БИОЛОГИЯ ПОЧВ

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ASSESSMENT OF BIOLOGICAL ACTIVITY AND SOIL HEALTH PARAMETERS OF VARIOUS SOIL TYPES IN THE ZHETYSU REGION

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Abstract. This study presents a comprehensive assessment of the biological condition of the humus horizons of meadow-serozem, foothill light chestnut, chestnut, and mountain dark chestnut soils in the Zhetysu Region of Kazakhstan, based on investigations conducted in virgin and cultivated areas during the spring and autumn seasons. The abundance of the main physiological and trophic groups of microorganisms was determined using culture-based methods, enzymatic activity was assessed using the fluorescein diacetate (FDA) hydrolysis assay, and structural and morphological characteristics were analyzed using Pfeiffer's circular chromatography method. Considerable seasonal variation and differences among soil types were observed in the biological indicators. The total microbial count decreased from 126.0×10^6 to 21.83×10^6 CFU/g of soil, while the maximum abundances of actinomycetes and bacteria utilizing mineral forms of nitrogen reached 180.0×10^5 and 174.67×10^5 CFU/g of soil, respectively. FDA hydrolytic activity reached 40.5 μg of fluorescein per gram of soil over a 3-hour incubation period. According to the circular chromatography results obtained in autumn, the central zone radius (CR) ranged from 21 to 27, the outer zone width (OZ) from 38 to 49, and the total radius (TR) from 96 to 110, with a relatively stable number of concentric rings (5–6). Virgin soils exhibited higher biological activity, whereas changes in biological indicators associated with land-use practices were observed in cultivated soils. These findings highlight the importance of integrating physical, chemical, and biological indicators when assessing soil condition and developing measures to preserve soil fertility.

Keywords: soil, microbial communities, enzymatic activity, bacteria, circular chromatography, soil fertility.

INTRODUCTION

Soil is a strategically important natural resource that supports food production. At the same time, degradation processes have affected approximately one-third of the world's agricultural soils [1], posing a serious threat to food security and sustainable development for both present and future generations. Kazakhstan ranks ninth in the world in terms of land area and is characterized by considerable soil diversity, comprising more than 700 soil types that differ in their chemical, physicochemical, and agrochemical properties, as well as in their degree of natural fertility. Soil formation in Kazakhstan occurs predominantly under arid and extreme climatic conditions. Consequently, these soils are particularly vulnerable to degradation and desertification and exhibit low resilience to anthropogenic disturbances [2].

In recent years, there has been a steady increase in the number of studies investigating soil microbial communities in the context of assessing soil biological health. Soil, as a habitat for microorganisms, is a complex heterogeneous system whose functioning is determined by a combination of interrelated and dynamically changing factors. Anthropogenic disturbances induce diverse responses in soil microbial communities, depending on their structural organization, taxonomic composition, and functional potential. Effective management of microbiological processes in soil requires reliable in-

formation on the quantitative and qualitative composition of its microbial communities [3]. However, a systematic understanding of the structure and diversity of soil microbial communities in Kazakhstan is currently lacking. In this regard, the microbiological characterization of different soil types is particularly important for studying, conserving, and sustainably utilizing ecosystem biodiversity [4].

One of the most pressing environmental issues of the past decade has been the deterioration of soil health, particularly the decline in soil organic matter content, largely driven by climate change [5]. The need for regular monitoring of soil organic matter content [6] is determined by its close functional relationship with soil properties such as acidity (pH), particle size distribution, moisture content, and mineral component content, as emphasized by Bot and Benites [7]. According to Graciano et al. [8], the high variability in the molecular structure of soil organic matter presents a significant methodological challenge in assessing humus content and microbiological activity. Furthermore, parameters such as pH, total organic matter content, electrical conductivity, nitrogen, phosphorus, and potassium concentrations, and moisture content are generally considered in isolation [9], making it difficult to obtain a comprehensive assessment of soil condition.

The aim of this study is to comprehensively assess the biological activity of Kazakhstan's soils as an integral indicator of their ecological and agroecological condition.

MATERIALS AND METHODS

The study focused on the humus horizons of subzonal soil subtypes in the foothill regions of Kazakhstan, including meadow-serozem, foothill light chestnut, chestnut, and mountain dark chestnut soils.

The bacterial composition of soils was investigated using samples collected from virgin and arable land plots at six test sites in the Zhetysu Region (Republic of Kazakhstan), representing Serozem (07 T/21), Light Chestnut Soil (12 T/21), and Dark Chestnut Soil (13 T/21) during different seasons (spring–autumn 2024).

Microbiological and enzymatic activities were determined using freshly collected soil samples in triplicate. Soil samples were collected using a soil auger from a depth of 0–10 cm at five points within each test site in accordance with ST RK ISO 18400-105-2018 and standard methods [10, 11]. Prior to analysis, the samples were stored at +4°C. Soil samples were prepared for microbiological analysis using generally accepted methods [12].

Microorganisms were enumerated using the plate count method on selective culture media: Sabouraud agar for filamentous fungi; universal medium (UM) for bacteria; starch-ammonia agar (SAA) for actinomycetes; Ashby's medium for nitrogen-fixing bacteria; and KBS medium for coryneform bacteria [12]. Additionally, the fluorescein diacetate (FDA) hydrolysis assay was performed [13]. Colonies were counted 3–7 days after inoculation, and plating was performed in triplicate. Incubation was carried out at 28–30 °C for 3–10 days. Microbial abundance was expressed as colony-forming units per gram of soil (CFU/g). Soil health was assessed using Pfeiffer's circular paper chromatography method [14], and the chromatograms were interpreted according to methodological guidelines [15].

The Pfeiffer method complements microbiological and FDA-based methods by providing an indirect characterization of the condition and structural organization of the soil organomineral complex based on the morphological features of chromatograms. Its application complements quantitative measures of microbial abundance and enzymatic activity by providing additional information on the structural and functional condition of the soil.

The research was conducted at the Microbiology Laboratory of the Depart-

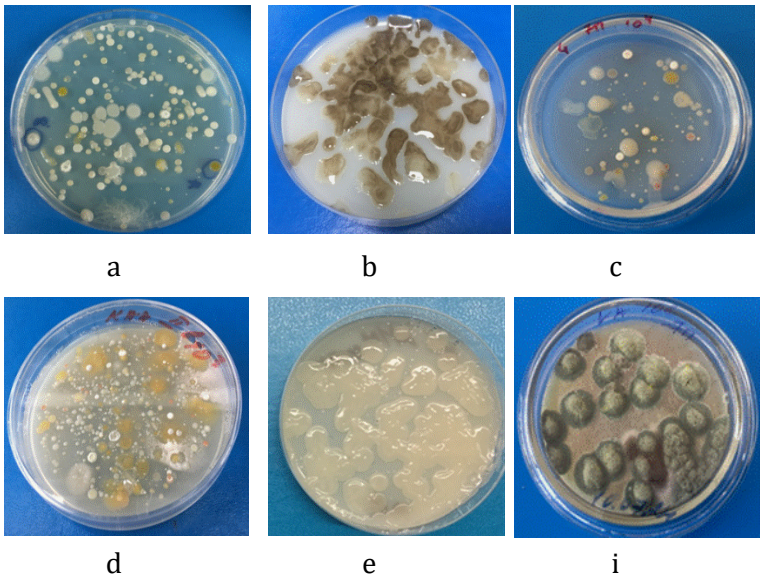
ment of Soil Fertility and Biology, U.U. Uspanov Kazakh Research Institute of Soil Science and Agrochemistry.

Data are presented as means ± standard error (SE). All analyses were performed in triplicate (n=3). Statistical analyses were conducted using Microsoft Excel. The standard deviation (SD) was calculated from the replicate measurements, and the standard error (SE) was calculated as $SD/\sqrt{n}$.

RESULTS AND DISCUSSION

During the study, various groups of microorganisms were isolated using standard methods, including fungi, bacteria, nitrogen-fixing microorganisms, coryneform bacteria, microorganisms capable of utilizing mineral forms of nitrogen, actinomycetes, and yeasts (figure 1).

During the spring season, high abundances of bacteria, actinomycetes, and coryneform microorganisms were recorded in virgin soils, whereas in autumn, a decrease in total microbial abundance and changes in the composition of the dominant taxonomic groups were observed (figure 2).



a – universal medium (UM); b – Ashby's medium; c – starch-ammonia agar (SAA); d – KBS medium; e – KG medium; i – Sabouraud medium.

Figure 1 – Morphology of microbial colonies grown on different selective culture media

Seasonal changes in the abundance and structure of microbial communities may be associated with fluctuations in temperature and moisture conditions and the availability of organic matter, potentially contributing to microbial activation in spring, followed by a decline in microbial abundance and changes in community structure during autumn.

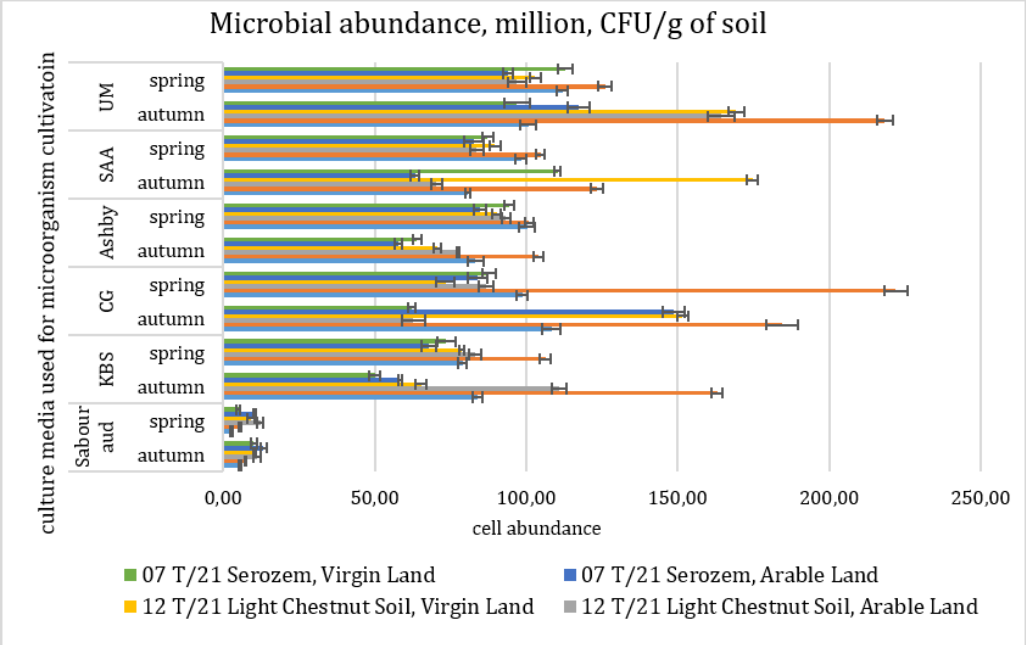


Figure 2 – Total microbial count in the experimental treatments: virgin and cultivated soils

The data presented in the figure indicate that bacteria and actinomycetes were the dominant groups of microorganisms in the soil samples, whereas fungal abundance was two orders of magnitude lower. A comparison of total microbial abundance showed that the number of colony-forming units per gram of soil was approximately the same across all samples. The distribution of the enumerated microbial groups was characterized by the following orders of magnitude: bacteria – $\times 10^5$, fungi – $\times 10^2$, and actinomycetes – $\times 10^4$.

Seasonal dynamics in soil microbial abundance showed considerable differences depending on soil type and sampling period. In spring, the highest abundance of bacteria and actinomycetes was recorded in 13 T/21 Dark Chestnut Soil, Virgin Land, at 222.0×10^5 CFU/g of soil, while the lowest abundance was observed in 12 T/21 Light Chestnut Soil, Virgin Land, at 73.33×10^5 CFU/g of soil. The higher microbial abundance in dark chestnut soil may have been associated with a higher organic matter content and favorable hydrothermal conditions.

In autumn, total microbial abundance decreased to 21.83×10^6 CFU/g of soil, compared with 126.0×10^6 CFU/g of soil in spring. This decrease may have been associated with a seasonal decline in soil temperature and reduced availability of readily decomposable organic substrates. Nevertheless, the highest abundance of actinomycetes was still recorded in 13 T/21 Dark Chestnut Soil, Virgin Land, reaching 180.0×10^5 CFU/g of soil, whereas the lowest abundance was observed in 07 T/21 Serozem at 62.33×10^5 CFU/g of soil.

The abundance of nitrogen-fixing microorganisms and coryneform bacteria was generally higher in virgin soils than in arable soils and serozem soils. Seasonal differences were observed in the abundance of bacteria utilizing mineral forms of nitrogen: in spring, the highest abundance was recorded in 13 T/21 Dark Chestnut Soil, Virgin Land, at 104.67×10^5 CFU/g of soil, whereas in autumn, it was recorded in 12 T/21 Light Chestnut Soil, Virgin Land, at 174.67×10^5 CFU/g of soil. The abundance of micromycetes also varied depending on the season and soil type. In spring, the

highest value was recorded in 12 T/21 Light Chestnut Soil, Arable Land, at 12.33×10^3 CFU/g of soil, whereas in autumn, it was recorded in 07 T/21 Serozem, Arable Land, at 13.33×10^3 CFU/g of soil.

Overall, virgin soils, particularly dark chestnut soils, were characterized by higher abundances of certain functional groups of microorganisms. The autumn decline in total microbial abundance was accompanied by changes in the relative proportions of individual functional groups, including an increase in the abundance of bacteria utilizing mineral forms of nitrogen in certain soil treatments. These findings indicate that the structure and abundance of soil microbial communities vary depending on soil type and seasonal conditions, which may influence the intensity of biological processes involved in maintaining soil fertility.

One of the informative methods for assessing soil biological activity is the fluorescein diacetate (FDA) hydrolysis assay, which is based on the enzymatic hydrolysis of fluorescein diacetate by soil microorganisms. The rate of hydrolysis reflects the overall hydrolytic activity of the soil microbial community. The results of the assessment of soil biological activity using the FDA hydrolysis assay are presented in figure 3.

Assessment of soil enzymatic activity using the FDA hydrolysis assay (μg of fluorescein/g of soil over 3 h) revealed pronounced seasonal changes in biochemical activity in virgin and arable soils. In 07 T/21 Serozem, Virgin Land, FDA activity was approximately 27.5 μg/g in spring, exceeding that in 07 T/21 Serozem, Arable Land (17.0 μg/g). By autumn, the values had decreased to 10.0 and 6.3 μg/g, respectively. The decrease in activity may have been associated with seasonal soil drying and elevated summer temperatures, which may limit the metabolic activity of soil microbiota.

In 12 T/21 Light Chestnut Soil, Virgin Land, FDA activity in spring was 34.5 μg/g, compared with 28.5 μg/g in 12 T/21 Light Chestnut Soil, Arable Land. In autumn, FDA activity in 12 T/21 Light Chestnut Soil, Virgin Land, increased to 40.5 μg/g, reaching the highest value among all the treatments studied, whereas it decreased to 21.3 μg/g in 12 T/21 Light Chestnut Soil, Arable Land. In 13 T/21 Dark Chestnut Soil, Virgin Land, FDA activity in spring was 33.5 μg/g, compared with 16.8 μg/g in 13 T/21 Dark Chestnut Soil, Arable Land.

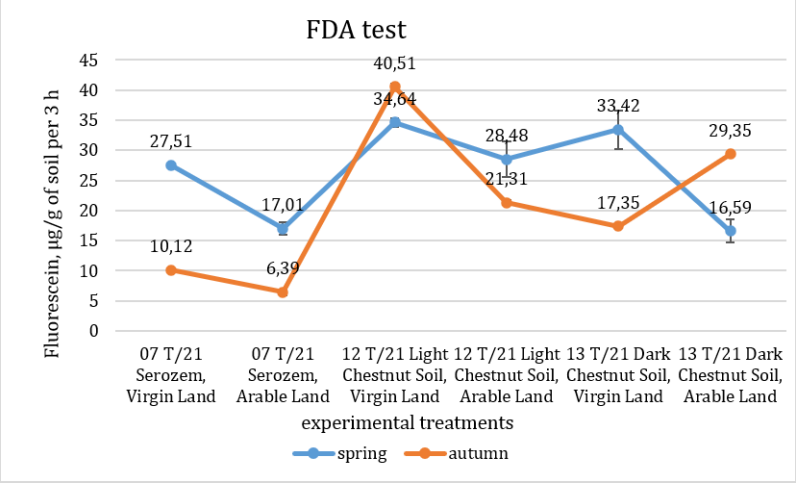


Figure 3 – Results of soil biological activity assessment using the FDA hydrolysis assay

In autumn, the value decreased to 17.3 μg/g in 13 T/21 Dark Chestnut Soil, Virgin Land, whereas it increased to 29.3 μg/g in 13 T/21 Dark Chestnut Soil, Arable Land. The increase in FDA activity in arable soil during autumn may have been associated with the input of fresh plant residues and changes in soil conditions following agricultural management practices, which may promote microbial activity.

Overall, the enzymatic activity of the investigated soils varied depending on soil genetic type, land use, and seasonal conditions. In spring, virgin soils exhibited higher FDA activity than arable soils.

This may be associated with the preservation of natural soil structure and greater organic matter inputs under undisturbed vegetation cover. Light chestnut and dark chestnut soils (12 T/21 Light Chestnut Soil and 13 T/21 Dark Chestnut Soil) exhibited higher FDA activity than serozem soil (07 T/21 Serozem). However, the pattern and magnitude of seasonal changes varied depending on soil genetic type and land use.

The Pfeiffer test is a method for the comprehensive assessment of the biological and functional condition of soils based on the morphometric and visual characteristics of circular chromatograms. Chromatogram interpretation takes into account the distribution of zones, radial channels, and spikes, as well as their color, size, shape, and degree of structural development. Together, these characteristics reflect patterns of organic matter distribution and provide a basis for assessing the structural and functional condition of soil microbial communities (figure 4).

Thus, the analysis of Pfeiffer chromatograms allows for a comprehensive characterization of soil condition based on a combination of morphological features, including the degree of development and spatial organization of zones, radial channels, and spikes, as well as their color and dimensional characteristics.

The intermediate zone is characterized by low levels of poorly integrated organic matter, whereas the outer zone indicates low enzymatic activity.

In virgin soils (07 T/21 Serozem, Virgin Land; 12 T/21 Light Chestnut Soil, Virgin Land; and 13 T/21 Dark Chestnut Soil, Virgin Land), the central zone was characterized by a low organic matter content in both spring and autumn.

However, the organic matter was integrated with mineral components, indicating high soil quality and good nutrient availability for microorganisms. Thus, the Pfeiffer test indicates higher soil quality and biological activity in virgin soils compared with arable soils and serozem soils.

For the interpretation of circular chromatograms, the radii of the zones forming the chromatogram were considered, including the overall radius (OR), the radius of the central zone, the widths of the intermediate and outer zones, and the numbers of channels, spikes, and concentric rings (figure 5), in accordance with the criteria proposed by Restrepo and Pinheiro [16].

A comparative analysis of soil samples collected from virgin and arable land plots during the spring and autumn seasons revealed pronounced seasonal variability in the investigated parameters, with the pattern and magnitude of these changes depending on soil type and land use. In all the investigated soils, a decrease in RCZ and an increase in outer zone width (OZ) were observed in autumn.

In 07 T/21 Serozem, Virgin Land, RCZ decreased from 50 to 25, while in 07 T/21 Serozem, Arable Land, it decreased from 55 to 23. Meanwhile, OZ increased from 21 to 47 and from 22 to 44, respectively. The overall radius (OR) increased from 105 to 110 in virgin soil but decreased from 107 to 97 in arable soil, accompanied by an increase in the number of channels and spikes in the chromatograms.

In 12 T/21 Light Chestnut Soil, Virgin Land, RCZ decreased from 65 to 27, while in 12 T/21 Light Chestnut Soil, Arable Land, it decreased from 58 to 26. Meanwhile, OZ increased from 18 to 38 and from 18 to 49, respectively. TR decreased from 110 to 96 in virgin soil and increased from 107 to 110 in arable soil, while the radial characteristics generally decreased.

In 13 T/21 Dark Chestnut Soil, Virgin Land, RCZ decreased from 58 to 25, while in 13 T/21 Dark Chestnut Soil, Arable Land, it decreased from 72 to 21.

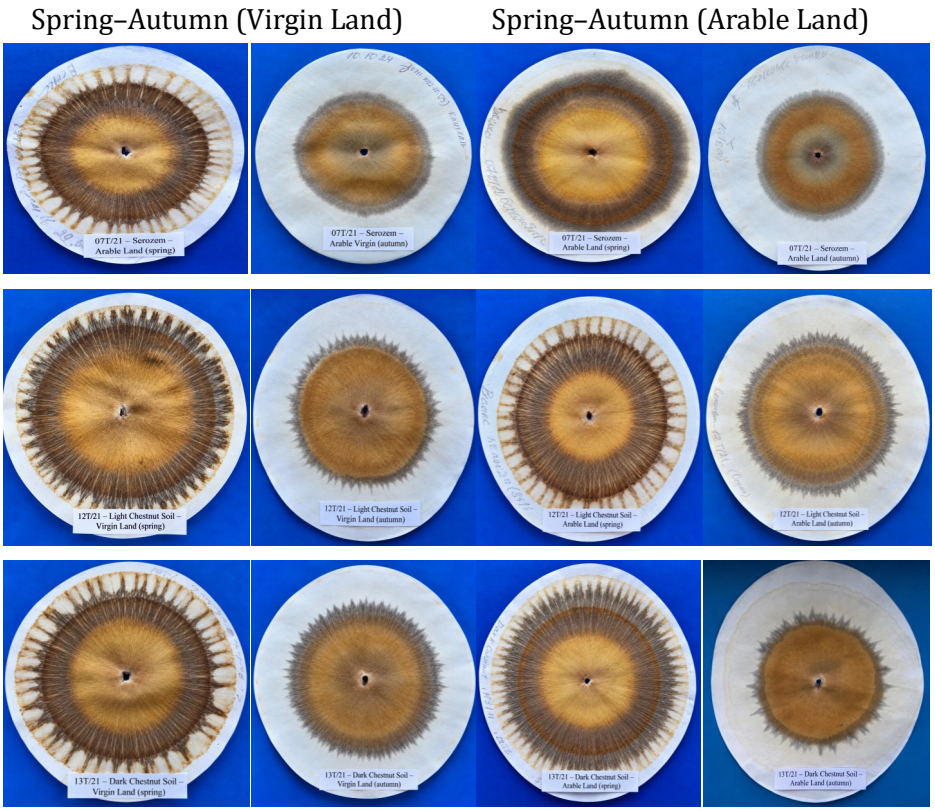


Figure 4 – Chromatograms of soil samples (virgin and cultivated soils) from agricultural crop cultivation sites

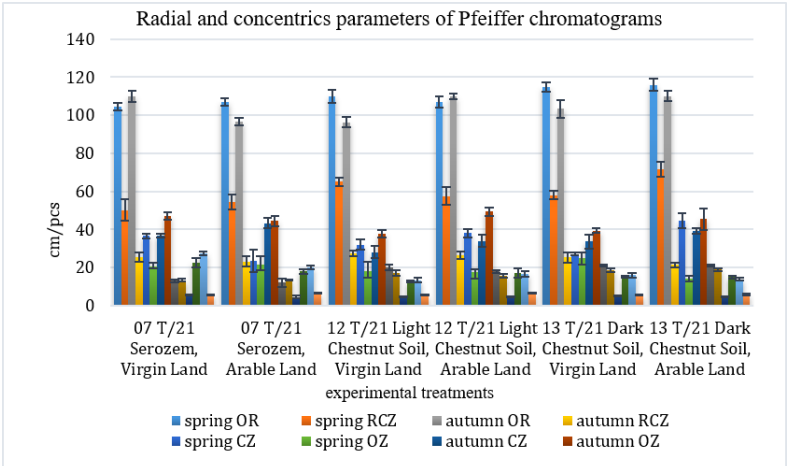


Figure 5 – Summary parameters of Pfeiffer chromatograms for the experimental treatments over the growing season (spring–autumn)

Meanwhile, OZ increased from 25 to 39 and from 14 to 45, respectively. TR decreased from 115 to 103 in virgin soil and from 116 to 110 in arable soil, accompanied by a reduction in the number of channels and spikes. The concentric characteristics of the chromatograms remained relatively stable, with 5–6 concentric rings.

Thus, the seasonal variation in the studied parameters may be associated with changes in soil moisture and temperature, microbial activity, and organic matter transformation. The observed differences between virgin and arable soils may be related to land-use practices. The combined use of culture-based methods, the FDA assay, and Pfeiffer circular paper chromatography provides complementary information on the abundance of specific microbial groups, overall hydrolytic activity, and chromatographic morphology, respectively, thereby enabling a more comprehensive assessment of the biological and structural-functional condition of the soil.

CONCLUSION

The study revealed differences in the seasonal dynamics of soil biological activity associated with soil type and land-use practices. Virgin soils, particularly dark chestnut soils, generally exhibited higher abundances of certain functional groups of microorganisms than arable soils. However, the magnitude and direction of seasonal changes varied among soil types and land-use treatments. In autumn, total microbial abundance decreased in most of the studied variants, whereas some functional groups, including mineral nitrogen-utilizing bacteria and micromycetes, maintained or increased their abundance. FDA activity also exhibited distinct seasonal patterns, with the direction and magnitude of changes varying among soil types and land-use treatments. Similar variability was observed in the morphological characteristics of the Pfeiffer chromatograms. Arable soils generally exhibited greater variability in individual microbiological indicators than their virgin counterparts. The most pronounced seasonal differences were observed in serozem and chestnut soils. These patterns may be associated with differences in soil type and land use that affect microbial community functioning, organic matter transformation, and the structural and functional condition of the soil.

Overall, the results indicate that soil biological activity varies according to soil type and land use, highlighting the importance of maintaining favorable biological and structural soil conditions. The identified patterns may be considered when developing adaptive land-use approaches aimed at maintaining biological activity and enhancing the resilience of arable soils.

The Pfeiffer circular paper chromatography method was used as an additional approach to assess soil condition. The morphological characteristics of the chromatograms complemented microbiological indicators and FDA activity measurements, providing an indirect characterization of the structural and functional condition of the soil organomineral complex.

FUNDING

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ТҮЙІН

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ЖЕТІСУ ОБЛЫСЫНЫҢ ӘРТҮРЛІ ТОПЫРАҚ ТҮРЛЕРІНІҢ БИОЛОГИЯЛЫҚ БЕЛСЕНДІЛІГІ МЕН ТОПЫРАҚ ДЕНСАУЛЫҒЫ КӨРСЕТКІШТЕРІН БАҒАЛАУ

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Зерттеу барысында Жетісу облысының шалғынды сұр, тау бөктерінің ашық қара-қоңыр, қара-қоңыр және таулы қара-қоңыр топырақтарының тың және жыртылған учаскелеріндегі гумусты горизонттардың биологиялық жай-күйіне көктемгі және күзгі кезеңдерде кешенді бағалау жүргізілді. Негізгі физиологиялық-трофикалық топтарға жататын микроорганизмдердің саны қоректік орталарда өсіру арқылы, ферментативтік белсенділік ФДА-тест көмегімен, ал құрылымдық-морфологиялық сипаттамалар Пфайффердің шеңберлік хроматография әдісімен анықталды. Зерттеу нәтижесінде биологиялық көрсеткіштердің айқын маусымдық және топырақ типіне байланысты өзгергіштігі анықталды: микроорганизмдердің жалпы саны $126{,}0\times10^6$-дан $21{,}83\times10^6$ КТБ/г топыраққа дейін төмендеді. Актиномицеттер мен минералдық азот қосылыстарын пайдаланатын бактериялардың ең жоғары саны тиісінше 180,0 және $174{,}67\times10^5$ КТБ/г топырақты құрады. ФДА-белсенділіктің ең жоғары мәні 3 сағат ішінде 40,5 мкг флуоресцеин/г топырақты құрады. Шеңберлік хроматография нәтижелері бойынша күзгі кезеңде CR көрсеткіші 21–27, OZ – 38–49, TR – 96–110 аралығында өзгерді, ал концентрлік сипаттамалар салыстырмалы тұрақтылық танытып, 5–6 сақинамен сипатталды. Тың топырақтар микроорганизмдердің жоғары биологиялық белсенділігімен сипатталды, ал жыртылған учаскелерде жерді пайдалану сипатына байланысты микробиологиялық көрсеткіштердің өзгеруі байқалды. Алынған нәтижелер топырақтардың биологиялық жай-күйін бағалау, олардың құнарлылығын сақтау және ұтымды пайдалану шараларын негіздеу кезінде микробиологиялық, физикалық және химиялық көрсеткіштерді кешенді түрде ескерудің маңыздылығын айқындайды.

Түйінді сөздер: топырақ, микробтық қауымдастықтар, ферментативтік белсенділік, бактериялар, дөңгелек хроматография, топырақ құнарлылығы.

РЕЗЮМЕ

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ОЦЕНКА БИОЛОГИЧЕСКОЙ АКТИВНОСТИ И ПОКАЗАТЕЛЕЙ СОСТОЯНИЯ ЗДОРОВЬЯ ПОЧВ РАЗЛИЧНЫХ ТИПОВ ОБЛАСТИ ЖЕТІСУ

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В работе проведена комплексная оценка биологического состояния гумусовых горизонтов лугово-серозёмных, предгорных светло-каштановых, каштановых и горных тёмно-каштановых почв Жетісуской области на целинных и пахотных участках в весенний и осенний периоды. Численность основных физиолого-трофических групп микроорганизмов определяли культуральными методами, ферментативную активность – с использованием ФДА-теста, структурно-морфологические характеристики – методом круговой хроматографии по Пфайфферу. Выявлена выраженная сезонная и почвенная вариабельность биологических показателей: общая численность микроорганизмов снизилась с $126{,}0\times10^6$ до $21{,}83\times10^6$ КОЕ/г почвы; максимальная численность актиномицетов и бактерий, использующих минеральные формы азота, составила 180,0 и $174{,}67\times10^5$ КОЕ/г почвы соответственно. ФДА-активность достигала 40,5 мкг флуоресцеина/г почвы за 3 ч. По данным круговой хроматографии осенью значения CR составляли 21–27, OZ – 38–49, TR – 96–

110 при относительной стабильности концентрических характеристик (5–6 колец). Целинные почвы характеризовались более высокой биологической активностью, тогда как на пахотных участках отмечались изменения показателей, связанные с характером землепользования. Полученные результаты свидетельствуют о необходимости комплексного учета физических, химических и биологических показателей при оценке состояния почв и разработке мероприятий по сохранению их плодородия.

Ключевые слова: почва, микробные сообщества, ферментативная активность, бактерии, круговая хроматография, плодородие.

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