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SOIL BIOACTIVITY STUDY THROUGH INNOVATIVE APPROACHES IN LANKARAN - ASTARA REGION, AZERBAIJAN

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SUMMARY

This paper presents a study aimed at determining the soil's biological activity using its enzymatic activity and the ability of urban soils to self-heal in various territories of the Lankaran Region, Azerbaijan. Results revealed microorganisms decreased to 2.0-1.0-0.8×10^6/g soil at the depth of 70-80-100 cm in light and ordinary subtypes of gray-brown soils. In cultivated variants of these soils under grain crops, a similar trend was dominant, with a decrease recorded in the total number of microorganisms from 4.8-4.0×10^6/g soil in upper horizons to 2.2-1.5×10^6/g soil in lower horizons. In the composition of microorganisms in the soil of virgin origin and cultivated cenoses, non-spore-forming bacteria (74.9% and 75.3%) and actinomycetes (24.8% and 24.3%) predominated. However, in small-sized fungi and spore-forming bacteria, there existed a slight difference of 0.5% and 0.3% and 18.8%–25.1%, respectively. In irrigated variants of these soils, the humus content decreased quite moderately, from 2.09% in the upper layers (0–10 cm) to 1.35% in the lower layers (50–70 cm). The average number of microbiota in the 0–100 cm layer of the studied gray-brown soils varies around 4.1-3.7-1.8-3.5×10^6/g soil.

Keywords: Anthropogenic factors, soil erosion, enzyme activity, cellulolytic activity, actinomycetes, small-sized fungi, spore-forming bacteria

Key findings: Cellulolytic activity is an important indicator of the intensity of destruction processes in the soil. Results showed that in individual horizons, the microbiota variations proved associated with the humus state of the studied soils.

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INTRODUCTION

Under increased anthropogenic load conditions on the planet's biosphere, the soil, being an element of the natural urban system and in dynamic balance with all other components, suffers degradation processes (AzStat, 2024). Erodibility of soil resources by area (ha/%) and total non-eroded areas are available in Figure 1. The flow of substances entering the soil because of human activities in natural cycles disrupts the soil biota's normal functioning. Among various biological criteria for assessing the anthropogenic impact on soils in the urban environment, the most promising biochemical indicators providing information about the critical enzymatic processes in the soil are the synthesis and decomposition of organic matter and nitrification (Aliyeva, 2023).

The soil biological activity and the qualitative and quantitative composition of soil microbiota immediately respond to any anthropogenic effect. Soils cover indicators reflecting the state of the environment (Mammadzade, 2024). The fiber decomposition process in the soil occurs under the aerobic and anaerobic conditions with the participation of special bacteria and fungi. Numerous studies revealed a close relationship between the intensity of biological processes, the microorganisms' composition and number, and the organic matter and biogenic elements in the soil**.** This phenomenon is currently the subject of contradictory opinions; however, in the global carbon cycle and balance, the crucial role of forests is undeniable.

Soil microorganisms have an enzymatic function, which performs various purposes in biogenic elements, as well as participates in soil formation and maintaining soil fertility (Bakshaliyeva *et al.*, 2020; Aliyeva and Mammadova*,* 2023). The detection of the soil microflora's biological activity can come from the decomposition degree of flax fabrics placed in a 5–25 cm soil layer. The soil's biological activity in mixed forests bore considerable effects from tree species, soil properties, and existing environmental conditions. Past research reported mixed forests generally exhibited the enhanced microbial activity and enzyme functions compared with the mono-species forest. The global warming is largely due to increasing concentrations of carbon dioxide and other greenhouse gases in the atmosphere. The study of microorganisms in different types of soils is vital in determining the influence of ecological and geographical factors on the distribution of soil microbes (Hasanova, 2015; Hasanova *et al.,* 2021).

In Southeast Azerbaijan, the economic region's location includes the shores of the Caspian Sea, the Talysh Mountains, and the Lankaran Plain. The territory begins from the border of the Shirvan-Salyan economic region in the north and extends to the Astara River in the southern economic region, which covers the administrative regions of Astara, Lankaran, Lerik, Jalilabad, Masalli, and Yardimli. The economic region's 8.7% territory and 4.1% of its population are within the mid-mountain belt at an altitude of 1001–1500 meters above sea level. The 6.9% territory of the Lankaran-Astara economic region, 1.6% of the population, and 6.4% of settlements comprised locations above 1501 meters.

Soils of various territories undergo continuous enhanced anthropogenic variations, attracting the researcher's attention beyond the capacity of the environment within vulnerable areas, leading to non-reinstatement of the ecosystem to its original state (Morgan, 2005; Mammadova *et al.,* 2017). Soil has a pertinent role in human life because of it being a special food source, providing 95%–97% of food resources to the world's population. The soil's role in preserving biological diversity is also at large (Mammadova and Mammadova*,* 2019*)*. The richness of the Lankaran Lowland flora, its physical and geographical conditions, the history of soil development and vegetation, and species composition also depended upon the anthropogenic and natural factors.

The Lankaran Region mountainous part has a rich flora and differed from other geographical regions of Azerbaijan in vegetation diversity, which is also quite the composition of the biocenoses in the ecosystem of the region. The study of subalpine-meadow, forest, undergrowth-meadow, mountain-xerophytic, mountain-

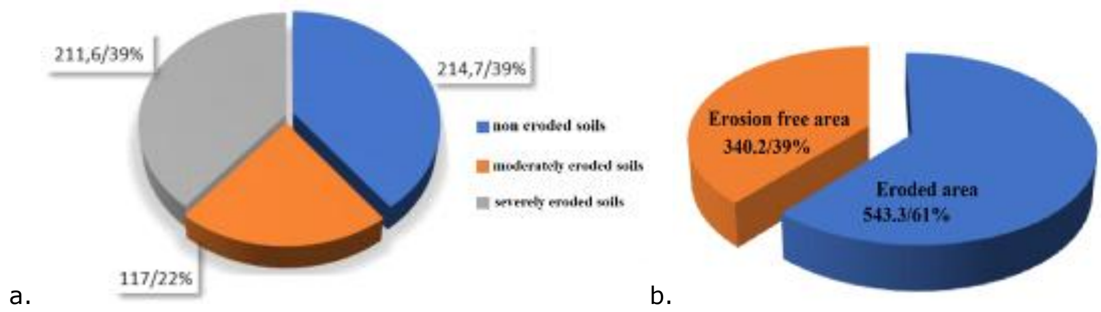


Figure 1. a) Erodibility of soil resources by area (ha/%), and b) total non-eroded area.

steppe, petrophytic, and marsh phytocenotypes transpired in the biodiversity of the Yardimli, Lerik, and Astara regions. In the mountain ecosystem, natural phytocenoses, especially relict forests, did not experience the glaciation of the third and fourth periods. The widespread species composition includes *Ruscus hyrcana Worono, Ilex hyrcana Pojark, Buxus hyrcana Pojark., Alcea lencoranica Iljin., Quercus macranthera* Fisch*., Celtis caucasica* Willd, *Quercus castaneifolia* C.A. Mey*, Fagus lucida, Astragalus leptophysus., Carex lasiocarpa, Vicia sativa* L*., Hieracium lachenalii, Trifolium pratense* L*., Potentilla alba, Euphorbia lactea, Scrophularia nodosa* L*., Orobanche lutea, Aster* L*., Verbascum sinuatum* L*., Geranium sanguineum, Geranium bohemicum, Geranium sylvaticum, Echinácea purpúrea, Veronica filiformis, Veronica persica,* and *Veronica longifolia*. The observed endemic species are *Gagea alexlencoana* Miscz*., G. caroli-kochii* Grossh*., Allium transcaucasicum A*. Grossh*., A. leucanthum* C. Koch., *Dianthus talyschensis* Boiss*., Grataegus caucasica* C. Koch*.,* and *Astragalus doktschaicus* Grossh*.*

In Azerbaijan, comprehensive soil studies based on climatic indicators, biological activity, erosion rate, population density, and enzyme activity took place for the first time in the selected territory. The comparative analysis revealed the enhancement in anthropogenic load has led to a decline in the biogenic substances. Within one belt, based on geographical factors, the soil parameters vary sharply. In this area, the drying process' justification may be due to the increase in anthropogenic impact. Overall, the total number of microorganisms remained at a more stable level than the natural variants. The decomposition process of organic matter is an essential integral link in the global biogeochemical cycle of elements and largely determines the soil fertility (Hasanov, 2017).

Cellulose decomposition affects the organic matter breakdown in general. Likewise, this indicator can become a quantitative measure of soil fertility, with pure cellulose as a model substrate for decomposition against the background to determine the effect of environmental factors and soil properties (Mammadova *et al.,* 2021; Mammadzade, 2022). The study of erosion processes also plays a vital role in the development of agriculture and tourism. By using agricultural land and increased settlement over the years, the erosion process has continually intensified (Bunyatova *et al.,* 2025). In the studied soils, the identification of uneroded, weak, and heavily eroded areas was successful (Figure 1).

MATERIALS AND METHODS

Subtropical podzolic yellow soil developed on gneiss eluvium on a ridge 1800 m high near the mountain Talysh. For the presented study, collected soil samples came from seven different locations in the Lankaran Region, Azerbaijan. For determining the enzymatic activities in soils, the indicators of cellulolytic, protease, and urease activity underwent analysis (Mammadova *et al.,* 2024). In soils, cellulolytic activity determination is used as the application method, while the protease activity is assessed by the microbiological breakdown of gelatin found in the emulsion layer photo paper. In identifying the urease activity in the soil, the study employed the express method of Aristovskaya T.V. and Chugunova M.V. The soil's biological activity assessment was through indicators of cellulolytic, protease, and urease activity. By considering these aspects of soil biological activity, it is possible to form a comprehensive picture of the soil microorganisms' activity in the study area.

Soil cellulolytic activity enables one to determine the fertility level and biogenicity. Determining the soil cellulolytic activity engaged the patch method, wherein placing a thin linen cloth measuring 5 cm × 5 cm occurred in a 100 g sample of soil. The soil taken from a forest outside the city served as the control sample. Based on the loss in tissue weight after 30 days of cultivation, studying the intensity of the fiber destruction succeeded by assessing the soil biological activity based on the intensity of fiber destruction, using the Zvyagintsev D.G. scale. The scale for fiber destruction intensity has the following levels: weakest (less than 10%), weak (10%–30%), medium (30%–50%), strong (50%–80%), and strongest (more than 80%) (Morgan, 2005). In maintaining ecological balance and preserving the potential for soils' self-purification and self-healing in urban areas under anthropogenic factors' influence causing degradation processes in the soil cover, it is necessary to conduct a constant monitoring of soil conditions (Rowell, 1999).

The data obtained makes it possible to assess the soil cover in urban areas and the soil biological activity assessment, which can help determine the intensity of soil processes and the nature of variations in urbanized areas. Soil microorganisms, in combination with other representatives of soil pedobionts, form complex biocenoses. Based on their symbiotic relationships, the transformation of organic residues and energy contained in them due to biological cycles progressed (Mammadova and Mammadova*,* 2020; Nasirova *et al.,* 2022). Calculating the intensity of photo paper decomposition in the studied soil samples relied on studying the protease activity of soils (Table 1).

RESULTS AND DISCUSSION

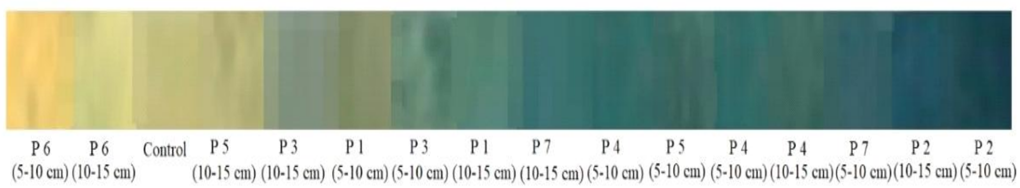
The results enunciated the fabric suffered the greatest destruction in samples 2 (10–15 cm), 4 (5–10 cm), and 7 (5–10 cm) (Macnunlu *et al.,* 2025). The intensity of the indicator color varied by determining the urease activity of anthropogenically polluted soils (indicator color determining soil urease activity (P – Test sample: Point) (Table 2). The highest activity of cellulase could be due to the richness of soils with nitrogen in these areas (points 4 and 7), mobile forms of potassium (points 2 and 7), and phosphorus (points 2 and 7), with the same as also confirmed in previous studies (Nasirova, 2024). At points 2 and 4, the highest degree of soil cellulolytic activity surfaced, as facilitated by the influx of intravital plant root exudates. However, at these points, the ultimate abundance of plant roots existed that developed a continuous network along the walls of the cut. The canvas in samples 3 (5–10 cm), 4 (10–15 cm), 6 (5–10 cm), and 6 (10–15 cm) had the lowest percentage of destruction. The soils in these areas were probably containing a small amount of microorganisms and organic matter, nitrogen deficiency, mobile forms of phosphorus and potassium, and a small amount of plant root exudates. Biological activity sustainability of contaminated soils is high compared with unpolluted soils, which indicates their considerable bioremediation potential and self-healing ability, revealing the ecosystem's stability (Nazim and Oqtay*,* 2024). The soils with the lowest biological activity can refer to the highest degree of anthropogenic factors (Rowell, 1999; Rzayeva, 2022).

Air temperature over the day and seasonal distribution of average relative humidity (%) appear in Figure 2. Proteases are mostly contributors in the activation of these processes. The determination of the protease activity of the soil used a photographic paper placed in a soil sample for four days, removing it daily, washing it from the soil under a weak stream of water, and drying it. The said method was relying on the microbiological breakdown of gelatin found in the emulsion layer of photographic paper. The results visually revealed the stronger the liquefaction

Table 1. Intensity of photo paper decomposition in the studied soil samples (Calculated based on studying the protease activity of soils).

Test sample	Sample coloring
Point 1 (5–10 cm)	Dark green color with white and yellow splashes
Point 1 (10–15 cm)	Dark green color with white and yellow splashes
Point 2 (5–10 cm)	Pale yellow color with pale green and white flecks
Point 2 (10–15 cm)	Dark green color with pale yellow splashes
Point 3 (5–10 cm)	Pale green with brown stripes
Point 3 (10–15 cm)	Pale yellow color
Point 4 (5–10 cm)	White color with pale yellow spots
Point 4 (10–15 cm)	Pale yellow color
Point 5 (5–10 cm)	Pale yellow with green spots
Point 5 (10–15 cm)	Gray color with isolated pale green areas
Point 6 (5–10 cm)	White color with blurry yellow spots
Point 6 (10–15 cm)	White color with blurry yellow spots
Point 7 (5–10 cm)	Dark orange color with white flecks
Point 7 (10–15 cm)	White color with dark orange splashes
Control	Pale orange with dark green and white spots

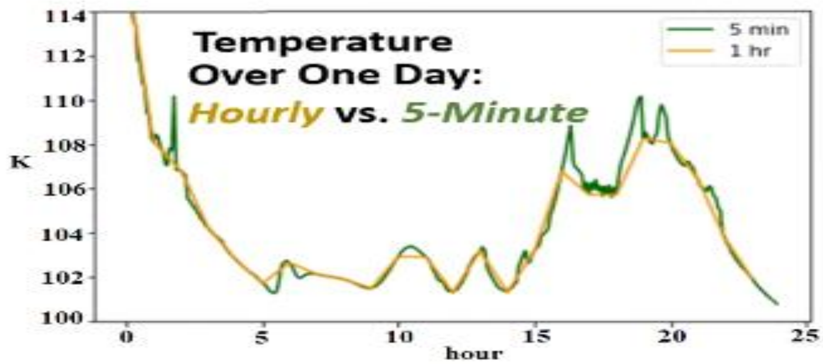
Table 2. The intensity of the indicator color variation by determining urease activity of anthropogenically polluted soils (Indicator color determining soil urease activity [P – Test sample: Point]).



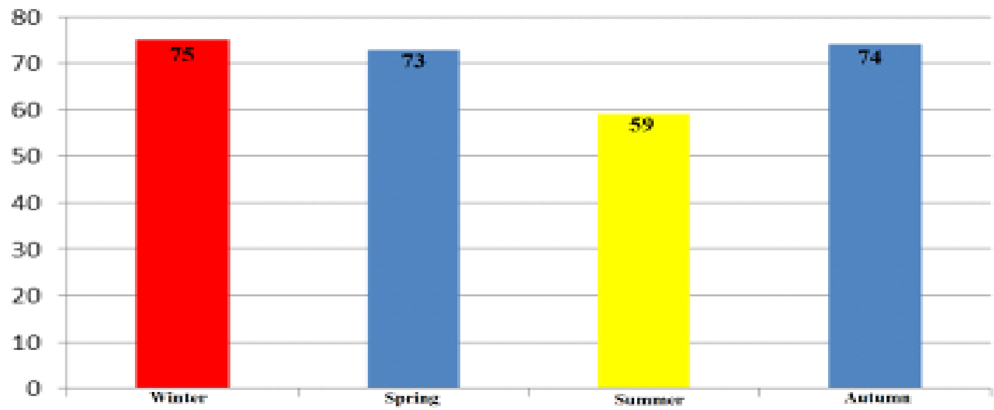
Test sample	Color after 24 hours	Color after three days
Point 1	Rich blue	Rich blue
Point 2	Dark blue	Rich blue
Point 3	Pale blue	Pale blue
Point 4	Light green	Blue
Point 5	Dark green	Dark green
Point 6	Light green	Partly yellow, blue
Point 7	Blue	Rich blue
Control (forest soil)	Dark green	Blue
Control (sand with wastewaters)	Pale green	Pale green
Control (with wastewaters)	Pale green	Pale green
Control (carbamide)	Pale green	Pale green

of the gelatin layer, the higher the protease activity of the soil, and such zones acquire a dark color. The highest protease activity was notable in samples 2, 5, 7, and the control, while the lowest was evident in samples 4 and 6. The urease activity can serve as an indicator of the soil's ability to natural bioremediation, self-purification, and soil resistance to inhibitory environmental factors. Urease activity emerged higher in fertile soils, and it enhanced in soils during periods of greatest biological activity during June to August. In soils, the protease enzymes determine the dynamics of nitrogen released in an accessible form with successive breakdown of proteins (Rzayeva, 2023).

The essence of the methodology was to change the color of the indicator located on the inner lid of the container with the test soil samples by adding urea. Plots in eroded,



a.



b.

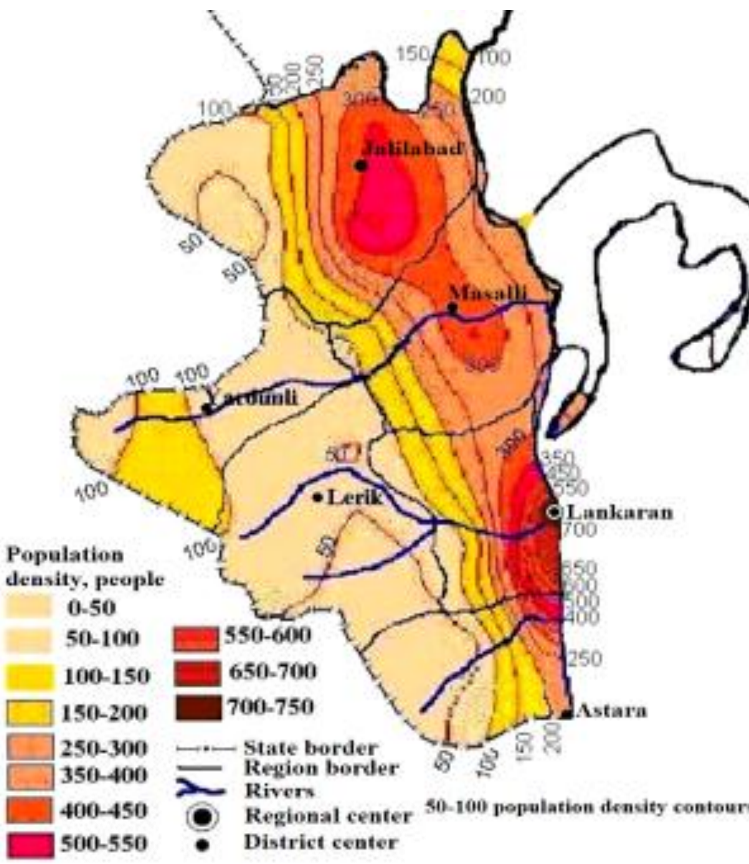
Figure 2. a) Air temperature over the day, and b) Seasonal distribution of average relative humidity (%).

moderately eroded, and non-eroded soils under agrocenoses reservoirs, the flat areas near Lankaranchay, and population density in the Lankaran-Astara economic region are visible in Figure 3. The control was the soil sample collected in a forest outside the city without adding urea, and a sample with urea dissolved in water without adding soil. The process comprised a strip of universal pH (0–12) indicator soaked in water and placed on the inside of the container lid. Then, observing the variations in color visually continued for 25 hours. For a more accurate assessment of these results, the study used the color scale, assigning a certain score to each color (from the weakest biological activity of the soil—yellow—to the strongest saturated blue). During the studies, it was also noteworthy that the most intense color (blue) prevailed at point 7. The color variation in the indicator was the same at both depths, indicating the highest soil biological activity at this point: a) air temperature over the day and b) seasonal distribution of average relative humidity (%). In the said soil sample, the ammonia release reaction proceeded much faster (two hours) than other studied soil samples (Nasirova, 2024).

Although the highest biological activity revealed by the soil sample was at point 1, the rate of color variation in the indicator was much lower (Bunyatova *et al.,* 2025). The highest biological activity of the soil samples at points 1 and 7 can be because of the soils of these areas connect within the city limits. Likewise, their surroundings comprised various potential sources of pollution, including car parking lots (releasing petroleum product residues into the environment), and experienced greater technogenic and anthropogenic load in contrast to the soil cover of parks and forests within the city. The soil pollution with small amounts of

a.

1,3,4 - non eroded soil on the plains (agrocenoces); 2,20,46 - moderately eroded soils with foothill relief; 5,16 - eroded soil under vegetables; 6,7,8,10,12,14,18,19,44 - moderately eroded soils under tea; 9,13,15,17,43,45 - non eroded soils under tea; 11 - undulating plain, not eroded.



b.

Figure 3. a) Plots in eroded, moderately eroded, non-eroded soils under agrocenoces reservoir, and the flat areas near Lankaranchay, and b) Population density in Lankaran-Astara economic region.

petroleum products coming from cars also stimulates the urease activity of soil microbiota. The reason for the soil with enhanced urease activity can be due to the activity of hydrocarbon-oxidizing microorganisms, as also confirmed by past studies. Coastal ecosystems of the Caspian Sea are subject to constant and intense anthropogenic impact (Nasirova, 2024). The soil's highest biological activity could be attributable to the fact that these points were less susceptible to anthropogenic influence, removed from the roadway, and practically not visited by people; points 4 and 5 were evidently deep in the forests, and point 2 was in a swampy part of the river. The lowest indicator of soil biological activity was apparently in the sample at point 6, and the indicator color was yellow and pale green.

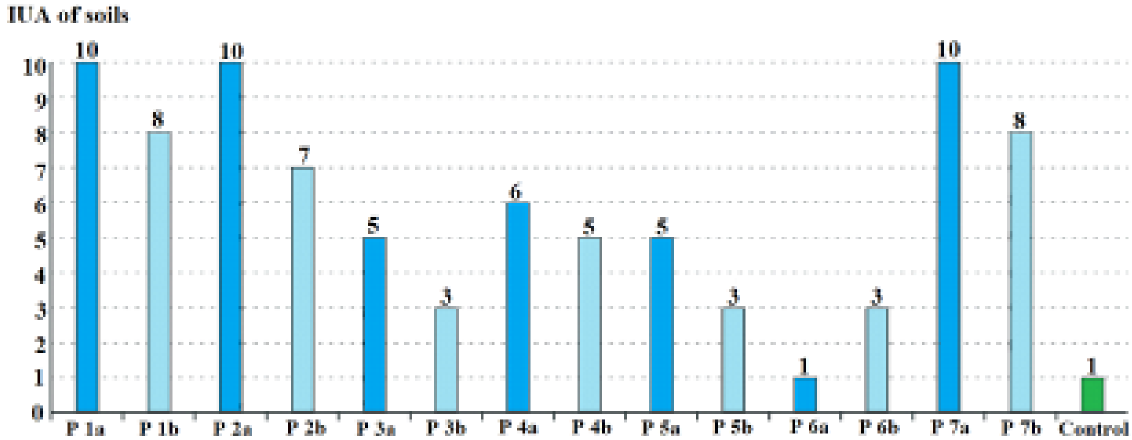
However, the reaction proceeded much slower than other soil samples. It took a longer time for the first signs to appear, and the soil sample at point 6 took 18 hours. In the studied area, the soil's lowest biological activity can be because of the highest degree of anthropogenic load, i.e., as the location of point 6 was next to a busy highway.

In the control sample, the color of the indicator remained unchanged, with no released ammonia. However, at the next stage, the biological activity of oil-contaminated soil incurred evaluation by determining the urease activity of the soil.

The study conducted the method of moistening the 100 g soil sample to 60% of its full moisture capacity with tap water and then adding oil at the concentration of 3%. Forest soil with oil (3%), sand with the addition of oil (3%), oil without the addition of urea became as controls, as well as soil (3 ml) and urea without soil and oil (1 g/100 g soil). Then, dissolving 1 g (per 100 g of soil) of urea in water, the study proceeded to add these into containers. A strip of universal pH (0–12) indicator, soaked in water and placed on the inside of the container lid, received observations for variations in its color. Observations went on for three days at a temperature of 26 °C, using a color scale to determine the intensity of the color variation. The results further revealed the greatest biological activity with oil pollution appeared in the soil samples numbered 1, 2, and 7, with a deep blue color observed in these samples. The lowest biological activity was distinct at point 3. After 24 hours of cultivation, the lowest soil biological activity also occurred at point 6 (Hasanova *et al.,* 2022).

In general, the most biologically active soils were prevalent at points 2 (riverbank) and 7 (residential area). On the riverbank, the soil's highest biological activity can refer to the lowest degree of anthropogenic load and, accordingly, not be influenced by the economic activities of microbiocenoses. At point 7, located in a residential area and close to the roadway, the bacterial activities acquire stimulation by the small doses of petroleum products. Under anthropogenic influence, the soils in various territories develop adaptive mechanisms, with increasing resistance to the adverse effects of human activities on the environment (Figures 4a and b). Among the ecological tourist centers of the region, the most popular are the forest areas around the Khanbulanchay, Azerbaijan (Hasanov, 2017).

Late summer and early autumn peaks of soil cellulolytic activity probably have associations with the massive influx of organic matter from leaf litter and aboveground herbaceous plants' residues, as well as the root systems of annual plants (The State of the World's Forests, 2020). At the end of June, when the temperature of the air and soil surface rises to 26 °C–28 °C, activating microscopic fungi transpires, positively influencing the course of cellulolytic processes. Their role in soil-forming activity increases with depth along with anaerobic bacteria—facultative anaerobes that exist without access to oxygen. A negative correlation was noticeable between the soil saprophytes' activity and the atmospheric precipitation (Figures 5a and b). The suppression of the soil biota's vital activity in the above-mentioned areas of the city has a connection with the significant anthropogenic load (Talibi and Hasanova, 2022; Sadigov *et al.*, 2024).



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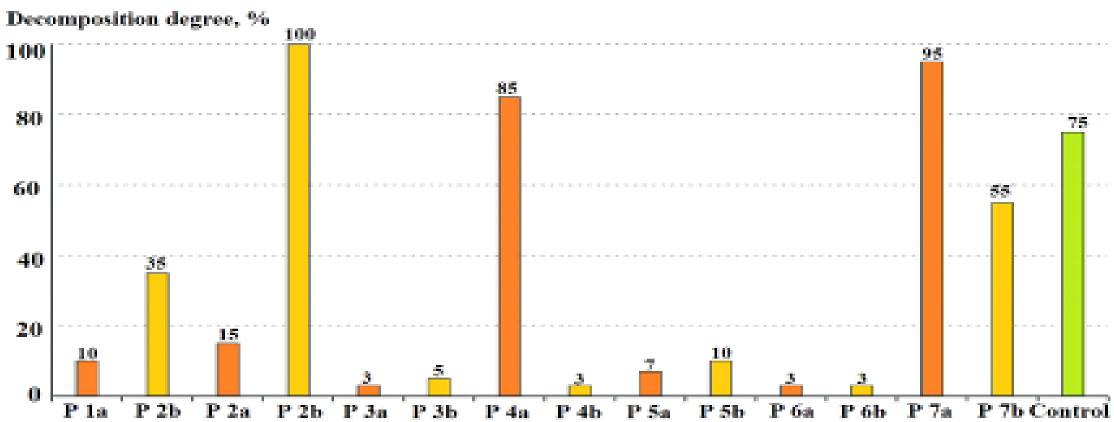
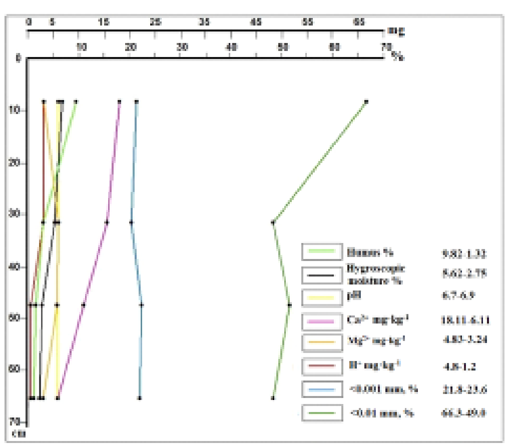
Note: IUA - Intensity of urease activity soils, points. P - Point; a - (5-10 cm), b - (10-15 cm).



b.
Note: P - Point; a - (5-10 cm), b - (10-15 cm).

Figure 4. a) Urease activity of the studied soil samples, and b) Cellulolytic activity of the studied soil samples.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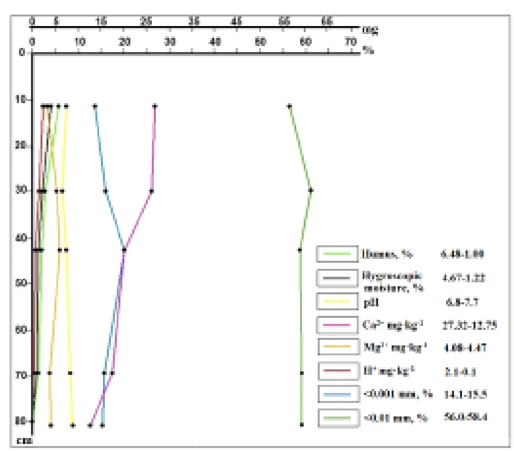
b.

Figure 5. a) Some parameters of soils of selected agrocenoses, and b) natural biotopes.

In studying the dark, ordinary, and light subtypes of these soils, the abundance decreases sharply, especially from the upper layers (0–10 cm) with an amount of 5.0×10^6/g of soil, to the lower layers (30–40 cm, 40–50 cm, and 50–60 cm) (Hasanova and Abasova, 2024). At a depth of 70-80-100 cm in light and ordinary subtypes of gray-brown soils, microorganisms decrease to $2.0\text{-}1.0\text{-}0.8\times10^6$/g soil. In soil cultivated variants under grain crops, a decrease resulted in the total number of microorganisms from $4.8–4.0\times10^6$/g of the soil (upper horizons) to $2.2–1.5\times10^6$/g of the soil (lower horizons). In microorganisms' composition of the natural cenoses soil and cultivated cenoses, non-spore-forming bacteria predominated (74.9% and 75.3%), and actinomycetes (24.8% and 24.3%). However, in small-sized fungi and spore-forming bacteria, a slight difference of 0.5% and 0.3% and 18.8-25.1% existed. In the soil irrigated variants, the humus content decreased quite moderately, from 2.09% in the upper layers (0–10 cm) to 1.35% in lower layers (50–70 cm), and the microorganisms remained at a more stable level than natural variants. In the studied gray-brown soils (0–100 cm), the average number of microbiota varies between $4.1–3.7–1.8–3.5\times10^6$/g soil. Microbiological analysis revealed the quantitative indicators of microorganisms significantly varied in individual layers of gray-brown soils (Mammadzade, 2024).

A comparative study of the physicochemical properties of natural cenoses and agrocenoses is vital for the involvement of wet soils in agriculture (Figure 8). Calcium has an advantage over absorbed cations by considering the analytical indicators in both sections. Thus, the calcium share in the total amount of absorbed bases in the alpine-meadow belt was 57.9–65.3%, followed by magnesium and hydrogen. In the subalpine belt, the calcium cation was still in first position. In the total absorbed cations, calcium makes up to 42.9% to 81.5%, magnesium varies from 12.2% to 27.1%, and hydrogen from 0.4% to 6.3% (The State, 2020; Sadigov *et al.,* 2024).

Based on the mechanical analysis in grassy mountain meadow soils of the alpine zone, particles smaller than 0.01 mm comprise the 49.0% to 66.3% of the profile. However, in the profile, the said indicator in the upper horizon decreases gradually downwards. In soils with the same subtype of the subalpine belt, the said indicator varies insignificantly and gradually enhances along with the profile. In both sections, the fractions with 0.05–0.01 mm in size predominate. The increase in particles with fewer fractions, in the direction of depth, showed an association with the intensity of the physical weathering, which occurred in the studied area. Along the profile, its quantity decreases sharply from the first layer to the second and then gradually decreases with depth (Hasanov*,* 2017).

Carbonation washed away these soils. The hygroscopic moisture profile at the top in different layers varies from 5.62 to 2.75. In other studies, these indicators showed that the hygroscopic moisture profile varies insignificantly and were also high (Talibi and Hasanova, 2022). The humus amount differs sharply along the profile and was relatively low. Soil cellulolytic activity was essentially an important indicator of the intensity of destruction processes (Kadhim and Hamza*,* 2021). The results showed that in individual horizons, microbiota variations emerged having linkages with the humus state of the studied soils.

CONCLUSIONS

In soils, determining the intensity of cellulose decomposition succeeded from the combined action of several factors: weather conditions, the nature of the vegetation cover, the volume of organic matter entering the soil, the soil type and its physical properties, and chemical composition. Low air temperature and heavy precipitation inhibited cellulolytic processes. In dark, ordinary, light, and irrigated gray-brown soils, the average number of microorganisms varies around $4.1–3.5\times10^6$/g soil. In microorganisms' composition of virgin soil and cultivated cenoses, non-spore-forming bacteria predominated, followed by actinomycetes, the spore-forming bacteria, and microscopic fungi.

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