

## Enzymatic activity of cryoconites from glaciers and high-altitude glacial soils in the Central Caucasus

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### Abstract

Nowadays, in light of research into glacial cryoconites and glacial soils has become particularly relevant due to ongoing global warming and the accelerated deglaciation of high-altitude glaciers. This study presents the research into enzyme activity in cryoconites from the Maly Azau Glacier and in glacial soils of the Central Caucasus. It was shown that the enzymes studied in the cryoconites exhibited virtually no activity, with the exception of a sample taken from a cryoconite cup, in which very weak invertase activity (1.71 mg glucose / (g day)) and weak phosphatase activity (8.62 mg TPF / (10 g day)). Vertical distribution of enzyme activity in the studied glacial soils indicated a decrease in the activity of all studied enzymes (dehydrogenase, catalase, invertase, phosphatase) down the profile. In the surface layer of the studied soils, redox enzymes exhibited relatively low activity. Thus, catalase (CAT) activity ranges from 0.50 to 3.63 ml O<sub>2</sub> / (g min)), and dehydrogenase activity from 0.07 to 1.52 mg TPF / (10 g day). In contrast to oxidases, hydrolytic enzymes exhibit high and very high activity, ranging from 27.98 to 81.94 mg glucose / (g day) for invertase and from 93.16 to 167.43 mg P<sub>2</sub>O<sub>5</sub> / (100 g h) for phosphatase. The results indicated that despite the adverse and extreme climatic conditions, biochemical processes occur within glacial cryoconites. Furthermore, as deglaciation continues, cryoconites-whether washed away or left behind following glacial retreat-may influence soil formation processes in areas newly freed from ice. Our research has confirmed that biological processes are actively taking place in high-altitude soils and as the climate warms and glaciers retreat, areas adjacent to glaciers in mountainous regions, located above 2.000 m a. s. l., can be used for agriculture.

**Key words:** periglacial soils, cryoconites, enzymatic activity, high-altitude regions, climate change, permafrost thaw

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## Introduction

Climate change is currently ongoing across the globe, leading to various adverse effects, such as changes in precipitation patterns, rising temperatures, permafrost degradation and glacier retreat in high-altitude regions (IPCC 2021<sup>[2]</sup>). According to scientific projections (Radić et al. 2014), glaciers in mountainous regions will lose 80% of their volume by the end of 2100, and some are expected to disappear within decades under current climatic conditions. Active deglaciation occurs in all mountainous regions, such as: Northern Eurasia (Khromova et al. 2014), Tibetan Plateau (Wang et al. 2024), the Andes (Caro et al. 2024), the Alps (Reinthal and Paul 2025) and many others. In the Caucasus, a steady decline in glaciation is being observed (Kotlyakov et al. 2023). Mikhaleiko (2020) reports the glaciers of the Greater Caucasus have lost almost 29% of their glacial area since the 1960s. This problem is particularly acute on the glaciers of the Central Caucasus, including those on Russia's highest mountain, Mount Elbrus. Thus, over the entire observation period since 1887, the glaciers of Elbrus have lost almost 20% of their ice cover area, shrinking from 147.5 km<sup>2</sup> to 120 km<sup>2</sup> (Nosenko et al. 2013, Rototaeva et al. 2019).

In addition to global warming, which is the main cause of glacial retreat, cryoconite deposits accumulating on their surfaces also play a significant role in the process (Lyu et al. 2024). Due to their dark colour, cryoconite deposits increase the albedo of glacier surfaces and accelerate their melting (Ferrario et al. 2017). Cryoconite consists mainly of bacteria, archaea, fungi and algae, with cyanobacteria making the greatest contribution to its formation; these form films and filaments during the ablation season, facilitating the formation of aggregates consisting of mineral deposits and organic matter (Wejnerowski et al. 2023). Ponieka et al. (2020) have

shown that cryoconites harbour microbial communities similar to those found in soils, which possess the ability to carry out photosynthesis, nitrification and denitrification, thereby influencing the biogeochemical cycles of glacial and periglacial zones. As glaciers retreat, cryoconite deposits may be transported and serve as an important source of microorganisms for primary succession and the initiation of soil formation processes (Zazovskaya et al. 2022).

Thus, changes in the nature of snow and ice cover, linked both to global warming and to the formation of cryoconites on glacier surfaces, have a significant impact on high-altitude soil ecosystems, leading to significant changes in the diversity and functions of soil microorganisms, which, in turn, affect the biogeochemical cycles of high-altitude soils (Donhauser and Frey 2018, Li et al. 2021).

Enzymes play a role in the biogeochemical cycles of carbon, nitrogen and phosphorus, as well as in the synthesis and decomposition of humic substances, the hydrolysis of organic compounds, and the conversion of the elements they contain into a form that is available for uptake by plants and microorganisms, *etc.* (Khaziev 2005, Liu et al. 2021). Soil enzymatic activity is a sensitive indicator of ecosystem health and an important factor in their functioning. The response of soil enzymes to rising temperatures partly influences carbon stability under climate change conditions (Vikram et al. 2024). Changes in climatic factors, such as rising temperatures and frequent cycles of soil wetting and drying, alter the composition of microbial communities in the soil, leading to a potential increase in enzyme activity and microbial biomass (Allison and Treseder 2008, Huang et al. 2022). As soil enzymes respond more rapidly than other soil parameters, they can be used as early, sensitive indicators of changes in soil biological

activity, vitality (Pramunadipta et al. 2026), and nutrients availability caused by global climate change (Zhang et al. 2015, Liu et al. 2021).

In the studies of glacial cryoconites carried so far, the main focus has been put on research into the microorganisms inhabiting them (Sehnal 2015, McCrimmon et al. 2018, Lutz et al. 2019), as well as their organic carbon and nitrogen content (Polyakov et al. 2022, Bagshaw et al. 2013). There is also a significant body of work assessing pollutants such as heavy metals (Łokas et al. 2019, Kushnov et al. 2026) and radionuclides (Baccolo et al. 2020, Tembotov et al. 2025) in glacial cryoconites. To date, only one paper (Abakumov and Tembotov 2021) has been published on enzyme activity in glacial cryoconites from the Caucasus. The article (Abakumov and Tembotov 2021) presents data on enzyme activity in cryoconites collected from two glaciers in the Central Caucasus – Shkhelda and Garabashi.

Regional soil literature (Soils of the Kabardino-Balkar ASSR 1984<sup>[3]</sup>, Kumakhov 2007, Molchanov et al. 2017) contains extensive data describing the morphological and physico-chemical properties of mountain soils in the Central Caucasus. There are also a number of studies on the biological properties of soils in the mountainous part of the Central Caucasus (Chadaeva et al. 2021, Gedgafova et al. 2023, Gorobtsova et al. 2024, Tembotov 2024).

The aim of this study was to assess enzyme activity in cryoconites from the Maly Azau Glacier and in glacial soils in the Elbrus region of the Central Caucasus. To achieve this aim, several the two following objectives were postulated:

1. to determine the activity of redox enzymes (catalase and dehydrogenase) in cryoconites and soils of the study areas;
2. to determine the activity of hydrolytic enzymes (invertase and phosphatase) in cryoconites and soils of the study areas.

## Material and Methods

### *Regional settings*

The sampling of cryoconites was carried out on the Maly Azau Glacier, located on Russia's highest mountain, Mount Elbrus (Fig. 1). The glacial soils studied are situated in the high-altitude region of the Central Caucasus, within the alpine and subnival zones of the Kabardino-Balkarian Republic, in the Elbrus region (Fig. 1).

Maly Azau is a typical cap glacier with a conical summit, situated on the southern slope of Mount Elbrus. The glacier begins at an altitude of 5.600 m and ends at 3.011 m; its area was 8.50 km<sup>2</sup> (Mikhaleenko 2020).

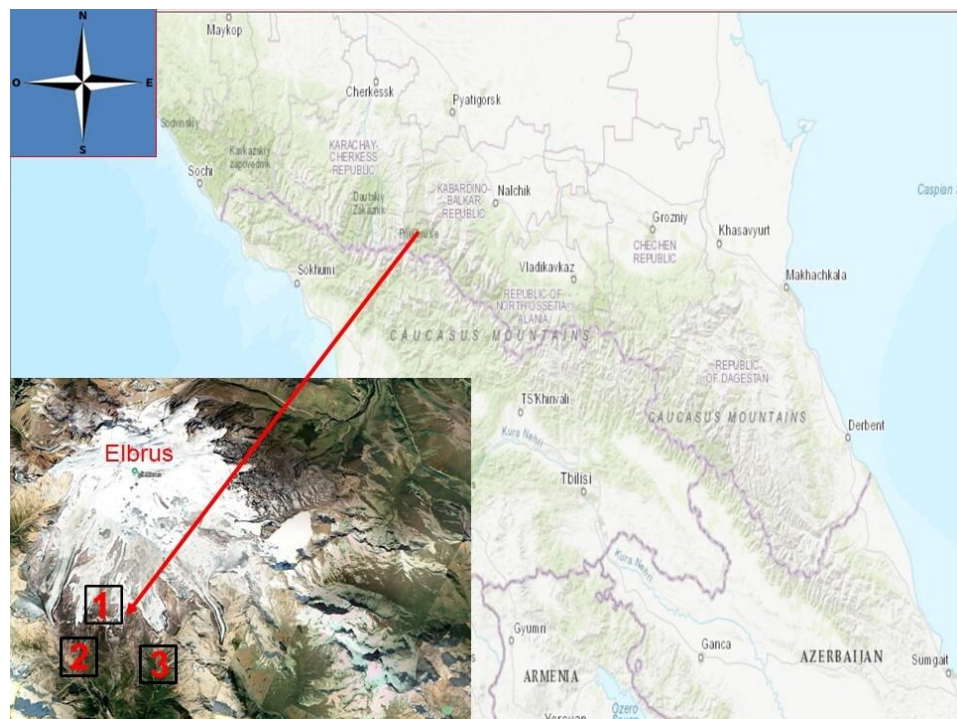
The studied glacial soils, classified as dark humus organo-accumulative (Classification and Diagnostics of Russian Soils

2004<sup>[1]</sup>) or Leptosol Umbric Hyperskeletal (WRB 2022<sup>[4]</sup>), are located in the Baksan and Terskol Gorges. The soils under study are situated in the high-mountain zone of the Elbrus region, on slopes of varying steepness and aspect, at altitudes of 2.580 and 2.642 meters above sea level. In these soils, the profile is weakly differentiated, with gradual transitions between horizons. The sod horizon is well developed, ranging in thickness from 3–5 cm to 7–10 cm. The humus profile, up to 20 cm thick, is dark brown in colour and has a powdery texture. The grain size distribution is mainly medium- and light-loamy, characterised by an abundance of stony inclusions from the surface and throughout the soil profile. The soil-forming material consisted pre-

dominantly of moraine deposits, carbonate clays and calcareous sandstones (Razumov 2002).

The climate of the study area is temperate continental, cold and humid, determined by the high-mountain relief and the

inflow of westerly air masses from the Atlantic (Fiapshev 1996). According to the Köppen–Geiger climate classification, the study area belongs to the ET category – an alpine climate (tundra climate) (Peel et al. 2007).



**Fig. 1.** Study area and sampling points in the Central Caucasus: 1 – cryoconite samples, 2 – surface soil samples, 3 – samples from soil profiles.

### *Sampling design*

Cryoconite samples were collected from three locations on the Maly Azau Glacier, at an altitude of 3.390 m above sea level. Coordinates: N 43.291723°, E 42.456567°.

Two samples were taken from the surface of the glacier, and one sample from a cryoconite cup (Fig. 2).



**Fig. 2.** Cryoconite sampling sites: **A** – the Maly Azau Glacier; **B** – a cryoconite collection cup on the Maly Azau Glacier.

In the high-altitude section of the Terskol Gorge, two profiles of Leptosol Umbric Hyperskeletal soil were established (Fig. 3), from which samples were

taken from the genetic horizons. A description of the sampling sites and the soil profiles is presented in the table (Table 1).



**Fig. 3.** Profiles of Leptosol Umbric Hyperskeletal soil on moraine deposits. *Note:* **A** – under a meadow, **B** – under a disturbed meadow.

| Sample ID              | Study site, coordinates                                   | Type of land use          | Soil name*                          | Horizon | Depth, cm |
|------------------------|-----------------------------------------------------------|---------------------------|-------------------------------------|---------|-----------|
| Terskol 1<br>(Fig. 3A) | Terskol Gorge,<br>N 43.289111°,<br>E 42.507684°<br>2642 m | Pasture, meadow           | Leptosol<br>Umbric<br>Hyperskeletal | Au      | 0-10      |
|                        |                                                           |                           |                                     | Aum     | 10-20     |
|                        |                                                           |                           |                                     | Ac      | 20-35     |
|                        |                                                           |                           |                                     | C       | 35-45     |
| Terskol 2<br>(Fig. 3B) | Terskol Gorge,<br>N 43.284650°,<br>E 42.510833°<br>2580 m | Pasture, disturbed meadow | Leptosol<br>Umbric<br>Hyperskeletal | H       | 0-7       |
|                        |                                                           |                           |                                     | Au      | 7-13      |
|                        |                                                           |                           |                                     | Ac      | 13-25     |

**Table 1.** Location and characteristics of the profiles within the Terskol Gorge in Kabardino-Balkaria. *Note:* \*WRB 2022<sup>[4]</sup>.

To investigate the spatial variability of the activity of the enzymes (*see* below), soil samples were collected from the 0-10 cm surface horizons at nine monitoring sites in the alpine and subnival zones at

altitudes ranging from 2.700 to 3.050 metres. Composite soil samples were collected at each monitoring site using the ‘envelope’ method.

**Soil enzymatic activity measurements**

Laboratory analytical tests were carried out in triplicate. Catalase activity (ml O<sub>2</sub> per 1 g per minute) was determined using the gasometric method; the activity of the other enzymes (dehydrogenase, mg TFF per 10 g per 24 hours; invertase, mg glucose per 1 g per 24 hours; phosphatase, mg P<sub>2</sub>O<sub>5</sub> per 100 g/1 h) – by the colorimetric

method (Kazeev et al. 2003). Enzyme activity levels were assessed using the Gaponyuk and Malakhov scale (Gaponyuk and Malakhov 1985). Similar methods for determining soil enzyme activity have been used in the studies by Guan et al. (1986) and Liu et al. (2021).

**Statistical processing**

Statistical processing was carried out using Excel and Statistica 12.0 programs. The descriptive statistic method was used at the significance level  $P \leq 0.05$  to identify

the average values, standard error, coefficient of variation, and error of the average in the sample.

## Results and Discussion

### *Enzymatic activity of cryoconites from the Maly Azau Glacier*

An investigation into enzyme activity in cryoconites from the revealed Redox enzymes catalase and dehydrogenase showed no activity in any of the Maly Azau Glacier samples examined (Table 2). In contrast, the hydrolytic enzyme invertase exhibited very low ( $>5$  mg glucose/(g day)) activity, whilst phosphatase showed low ( $5\text{--}15$  mg  $\text{P}_2\text{O}_5$ /(100 g h)) activity. It is worth noting that the activity of hydrolytic enzymes was detected only in the sample taken from the cryoconite cup. Previous study (Abakumov and Tembotov 2021) focused on the activity of enzymes in cryoconites from Caucasus glaciers showed that enzyme activity in cryoconites depended on the extent of recreational impact on the glacier. It was thus found that

in cryoconites from the Shkhelda Glacier, which is virtually untouched by human activity due to its inaccessibility, no enzyme activity was detected. Contrastingly, activity was observed for all the enzymes studied in cryoconites from the Garabashi Glacier, which is subject to significant recreational impact. It suggests that, despite the extreme climatic conditions, various biochemical processes take place in the cryoconites of high-altitude glaciers in the Central Caucasus and, consequently, microorganisms are present. The presence of bacteria in glacial cryoconites has also been reported in studies from the Arctic (Singh et al. 2014), Antarctica (Christner et al. 2003) and the Alps (Lee et al. 2011).

| Sample ID | Dehydrogenase activity, mg TPF/(10 g day) | Catalase activity, ml $\text{O}_2$ /(g min) | Invertase activity, mg glucose/(g day) | Phosphatase activity, mg $\text{P}_2\text{O}_5$ /(100 g h) |
|-----------|-------------------------------------------|---------------------------------------------|----------------------------------------|------------------------------------------------------------|
| Azau      | 0                                         | 0                                           | 0                                      | 0                                                          |
| Azau      | 0                                         | 0                                           | 0                                      | 0                                                          |
| Azau      | 0                                         | 0                                           | 1.71                                   | 8.62                                                       |

**Table 2.** Enzymatic activity of cryoconites from the Maly Azau Glacier.

### *Enzymatic activity of soils in the Elbrus region*

Vertical distribution of the activity of the enzymes examined in the periglacial soils of the Elbrus region showed that maximum activity was observed in the surface layers, the activity, however, decreased with depth (Table 3). It was found that the activity of the redox enzyme dehydrogenase, which already exhibits low values in the surface layer, shows no activity

at all in the lower horizons. Catalase (CAT) activity manifested differently in the two soil profiles. In the Terskol 1 profile, CAT activity was moderate in the upper horizons and transitions to the weak category in lower ones. In the second profile, Terskol 2, activity was already weak in the upper horizons, transitioning to the very weak category in the lower horizon.

| Sample ID | Horizon | Dehydrogenase activity, mg TPF/(10 g day) | Catalase activity, ml O <sub>2</sub> /(g min) | Invertase activity, mg glucose/(g day) | Phosphatase activity, mg P <sub>2</sub> O <sub>5</sub> /(100 g h) |
|-----------|---------|-------------------------------------------|-----------------------------------------------|----------------------------------------|-------------------------------------------------------------------|
| Terskol 1 | Au      | 0.19                                      | 3.6                                           | 57.3                                   | 67.6                                                              |
|           | Aum     | 0                                         | 3.2                                           | 30.3                                   | 35.3                                                              |
|           | Ac      | 0                                         | 2.4                                           | 0                                      | 3.29                                                              |
|           | C       | 0                                         | 0                                             | 0                                      | 3.17                                                              |
| Terskol 2 | H       | 0.13                                      | 1.7                                           | 84.9                                   | 34.4                                                              |
|           | Au      | 0                                         | 1.3                                           | 82.7                                   | 33.9                                                              |
|           | Ac      | 0                                         | 0.6                                           | 0                                      | 11.2                                                              |

**Table 3.** Enzymatic activity in the genetic horizons of the Elbrus region soils.

Unlike redox-sensitive soils, the soils studied exhibited relatively high levels of hydrolytic enzymes (Table 3). Invertase activity in the surface layer of both sections ranged from high to moderate, whilst it is completely absent in the lower horizon. Phosphatase activity in the surface horizon of the Terskol 1 section was high, gradually decreasing to moderate in the lower horizons, and then dropping sharply to very low. The Terskol 2 site exhibited moderate phosphatase activity in the surface layers, which decreased to low activity found further down the profile. This is consistent with data of Gorobtsova et al. (2015), Dadenko et al. (2016), which indicate a decrease in enzyme activity following a reduction in organic matter occurring in the lower soil horizons. Similar results were obtained by Shchelchkova (2002) for

permafrost and long-term frozen soils in Southern Yakutia, which showed that between 56 and 100% of the soil enzyme pool is concentrated in the top 10–20 cm layer of soil. In the soils of the wet meadows of the Qinghai-Tibet Plateau, the activity of the enzymes urease, catalase and amylase also decreased significantly with soil depth and demonstrated clear ‘surface aggregation’ (Wu et al. 2020). A decrease in the activity of invertase and urease in the lower horizons of permafrost soils in Northern China was reported by Liu et al. (2021).

Given that the studied enzymes exhibited the highest activity in the upper horizon, samples of the surface layer (0 – 10 cm) were collected from nine points in the high-altitude part of the Elbrus region. The results of the data obtained are presented in the Table 4.

| Points № | Dehydrogenase activity, mg TPF/(10 g day) | Catalase activity, ml O <sub>2</sub> /(g min) | Invertase activity, mg glucose/(g day) | Phosphatase activity, mg P <sub>2</sub> O <sub>5</sub> /(100 g h) |
|----------|-------------------------------------------|-----------------------------------------------|----------------------------------------|-------------------------------------------------------------------|
| 1        | 0.62                                      | 1.50                                          | 74.85                                  | 167.43                                                            |
| 2        | 1.25                                      | 1.63                                          | 81.94                                  | 150.75                                                            |
| 3        | 1.52                                      | 3.63                                          | 76.95                                  | 93.16                                                             |
| 4        | 0.21                                      | 0.95                                          | 43.08                                  | 154.68                                                            |
| 5        | 0.87                                      | 1.13                                          | 73.46                                  | 154.24                                                            |
| 6        | 0.12                                      | 1.05                                          | 71.88                                  | 112.36                                                            |
| 7        | 0.07                                      | 0.83                                          | 61.22                                  | 134.76                                                            |
| 8        | 0.55                                      | 0.80                                          | 43.14                                  | 165.01                                                            |
| 9        | 0.56                                      | 0.50                                          | 27.98                                  | 96.07                                                             |

**Table 4.** Enzymatic activity of the upper horizons (0–10 cm) of the soils of the Elbrus region.

The data obtained (Table 4) showed that in periglacial soils, CAT activity, according to the classification used (Gaponyuk and Malakhov 1985), is generally very low or low, with the exception of the sample from monitoring site No. 3, in which moderate activity was detected. Dehydrogenase activity in all soil samples is characterised by very low values (Table 2). The results of the study (Table 2) showed that in the soils, invertase activity exhibited moderate and high values. Phosphatase was also quite active and is characterised by high and very high values. High soil enzyme activity has also been observed in the high-altitude regions of south-western China. A study (Cai et al. 2025) demonstrated that enzyme activity increased with altitude, a phenomenon attributed to the stimulation of microbial metabolic activity resulting from higher organic matter content. But this increase occurs up to a certain height and depends on the exposure of the slope. So in the work (Tembotov 2024) it is shown that with an increase in absolute height, from the foothill soils to the soils of the sub-alpine zone, the values of microbiological indicators and the content of organic matter in the surface horizons (0 – 10 and 0 – 20 cm) significantly increase, and then at the maximum height – in the alpine zone significantly decreases. The study of Xu et al. (2018) conducted in the Tibetan Plateau also showed that soil invertase activity correlated significantly with the organic carbon content in the soil (Xu et al. 2018).

Our study confirmed that the activity of redox enzymes in glacial soils of the Central Caucasus was relatively low, which is consistent with Galstyan (1974), and Dadenko et al. (2016), who demonstrated that in mountain soils, the catalytic activity

of oxidoreductases is suppressed. Similarly, low values were also obtained in previous studies of the activity of redox enzymes in mountain soils of the Central Caucasus (Khakunova et al. 2018, Gedgafova et al. 2022). With regard to hydrolytic enzymes, the study by Kazeev et al. (2003) notes that the high activity of hydrolases in mountain-meadow (organo-accumulative dark humus) soils is due to  $H^+$  ions, which account for up to 95% of exchangeable acidity. The high activity of hydrolytic enzymes is also associated with the high organic matter content in these soils (Gedgafova et al. 2023). Thus, our study demonstrated that redox and hydrolytic enzymes exhibit divergent activity in the high-altitude soils of the Central Caucasus. Similar conclusions have been drawn in previous studies of high-altitude soils in the Caucasus (Uligova et al. 2019, Gedgafova et al. 2022, 2023), which demonstrated that, due to the significant amount of organic matter, the soils in these regions exhibited high hydrolase activity, whilst the catalytic action of oxidoreductases was suppressed. This is also consistent with the results obtained for the Qinghai-Tibet Plateau, where it was shown that as the activity of hydrolytic enzyme – urease – increased, the activity of the redox enzyme – catalase – decreased (Wu et al. 2020).

Statistical analysis of the data (Table 5) showed that catalase activity in the soils under investigation is highly variable, with a coefficient of variation of 70%. Dehydrogenase activity in the soils, like CAT activity, is also highly variable, with a coefficient of variation of 78%. The hydrolytic enzymes studied showed less variability, with a coefficient of variation of 31% for invertase and 21% for phosphatase.

| Enzyme                                                                         | Statistical indicators |         |         |                    | Coefficient of variation, % | Mean error |
|--------------------------------------------------------------------------------|------------------------|---------|---------|--------------------|-----------------------------|------------|
|                                                                                | Mean                   | Minimum | Maximum | Standard deviation |                             |            |
| <b>Dehydrogenase activity,</b><br><i>mg TPF/(10 g day)</i>                     | 0.64                   | 0.07    | 1.53    | 0.50               | 77.99                       | 0.17       |
| <b>Phosphatase activity,</b><br><i>mg P<sub>2</sub>O<sub>5</sub>/(100 g h)</i> | 136.50                 | 93.16   | 167.43  | 28.98              | 21.23                       | 9.66       |
| <b>Invertase activity,</b><br><i>mg glucose/(g day)</i>                        | 61.61                  | 27.98   | 81.94   | 18.99              | 30.83                       | 6.33       |
| <b>Catalase activity,</b><br><i>ml O<sub>2</sub>/(g min)</i>                   | 1.34                   | 0.50    | 3.63    | 0.93               | 69.49                       | 0.31       |

**Table 5.** Statistical indicators of enzyme activity in the soils of the Elbrus region.

## Conclusions

Analysis of cryoconites from the Maly Azau Glacier revealed that the hydrolytic enzymes invertase and phosphatase exhibit very low and low activity, respectively. The redox enzymes catalase (CAT) and dehydrogenase did not exhibit any activity in the cryoconite samples studied.

Analysis of the data obtained showed that the activity of redox enzymes in the high-altitude soils of the Central Caucasus was not high, indicating a low rate of redox processes in the soils studied. Hydrolytic enzymes, in turn, exhibited high and very high activity in the soils studied, which is consistent with the literature, which reports high activity of hydrolytic enzymes

in mountain soils due to the significant amount of organic matter characteristic of these soils.

Despite the extreme climatic conditions, biochemical processes occur within the cryoconites of high-altitude glaciers. As deglaciation continues, cryoconites washed away or left behind after the retreat of glaciers may influence soil formation processes in areas freed from ice, and high-altitude soils possess high biological activity; as the climate warms and glaciers retreat, the areas adjacent to glaciers in mountainous regions may be incorporated into a sort of agricultural use.

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