georgian military road for the purpose of arrangingan innovative snow avalanche construction

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#### ABSTRACT

The paper presents the sensitive areas of snow avalanches at Kobi - Gudauri section of the Georgian military road in the appropriate GPS coordinates, the areas are marked on a digital map using GIS technologies, based on the field surveys and theoretical studies. The statistical material for snow avalanches formation at Kobi - Gudauri section is assessed and a brief description of the geographical, hydrological and hydraulic parameters of snowpack and avalanche formation is proposed. Using the mentioned material, a geographical location has been selected for the arrangement of the innovative snow avalanche construction, which is located at Kobi-Gudauri section of the Georgian military road, in the vicinity of the so-called Panorama at an average mark of 2500 m above sea level with an average inclination of the slope 24°.

*Keywords:* Snow avalanche, Kobi-Gudauri section, Georgian military road, Innovative snow avalanche construction, Anomalies affecting, Slope inclination.

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#### 1. Introduction

Among the anomalies affecting the infrastructure in the mountainous regions of Georgia, snow avalanches are the most dangerous ones, having a particular frequency to the extent of the consequences of the damage incurred and they have been increased geometrically in the last ten years in terms of the climate change in the world.

Mtskheta-Mtianeti region is taken as the survey target, it is located in the northern part of Eastern Georgia and its area constitutes 8.3% of the total country. The region has the most significant strategic purpose for our country.

As scientific studies have shown, the most avalanche-prone regions in Georgia are Racha-Lechkhumi and Kvemo Svaneti (74%), followed by Mtskheta-Mtianeti and Adjara (66-66%). It is interesting that Mtskheta-Mtianeti is the leader in the

high-risk avalanching sites, which was probably expectable, taking into consideration great absolute altitudes, slope inclination, forest cover, etc. [1].

According to the data of the National Environment Agency, there are more than 5,000 identified avalanche catchments in Georgia. Updatable data constantly, almost automatically allows the changes in nature to be quickly reflected in the database, processed and thousands of avalanches to be quickly mapped [2].

Due to the natural conditions of Georgia, we believe that it is very important to pay more attention to the study of snow avalanches and the use of modern technologies in the process. Snow avalanche and its danger isolates some municipalities of Georgia for almost ten months, and given that these regions (Tusheti, Khevsureti-Arkhoti, River Arghuni gorge, Mighmakhevi, periodically Kazbegi Municipality) are bordering to the Russian Federation, it becomes

clear that this is the serious problem for safety of the state of Georgia. Figure 1 presents the risk map of snow avalanching sites of Georgia according to the relevant classification [2], and Table # 1 shows the data of the Faculty of Geography of Lomonosov Moscow State University – percentage data of snow avalanche formation on the central range of mountains of Caucasus (including in Georgia) according to absolute altitudes [website].

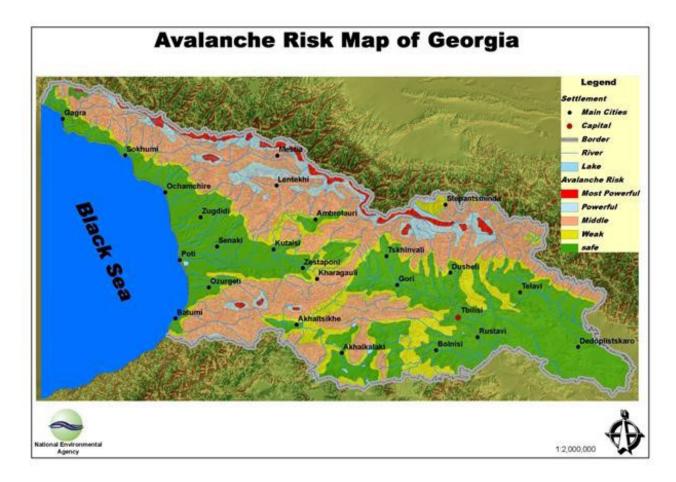


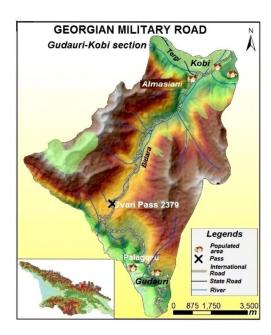
Fig. 1. Avalanche risk map of Georgia (Scale 1:2000000)

The study of this natural disaster process is especially important on highways, playing a major role not only within the country but also in transnational movement.

Table 1. Percentage values of snow avalanche formation at the Central Caucasus Mountain Range

$N_{\underline{0}}$	Absolute marks above sea level (m)	Percentage values of snow avalanche
		formation (%)
1	From 1000 m - below	2,2
2	1000 - 1500	10,3
3	1500 - 2000	13,9
4	2000 - 2500	19,5
5	2500 - 3000	31,5
6	From 3000 m - above	22,1

Exactly such is the case with the Mtskheta-Mtianeti region highway, known as the Georgian Military Road (Fig. 2). This road has had and still has a transit, trade-economic, cultural, tourist-recreational function both for the Mtskheta-Mtianeti region and for Georgia. This is the road that connects the South Caucasus with the North Caucasus and Russia. The importance of this road is further enhanced by the gas pipeline, which is supplied from Russia to Armenia via a military road. Avalanches are frequent along the mentioned road, especially at Kobi-Gudauri section, which temporarily hinders the traffic during the season [3].



**Fig. 2.** Kobi-Gudauri section of the Georgian military road

Snow avalanches are typical for the medium and high mountain zones of Georgia and its formation and dissemination are mainly preconditioned by the large slope of the relief surface, separation, features of green cover and meteorological elements:

- In 1985-1987, relatively long-term stationary observations of the avalanche regime on the territory of Georgia took place only at Jvari Mountain Pass avalanche research station. According to these data, 18-20 avalanches have been recorded from one and the same avalanche catchment. It should also be noted that a significant proportion of new snow avalanches on the steep slopes of complex avalanche sites stopped at the top of the avalanche catchment so that they could not reach the highway [4];
- In February 1992, 15 avalanches set off at Kobi-Kazbegi motor road section and traffic was in-

terrupted for 4 days and nights. According to the relevant state service data, in 1996, snow-avalanche set off at Gudauri-Kobi section 149 times, as a result of which the motor road was closed for 42 days. In December of the same year, 21 people were lost in an avalanche falling down from the White Mountain (Georgian military road);

- In 1997, a snow-avalanche set off 120 times at Gudauri-Kobi section, killing 5 people, and closing the road for 40 days;
- In 1998, avalanches set off at one and the same section 54 times and traffic was interrupted for 22 days.

The statistics of the loss caused by the snow avalanches at Gudauri-Kobi section in 2020 are as follows:

- In Gudauri, on Stepantsminda-Kobi road, an avalanche set off and carried one car down into the ravine. According to the Emergency Management Service, 3 people were in the car. One was rescued unharmed, one was injured and one citizen was killed (Fig. 3);
- A Russian citizen lady died as a result of a snow avalanche in Gudauri. Two people were rescued, one of whom, a Georgian citizen, had a fracture, and the other, an Armenian citizen, was rescued to a safe place unharmed. Their lives are not in danger (Fig. 4);
- On 15 February 2020 at around 12:00 13:00 an avalanche set off on Kobi-Gudauri road on the Sadzele territory in the ski prohibited zone. There were 4 tourists on the site, three of them managed to leave the territory in time, and one tourist died (Fig. 5). Georgia is a mountainous country and the share of its mountains and foothills is 54%. The infrastructure in these areas is constantly changing due to the events of disturbances of sustainability and ecological balance (Fig. 6).

Among the anomalies affecting the infrastructure in the high mountainous regions of Georgia, snow avalanches are the most dangerous ones, having a particular frequency to the extent of the consequences of the damage incurred and it has been increased geometrically in the last ten years in terms of the climate change in the world [5].

There are three main avalanche-prone sections of the Georgian military road - Zhinvali-Mleta, Gudauri-Kobi and Almasiani-Dariali. The first section (Zhinvali-Mleta) and the third section (Almasiani-Dariali) are weak, while the second section (Gudauri-Kobi) which is our survey target (Fig. 7) is characterized by particularly strong avalanche hazard.



Fig. 3. 2 February 2020



Fig. 4. 10 February 2020



Fig. 5. 15 February 2020



Fig. 6. Gudauri skiing lodge





Fig. 7. Snow avalanche at Kobi-Gudauri section of the Georgian military road

Due to the western air masses, there is humid climate along the Georgian military road, the amount of precipitation in November-April is 1300 mm. The maximum height of snowpack recorded in 1968 was 373 cm. Snow height of more than 100 cm was recorded 16 times in 42 years. Snowpack with the height of 200 cm and more was recorded 23 times and more than 300 cm - 2 times.

There are 52 avalanche catchments at Zhinva-li-Mleta section and avalanches are expected during heavy precipitations (more than 70 mm precipitation in 18 hours). Most avalanches cover the road in heavy snowy winters. The exception is three sporadic avalanches of Jinwali-Mleta section, which come out to the road in anomalous winter conditions. The volume of snow taken from avalanches is from 1 to 100 thousand m3. Maximum volume was observed in the extremely snowy winter of 1986/87. At this section, there are 7 avalanche catchments in Pasanauri territory [6, 7].

There are 27 avalanches at Almasiani-Dariali section, which completely block the road during

heavy precipitations (50 mm in 18-36 hours), and the volume of snow mass taken on the highroad was 76 thousand m3 in January 1987.

There are 59 avalanche catchments at Gudauri-Kobi section, out of which 41 avalanches come out on the road. The avalanche tunnels and gallery on this section fully or partially protect part of the road from only 17 avalanches.

The annual amount of precipitation on the Georgian military road increases with the increase of the altitude of the place from 739 mm to 1733 mm. Precipitation in the cold period of the year is 27-37% of the annual norm. The number of rainy days ranges from 122 to 183. The snowpack is unevenly distributed. Especially in extremely snowy winters, the maximum height of snowpack at Gudauri and Jvari Mountain Pass is 386-373 cm, respectively. During one snowfall, the height of snowpack increases by 210-270 cm, and the day-night increase is 100-120 cm, the number of snowy days is up to 1300 m, from 50 to 95 days, and at 1900-2400 m it is for 131-218 days. The maximum height and repeatability of snowpack are presented in the table (Table 2).

Table 2. Maximum height and repeatability of snowpack [8]

		May beight May			peatabil	ity		
#	Meteorological station	Max. height (m)/years of observance	Max. height (cm)/ year	>50-	101-200	201-	301-	>400
1	Pasanauri	1070/79	148/1939	31	8	-	-	-
2	Mleta	1580/60	360/1991	18	28	1	2	-
3	Stepantsminda	1744/75	112/1974	29	4	-	-	-
4	Kobi	1962/19	176/1956	5	9	-	-	-
5	Gudauri	2194/59	386/1987	-	38	18	3	-
6	Jvari Mountain Pass	2395/42	373/1968	-	16	23	2	-
7	Kazbegi (high mountainous)	3653/48	165/1936	33	8	-	-	-

Most of the avalanche catchments in the vicinity of the Georgian military road - 31 avalanches (45%) cover an area of less than 0.5 ha, while the surface slope is mostly 31-350 (23 avalanches), the maximum speed of 26 avalanches is 31-20 m/s (Table 3).

Area			Slope			Maximum speed			
hec	Amount	%	Degree Amount % 1		m/sec	Amount	%		
<0,5	31	45	<25	6	9	<20	12	17	
0,5-1,0	16	23	26-30	15	22	21-30	25	36	
1,1-10,0	18	26	31-35	23	33	31-40	26	38	
>10	4	6	36-40	15	22	>40	6	9	
			>40	10	14				

Table 3. Distribution of avalanche catchments by area, surface slope of avalanche sites and maximum avalanche speed

Hazardous avalanche catchments for the Georgian military road are located in all three low-medium- and high mountain zones. The weakest maximum impact force of the existing avalanches is  $4\ t/m^2$ , and the strongest is  $97\ t/m^2$  [9].

# 2. Outcomes of field survey at Kobi-Gudauri area as of 2020 – 2021

Kobi-Gudauri military road section for the study region is characterized by frequent natural disasters, among which snow avalanches play an important role.

Facts of catastrophically increased snow avalanche intensity and frequency, demographic change, unplanned urbanization, insufficient control over safety requirements protection, socio-economic inequality, environmental degradation, climate change and, in the light of all this, planning the preventive measures, taking into account all possible natural hazards, need an active approach.

Mitigation of the risk of snow avalanche disasters is a multidisciplinary complex issue and requires the support at the political and legislative levels, proper development of appropriate scientific knowledge, planning, selection of innovative regulatory and engineering measures, disaster preparedness, effective response mechanisms and public awareness.

In view of all the above, the grant project leader and project implementers carried out field trips and expeditions to identify the sensitive areas of snow avalanche formation at Kobi-Gudauri section of the Georgian military road in the winter and spring of 2020-2021 for the purpose of conducting field-scientific research.

Sensitive points of snow avalanche were recorded on site using GPS and GIS digital maps, the quantitative indicators of which: absolute height of the point (m), GPS coordinates, point numbers on the Georgian military road and lengths of sections are given in Table # 4 and in parallel marked on the digital map (Fig. 8).

In order to arrange an innovative antiavalanche structure [9], to select a representational place, inJanuary-March 2021 with the involvement of (N)LE Ecocenter for Environmental Protection and the staff of the Hydrometeorology Department of theNational Environmental Agency, the field recon-naissance surveys were carried out at Kobi-Gudaurisection (Fig. 8). Analysis and evaluation of thesefield surveys revealed two avalanche catchmentsfor the arrangement of an innovative antiavalanche structure, the indicators of which are shown on the map (Fig. 9). The mountain inclination avalanche slope thefirst catchment is 240, and that of the sec-ond avalanche catchment is 200.

Table 4. Numerical indicators of field - scientific researches

Geographical values of avalanche-prone points at Kobi-Gudauri section of the Georgian
Southern Road

N	Point numbers	Absolute mark	Coordinates Noau Ro		Road co	ordinates	Distance between point and road	
		(H)	X	Y		X Y		(m)
1	16	2035	458341	4711074	16.1	458485	4710988	181.27
2	15	2054	458228	4710769	15.1	458331	4710753	110.76
3	1	2122	458099	4710062	1.1	458080	4710083	29.8
4	3	2170	457649	4709686	3.1	457636	4709726	40.74
5	14	2192	456988	4709635	14.1	457093	4709573	127.18
6	5	2220	456872	4709189	5.1	456847	4709212	36.25
7	6	2223	456841	4709142	6.1	456810	4709161	40.01
8	7	2265	456545	4708595	7.1	456466	4708584	81.52
9	8	2274	456545	4708311	8.1	456441	4708348	110.92
10	9	2312	456549	4707951	9.1	456440	4708078	156.98
11	10	2335	456418	4707677	10.1	456255	4707684	179.04

The geological conditions of both avalanche catchments are homogeneous and they are cretaceous marls, limestones and argillaceous slates. Subaerial volcanites of the fourth age with the dacite-andesite composition are also found in the vicinity of the avalanches [10].

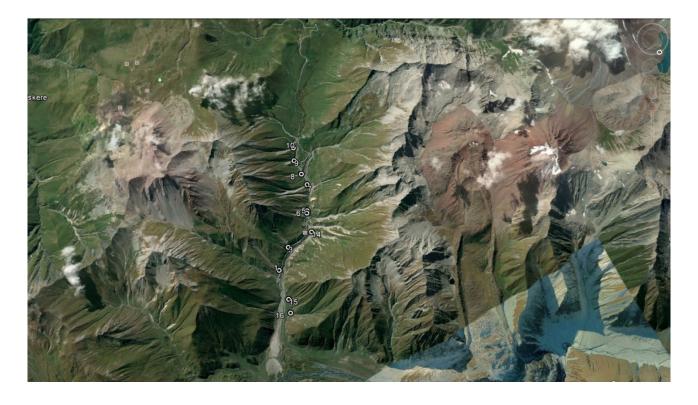


Fig. 8. Map of the Sensitive Areas of Kobi - Gudauri of the Georgian Military Road (29 October 2020)

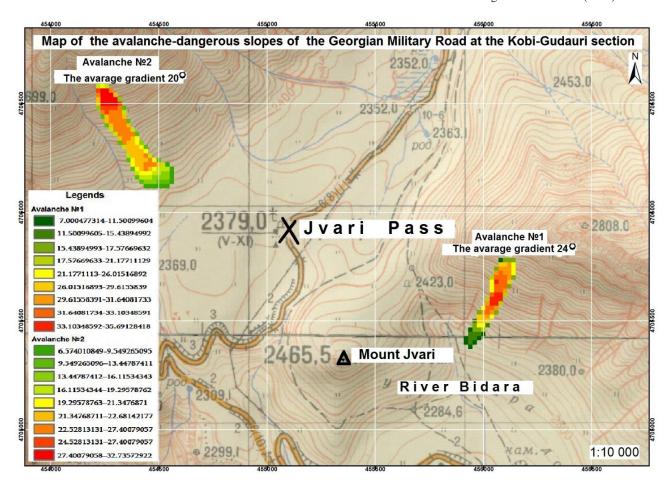


Fig. 9. Snow-catchment areas for arrangement of antiavalanche structure

Photo material reflecting the outcomes of the field survey conducted at Kobi-Gudauri area is given at the 10th figures.



a) Snow Avalanche # 2 (Jvari Mountain Pass. Absolute mark 2699 (m) Inclination of the mountain slope  $20^{\circ}$ 



 b) Snow Avalanche #1 (Panorama surrounding territory) Absolute mark 2570 (m)
 Inclination of the mountain slope 24°

**Fig. 10.** Field surveys at Kobi - Gudauri section of the Georgian Military Road (16 – 22 March 2021)

To arrange the innovative snow avalanche construction at Kobi-Gudauri section of the Georgian military road, we selected snow avalanche catchment # 1, with a slope inclination of 240 (see Fig. 9 and Fig. 10 - b), located in the vicinity of Panorama at absolute height 2570 (m), with coordinates X = 4560433; Y = 4705596. Thereafter, based on the conducted scientific field surveys and theoretical studies, it has been established that due to the scale of the avalanches, notwithstanding the existing models and control measures, the catastrophic consequences cannot be avoided. Snow avalanches are a terrifying phenomenon among natural disasters and the creation of an innovative type of the regulatory structures is related to their genesis and dynamics.

Taking into consideration the foregoing, the design of innovative antiavalanche structure [10] has already been developed, the implementation of which is planned for the snow avalanche catchment #1 at the sensitive area of Kobi-Gudauri of the Georgian military road (Fig. 10-b).

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