Bulgarian Journal of Agricultural Science, 32 (No 2) 2026, 278–285

Modern approaches in the study of soil bioactivity in Katekh and Garakli village (Azerbaijan) irrigated alluvial meadow and mountain forest brown soils

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Abstract

Mammadova, G., Salimova, Sh., Aliyeva, G., Isagova, V. & Hasanova, T. (2026). Modern approaches in the study of soil bioactivity in Katekh and Garakli village (Azerbaijan) irrigated alluvial meadow and mountain forest brown soils. *Bulg. J. Agric. Sci., 32*(2), 278–285

The aim of our study was to assess the biological activity of soils in various mountainous areas using the method of determining the enzymatic activity of soils, to study their ability to self-recovery. For the first time in the Republic of Azerbaijan, comprehensive soil studies (biological activity, erosion rate, enzyme activity) were conducted in the selected area. From the comparative analysis it follows that the increase in anthropogenic load led to a decrease in the content of biogenic substances. Within one belt, depending on geographical factors, a number of soil parameters change sharply. Along with the drying process in this area, an increase in anthropogenic impact can be justified. Comprehensive studies using bioindication indicators, in particular, soils of agroecosystems, have not yet acquired a systematic form, and existing studies are fragmentary. In Azerbaijan, a unified, generally accepted system for biomonitoring soils of natural and anthropogenically modified ecosystems based on biological indicators has not been developed. Despite the relevance of the problem of balanced use of natural resources and environmental protection, there is still no unified system of biodiagnostic indicators of biochemical and microbiological processes that could be used to conduct a preventive assessment and monitoring of negative changes and phenomena in the soil under anthropogenic impact. Research conducted in this direction is considered to be very relevant. This is one of the important issues for soil scientists and biologists.

Keywords: ecological state; anthropogenic factors; biological activity; vegetation; Azerbaijan

Introduction

The increasing anthropogenic impact on ecosystems requires new effective methods for monitoring them and diagnosing the state of environmental objects. Recently, when developing approaches to assessing the ecological state of soils, scientists are increasingly focusing on methods based on diagnosing changes in biotic and abiotic components of soils (Aristovskaya et al., 1988). However, in Azerbaijan, comprehensive studies using bioindication indicators, in particular, soils in agroecosystems, have not yet acquired a systematic form, and existing studies are fragmentary. The basis of the modern approach to assessing the quality of environmental objects is the principle of “balanced functioning” of the ecosystem, based on taking into account the relationship between the components of the biocenoses and their interaction with the soil. The main indicators of this approach are the quantity and quality of manufactured products, biological diversity. It is impossible to overestimate the ecological function of soil in the biosphere. Soil has always been and is at the center of almost all biosphere processes. It is a link between the biological and geological cycles (Akhundova et al., 2025; Akbarova and Mammadova, 2024; Bloem et al., 2006). Sheki – Zagatala economic region include Balaken, Gakh, Gabala, Oghuz, Sheki and Zagatala regions. Currently, the soils of various territories are undergoing increasing anthropogenic changes, which is attracting close attention from researchers. Beyond the capacity of the environment within vulnerable areas can lead to the impossibility of returning the ecosystem to its original state (Ismayil et al., 2025; Kadhim and Hamza, 2021). Its role in human life is based on the fact that soil is a special food source, providing 95 – 97% of food resources for the world’s population. The role of soil in preserving biological variety of plants is also large. As can be seen from the analysis of scientific research, the richness of the flora of the Katekh and Garakli villages, its physical and geographical conditions, the history of the development of soil and vegetation cover, species composition and structure also depend on the influence of anthropogenic and natural factors. The mountainous part of Balaken region has a rich flora and differs from other botanical and geographical regions of the Azerbaijan Republic in the diversity of vegetation, which is considered the composition of the biocenoses in the ecosystem of the region (Mirzezadeh et al., 2025; Macnunlu et al., 2025; Mammadova et al., 2024). Subalpine-meadow, forest, undergrowth-meadow, mountain-xerophytic, mountain-steppe, petrophytic and marsh phytocenotypes were studied in the biodiversity of the Balaken, Zagatala, Gakh, Sheki administrative districts. In the mountain ecosystem, natural phytocenoses, especially relict forests, were not subject to the glaciation of the third and fourth periods (Ismayil et al., 2025). The predominant species on highly humidic soils are: Polytrichum, Pleurozium, Sphagnum, Cladonia, which form a continuous cover, Vaccinium myrtillus L. In the most heavily swamped areas, against the general background of the moss cover, the following appear: *Vaccinium uliginosum* L., *Ledum palustre* L., *Molinia coerulea* L., *Asarum europaeum* L., Centaurea marschalliana, *Koelleria cristata* L. *Pers*., *Trifolium montanum* L., *Achillea millefolium*, *Geranium sanguineum* L., *Calamagrostis epigeios* L. *Roth*. and occasionally, but not everywhere, *Oxycoccus palustris Pers*., *Oxalis acetosella* L. In the undergrowth, there is *Rubus caesius* L., *Viburnum opulus* L. In the grass cover there is a lot of nettle *Urtica dioica* L., fern *Matteuccia struthiopteris* L., *Equisetum sylvaticum* L, *Phragmites australis*, *Convallaria majalis* L., *Polygonatum odoratum* (Mill.) *Druce*.

Under conditions of increased anthropogenic load on the planet’s biosphere, soil, being an element of the natural urban system and being in dynamic balance with all other components, is subject to degradation processes. Flows of substances entering the soil as a result of activities humans are included in natural cycles, disrupting the normal functioning of the soil biota and, as a consequence, the entire soil system (Mammadova et al., 2024; Nasirova et al., 2026). Among the various biological criteria for assessing the anthropogenic impact on soils in the urban environment, the most biochemical indicators are operational and promising, providing information about the dynamics of the most important enzymatic processes in the soil: synthesis and decomposition of organic matter, nitrification and other processes (Mammadova et al., 2024). The biological activity of the soil, the qualitative and quantitative composition of the soil microbiota immediately respond to any anthropogenic impact. Soil cover indicators can be considered as reliable and informative indicators reflecting the state of the environment. The process of decomposition of fiber in the soil occurs under both aerobic and anaerobic conditions with the participation of special groups of bacteria and fungi. The process of decomposition of organic matter is an important integral link in the global biogeochemical cycle of elements and largely determines soil fertility (Nazim and Oqtay, 2024). The rate of cellulose decomposition affects the rate of decomposition of organic matter in general. This indicator can be considered as a quantitative measure of soil fertility, and pure cellulose can be considered as a model substrate for decomposition, against the background of which it is possible to determine the effect of environmental factors and study the properties of the soil. The study of erosion processes is also important for the development of agriculture and tourism. As a result of agricultural land use and increased settlement over the years, the erosion process has been intensifying. The soils of the area are not eroded, weak and heavily eroded areas were identified (Shukurov et al., 2025; Sun et al., 2020).

Data and Methods

For the research, soil samples were taken from seven points in the region in the Katekh (41°38′40″E; 46°32′32″N) and Garakli village (41°48′40″E; 46°22′32″N). The authors used a method for determining the enzymatic activity of soils, which was assessed using indicators of cellulolytic, protease and urease activity. The cellulolytic activity of the soil was determined by the application method, and the protease activity by a method based on the microbiological breakdown of gelatin present in the emulsion layer photo paper. To determine the urease activity of soil, the express method was used (Aristovskaya et al., 1988). The biological activity of soils in the study areas was assessed through indicators of cellulolytic, protease and urease activity. Consideration of these aspects of the biological activity of soils makes it possible to form a comprehensive picture of the activity of soil microorganisms in the study areas. The cellulolytic activity of the soil was determined by the patch method. To do this, a thin linen cloth measuring 5 × 5 cm was placed in a 100 g sample of soil. Based on the loss in tissue weight after 30 days of cultivation, to assess the biological activity of soils based on the intensity of fiber destruction, used the scale (percentage of decomposed tissue): very weak – less than 10%, weak – 10 – 30%, medium – 30 – 50%, strong – 50 – 80%, very strong – more 80% (Bloem et al., 2006). To maintain ecological balance and preserve the potential for self-purification and self-healing of soils in urban areas under the influence of anthropogenic factors causing degradation processes in the soil cover, it is necessary to conduct constant monitoring of their condition. The data obtained makes it possible to assess the soil cover urban area (Verdiyeva et al., 2025). Conducted assessment biological activity of soils can be used for rapid diagnostics of the intensity of soil processes and the nature of their changes in urbanized areas. Soil microorganisms, in combination with other representatives of soil pedobionts, form complex biocenoses. Based to their symbiotic relationships, the transformation of organic residues and the transformation of the energy contained in them of the biological cycle is carried out. Two-way nested analysis of variance (ANOVA) was conducted with SPSS to evaluate the effects of forest composition, vertical soil depths profiles, and their interactions on microbial and chemical properties. A correlation analysis was performed to explore the associations between different pairs of soil variables. Before conducting the statistical analyses, soil microbial and chemical variables data were inspected for normality check, outliers check (box plot method), and homogeneity of variances check, and no transformations were found necessary. The statistical significance level was set at $p < 0.05$, and the mean that shows a significant difference was compared using test (Tukey's). Soil microbial biomass fractions (MBC and MBN) were determined using the chloroform fumigation-extraction method. For each plot, half of the prepared samples were fumigated with ethanol-free chloroform in an evacuated extractor at 25 °C for 24 hours. The remaining soil samples were treated as control (CK). Briefly, ~10 g per moist soil (dry weight equivalent) of fumigated and non-fumigated samples were extracted with 50 mL (0.5 mol L^{-1}) K_2SO_4 soil:extractant = 1:4 after shaking for approximately 60 min on a reciprocal shaker. The extracts were filtered through a 7 cm diameter filter paper and stored at – 15 °C for further analysis. Total organic carbon (TOC) and total N (TN) in the extracts were determined with a TOC/TN analyzer.

Results

Since soil microbial biomass and enzymatic activity integrate the physicochemical properties of the soil, they can be considered as suitable biological forms of soil quality indicators. Naturally restored forest and mixed forest plantations will have significantly higher soil microbial activity (MBC, MBN, MBN/TN and microbial ratio (MBC/SOC) and enzymatic activity (urease, sucrose, protease and catalase), than coniferous monoculture plantations due to poorer litter quality. The soil microbial biomass fractions (MBC and MBN) in mixed forest and NBF stands were remarkably higher in the 0 – 10 cm soil layer than HMP (hazelnut monoculture plantations) stands. In the remaining two layers, they were also significantly different, but the pattern was not clear (all $p < 0.001$) (Fig. 1a-d).

As a result of the experiment, it was shown that the fabric suffered the greatest destruction in samples 2 (12 – 16 cm), 4 (6 – 12 cm), and 7 (6 – 12 cm). The high activity of cellulase here is explained by the richness of the soils of these areas in N (points 5 and 6), mobile forms of K (points 2 and 7) and P (points 3 and 6) – this was established by us in the course of previously conducted experiments. At points 2 and 4, there is a high degree of cellulolytic soil activity, which is facilitated by the influx of intravital plant root exudates into them, which stimulates the activity of cellulolytic. At these points, there is a high abundance of plant roots. The roots create a continuous frame network along the walls of the cut. The canvas in samples 3 (6 – 12 cm), 4 (12 – 16 cm), 6 (5 – 12 cm) and 6 (12 – 16 cm) had the lowest percentage of destruction, since the soils of these areas are probably characterized by a small amount microorganisms and organic matter, nitrogen deficiency, mobile forms of phosphorus and potassium, a small amount of plant root exudates. Proteases are involved in the activation of this process. The protease activity of the soil was determined using photographic paper, which was placed in a sample of soil for four days, removing it every day, washing it from the soil under a weak stream of water and drying it. The method is based on the microbiological breakdown of gelatin present in the emulsion layer of photographic paper. The result was assessed visually: the stronger the liquefaction

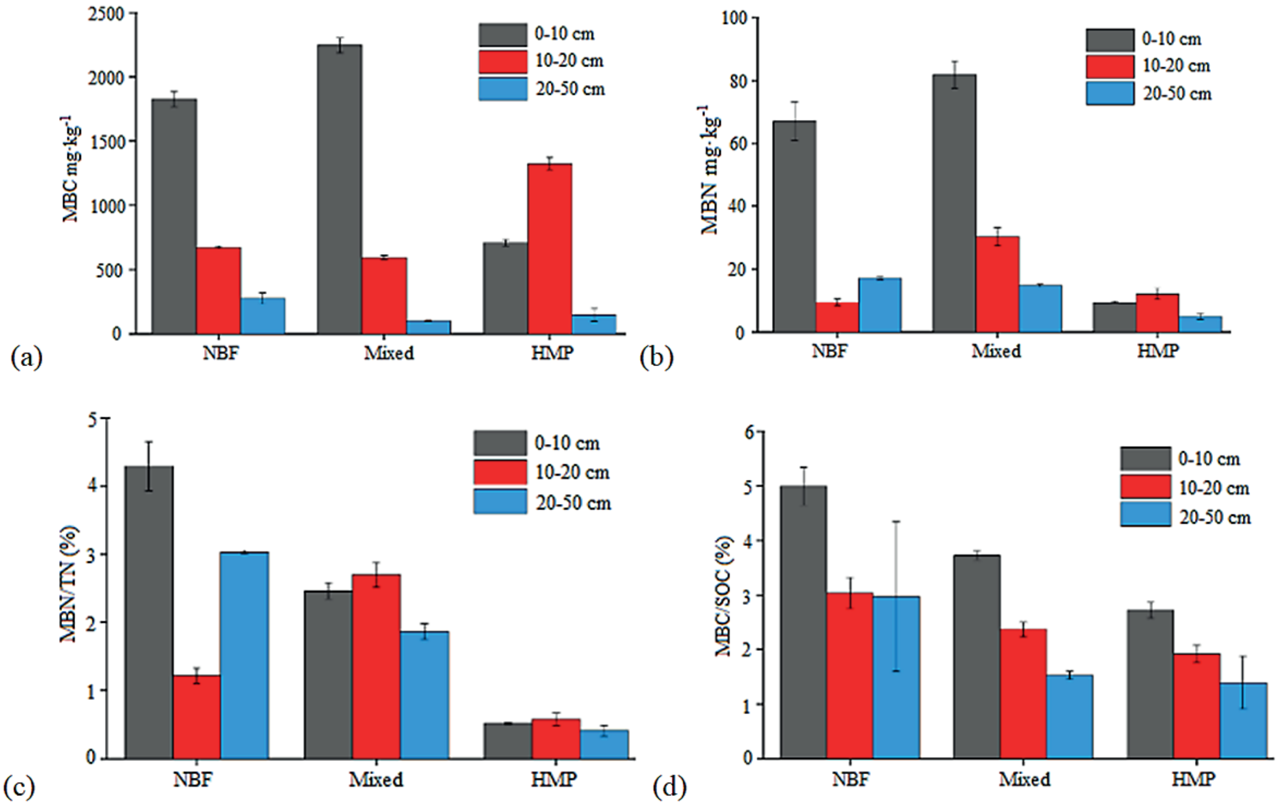

Fig. 1. (a) MBC, (b) MBN, (c) MBN/TN, and (d) MBC/SOC at three soil depths along NBF, mixed forest (Forest biotopes), and HMP stands

Source: Authors' own elaboration

of the gelatin layer, the higher the protease activity of the soil (such zones acquire a dark color). The highest protease activity was recorded in samples 2, 5, 7 and control. Low protease activity in samples 4 and 6. Urease activity can be used as an indicator of the soil's ability to natural bioremediation, self-purification, and soil resistance to inhibitory environmental factors (Sun et al., 2020; Shukurov et al., 2025). Urease activity is higher in fertile soils. It increases in all soils during periods of their greatest biological activity in July – August. The essence of the method is to change the color of the indicator located on the inner lid of the container, with the test soil sample with the addition of urea. The control was a soil sample taken in a forest outside the city, without adding urea, and a sample with urea dissolved in water, without adding soil. A strip of universal pH 0 – 12 indicator was soaked in water and placed on the inside of the container lid. Then we visually observed the change in the color of the indicator for 25 hours. To more accurately determine the result of the experiment, we used a color scale and assigned a certain score to each color (from the weakest biological activity of the soil – yellow to the strongest saturated blue). During the experiment, it was found that the most intense color (blue) was recorded at point 7, and the rate of color change of the indicator was the same at both depths, which indicates high biological activity of the soil at this point. For soil taken from this area, the ammonia release reaction proceeded much faster (2 hours) compared to other studied samples. Also, high biological activity of soils was noted at point 1, but the rate of color change of the indicator here is much lower. The high biological activity of the soils taken at points 1 and 7 can be explained by the fact that the soils of areas located within the city limits and surrounded by various potential sources of pollution, including car parking lots (which release petroleum product residues into the environment), experience greater technogenic and anthropogenic load in contrast to the soil cover of parks and forests within the city. The high biological activity of soils is explained by the fact that these points are less susceptible to anthropogenic influence, since they are removed from the roadway, and are practically not visited by people. Points 4 and 5 are located deep in the forest, and point 2 is in a swampy part of the river. Low indicators of biological activity of soils were observed at point 6. The color of the indicator is yellow, pale green. The reaction proceeded much slower than in other samples. It took much longer for the first signs to appear, for example at point 6 – 18 hours. The low biological activity of soils in this area can be explained by the high degree of anthropogenic load: point 6 is located next to a busy highway. In the control sample, the color of the indicator remained unchanged, and no ammonia was released. At the next stage, the biological activity of oil-contaminated soil was assessed by determining the urease activity of the soil. Maintaining or increasing the biological activity of contaminated soil compared to soils, where there is no pollution, this may indicate their high biological activity and significant bioremediation potential, the ability to self-heal, which indirectly indicates the stability of the ecosystem in the study area. To conduct the study, we took a 120 g sample of soil, moistened it to 60% of its full moisture capacity with tap water, and added oil at a concentration of 3%. Forest soil with oil (3%), sand with the addition of oil (3%), oil without the addition of urea were used as controls and soil (3 ml) and urea without soil and oil (1 g/100 g soil). Then 1 g (per 100 g of soil) of urea dissolved in water was added to the containers. A strip of universal pH 0 – 12 indicator was soaked in water and placed on the inside of the container lid. Observed the change in the color of the indicator. The observation was carried out for three days at a temperature of 27 °C. A color scale was also used to determine the intensity of the indicator color change. As a result of the experiment, it was found that the greatest biological activity under conditions of oil pollution soil samples numbered 1, 2 and 7 are present. In these samples, a deep blue color of the indicator was observed. The lowest indicators of biological activity of soils were noted at point 3. After 24 hours of cultivation, low biological activity of soils was noted at point 6. In general, the most biologically active soils are located at points 2 (river bank) and 7 (residential area) – these samples were identified in all experiments. On the river bank, the high biological activity of soils can be explained by the low degree of anthropogenic load and, accordingly, practically unchanged by economic activities microbiocenosis. At point 7, located in a residential area in close proximity to the roadway, activity bacteria is stimulated by the introduction of small doses of petroleum products. Under anthropogenic influence, the soils of various territories develop adaptive mechanisms, the action of which is aimed at increasing resistance to the adverse effects of humans on the environment.

The population mainly lives in Katekh, Garakli, Gullar and Tulu villages. Among the ecological tourist centers of the region, the most popular are the forest areas around the Alazan river and Balaken Central Park. Based on a combined dataset for three forest types and three soil depths, strong positive linear relationships were observed for SOC and soil TN with MBC and MBN. The association values were: between SOC and MBC ($R = 0.73$, $p = 0.01$), and MBN ($R = 0.46$, $p = 0.02$), (Figure 2a,b), and soil TN and MBC ($R = 0.65$, $p = 0.01$), and MBN ($R = 0.38$, $p = 0.02$), (Figure 3a,b).

Late summer and early autumn peaks of cellulolytic activity of soils are probably associated with the massive influx of organic matter into it in the form of leaf litter, above-

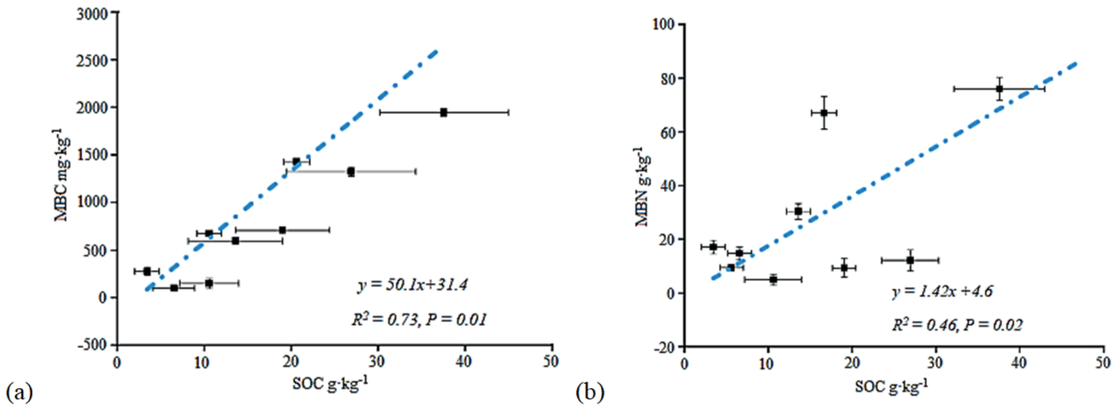

Fig. 2. Linear relationship between soil parameters: SOC and (a) MBC and (b) MBN across soil depth and mixed forest types in northwest Azerbaijan Republic (Balaken region)

Source: Authors' own elaboration

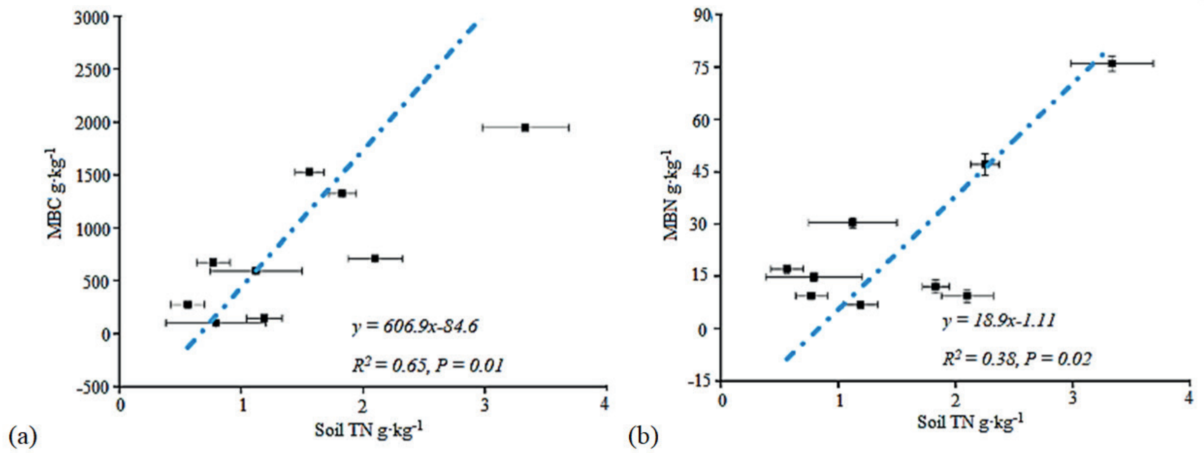

Fig. 3. A linear relationship between soil parameters: TN and (a) MBC, and (b) soil MBN across the soil depths and mixed forest types in Azerbaijan Republic (Balaken region)
Source: Authors' own elaboration

ground parts of herbaceous plants, which died at the end of the growing season, as well as root systems of annual plants. At the end of July, when the temperature of the air and soil surface rises to 26 – 28 °C, microscopic fungi are activated, positively influencing the course of cellulolytic processes. Their role in soil-forming activity increases with depth along with anaerobic bacteria, facultative anaerobes that can exist without access to O_2. The suppression of the vital activity of soil biota in the above-mentioned areas of the city is associated with significant anthropogenic load. Microbiological analyzes revealed that the quantitative indicators of microorganisms change significantly in individual layers of irrigated alluvial meadow and mountain forest brown soils. It was found that in the studied dark, ordinary and light subtypes of these soils, their abundance decreases sharply, especially from the upper layers (0 – 10 cm) with an amount of $5.0 \cdot 10^6$/g of soil, to the lower layers (30 – 40 cm; 40 – 50 cm; 50 – 60 cm). At a depth of 70 – 80 – 100 cm in light and ordinary subtypes of gray-brown soils, the number of microorganisms decreases to $2.0 – 1.0 – 0.8 \cdot 10^6$/g soil. In the cultivated variants of these soils under grain crops, a similar trend was observed, a decrease in the total number of microorganisms from $4.8 – 4.0 \cdot 10^6$/g of soil in the upper horizons to $2.2 – 1.5 \cdot 10^6$/g of soil in the lower horizons. In the group composition of microorganisms in the soil of natural and irrigated soils, non-spore-forming bacteria predominated, 75.9% and 76.3%, actinomycetes 24.9% and 24.6%, in the number of small-sized fungi and spore-forming bacteria, there was a slight difference of 0.5% and 0.3% and 18.8 – 25.1%. (Fig. 4a and b). In the irrigated variants of these soils, the humus content decreased quite moderately, from 2.09% in the upper (0 – 10 cm) layer to 1.35% in the lower (50 – 70 cm) layers. The average number of micro biota in the 0 – 100 cm layer of the studied soils varies between $5.2 – 3.9 – 1.9 – 4.6 \cdot 10^6$/g. soil. Ca has an advantage over absorbed cations, when taking into account the analytical indicators in both sections. Thus, the share of Ca in the total amount of absorbed bases in the forest meadow soils is 57.9 – 65.3%. In the subalpine belt, according to the same indicators, the calcium cation is still in first place. Ca makes up 42.9 – 81.5% of the total amount of absorbed cations. The amount of Mg varies from 12.4 to 28.1%, H from 0.4 to 6.3%. According to the results of mechanical analysis in grassy mountain meadow soils of the alpine zone, particles smaller than 0.01 mm make up from 49.0 to 66.3% of the profile. This indicator on the profile from the upper horizon decreases downwards. In soils of the same subtype of the subalpine belt, this indicator changes insignificantly and gradually increases along the profile. In both sections, fractions of 0.05 – 0.01 mm in size predominate. The increase in particles consisting of less fractions, in the direction of depth, is associated with the intensity of the physical weathering process occurring in the area. Along the profile, its quantity decreases sharply from the first layer to the second, and then gradually decreases with depth. These soils were washed away by carbonation. The hygroscopic moisture profile at the top from layer to layer it varies from 5.62 to 2.75. These indicators show that in other studies the hygroscopic moisture profile changes insignificantly and is also high. The amount of humus varies sharply along the profile and is relatively low.

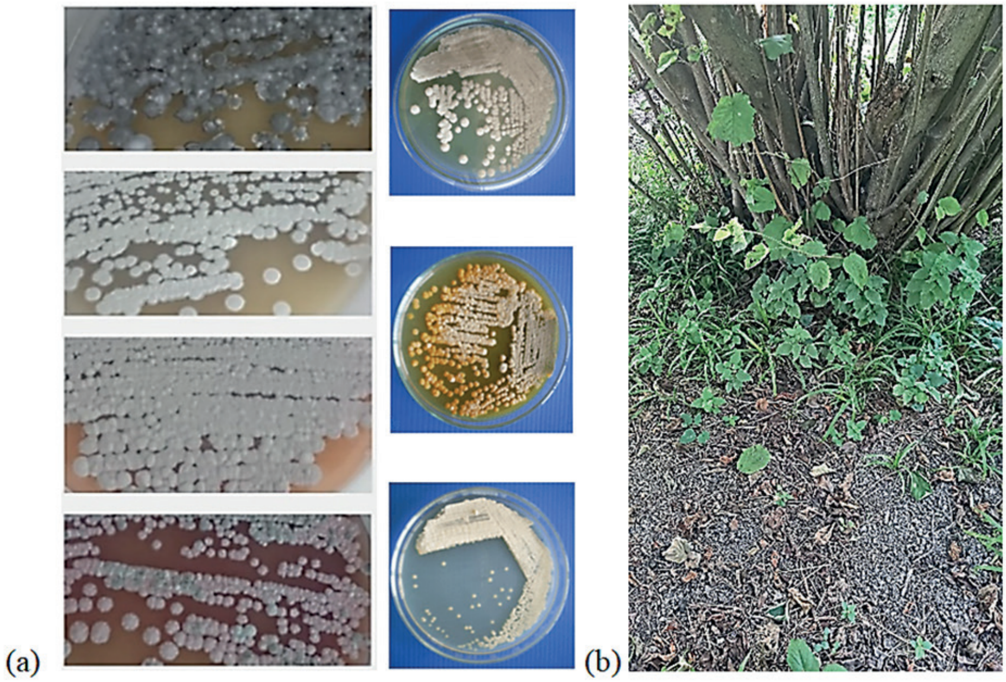

Fig. 4. Actinomycetes activity (a) actinomycetes colones; (b) mountain forest brown soils under hazelnut (41°38′40″E; 46°32′32″N)
Source: Authors' own elaboration

Conclusion

Cellulolytic activity is an important indicator of the intensity of destruction processes in the soil. The intensity of cellulose decomposition in the soil is determined by the combined action of several factors: weather conditions, the nature of the vegetation cover, the volume of organic matter entering the soil, the type of soil, its physical properties, and chemical composition. Cellulolytic processes are inhibited by low air temperatures ($r = -0.9$) and heavy precipitation ($r = -0.8$). The results of the study showed that changes in the micro biota in individual horizons were associated with the humus state of the studied soils. At a depth of 70 – 80 – 100 cm in light and ordinary subtypes of gray-brown soils, the number of microorganisms decreases to 2.0 – 1.0 – $0.8 \cdot 10^6$/g soil. In the cultivated variants of these soils under grain crops, a similar trend was observed, a decrease in the total number of microorganisms from 4.8 – $4.0 \cdot 10^6$/g of soil in the upper horizons to 2.2 – $1.5 \cdot 10^6$/g of soil in the lower horizons. In the group composition of microorganisms in the soil of virgin and cultivated cenoses, non-spore-forming bacteria predominated, 74.9% and 75.3%, actinomycetes 24.8% and 24.3%, in the number of small-sized fungi and spore-forming bacteria, there was a slight difference of 0.5% and 0.3% and 18.8-25.1%. In the irrigated variants of these soils, the humus content decreased quite moderately, from 2.09% in the upper (0 – 10 cm) layer to 1.35% in the lower (50 – 70 cm) layers. The total number of microorganisms remained at a more stable level compared to virgin variants. The average number of micro biota in the 0 – 100 cm layer of the studied gray-brown soils varies between 4.1 – 3.7 – 1.8 – $3.5 \cdot 10^6$/g soil.

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Received: **September, 20, 2024**; *Approved*: **October, 24, 2024**; *Published*: **April, 2026**