How to Cite: Dolgireva, V.E., Belyaev, A.M., Belyaev, I.A., Amirkhanova, Z.T., Drobchenko, Y.A. (2025). Assessment of the cellulose-decomposing potential of soil biota near the railway tracks of the city of Karaganda. *Fundamental And Experimental Biology, 30*, 4(120), 55-64. https://doi.org/10.31489/2025FEB4/55-64

Research Article

https://doi.org/10.31489/2025FEB4/55-64

UDC 631.427

Received: 3.06.2025 | Accepted: 15.10.2025 | Published online: 31 December 2025

V.E. Dolgireva*, A.M. Belyaev, I.A. Belyaev, Z.T. Amirkhanova, Y.A. Drobchenko

Karaganda Medical University, Karaganda, Kazakhstan
**Corresponding author: gubskaya_lera@mail.ru*

Assessment of the cellulose-decomposing potential of soil biota near the railway tracks of the city of Karaganda

The aim of the study was to assess the cellulose-degrading potential of soil biota in the areas adjacent to the railway tracks of the city of Karaganda. Soil samples were collected along the railway line between the stations *Vishnyaki* and *O.P. 721 km* using the envelope method. Agarized Getchinson's medium was used to isolate cellulose-degrading microorganisms. The total cellulolytic activity was evaluated by the level of acid formation in liquid Getchinson's medium. A gradient of decreasing cellulolytic activity was observed from points 50 meters away from the railway to those at a distance of 10 meters. Regardless of soil composition, an increase in cellulose-degrading microorganisms was found at the points further from the railway. The change in total cellulose-degrading activity in the studied points was accompanied by a variation in the structure of autochthonous microbiota capable of cellulose decomposition. The findings highlight the need for further investigation of soils in areas adjacent to railway lines.

Keywords: railway, cellulolytic microorganisms, soil, cellulase, microbial ecology, cellulolytic activities

Introduction

Soil is a complex natural entity composed of organic and inorganic substances. A significant part of the soil is made up of living organisms that substantially influence its composition and properties. In addition to seasonal climatic changes, soil is subject to anthropogenic influences. To counteract these impacts, soil possesses its own capacity for neutralizing and degrading pollutants and this process is entirely dependent on the biological and enzymatic activity of soil biota. One of the properties of these soil microorganisms is their ability to break down cellulose [1]. Sources of cellulose include plant residues, wild and domestic animal manure, packaging materials, and other paper-based products of anthropogenic origin. Cellulolytic activity is predominantly exhibited by autochthonous soil bacilli, actinomycetes, and fungi [2]. Research into the genetic foundations of these microorganisms has shown that there are four different methods of cellulose degradation: hydrolysis by C1 enzymes, hydrolysis by β-1,4-glucanase, hydrolysis by β-1,4-glucosidase, and glucose metabolism [3]. As microorganisms are capable of producing a number of different enzymes, they can act synergistically or independently of each other [4]. During soil formation, cellulose is broken down into glucose or the disaccharide cellobiose, which can be used by other parties of the soil biocenosis for structural and energy purposes [5].

Currently, there is a vast amount of literature available on methods of isolation, structure, and functions of cellulolytic bacterial communities [6–8]. However, an assessment of how different modes of transportation affect the community of cellulolytic soil bacteria and their activity has not been conducted.

Therefore, the aim of our research was to evaluate the cellulolytic activity of soil biota in selected railway tracks areas.

Materials and methods

Four sites were selected along the railway between the city of Karaganda and the village of Karabas for soil sample collection (Figs. 1-2). To assess the gradient of railway impact on soil biocoenosis, samples were taken symmetrically from both sides at distances of 10 and then 50 meters as control points.

Figure 1. General map of soil sampling sites (1-2)

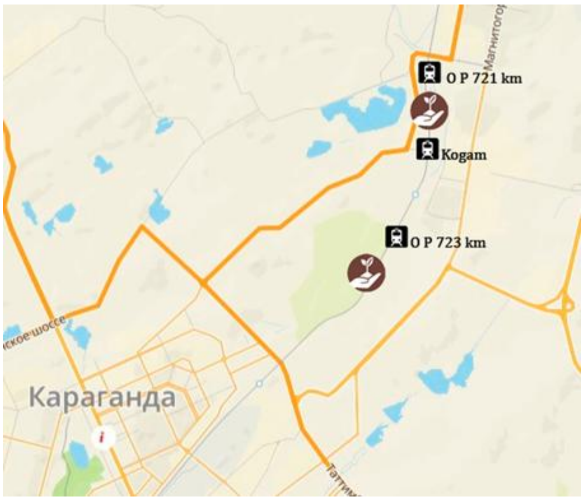

Figure 2. General map of soil sampling sites (3-4)

Site 1. Station *Kosmos*

This site is located 300 meters north of the *Kosmos* station (49.71401710462086 N, 73.02334027920278 E) with the Sokyr River 1.25 km to the west of the sampling site (Fig. 3). A settlement of small individual gardens is situated at a distance of 226 m northeast from the sampling site. The predominant soil type is chestnut, with plants from family the Poaceae (Nardus stricta, Digitaria ischaemum) and from family the Umbelliferae (Eryngium).

Figure 3. Station *Kosmos*

Site 2. Station *Vishnyaki*

The next site is located 255 meters north of the *Vishnyaki* station (49.695128 N, 73.014013 E), (Fig. 4). A settlement of small individual gardens is situated a distance of 209 m northeast. The predominant soil type is chestnut, with plants from family the Asteraceae (Centaurea orientalis, Galatella angustissima).

Figure 4. Station *Vishnyaki*

Site 3. Station *OP 723 km*

The sampling location for soil samples is located 2.4 km south of the *OP 723 km* station (49.843244 N, 73.166963 E) with a park zone at a distance of 390.9 m to the west (Fig. 5). The predominant soil type is chestnut, with woody plants (Acer, Ulmus parvifolia, Populus) and plants from family the Poaceae (*Hordeum jubatum*).

Figure 5. Station *OP 723 km*

Site 4. Station *OP 721 km*

This site is located 177.3 meters north of the *Kogam* station (49.881081 N, 73.180029 E) Solonka River at a distance of 129.5 m to the west (Fig. 6). Residential buildings are situated 353 m east of the sampling site. The predominant soil type is chestnut, with plants from family the Poaceae.

Figure 6. Station *OP 721 km*

Soil sampling from the listed sites was conducted in accordance with the requirements of GOST 17.4.4.02-2017 "Methods of soil sampling and sample preparation for chemical, bacteriological, helminthological analysis" [GOST 17.4.4.02-2017] [9]. The "envelope" method was used, where 5 point samples were taken from the soil horizon to a depth of 10–15 cm from a sampling area of 25x25.

All samples were collected using a sterile instrument into sterile polyethylene bags. After labeling, the samples were transported to the laboratory of the Karaganda Medical University. Aseptic conditions were maintained during sampling and transportation.

In the laboratory, soil from each of the 5 selected points on the sampling area was mixed to form an average sample weighing 200–250 grams each. From this combined average sample, ten-fold serial dilutions were prepared (Fig. 7).

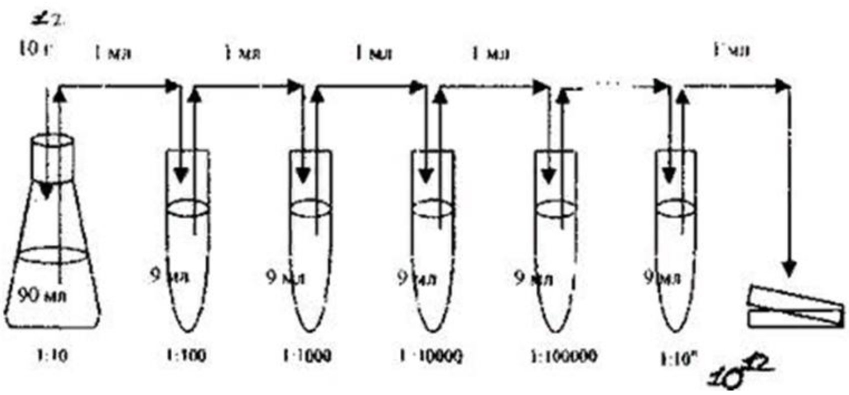

Figure 7. Carrying out the dilution method

The obtained serial ten-fold dilutions of soil were used for qualitative and quantitative assessment of soil biota by inoculating into selective and differential media:

1. To assess the general biological contamination of soils, the number of mesophilic aerobic flora (MAF) and thermophilic bacteria was determined at an incubation temperature of 60 °C.

2. Getchinson's medium was used for the detection of cellulolytic organisms, both in agarized and liquid forms. In this medium, the sole carbon source is cellulose from filter paper or cotton wool [10]. The results on agarized Getchinson's medium were counted visually by observing colony growth, with colony-forming units (CFU) per gram calculated.

3. Colonies grown on agarized medium were subcultured after assessing morphological characteristics and Gram staining onto other media for further study of the biological properties of pure cultures.

4. The total cellulolytic activity was evaluated on liquid Getchinson's medium by the level of acid formation. An indicator, bromothymol blue, was added to determine the level of acid production. The color changed from green (neutral pH) to yellow (pH 6.2 and below). For the negative control, optical density of Getchinson's medium with a pH of 7.4 and added bromothymol blue (olive-green coloration) was used. For

the positive control, Getchinson's medium with a pH of 5.3 and added solution of citric acid (strong yellow color) was used. The intensity of yellow coloration correlated with the total cellulolytic activity and was determined by measuring optical density at a wavelength of 405 nm using a semi-automatic plate photometer (Stat Fax 2100). The mean optical density of the negative control, determined in 6 replicates, was 0.1286 with a standard deviation of 0.0018. The mean optical density of the positive control, similarly determined, was 0.6112 with a standard deviation of 0.0016.

The statistical analysis of the research results was conducted using STATISTICA 7.0 software. Descriptive statistics (mean, M ± standard deviation, SD) were calculated for quantitative variables, while frequency analysis was performed for qualitative variables. The distribution of variables was assessed using the Shapiro-Wilk test. For comparing qualitative variables, the Z-test was used, and for quantitative variables, the Student's t-test was employed. A significance level of $p < 0.05$ was considered statistically significant for all variables.

Results and Discussion

Based on the results of the study of the total cellulolytic activity of soil biota, a gradation of decreasing activity from points 50 meters away from the railway to points at a distance of 10 meters was identified. The degree of decrease in activity depending on the distance from the railway varied in severity (Figs. 8–11).

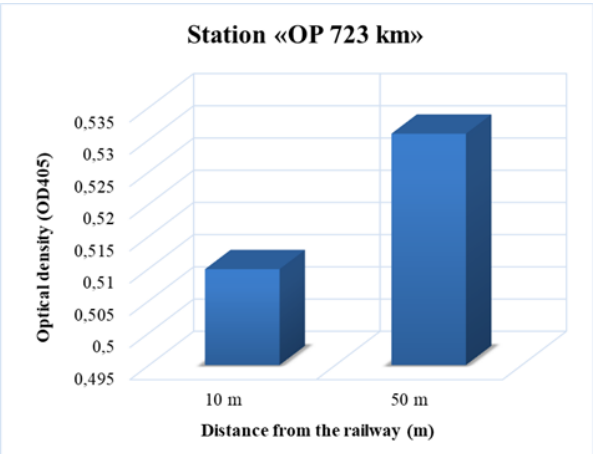

Figure 8. Cellulolytic activity on the Station *OP 723 km*

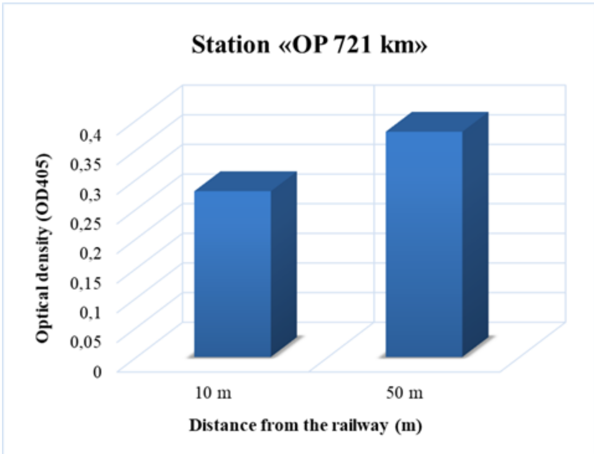

Figure 9. Cellulolytic activity on the Station *OP 721 km*

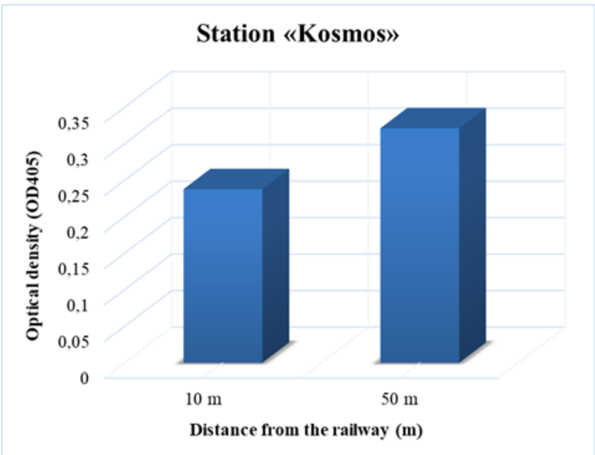

Figure 10. Cellulolytic activity on the Station *Kosmos*

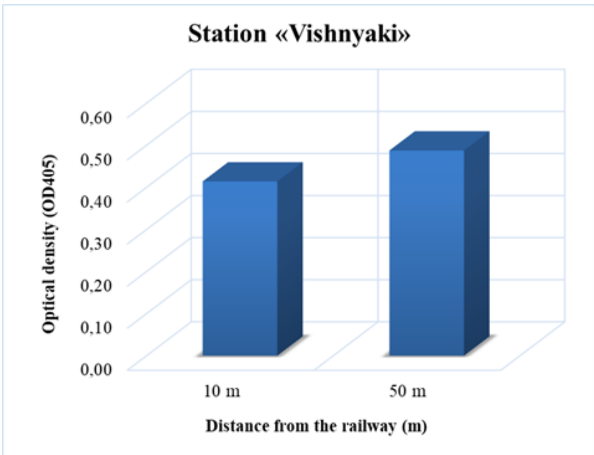

Figure 11. Cellulolytic activity on the Station *Vishnyaki*

So, the highest cellulolytic activity was found at Station *OP 723 km* with a value of 0.53091. This station had the richest source of cellulose due to its developed vegetation (trees, shrubs, and grass) [11]. The lowest cellulolytic activity was observed at Station *OP 721 km* and Station *Kosmos* with values of 0.38007 and 0.32130. Station *Vishnyaki* had a relatively high cellulolytic activity of 0.48738, likely due to its pronounced grassy cover and almost absence of woody vegetation.

According to the statistical data, significant differences were found at all stations except Station *OP 723 km*, which can be explained by the uniformity of the selected sampling area and the abundant vegetation along all 50 meters from the railway (Tab.).

Table

Total cellulolytic activity

Soil's samples	10 m, M (SD)	50 m, M (SD)	Значение p
Station *OP 723 km*	0.50989 (0.045642769)	0.53091 (0.040807636)	0.348
Station *Vishnyaki*	0.41392 (0.032224079)	0.48738 (0.071453222)	0.025
Station *OP 721 km*	0.27966 (0.022094188)	0.38007 (0.014789072)	0.0001
Station *Kosmos*	0.23788 (0.033181147)	0.32130 (0.057072986)	0.004

In addition to changes in the total cellulolytic activity in the studied points, there was observed a change in the structure of autochthonous microbiota capable of cellulose decomposition. This group included Bacillus, Actinomycetes, and Fungi.

At the *OP 723 km* station, the specific weight of cellulose-degrading Bacillus is 310 CFU/g, which constitutes 20.4 % of the total Bacillus count. At a distance of 50 meters, this value increases to 334 CFU/g (21.5 %). A growth trend is also observed in the calculation of cellulose-degrading Actinomycetes, where at 10 meters their specific weight was 25.5 %, and at 50 meters it was 28.7 % (Fig. 12). However, the results of the statistical analysis showed insignificant differences between the groups of Bacillus and Actinomycetes regarding the studied parameter. This may be due to the limited sample size, which did not provide sufficient statistical power to detect real differences.

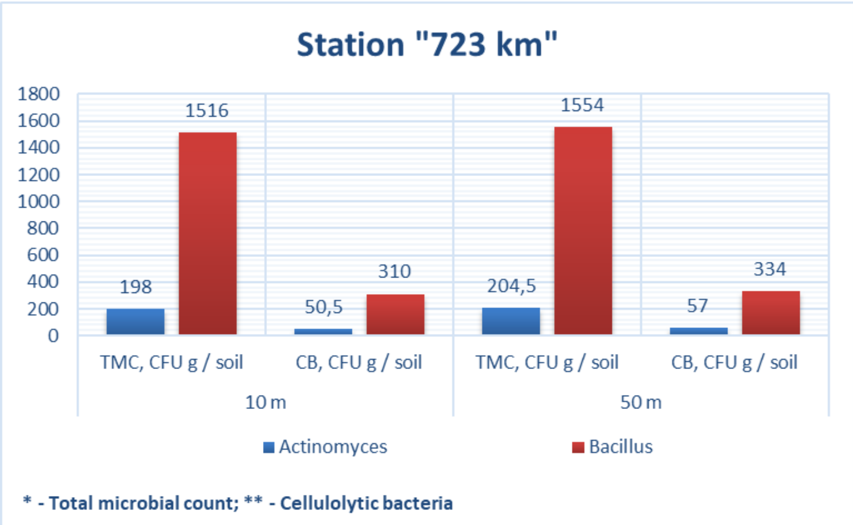

Figure 12. Specific gravity of cellulose-decomposing microorganisms at Station *OP 723 km*

At the *OP 721 km* station, the specific weight of cellulose-degrading Bacillus is 164 CFU/g, which constitutes 19.8 % of the total Bacillus count. It is worth noting that at a distance of 50 meters, the increase in the specific weight of Bacillus does not lead to an increase in the percentage of cellulose-degrading species, which remains at 16.7 %. Similarly, with cellulose-degrading Actinomycetes, their specific weight changes from 36.5 CFU/g to 42.5 CFU/g, which corresponds to 28.6 % and 21.8 % respectively (Fig. 13). However, the results of the statistical analysis showed insignificant differences in the group of Actinomycetes regarding the studied parameter, which may be attributed to the limited sample size. Statistically significant results were found in the group of Bacillus ($p < 0.001$).

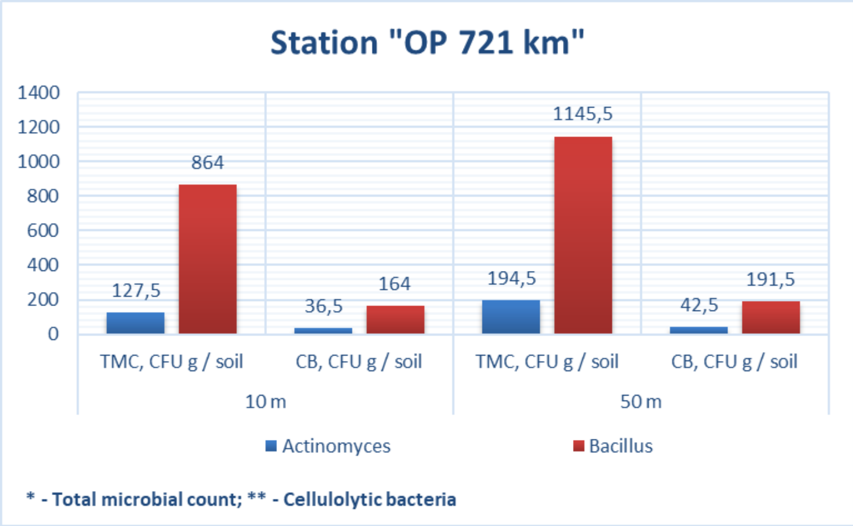

Figure 13. Specific gravity of cellulose-decomposing microorganisms at Station *OP 721 km*

It should be noted that at the *Vishnyaki* station, at a distance of 10 meters, the percentage of cellulose-degrading Bacillus and cellulose-degrading Actinomycetes is 39.1 % and 39.6 % respectively. However, at a distance of 50 meters, despite the increase in the specific weight of the total count and the number of cellulolytic representatives, the percentage of cellulose-degrading Actinomycetes increases to 44.6 %, while the percentage of cellulose-degrading Bacillus decreases to 23.8 % (Fig. 14). The results of the statistical analysis indicated insignificant differences in the group of Actinomycetes concerning the studied parameter, which may be attributed to the limited sample size. Statistically significant results were found in the Bacillus group ($p < 0.001$).

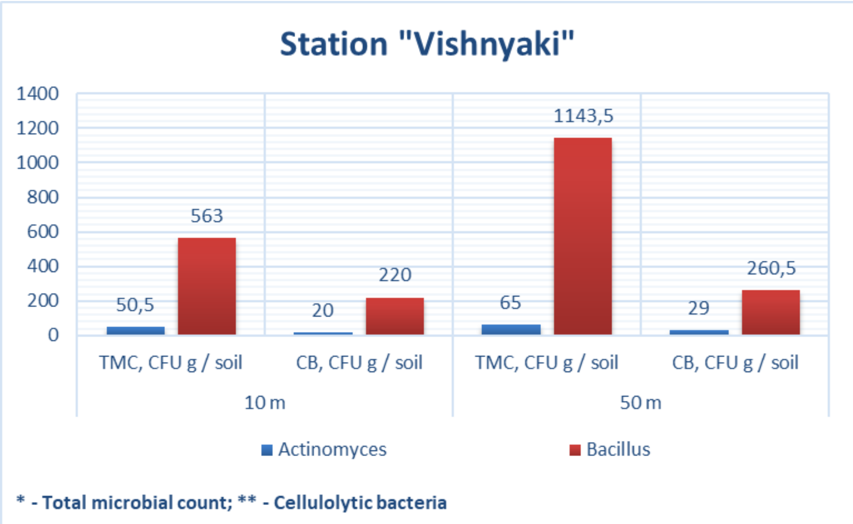

Figure 14. Specific gravity of cellulose-decomposing microorganisms at the Station *Vishnyaki*

At the *Kosmos* station, the specific weight of cellulose-degrading Bacillus is 200 CFU/g, which constitutes 15.6 % of the total Bacillus count. It is worth noting that at a distance of 50 meters, the specific weight of cellulose-degrading Bacillus increases to 215 CFU/g, but the percentage of Bacillus from the total count decreases to 14.3 %. On the other hand, cellulose-degrading Actinomycetes increase from 30.3 % to 31.1 % (Fig. 15). However, the results of the statistical analysis showed insignificant differences between the groups of Bacillus and Actinomycetes regarding the studied parameter. This may be due to the limited sample size, which did not provide sufficient statistical power to detect real differences.

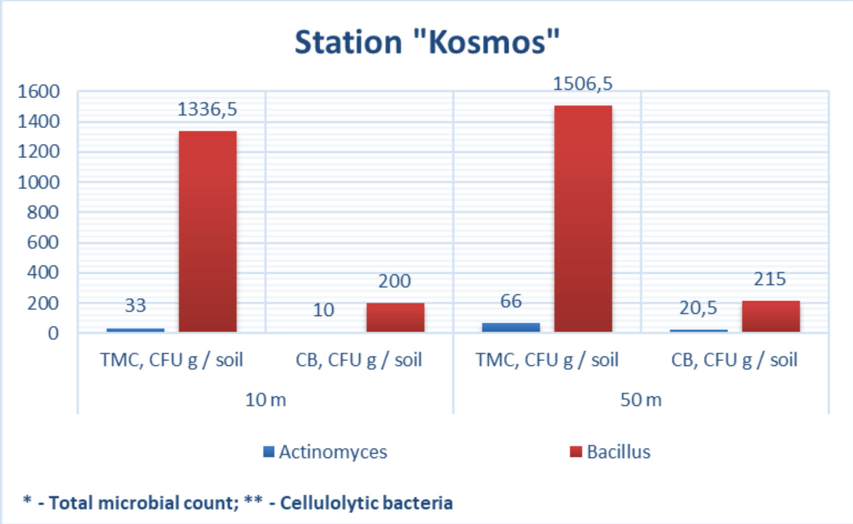

Figure 15. Specific gravity of cellulose-decomposing microorganisms at the Station *Kosmos*

It is worth noting separately the fungi, as there is a complete absence of fungal flora at a distance of 10 meters from the railway on all sites. The exception was the *Kosmos* station, where at a distance of 10 meters, the specific weight of cellulose-degrading fungi was 1.5 CFU/g, which corresponds to 12.5 %. At a distance of 50 meters, fungi are found on all investigated sites, however, at the *OP 721 km* station, cellulose-degrading fungi are absent (Fig. 16). The results of the statistical analysis showed that the differences in the Fungi group are not significant, despite their absence near railway tracks. This may be due to the limited sample size, which did not provide sufficient statistical power to detect real differences.

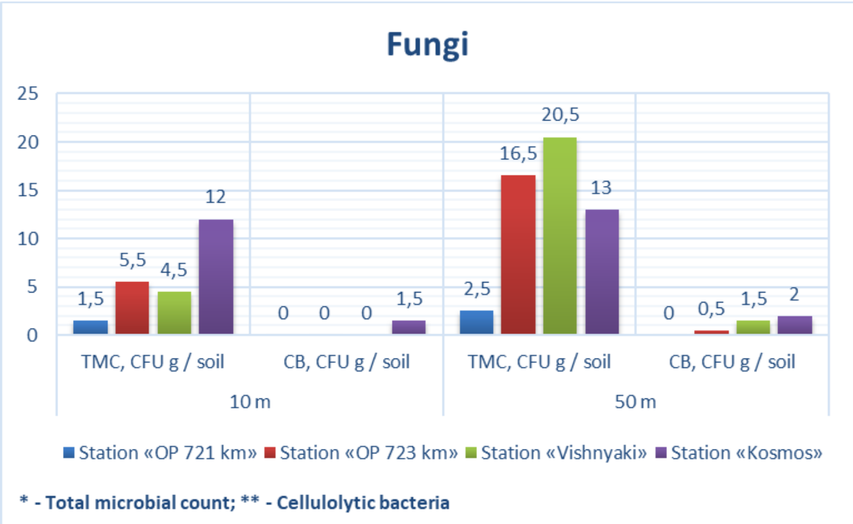

Figure 16. Specific gravity of cellulose-decomposing fungi

The study of the soil community structure revealed that in the studied areas the leading role in cellulose decomposition belongs to Bacillus and Actinomycetes, the number of which increases at the point of distance from the railroad compared to the ten-meter distance. Previously, the high cellulolytic potential of Bacillus and Actinomycetes has been identified in the production of biopreparations [12]. Irrespective of the plots with different soil composition, an increment of cellulolytic microorganisms was observed at the point of distance from the railroad (50 meters). At a distance of 10 meters from the railroad, a decrease in the total number of saprophytic fungi and the absence of their cellulolytic activity were detected.

Conclusions

Thus, a decrease in total cellulolytic activity was observed at three stations, along with a significant reduction in the abundance of Bacillus at the *Vishnyaki* and *OP 721* km stations. The statistically insignificant results in the groups of Actinomycetes and Fungi can be attributed to the small sample size, which did not provide sufficient statistical information to detect real differences.

It is worth noting that spatial heterogeneity of microbial communities is an inherent characteristic of soils and correlates with gradients of terrain and soil characteristics, including abiotic factors (density, structure, moisture, oxygen concentration, pH, organic matter content in soil), biotic factors (interaction of food webs, vegetation dynamics), and anthropogenic factors (land use systems) [13]. Therefore, the insignificance of the results obtained at the *Kosmos* and *OP 723 km* stations may be due to the undisturbed steppe area and the presence of abundant vegetation compared to the other two stations, which are characterized by proximity to residential areas. In this case, it can be hypothesized that the railway does not exert a strong influence on the soil biocoenosis but rather acts as a contributing factor inhibiting microbial activity.

To gain a more precise understanding of the situation, further research with larger samples and analysis of multiple factors is necessary, as statistical insignificance does not rule out the possibility of real differences and indicates the need for further investigation with adjusted research approaches.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Dolgireva V.E.** — conceptualization, data curation, investigation, visualization, writing — original draft; **Belyaev A.M.** — project administration, resources, supervision; **Belyaev I.A**. — writing — review & editing; **Amirkhanova Z.T.** — methodology; **Drobchenko Y.A.** — formal analysis, validation.

References

1 Patagundi, B.I., Shivasharan, C.T., & Kaliwal, B.B. (2014). Isolation and characterization of cellulase producing bacteria from soil. *International Journal of Current Microbiology and Applied Sciences, 3*(5), 59–69.

2 Mahmood, R., Afrin, N., Jolly, S.N., & Shilpi, R.Y. (2020). Isolation and identification of cellulose-degrading bacteria from different types of samples. *World, 9*(2), 8–13.

3 Brumm, P.J. (2013). Bacterial genomes: what they teach us about cellulose degradation. *Biofuels, 4*(6), 669–681. DOI: 10.4155/bfs.13.44

4 Qin, W. (2016). Recent developments in using advanced sequencing technologies for the genomic studies of lignin and cellulose degrading microorganisms. *International Journal of Biological Sciences, 12*(2), 156. DOI: 10.7150/ijbs.13537

5 Yadav, S., Reddy, B., & Dubey, S. K. (2020). De novo genome assembly and comparative annotation reveals metabolic versatility in cellulolytic bacteria from cropland and forest soils. *Functional & Integrative Genomics, 20,* 89–101. DOI: 10.1007/s10142-019-00704-0

6 McDonald, J.E., Rooks, D.J., & McCarthy, A.J. (2012). Methods for the isolation of cellulose-degrading microorganisms. In *Methods in Enzymology, 510,* 349–374. Academic Press. DOI: 10.1016/B978-0-12-415931-0.00019-7

7 Gupta, P., Samant, K., & Sahu, A. (2012). Isolation of cellulose-degrading bacteria and determination of their cellulolytic potential. *International Journal of Microbiology, 2012.* DOI:10.1155/2012/578925

8 Kimeklis, A.K., Gladkov, G.V., Orlova, O.V., Afonin, A.M., Gribchenko, E.S., Aksenova, T.S., & Andronov, E.E. (2023). The Succession of the Cellulolytic Microbial Community from the Soil during Oat Straw Decomposition. *International Journal of Molecular Sciences, 24*(7), 6342. DOI: 10.20944/preprints202212.0246.v1

9 (2017). Nature protection. Soils. Methods of soil sampling and sample preparation for chemical, bacteriological, helminthological analysis: HOST 17.4.4.02-2017. Interstate Standards. Standardinform, Russian.

10 Smirnova I. E., Fayzulina E.R., Tatarkina L.G., & Babaeva Sh.A. (2020). Isolation of cellulolytic bacteria that are promising for creating biofertilizers for forage and legume crops. *Microbiology and Virology, 4*(31), 13–23.

11 Stursova, M., Zifcakova, L., Leigh, M.B., Burgess, R., & Baldrian, P. (2012). Cellulose utilization in forest litter and soil: identification of bacterial and fungal decomposers. *FEMS Microbiology Ecology, 80*(3), 735–746. DOI: 10.1111/j.1574-6941.2012.01343.x

12 Wita, A., Białas, W., Wilk, R., Szychowska, K., & Czaczyk, K. (2019). The influence of temperature and nitrogen source on cellulolytic potential of microbiota isolated from natural environment. *Polish Journal of Microbiology, 68*(1), 105–114. DOI: 10.21307/pjm-2019-012

13 Serita, D. Frey (2015). The Spatial Distribution of Soil Biota. *In book: Soil Microbiology, Ecology and Biochemistry.* DOI: 10.1016/B978-0-12-415955-6.00008-6.

В.Е. Долгирева, А.М. Беляев, И.А. Беляев, Ж.Т. Амирханова, Е.А. Дробченко

Қарағанды қаласының теміржол маңындағы топырақ биотасының целлюлозаны ыдырату шамасын бағалау

Зерттеудің мақсаты — Қарағанды қаласындағы теміржол бойындағы топырақ биотасының целлюлозаны ыдырату әлеуетін бағалау. Топырақ үлгілері «Вишняки» мен «О.П. 721 км» станциялары аралығындағы теміржол бойынан «конверт» әдісімен жиналды. Целлюлозаны ыдырататын микроорганизмдерді бөлу үшін Гетчинсон агарлы ортасы қолданылды. Жалпы целлюлолитикалық белсенділік Гетчинсон сұйық ортасындағы қышқыл түзілу деңгейі бойынша бағаланды. Теміржолдан 50 метр қашықтықтағы нүктелерден 10 метр қашықтықтағы нүктелерге дейін целлюлолитикалық белсенділіктің төмендеу градациясы анықталды. Құрамында әртүрлі топырақ бар учаскелерге қарамастан, теміржолдан алыстаған сайын целлюлозаны ыдырататын микроорганизмдердің көбеюі анықталды. Зерттелген нүктелердегі жалпы целлюлозаны ыдырату белсенділігінің өзгерісі аутохтонды микробиотаның құрылымының өзгеруімен қатар жүрді. Мақаланың нәтижелері теміржол бойындағы аумақтардағы топырақты одан әрі зерттеудің қажеттілігін растайды.

Кілт сөздер: теміржол, целлюлозаны ыдыратушы микроорганизмдер, топырақ, целлюлаза, микробтық экология, целлюлолитикалық белсенділік

В.Е. Долгирева, А.М. Беляев, И.А. Беляев, Ж.Т. Амирханова, Е.А. Дробченко

Оценка целлюлозоразлагающего потенциала почвенной биоты вблизи железнодорожных путей города Караганды

Целью исследования являлась оценка целлюлозоразлагающего потенциала почвенной биоты в зоне прилегания железнодорожных путей города Караганды. Почвенные образцы были отобраны вдоль железной дороги между станциями «Вишняки» и «О.П. 721 км» методом «конверта». Для выделения целлюлозоразлагающих микроорганизмов была использована агаризованная среда Гетчинсона. Суммарная целлюлолитическая активность оценивалась по уровню кислотообразования в жидкой среде Гетчинсона. Выявлена градация снижения целлюлолитической активности точек на расстоянии 50 метров от железной дороги к точкам на дистанции 10 метров. Независимо от участков с различным почвенным составом, установлено приращение целлюлозоразлагающих микроорганизмов в точке удаления от железной дороги. Изменение суммарной целлюлозоразлагающей активности в изучаемых точках сопровождалось переменой структуры аутохтонной микробиоты, способной к разложению целлюлозы. Выводы статьи подтверждают необходимость дальнейшего исследования почв в зонах, прилегающих к железным дорогам.

Ключевые слова: железная дорога, целлюлозоразлагающие микроорганизмы, почва, целлюлаза, микробная экология, целлюлолитическая активность

Information about the authors

Valeriya Evgenevna Dolgireva — Master's student at the School of Biomedicine, Karaganda Medical University, Karaganda, Kazakhstan; e-mail: gubskaya_lera@mail.ru; https://orcid.org/0000-0003-1893-9860

Andrei Mihailovich Belyaev — Associate Professor of the Department of Biomedicine, Candidate of Medical Sciences, Karaganda Medical University, Karaganda, Kazakhstan; e-mail: Belyaev.A@kqmu.kz; https://orcid.org/0000-0002-1527-6080

Ilya Andreevich Belyaev — Associate Professor of the Department of Biomedicine, Doctor of Philosophy, PhD, Karaganda Medical University, Karaganda, Kazakhstan; e-mail: I. Belyaev@qmu.kz; https://orcid.org/0000-0003-3373-2059

Amirkhanova Zhanerke Talgatkyz — Assistanat Professor of the Department of Biomedicine, Karaganda Medical University, Karaganda, Kazakhstan; e-mail: janerkeamir@mail.ru; https://orcid.org/0000-0002-5487-3574

Drobchenko Yelena Alexandrovna — Lecturer at the Department of Informatics and Biostatistics, Karaganda Medical University, Karaganda, Kazakhstan; e-mail: Drobchenko@qmu.kz; https://orcid.org/0000-0001-9291-7585