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Genetic Diversity of *Hippophae rhamnoides* in Technogenic Areas of Northern Kazakhstan

Genetic diversity is a key indicator of a species' evolutionary development, adaptability to environmental changes, and long-term survival. Globally, the sustainable use and conservation of genetic resources — including *in situ* protection and population-level genetic studies — is a major scientific priority. Sea buckthorn (*Hippophae rhamnoides* L., Elaeagnaceae) has been traditionally used for centuries but has only recently been recognized for its significant economic value. Its berries are rich in biologically active compounds, making the species increasingly important in medicine, agriculture, food production, and cosmetics across Europe and Asia. This study evaluates the genetic diversity of *Hippophae rhamnoides* in technogenic zones of Northern Kazakhstan using ISSR markers. Seed samples were collected from two plant populations in the Prigorodny Forest District, Kostanay Region, for molecular analysis. ISSR analysis revealed high within-population polymorphism (87%) and lower between-population polymorphism (13%) in Hippophae *rhamnoides*. UPGMA clustering indicated genetic differentiation both between and within populations, suggesting distinct genetic origins and potential cross-pollination. The observed high within-population polymorphism and clustering pattern highlight genetic differentiation of Hippophae *rhamnoides* forms according to their origin. This study is preliminary, and further research should include populations from other technogenically affected regions, as well as comparative analyses with populations from protected areas.

Keywords: ISSR markers, molecular analysis, polymorphism, population, *Hippophae rhamnoides*

Introduction

Genetic diversity or variability of living organisms is an important indicator of the evolutionary development of any species, its ability to adapt to environmental conditions, and its preservation as a species by forming a phylogenetic tree.

Monitoring this indicator is especially important for species that are rare or endangered, have small populations, as well as for species that are domesticated or grown in special botanical gardens. Currently, in all countries of the world, the sustainable use of natural genetic resources, including the study of the genetic diversity of various populations in their range and the conservation of endangered species in situ, is one of the most important issues [1].

Although people have used sea buckthorn (*Hippophae* L., Elaeagnaceae) for thousands of years, its significant economic importance has only recently been appreciated. *Hippophae rhamnoides* (sea buckthorn) is a member of the berry family and its fruits contain a high level of active biological compounds [2–5]. It is currently used in many areas of medicine, food, agriculture, and cosmetology in Europe and Asia [6].

In addition, sea buckthorn is currently the subject of food security and sustainable use of genetic resources programs [7-8]. Therefore, it is necessary to take measures to preserve and rationally use agrobiologically important sea buckthorn species in their natural habitat.

Despite the widespread use of sea buckthorns in our country, research on the distribution of its species and their genetic diversity is much less than research on the phytochemical composition of sea buckthorns. In Kazakhstan, detailed information is available only for a single species of sea buckthorn and its subspecies [9-10]. Therefore, investigating populations of this highly valuable and climate-resilient member of the Elaeagnaceae family using ISSR (Inter Simple Sequence Repeat) markers will allow for the assessment of its genetic diversity and distribution in Kazakhstan, reconstruction of its phylogenetic relationships, and the establishment of a DNA bank.

The aim of our study is to evaluate the genetic diversity of *Hippophae rhamnoides*, a poorly studied sea buckthorn species distributed across technogenic zones in Kazakhstan, using ISSR markers.

Experimental

Plant seed samples were collected during a field botanical study conducted in the spring–autumn period of 2023-2024 in the Prigorodny Forest District, Kostanay Forestry of the Kostanay region, located in Northern Kazakhstan.

The research was carried out in the Laboratory of Molecular Genetics and Plant Biotechnology at the Institute of Botany and Phytointroduction under the Committee of Forestry and Wildlife, Ministry of Ecology and Natural Resources of the Republic of Kazakhstan.

A total of six seed and fruit samples were collected, representing two plant populations. The first population included three forms: Hr 80, Hr 81, and Hr 82; the second population comprised three forms as well: Hr 78, Hr 32, and Hr 34. The map shows sample collection points (Fig. 1).

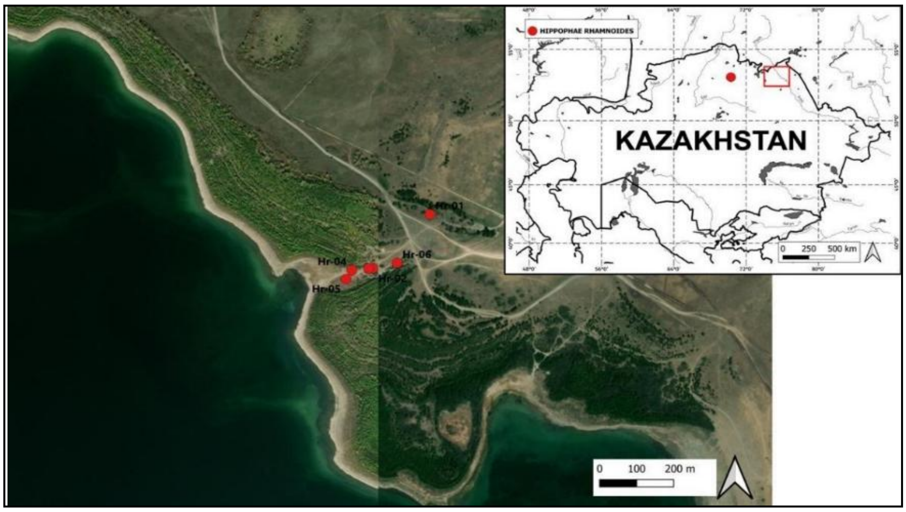

Figure 1. Area for collecting of *Hippophae rhamnoides* forms

Obtaining seedlings as biological material from Hippophae rhamnoides seed samples

To obtain seedlings from *Hippophae rhamnoides* seeds, a special coconut substrate was used. Initially, the seed samples were rinsed with distilled water to remove various contaminants or residual substances. After rinsing, the seeds were placed in a refrigerator at +5 °C for 30–40 minutes. While the seeds were undergoing cold stratification, 100 g of dense, solid coconut substrate was softened using tap water and left to swell. The prepared substrate was then divided into six plastic containers. The chilled seed samples were placed on the surface of the substrate, avoiding deep placement, as light exposure is essential for sea buckthorn seed germination. The containers were covered and stored in a dark place at a temperature of 20–25 °C for one week, until germination began. Seed germination was observed after one week, with the appearance of the first leaves. The plastic containers were placed in a special room with a lamp, without a cover. The substrate was monitored every 4-5 days and watered with 5–10 mL of tap water as needed. Within two weeks, the first seedlings were obtained for use as biological material.

DNA extraction from seedling samples

For DNA extraction, seedling samples were carefully removed from the coconut substrate using sterilized tweezers and rinsed thoroughly with distilled water. DNA was extracted using the DiamondDNA^{TM} Plant DNA Extraction Kit (Altaibiotech, USA), specifically designed for isolating genomic DNA from plant tissues.

To determine the exact concentration of DNA molecules extracted from the plant, a MAXLIFE fluorimeter from Diamond DNA^{TM} was used. DNA concentrations were measured between 0.5 and 2000 ng/μl (Tab. 1).

T a b l e 1

Concentration of DNA molecules extracted from *Hippophae rhamnoides* seeds, measured using the MAXLIFE fluorimeter

Form	Population	DNA concentration (0,5-2000 ng/μl)
Hr80	1	56.0
Hr81	1	56.0
Hr82	1	53.3
Hr78	2	40.0
Hr32	2	66.6
Hr34	2	40.0

The presence or absence of DNA fragments was verified using 1.5 % agarose gel electrophoresis in a horizontal electrophoresis chamber at 45 V and 0.45 A for 20 minutes (Fig. 2). For visualization of DNA on the gel, 2.5 μL of the DNA sample was mixed with 1.5 μL of a specialized 6x DNA Loading Buffer (Glycerine, Xylencyanol) and 7 μL of ddH_2O. As a control, *Thermo Scientific 6x DNA Loading Dye* was used to ensure proper migration and comparison.

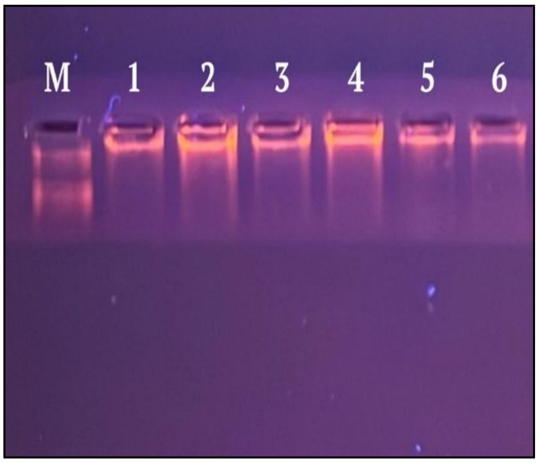

Figure 2. Electrophoresis of DNA isolated from sea buckthorn seedlings

Polymerase Chain Reaction

PCR was performed on a VeritiPro thermocycler (Thermo Fisher Scientific, USA). For 20 μl of the ISSR-PCR solution, a mixture was prepared containing: 1 μl of DNA, 10 μl of 2x HS Taq Red Mix, 1.3 μl of 10 pM primer, 7.7 μl of ddH2O. ISSR-PCR mode: initial denaturation — 94 °C, 01:30 min., denaturation — 94 °C, 00:40 min., primer annealing — 45 °C, 00:45 min., elongation — 72 °C, 01:30 min x 36 cycles, final elongation step — 72 °C for 6 min. The names and nucleotide sequences of the ISSR primers used in the study, along with information on their annealing temperatures, are shown in Table 2.

T a b l e 2

ISSR primer sequences used in the study

№	Primer Name	Primer Sequence (5′ → 3′)	Tm, ^{0}C
1	UBC-810	5'- GAG AGA GAG AGA GAG AT -3'	45
2	UBC-812	5'- GAG AGA GAG AGA GAG AA -3'	45
3	ISSR-3A37	5'- CAC ACA CAC ACA CAT GA -3'	45
4	HB-12	5'-CAC CAC CAC GC -3'	45
5	17899B	5'-CAC ACA CAC ACA GG-3'	45
6	X10	5'-AGC ACG ACG ACG ACG ACG C -3'	45
7	814	5'-CTC TCT CTC TCT CTC TTG-3'	45
8	MAO	5'-TCA GAC GAT GCG TCA TCT CCT CCT CCT CRC -3'	45

Horizontal agarose gel electrophoresis

To confirm the successful synthesis of specific marker loci in the PCR products, electrophoretic analysis was performed. A 1.5 % agarose gel was prepared using 3 g of agarose, 200 mL of 0.5x TBE buffer, and 35 μL of ethidium bromide solution. The PCR products (10 μL) were loaded onto the gel, and electrophoresis was carried out in 0.5x TBE buffer for 2 hours and 15 minutes. The resulting gel was visualized using a UV transilluminator to detect DNA bands.

Results and Discussion

Analysis of genetic diversity of Hippophae rhamnoides using ISSR markers

To select the most polymorphic ISSR-PCR primers, a preliminary screening of eight ISSR primers, selected according to previously published studies [11-12], was performed on six samples of Hippophae rhamnoides. The selected primers proved to be effective in our research [13]. One of the advantages of ISSR markers is that their application does not require prior knowledge of the genome's nucleotide sequence [14-15].

Among the tested markers, four primers—ISSR-3A37 (Fig. 3), HB-12, UBC-810, and UBC-812 were identified as the most informative and were selected for further analysis. A total of 197 ISSR DNA fragments were identified from the DNA fragments studied, of which 39 fragments were polymorphic for the ISSR-3A37 marker, 38 fragments were polymorphic for the HB-12 marker, 44 fragments were polymorphic for the UBC-810 marker, and 76 fragments were polymorphic for the UBC-812 marker. The most polymorphic marker was the UBC-812 marker.

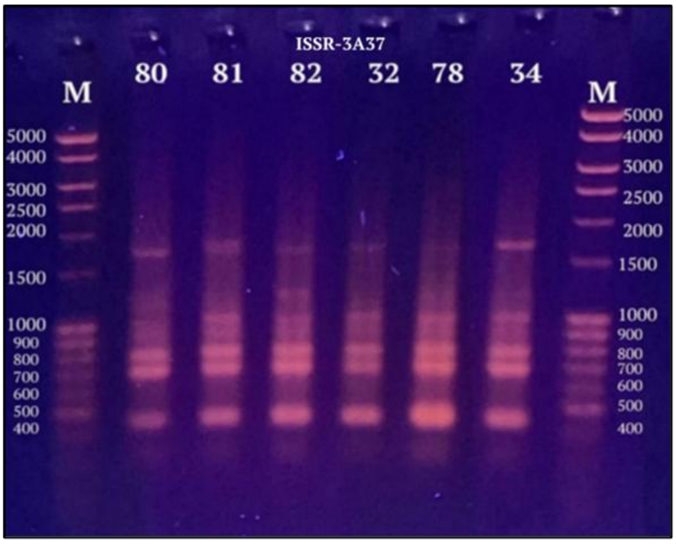

Figure 3. Electrophoregram of the ISSR-PCR product for the ISSR-3A37 marker

Based on the frequencies of DNA fragments, key indicators of genetic diversity for two *Hippophae rhamnoides* populations collected from compartments 62 and 66 of the Prigorodny Forest District (Kostanay Region) were calculated using GenAlEx 6.5 software.

For ISSR markers, the mean number of alleles (Na) typically varies between 0 and 1, though in some cases it may exceed 1. In this study, the Na value for the first population was 0.957 ± 0.08, while for the second population, it was 1.304 ± 0.06. The overall mean Na across both populations was 1.130 ± 0.058.

The effective number of alleles (Ne) reflects not only the number of alleles per locus but also their evenness in distribution. According to the results, Ne was 1.122 ± 0.04 in the first population and 1.243 ± 0.05 in the second population, demonstrating consistency between the effective and average allele numbers.

Shannon information index (I) [16] is one of the indices that assess the diversity of species in an ecosystem. It calculates whether the research population is always full and whether there are many or few species. The Shannon information index (I) for the first population is 0.097 ± 0.034. The Shannon information index (I) for the second population is 0.194 ± 0.044. The Shannon information index (I) for the two populations in total was 0.145 ± 0.028.

In addition, the unbiased diversity (uh) index based on alleles was considered. This index allows us to assess genetic diversity based on allele frequencies and the size of the analyzed samples. The values of this index range from 0 to 1, where 0 indicates low diversity and suggests that all analyzed samples belong to the same species. A value of 1 indicates the presence of multiple species. For the first population, the unbiased diversity index (uh) was 0.101 ± 0.036. For the second population, the unbiased diversity index (uh) was 0.203 ± 0.046. The overall unbiased diversity index (uh) across the two populations of sea buckthorn was 0.152 ± 0.029. These results suggest that the plant samples from both populations are likely to belong to the same species, *Hippophae rhamnoides*. If the uh index is close to 1, it can be assumed that there are subspecies of the species *Hippophae rhamnoides*.

The Shannon diversity index and the unbiased diversity index (uh) are similar to each other. This is because both consider the diversity of species in the study area. The main difference between these indices is that the unbiased diversity (uh) based on alleles is used to analyze genetic diversity, while the Shannon information index is used to assess species diversity in an ecosystem.

The sea buckthorn plant samples exhibited 87 % within-population variation, while the between-population variation accounted for 13 % (Fig. 4).

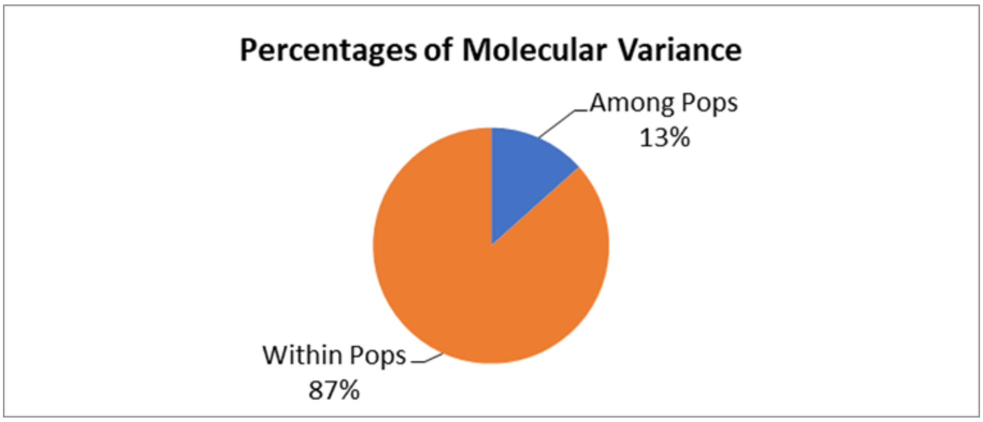

Figure 4. Within-population and between-population variation in *Hippophae rhamnoides*

As a result of the UPGMA cluster analysis, the studied forms of the sea buckthorn plant were divided into two separate groups: the first population and the second population.

The forms in the first group were further divided into three clusters. The first cluster included the Hr81 and Hr82 forms. These forms belong to the same population, which suggests a close genetic origin. The second cluster consisted solely of the Hr32 form. Although Hr32 belongs to the second population, its clustering with the first group may be explained by cross-pollination. The third cluster contained only the Hr80 form, which was genetically distinct from Hr81 and Hr82, likely due to differences in origin. The second group comprised the Hr78 and Hr34 forms, both belonging to the second population. This group was divided into two separate clusters, indicating that despite originating from the same population, these forms exhibit signs of genetic differentiation (Fig. 5).

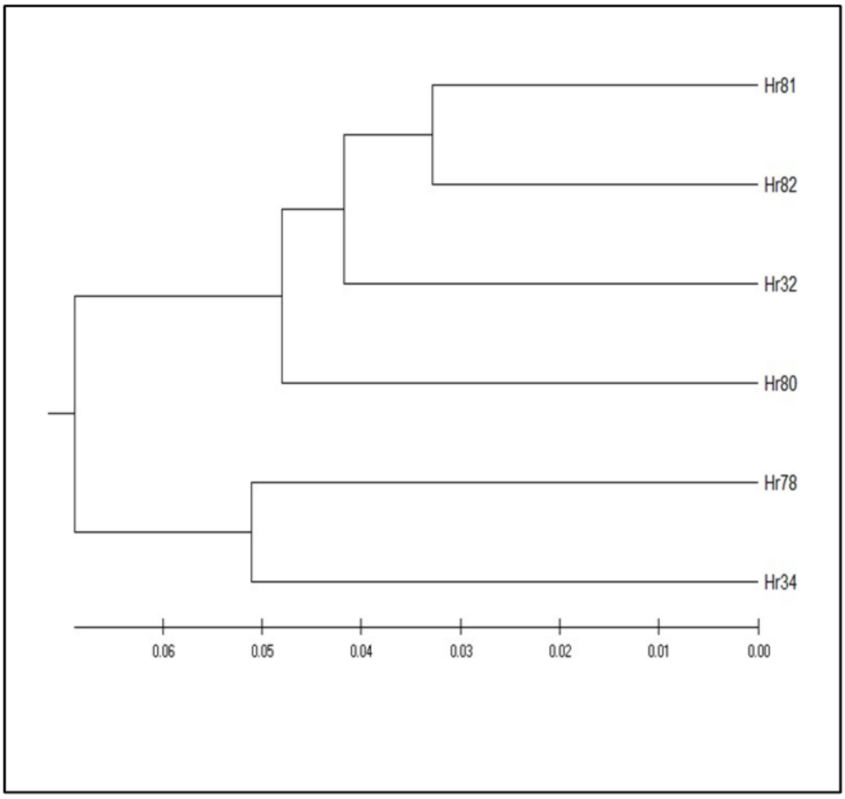

Figure 5. UPGMA dendrogram of the plant forms of the sea buckthorn using the program MEGA 7

Conclusion

Based on the obtained ISSR DNA fragments, the level of within-population polymorphism was 87 %, while the between-population polymorphism was 13 %.

The UPGMA cluster analysis revealed that the studied forms of *Hippophae rhamnoides* were divided into two separate groups corresponding to the first and second populations. The forms in the first group were further separated into three clusters. The first cluster included the Hr81 and Hr82 forms. The second cluster consisted solely of the Hr32 form, which belongs to the second population. Its placement in the first group is explained by the possibility of cross-pollination. The third cluster included only the Hr80 form.

The second cluster group consisted of the Hr78 and Hr34 forms of the second population, which formed separate individual clusters.

The high level of within-population polymorphism (87 %) and the clustering pattern indicate that the *Hippophae rhamnoides* forms exhibit genetic differentiation associated with their origin. However, this study represents only a preliminary stage in the genetic assessment of sea buckthorn populations. To obtain a more comprehensive understanding of the species' genetic diversity and adaptive potential, it is necessary to expand the analysis to include populations from other technogenically-transformed regions, as well as to compare them with populations growing in protected areas.

Acknowledgements

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Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript: **Shadmanova L.Sh. —** conceptualization, data curation, investigation, methodology, editing; **Kulboldin T.S. and Token A.I. —** data curation, formal analysis, supervision, writing draft; **Kanapin Ch.B., Mukan G.S., Akhatov K.Zh., Yeszhanova A.S. and Razhanov M.R. —** conducting the fieldwork and sample collection.

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Л.Ш. Шадманова, Т.С. Кульболдин, А.И. Төкен, К.Б. Канапин, К.Ж. Ахатов, Г.С. Мұқан, А.С. Есжанова, М.Р. Ражанов

Солтүстік Қазақстанның техногендік экожүйелеріндегі *Hippophae rhamnoides* популяцияларының генетикалық әртүрлілігі

Генетикалық әртүрлілік түрдің эволюциялық дамуының, қоршаған орта өзгерістеріне бейімделуінің және ұзақ мерзімді сақталуының негізгі көрсеткіші. Әлемдік деңгейде генетикалық ресурстарды орнықты пайдалану мен сақтау, соның ішінде *in situ* қорғау және популяциялық-генетикалық зерттеулер басты ғылыми басымдықтардың бірі саналады. Итшомырт шырғанақ (*Hippophae rhamnoides* L., Elaeagnaceae) ғасырлар бойы дәстүрлі түрде қолданылып келген, алайда оның жоғары экономикалық құндылығы тек соңғы уақытта мойындалды. Оның жемістері биологиялық белсенді қосылыстарға бай, сондықтан бұл түр Еуропа мен Азияда медицинада, ауыл шаруашылығында, тамақ өнеркәсібінде және косметологияда барған сайын маңызға ие болуда. Осы зерттеуде Солтүстік Қазақстанның техногендік аймақтарында өсетін итшомырт шырғанақтың (*Hippophae rhamnoides*) генетикалық әртүрлілігі ISSR-маркерлерді қолдану арқылы бағаланды. Молекулалық талдау үшін Қостанай облысындағы Пригородный орманшылығынан екі популяциядан тұқым үлгілері жиналды. ISSR-талдау *Hippophae rhamnoides* үшін жоғары деңгейдегі ішкіпопуляциялық полиморфизмді (87 %)

және төмен деңгейдегі аралық популяциялық полиморфизмді (13 %) көрсетті. UPGMA кластерлеуі популяциялар арасында және олардың ішінде генетикалық дифференциацияны анықтады, бұл әртүрлі генетикалық шығу тегі мен ықтимал айқас тозаңдануды көрсетеді. Жоғары ішкіпопуляциялық полиморфизм және кластерлеу сипаты *Hippophae rhamnoides* формаларының шығу тегіне байланысты генетикалық дифференциациясын айқындайды. Бұл зерттеу алдын ала сипатқа ие, әрі қарайғы жұмыстар басқа техногендік аймақтардағы популяцияларды және қорғалатын аймақтарда өсетін популяциялармен салыстырмалы талдауды қамтуы тиіс.

Кілт сөздер: ISSR-маркерлер, молекулалық талдау, полиморфизм, популяция, *Hippophae rhamnoides*

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Генетическое разнообразие популяций *Hippophae rhamnoides* в техногенных экосистемах Северного Казахстана

Генетическое разнообразие является ключевым показателем эволюционного развития вида, его адаптивности к изменениям окружающей среды и долгосрочного выживания. Во всем мире устойчивое использование и сохранение генетических ресурсов, включая *in situ* охрану и популяционно-генетические исследования, является важнейшим научным приоритетом. Облепиха крушиновидная (*Hippophae rhamnoides* L., Elaeagnaceae) традиционно используется на протяжении веков, но лишь недавно была признана видом с высоким экономическим потенциалом. Ее ягоды богаты биологически активными соединениями, что делает вид все более востребованным в медицине, сельском хозяйстве, пищевой промышленности и косметологии в Европе и Азии. В данном исследовании оценено генетическое разнообразие облепихи (*Hippophae rhamnoides*), произрастающей в техногенных зонах Северного Казахстана, с использованием ISSR-маркеров. Для молекулярного анализа были собраны семена из двух природных популяций Пригородного лесничества Костанайской области. ISSR-анализ показал высокий уровень внутрипопуляционного полиморфизма (87 %) и низкий уровень межпопуляционного полиморфизма (13 %) у *Hippophae rhamnoides*. Кластеризация методом UPGMA выявила генетическую дифференциацию между и внутри популяций, что указывает на различное генетическое происхождение и возможное перекрестное опыление. Высокий уровень внутрипопуляционного полиморфизма и характер кластеризации указывают на генетическую дифференциацию форм *Hippophae rhamnoides* в зависимости от их происхождения. Данное исследование носит предварительный характер и является лишь первым шагом в генетической оценке популяций облепихи. Для более полного понимания генетического разнообразия и адаптивного потенциала вида необходимо расширить анализ, включив в него популяции из других техногенно трансформированных регионов, а также провести сравнение с популяциями, произрастающими в охраняемых зонах.

Ключевые слова: ISSR-маркеры, молекулярный анализ, полиморфизм, популяция, *Hippophae rhamnoides*

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Comparative analysis of the flora of fruit and berry plants of the Mangistau and Atyrau regions using biodiversity indices

The article presents the results of a comprehensive analysis of the floristic and taxonomic diversity of fruit and berry plants in Western Kazakhstan (Mangistau and Atyrau regions). Field studies conducted in 2024-2025 revealed more than 60 species, mainly belonging to the families *Rosaceae*, *Caprifoliaceae*, *Elaeagnaceae* and *Grossulariaceae*. The dominance of the Rosaceae family was confirmed; the genera *Crataegus, Rosa*, *Lonicera* and *Ribes* form the basis of shrub-tree communities in the region. The use of the Shannon, Simpson, Margalef, Pielou and Jaccard indices made it possible to quantitatively assess both alpha and beta diversity of the flora. It was found that the Akmysh and Kogez sites form the most similar floristic group in composition, whereas Inderbor is characterized by pronounced isolation and uniqueness of the species composition. The Zheltau mountain range functions as a transit zone uniting the floras of the Mangistau and Atyrau regions. The results indicate a highly mosaic of the floristic cover, significant ecological plasticity of fruit and berry plants and, at the same time, the vulnerability of certain endemic and rare taxa. They are of great importance for developing strategies for the protection of biodiversity, preserving the gene pool and the prospective use of economically valuable species in breeding and introduction programs.

Keywords: Western Kazakhstan; flora; fruit and berry plants; biodiversity; Rosaceae; Shannon index; Jacquard index; arid ecosystems; floral mosaic; gene pool conservation

Introduction

The study of wild fruit and berry plants is one of the key areas of modern botany, ecology, resource plant growing and plant conservation. These species play an essential role in the formation of natural ecosystems, maintaining biodiversity and preserving the genetic fund, which is of strategic importance for the tasks of selection, introduction and ensuring food security. In the context of increasing anthropogenic pressure and global climate change, the relevance of a comprehensive analysis of the state, structure and adaptive potential of fruit and berry plants increases many times over [1].

Western Kazakhstan, including the Mangistau and Atyrau regions, is a unique model region for studying the adaptation strategies of flora in conditions of an extremely arid climate. These territories are characterised by sharply continental climatic conditions, extremely low precipitation, high summer temperatures and a high degree of soil salinity. Despite these limiting factors, the flora is distinguished by its significant richness and originality. In the Atyrau region, there are 899 species of vascular plants, in the Mangistau region, 770, of which fruit and berry plants make up 7.8 % and 16.7 %, respectively [2].

The taxonomic structure of fruit and berry plants in the region shows the dominance of the Rosaceae family, which unites the most significant number of economically valuable species, while representatives of the Caprifoliaceae, Elaeagnaceae, Grossulariaceae, Moraceae, Nitrariaceae and Peganaceae families also participate in the formation of communities. Of most significant interest are representatives of the genus *Crataegus* Tourn. Ex L., *Rosa* L., *Ribes* L. and *Lonicera* L., confined mainly to mountain and foothill biotopes. Among them, there are both widespread species (*Crataegus sanguinea* Pall., *Lonicera tatarica* L., *Rosa laxa* Retz.), and narrow-range endemics, *Crataegus ambigua* CA. Mey. ex A.K. Becker, *Rosa iliensis* Chrshan.), as well as species with limited competitiveness (*Ribes aureum* Pursh) [3].

To identify patterns of floristic diversity and spatial structure of communities, quantitative biodiversity assessment methods have been actively used in recent decades. The most common indicators are the Shannon index (characterising species richness and uniformity of distribution of individuals), the Simpson index (assessment of dominance of individual taxa), as well as the Margalef index and the Pielou index (measures of alpha diversity). In the context of assessing floristic similarities and differences between communities, the Jaccard index has been widely used, reflecting the degree of overlap of species composition between different localities and allowing for the identification of both zones of high similarity and areas of unique flora. The use of these indices provides an objective statistical basis for analysing the stability of communities, their population dynamics and ecological-cenotic structure [4].

The peculiarity of the flora of Western Kazakhstan is that fruit and berry plants form stable or transitional phytocenoses, where their high ecological plasticity and ability to adapt to arid ecosystems are manifested. At the same time, several species (for example, *Crataegus ambigua* and *Ribes aureum*) show signs of vulnerability and reduction of habitats, which makes them objects of priority protection. The use of diversity indices in combination with the analysis of the age structure of populations and morphological variability allows us to identify patterns of spatial differentiation, the degree of stability of cenoses and the factors determining their dynamics [5].

Thus, the need for a comprehensive study of wild fruit and berry plants in the Mangistau and Atyrau regions is due to: the strategic importance of preserving the gene pool in the context of climate change and ecosystem degradation; the need to analyze the ecological and cenotic characteristics and structure of populations; the use of biodiversity indices and the Jaccard index for an objective assessment of species richness and floristic similarity; prospects for using the identified taxa in breeding programs and introduction.

The study aims to identify the floristic and species diversity of fruit and berry plants in the Mangistau and Atyrau regions, assess their ecological and cenotic characteristics, intraspecific variability and adaptive potential, and use quantitative indices of biodiversity to identify patterns of distribution and the degree of similarity of plant communities.

Experimental

Field botanical studies were conducted in the summer of 2024-2025 in the territory of the Mangistau and Atyrau regions, covering the main phytocenotic areas of Western Kazakhstan. Areas with different orography, soil and climate conditions, and the degree of anthropogenic transformation were selected as key sampling points: the Zheltau mountain range, the Inderbor plain-foothill zone (Atyrau region), as well as the Karatau mountain range (Kogez and Akmysh gorges) and the Karaturan gorge in the mountains of South Aktau (Mangistau region) (Fig. 1–4).

A B

Figure 1. *Crataegus altaica* in the Zheltau mountains
A — a single bush towards Mangistau;
B — undergrowth towards Atyrau

A B

Figure 2. Hawthorn dubious in the gorges of Akmysh (A) and Zhemsemsay (B)

A B

Figure 3. Hawthorn dubious in the gorges of Samal (A) and Kogez (B)

A B

Figure 4. Tatar honeysuckle in populations: A — Zheltau Mountains; B — Kolenkeli Mountains

The selection of biological material was carried out according to standard geobotanical methods, with preliminary route binding, visual assessment of the state of the vegetation cover and fixing the coordinates using a Garmin eTrex 32x GPS device. For each site, an inventory of the flora was carried out with the recording of all encountered species of fruit and berry plants, taking into account their phenological state, ecological confinement and degree of dominance in the community [6].

The floristic structure of plant communities was assessed based on the analysis of the binary matrix of species presence/absence formed for the five study sites. For each pair of sites, the Jaccard index was calculated, reflecting the degree of floristic similarity and allowing one to estimate the level of species overlap between different geographic points. The Jaccard index was calculated using the formula:

$$J = \frac{a}{(a+b+c)}$$

Where a is the total number of species in the first plot, b is in the second plot, and c is the number of common species. Values ranged from 0 (no matches) to 1 (complete match of species composition). Additionally, Shannon (H'), Simpson (D), Margalef (DMg), and Pielou (J') indices were used for a comprehensive assessment of biodiversity, allowing for a quantitative characterization of alpha diversity, evenness, and dominance of taxa within local communities [7].

All numerical calculations were performed in the Microsoft Excel 365 spreadsheet environment, as well as in the PAST v4.13 statistical program. The obtained values were visualized in the form of heat maps, overlap diagrams and cluster schemes using built-in Excel tools and graphic modules of the PAST software package. Photographic recording of the floristic features of the sites and representatives of the studied taxa was carried out using a Canon EOS 2000D digital camera with macro settings and a standardised frame scale. Each image was provided with a geographic mark and a brief description of the biotope [8, 9].

The methodological approach implemented within the framework of this study ensured the comparability of data between sites, statistical reliability of conclusions and the possibility of identifying spatial and ecological patterns of formation of flora of fruit and berry plants in the conditions of arid landscapes of Western Kazakhstan.

Results and Discussion

Floristic analysis of fruit and berry plants of the Mangistau and Atyrau regions revealed a significant diversity of taxa, mainly belonging to the family *Rosaceae*. Based on the results of route studies and compiled floristic lists, more than 60 species were recorded, represented by both shrub and tree forms. Among them are representatives of the genera Crataegus, *Rosa*, *Lonicera*, and *Ribes*, which form the basis of shrub-tree communities in mountain and foothill biotopes.

Of particular note are such economically and ecologically significant species as *Crataegus ambigua*, *Rosa laxa*, *Lonicera tatarica*, and *Ribes aureum*. Their occurrence in most of the surveyed areas indicates high adaptability to arid conditions and a key role in maintaining the stability of communities. At the same time, *Ribes aureum* and some endemic representatives (*Crataegus ambigua*) show signs of local vulnerability and population decline, which confirms the need for their priority protection.

A comparative analysis of the species lists (Tab. 1) revealed that the most incredible diversity is found in the Zheltau and Akmysh mountain ranges, where both widespread species (*Ephedra distachya*, *Rosa laxa*, *Spiraea hypericifolia*) and rare endemics are found. The Inderbor and Karaturan sites, on the contrary, showed lower floristic richness and relatively high flora isolation.

Table 1

Species composition

Species	**Gorges**				
	Zheltau	**Akmysh**	**Kogez**	**Inderbor**	**Karaturan**
Achillea nobilis	+				
Agropyron desertorum	+				
Agropyron fragile				+	+
Alhagi pseudalhagi					+
Allium sabulosum				+	
Alyssum dasycarpum	+				
Anabasis Salsa	+				
Armeniaca vulgaris		+	+		
Artemisia austriaca		+	+		
Artemisia lercheana				+	
Artemisia lessingiana					+
Artemisia terrae albae	+				
Atraphaxis replicata	+	+			+

Continuation of Table 1

Species	Gorges				
	Zheltau	Akmysh	Kogez	Inderbor	Karaturan
Atraphaxis spinosa	+				
Bromus tectorum	+				
Camelina sylvestris			+		
Caragana grandiflora		+	+		
Centaurea adpressa	+				
Centaurea squarrosa	+				
Chorispora tenella			+		
Cichorium intybus			+		
Convolvulus fruticosus		+			
Crataegus altaica	+				
Crataegus ambigua	+	+	+		
Echinops ritro	+				+
Ephedra distachya	+				+
Ephedra lamotolepis				+	
Eremopyron bonaepartis					+
Euphorbia seguieriana				+	
Haplophyllum obtusifolium					+
Herb variety	+	+	+		+
Inula britannica			+		
Kochia prostrata	+			+	
Lactuca serriola				+	
Lagochilus acutilobus		+			
Lavatera thuringiaca				+	
Limonium gmelinii	+				
Lonicera tatarica	+				
Malacocarpus critmifolium	+				+
Malus sieversii			+		
Marrubium vulgare	+			+	
Medicago caerulea					+
Melilotus albus			+		
Mentha longifolia		+			
Meristotropis triphylla		+			
Morus alba					+
Nepeta cataria	+		+	+	+
Plantago salsa				+	
Poa bulbosa	+			+	+
Potentilla pedata				+	
Potentilla supina					+
Prunus spinosa			+		
Rhamnus sintenesii	+	+	+		+
Ribes aureum			+		+
Rosa acicularis			+		
Rosa iliensis			+		
Rosa laxa	+	+	+	+	
Rose iliensis			+		
Rubus caesius				+	
Salix alba			+		
Scandix stellata			+		
Schumannia karelinii					+
Silene suffrutescens	+				
Spiraea hypericifolia	+			+	
Stellaria media			+		
Stipa capillata	+				
Teucrium polyum		+	+		
Verbascum soongaricum				+	

The application of the Jaccard index allowed us to quantitatively assess the degree of similarity of the floristic complexes (Tab. 2).

T a b l e 2

Jaccard index

Region 1	Region 2	Jaccard index
Zheltau	Akmysh	0.14
Zheltau	Cohesion	0.11
Zheltau	Inderbor	0.15
Zheltau	Karaturan	0.21
Akmysh	Cohesion	0.28
Akmysh	Inderbor	0.03
Akmysh	Karaturan	0.10
Cohesion	Inderbor	0.05
Cohesion	Karaturan	0.10
Inderbor	Karaturan	0.09

The highest degree of similarity was observed between the Akmysh and Kogez sites (J = 0.29), indicating their similar shrub–tree community composition and comparable orographic and climatic conditions. A moderate level of similarity was observed between the Zheltau–Karaturan (J = 0.22) and Zheltau–Inderbor (J = 0.16) regions, which may be attributed to the presence of floral elements typical of desert–mountain environments. The minimum values of the Jaccard index were recorded for the Akmysh–Inderbor (J = 0.03) and Kogez–Inderbor (J = 0.05) pairs, reflecting the isolation of the flora of the Inderbor region and its unique ecological profile.

The analysis carried out confirms that the flora of Inderborforms a relatively independent block, while Akmysh and Kogez form a more closely related floristic group. Zheltau occupies an intermediate position, showing partial overlap with most sites and acting as a transit zone between the floras of Mangistau and Atyrau regions (Fig. 5).

The clustering dendrogram (Fig. 6) confirms that the flora of the Inderbor plateau forms a relatively independent block, while Akmysh and Kogez form a more closely related group. Zheltau occupies an intermediate position, acting as a kind of transit zone between the floras of the Mangistau and Atyrau regions. Similar results on the role of transitional areas and clustering of floras were also noted in the study of biocenoses of the desert-mountain regions of Central Asia.

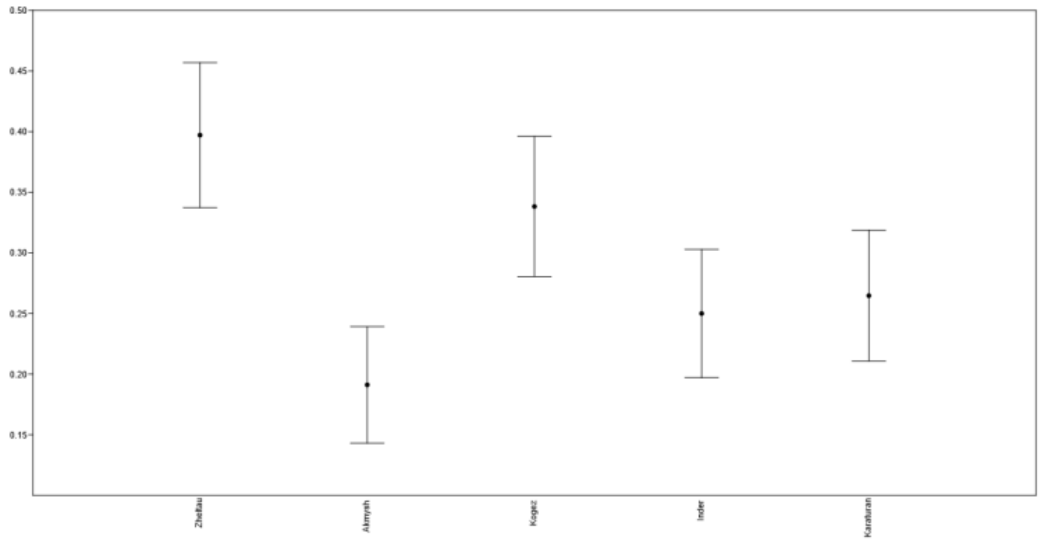

Figure 5. Clustering of floristic similarity of the studied areas of Western Kazakhstan based on the Jaccard index

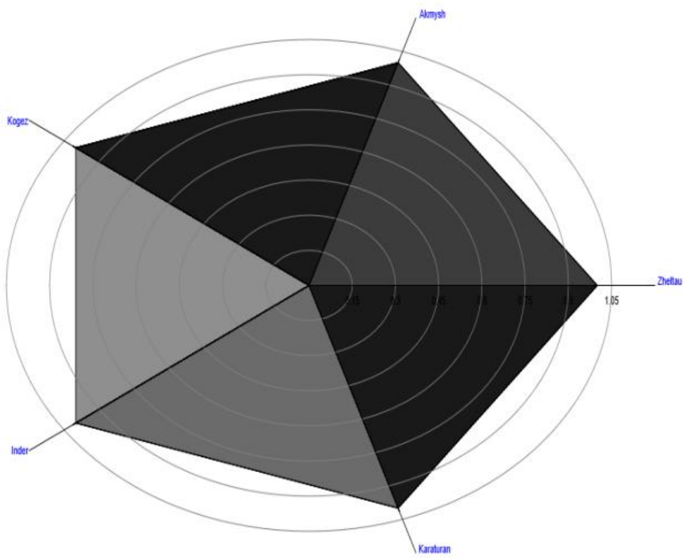

Figure 6. Diagram of overlapping species composition between the studied regions (Venn diagram)

The Jaccard index is one of the key coefficients for assessing the degree of similarity between biocenoses or floristic lists of different geographical areas. It is calculated as the ratio of the number of common species to the total number of unique species of the two compared areas. Its values vary from 0 (complete difference) to 1 (complete coincidence). Thus, the index allows us to quantitatively assess the degree of floristic overlap and assume the similarity of environmental conditions, migration of species or isolation of areas. Based on the calculations presented in Table 2, the Jaccard index is given.

Figure 7 shows a heat map of the Jaccard indices between regions. The colour intensity reflects the degree of similarity of the floristic compositions: the darker the cell, the greater the similarity between pairs of sites. The greatest similarity: Akmysh–Kogez (J = 0.29). The minimum similarity: Akmysh–Inderbor (J = 0.03).

Figure 7. Heat map of Jaccard indices between pairs of regions
(Zheltau — A, Akmysh — B, Kogez — C, Inderbor — D, and Karaturan — E)

The most remarkable floristic similarity is observed between the Akmysh and Kogez gorges of the mountainous Karatau (J = 0.2857). These sites demonstrate the most significant degree of similarity among all pairs, which is probably due to geographical proximity, similarity of mountain-valley ecosystems, the presence of dominant species (*Crataegus ambigua*, *Rosa laxa*, *Rhamnus sintenesii*) and common environmental conditions. Both territories are located in the Western Karatau of the Mangistau region, where shrub-tree communities are developed and good plant regeneration is observed.

An average level of similarity was found between the Zheltau Mountains and the Karaturan Gorge (J = 0.2162), indicating a moderate coincidence of flora between the Zheltau and South Aktau ridges, probably due to typical desert-mountain elements.

The flora of Mount Zheltau and Mount Inderbor (J = 0.1579), as well as Mount Zheltau and the Akmysh gorge (J = 0.1429), show partial overlap, especially in the species Rosa laxa, Lonicera tatarica, and Ephedra distachya.

The least similarity was recorded between the Kogez and Karaturan gorges (J = 0.1081). Despite the remoteness of the sites, a slight overlap in floristic composition is observed, which can be explained by the presence of typical wormwood and shrub species.

Minimal similarity: Akmysh–Inderbor (J = 0.0345), Kogez–Inderbor (J = 0.0526), Inderbor–Karaturan (J =0.0938). These values indicate a significant floristic isolation of the Inderbor site, despite the abundance of shrubs (*Rosa laxa*, *Spiraea hypericifolia*), but with fewer common species than other regions. Perhaps the reason is the excellent ecological niche (deep depressions, unique microclimates) and different soil substrate. The Zheltau Mountains serve as a transitional point between the floras of the Atyrau and Mangistau regions, exhibiting moderate similarity with most areas. Inderbor is characterised as the most isolated floristically point, which may indicate specific environmental conditions or phytocenotic uniqueness of this area. Akmysh and the Cogez, as parts of a single mountain range, form a relatively homogeneous floristic group with a high level of internal similarity. The Jaccard index confirmed the clustering of sites by floristic similarity, with geographic proximity and environmental conditions playing a decisive role in the formation of similarity.

Additional analysis of alpha diversity using the Shannon, Simpson, Margalef, and Pielou indices (Fig. 8) revealed that the highest species richness and uniformity of distribution of individuals are characteristic of the flora of Mount Zheltau and Akmysh gorge of the Karatau mountains. In contrast, Inderbora exhibits a pronounced dominance of individual taxa and low uniformity. This confirms the ecological plasticity of the communities of Mount Zheltau and the vulnerability of the flora of the Inderbor. Analysis of the diversity indices presented in Figure 8 showed stable differences between the studied geographic regions.

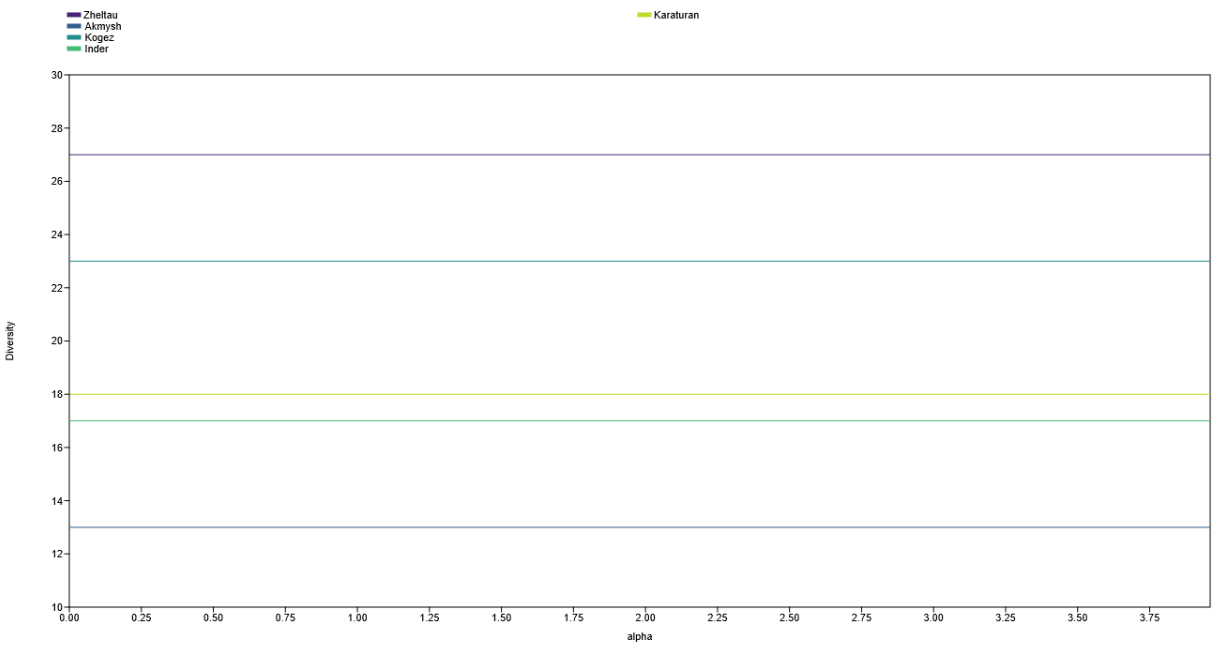

Figure 8. Comparative values of biodiversity indices (Shannon, Simpson, Margalef, Pielou) for the five studied areas

Regardless of the change in the parameter α, the index values remained constant, indicating the stability of the species diversity assessment when using this approach. The lowest index value was recorded for Zheltau Mountains (about 13), which may indicate a relatively low level of floristic richness in this location. Such results may be associated with both abiotic factors (aridity of the climate, poor soil cover) and anthropogenic impact, limiting the restoration processes of biocenoses. Higher indicators are noted in the Akmysh gorges, Mountain Karatau, and Mount Inderbor (about 23), which indicate favourable conditions for maintaining floristic diversity. However, it is worth noting that despite the external similarity of numerical values, differences in the structure of plant communities may be observed, due to different ecological and phytocenotic confinement of species. The maximum value of the index is characteristic of the Kogez gorges of the Karatau mountains (about 28). This result indicates the highest saturation of flora. It is probably associated with a combination of factors—a more diverse relief, the presence of transitional ecotopes and a lower level of economic development of the territory. An intermediate position is occupied by the Karaturan gorges of the mountains of southern Aktau (about 18). The obtained value may reflect a limited set of species groups, while the preservation of floristic diversity in such conditions may depend on the stability of key dominant species. Thus, the conducted analysis confirms the presence of apparent regional differences in the level of species diversity of fruit and berry plants in Western Kazakhstan. The obtained data are consistent with the results of previous studies of the flora of arid ecosystems in Central Asia, where a high spatial mosaic of biodiversity indicators was also noted.

A comparative assessment of the taxonomic structure (Fig. 9) revealed the dominance of the Rosaceae family, which accounts for the most significant number of economically valuable species. Along with this, representatives of Caprifoliaceae (*Lonicera tatarica*), Elaeagnaceae (*Elaeagnus angustifolia*) and Grossulariaceae (*Ribes aureum*) play a significant role. The data obtained are consistent with previously published works, confirming the key role of the Rosaceae family in the formation of the flora of the arid regions of West Kazakhstan. Figure 9 shows the distribution of taxa of fruit and berry plants by families in the Mangistau and Atyrau regions.

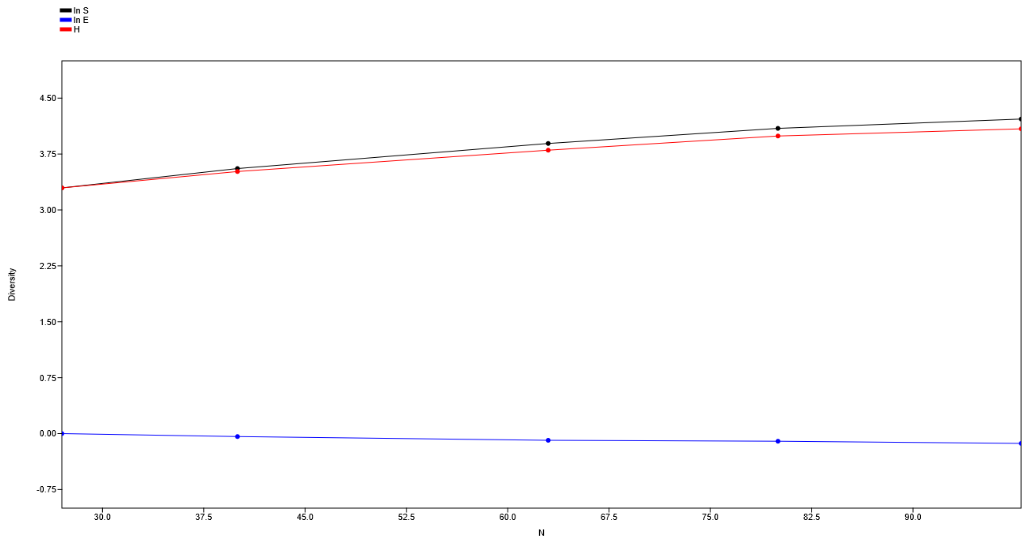

Figure 9. Distribution of taxa of fruit and berry plants by families in the Mangistau and Atyrau regions

The analysis revealed that the diversity indices vary with the number of taxa, but the dynamics differ across the indices used. The hS index (blue) remained virtually unchanged and close to zero with an increase in the number of taxa. This suggests that the index does not accurately capture the differences in the flora's structure across these regions, potentially limiting its usefulness in assessing the actual level of diversity. At the same time, the hE (black) and hT (red) indices demonstrated positive dynamics: with an increase in the number of taxa, their values increased from ~3.2 to ~4.0. Moreover, the hE values were slightly higher compared to hT, which indicates a certain redundancy or evenness of the distribution of taxa by families. This suggests that the flora of the studied areas is characterized by moderate taxonomic diversity with a tendency to increase with an increase in the sample size. Thus, the analysis of taxa distribution shows that the flora of

Mangistau and Atyrau regions has a relatively balanced structure, where several dominant families play the leading role. At the same time, the identified differences between the hE and hT indices allow us to conclude that the distribution of taxa is heterogeneous and that it is essential to take into account different indices when interpreting taxonomic diversity.

The spatial differentiation scheme of flora (Fig. 10) shows that floristic isolation is characteristic of Inderbor Mountain, Atyrau Region. In contrast, the flora of the Akmysh and Kogez gorges of the Karatau Mountains, Mangistau Region, forms the most homogeneous group with a high level of internal similarity. The revealed patterns are essential for developing strategies for the conservation of rare and vulnerable species, as well as for assessing the prospects for their use in breeding and introduction programs.

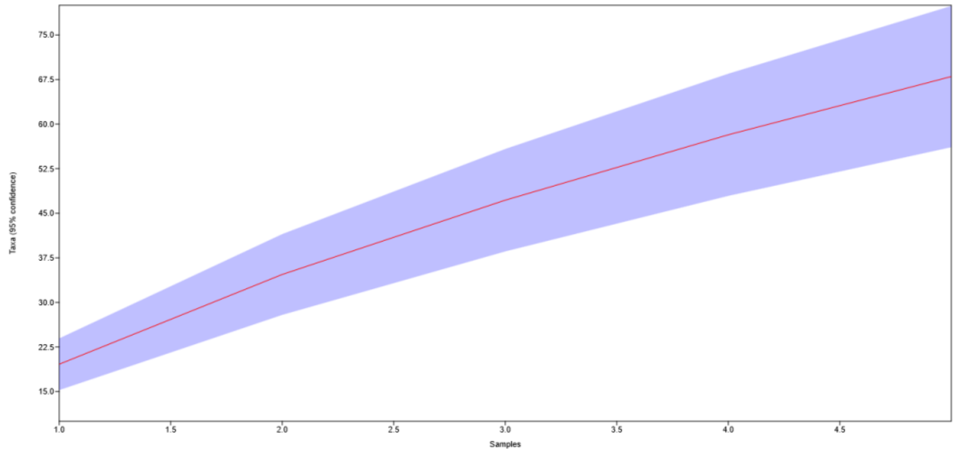

Figure 10. Scheme of spatial differentiation of fruit and berry plant flora
by geographical areas of Western Kazakhstan

As can be seen in Figure 10, with an increase in the number of studied samples, a stable trend towards an increase in the β-diversity index is observed. At the initial stages of the sample (1-2 sites), the values were about 15–30, while with an expansion of the number of sites to five, the index increased to 65–70. Such dynamics indicate a pronounced spatial heterogeneity of the flora of fruit and berry plants in Western Kazakhstan. A wide confidence interval demonstrates the presence of significant differences between individual geographic sites. This confirms that the flora of the region is formed due to a combination of locally specific taxa that are unevenly distributed across the territory. Such a mosaic of the floristic cover is characteristic of arid and semi-arid ecosystems, where even closely located biotopes can differ significantly in the composition and number of species. Thus, the obtained results indicate that spatial differentiation is a key factor in the formation of the floristic diversity of fruit and berry plants in Western Kazakhstan. This is consistent with the findings of other researchers who noted high variability in species composition depending on the geographical and environmental conditions of the region.

Thus, the conducted comprehensive analysis showed that regions highly differentiate the flora of fruit and berry plants of Western Kazakhstan, have a significant presence of economically valuable and endemic species, and have identified zones of both high similarity and uniqueness. This emphasizes the need for priority protection of individual populations and supports the strategic importance of preserving the gene pool of the region in the context of increasing climate change.

Conclusion

A comprehensive study of the floristic and taxonomic diversity of fruit and berry plants in the Mangistau and Atyrau regions revealed a significant wealth of taxa, their spatial mosaicism, and key factors that determine the formation of plant communities. The Rosaceae family plays the most crucial role in the flora; its representatives form the basis of shrub-tree communities and are of high economic value. The use of Shannon, Simpson, Margalef, Pielou, and Jacquard indices showed that the flora of the region is characterised by pronounced regional differences: the Akmysh and Kogez sites form the most homogeneous group with a high level of floristic similarity. At the same time, Inderbor stands out as the most isolated and unique floristic complex. Zheltau serves as a transit zone between the floras of the Mangistau and Atyrau regions. A comparative analysis of the spatial distribution of the flora confirmed that β-diversity increases with an increase in the number of samples, reflecting the high heterogeneity of the floristic cover in arid conditions. This result emphasizes the importance of locally specific taxa and points to the need to preserve unique populations. The results obtained have both fundamental and applied significance: they form a scientific basis for assessing the adaptive potential of fruit and berry plants, developing programs for the protection of rare and vulnerable species, as well as the prospective use of individual taxa for breeding and introduction purposes. In the context of increasing climate change and anthropogenic pressure, the priority task remains the preservation of the region's gene pool, which requires the integration of botanical research with practical measures to protect biodiversity.

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Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was prepared with the contributions of all authors, who have given their approval to the final version. **A.A. Imanbayeva** — conceptualization, project administration, writing, review and editing; **N.I. Duysenova —** investigation, data curation, and plant material collection; **A. Lukmanov** — methodology, formal analysis, and visualization; **G. Bekbosyn** — data curation, statistical analysis.

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Маңғыстау және Атырау облыстарының жеміс-жидек өсімдіктері флорасын биоалуантүрлілік индекстері арқылы салыстырмалы талдау

Мақалада Батыс Қазақстанның (Маңғыстау және Атырау облыстары) жеміс-жидек өсімдіктерінің флоралық және таксономиялық алуантүрлілігін кешенді талдау нәтижелері ұсынылған. 2024–2025 жылдары жүргізілген далалық зерттеулер 60-тан астам түрді анықтады, олардың басым бөлігі Rosaceae, Caprifoliaceae, Elaeagnaceae және Grossulariaceae тұқымдастарына жатады. Rosaceae тұқымдасының басымдылығы расталды; *Crataegus*, *Rosa*, *Lonicera* және *Ribes* туыстары аймақтың бұталы-ағашты қауымдастықтарының негізін құрайды. Шеннон, Симпсон, Маргалеф, Пилу және Жаккар индекстерін қолдану флораның альфа және бета алуантүрлілігін сандық тұрғыда бағалауға мүмкіндік берді. Акмыш пен Көгез учаскелері түрлік құрамы бойынша ең ұқсас флоралық топты құрайтыны, ал Индер ерекше оқшауланған және бірегей түр құрамымен ерекшеленетіні анықталды. Желтау жотасы Маңғыстау мен Атырау облыстары флорасын біріктіретін транзиттік аймақ рөлін атқарады. Алынған нәтижелер флора жамылғысының жоғары мозаикалылығын, жеміс-жидек өсімдіктерінің экологиялық икемділігін және кейбір эндемик пен сирек таксондардың осал екенін көрсетеді. Бұл деректер биоалуантүрлілікті сақтау стратегияларын әзірлеу, генофондты қорғау және шаруашылық маңызы бар түрлерді селекция мен интродукция бағдарламаларында болашақта пайдалану үшін маңызды.

Кілт сөздер: Батыс Қазақстан, флора, жеміс-жидек өсімдіктері, биоалуантүрлілік, Rosaceae, Шеннон индексі, Жаккар индексі, қуаң экожүйелер, флоралық мозаика, генофондты сақтау

А. Иманбаева, Н. Дуйсенова, А. Лукманов, Г. Бекбосын

Сравнительный анализ флоры плодово-ягодных растений Мангистауской и Атырауской областей с использованием индексов биоразнообразия

В статье представлены результаты комплексного анализа флористического и таксономического разнообразия плодово-ягодных растений Западного Казахстана (Мангистауской и Атырауской областей). Полевые исследования, проведенные в 2024–2025 гг., выявили более 60 видов, преимущественно относящихся к семействам Rosaceae, Caprifoliaceae, Elaeagnaceae и Grossulariaceae. Подтверждено доминирование семейства Rosaceae; роды *Crataegus*, *Rosa*, *Lonicera* и *Ribes* формируют основу кустарниково-древесных сообществ региона. Применение индексов Шеннона, Симпсона, Маргалефа, Пилу и Жаккара позволило количественно оценить альфа- и бета-разнообразие флоры. Установлено, что участки Акмыш и Когез формируют наиболее сходную флористическую группу по видовому составу, тогда как Индер отличается выраженной изолированностью и уникальностью флоры. Горный массив Желтау выполняет роль транзитной зоны, объединяющей флору Мангистауской и Атырауской областей. Полученные результаты свидетельствуют о высокой мозаичности флористического покрова, значительной экологической пластичности плодово-ягодных растений и одновременно уязвимости отдельных эндемичных и редких таксонов. Они имеют большое значение для разработки стратегий охраны биоразнообразия, сохранения генофонда и перспективного использования хозяйственно-ценных видов в селекционных и интродукционных программах.

Ключевые слова: Западный Казахстан, флора, плодово-ягодные растения, биоразнообразие, Rosaceae, индекс Шеннона, индекс Жаккара, аридные экосистемы, флористическая мозаичность, сохранение генофонда

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Composition and biological activity of essential oil from aboveground parts of *Artemisia messerschmidtiana*

The study of the chemical composition of biologically active compounds from plants of the natural flora is a promising direction for investigating their biological activity and potential use as sources for the medicinal, pharmaceutical, and cosmetic industries. This article presents the results of an evaluation of the composition and biological activity of essential oil isolated from *Artemisia messerschmidtiana* from the Far East. The essential oil was extracted by hydrodistillation. The obtained essential oil was tested for cytotoxic, fungicidal, antimicrobial, antimalarial, tuberculostatic, and antileishmanial activities. The composition of the extracted essential oil was analyzed by GC–MS methods. The total composition of the essential oil included 43 components, with the highest content observed for 1,8-cineole, camphor, and α-campholenal. Testing of the essential oil revealed weak antimicrobial, fungicidal, and antimalarial activities, and high cytotoxic activity. No antituberculosis or antileishmanial activity was detected in the essential oil. The results contribute to the understanding of the biological activity of components of the genus *Artemisia* L.

Keywords: Artemisia messerschmidtiana, essential oil, biological activity, chromatography-mass spectrometry

Introduction

The study of the composition of essential oils and the biological activity of medicinal plants is an important task for science. Numerous studies have shown that plants of the genus Artemisia L. differ within sections and species in terms of the content of many groups of biologically active substances, including the composition of essential oils. These differences are influenced by various factors, such as soil conditions, differences in moisture, solar insolation, temperature, altitude above sea level, and age characteristics. The composition and quantitative accumulation of individual components are influenced by the timing of raw material collection, fertility and fertilization, drying conditions, and the genetic characteristics of individual plant populations. Differences in chemical composition depend on the plant organ, and the extraction method. A review of the literature on the evaluation of essential oils of the genus *Artemisia* and compounds isolated from them shows that in recent years, numerous studies have been conducted on their antibacterial, antifungal, antiviral, and other anti-infective properties [1].

Despite extensive research on the genus *Artemisia*, not all species have been studied sufficiently, which creates potential for the search for new medicinal compounds in the natural flora.

Artemisia messerschmidtiana Besser (=*Artemisia gmelinii* var. *messerschmidtiana* Poljakov) is a semi-shrub 60–80 cm high of the *Asteraceae* family. It grows in Buryatia, Irkutsk and Chita regions, Krasnodar Krai, and Mongolia (Fig. 1) on slopes with shrubby meadow-steppe vegetation and forest edges [2].

Figure 1. Areas of *A. messerschmidtiana*
(from Artemisia gmelinii var. messerschmidtiana Poljakov | Plants of the World Online | Kew Science)

As a continuing our research on essential oils of wormwood from the Far East [3–6] and Kazakhstan [7–9], we investigated the chemical composition and some kinds of biological properties of *A. messerschmidtiana's* essential oil from aboveground parts.

Previously, South Korean scientists isolated methyl esculin, daphnetin, 6-methyl esculetin, dimethyl daphnetin, esculin, and umbelliferone from *A. messerschmidtiana* raw materials [10].

The study of the aerial part *of A. messerschmidtiana* allowed the isolation of scopoletin, whose structure was established by spectral methods. The antibacterial and cytostatic activity of the dry extract and essential oil was studied. The content of 43 chemical elements in the plant was determined by atomic emission spectrometry. The anatomical structure of *A. messerschmidtiana* Bess. was also studied [11].

Experimental

Collection of raw materials. Raw materials of *A. messerschmidiana* for research were collected in the second decade of September 2017, in an oak forest on the southern steep slope on the left bank of the Razdolnaya (Suifun) River, near the village of Chernyatino, Oktyabrsky District, Primorsky Krai (Russian Federation) (Fig. 2).

Figure 2. A. messerschmidtiana

The herbarium code for *A. messerschmidtiana* sample was 103564. Plant samples were collected into herbarium fund of G.B. Eljakov Pacific Institute of Bioorganic Chemistry, laboratory of chemotaxonomy (Far East Branch of RAS, Vladivostok, Russian federation).

Obtaining essential oil. Essential oil was obtained by hydro distillation methods from aboveground parts with a Clevenenger apparatus, period of extraction was 2 hours [12], using hexane trap.

The essential oil of *A. messerschmidtiana* was tested on anti microbial and fungicidal activity, using the standard strains: *Aspergillus fumigates* ATCC 204305, *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Staphylacoccus aureus* ATCC 29213, MRSATCC 33591, *Pseudomonas aeruginosa* ATCC 27853, *Candida glabrata* ATCC 90030, *Cryptococcus neoformans* ATCC 90113, *Escherichia coli* ATCC35218, and *Mycobacterium intracellulare* ATCC 23068 [13]. The reference preparations were amphotericin B and ciprofloxacin.

Primary screening was performed using a dose 50 μg/ml twice. Inhibition of bacterial and fungal growth (% Ing.) was taken into account in comparison with positive and negative controls. In secondary screening, samples were tested at concentrations ranging from 2 to 50 μg/ml of a suspension of 9 strains of microorganisms, and IC50 was calculated based on the results.

The anti malarial activity of essential oil sample was tested as inhibition strains of *Plasmodium falciparum* (chloroquine-sensitive — D6, and chloroquine-resistant — W2) using negative and positive control. The preparation chloroquine was applied as a negative control. Also the essential oil was tested on the mammalian cellular line VERO. The selectivity index (SI) was assessed as the ratio IC_{50} VERO to IC_{50} D6 or W2.

Study of cytotoxic activity was conducted on nauplii of *Artemia salina* [14]. Mortality and survival rates of larvae after exposure to different concentrations of essential oil were recorded. Mortality (P) index was calculated by the formula:

$$P = (A — B — C) / N \times 100\ \%$$

where, A is the number of dead larvae after 24 hours; B is the number of larvae that died before the start of the test; C is the average number of larvae that died in the negative control; N is the total number of larvae.

Mycobacteria testing was performed using the REMA method [15–18]. Working solutions of essential oil were diluted in Middle Brook 7H9 culture medium with the addition of OADC. 100 mL of Middle Brook 7H9 and essential oil were added to all test wells, with control wells containing no essential oil samples. Activity was assessed by color: blue color indicated no growth of mycobacteria, pink color indicated growth [19]. Isoniazid, amphotericin B, and chlorhexidine dihydrochloride were used as negative controls.

Testing for anti-leishmaniasis activity. The activity of essential oil was evaluated *in vitro* against *L. donovani* promastigotes (Pms) by flow cytometry (FACS). FACS analysis was performed to quantitatively assess fluorescence levels in treated and untreated groups. A decrease in fluorescence intensity indicated inhibition of parasite growth. J774 macrophages ($5x10^5$ cells per well) in 12-well culture plates were infected with Pms at a ratio of 10:1. The infection level in infected macrophages before and after treatment with the preparation was measured using FACS.

All tests were performed in triplicate. The activity of the samples was also evaluated by Gimza staining [20].

Results and Discussion

Gas Chromatography-mass spectrometry (GC/MS) is a highly accurate analytical method that allows the qualitative and quantitative composition of essential oils to be determined. Using GC/MS, it is possible to characterize in detail all components present in essential oils, including major and minor components, which may also have biological activity and influence the aroma and properties of the oil.

GH/MS analysis of essential oils was conducted by literature data [4]. Retention indices were recalculated relative to normal hydrocarbons C_8-C_{32}.

In essential oil of *A. messerschmidtiana* was determined 43 components. The main components are 1,8-cineole (29.1 %), camphor (24.8 %), and α-campholinal (4.9 %) (Tab. 1).

Table 1

Component composition of the essential oil of *A. messerschmidtiana*

R_{calc}	Component	Area, %	R_{calc}	Component	Area, %
797	Hexanal	0,3	116	Borneol	2.3
843	2-Hexenal	0.2	1167	α-Santolin alcohol	1.9
916	Tricyclene	0.3	1168	*Unidentified 2*	0.8
926	α-Pinene	0,6	**1169**	**α-Campholenal**	**4.9**
941	Camphene	2.3	1174	Terpinene-4-ol	0.9

Continuation of Table 1

R_{calc}	Component	Area, %	R_{calc}	Component	Area, %
963	4(10)-Thujone	0.1	1182	*p*-Cymene-8-ol	0.9
985	1-Octen-3-ol	0.8	1188	α-Terpineol	0.4
1010	3,5,5-Tetramethyl-1,3-cyclohexadiene	0.3	1200	*trans*-Piperitol	0.2
1016	*o*-Diethylbenzene	0.2	1224	*trans*-Chrysotenyl acetate	2,0
1020	*o*-Cymene	0.2	1278	Bornyl acetate	1.3
1033	**1,8-Cineole**	**29.1**	1288	Eucarvon	0.4
1052	γ-Terpinene	0.2	1307	5-Isopropenyl-2-methylcyclopent-1-ene-carboxaldehyde	0.1
1065	*cis*-Linalool oxide	0.1	1468	β-Eudesmene	0.2
1100	6-Methyl-3,5-heptadien-2-one	0.2	1562	Spatulenol	0.8
1100	***Unidentified 1***	**8.1**	1565	Caryophyllene oxide	1.2
1116	Chrysanthenone	0.4	1592	Gumulen-1,2-epoxide	0.1
1130	*cis*-2-p-Menten-1-ol	0.3	1646	Neointermedium	0.3
1133	*cis*-Chrysanthenol	0.5	1651	5β,10α-Eudesm-11-en-4-ol	0.6
1147	**Camphor**	**24.8**	1680	(*1R,7S, E*)-7-isopropyl-4,10-dimethylenecyclode-5-enol	0.2
1148	*p*-Mentha-1,5-dien-8-ol	0.2	1794	Methyl ester of isocostonic acid	0.5
1155	Pinacurvone	1.0	1968	*n*-Hexadecanoic acid	0.3
1161	Isogeranial	0,2		**Total**	**90,7**

Testing on the anti microbial and fungicidal activity showed weak activity (Tab. 2).

Table 2

Anti microbial and fungicidal activity of *A. messerschmidtiana* essential oil

Essential oils / comparison preparation	*C. albicans*	*C. glabrata*	*C. krusei*	*A. fumigatus*	*C. neoformans*	*S. aureus*	MRS	*E. coli*	*P. aeruginosa*	*M. intracellulare*	Test concentration (μg/ml)
Amphotericin B	10	99	100	99	100	-	-	-	-	-	5
Ciprofloxacin	-	-	-	-	-	89	96	98	97	85	1
Essential oil	0	5	3	0	6	0	0	2	11	5	0

The essential oil from this plant has also been studied for its antimalarial activity and showed low activity with 40 % inhibition against *Plasmodium falciparum* D6 compared to the reference drug chloroquine (Tab. 3).

Table 3

Antimalarial activity of *A. messerschmidtiana* essential oil

Essential oils/comparison drug	*P. falciparum* D6 % Inh.
Chloroquine	9
Essential oil of *A. messerschmidtiana*	4

The essential oil of the plant was also studied for cytotoxic activity. All concentrations of essential oil demonstrated acute lethal toxicity, so, all larvae died.

The testing on anti tuberculosis activity (Tab. 4, 5) showed no significant effect.

Table 4

Activity of *A. messerschmidtiana* essential oil against mycobacteria (anti-tuberculosis activity) and yeast

Essential oils/comparison preparation	*M. tuberculosis* H37Rv ATCC 27294	*M. avium* ATCC 25291	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019
Essential oil *A. messerschmidtiana*	2000	>2000	>3000	>3000
Positive control	0.06Isoniazid	>1.0 Isoniazid	1.0 Amphotericin B	0.5 Amphotericin B
Test concentration against *Mycobacterium* sp from 31.25 μg/ml to 2000 μg/ml. Concentration of reference preparations against *Mycobacterium* sp from 0.015 μg/ml to 1.0 μg/ml				

Table 5

Activity of *A. messerschmidtiana* essential oil against bacteria

Essential oils/comparison preparation	*E. faecalis* ATCC 4082	*S. salivarius* ATCC 25975	*S. mitis* ATCC 49456	*S. mutans* ATCC 25175	*S. sanguinis* ATCC 10556	*S. sobrinus* ATCC 33478	*L. casei* ATCC 11578
Essential oil of *A. messerschmidtiana*	400	400	400	400	>400	400	400
Chlorhexidine dihydrochloride	7.375	1,844	7.375	0.922	3.688	1,844	3.688
Test concentration against yeast: 1.46 μg/ml to 3000 μg/ml. Concentration of reference products against yeast: 0.031 μg/ml to 16.0 μg/ml. Control: C. *parapsilosis* ATCC 22019—MIC value: 0.25–1.0 μg/ml. C. *krusei* ATCC 6258—MIC value: 0.25–2.0 μg/ml							

The latest series of tests on *A. messerschmidtiana* essential oil showed no anti-leishmaniasis activity compared to existing drugs.

Conclusion

Thus, the studies conducted show that 43 components were found in the essential oil of *A. messerschmidtiana*. The major components were camphor, 1,8-cineole, and α-campholenal. Analysis of biological activities showed that the tested essential oil exhibited weak fungicidal, antimicrobial, and antimalarial effects, but high cytotoxic activity. No antituberculosis or antileishmanial activity was detected in the essential oil.

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Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Suleimen Ye.M.** — investigation, methodology, funding, writing-review & editing; **Mamytbekova G.K.** — conceptualization, essential oil isolation, antimicrobial and antifungal activity, data curation; **Serikbai G.** — antimicrobial activity; **Birimzhanova D.A.** — data curation, GS/MS data interpretation; **Doudkin R.V. —** investigation, methodology, plant material collection; **Gorovoy P.G. —** investigation, methodology, plant material collection; **Ross S. —** GC/MS analysis; **Martins C.H.G. —** investigation of antituberculosis activity.

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Artemisia messerschmidtiana эфир майының құрамы мен биологиялық белсенділігі

Дәрілік өсімдіктердің жаңа көздерін іздеу аясында эфир майларының химиялық құрамын зерттеу және олардың биологиялық белсенділігін бағалау маңызды міндет. Мақалада Қиыр Шығыстың *Artemisia messerschmidtiana* флорасынан бөлінген эфир майының құрамы мен биологиялық белсенділігін бағалау нәтижелері талқыланған. Эфир майы гидродистилляция арқылы бөлініп алынды. Алынған эфир майының микробқа, зеңге, безгекке, цитотоксикалық, туберкулезге және лейшманриозға қарсы белсенділігі тексерілді. Эфир майының құрамын хроматография-масс-спектрометрия көмегімен талдау 43 компоненттің бар екенін көрсетті. Максималды құрам 1,8-цинеол, камфора және α-камфоленал үшін белгіленді. Эфир майын сынау әлсіз микробқа, фунгицидтік пен безгекке қарсы және жоғары цитотоксикалық белсенділікті анықтауға мүмкіндік берді. Эфир майының туберкулезге қарсы немесе лейшманияға қарсы белсенділігі анықталған жоқ. Алынған нәтижелер *Artemisia* L. туысының компоненттерінің биологиялық белсенділігін білуге ықпал етеді.

Кілт сөздер: Artemisia messerschmidtiana, эфир майы, биологиялық белсенділік, хроматографиялық масс-спектрометрия

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Состав и биологическая активность эфирного масла *Artemisia messerschmidtiana*

Изучение химического состава эфирных масел и оценка их биологической активности является важной задачей в свете поиска новых источников лекарственных растений. В настоящей статье рассматриваются итоги оценки состава и биологической активности эфирного масла, выделенного из *Artemisia messerschmidtiana* флоры Дальнего Востока. Эфирное масло было выделено методом гидродистилляции. Полученное эфирное масло было исследовано на антимикробную, антифунгальную, противомалярийную, цитотоксическую, антитуберкулезную и антилейшманиозную активность. Анализ состава эфирного масла с помощью хромато-масс-спектрометрии показал присутствие 43 компонентов. Максимальное содержание отмечено для 1,8-цинеола, камфоры и α-камфоленаля. Тестирование эфирного масла позволило установить слабую антимикробную, фунгицидную и противомалярийную активность, а также высокую цитотоксическую. Противотуберкулезная и противолейшманиозная активность эфирного масла не выявлена. Полученные результаты вносят вклад в знание биологической активности компонентов рода *Artemisia* L.

Ключевые слова: Artemisia messerschmidtiana, эфирное масло, биологическая активность, хромато-масс-спектрометрия

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Identification of important plant areas in the Mountainous Eastern Karatau (Mangystau)

The identification of Important Plant Areas (IPAs), which are of particular significance for the conservation of biological diversity, is one of the international programs implemented under the Convention on Biological Diversity. The aim of this study was to identify IPAs in the Eastern Karatau Mountains. Field research was conducted using the traditional route reconnaissance method, covering the most diverse biotopes (landscape and ecological conditions) and their characteristic phytocoenoses. The vegetation cover of the identified areas was studied using standard geobotanical methods, including the description of plant communities, as well as the assessment of species diversity and vegetation condition. The flora of the Mangystau Region is characterized by a relatively low level of species diversity, and the vegetation cover is typical of desert regions. Based on criteria A–C, eight IPAs were identified: Kurkeruek, Kirezhyk Zheke, Eskildi Aday, Shili, Kaskyr Sai, Kaskyr Sai 2, Bakshy, and Agashty. The locations of ten species with IUCN conservation statuses 2(U), 3(R), and 4(I) were recorded: *Armeniaca vulgaris*, *Tulipa sogdiana*, *Capparis herbacea*, *Crambe edentula*, *Ephedra aurantiaca*, *Onosma staminea*, *Rhamnus sintenisii*, *Rubus caesius*, *Verbascum blattaria*, and *Crataegus ambigua*. For each Important Plant Area, a passport was compiled, threats to vegetation were identified, and recommendations for biodiversity conservation were developed.

Keywords: flora, Eastern Karatau Mountains, rare, endangered species, endemics, Red Book of Kazakhstan, important plant areas

Introduction

Import Plant Areas (IPAs) are an internationally recognized expert approach developed in the UK by Plantlife [1]. It aims to identify and conserve the richest areas in terms of plant life, possibly within existing protected areas. The designation of IPAs is necessary in order to combine efforts to conserve important wild plant populations in these areas and is an important complement to the Key Biodiversity Areas designated in a broader context [2].

The identification and designation of IPAs is based on three criteria, which take into account floristic richness, the number of rare and endemic species in need of protection, species listed in the Red Data Books, or species whose current status is of great importance for the evolution and preservation of life-supporting biosphere systems, as well as the presence of rare and threatened plant communities (habitats) [3–5].

The Mangystau region is an important industrial region with a significant range of environmental problems, as well as poorly studied areas in terms of assessing their condition and the presence of protected plant species. This problem is particularly acute in light of global climate change and the increasing anthropogenic pressure on natural areas [6–8]. The Mountainous Eastern Karatau region in Mangyshlak is one such poorly studied area.

The choice of the research area was determined by its relatively good floristic study, the availability of a large array of herbarium collections over a period of more than 50 years, the significant species diversity of the natural flora, and the presence of a number of endangered, rare, and endemic species in the area, the preservation of which is of not only national but also important international significance.

The aim of this study is to identify important plant areas for the Eastern Karatau Mountains.

Experimental

Surveys were conducted using route reconnaissance methods, covering different seasons of the 2024 growing season. The coordinates of plant species locations were determined using a GPS navigator. The geo referencing of historical herbarium sample collection points was carried out using Google Earth.

The following criteria were used to identify key botanical areas:

Criterion *A*. The site contains important populations of one or more plant species that are of high value on a global or European scale. There are four categories under criterion A.

A(i) — plant species recognized as being threatened with global extinction. This includes plants from the International Union for Conservation of Nature (IUCN) Red List.

A(ii) — plant species recognized as being threatened with extinction in Europe.

A(iii) — endangered endemic species not included in A(i) or A(ii). According to the guidelines for identifying KBTs, this category includes areas with national endemic species whose range does not extend beyond the borders of Kazakhstan and which are listed in the national Red Book.

A(iv) — endangered sub-endemic species with a narrow range, not included in A(i) or A(ii) and distributed in neighboring countries.

A(v) — species that are rare, endangered, and in need of protection within Kazakhstan and the Mangystau region.

Criterion *B* — the site is distinguished by its floristic richness or richness in plant species of special significance.

Criterion *C* — represents a unique or rare type of ecosystem, the presence of threatened habitats.

The main criterion is designated as A, as it includes Red Book species, the protection of which is specified by the legislation of the Republic of Kazakhstan.

The list of IPA trigger species and their distribution was compiled using data from field expeditions and herbarium collections stored in the herbarium of the Mangyshlak Botanical Garden (MANG), the Institute of Botany and Phytointroduction (AA), the Herbarium of the Botanical Institute of the Russian Academy of Sciences in St. Petersburg (LE), and the Herbarium of Moscow State University (MW). The collected herbarium material was identified using fundamental summaries: Flora of Kazakhstan [9], Illustrated Plant Guide of Kazakhstan [10], and the Plant Guide of the Mangystau Region [11]. The nomenclature of each taxon was carried out in accordance with POWO source [12]. The conservation status of each species was determined in accordance with the Red Book of Kazakhstan [13] and the regional Mangystau Red Book [14].

Results and Discussion

The Eastern Karatau Range is the highest massif of the Mangyshlak Mountains [15]. Its elevation ranges from 380 to 480 m above sea level. The highest point is Mount Beschoku, rising 555 m above sea level. The ridge stretches for 45 km and is about 10 km wide. Its summit is a peneplanated, hilly surface with ridges of dense rock, stretching parallel to each other and rising 5–10 m above the plain. Individual cone-shaped peaks rise 50–100 m above the peneplain (the Dzhipakhchi, Beschoku, and other mountains). Eastern Karatau has very steep slopes, almost vertical in places. The slopes are deeply cut by deep ravines. The northern and southern macroslopes of the ridge are very steep, rocky and gravelly, with outcrops of bedrock, cut by numerous canyon-like gorges, deep ravines, and gullies. The lithological composition of the rocks that make up Eastern Karatau is very diverse. It is represented by various sandstones, siltstones, and shales, with one or another type of rock predominating.

There are 9 rare species in Eastern Karatau, whose numbers are declining, of which 3 species are included in the Red Book of Kazakhstan [13], and 6 are included in the Red Book of the Mangystau Region [14] (Tab.).

T a b l e

Vascular plant species of Eastern Karatau included in the Red Data Books

Species	**No. IPAs**	**Status (according to IUCN Red List)**	**Population status**	**Note**
Armeniaca vulgaris Lam.	8	3 (R)	Rare species with declining numbers	Listed in the Red Book of Kazakhstan
Capparis herbaceae Sp.	8	2 (U)	Rare species	Listed in the Red Book of Mangystau Region

C o n t i n u a t i o n   o f   T a b l e

Species	No. IPAs	Status (according to IUCN Red List)	Population status	Note
Crambe edentula Fisch.	7, 8	2 (U)	Rare species	Listed in the Red Book of Mangystau Region and Kazakhstan
Ephedra aurantiaca Takht. et Pachom.	1	4 (I)	Uncertain species	Listed in the Red Book of Mangystau Region
Onosma staminea Ledeb.	8	2 (U)	Rare species	Listed in the Red Book of Mangystau Region
Rhamnus sintenisii Rech.	1–8	4 (I)	Uncertain species	Listed in the Red Book of Mangystau Region
Rubus caesius L.	6–8	4 (I)	Undetermined species	Listed in the Red Book of Mangystau Region
Verbascum blattaria L.	8	2 (U)	Rare species	Listed in the Red Book of Mangystau Region
Tulipa sogdiana Bunge	8	5 (co)	Rare species	Listed in the Red Book of of the Republic of Kazakhstan and Mangystau Region

Survey of the territory showed an extreme degree of vegetation transformation, which allowed us to identify 8 IPAs: 1). Kurkeruek; 2). Kirezhyk zheke; 3). Eskildi adai; 4). Shili Gorge; 5). Kaskyr sai; 6). Kaskyr sai 2; 7). Bakshy; 8). Agashty (Fig. 1).

Figure 1. Locations of important plant areas in the Eastern Karatau Mountains

<u>The important plant area Kurkeruek</u> (N 44°04'200"; E 052°36'365", altitude — 156–361 m above sea level) is located in the foothills of the Mountainous Eastern Karatau Range. The relief is low-mountainous, with very steep slopes, almost vertical in places. The slopes are deeply cut by ravines. The water regime is automorphic, characterized by seasonal atmospheric moisture in the area, dry gorges, and no water. They consist of gray-brown soil cover.

The floral diversity offered by IPA Kurkeruek consists of 49 species of vascular plants. The vegetation cover includes species listed in the Red Book of Mangystau Region, *Rhamnus sintenesii* and *Ephedra aurantiaca*, whose presence meets criterion *A(iii).*

The territory is partially used for grazing. The main part of the vegetation cover of the territory is assessed as background with patches of slight disturbance. The low density of settlements contributes to the preservation of beautiful flowering ornamental and medicinal species. Types of anthropogenic impacts are grazing and roads. The degree of anthropogenic impact ranges from low to moderate. Part of the site (dry slopes with rock outcrops) requires a complete ban on grazing.

Important plant area Kirezhyk Zheke Gorge (N 44°04'200"; E 052°36'365", altitude — 304–320 m above sea level). The proposed Kirezhyk Zheke IPA is located in the foothills of the Eastern Karatau Mountains, on a salt marsh depression in the foothill plain. The water regime is automorphic, characterized by atmospheric moisture, semi-hydromorphic, characterized by the influence of closely occurring groundwater, and hydromorphic, caused by the influence of surface water from springs. The soil profile is characterized by a poor humus horizon and a slightly lumpy structure.

According to criterion *A(iv)*, two Red Book species have been identified: *Rhamnus sintenesii* and *Verbascum blattaria,* which are listed in the regional Red Book [14]. According to criterion *B*, the site is characterized by a fairly complete range of flora typical of sub-mountain medium deserts. Thirty-seven species of vascular plants have been identified here. According to criterion *C*, grazing livestock poses a threat to the preservation of the vegetation of the proposed IPA.

The important plant area Eskildi Adai (N 44°00'332"; E 052°42'590", elevation 135–164 m above sea level). The IPA is located on the foothill plain of the southwestern slope of the Mountainous Eastern Karatau ridge. The relief is rocky and heavily dissected. The water regime is automorphic, characterized by atmospheric moisture. The gently undulating foothill plain is characterized by the spread of gray-brown desert soils.

The proposed IPA Eskildi is botanically interesting as an example of a foothill plain with complex vegetation cover. The floristic diversity of the proposed site is demonstrated by a list of 47 species of vascular plants. There is also a species listed in the catalog of rare and endangered plant species of Mangystau — *Rhamnus sintenesii,* which meets criterion A*(iii).* Criterion *B* — the high floristic diversity of IPA is demonstrated by 42 plant species. Criterion *C* — threats to the habitat of plants in the proposed Eskildi IPA include grazing, plant collection, and steppe fires.

Overall, the condition of the vegetation cover is satisfactory. The remoteness from populated areas ensures the preservation of the flora. It is recommended that this area be given the status of a specially protected area as a botanical reserve.

The important plants area Shili Gorge (N 44°28'40"; E 052°38'450", altitude — 212 m above sea level). The site is located in the Kus Konbas area of Eastern Karatau. There is a mineral water spring within the site. The site is characterized by specific conditions that allow for a unique combination of communities from different ecological and geographical groups (desert and mountain-desert) in the local area. The value of the site also lies in the presence of the Red Book species *Rhamnus sintenesii,* which meets criterion *A(iii).* Criterion C — The originality of the site lies in its fairly diverse species composition. The proposed IPA may be subject to anthropogenic influences associated with mineral extraction and intensive grazing of domestic livestock.

The important plant area Kasqyr Sai (N 44°02'325"; E 052°37'291", elevation — 258 m above sea level). The area is occupied by rocky lowlands with short, rugged foothill valleys of dry ravines covered with stones and scree. The soils of plant communities are rocky. The vegetation consists mainly of petrophytic communities, reflecting various stages of overgrowth of rocky mountain rocks in the process of their weathering.

The botanical value of the site lies in its significant species diversity — 53 species — and the presence of the rare species *Rhamnus sintenesii* and *Verbascum blattaria*, which are listed in the catalog of rare and endangered plant species of Mangystau [14]. Criterion A *(iii)* — rare species: *Rhamnus sintenesii* and *Verbascum blattaria.* Criterion B — Great diversity of habitats and floristic richness. Criterion C — Groups of petrophytes on rocks and scree slopes are rare in the desert zone as a whole.

Overall, the condition of existing plant communities, rare species, and their habitats can be assessed as stable, with livestock grazing posing a threat.

The important plant area Kaskyr sai 2 (N 44°02'378"; E 052°36'101", elevation — 229 m above sea level). The site has a fragmented relief, with large rocky cliffs on all sides, stretching for about 1.2 km with a width of 30–60 m. The entrance to the gorge is not wide, 25–30 m, and narrows even more towards the bottom to 15–20 m. The communities proposed for designation occupy the rocky and stony slopes, the lower part and the bottom of the gorge. The substrate is stony, gray-brown and clayey at the foot.

The site represents typical mountain desert vegetation characteristic of the middle and lower parts of the gorges of the Eastern Karatau Mountains. The vegetation cover is slightly disturbed, and the floristic diversity of the communities is fairly well represented. Criterion *A (iii)* — the presence of rare species listed in the Red Book of Kazakhstan: *Rubus caesius*, *Rhamnus sintenesii*, included in the catalog of rare and endangered plant species of Mangistau. Criterion B — high overall species diversity of plants (43 taxa) characteristic of mountainous desert areas represented in this key area. The condition of the vegetation cover in the area described is good but unstable.

The important plant area Bakshy (N 44°03'229"; E 051°30'064", elevation — 290 m above sea level) is located at the entrance to the gorge. It features a private garden, an artificial apricot plantation, and a reservoir. The gorge has a dissected relief, with large rocky slopes on all sides, stretching for about 3 km with a width of 40–80 m. The entrance to the gorge is wide, but after 25 m it narrows to 20 m. The communities proposed for designation occupy the slopes, lower part, and bottom of the gorge. The substrate is large and rocky, with gray-brown soil.

Criterion A (iii) — presence of Red Book species — *Crambe edentula*, *Rhamnus sintenisii* (Fig. 2), *Armeniaca vulgaris*, *Artemisia gurganica*, *Rubus caesius*, *Teucrium polium*. Criterion *B* is high floristic diversity of IPA, including 58 species of higher plants. Criterion C — the habitat is occupied by slightly disturbed communities of low-mountain vegetation.

A B

Figure 2. Teucrium polium (A) and Rhamnus sintenesii (B)

The status of rare species is stable. They are threatened by recreational pressure, mudslides, and livestock grazing. The degree of transformation is estimated at 20–25 %. The area needs restrictions on livestock grazing.

The important plant area Agashty (N 44°03'318"; E 051°30'416", altitude — 288 m above sea level). The site is rocky and stony, with steep, rocky slopes on all sides, stretching for about 1.2 km and 20–40 m wide. The entrance to the gorge is not wide, 25–30 m, and narrows even more towards the bottom to 15–20 m. The communities proposed for designation occupy the rocky and stony slopes, the lower part and the bottom of the gorge. The substrate is stony, gray-brown and clayey at the foot.

The site represents typical mountain desert vegetation characteristic of the middle and lower parts of the gorges of the Eastern Karatau Mountains. The vegetation cover is slightly disturbed, and the floristic diversity of the communities is fairly well represented, including 80 species of vascular plants. Criterion *A (iii)* is represented by species listed in the Red Book of Kazakhstan and the catalog of rare and endangered plant species of the Mangystau region: *Armeniaca vulgaris, Verbascum blattaria, Salix alba, Rubus caesius, Rhamnus sintenesii*, *Crambe edentula, Tulipa sogdiana*, and *Onosma staminea* (Fig. 3). Criterion B is the overall high species diversity of plants, characteristic of mountain desert areas, represented in this IPA. The area is subject to anthropogenic impact and livestock grazing.

A B

Figure 3. Rubus caesius (A) and Tulipa sogdiana (B)

Conclusion

Thus, as a result of studies of the flora of the Mountainous Eastern Karatau of the Mangystau region, eight IPAs were identified that meet the international criteria for IPA: Kurkeruek; Kirezhyk zheke; Eskildi adai; Shili Gorge; Kaskyr Say; Kaskyr Say 2; Bakshy and Agashty. The communities were described and the species composition of the communities in these areas was determined.

The locations of 10 species with IUCN status 2(U), 3(R), 4(I) have been identified: *Armeniaca vulgaris, Capparis herbaceae, Crambe edentula, Ephedra aurantiaca, Tulipa sogdiana, Onosma staminea*, *Rhamnus sintenisii*, *Rubus caesius, Verbascum blattaria*. A passport has been created for each key botanical territory, factors threatening the condition of vegetation have been identified, and proposals for biodiversity conservation have been made.

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Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions from all authors. All authors have given approval to the final version of the manuscript. **Adamzhanova Zhanna** — mapping of IPA, investigation, visualization, manuscript writing; **Mukhtubaeva Saule** — investigation, methodology; **Duisenova Nurzaugan** — identification of criteria according to IPA; **Lukmanov Akimzhan** — compilation of a list of vascular plant species.

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Таулы Шығыс Қаратаудың (Маңғыстау) негізгі ботаникалық аумақтарын бөлу

Биологиялық алуантүрлілікті сақтау үшін ерекше маңызы бар аумақтарды (сирек өсімдіктер кездесетін негізгі ботаникалық аумақтарды қоса алғанда) анықтау — биологиялық алуантүрлілік жөніндегі конвенция шеңберінде жүзеге асырылатын халықаралық бағдарламалардың бірі. Зерттеудің мақсаты — Шығыс Қаратау таулы өңірінің негізгі ботаникалық аумақтарын айқындау. Зерттеу экспедициялары маршруттық рекогносцирлеу әдісімен жүргізіліп, алуан түрлі биотоптар (ландшафтық-экологиялық жағдайлар) мен оларға тән фитоценоздарды қамтыды. Негізгі ботаникалық аумақтардың өсімдік жамылғысын зерттеу дәстүрлі геоботаникалық әдістермен жүргізілді, оған өсімдік қауымдастықтарын сипаттау, түр алуандығымен өсімдіктердің жағдайына баға беру кірді. Маңғыстау облысының флорасы түрлік алуандықтың төмендігімен ерекшеленеді, ал оның өсімдік жамылғысы шөлді аймақтарға тән. А-С критерийлерін қолдана отырып, Құркеруек, Кірежік Жеке, Ескілді Адай, Шілі, Қасқырсай, Қасқырсай 2, Бақшы және Ағашты сияқты 8 негізгі ботаникалық аумақ анықталды. МСОП бойынша мәртебесі бар 10 түрдің мекендері тіркелді, олар: *Armeniaca vulgaris, Tulipa sogdiana, Capparis herbaceae, Crambe edentula, Ephedra aurantiaca, Onosma staminea, Rhamnus sintenisii, Rubus caesius, Verbascum blattaria, Crataegus ambigua*. Әрбір негізгі ботаникалық аумаққа паспорт жасалды, өсімдік жамылғысына қауіп төндіретін факторлар айқындалды және биоалуантүрлілікті сақтау бойынша ұсыныстар берілді.

Кілт сөздер: флора, Шығыс Қаратау таулы өңірі, сирек, жойылып бара жатқан түрлер, эндемиктер, Қазақстанның Қызыл кітабы, негізгі ботаникалық аумақтар

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Выделение ключевых ботанических территорий Горного Восточного Каратау (Мангистау)

Выявление территорий, имеющих особое значение для сохранения биологического разнообразия (включая ключевые ботанические территории с редкими растениями), является одной из международных программ, реализуемых в рамках Конвенции о биологическом разнообразии. Целью настоящего исследования являлось выделение ключевых ботанических территорий Горного Восточного Каратау. Экспедиционные исследования объектов производились традиционным методом маршрутной рекогносцировки с охватом наиболее разнообразных биотопов (ландшафтно-экологических условий) и свойственных им фитоценозов. Изучение растительного покрова ключевых ботанических территорий осуществлялось традиционным методом геоботанических исследований, включающим описание растительных сообществ, а также оценку видового разнообразия и состояния растительности. Флора Мангистауской области отличается невысоким уровнем видового разнообразия, растительный покров этого региона типичен для пустынных регионов. При использовании критериев А-С было выделено 8 ключевых ботанических территорий: Куркеруек, Кірежік Жеке, Ескілді Адай, Шилі, Каскырсай, Каскырсай 2, Бакшы и Ағашты. Выявлены местонахождения 10 видов, имеющих статус по МСОП 2(U), 3(R), 4(I): *Armeniaca vulgaris, Tullipa sogdiana, Capparis herbaceae, Crambe edentula, Ephedra aurantiaca, Onosma staminea, Rhamnus sintenisii, Rubus caesius, Verbascum blattaria, Crataegus ambigua*. Для каждой ключевой ботанической территории составлен паспорт, определены факторы, угрожающие состоянию растительности, и сформулированы предложения по сохранению биоразнообразия.

Ключевые слова: флора, горный Восточный Каратау, редкие, исчезающие виды, эндемики, Красная книга Казахстана, ключевые ботанические территории

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Study of lichen species growing in the "eastern part" of Kazakh small mountain hills

The rapid development of industry at the present stage, the accumulation of harmful waste, and the emergence of large settlements—all these anthropogenic factors cause climate change around the world. In this regard, it is known that a decrease in atmospheric moisture has a significant impact on the expansion of desert areas. The above-mentioned global changes directly affect living organisms on Earth and alter the species composition of both flora and fauna. Therefore, scientists must pay special attention to the natural changes occurring in local flora. In this study, lichens were chosen as the object of research, as they form an essential part of the local flora, inhabit various biomes, and are resistant to climatic changes due to their structural features. In recent years, scientific publications on the plant flora of Kazakhstan have included lichenological studies providing data on the biological characteristics, ecology, and indicator value of species growing in specific regions. However, there are no dedicated studies covering the lichen species found in the flora of the Chingistau, Kalbatau, Kokentau, and Abraly mountains, which belong to the eastern part of the Kazakh Small Hills. In the present study, a comparative analysis of the species composition was carried out, since the mountain ranges collectively known as the Small Hills of the East are located far from each other. As a result, 11 species belonging to 5 genera and grouped into 6 families were identified as growing across all these hills.

Keywords: Kazakh small hills, epilyticlichen, epigeallichen, indiffent lichen, Drude scale

Introduction

The "eastern part" of the Kazakh small hills occupies the southwestern part of the land sections of the Abay region. This territory is distinguished by a strongly sliced low-mountainous, small-hilly terrain. The Chingis Mountains occupy a large area, stretching from northwest to southeast [1]. Most of all, small hills in the eastern part of Kazakhstan are located connecting the territories of two large regions of Kazakhstan. The part that includes the Abraly mountains, the eastern part of Saryarka includes small hills located on the territory of the Karaganda region, Karkaraly district and Abay district [2].

In connection with the topic of the research work, the importance of the emergence of various types of lichens, based on the diversity of photo—and mycobiontes in the symbioses of lichens in rocks, is shown in the research works of foreign scientists. Lichens are sensitive to climate change and have been found to be at risk of extinction in the highlands. Temperature and precipitation, the effects of the altitude gradient change the structure and phylogenetic diversity of lichen associations [3–5]. In addition, data are presented on the structure of lichen thalli and their changes depending on climatic conditions. It has been established that a certain lichen consists of several groups of algae and also fungi, including ascomycetes and two phylogenetic basidiomycetes [6–8]. The frequency of occurrence and distribution of lichens is determined by considering their functional features. Changes in the external morphology of lichens are considered to be related to its level of hydration and the course of photosynthetic activity. The vast majority of lichens are adapted to grow in the high latitudes of the mountains, in temperate and subtropical zones [9].

In recent years, research work on lichenology in Kazakhstan shows data on the species of lichens distributed in the small hilly, mountainous areas of central Kazakhstan and their relationship with the environment [10–12].

On the rocks of small hilly mountain slopes, the main plant associations, painted in different colors, are formed by lichens. In some domestic works, it is given that among the vegetation cover in the Kazakh small hills, lichens belonging to the genus Cladonia, Alectoria, Peltigera are found from the lower plants [2]. In literary sources, the biology, ecology and types of lichens that need protection are studied in the biomes of the Burabay Park [13-14].

In addition, among the published scientific works in accordance with the plant flora of Kazakhstan, one can find General Data related to the distribution, morphological and environmental characteristics of lichen species. But, in particular, due to the species of lichen growing in the mountains of Chingistau, Kalbatau, Kokentau, Abraly, which belong to the small hills and low mountains of Abay region, information is very rare. These mountains are known as the spurs of the Tarbagatai and Saryarka mountains of the East, which are located on the territory of the Abay region at a certain distance from each other. The species of higher plants that grow in the study areas are very inhospitable in the mountain belts. This is due to the fact that the soil cover is gravel, and the moisture content is very small, there are often associations of real xerophytic plants. As the dominant plant species characteristic of all research areas, plant species such as Festuca, Artemisia, Stipa, Spiraea, Juniperus, Allium, Orostachys and others grow. In accordance with the main goal of the research work, the above-mentioned species of lichen growing on small hills and low mountains were studied. In the regions surveyed, the route expedition was carried out in different seasons of 2024-2025.The lichenological study was carried out using geobotanical methods.

Experimental

Identification of lichen species settled in the mountains of Abraly ($49^0$10N $77^0$25E), Kokentau (Semeytau) ($50^0$10N $79^0$43E), Kalbatau ($49^0$22N $81^0 29^{|}$30E) and Chingistau ($48^0$52N $79^0$15E), grouping into systematical taxa and meeting with the environment communication research was carried out.

a) **b)** **c)** **d)**

Figure 1. Map-scheme of the regions where the route expedition to the small mountain hills of Abay region was carried out a) Abraly mountains b) Chingistau c) Kokentau (Semeytau) d) Kalbatau

Among the geobotanicalmethods, the determination of quantitative indicators of species in plant associations living in research areas is of great importance. To determine the number of lichens settled at the research sites, the number, density and variety of lichens within 1 $м^2$ of wooden Squaresticks were evaluated in advance. In the study, indicators were obtained depending on the variety, number and covering projection of lichens within 1 $м^2$ Squaresticks in the mountains of Abraly, Chingistau, Kalbatau, Kokentau. To determine the quantitative and covering projection in the research areas, the obtained Squaresticks were carried out by throwing them randomly (10–15 times with a repetition) at the research sites. The accuracy of the numerical values obtained from the test sites directly depends on the repeated implementation of the experiment. This is because it must be taken into account that the types of epilytic lichen have their own characteristics in the research methods compared to epiphytic and epigeal lichen. Especially in some cases, factors such as the surface shapes of different rocks (steep, rocky) and the growth of lichen individuals settled on them, covering each other, cause difficulties in determining their quantitative indicator. To find quantitative indicators of very rare (Sol), rare (Sp), often (Cop) very numerous (Soc) species of lichens, the Drude scale was used as a geobotanical method [15].

Determination of the types of lichen taken as samples was carried out using the 3-volume determinant of E.I. Andreeva "Flora of spore plants of Kazakhstan" [16]. Identification of lichens collected during the route expedition was carried out using the "Low vacuum raster electron microscope (rem)" JSM-6390 LV JEOL (Japan). In connection with the research work, collections of collected types of lichens were created.

Results and Discussion

The route expedition was carried out in the last months of autumn, spring and summer. The GIS map of the regions where the route expedition was conducted was taken, and latitude and longitude indicators were given (map).

As shown on the map, certain points of the small hills of the East were taken. During the guided expedition on the Chingistau, Kalbatau, Kokentau and Abraly mountains, which are part of the Kazakh small and low-lying mountain ranges, the collected types of lichen were identified and classified into systematic groups. The identified species were analyzed depending on the form of life, ecology and frequency of occurrence (Tab. 1).

Table 1

Biology and ecological features of lichen species settled in the small hills of Abay region

№	Genus	Frequency of meetings	Lifeform	Ecology
Family *– Verrucariaceae*				
1	*Genus Verrucaria* *Species V.nigrescens*	(Cop)	scale lichens	epilytic
Family *–Acarosporaceae*				
2	*Genus Acarospora* *Species A. chlorophana*	(Sol)	scale lichens	epilytic
Family *– Caloplacaceae*				
3	*Genus Caloplaca* *Species C. viridirufa*	(Sol)	scale lichens	epilytic
4	*Genus Caloplaca* *Species C. decipiens*	(Sp)	scale lichens	epilytic
Family *– Lecanoraceae*				
5	*Genus Placolecanora* *Species P. alphoplaca*	(Sol)	scale lichens	epilytic
6	*Genus Lecanora* *Species L. allophana*	(Sp)	scale lichens	epilytic
7	*Genus Lecanora* *Species L. frustulosa*	(Sp)	scale lichens	epilytic
Family *– Parmeliaceae*				
8	*Genus Parmelia* *Species P. tominii*	(Cop)	leafylichens	epilytic
9	*Genus Parmelia* *Species P. conspersa*	(Sp)	leafylichens	epilytic
10	*Genus Parmelia* *Species P. vagans*	(Soc)	leafylichens	epigeal
11	*Genus Parmelia* *Species P. sulcata*	(Sol)	leafylichens	indiffent

Of the 11 species identified depending on the Life Form, 4 species were grouped into leafy lichens and 7 species of scaly (sometimes small-leaved) lichens. Due to their ecology, 9 out of 11 species were classified as epilytic lichen, 1 as epigeal, and one species as indifferent species (Tab. 1). In the course of the study, the settlement of lichens at different heights of the mountains was directly related to the mountain slopes. In comparison with the slopes of the mountains, epilytic lichen species are most often settled on sunny slopes, where there is plenty of sunlight. In addition, the lichens that settled on the sunny side of the mountain differed due to their morphological structures and colors. For example, species such as Acarospora chlorophana, Caloplaca viridirufa, Caloplaca decipiens, Lecanora Allophana, which have different frequency of occurrence, and most importantly, have a beautiful bright color. And on the negative surfaces of the mountain, it was noticed that lichen species Verrucaria nigrescens, Lecanora frustulosa, Parmelia tominii, Parmelia conspersa often grow in black and gray color.

T a b l e 2

Indicators of lichen species settled in small and low Kazakh mountains, depending on the quantitative and covering projection

№	Lichen species	Chingistau		Kalbatau		Kokentau		Abraly mountains		Average indicators of all types of lichens	
		Ranks	coverprojection %	Ranks	coverprojection %	Ranks	coverprojection %	Ranks	coverprojection %	Ranks	coverprojection %
1	*Verrucaria nigrescens*	1, 8	2,2	6,1	3,1	22,2	20,4	24,9	25	13,8	12,7
2	*Acarospora chlorophana*	-	-	1,8	1,7	2,5	0,5	10,6	2,2	3,7	1,1
3	*Caloplaca viridirufa*	-	-	4,1	2,1	-	-	-	-	1,0	0,5
4	*Caloplaca decipiens*	2,2	2,0	4	1,6	8,2	1,8	12,9	8,2	6,8	3,4
5	*Lecanora allophana*	1,4	0,8	13,5	5,3	5,6	3,4	6,3	4,5	6,7	3,5
6	*Lecanora frustulosa*	2,9	2,7	2	1,5	2,5	0,9	19	9,5	6,6	3,7
7	*Parmelia tominii*	1,3	1,3	27,7	11,5	12	6	21,7	10	15,7	7,2
8	*Parmelia conspersa*	6,8	2,7	3,5	2,6	8	3,7	2,1	3	5,1	3
9	*Parmelia vagans*	55,1	47,2	76,4	45,4	2	1,1	-	-	33,4	23,4
10	*Placolecanora alphoplaca*	1,8	2,7	-	-	4,1	2,7	-	-	1,5	1,3
11	*Parmelia sulcata*	0,3	0,1	-	-	-	-	-	-	0,07	0,02

In terms of the number of identified lichen species grows in Chingistau (9), Kalbatau (9), Kokentau (9), and Abraly mountains (7). Due to the covering projection, the species Parmelia vagans showed greater values in the Chingistau and Kalbatau mountains (47.2 % and 45.4 %). In the process of comparing their averages with the diversity of lichens settled in the mountains obtained in each study, it was found that, depending on the number and covering projection, the dominant species are Parmelia vagans, Parmelia tominii and Verrucaria nigrescens. In addition, in the course of the study, the species Parmelia sulcata, Caloplaca viridirufa and Placolecanora alphoplaca, which are very small in number and have a very low incidence, were classified as very rare species (Tab. 2, Diagrams 1-2).

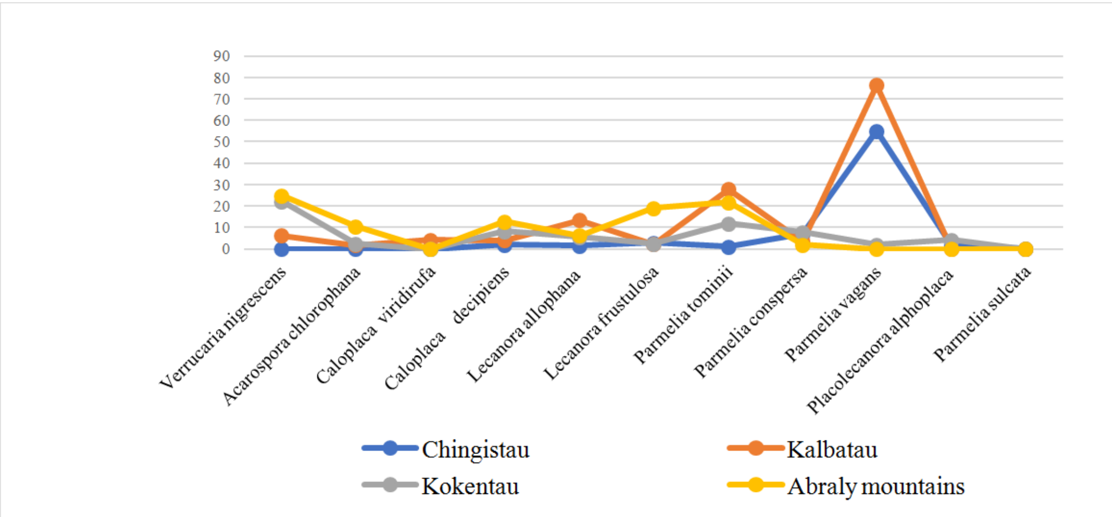


Diagram-1. Quantitative indicators of lichen species settled in Kazakh small mountain ranges

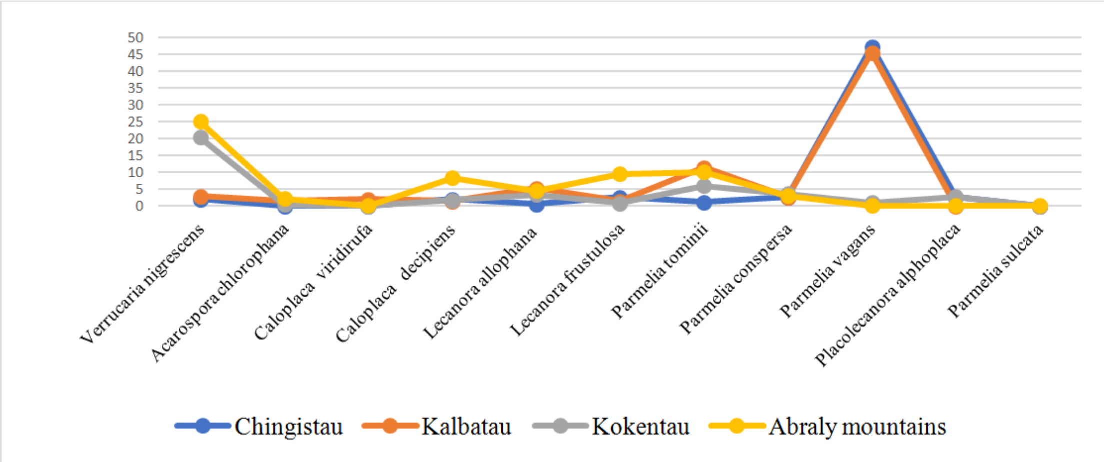
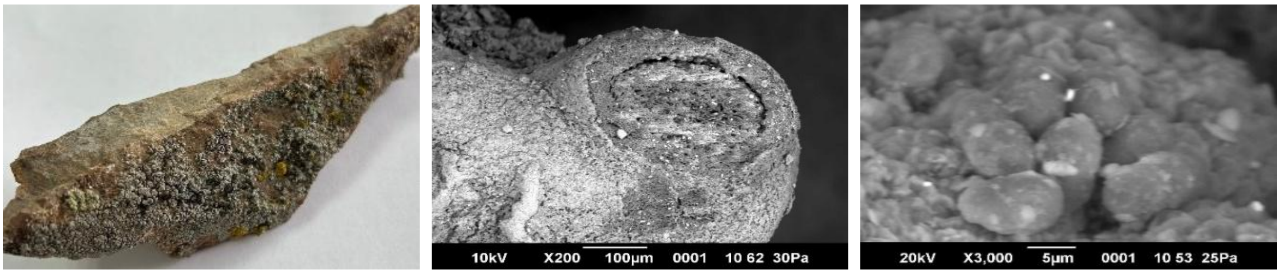


Diagram-2. Indicators of lichen species settled in Kazakh small mountain ranges depending on the covering projection

The following is a morphological brief description based on the settlement of lichens on substrates and photo images obtained under a scanning microscope.

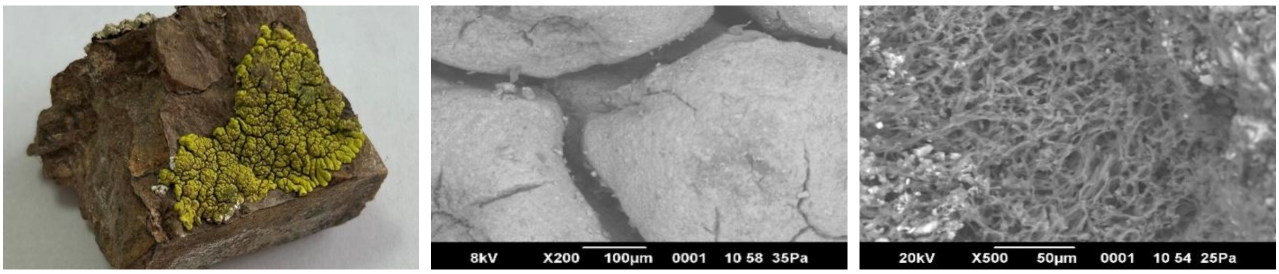
1. *Verrucariaceae*–Verrucaria nigrescens—frequent, scalylichen. Belongs to the epilytic type, adapted to growth in the stone. The lichen thallus is brownish-gray in color, very thin, the underside of the thallus is black. Many perithecia, black in color, are located inside the thallus, penetrating it. The spores are of different shapes, arranged in several rows. Pycnidium are formed by spreading over the lichen in the form of a black dot.



a) b) c)

Figure 2. a) General view of the lichen Verrucaria nigrescens settled on the stone surface;
b) Aseparate perithecia and a black pycnidium part of the species;
c) Manifestation of lichen spores formed in the peritoneum

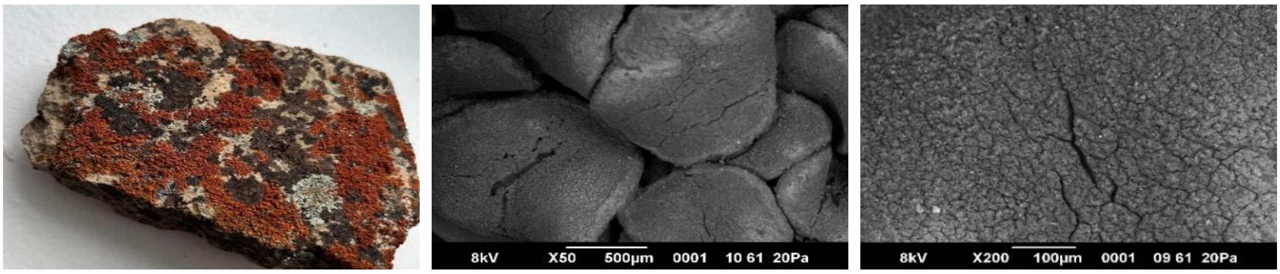
2. *Acarosporaceae*–Acarospora chlorophana—very rare, epilyticlichen. The surface of the thallus is uniformly scaly, thin. The areoles are smooth, slightly fleshy, light yellow-green in color. In close contact with each other, in the form of a whole coating, sometimes separated, scattered among other types of lichen is formed. The middle layer of the thallus is white, dense, the undercoat is black, clearly noticeable.



a) b) c)

Figure 3. a) The period when the species Acarospora chlorophana grew in the substrate;
b) Angular formation of apothecium disks; c) View of the corepart of the lichen

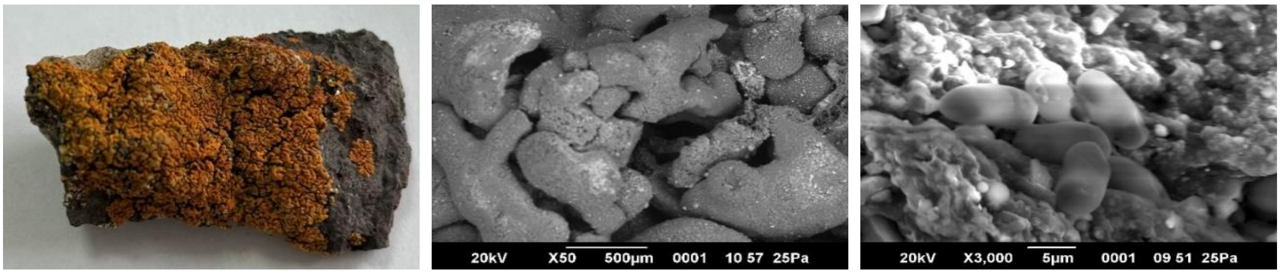
3. *Caloplacaceae*–Caloplaca viridirufa—very rare, scaly lichen. The color is dark red or red-brown, with epilytic lichens settling on the surface of the stones.The thallus on the surface of the stone is formed by a thin, hard sticking. The apothecia is located in large numbers in the middle part of the thallus, forming singly, but in close contact with each other. The apothecia is oval or round in shape, with a whole edge. Eight ascospores are formed in the ascus.



a) b) c)

Figure 4. a) Location of the species Caloplaca viridirufa in the stone;
b) Representation of apotheias of different shapes; c) The surface part of the individual apothecary

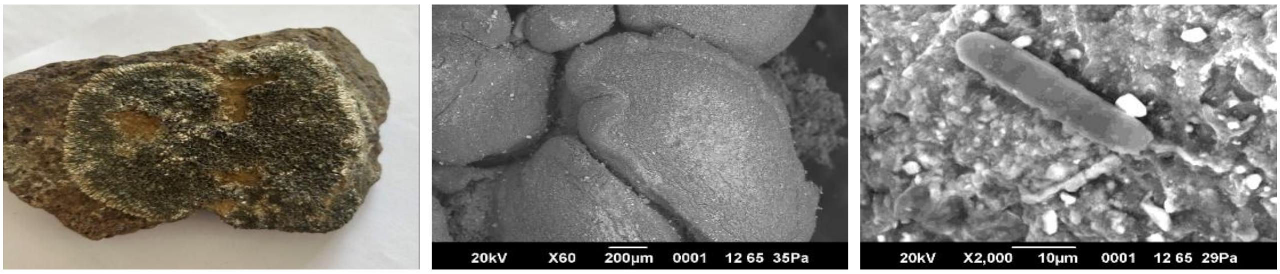
4. *Caloplacaceae*–Caloplaca decipiens (Gasparrinia decipiens)—the most common is epilytic, scaly lichen. The surface of the thallus is rough, in the form of grains. The color of the surface layer is orange-red. Flatshape, color orange-yellow. The undercoat of the thallus is dark gray in color, not clearly noticeable. The incipient apothecia is sessile and sparsely located. The edges of the apothecia disks are intact but, branched. The spores are two-celled, bipolar, and consist of 8 elliptical spores.



a) b) c)

Figure 5. a) Caloplaca decipiens lichen species; b) Manifestation of the apothecia part of the lichen; c) Elliptical spores

5. *Lecanoraceae*–Placolecanora (Lobothallia) alphoplaca—the extreme edges of the areoles of different shapes and sizes are clearly formed, the surface part of the thallus is rough. The color of the thallus is white-gray, in the middle part there are groups of black apotheosis.The shapes of the apothecia are convex and formed in a protruding state. 4 or 8 spores are located in special pockets.

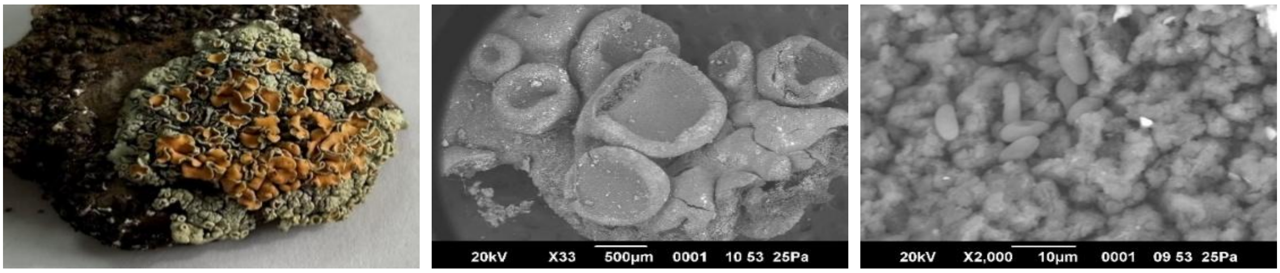


a) b) c)

Figure 6. a) General view of the species Placolecanora (Lobothallia) alphoplaca;
b) The middle part of the convex apothecia part; c) The stage of formation of 8 ellipse-shaped spores

6. *Lecanoraceae*–Lecanora allophana—rare, epilyticlichen. This species is known as an epiphytic species, which grows not only on stone surfaces, but also clinging to forest trees in certain regions of Kazakh-

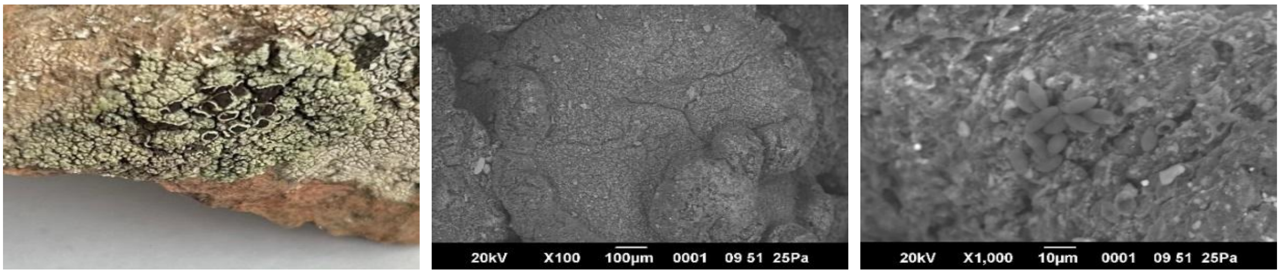
stan. The scaly color of lichens thallus is light gray or pale green. In the middle part, the color is pale yellow, and the edges are pale green, numerous apothecia are formed as a whole or singly. Apothecia are located on the substrate with short ridzines.



a) b) c)

Figure 7. a) General view of the epilytic species Lecanora Allophana;
b)Convex edge apothecarypart; c) The period of formation of ellipse-shaped spores

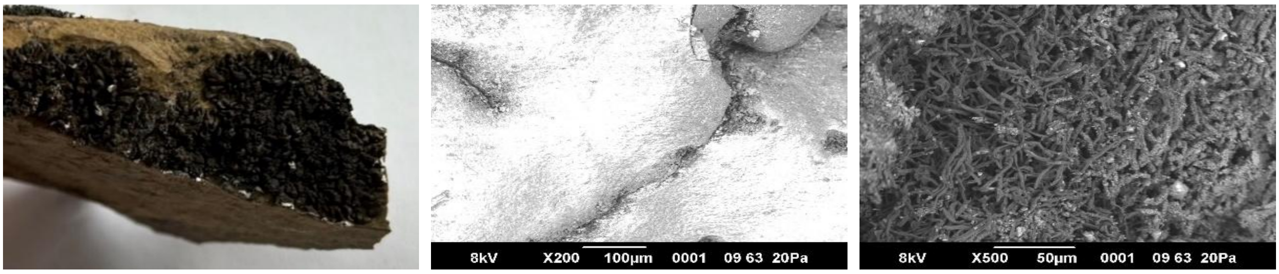
7. *Lecanoraceae*–Lecanora frustulosa—the meetingvest is rarelye pilytic lichen. The surface of the thallus is scaly, the color is greenish-yellow or greenish-gray, the underside is black. While the extreme thalli on the surface of the Stone are formed in an elevated form, tightly adjacent to each other, the thalli of the middle apothecia part are formed, forming a relatively thin layer. The apothecia of the settled black color is numerous, arranged in groups. The apothecia discs are rounded, with jagged edges, and then the flesh changes to a lush state.The shapes of the unicellular colorless spores are ovoid.



a) b) c)

Figure 8. a) Species Lecanora frustulosa with black apothecia;
b) Expression of apothecia with sliced edges; c) The stage of spore formation of the lichen Lecanora frustulosa

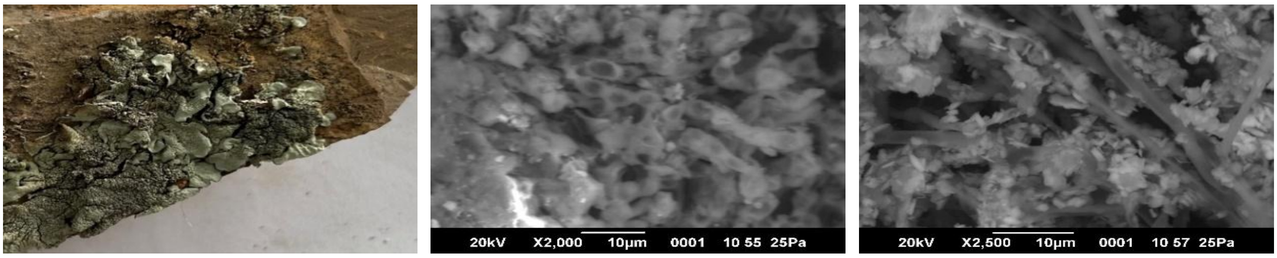
8. *Parmeliaceae*–Parmelia tominii—in the study areas, meetings were frequent. Lichen thallus consists of short ridzines that are not tightly attached to the substrate, of various shapes, the surface is brownish-black in color, with folds, the underside is white, reaching the edge of the thallus. There are no soredia and Isidis. The edges are formed with fleshy thalli tightly touching and covering each other. Thalli located at the edgesare arranged dichotomously or radially branched. The disks of their apothecia, scattered or grouped, are flat, brown in color.



a) b) c)

Figure 9. a) Appearance of lichen Parmelia tominii on the substrate;
b) The apotheos is of the disc is not obvious; c) The lower ridzinal section of the lichen

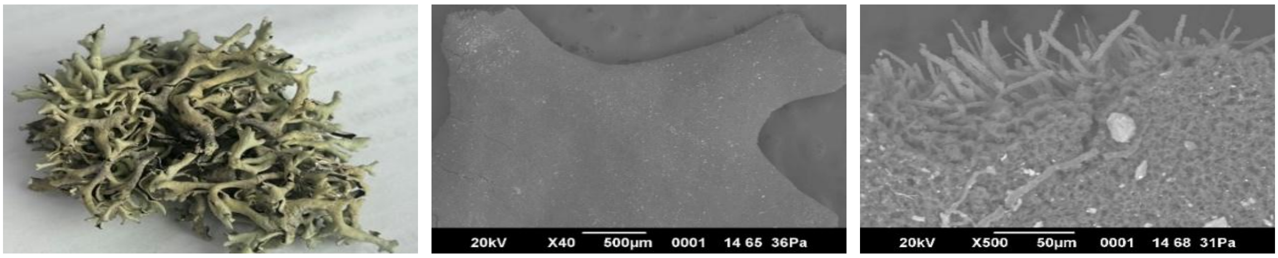
9. *Parmeliaceae*–Parmelia conspersa—rare in research areas. In the middle parts, leaf-shaped thalli are formed by sticking to the surface of the stone, while the extreme edges are loosely raised. The petals of the thallus, cut off from the part, are collected in a dense touch with a wavy spin. The surface is greenish-gray in color, with a large number of isidiums formed on the periphery. Apothecia of black-brown color is formed in a raised form on a set or short-term leg. The middle part of the thallus is whitish, loose, formed by filaments. There are many pycnidis in the form of black spots, spread all over their thallus.



a) b) c)

Figure 10. a) Parmeliaconspersa; b) Section of the apothecary thallus; c) Reflection of isidia formed in groups

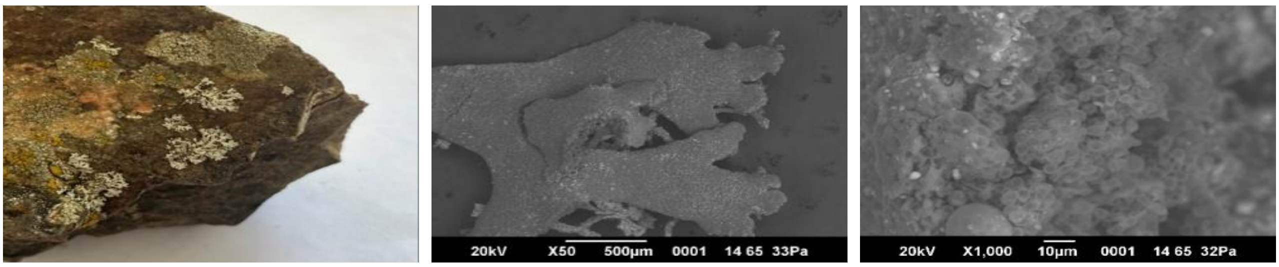
10. *Parmeliaceae*–Parmelia vagans—lichen has been identified as an epigeal species that grows in research areas. The meeting is high. The thallus is loosely arranged on a deciduous substrate, with dichotomous or dorsoventrally branched marginal edges wrapped in a tube and formed by the edges pointing downwards. The surface of the thallus is yellowish-green, the surface is smooth. Soredium, isidium, and apotheosis do not exist.



a) b) c)

Figure 11. a) Parmelia vagans; b) Sectionof the edges of the thallus;
c) Manifestation of the anatomical structure of the thallus

11. *Parmeliaceae*–Parmeliasulcata—indiffent (in the bark of a stone and woody plant), very rare, leaf-shaped lichen with sliced edges. Tightly attached to the substrate, the upper surface of the thallus is bluish-gray in color, arranged in groups. Docked apothecias are rarely formed. Soredia of whitish or greenish color are located covering the surface of the thallus as a whole. Pycnidis are formed at the ends of the slicked edges of the obtuse shape of the lichen thallus. Reproduction is carried out by pycnoconidiums.



a) b) c)

Figure 12. a) Indiffent Parmeliasulcata, which grew scattered on the surface of the stone lichen;
b) Part of the lichen thallus with pycnidia formed along the edges;
c) Soredium appearance, formed as a whole in the surface part of the lichen thallus

The study examined 11 species of lichens growing in the region, combined into 6 genus in 5 families.

Conclusion

Identification of lichen species settled in the mountains of Abraly ($49^0$10N $77^0$25E), Kokentau (Semeytau) ($50^0$10N $79^0$43E), Kalbatau ($49^0$22N $81^0$29|30E) and Chingistau ($48^0$52N $79^0$15E), occupying the eastern part of the Kazakhs hills, belonging to the territory of Abay region, along with the determination of quantitative indicators and covering projects, environmental and biological features were considered.

Of the 11 species, united in 5 identified families, 6 genus, 9 were identified as epilytic lichens growing on the surface of the stone, one species as epigeal, and one species as indifferent (settled on different substrates).

It was found that despite the remoteness of the Abraly, Kokentau (Semeytau), Kalbatau and Chingistau mountains, belonging to the eastern small hills and low mountains of the Kazakhs, lichen species Verrucaria nigrescens, Caloplaca decipiens, Lecanora allophana, Lecanora frustulosa, Parmelia tominii, Parmelia conspersa grow in all mountains. According to the Drude scale, the species Parmelia sulcata, Caloplaca viridirufa, Acarospora chlorophana, Placolecanora alphoplaca were attributed to lichens that are small in number and have a very low incidence.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Zhumaniyazova A.Zh**. — performed the experimental part of the article, analyzed the results. **Bukabayeva Zh.T**. — substantiated the relevance of the topic, conducted a literary review, determined the direction of research, participated in writing annotations and keywords; **Silybayeva B.M. —** laid the theoretical basis of the study, conducted a comparative analysis of scientific works and revealed the scientific novelty of the research problem; **Kunanbayeva N.S**. — prepared diagrams and tables, drew up illustrative materials; **Anuarbekova A.N**. — edited the scientific text, brought it in line with the requirements of the international publication. She made linguistic and stylistic adjustments and ensured the preparation of the article for publication.

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Қазақтың ұсақ тау шоқыларының «шығыс бөлігінде» өсетін қына түрлерін зерттеу

Қазіргі кезеңдегі индустрияның қарқынды дамуы, зиянды қалдық заттардың үйіндісінің артуы, ірі елдімекендердің пайда болуы және т.б. антропогендік факторлардың әсерлері дүниежүзінде климаттың өзгеруін туғызуда. Осыған байланысты атмосфералық ылғалдың азаюы шөл және шөлейтті аймақтардың көлемінің артуына әсері жоғары екендігі белгілі. Жоғарыда аталған жаһандық өзгерістер, жер бетіндегі тірі организмдерге тікелей әсер ете отырып, кез келген флора мен фаунаның түрлік құрамын өзгерістерге ұшыратады. Осыған орай, қазіргі уақытта ғалымдардың жергілікті флорадағы туындаған табиғи өзгерістерге ерекше көңіл бөлуі қажет. Зерттеу жұмысында жергілікті флораның негізгі бір бөлігін құрайтын, әртүрлі биомдарда қоныстанған, өзінің құрылыс ерекшеліктерімен сыртқы ортаның климаттық өзгерістеріне төзімді қына түрлері зерттеу объектісі ретінде қарастырылды. Соңғы жылдарда Қазақстанның өсімдіктер флорасына сәйкес, лихенологиялық жарияланған ғылыми зерттеулерде белгілі бір жерлерде өсетін түрлердің биологиялық ерекшеліктері, экологиясы және индикациялауда қолдануға байланысты деректерді кездестіруге болады. Қазақтың ұсақ тау шоқылырының «шығыс бөлігіне» жататын Шыңғыстау, Қалбатау, Көкентау және Абралы тауларының флорасында кездесетін қына түрлерін қамтитын нақтылы зерттеу жұмыстары жоқ. Зерттеу жұмысында зерттеу объектісі ретінде қарастырылған шығыстың ұсақ тау шоқылары ретінде жалпы атаумен берілген таулар бір-бірінен алыс қашықтықта орналасқандықтан түрлік құрамына байланысты салыстырмалы түрде талдау жасалынды. Барлық ұсақ шоқылы тауларда өсетін 5 тұқымдасқа жататын, 6 туысқа біріктірілген 11 түр анықталды.

Кілт сөздер: Қазақтың ұсақ шоқылары, эпилитті қыналар, эпигейлі қыналар, индиффентті қыналар, Друде шкаласы

Ә.Ж. Жұманиязова, Б.М. Силыбаева, Ж.Т. Букабаева,
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Изучение видов лишайников, произрастающих в «восточной части» Казахских мелкосопочников

Динамичное развитие промышленности на современном этапе, увеличение количества вредных отходов, появление крупных населенных пунктов и, в том числе, воздействие антропогенных факторов вызывает изменение климата во всем мире. В связи с этим известно, что уменьшение атмосферной влаги оказывает большое влияние на увеличение размеров пустынных и полупустынных зон. Вышеупомянутые глобальные изменения, оказывая непосредственное влияние на живые организмы на Земле, вызывают изменения видового состава любой флоры и фауны. В связи с этим в настоящее время ученым необходимо уделять особое внимание естественным изменениям, происходящим в местной флоре. В исследовательской работе объектом исследования рассматривались виды лишайника, составляющие основную часть местной флоры, обитающие в различных биомах, устойчивые к климатическим изменениям внешней среды, со своими особенностями строения. В последние годы, согласно флоре растений Казахстана, в лихенологически опубликованных научных исследованиях можно встретить данные, связанные с биологическими особенностями, экологией и использованием в индикации видов, произрастающих в определенных местах. Нет конкретных исследований, включающих виды лишайника, встречающиеся во флоре гор Абралы, Чингистау, Калбатау, Кокентау, относящихся к мелкому горному хребту востока. В исследовательской работе был проведен сравнительный анализ, связанный с видовым составом, поскольку горы, названные общим названием как небольшие горные холмы востока, рассматриваемые как объект исследования, расположены на большом расстоянии друг от друга. Было идентифицировано 11 видов, объединенных в 7 родов, принадлежащих к 5 семействам, произрастающим во всех небольших холмистых горах.

Ключевые слова: Казахские мелкосопочники, эпилитные лишайники, эпигейные лишайники, индифферентные лишайники, шкала Друде

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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)

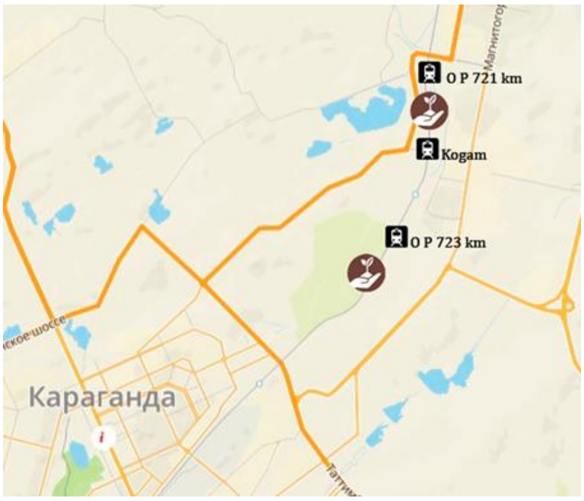

Figure 2. General map of soil sampling sites (3-4)

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).

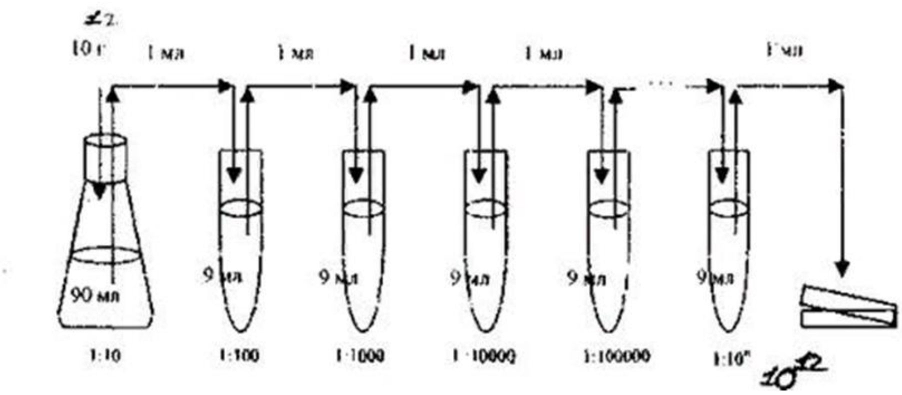

Figure 7. Carrying out the dilution method

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).

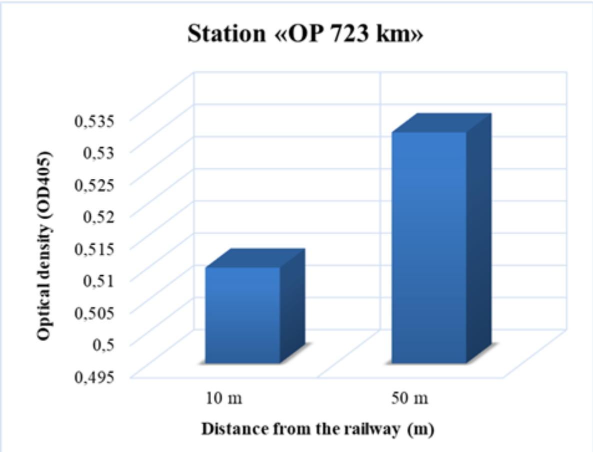

Figure 8. Cellulolytic activity on the Station *OP 723 km*

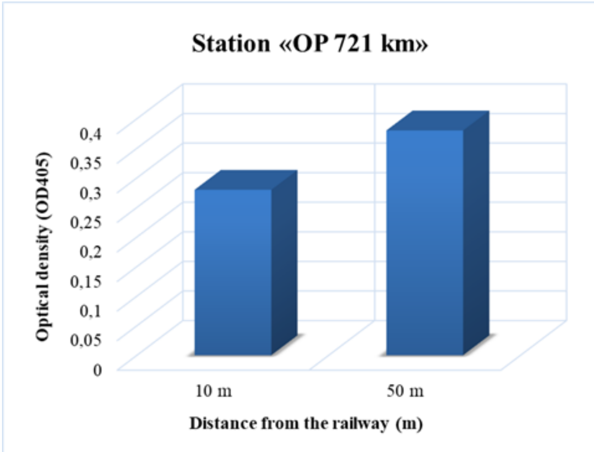

Figure 9. Cellulolytic activity on the Station *OP 721 km*

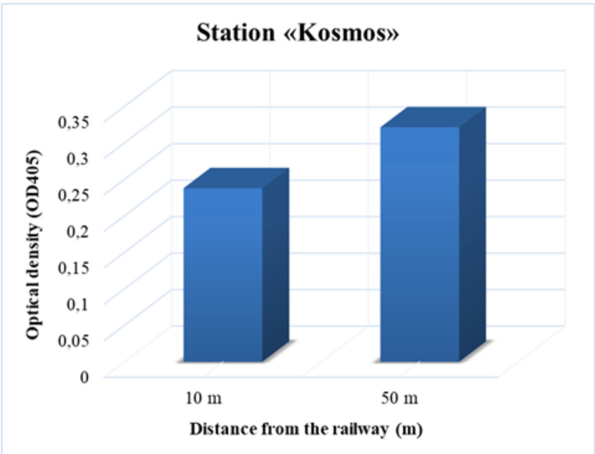

Figure 10. Cellulolytic activity on the Station *Kosmos*

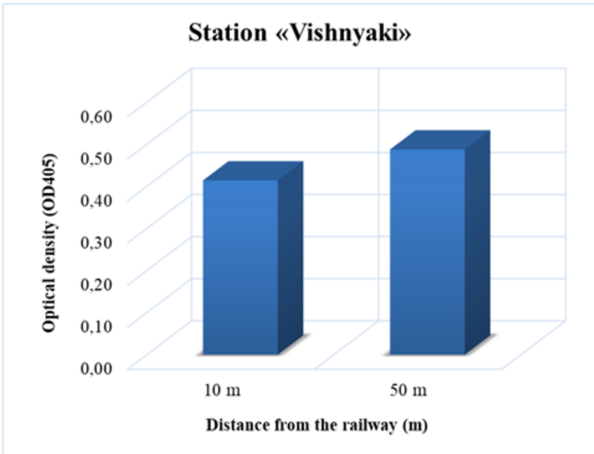

Figure 11. Cellulolytic activity on the Station *Vishnyaki*

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.

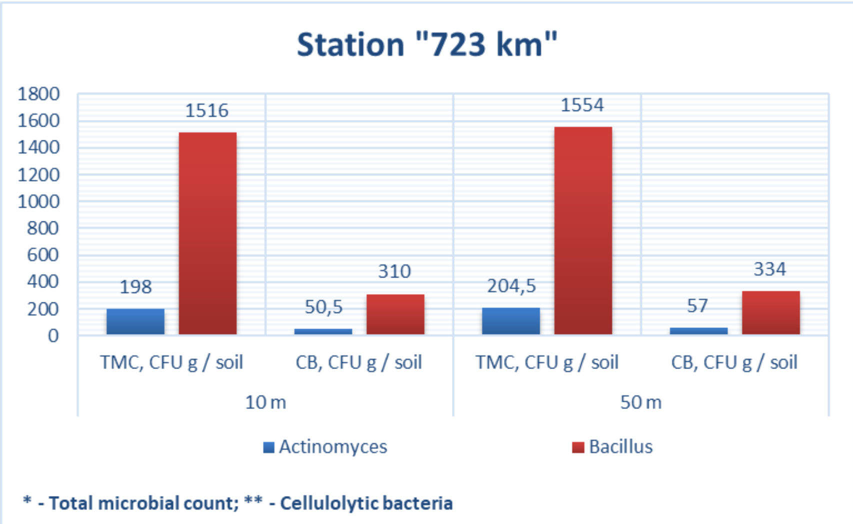

Figure 12. Specific gravity of cellulose-decomposing microorganisms at Station *OP 723 km*

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 ($p < 0.001$).

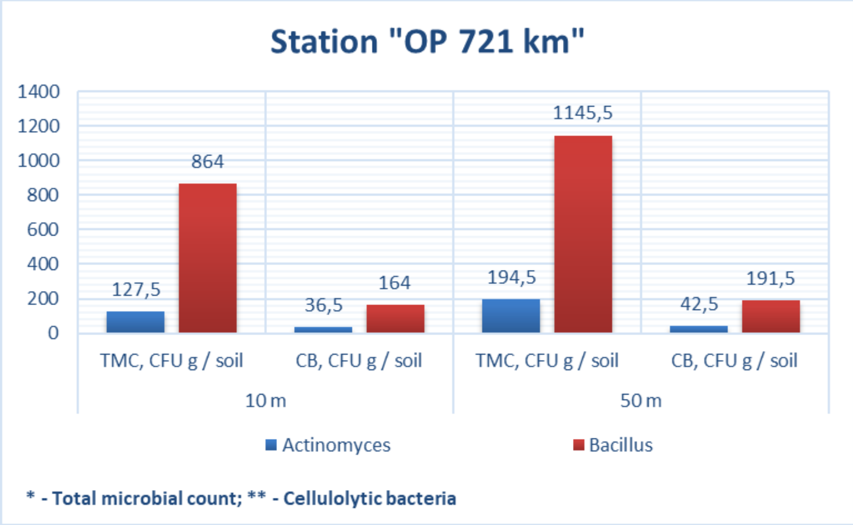

Figure 13. Specific gravity of cellulose-decomposing microorganisms at Station *OP 721 km*

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 ($p < 0.001$).

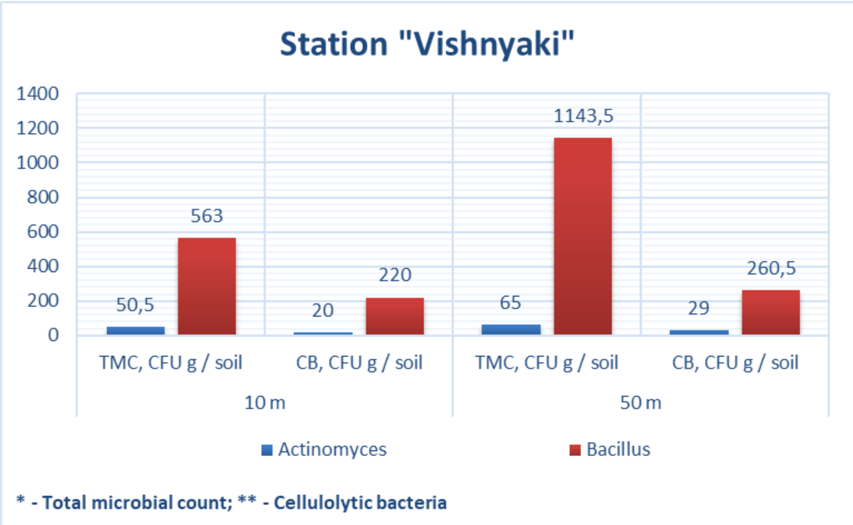

Figure 14. Specific gravity of cellulose-decomposing microorganisms at the Station *Vishnyaki*

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.

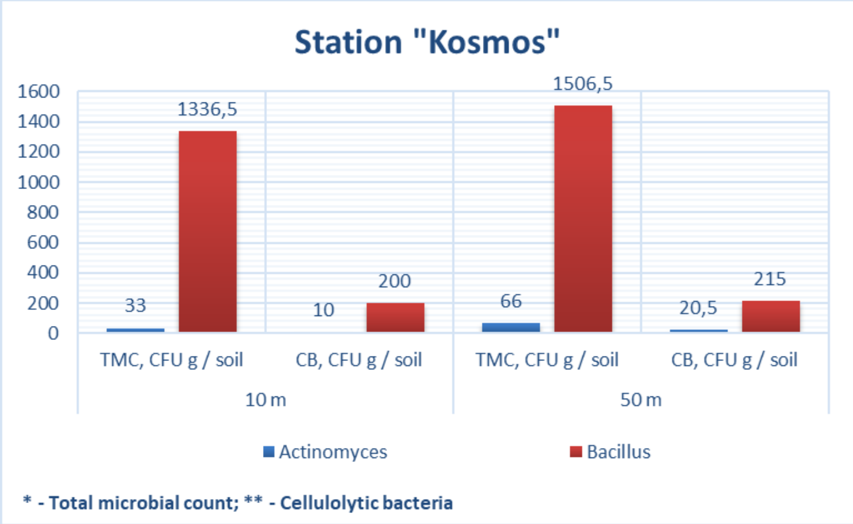

Figure 15. Specific gravity of cellulose-decomposing microorganisms at the Station *Kosmos*

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.

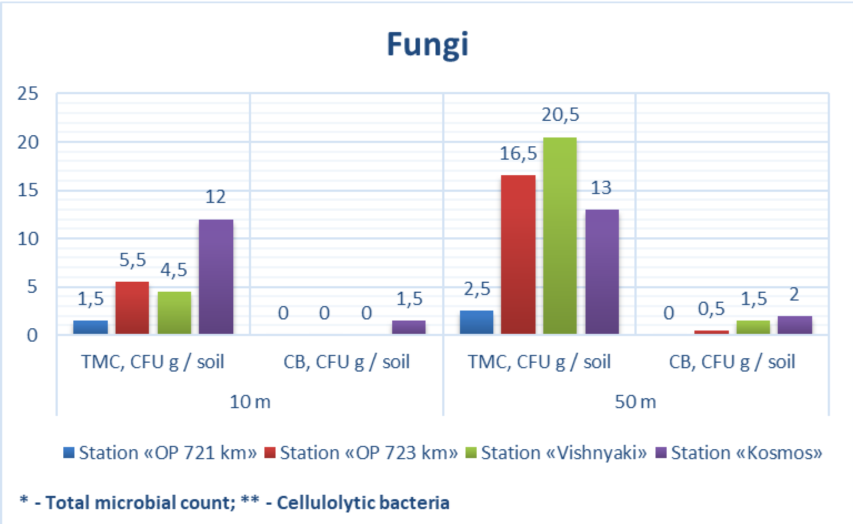

Figure 16. Specific gravity of cellulose-decomposing fungi

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.

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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

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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

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Morphological and biochemical analysis of hawthorn and rosehip of the Ulytau region

The study investigates the morphological and biochemical profiles of two forms of *Crataegus chlorocarpa* and five forms of *Rosa* species (*R. laxa, R. acicularis, R. spinosissima*) from natural populations in the Zhanaarka district, Ulytau region, Kazakhstan. Geographical coordinates, shrub height, age, crown shape, yield, fruit size, color, and taste were assessed. Biochemical analyses quantified vitamin C, anthocyanins, leucoanthocyanidins, catechins, sugars, pectins, and titratable acidity using standardized methods. *Rosa acicularis*-058 showed the highest vitamin C content (576.4 mg/100 g FW), while *Rosa spinosissima*-064 excelled in anthocyanins (1259.3 mg/100 g FW). *Crataegus* forms were notable for their catechin content (up to 242.3 mg/100 g FW). Pearson's correlation revealed strong positive correlations between vitamin C and leucoanthocyanidins (r=0.83, p=0.02), and between titratable acidity and sugars (r=0.86, p=0.01).UPGMA cluster analysis identified three distinct groups based on biochemical traits. These findings highlight the potential of these wild plants for nutraceutical development and breeding, as they surpass some conventional crops in bioactive compounds.

Keywords: Ulytau region, *Crataegus chlorocarpa*, *Rosa* species, morphology, biochemistry, vitamin C, anthocyanins, cluster analysis

Introduction

The vegetation cover of the Ulytau region ranges from steppe to desert, with plants such as *Artemisia, Stipa*, and *Salsola* dominating. The climate of the region is dry and continental, characterized by a hydrothermal coefficient of approximately 0.5, an average annual air temperature ranging from 2.8 to 3.6 °C, and a summer temperature peak of 23-24 °C. Significant temperature fluctuations and limited precipitation create difficult conditions for plant life, resulting in vegetation that is typical of northern deserts and that varies from north to south depending on humidity and temperature. The tree and shrub flora of Ulytau is mainly from the *Rosaceae* family and is widely used for landscaping in the industrial regions of Karaganda and Zhezkazgan. Among them, the most common are *Lonicera tatarica* L., *Elaeagnus angustifolia* L., and *Elaeagnus oxycarpa* Schlecht., *Padus avium* Mill., *Viburnum opulus* L., *Rosa laxa*, and *Crataegus chlorocarpa* [1].

The genus *Rosa* comprises approximately 400 species worldwide, with 21 species native to Kazakhstan, four of which are endemic [2–4]. This genus is of growing interest due to its fruits used in the pharmaceutical industry and folk medicine [5]. Rosehips are rich in vitamins, minerals, and phytochemicals, which are confirmed by modern scientific research [6–8]. They are used for the production of drinks, jams, jellies, tea, and wine [9, 10].

The genus *Crataegus* is represented by more than 300 species, seven of which grow in the mountainous and steppe regions of Kazakhstan [2–4]. Hawthorn fruits are valued for their cardiotonic properties and are widely used in official medicine in the treatment of cardiovascular diseases [11]. In addition to medicinal use, they are in demand in the food industry for the preparation of jams, jellies, and jelly. In several countries, such as China, Turkey, and Iran, hawthorn is cultivated as a fruit crop [12–14].

Growing interest in nutraceuticals is driving the selection of forms high in phenols and antioxidants that are superior to traditional crops such as blueberries and sea buckthorn [15–18].

In the Ulytau region, rosehips and hawthorns grow mainly in amateur gardens and are used as border plants, although their commercial cultivation is not yet developed.

This study aimed to determine the morphological characteristics of the fruits, as well as the biochemical indicators of selected forms of hawthorn and rosehip from the Zhanaarka district of the Ulytau region.

Experimental

The study was carried out in the natural populations in the Zhanaarka district of the Ulytau region. Two forms of *Crataegus* (*Crataegus chlorocarpa*: Cr-049, Cr-050) and five forms of rosehips (*Rosa laxa*: *R.laxa*-049, *R.laxa*-051; *R. acicularis*: R.acic-058, R.acic-063; *R. spinosissima*: R.spin-064) were investigated.

The coordinates and altitudes of the growing sites were determined using a Garmin eTrex 10 GPS navigator (Tab. 1). For each bush, the origin (seed or sprout), age (using an increment borer [19]), height, crown shape, and yield (on a 5-point scale) were recorded. The fruits were harvested at the end of August, transported in plastic containers, and stored at -20°Cuntil analysis.

Table 1

Coordinates of the localities of the selected forms of *Crataegus chlorocarpa*and species of the genus *Rosa* (*R. laxa, R. acicularis, R. spinosissima*)

Forms	Forestry	Coordinates		Altitude m
		North	East	
Cr-049	Zhanaarka	48º55′6815″	070°54′9710″	567
Cr-050	Zhanaarka	48º55′6782″	070°55′0079″	518
R.laxa-049	Zhanaarka	48º55′6815″	070°54′9710″	567
R.laxa-051	Zhanaarka	48º55′6754″	070°55′0154″	517
R.acicularis-058	Karazhal	47º59′6157″	070°48′3381″	490
R.acicularis-063	Boltai	47º47′0221″	071°31′8400″	645
R.spinosissima-064	Boltai	47º46′9958″	071°31′8493″	644

Morphological analysis. The size of the fruits was measured with a caliper with an accuracy of 0.1 mm. Length, width, shape, color of the peel and pulp, taste, number of seeds, and ripening time were determined.

Biochemical analysis. Vitamin C content was determined by the iodometric method [20]. Leucoanthocyanidins (LAC) were analyzed using the butanol-HCl method [21]. Anthocyanins were extracted with a 1 % HCl solution in 96 % ethanol [22]. Catechins were determined by the vanillin method [23]. Titratable acidity (TA) was measured by titration with 0.1 N NaOH. The content of sugars was determined colorimetrically by the Bertrand method [24], and water-soluble pectin (WSP) and water-insoluble pectin (WIP) were determined by the carbazole method with calibration by galacturonic acid [25]. The results were expressed in mg/100 g of fresh weight (FW).

Statistical analysis. Mean values and standard deviations were calculated in *Microsoft Excel* 2019. P-value and confidence intervals adjusted for comparing a family of 7 estimates (confidence intervals corrected using the Tukey method).

Cluster analysis was performed using the unweighted pair-group method with arithmetic mean (UPGMA) with Euclidean distance using Past Software.

Results and Discussions

Table 1 shows that most of the forms of fruit plants are concentrated in the Zhanaarka forest. The northernmost points are represented by forms from Zhanaark (latitude coordinates 48° and above). Forms Karazhal and Boltay are represented at more southern latitudes (47°). The forestry of Zhanaarka is represented by four forms (Cr-049, Cr-050, *R.laxa*-049, *R.laxa*-051), which are geographically very close to each other. Karazhal is represented by one form (*R.acicularis*-058). Boltai is represented by two forms (*R.acicularis*-063, *R.spinosissima*-064) with similar coordinates.

The minimum altitude is recorded in Karazhal (490 m). The maximum altitude is in Boltay (645 and 644 m). Zhanaark sections are located at altitudes of 517–567 m. Altitude range: 490–645 m. The data are conducive to a comparative analysis of ecological and geographical conditions at different points of growth of fruit plant species in the Ulytau region.

As part of the study of economically valuable traits, the morphological features of shrubs and fruits of the selected forms of fruit plants growing in the Zhanaarka district of the Ulytau region were analyzed (Fig. 1).

Figure 1. A — *Crataegus chlorocarpa*-049; B — *Crataegus chlorocarpa*-050; C — *R.laxa*-049;
D — *R.laxa*-051; E — *R.acicularis*-058; F — *R.acicularis*-063; G — *R.spinosissima*-064

Two forms of *Crataegus chlorocarpa*have seed origins, aged 8 years, with a height of 3.5 m (049) and 4.0 m (050). A spreading crown was noted in the *Crataegus chlorocarpa* (Cr-049), and a spherical in the *Crataegus chlorocarpa* (Cr-050) form. The yield of both forms is high — 5 points (Tab. 2).

All selected 5 forms of rosehips have a rootsucker origin. The age of the bushes varies from 6 to 10 years. The height of the bushes ranges from 1.5 m (*R-laxa* 051, *R.spinosissima*-064) to 3.0 m (*R.acicularis*-063). The crown shape is spherical (*R. laxa*-049) and spreading (in other forms). The yield is excellent: 5 points (*R.laxa*-049, *R.laxa*-051, *R.acicularis*-058), 4 points (*R.acicularis*-063, *R.spinosissima*-064) (Tab. 2).

Table 2

Morphological description of the selected forms of fruit plants in the Zhanaarka district of the Ulytau region

Form	Origin	Shrub age	Shrub height, m	Crown form	Productivity, points
Cr-049	Seed	8	3,5	Spreading	5
Cr-050	Seed	8	4,0	Spherical	5
R.laxa-049	Rootsucker	6	2,0	Spherical	5
R.laxa-051	Rootsucker	10	1,5	Spreading	5
R.acicularis-058	Rootsucker	8	2,5	Spreading	5
R.acicularis-063	Rootsucker	10	3,0	Spreading	4
R.spinosissima-064	Rootsucker	8	1,5	Spreading	4

The fruits of the two selected forms of *Crataegus chlorocarpa* are round and orange, with orange pulp. The taste of the fruits is sweet. The ripening period of fruits was observed in the 3rd decade of August (Tab. 3).

T a b l e 3

Main economically valuable traits of selected forms of fruit plants in the Zhanaarka district of the Ulytau region

Form	Fruit length (cm)	Fruit width (cm)	Fruit shape	Peel color	Pulp color	Fruit taste	Quantity of seeds	Ripening period
Cr-049	1.05±0,02	1.05±0,02	Round	Orange	Orange	Sweet	3.6±0,54	3 decade of August
Cr-050	1.1±0,02	1.1±0,02	Round	Orange	Orange	Sweet	4.74±0,5	3 decade of August
R.laxa-049	2.04±0,04	1.14±0,06	Pear-shaped	Red	Orange	Sweetish-sour	45.6±2,4	3 decade of August
R.laxa-051	2.28±0,05	1.16±0,03	Pear-shaped	Red	Orange	Astringent	12.8±0,8	2 decade of Sept.
R.acic-058	1.48±0,04	1.09±0,03	Pear-shaped	Red	Orange	Sweetish-sour	7.8±0,83	1 decade of Sept.
R.acic-063	1.55±0,07	1.28±0,03	Pear-shaped	Dark red	Orange	Sweet	7.8±0,83	1 decade of Sept.
R.spin-064	0.74±0,03	0.98±0,02	Flat-globose	Black	Black	Sweetish-sour	3.4±1,51	2 decade of Sept.

The maximum fruit size is found in *R. laxa*-049 (2.04 cm) and *R. laxa*-051 (2.28 cm), the minimum in *R. spinosissima*-064 (0.74 cm). The shape of the fruits is pear-shaped (*R.laxa*-049, *R.laxa*-051, *R.acicularis*-058, *R.acicularis*-063) and flat-globose (*R.spinosissima*-064). The color of the peel is red in the forms *R. laxa*-049, *R. laxa*-051, *R. acicularis*-058, dark red in the form *R. acicularis*-063, and black in *R. spinosissima*-064. The pulp is mostly orange in all forms of rosehips, except for the *R. spinosissima*-064 form, which is black. Taste: sweetish-sour (*R.laxa*-049, *R.spinosissima*-064), astringent (*R.laxa*-051), sweetish-sour (*R.acicularis*-058), sweet (*R.acicularis*-063). Number of seeds: from 3.4 (*R.spinosissima*-064) to 45.6 (*R.laxa*-049). Ripening period was 3rd decade of August (*R.laxa*-049), 1-2nd decade of September (other forms).

The analysis revealed the morphological diversity of the selected forms of fruit plants. Seed forms of *Crataegus chlorocarpa* are characterized by high yields, considerable height, and rounded orange fruits with a sweet taste, which makes them suitable for fresh consumption. Wild rose forms are distinguished by a wide variety of shapes, colours, and taste qualities of fruits, which allows them to be used in breeding for various purposes (food, technical, and processing). These forms can serve as valuable material for creating new varieties with high yields, original fruit characteristics, and adaptation to regional conditions.

Table 4 contains comparative data on the biochemical indicators of the selected forms of *Crataegus chlorocarpa* and species of the genus *Rose*. The content of vitamin C, leucoanthocyanidins, catechins, anthocyanins, titratable acidity, total sugars, water-soluble pectin, and water-insoluble pectin was measured.

T a b l e 4

Biochemical indicators of the selected forms of fruit plants in the Zhanaarka district of the Ulytau region

Form	Vitamin C mg/% FW	LAC mg/% FW	Catechin mg/% FW	Antocyanin mg/% FW	TA % FW	Sugars % FW	WSP % FW	WIP % FW
Cr-049	2.99±0.07^{a}	193.7±0.2^{d}	180.0±0.2^{b}	30.7±0.3^{d}	0.64±0.01^{a}	4.2±0.4^{a}	0.55±0.02^{a}	0.36±0.06^{a}
Cr-050	3.34±0.02^{a}	140.4±0.2^{f}	242.3±0.1^{a}	16.8±0.3^{e}	0.56±0.01^{a}	3.6±0.15^{a}	0.95±0.03^{b}	0.84±0.02^{b}
R.laxa-049	140.4±0.3^{d}	166.3±0.4^{e}	164.4±0.1^{c}	7.92±0.03^{g}	2.4±0.1^{b}	13.8±0.4^{b}	2.08±0.03^{d}	1.49±0.01^{c}
R.laxa-051	82.36±0.2^{b}	70.56±0.3^{g}	89.13±0.1^{d}	42.6±0.2^{c}	1.8±0.1^{b}	19.2±0.15^{c}	2.44±0.05^{e}	2.28±0.01^{e}
R.acic-058	576.4±0.2^{f}	435.6±0.4^{a}	29.52± 0.03^{e}	52.5±0.2^{b}	3.9±0.2^{c}	24.6±0.3^{d}	1.41±0.01^{c}	1.73±0.01^{d}
R.acic-063	153.1±0.4^{e}	200.16± 0.2^{c}	182.4±0.2^{b}	12.87±0.03^{f}	3.2±0.2^{c}	21.6± 0.15^{cd}	1.39±0.03^{c}	5.73±0.02^{d}
R.spin-064	9.50±0.1^{c}	228.2±0.1^{b}	4.03±0.02^{f}	1259.3± 0.35^{a}	2.24±0.07^{b}	24.9±0.15^{d}	2.14±0.01^{d}	2.24±0.02^{e}

Note – Different letters in the same column indicate significant differences at P≤0.05; Abbreviations: TA-titratable acidity; WSP-water-soluble pectin; WIP-water-insoluble pectin; LAC — leucoanthocyanidin

The highest content of vitamin C was recorded in *Rosa acicularis*-058 at 576.4 mg/100 g, which is an order of magnitude higher than the rest of the samples. The minimum values for the forms of *Crataegus chlorocarpa* (Cr-049, Cr-050) are less than 4 mg/100 g.

The richest forms of leucoanthocyanidin are *Rosa acicularis*-058 (435.6 mg/100 g) and *Rosa spinossisima*-064 (228.2 mg/100 g). Among hawthorns, the level of leucoanthocyanidins is lower (about 140–194 mg/100 g). The maximum values of catechins were found in *Crataegus chlorocarpa*-050 (242.3 mg/100 g) and *Rosa acicularis*-063 (182.4 mg/100 g).

In terms of anthocyanin content, *Rosa spinosissima*-064 (1259.3 mg/100 g) is the absolute leader; the closest competitor in terms of anthocyanin content is inferior tenfold—for *Rosa acicularis*-058, *the* value is only 52.5 mg/100 g, and the rest of the samples are even lower.

The highest titrable acidity is in *Rosa acicularis*-058 (3.9 g/100 g), followed by *Rosa acicularis*-063 (3.2 g/100 g). The lowest values were observed in two forms of hawthorn–0.56-0.64 %.

Sugars are the most important component of fruits and berries. The content of sugars in fruits is a feature of the species: their high or low levels, inherent in a botanical species, usually remain quite stable over many years, similar in climatic conditions and in different habitats [25]. Hawthorn fruits contain mainly fructose, as well as small amounts of glucose and sucrose [26]. The clear leaders in sugars are various forms of the genus *Rosa* (especially *R. spinosissima* and *R. acicularis* — more than 24 g/100 g), hawthorn has much less sugar (3.6–4.2 g/100 g).

The largest amount of water-soluble pectin was found in *Rosa laxa*-051 (2.44), and water-insoluble pectin in *Rosa acicularis*-063 (5.73).

In all cases, the fruits of the genus *Rosa* are significantly superior to hawthorn in terms of vitamin C, sugars, and acidity. The form *R. spinosissima*-064 stands out for its ultra-high anthocyanin content. *Crataegus chlorocarpa* is promising in terms of catechin content, but its fruits are insignificant in most other parameters. For use in the food industry or medicine, the fruits of *Rosa acicularis*-058 are optimal in terms of vitamin C content.

To obtain anthocyanins in large quantities, *Rosa spinosissima*-064 should be chosen. *Crataegus* fruits are mainly suitable for the production of catechins, but are inferior in other respects. The data allow us to recommend different forms for targeted needs—for example, *Rosa acicularis*-058 for the production of vitamin concentrates, *Rosa spinosissima*-064 for anthocyanin extract, and hawthorn as a source of catechins.

Table 5 shows Pearson's correlation coefficients between nine variables measured in the fruits of various species of rose hips and Altai hawthorn. The data are based on seven samples collected at different altitudes (from 490 to 645 m).

Table 5

Pearson's correlation matrix between nine variables of different forms of rosehip species (*R. laxa, R. acicularis, R. spinosissima*) and two forms of C.chlorocarpa

Compound	Vitam C	LAC	Catechin	Anthocyan	TA	Sugar	WSP	WIP	Altitude
Vitamin C	1.00 (0.00)	**0.83 (0.02)**	-0.43 (0.33)	-0.26 (0.57)	**0.80 (0.03)**	0.52 (0.23)	0.03 (0.94)	0.13 (0.79)	-0.44 (0.32)
LAC	**0.83 (0.02)**	1.00 (0.00)	-0.50 (0.25)	0.11 (0.82)	0.66 (0.11)	0.45 (0.32)	-0.23 (0.62)	0.00 (1.00)	-0.15 (0.74)
Catechin	-0.43 (0.33)	-0.50 (0.25)	1.00 (0.00)	-0.64 (0.12)	-0.56 (0.19)	**-0.81 (0.03)**	-0.56 (0.19)	-0.05 (0.91)	-0.04 (0.93)
Anthocyan	-0.26 (0.57)	0.11 (0.82)	-0.64 (0.12)	1.00 (0.00)	0.06 (0.90)	0.45 (0.32)	0.37 (0.41)	0.03 (0.95)	0.55 (0.20)
TA	**0.80 (0.03)**	0.66 (0.11)	-0.56 (0.19)	0.06 (0.90)	1.00 (0.00)	0.86 (0.01)	0.38 (0.40)	0.59 (0.17)	0.11 (0.82)
Sugare	0.52 (0.23)	0.45 (0.32)	**-0.81 (0.03)**	0.45 (0.32)	0.86 (0.01)	1.00 (0.00)	0.65 (0.12)	0.59 (0.16)	0.27 (0.56)
WSP	0.03 (0.94)	-0.23 (0.62)	-0.56 (0.19)	0.37 (0.41)	0.38 (0.40)	0.65 (0.12)	1.00 (0.00)	0.26 (0.57)	0.11 (0.81)
WIP	0.13 (0.79)	0.00 (1.00)	-0.05 (0.91)	0.03 (0.95)	0.59 (0.17)	0.59 (0.16)	0.26 (0.57)	1.00 (0.00)	0.58 (0.17)
Altitude	-0.44 (0.32)	-0.15 (0.74)	-0.04 (0.93)	0.55 (0.20)	0.11 (0.82)	0.27 (0.56)	0.11 (0.81)	0.58 (0.17)	1.00 (0.00)
In brackets: p-values (p<0.05)									

A significant and strong correlation was observed between vitamin C and leucoanthocyanidins (LAC) (r=0.83, p=0.02). In rose hips, vitamin C often correlates with other antioxidants, as shown in studies [27], where the content of ascorbic acid varies with genotype and growing conditions.

Titratable acidity (TA) is associated with increased vitamin C content (r=0.80, p=0.03), which is typical for fruit plants: organic acids stabilize vitamin C and promote its accumulation. A correlation was found between titratable acidity and sugars (r=0.86, p=0.01). Acidity and sugars often balance each other in fruits (the sugar/acid ratio affects the taste). In rose hips, sugars (fructose, glucose) correlate with ripeness and acids. A moderate correlation was observed between sugars and water-soluble pectins (WSP) (r=0.65, p=0.12). WSP often increases with sugar content, as both are associated with fruit ripening and cell wall hydrolysis.

Other moderate correlations were also noted: LAC and TA (r=0.66), Sugars and WIP (water-insoluble pectin) (r=0.59), and TA and WIP (r=0.59). These relationships indicate a group of variables related to acidity and carbohydrates (TA, Sugars, pectins), which reflects fruit metabolism. In studies of the flora of Transylvania [28], vitamin C and acids contained in *Rosa canina* correlate positively, as they accumulate in response to stress (cold, altitude).

A significant negative correlation was observed between catechin and sugars (r=-0.81, p=0.03). Catechins (polyphenols) decrease with an increase in sugars, which may indicate different metabolic pathways: catechins are synthesized in the early stages, while sugars are synthesized during ripening. Catechin and anthocyanin (r=-0.64, p=0.12)—moderate negative. Anthocyanins and catechins are both flavonoids, but their synthesis may compete (e.g., under the influence of light or temperature).

A moderate positive correlation was found between altitude and anthocyanin content (r=0.55, p=0.20). Anthocyanins often increase at altitude due to increased UV radiation (stress protection), as in studies of *Rosa canina* [27]. WIP and Altitude (r=0.58, p=0.17), insoluble pectins may increase at altitude for structural adaptation (thicker cell walls). This is consistent with the literature: in rosehips at high altitudes, anthocyanins and pectins increase for protection, while vitamin C varies by genotype [28].

Thus, the correlation matrix reveals key patterns in the biochemistry of rosehips and hawthorn (Fig. 2): strong positive correlations between Vitamin C, LAC, TA, and sugars suggest synergistic metabolism of antioxidants and carbohydrates associated with fruit maturity and quality.

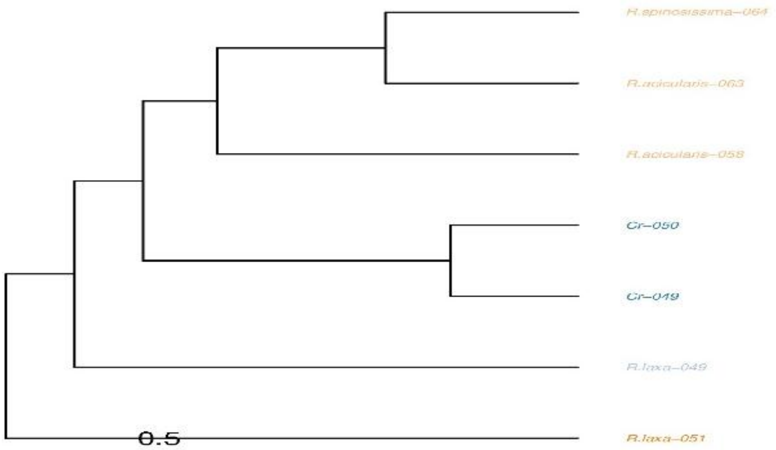

Figure 2. Cluster analysis of selected fruit plants by biochemical characteristics

Negative correlations with Catechin indicate alternative pathways for flavonoid biosynthesis. The altitude at which fruit plants grow in Ulytau has a weak effect, but may stimulate anthocyanins and pectins as an adaptation to stress, as confirmed by studies in Turkey [29], where in *Rosa canina* genotypes, anthocyanin content increases with altitude due to UV.

Based on the structure and the association distances, the following key points are distinguished: Cluster 1: *R.spinosissima*-064, *R.acicularis*-063, *R.acicularis*-058—these elements are combined at a short distance, which indicates their high similarity. Cluster 2: Cr-050, Cr-049—also close elements, but different from Cluster 1. Cluster 3: *R.laxa*-049, *R.laxa*-051–coalesced at a distance close to 0.5 and more distant from other clusters. Distances and similarities: Mergers within each cluster occur at a distance of less than 0.5, suggesting a close relationship within the groups. Clusters 1 and 2 join each other at a distance greater than 0.5, showing moderate similarity, while Cluster 3 joins them at an even greater distance, indicating a significant difference. The distance threshold of 0.5 separates the three main clusters. Color coding: Orange markings: *R.spinosissima*-064, *R.acicularis*-063, *R.acicularis*-058, *R.laxa*-051**—**belong to the genus *Rosa*. Blue markings: Cr-050, Cr-049**—**indicate the genus *Crataegus*. Light blue mark: *R.laxa*-049**—**also belongs to the genus *Rosa*, but highlighted separately, possibly due to the characteristics of the species.

Thus, the dendrogram distinguishes three main clusters: Cluster 1 (*R.spinosissima*-064, *R.acicularis*-063, *R.acicularis*-058), Cluster 2 (Cr-050, Cr-049), Cluster 3 (*R.laxa*-049, *R.laxa*-051). Clusters 1 and 2 are more similar to each other than to Cluster 3, as indicated by the union distances. A threshold of 0.5 effectively separates these groups. Color coding (orange for the genus *Rosa*, blue for *Crataegus*) suggests taxonomic differences between clusters.

Conclusions

In conclusion, this comprehensive analysis of two *Crataegus chlorocarpa* forms and five Rosa species from the Ulytau region reveals substantial morphological diversity and biochemical richness, adapted to the arid continental climate. Key highlights include the superior vitamin C in *Rosa acicularis*-058, exceptional anthocyanins in *Rosa spinosissima*-064, and high catechins in Crataegus forms, with Rosa species generally outperforming hawthorn in vitamins, sugars, and acidity. Correlation and cluster analyses underscore synergistic metabolic pathways and taxonomic groupings, influenced minimally by altitude. These results position Ulytau's wild fruits as valuable resources for nutraceuticals, pharmaceuticals, and food products, potentially exceeding traditional antioxidants like blueberries. The data inform targeted breeding for resilient varieties, supporting sustainable use under Kazakhstan's Flora Law. Future studies should incorporate genetic sequencing, multi-year environmental monitoring, and expanded sampling to validate and extend these findings for commercial applications.

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Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was prepared with the contributions of all authors, who have given their approval to the final version. **Mukan G.S.** — conceptualization, project administration, writing, review and editing; **Sankaibayeva A.G. —** investigation, data curation, and plant material collection; **Kidarbek T.** — methodology, formal analysis, and visualization; **Baimaganbetova M.M. and Dostemessova A.B.** — data curation, statistical analysis.

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Ұлытау облысындағы долана мен итмұрынның морфологиялық және биохимиялық талдауы

Зерттеу Қазақстанның Ұлытау облысы Жаңаарқа ауданында өсетін Алтай доланасының (*Crataegus chlorocarpa*) екі түрі мен итмұрынның (*Rosa laxa, R. acicularis, R. spinosissima*) бес түрінің морфологиялық және биохимиялық сипаттамаларын анықтауға арналған. Зерттеу барысында өсімдіктердің географиялық координаттары, өсу биіктігі, жасы, бөрікбасының пішіні, өнімділігі, сондай-ақ жемістерінің мөлшері, түсі мен дәмі тіркелді. Биохимиялық талдау құрамында С дәрумені, антоцианиндер, лейкоантоцианидиндер, катехиндер, қанттар, пектиндер және титрленетін қышқылдық көрсеткіштері анықталды. *Rosa acicularis*–058 С дәруменінің жоғары мөлшерімен (576,4 мг/100 г) ерекшеленсе, *R. spinosissima*–064 антоцианиндердің көп мөлшерімен (1259,3 мг/100 г) көзге түсті. Доланының зерттелген формалары катехиндер көзі ретінде перспективалы екені анықталды. Кластерлік талдау нәтижесінде әртүрлі биохимиялық профилі бар үш топқа жататын өсімдіктер айқындалды. Алынған мәліметтер жаңа сорттарды іріктеу және функционалдық тағам өнімдерін әзірлеу үшін пайдалануға болады.

Кілт сөздер: Ұлытау облысы, *Crataegus chlorocarpa*, Rosa түрлері, морфология, биохимия, С витамині, антоцианиндер, кластерлік талдау

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Морфологический и биохимический анализ боярышника и шиповника Улытауской области

Исследование посвящено изучению морфологических и биохимических характеристик двух форм боярышника алтайского (*Crataegus chlorocarpa*) и пяти форм шиповника (*Rosa laxa, R. acicularis, R. spinosissima*), произрастающих в Жанааркинском районе Улытауской области Казахстана. Определены географические координаты, высота произрастания, возраст, форма кроны, урожайность, а также размеры, окраска и вкус плодов. Биохимический анализ включал определение содержания витамина С, антоцианов, лейкоантоцианидинов, катехинов, сахаров, пектинов и титруемой кислотности. Выявлено, что *Rosa acicularis-058* отличается высоким содержанием витамина С (576,4 мг/100 г), а *Rosa spinosissima-064* – повышенным содержанием антоцианов (1259,3 мг/100 г). Формы боярышника перспективны как источник катехинов. Результаты кластерного анализа показали три группы растений с различными биохимическими профилями. Полученные данные могут быть использованы для селекции новых сортов и разработки функциональных продуктов.

Ключевые слова: Улытауская область, *Crataegus chlorocarpa*, виды *Rosa*, морфология, биохимия, витамин С, антоцианы, кластерный анализ

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Vegetation cover of the Kulaly Islands in the Kazakhstan region of the Caspian Sea

This study presents a comprehensive analysis of the floristic and ecological diversity of vascular plants on Kulaly Island, located in the Kazakhstani sector of the Caspian Sea. Field surveys documented 149 species, predominantly annual therophytes (47.0%) and perennial xerophytes, reflecting adaptations to arid climates, high soil salinity, and unstable substrates. Most taxa are associated with sandy and solonchak habitats, while wetland and aquatic ecosystems support comparatively fewer species. A quantitative assessment of alpha diversity using the Shannon (H′ = 1.55), Simpson (D = 0.71), and Pielou (J = 0.79) indices revealed a relatively high level of structural heterogeneity, with a balanced distribution of ecological groups. Canonical Correspondence Analysis (CCA) identified salinity, moisture, and substrate mobility as the primary environmental gradients structuring vegetation, with clear separation of halophytic, psammophytic, and hydrophilic communities. These findings demonstrate both the ecological plasticity of the flora and the vulnerability of rare and localised taxa to hydrological fluctuations of the Caspian Sea and anthropogenic pressures. The results provide a crucial scientific foundation for biodiversity monitoring, the conservation of genetic resources, and the sustainable management of fragile arid ecosystems in western Kazakhstan.

Keywords: Kulaly Island, Caspian Sea; vascular plants; biodiversity; halophytes; psammophytes; Shannon index; Jacquard index; arid ecosystems; gene pool conservation

Introduction

The Kazakhstan sector of the Caspian Sea occupies the northeastern and eastern parts of the planet's largest enclosed body of water and is of high ecological significance. It encompasses several islands and island groups, the largest of which is the Tyuleniy Islands archipelago, administratively part of the Mangystau region. The archipelago spans approximately 130 square kilometres and comprises five islands: Kulaly, Morskoy, Podkovka, Rakushechny, and Zhilany. These islands form unique natural complexes where, despite the harsh climate and unique geomorphology, distinctive floral and faunal communities have been preserved [1].

The islands' geological structure is dominated by sand, shell, and clay deposits, the surface of which is subject to wind erosion and abrasion. The terrain is low-lying and flat, with absolute elevations not exceeding 5-6 meters above sea level. These conditions make the island ecosystems vulnerable to fluctuations in the Caspian Sea level and the impact of ice masses. The climate is sharply continental with pronounced seasonal contrasts: in summer, air temperatures reach 40°C, and soil warms up to 65–70°C; in winter, temperatures drop to –10°C. High humidity, frequent fog, and soil salinity create a stressful environment that limits the growth of vegetation [2].

The islands of the Tyuleniy Archipelago are critical biodiversity hotspots in the Kazakhstan sector of the Caspian Sea. They are designated as Important Bird Areas (IBAs) because they provide crucial habitats for nesting and migratory stopovers for numerous bird species. Rare and endemic species inhabit the area, including the Caspian seal (*Pusa caspica*), which is listed in the Red Data Book of Kazakhstan and the IUCN Red List of Threatened Species. The islands' flora serves as a biogeocenotic framework, preventing deflation and erosion, stabilising loose substrates, forming microbiotopes, and acting as an indicator of climate change.

Of particular importance in the archipelago is Kulaly Island—the largest in area (approximately 38 km²) and the longest (34 km long, 2–4 km wide). It is distinguished by its crescent-shaped form and mosaic landscapes: dunes, salt marshes, lagoons, and coastal shoals. Kulaly is the only island where a permanent human presence remains, associated with fishing. This makes the island's ecosystems more vulnerable to anthropogenic impact, but simultaneously opens up opportunities for observation and regular monitoring. The most diverse floristic complexes of desert vegetation are represented here, including halophytes (*Halocnemum strobilaceum, Salicornia europaea*), psammophytes (*Agriophyllum squarrosum, Carex physodes*), xerophytes and salt-tolerant shrubs (*Tamarix ramosissima, Kalidium caspicum*) [3].

Despite the importance of the island ecosystems, the level of knowledge of Kulala's flora and fauna remains extremely low. The main problems can be summarised as follows: Systematic floristic surveys have been conducted only sporadically; the most notable expedition took place in 2013, with the participation of the Mangyshlak Experimental Botanical Garden and scientific organisations from Dagestan. However, the obtained data are of a general nature and do not encompass the full diversity of the island's vascular plants [4].

Inaccessibility and extreme conditions. The harsh climate, high humidity, isolation, and complex transport logistics limit the possibility of conducting long-term and regular expeditions. This leads to a shortage of herbarium materials, complicating the creation of a comprehensive flora inventory. Fishing, military outposts, and uncontrolled human impacts (such as waste disposal and trampling of vegetation) have a localised but significant effect on biodiversity. Fluctuations in the Caspian Sea level, increased frequency of extreme weather events, and changes in the hydrological regime directly affect the composition and structure of vegetation.

The flora of the mainland part of the Mangystau region has been relatively well studied, comprising 770 species of vascular plants from 73 families and 333 genera [5, 6]. The Chenopodiaceae, Asteraceae, Brassicaceae, Poaceae, Fabaceae, and other families play a significant role, which is typical of the Irano-Turanian floristic province. However, the flora of island ecosystems remains poorly studied, with no comprehensive reports and limited data on the dynamics of plant communities and their role in ecosystem processes. This significantly complicates the development of conservation and monitoring programs [7].

Studying the flora of Kulaly Island helps fill gaps in our knowledge of the island ecosystems of the Caspian region. The data obtained will contribute to: Identifying plant adaptation strategies to extreme conditions (salinity, aridity, wind load, ice processes); Creating indicator species groups for monitoring climate change; Assessing the conservation potential of island biocenoses; and Developing scientifically based measures for the conservation of biodiversity and sustainable use of the region's natural resources. The objective of this study is to analyse the vascular plants of Kulaly Island, identify their floristic composition, and assess the role of vegetation in maintaining the stability of island ecosystems in the Kazakhstan sector of the Caspian Sea.

Experimental

Field studies of the vegetation cover of Kulaly Island were conducted during the summer of 2013 as part of a comprehensive expedition by the Mangyshlak Experimental Botanical Garden. The work covered the central, northeastern, and coastal parts of the island, allowing for the spatial heterogeneity of plant communities to be taken into account. Floristic data were collected using a route-reconnaissance method across the main habitat types, including dune sands, salt marshes, coastal lagoons, and lowland meadows. Along each route, vascular plant species encountered were recorded, linked to the biotope, moisture level, and substrate mobility. Their life form, ecological-coenotic role, and relative frequency were also noted. The collected specimens were herbarized using standard botanical methods and are stored in the herbarium of the Mangyshlak Experimental Botanical Garden (MEBG). Each plant was accompanied by a label indicating the coordinates, collection date, ecotope type, and the name of the collector. Species were identified using the "Flora of Kazakhstan" (1956–1966) and regional identification guides, such as "Identification Guide to Plants of Central Asia" (1968). An international database, Plants of the World Online (POWO, 2021), was also utilised to update the nomenclature. Standard biostatistical approaches were used to quantify biodiversity and the ecological-cenotic structure of the flora: analysis of species distribution by life form according to Raunkiaer's classification, grouping by salt tolerance, moisture availability, and substrate types (sand, silt, clay, gravel, and salt marshes), and calculation of the Shannon, Simpson, Margalef, and Pilou indices to characterize alpha diversity. Data processing and graphic creation were performed using PAST 4.0 and Microsoft Excel 2019 [8].

Canonical correspondence analysis (CCA) was employed to investigate the impact of abiotic factors on the distribution of vascular plants. This multivariate statistical approach enables the simultaneous analysis of a matrix of species composition and environmental parameters, identifying the primary ecological gradients that govern the spatial structure of the flora. The study was conducted in R (version 4. x) using the vegan package. The input data included a species occurrence matrix (comprising 149 taxa) and a matrix of environmental factors (including salinity, humidity, substrate mobility, and life form). The results are visualised as biordination diagrams, where the CCA axes reflect the contribution of environmental factors, and the position of each species indicates its ecological preferences [9].

Results and discussion

A study of the flora of Kulaly Island revealed a significant diversity of vascular plants, reflecting the adaptation of communities to arid conditions and high soil salinity. A total of 149 species representing various life forms and ecological strategies were recorded. The most significant number of taxa are represented by annual therophytes and perennial xerophytes, indicating the dominance of short-lived forms capable of quickly completing their life cycles under conditions of moisture deficiency. Most of the identified species are associated with sandy and saline habitats, emphasising the leading role of these biotopes in maintaining the island's floristic diversity. Fewer species characterise wetland and meadow areas, but their importance for the overall flora structure is demonstrated by the formation of specific communities that serve as indicators of increased humidity.

To clearly present the floristic composition and its ecological characteristics, a summary table was compiled, including information on the life form of each species, its salt tolerance, humidity requirements, type of substrate, and its central role in the community (Tab. 1).

Table 1

Ecological structure and floristic composition of vascular plants of Kulaly Island

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
	Equisetaceae					
1	***Equisetum ramosissimum***	Perennial. Hemicryptophyte.	Saline and sandy soils	Dry to moderately humid conditions	Mobile sandy and rocky substrates	Soil-strengthening, participant in xerophytic communities
	Ephedraceae					
2	***Ephedra distachya***	Perennial. Chamaephyte	Sandy soils, slightly saline	Dry conditions	Shifting sands	Forms sparse communities, a food source for animals
	Typhaceae					
3	***Typha angustifolia***	Perennial. Hemicryptophyte, geocryptophyte	Fresh and slightly brackish waters	High humidity (shores of reservoirs)	Stable, silty substrates	Forms coastal thickets and acts as a filter feeder
4	***Typha latifolia***	Hemicryptophyte, helocryptophyte	Fresh water	High humidity	Stable shores	Strengthening the banks, creating habitats
	Potamogetonaceae					
5	***Stuckenia filiformis***	Perennial. Hydrocryptophyte.	Fresh and brackish water (up to 11%)	Aquatic environment	Silts, sandy bottom sediments	Forms underwater communities and stabilises bottom soils
6	***Stuckenia pectinata***	Perennial. Hydrocryptophyte.	Sea bays (slightly salty)	Aquatic environment	Sandy and silty soils	An essential component of underwater communities
7	***Zannichellia palustris subsp. major***	Perennial. Hydrocryptophyte.	Fresh and brackish waters	Aquatic environment	Ily	Forms underwater thickets, an indicator of brackish biotopes
8	***Zannichellia palustris***	Perennial. Hydrocryptophyte.	Fresh and brackish waters	Aquatic environment	Silts, soft soils	Participant in underwater phytocenoses
	Ruppiaceae					
9	***Ruppia maritima***	Perennial. Hydrocryptophyte.	Brackish waters	Aquatic environment	Sandy and Shelly's bottom soils	The primary component of underwater vegetation serves as a food source for waterfowl.

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
10	***Ruppia cirrhosa***	Perennial. Hydrocryptophyte.	Brackish and salt waters	Aquatic environment	Sandy and shell-sandy soils	Forms dense underwater thickets, an indicator of salty biotopes
	Juncaginaceae					
11	***Triglochin palustris***	Perennial. Helocryptophyte	Lightly saline meadows	Wet, damp conditions	Stable silt and meadow substrates	Forms coastal and meadow communities, an indicator of dampness
	Poaceae					
12	***Aeluropus littoralis***	Perennial. Hemicryptophyte.	Salt marshes, salted sands	Dry conditions	Mobile sandy and saline soils	Soil conditioner, dominant in solonchak communities
13	***Alopecurus arundinaceus***	Perennial. Geocryptophyte.	Saline meadows	Wet, coastal conditions	Stable floodplain and meadow substrates	Forms dense turf, fodder value
14	***Aristida adscensionis***	Annual. Therophyte	Non-saline dry sands	Dry conditions	Mobile sandy and rocky substrates	Ephemeral of desert communities, it fixes the sands
15	***Bromus tectorum***	Annual. Therophyte	Non-saline sands	Dry conditions	Light, sandy, and gravelly soils	Ephemeral component of desert communities
16	***Bromus squarrosus***	Annual. Therophyte	Non-saline sands	Dry conditions	Shifting sands	Participant in ephemeral deserts
17	***Sporobolus aculeatus***	Annual. Therophyte	Saline and salt marshes	Wet, damp habitats	Silts, damp sands	Forms thickets on salt marshes, with food value
18	***Sporobolus alopecuroides***	Annual. Therophyte	Solonetzic soils	Wet, damp conditions	Sandy-clayey substrates	Member of meadow-saline communities
19	***Digitaria sanguinalis***	Annual. Therophyte	Non-saline soils	Moderately humid (along the ditches)	Sandy and alluvial soils	A weed species in agrocenoses, a soil fixer
20	***Eremopyrum bonaepartis***	Annual. Therophyte	Non-saline soils	Dry conditions	Sands, clayey substrates	Ephemeral desert communities
21	***Eremopyrum triticeum***	Annual. Therophyte	Solonetzic, gravelly soils	Dry conditions	Dry substrates (sand, crushed stone)	Xerophytic ephemeral, an indicator of desert conditions
22	***Lolium arundinaceum***	Perennial. Hemicryptophyte.	Solonetzic and slightly saline soils	Wet conditions (shores, meadows)	Clayey, meadow substrates	Forms turf, valuable forage species
23	***Leymus racemosus***	Perennial. Hemicryptophyte.	Non-saline sands, sea coasts	Moderately humid (shores)	Shifting sands	Strengthening sandy banks, forming turf
24	***Leymus ramosus***	Perennial. Hemicryptophyte.	Solonetzic steppes, river banks	Moderately humid	Sandy-clayey, solonetzic	Soil strengthener, weed species in agrocenoses
25	***Stipa caucasica***	Perennial. Hemicryptophyte.	Non-saline sands and gravelly slopes	Dry conditions	Shifting sands, rocky substrates	Xerophyte forms feather grass communities
26	***Stipa capillata***	Perennial. Hemicryptophyte.	Non-saline gravelly and sandy soils	Arid	Slopes, scree	Indicator of dry steppes and deserts
27	***Stipa sareptana***	Perennial. Hemicryptophyte.	Clayey saline depressions, sands	Arid	Rocky, clayey, sandy	Member of feather grass-wormwood communities
28	***Stipa hohenackeriana***	Perennial. Hemicryptophyte.	Non-saline soils	Dry	Loess, gravelly slopes	Forms feather grass in the foothills
29	***Puccinellia distans***	Perennial. Hemicryptophyte/helocryptophyte	Slightly saline meadows	Wet, swampy	Sandy-clayey, pebble	Dominant of wet meadows, an indicator of salinity

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
30	***Puccinellia poecilantha***	Perennial. Hemicryptophyte/helocryptophyte	Slightly salty waters, wet salt marshes	Raw conditions	Sandy-loamy, grussy	Forms a grass stand, an indicator of water-logged soils
31	***Phalaris arundinacea***	Perennial. Geocryptophyte.	Non-saline floodplains	Raw conditions	Stable floodplain soils	Creates dense turf and strengthens banks
32	***Phragmites australis***	Perennial. Hemicryptophyte/geocryptophyte	Slightly saline and non-saline waters	Raw conditions	Coastal and silty soils	Forms thickets and stabilises banks
33	***Polypogon monspeliensis***	Annual. Therophyte	Solonetzic soils	Raw conditions	Moist soils	Fast-growing, ephemeral, waterlogging indicator
34	***Tragus racemosus***	Annual. Therophyte	Non-saline sands	Dry conditions	Shifting sands	A weedy species, a member of sandy communities
	Cyperaceae					
35	***Carex physodes***	Perennial. Hemicryptophyte.	Non-saline sands	Dry conditions	Mobile and fixed sands	The primarystabiliser of sands fixes dunes
36	***Bolboschoenus maritimus***	Perennial. Geocryptophyte.	Salt meadows, swamps	Raw	Silts, wet sands	Forms coastal thickets and filters water
	Asparagaceae					
37	***Asparagus breslerianus***	Perennial. Hemicryptophyte.	Salt marshes, salt lakes	Arid	Clayey, chalky soils	A semi-desert species, an element of xerophytic communities
	Polygonaceae					
38	***Atraphaxis spinosa***	Shrub. Phanerophyte.	Non-saline soils	Dry	Clay and gravel substrates	Forms sparse forests and shrub communities
	Amaranthaceae					
39	***Agriophyllum pungens***	Annual. Therophyte	Non-saline sands	Arid	Dune and hummocky sands	Psammophyte, sand fixer
40	***Anabasis eriopoda***	Perennial. Hemicryptophyte.	Sandy-clay deserts	Arid	Clay and gravel substrates	Dominant of desert communities
41	***Anabasis aphylla***	Subshrub. Chamaephyte	Salt marshes, solonetz	Moderately humid (with close ground-water)	Clay and sandy substrates	Member of salt marsh communities
42	***Atriplex aucheri***	Annual. Therophyte	Salt marshes	Moderately humid	Clay and sandy substrates	An inhabitant of salt marshes, a weed species
43	***Atriplex dimorphostegia***	Annual. Therophyte	Hilly sands, outskirts of Takyrs	Arid	Rubble and sandy soils	Ephemer, a member of desert communities
44	***Atriplex patens***	Annual. Therophyte	Wet salt marshes	Wet	Coastal solonetzic soils	Member of salt marsh communities
45	***Atriplex tatarica***	Annual. Therophyte	Salt marshes, solonetz	Moderately humid	Floodplain and meadow soils	A weedy species, an indicator of salt marshes
46	***Bassia hyssopifolia***	Annual. Therophyte	Salt marshes, salted sands	Arid	Sandy and loamy substrates	An inhabitant of salt marshes, a weed species
47	***Bassia odontoptera***	Annual. Therophyte	Salt marshes, clay slopes	Arid	Rocky and clayey substrates	A desert species, an indicator of saline habitats
48	***Bassia prostrata***	Perennial. Hemicryptophyte.	Salt marshes, solonetz	Arid	Rocky and sandy substrates	Forage species, soil fixer
49	***Bienertia cycloptera***	Annual. Therophyte	Gypsum-bearing salt marshes, shores of salt lakes	Moderately moist (occasionally damp)	Clayey and saline substrates	An indicator of salt marsh habitats, an ephemeral of desert communities

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
50	***Corispermum laxiflorum***	Annual. Therophyte	Non-saline sands	Arid	Shifting sands, dunes	Psammophyte, a member of desert communities, a sand fixer
51	***Corispermum aralocaspicum***	Annual. Therophyte	Saline sands, saline coasts	Moderately wet/dry	Hilly sands, clayey and peb-ble soils	Psammophyte and halophyte species areindicators of arid habitats
52	***Corispermum squarrosum***	Annual. Therophyte	No data for the region	—	—	A rare alien species, a weed (in the flora of Kulala)
53	***Ceratocarpus arenarius***	Annual. Therophyte	Non-saline sands and sandy loams	Arid	Sandy and rocky-gravelly substrates	Ephemeral desert and steppe communities
54	***Chenopodium album***	Annual. Therophyte	Weedy, non-saline places	Moderately humid	Various substrates (sand, loam)	A weed species, an indicator of anthropo-genic habitats
55	***Climacoptera kasakorum***	Annual. Therophyte	Clayey salt marshes	Wet (occasionally damp)	Plump clay substrates	Halophyte, a member of salt marsh commu-nities
56	***Climacoptera lanata***	Annual. Therophyte	Salt marshes, shores of salt lakes	Damp, salty conditions	Clay and saline substrates	Halophyte, dominant in saline communities
57	***Caroxylon scleranthum***	Annual. Chamaephyte	Non-saline sands, less often, gravelly places	Arid	Sandy and rocky substrates	Psammophyte, indica-tor of dry habitats
58	***Caroxylon nitrarium***	Annual. Therophyte	Takyrs, salt marshes, salted sands	Moderately humid (salt marshes) and dry	Clayey-gravelly and gypsum-bearing sub-strates	Halophytes, which are indicators of saline soils, are integral to semi-desert communi-ties
59	***Grubovia sedoides***	Annual. Therophyte	Solonchaks, solonetz, solonetzic steppes	Moderately dry	Clayey and compacted soils	A member of halo-phytic communities, a weed species
60	***Halanthium kulpianum***	Annual. Therophyte	No data (absent in the KM flo-ra)	—	—	A rare alien species
61	***Halimocnemis sclerosperma***	Annual. Therophyte	Takyrs, salt marshes, serozems	Arid and semi-humid	Clayey and compacted soils	Halophyte, a member of salt marsh commu-nities
62	***Halocnemum strobilaceum***	Shrub. Chamaephyte	Salt marshes, wet and puffy salt marshes, and the shores of salt lakes	Wet	Clay and silt substrates	Dominant of solonchak communi-ties, an indicator of saline depressions
63	***Halogeton glomeratus***	Annual. Therophyte	Salt marshes, gravelly desert slopes	Arid	Crushed rock and clay soils	Obligate halophyte, indicator of desert salt marshes
64	***Halostachys caspica***	Shrub. Phanerophyte.	Wet and puffy salt marshes, sea coasts	Wet	Clay and silt soils	Dominant of coastal and salt marsh com-munities
65	***Halothamnus turcomanicus***	Shrub. Chamaephyte	No data (absent in the KM flo-ra)	—	—	Rare species
66	***Kalidium caspicum***	Shrub. Chamaephyte	Salt marshes, salt marshes, banks of salt lakes and rivers	Wet and semi-wet	Clay substrates	Halophyte, coastal stabiliser

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
67	***Caviria gossypina***	Annual. Therophyte	Chalk outcrops, gypsum-bearing clays, salt marshes	Arid	Chalk and clay substrates	Indicator of saline and gypsum-bearing soils
68	***Lipandra polysperma***	Annual. Therophyte	Sandy shores, cliffs, estuaries, and garbage dumps	Moderately humid	Sandy and loose soils	A weed species, an inhabitant of anthropogenic habitats
69	***Oxybasis glauca***	Annual. Therophyte	Salt marshes, river and lake banks, and vegetable gardens	Wet and semi-wet	Sandy and clayey substrates	A weedy species, an indicator of saline habitats
70	***Pyankovia affinis***	Annual. Therophyte	Clayey solonchaks, takyrs, rubble-clayey trails	Arid and semi-humid	Clay and gravel substrates	Halophyte, a member of desert communities
71	***Pyankovia brachiata***	Annual. Therophyte	Solonetz, highly solonetzic soils	Arid	Clayey-gravelly substrates	Member of halophytic communities
72	***Petrosimonia brachiata***	Annual. Therophyte	Salt marshes, less commonly solonetz	Moderately humid	Clay and silt soils	Forms clumps on salt marshes, an indicator of wet halophytic habitats
73	***Petrosimonia glaucescens***	Annual. Therophyte	Salt marshes, solonetz	Moderately dry	Clay compacted soils	Member of halophytic communities
74	***Salicornia europaea***	Annual. Therophyte	Wet salt marshes, sors, sea coasts, and shores of salt lakes	Wet	Silty and saline substrates	Halophyte, an indicator of waterlogged salt marshes
75	***Salsola australis***	Annual. Therophyte	Sandy areas of river valleys, saline places	Dry and moderately dry	Sandy and sandy loam substrates	A weed species, an indicator of anthropogenic disturbances
76	***Salsola paulsenii***	Annual. Therophyte	Solonetzic sandy soils, desert steppes	Arid	Sandy and sandy loam	Member of halophytic desert communities
77	***Salsola foliosa***	Annual. Chamaephyte	Salt marshes, salt licks, takyrs	Dry and moderately dry	Clay and saline	Weed and halophytic species
78	***Suaeda acuminata***	Annual. Therophyte	Salt marshes, salt marsh meadows, wastelands	Semi-moist and moist	Clayey and saline	Halophyte, an indicator of salt marsh habitats
79	***Suaeda kossinskyi***	Annual. Therophyte	Salt marshes, sors in sandy areas	Semi-moist	Saline sandy substrates	Member of salt marsh communities
80	***Suaeda salsa***	Annual. Therophyte	Coastal and continental salt marshes	Wet	Silt and salt marshes	Halophyte, an indicator of coastal habitats
	Caryophyllaceae					
81	***Holosteum umbellatum subsp. glutinosum***	Annual. Therophyte	Non-saline clay and rocky slopes	Dry	Clayey and stony	Ephemeral, a member of xerophytic communities
82	***Psammophiliella muralis***	Annual. Therophyte	Weedy areas, arable land, wastelands, salt marshes	Moderately humid	Various substrates	A weed species that occurs in disturbed habitats

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
83	***Gypsophila paniculata***	Perennial. Hemicryptophyte.	Meadow and sandy steppes, salt marshes	Moderately dry and semi-moist	Sandy loam and sierozem soils	Forms clumps, an indicator of steppe habitats
84	***Gypsophila perfoliata***	Perennial. Hemicryptophyte.	Sands, pebbles, salt meadows	Semi-moist	Sandy and pebble substrates	Member of meadow and floodplain com-munities
85	***Silene wolgensis***	Biennial. Therophyte	Meadow and grassy slopes	Moderately humid	A variety of grassy soils	Member of the mead-ow and forest edge communities
86	***Silene cyri***	Biennial. Therophyte	Non-saline rocky slopes	Arid	Rocky and gravelly substrates	An element of xerophytic communi-ties, an indicator of the foothills
87	***Spergularia diandra***	Annual. Therophyte	Solonetzic meadows, salt marshes	Moderately humid	Clay and silt substrates	Halophyte, an indica-tor of wet habitats
88	***Spergularia mediasubsp. media***	Perennial. Hemicryptophyte.	Saline soils	Wet	Saline substrates	Coastal halophyte
89	***Spergularia rubra***	Annual. Therophyte/hemicryptophyte	Saline and sandy soils, weedy areas	Moderately dry	Sandy and clayey soils	Weed and halophytic species
	Ceratophyllaceae					
90	***Ceratophyllum demersum***	Perennial. Hydrocryptophyte.	Fresh and slightly salty water bodies	Aquatic environment	Silty bottom sediments	Forms underwater communities and sta-bilizes the bottom soils
	Ranunculaceae					
91	***Adonis scrobiculata subsp. scrobiculata***	Annual. Therophyte	Saline and wet sands, shrub thickets	Wet and semi-wet	Sandy and sandy loam substrates	Ephemeral, an indica-tor of disturbed habi-tats
92	***Ranunculus falcatus***	Annual. Therophyte	Steppes and deserts, clay and sandy soils	Dry	Clay, sand and pebble substrates	Ephemer, a member of desert communities
93	***Ranunculus testiculatus***	Annual. Therophyte	Deserts, clayey and sandy soils	Arid	Sandy and clayey soils	Ephemeral desert habitats
94	***Delphinium camptocarpum***	Annual. Therophyte	Fixed and semi-fixed sands	Dry	Sandy substrates	Member of ephemeral communities on the sands
	Papaveraceae					
95	***Papaver laevigatum***	Annual. Therophyte	Rocky and gravelly slopes, pebbles	Arid	Crushed rock and variegated rocks	Ephemeral, an indica-tor of rocky habitats
	Brassicaceae					
96	***Alyssum turkestanicum***	Annual. Therophyte	Rubbly, stony and slightly saline soils	Arid	Loamy and sandy loam sub-strates	Ephemeral, a member of xerophytic commu-nities
97	***Kakile maritimasubsp. euxina***	Annual. Therophyte	Sea coasts (presumably)	Wet	Coastal sands	Coastal halophyte, rare alien species
98	***Chorispora tenella***	Annual. Therophyte	Steppe slopes, banks of reser-voirs	Moderately humid	Clay and sandy loam substrates	Ephemeral, an indica-tor of disturbed habi-tats
99	***Descurainia sophia***	Annual. Therophyte	Salted areas	Moderately dry	Clayey and sandy loam	A weedy species, an indicator of saline areas
100	***Goldbachia laevigata***	Annual. Therophyte	Hilly sands, sandy-rocky and clayey substrates	Arid and semi-humid	Shifting sands, sandy loams	Ephemer, a member of desert communities

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
101	***Meniocus linifolius***	Annual. Therophyte	Sands and outcrops of Tertiary clays	Arid	Sandy and clayey substrates	Ephemeral indicator of clay outcrops
102	***Strigosella brevipes***	Annual. Therophyte	Sands (often saline), gypsum clays, pebbles	Arid	Sandy and gravelly	Ephemeral, a member of halophytic and desert communities
103	***Strigosella circinata***	Biennial. Therophyte	Sands, alluvial deposits, sandy loams	Arid	Sandy and sandy loam	Ephemeral, a rare species on the plain
104	***Syrenia siliculosa***	Biennial. Therophyte	Steppe meadows on sandy soils	Moderately humid	Sandy and sandy loam	Member of meadow communities
	Fabaceae					
105	***Alhagi pseudalhagisubsp. persarum***	Perennial. Hemicryptophyte.	Slightly saline clay soils, sands	Moderately humid	Clayey and sandy	Halophyte, a depression indicator
106	***Alhagi pseudalhagi***	Perennial. Hemicryptophyte.	Slightly saline sandy soils, edges of fixed sands	Moderately dry	Sandy	Weed species, forage shrub
107	***Astragalus karakugensis***	Subshrub. Chamaephyte	Semi-fixed sands	Arid	Shifting sands	Psammophyte, sand fixer
108	***Astragalus ammodendron***	Subshrub. Chamaephyte	Sandy soils	Arid	Shifting sands	Forage and soil-strengthening species
	Geraniaceae					
109	***Erodium ciconium***	Annual. Therophyte	Loamy soils, variegated flowers	Moderately dry	Loams and stony soils	Ephemeral, an indicator of disturbed habitats
	Nitrariaceae					
110	***Tetradiclis tenella***	Annual. Therophyte	Wet saline soils	Wet	Clayey and saline	Halophyte, an indicator of damp salt marshes
111	***Peganum harmala***	Perennial. Hemicryptophyte.	Sands, weedy places	Arid	Sandy	Weed species, medicinal plant
	Zygophyllaceae					
112	***Zygophyllum fabago***	Perennial. Hemicryptophyte.	Sands, salt marshes	Arid	Loamy and sandy	Halophyte, weed species
	Rutaceae					
113	***Haplophyllum obtusifolium***	Subshrub. Chamaephyte	Sand and gravel deposits, rocky ridges	Arid	Pebbles and sand	Elements of xerophytic communities
114	***Haplophyllum bungei***	Perennial. Hemicryptophyte.	Margins and depressions of hummocky sands	Arid	Sandy	Desert habitat indicator
	Euphorbiaceae					
115	***Euphorbia helioscopia***	Annual. Therophyte	Weedy and fallow areas	Moderately humid	Loamy and garden soils	Weed, an indicator of disturbed habitats
	Malvaceae					
116	***Malva pusilla***	Annual/biennial. Therophyte	Fine-grained and gravelly slopes, pebbles	Moderately dry	Rubble and sandy soils	
	Frankeniaceae					
117	***Frankenia hirsuta***	Subshrub. Chamaephyte	Wet banks, clay and rocky deserts	Wet	Clay and stony soils	Halophyte, an indicator of wet habitats

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
	Tamaricaceae					
118	***Tamarix gracilis***	Shrub, phanerophyte	Saline soils (solonetz, solonchaks)	High (groundwater close)	Sandy, clayey	Forms coastal thickets and strengthens banks
119	***Tamarix ramosissima***	Shrub, phanerophyte	Solonetz, salt marshes	Average	Sands, chalk hills	Substrate stabilisation, habitat for birds
120	***Tamarix laxa***	Shrub, phanerophyte	Solonetzes, solonchaks, takyrs	Variable	Sands	Dominant of solonetz communities
	Elaeagnaceae					
121	***Elaeagnus angustifolia***	Shrub/low tree, phanerophyte	Slightly saline sands	Low	Hummocky sands	
	Apiaceae					
122	***Ferula nuda***	Perennial, hemicryptophyte	Slightly saline sands	Average	Semi-fixed sands	Elements of psammophytic groups
123	***Ferula foetida***	Perennial, hemicryptophyte	Arid habitats	Low	Sandy hills	Dominant of dry communities, medicinal value
124	***Zosima absinthiifolia***	Perennial/biennial, hemicryptophyte/therophyte	Not salted	Dry	Rocky slopes	Member of xerophytic communities
	Primulaceae					
125	***Androsace maxima***	Annual, therophyte	Slightly saline	Low	Rocky, gravelly slopes	Episodic component of rock and steppe phytocenoses
	Plumbaginaceae					
126	***Limonium gmelinii***	Perennial, hemicryptophyte	Salt marshes, salt marsh meadows	High	Salt-laden depressions, river valleys	A weed species, an element of anthropogenic habitats
127	***Limonium caspium***	Perennial, hemicryptophyte	Salt marshes	High	Damp meadows, depressions	Indicator of saline and wetland habitats
	Gentianaceae					
128	***Centaurium pulchellum***	Annual, therophyte	Saline substrates	Average	Sands, stream banks	Indicator medicinal plant
	Apocynaceae					
129	***Cynanchum acutum subsp. sibiricum***	Perennial, hemicryptophyte	Slightly saline	Average	Rubble-rocky slopes, variegated flowers	Weedy appearance
	Convolvulaceae					
130	***Convolvulus persicus***	Perennial, hemicryptophyte	Salt-laden sands	Average	Coastal sands (200 m from the sea)	A rare protected species, an indicator of coastal communities
131	***Convolvulus erinaceus***	Subshrub, chamaephyte	Slightly saline	Low	Hilly and flat sands	Sand stabilizer
132	***Convolvulus arvensis***	Perennial, hemicryptophyte	Wide range	From dry to wet	Miscellaneous (vegetable gardens, wastelands, fields)	A weed species with a broad ecological range
	Boraginaceae					
133	***Tournefortia sibirica***	Perennial, hemicryptophyte	Lightly saline coasts	Average	Shell rocks, boulders, pebbles, storm surges	Coastal stabiliser forms protective communities
134	***Heliotropium ellipticum***	Perennial, hemicryptophyte	Non-salted or slightly salted	Average	Rocky shores, mountain trails	A common component of coastal communities

Continuation of Table 1

№	View	Life form	Habitat salinity	Humidity	Substrate mobility	Primary role in the community
135	***Lappula barbata***	Annual or biennial, therophyte	Unsalted	Low	Rocky and gravelly slopes	Ephemeral desert and mountain communities
136	***Lappula patula***	Annual, therophyte	Unsalted	Low	Sands, sandy loams, pebbles	A weedy and ephemeral species of desert steppes
	Lamiaceae					
137	***Lamium amplexicaule***	Annual or biennial, therophyte	Unsalted	Average	Rocky and gravelly slopes, forest edges, fields, and vegetable gardens	A weed species, an indicator of disturbed habitats
	Orobanchaceae					
138	***Orobanche amoena***	Perennial, hemicryptophyte	Solonetzic steppes	Average	Clay and sandy substrates	A parasitic plant affects the structure of communities
	Asteraceae					
139	***Artemisia arenaria***	Subshrub, chamaephyte	Salted coasts, sands	Low	Sandy substrates	Dominant desert vegetation
140	***Artemisia austriaca***	Perennial, chamaephyte	Steppe and solonetzic meadows	Average	Sandy and sandy loam soils	A widespread species, it grows as a weed.
141	***Artemisia lercheana***	Subshrub, chamaephyte	Light chestnut soils, chalk outcrops	Low	Rocky, gravelly, sandy substrates	Member of cereal-wormwood communities
142	***Artemisia abrotanum***	Hamephyte, hemicryptophyte	Saline and moist meadows	Medium-high	Banks of rivers, lakes, and ravines	Medicinal species, found in agrocenoses
143	***Artemisia scoparia***	Annual, therophyte	Weed and steppe communities	Average	Sandy loam, sandy, loess slopes	A weedy species that quickly colonises disturbed lands.
144	***Artemisia marschalliana var. marschalliana***	Subshrub, chamaephyte	Sands, salted coasts	Low	Sandy soils	Dominant of local desert communities
145	***Cicerbita macrophylla***	Perennial, hemicryptophyte	Not salted	Moderate	Forest and meadow habitats	Potential component of wetland communities
146	***Lactuca serriola***	Annual/biennial, therophyte	Slightly saline soils, anthropogenic places	Average	Sands, vegetable gardens, roads	A common weed species
147	***Lactuca tatarica***	Perennial, hemicryptophyte	Salt marshes, saline clays and sands	Average	Pebbles, sandstone outcrops	Indicator of saline habitats
148	***Microcephala lamellata***	Annual, therophyte	Solonchaks, clayey, takyr-like soils	Average	Sandy and gravelly substrates	Ephemeral desert communities
149	***Senecio glaucus subsp. coronopifolius***	Annual, therophyte	Salt marshes, solonetzic meadows	Medium-high	Salt meadows	An ephemeral species in salt marsh communities

Floristic composition analysis revealed a diverse range of life forms and ecological strategies among the plants represented in the studied sample (149 species). The most significant proportion is made up of annual therophytes (70 species, 47.0%), which form the basis of ephemeral and ephemeroid communities characteristic of desert and semi-desert habitats. Perennial hemicryptophytes (30 species, 20.1%), represented by steppe and meadow species that form dense sods and ensure the stability of the vegetation cover, are of significant importance. Hydrocryptophytes (10 species, 6.7%), confined to aquatic and coastal biotopes, chamaephytes (10 species, 6.7%), and geocryptophytes (5 species, 3.4%) were also noted. Phanerophytes (7 species, 4.7%) are represented by shrubs and small trees that play a key role in stabilising the substrate (e.g., Tamarix, Halostachys). Mixed life forms comprise 11.4% of the flora.

In terms of habitat salinity, non-saline biotope species predominate, comprising 40 species (26.8%). However, a significant portion of the flora is formed by halophytes: solonchaks (30 species, 20.1%), solonetzic (20 species, 13.4%), and slightly saline habitats (20 species, 13.4%). Another 15 species (10.1%) are associated with brackish and saline water bodies, while 12 species (8.1%) inhabit freshwater environments. Thus, almost half of the flora is represented by halophytes, emphasising the adaptation of the vegetation to the extreme conditions of saline and arid ecosystems.

The calculation of diversity indices revealed that the flora is characterised by a relatively high level of species and structural diversity (Tab. 2).

Table 2

Biodiversity indices

Index	Meaning
Shannon H'	1.55
Simpson D	0.71
Saw J	0.79

The Shannon index was H' = 1.55, reflecting the presence of several major life-form groups dominated by therophytes. The Simpson index was D = 0.71, indicating a relatively high probability of encountering different groups if randomly selected. The Pilou evenness index (J = 0.79) indicates a relatively balanced distribution of species among the various life forms.

Overall, the results of the analysis confirm that the flora of the studied area is ephemeral and ephemeroid in nature, but is also distinguished by a pronounced mosaic: along with ephemerals, perennial halophytes, shrubs, and hydrophytes play a significant role, forming a stable and diverse vegetation cover adapted to sharp fluctuations in humidity and salinity of habitats.

Canonical correspondence analysis (CCA) of the Kulaly Island flora revealed key ecological gradients that determine the spatial distribution and structural organisation of plant communities. Factors such as substrate salinity, moisture level, and soil mobility were included as key predictors in the analysis, allowing for a comprehensive characterisation of the ecological niche of each species (Fig.).

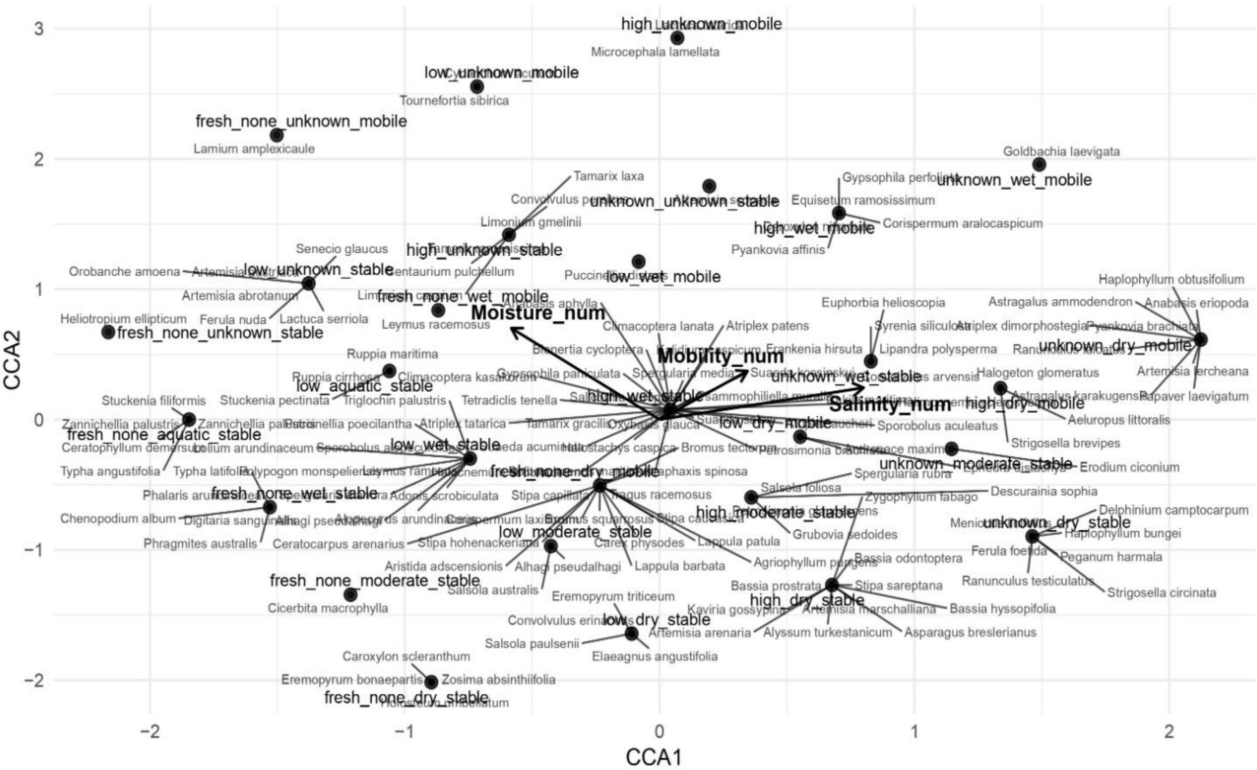

Figure. Canonical analysis of the environment and biodiversity flora of Kulaly Island

The distribution of objects on the diagram showed that the CCA1 axis best reflects the salinity gradient, which is closely related to moisture conditions. Typical halophytes from the Chenopodiaceae and Amaranthaceae families are located at the positive pole of the axis. *Suaeda salsa*, *Halocnemum strobilaceum*, *Kalidium caspicum*, *Halogeton glomeratus*, *Atriplex dimorphostegia*, *Salsola paulsenii, and* others. These species are confined to salt marshes and areas with high salt concentrations, forming distinct communities that are resistant to the extreme conditions of mineralised soils. On the opposite side of the axis are hydrophytes and coastal species—*Typha angustifolia*, *Typha latifolia*, *Stuckenia filiformis*, *Zannichellia palustris*, *Ruppia maritima*, *Ceratophyllum demersum*, reflecting freshwater aquatic habitats. Thus, the CCA1 axis represents the primary ecological-geochemical contrast in the island's flora, ranging from fresh-water to saline ecotopes.

The CCA2 axis broadlycharacterises the influence of substrate mobility and moisture gradients. The upper part of the diagram contains species associated with dune and sand ecotopes characterised by high substrate dynamics—Agriophyllum pungens, Aristida adscensionis, Carex physodes, Corispermum aralocaspicum, and Anabasis eriopoda. Their spatial displacement indicates the significant role of edaphic instability and wind activity in the formation of phytocenoses. Species that prefer more stable substrates, such as Suaeda acuminata, Artemisia arenaria, and Atriplex tatarica, are concentrated closer to the centre of the diagram, demonstrating a broad ecological amplitude range and plasticity in relation to substrate conditions.

Coastal hygrophilous species occupy a special position (*Phragmites australis*, *Bolboschoenus maritimus*, *Polypogon monspeliensis*), which form a cluster in the lower left quadrant of the ordination. Their placement confirms that humidity is the second most crucial factor structuring the island's vegetation cover. In contrast, xerophytes (*Astragalus ammodendron*, *Artemisia scoparia*, *Ephedra distachya*, *Haplophyllum*bungei) are shifted to the right pole of the diagram, which reflects their adaptation to arid conditions and moisture-poor substrates.

Thus, the CCA results indicate that the primary ecological determinant of Kulaly Island's vegetation cover is salinity, which shapes the fundamental division of the flora into hydrophilic and halophytic complexes. A secondary, but significant, role is played by the moisture gradient, which creates a contrast between coastal and dry arid communities. Finally, substrate mobility determines the specificity of dune and psammophyte assemblages, increasing their ecological selectivity and limiting species composition. The observed species distribution confirms the high ecological mosaicism of Kulaly Island's flora, where each species or group of species occupies a strictly defined position within the spatial distribution of environmental axes. This spatial structure highlights the adaptive strategies of plants in the extreme conditions of arid-salt marsh landscapes in the northeastern Caspian.

Conclusion

The flora of Kulaly Island is characterised by a significant diversity of vascular plants—149 species—reflecting a wide range of life forms and ecological strategies. The most abundant taxa are annual therophytes (47.0%) and perennial xerophytes, indicating the vegetation's adaptation to the extreme conditions of an arid climate, high salinity, and dynamic substrate. Perennial halophytes and shrubs play a crucial role in stabilising ecosystems, strengthening soil substrates, and forming resilient communities.

Calculation of biodiversity indices confirmed the high structural mosaicity of the flora. The Shannon index (H' = 1.55) and Simpson index (D = 0.71) indicate a relatively high level of alpha diversity. In contrast, the Pilou evenness index (J = 0.79) reflects a balanced distribution of taxa across different ecological groups. This indicates the presence of a stable floristic structure even under conditions of sharp fluctuations in habitat humidity and salinity.

Canonical correspondence analysis (CCA) identified three key ecological gradients determining the spatial distribution of flora: salinity, humidity, and substrate mobility. The primary division of flora is determined by the contrast between freshwater and saline biotopes, while secondary factors structure coastal, arid, and dune communities. This spatial organisation confirms the high ecological flexibility of the Kulala flora and its ability to form stable phytocoenoses in the extreme conditions of the arid-salt marsh landscape.

At the same time, the vulnerability of rare and locally distributed taxa to anthropogenic impacts (fishing, economic use) and fluctuations in the Caspian Sea level was revealed. This highlights the need for systematic monitoring of the island's flora, the implementation of environmental measures, and the development of long-term biodiversity conservation programs. Data obtained from a scientific basis for assessing the dynamics of island ecosystems can be used to develop strategies for the sustainable use of the region's natural resources.

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Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was prepared with the contributions of all authors, who have given their approval to the final version. **Sagybdykova M.S**. — conceptualisation, project administration, writing, review and editing. **Gassanova G.G**. — investigation, data curation, and collection of plant material; **Imanbaeva A.A**. — methodology, formal analysis, and visualization, **Orazov A.E**. — data curation, statistical analysis, and writing – original draft.

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М.С. Сагындыкова, Г.Г. Гасанова, А.А. Иманбаева, А.Е. Оразов

Каспий теңізінің Қазақстан аймағындағы Құлалы аралдарының өсімдік жамылғысы

Зерттеуде Каспий теңізінің қазақстандық секторында орналасқан Құлалы аралының тамырлы өсімдіктерінің флоралық және экологиялық алуан түрлілігінің кешенді талдауы ұсынылған. Далалық зерттеулер барысында барлығы 149 түр тіркелді, олардың ішінде басым бөлігі біржылдық терофиттер (47,0%) мен көпжылдық ксерофиттер болды. Бұл флораның аридті климатқа, топырақтың жоғары тұздылығына және тұрақсыз субстраттарға бейімделуін көрсетеді. Түрлердің негізгі бөлігі құмды және сор топырақты биотоптармен байланысты, ал сулы-батпақты және су экожүйелерінде салыстырмалы түрде аздаған таксондар ғана кездесті. Альфа-алуантүрлілікті сандық бағалау нәтижесінде Шеннон (H′ = 1,55), Симпсон (D = 0,71) және Пилу (J = 0,79) индекстері флора құрылымының жоғары мозаикалылығын және экологиялық топтардың салыстырмалы түрде теңгерімді таралуын көрсетті. Канондық корреспонденттік талдау (CCA) өсімдік жамылғысын құрылымдайтын негізгі экологиялық градиенттерді — топырақтың тұздылығын, ылғалдылықты және субстраттың қозғалмалылығын анықтады. Бұл факторлар галофиттік, псаммофиттік және гидрофильді қауымдастықтарды айқын ажыратады. Зерттеу нәтижелері флораның жоғары экологиялық икемділігін, сонымен қатар сирек және шектеулі таралған таксондардың Каспий теңізі деңгейінің гидрологиялық ауытқуларына және антропогендік әсерлерге осалдығын көрсетті. Алынған деректер биоалуантүрлілікті мониторингтеу, генетикалық ресурстарды сақтау және Батыс Қазақстанның нәзік аридті экожүйелерін тұрақты басқару үшін маңызды ғылыми негіз болып табылады.

Кілт сөздер: Құлалы аралы, Каспий теңізі, тамырлы өсімдіктер, биоалуантүрлілік, галофиттер, псаммофиттер, Шеннон индексі, Жаккар индексі, аридті экожүйелер, генқорды сақтау

М.С. Сагындыкова, Г.Г. Гасанова, А.А. Иманбаева, А.Е. Оразов

Растительный покров острова Кулалы в Казахстанском секторе Каспийского моря

В статье представлены результаты комплексного анализа флористического и экологического разнообразия сосудистых растений острова Кулалы, расположенного в казахстанском секторе Каспийского моря. В ходе полевых исследований было выявлено 149 видов, среди которых преобладают однолетние терофиты (47,0%) и многолетние ксерофиты, что отражает адаптацию флоры к аридному климату, высокому уровню засоления почв и подвижным субстратам. Основная часть видов приурочена к песчаным и солончаковым биотопам, тогда как водно-болотные и аквальные экосистемы характеризуются меньшим числом таксонов. Количественная оценка альфа-разнообразия показала следующие значения: индекс Шеннона (H′ = 1,55), индекс Симпсона (D = 0,71) и индекс равномерности Пилу (J = 0,79), что свидетельствует о выраженной структурной мозаичности и сбалансированном распределении экологических групп. Канонический корреспондентный анализ (CCA) выявил три ведущих экологических градиента — засолённость, влажность и подвижность субстрата, которые определяют пространственное распределение флоры и формирование галофитных, псаммофитных и гидрофильных сообществ. Полученные данные демонстрируют высокую экологическую пластичность растительности, а также уязвимость редких и локально распространённых таксонов к гидрологическим колебаниям уровня Каспийского моря и антропогенному воздействию. Результаты исследования служат важной научной основой для мониторинга биоразнообразия, сохранения генетических ресурсов и устойчивого управления аридными экосистемами Западного Казахстана.

Ключевые слова: остров Кулалы, Каспийское море, сосудистые растения, биоразнообразие, галофиты, псаммофиты, индекс Шеннона, индекс Жаккара, аридные экосистемы, сохранение генофонда

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Review

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The impact of heavy metals on plant organisms and methods of their analysis: an overview

This review provides a detailed analysis of the impact of heavy metals on plant organisms, with a focus on the specific issue of aluminum toxicity in Kazakhstan's industrial regions. The rapid expansion of mining and metallurgical industries has resulted in elevated pollutant emissions, with aluminum posing a significant environmental risk. Unlike other metals, its phytotoxicity manifests indirectly through soil acidification caused by acid rain (resulting from SO_2 and NO_x emissions), which mobilizes toxic Al^{3+} ions from aluminosilicates. The Pavlodar region serves as a case study to examine secondary aluminum contamination and its major effects on plant roots, including growth inhibition, cytoskeleton disruption, mineral nutrient imbalance, and oxidative stress. The review compiles data on heavy metal accumulation in plants across Kazakhstan and critically evaluates advanced analytical techniques (ICP-MS, XAS, EXAFS) that are essential for determining aluminum bioavailability and toxicity. It also highlights the role of plants as bioindicators and the potential of phytoremediation technologies. Based on current research, the review recommends adaptive measures for Kazakhstan, including soil liming, the use of aluminum-tolerant plant species, and implementation of modern environmental monitoring to reduce ecological risks and maintain ecosystem productivity.

Keywords: heavy metals, aluminum, phytotoxicity, industrial pollution, acidic soils, environmental monitoring, bioavailability, phytoremediation

Introduction

The rapid pace of world development and its inevitable consequences significantly influence the environment, state, and stability which are closely related to human health and life. The transition from socioeconomic development to industrialization in the past century led to the predominance of industrial production in the economy. The consequences of past industrial development are reflected in the current state of resources and the sustainable development of the environment. Despite modern society's transition to a new stage of industrialization based on engineering, intellect, and automated IT technologies, the impact of industry and the degree of anthropogenic influence reach critical levels every year [1].

Kazakhstan is one of the largest producers and exporters of nonferrous and rare metals, including lead, copper, and zinc, as well as coal and oil. The rapid growth of industry, not always accompanied by adequate measures for environmental protection, leads to the accumulation of toxic chemical elements present in emissions in the soil, water, and vegetation, which has significant long-term ecological consequences. High levels of pollution are observed in areas where metal mining and processing are actively conducted, such as lead, cadmium, zinc, copper, chromium, and others. These elements enter the environment through atmospheric emissions, wastewater, and industrial waste, leading to their accumulation in vegetation, soil, and water [2]. Special attention is given to the stress exerted on the environment, where heavy metals (HMs) are considered a significant factor [1]. Overall, HMs are natural components of the Earth's crust, but anthropogenic activities result in a radical alteration of their biochemical balance and geochemical cycle [3]. As a result of being released into the atmosphere, they can travel long distances, with air masses settling on vegetation and soil. Under the influence of abiotic factors, HMs penetrate plant tissues initially due to plants' need for certain chemical elements. However, excessive accumulation later leads to a negative impact on plant life. The problem of heavy metal pollution is particularly acute in areas concentrated with large enterprises in the mining and metallurgical industries, as well as along major transportation routes.

Kazakhstan is facing a serious ecological situation related to high concentrations of heavy metals in various country regions. In particular, it is known that five settlements in Kazakhstan (Karaganda—high concentration of coal industry, metallurgical plants, and intense automotive traffic; Astana—automotive transport, heating (coal and gas), construction, and overall urbanization; Talgar—agriculture, increasing traffic flow, and construction in the suburbs of Almaty; Aktobe—intensive development of chemical and metallurgical industries, as well as increased vehicle traffic; Aksai—industrial development, transportation load, and local heating) are classified as cities with very high air pollution. Additionally, another 21 cities are classified as having high pollution levels, and 28 cities have elevated pollution levels. According to data from the Department of Environmental Monitoring of the Ministry of Ecology and Natural Resources of the Republic of Kazakhstan over the past five years (from 2020 to 2024), a consistently high level of air pollution has been observed in Astana and Karaganda. The main pollutants for these cities are suspended particles (dust), PM-2.5 and PM-10 suspended particles, carbon monoxide, nitrogen oxides, and hydrogen sulfide [4].

Despite the traditional association of heavy metal pollution with elements such as lead, cadmium, and mercury, the issue of aluminum (Al) toxicity is becoming increasingly relevant in the context of intensive industrial development. This is particularly true for the Pavlodar region of Kazakhstan—a key industrial hub that hosts major energy facilities (Ekibastuz GRES), aluminum production, and oil refining enterprises. The paradox of aluminum pollution lies in the fact that its main source is not direct emissions, but secondary contamination processes. Massive emissions of sulfur dioxide (SO_2) and nitrogen oxides (NO_x) from coal combustion and industrial activities lead to acid rain. Acidification of the soil environment (pH < 5.0) causes the dissolution of natural aluminosilicates, resulting in the release of Al^{3+} ions into the soil solution—the most toxic form of aluminum for plants [5–7]. Thus, although aluminum is not a platinum-group metal in terms of density, it represents a classic example of an “ecotoxicant”, whose negative impact on ecosystems is directly linked to anthropogenic activities and constitutes a dominant stress factor for plants in industrial regions with acidic soils.

Pollution by heavy metals has long-term negative consequences for human health. The risk of heavy metal poisoning can arise through several pathways: inhalation of polluted air, consumption of contaminated food (especially vegetables and fruits that accumulate toxic substances from the soil), and through contaminated drinking water, into which chemical elements often enter through air masses. The impact of this pollution on ecosystems primarily manifests itself in the disruption of biochemical and physiological processes in plants. Heavy metals have the ability to accumulate in plant tissues, leading to reduced growth, productivity, and photosynthesis capacity. Soil forms a close connection with plant organisms. Excessive accumulation of pollutants in plants often depends on soil properties and conditions, which, after reaching certain thresholds, stimulate the mobility of pollutants from soil to plants [3]. The entry of toxic substances into the food chain can harm not only vegetation but also animals and humans. The urgency of the problem lies in the fact that heavy metals have a cumulative effect, meaning they accumulate in the body and can cause chronic diseases, including severe damage to the kidneys, liver, and nervous system, as well as increase the risk of developing cancer [8]. The problem of aluminum toxicity, while distinct in its mechanism, adds another layer of urgency to this issue. Its impact is not through direct accumulation in the food chain like cadmium or lead, but through the large-scale degradation of the very basis of agricultural and natural ecosystems—the soil itself. In regions like Pavlodar, this leads to a silent but steady decline in soil fertility and plant productivity, posing a direct threat to food security and environmental health [9].

In Kazakhstan, particularly in areas exposed to pollutants, there has been an increase in morbidity among the population, including a rise in cases of diseases associated with the accumulation of aluminum, lead, cadmium, and other toxic substances in the body. Furthermore, chronic exposure to bioavailable aluminum (Al), mobilized from soils by industrial acidification, has been linked to neurodegenerative disorders and bone diseases, adding a significant layer of public health concern in regions affected by acid rain deposition. Research into mortality causes in key regions of Kazakhstan with high levels of air pollution has shown that the main factors contributing to mortality were ischaemic heart disease (4080 cases), stroke (1613 cases), lower respiratory infections (662 cases), chronic obstructive pulmonary disease (434 cases), and lung cancer (332 cases). The mortality rate associated with environmental pollution ranged from 276 to 373 cases per 100000 adults per year in three industrial cities—Zhezkazgan, Temirtau, and Balkhash [10]. According to the Bureau of National Statistics of the Agency for Strategic Planning and Reforms of the Republic of Kazakhstan and the National Report on the State of the Environment and Use of Natural Resources of the Republic of Kazakhstan, it was found that between 2017 and 2022, emissions of pollutants into the atmosphere from stationary sources in the country decreased by only 1.82% (from 2357.8 to 2314.8

thousand tons) [11]. The maximum peak during this period occurred in 2019 (2483.1 thousand tonnes). Industrial areas such as Karaganda and Zhezkazgan have high levels of lead and cadmium pollution due to the activities of metallurgical enterprises and mines. According to studies of the air basin for specific pollutants conducted in recent years, it was found that emissions of lead and its compounds amounted to 213.4 tonnes; 54.1 tonnes were attributed to arsenic, 56.6 tonnes to chlorine, 103.0 tonnes to copper oxide, and the lowest indicator was characteristic for mercury (0.2 tonnes) [12]. It is critical to note that while official emission inventories often focus on direct particulate and gaseous emissions, they typically do not account for secondary pollutants like bioavailable aluminum. The mobilization of Al^{3+} from soils, resulting from the acidification caused by prior SO_2 and NO_x emissions, represents a significant and underreported pathway of ecosystem contamination and human exposure in industrial regions such as Pavlodar and Temirtau [5–7]. Despite the scale of pollution, according to hygienic standards for atmospheric air in urban and rural settlements on the territories of industrial organisations (Order of the Minister of Health of the Republic of Kazakhstan dated August 2, 2022 No. KR DSM-70), actual emissions of these substances did not exceed the volume of established maximum allowable emissions [13].

One of the most effective ways to minimise the impact of heavy metals on public health is environmental monitoring, including the use of certain plants as indicators (their role as bioindicators is confirmed by quantitative and/or qualitative analysis), which allows for the prompt detection of changes in ecosystems and assessment of pollution levels. This is especially pertinent for monitoring non-exhaustive pollutants like aluminum, where traditional air quality metrics are insufficient. Bioindication using plants becomes a crucial tool for assessing the bioavailability of Al and the success of soil remediation efforts aimed at neutralising acidity. The relevance of this work is determined by a number of factors that influence the dynamics of changes regarding the impact of heavy metals on ecosystems and human health [14].

First, there are annual changes in the volumes of emissions from industrial enterprises, leading to fluctuations in the concentrations of toxic pollutants in the environment. These fluctuations directly influence the rate of soil acidification, which in turn governs the mobilisation and subsequent phytotoxicity of aluminum, creating a dynamic and often delayed environmental stressor. Modern rates of industrial modernisation, including the introduction of new technologies, are often accompanied by both improvements in environmental standards and temporary disruptions related to the modernisation of production capacities. Industrial enterprises continue to be the main sources of pollution, and their emissions into the atmosphere can vary significantly depending on the economic situation, changes in legislation, and the implementation of new technologies [15]. Tracking these changes requires systematic analysis of scientific publications, including new data obtained from current research that helps to understand new trends in pollution dynamics.

Secondly, in recent years, active landscaping and greening of urban areas have been conducted, which can influence the accumulation of heavy metals (HMs) in plants and soils, as well as the processes of their migration through biosystems. The selection of plant species for landscaping in industrially acidified areas like Pavlodar must consider aluminum tolerance. Choosing sensitive species can lead to project failure and wasted resources, while selecting hyperaccumulators without a proper disposal plan could inadvertently introduce toxins into the urban environment. Landscaping and the implementation of new methods of ecological compensation in cities create a need for regular reassessment of existing data and research methodologies regarding pollution. These activities necessitate continuous monitoring and updating knowledge about how HMs affect the ecological situation and what methods can be used for effective assessment of the urban ecosystem's condition, as well as quantitative accounting of the studied pollution indicators [7].

Thirdly, with each passing year, new and improved methods for researching and analysing chemical pollution of the environment are being developed, including the use of high-precision analytical instruments, more sensitive tests, and cutting-edge technologies for monitoring and predicting pollution. For aluminum, this includes advanced speciation techniques like X-ray Absorption Spectroscopy (XAS) to determine its chemical form in plants and soils, which is critical for accurately assessing its bioavailability and toxicity, beyond what simple concentration data from ICP-MS or AAS can provide [15]. Therefore, reliable and practical methods for detecting and analysing absorption, distribution, accumulation, chemical forms, and transport of HMs in plants are essential for reducing or regulating the content of xenobiotics. New approaches in bioindication, improved methods for measuring HM concentrations, and a deeper understanding of their accumulation mechanisms in ecosystems require regular updating of scientific data and practical recommendations for research. This enables the study of plant responses to pollutants and the identification of differences in HM content among individual plant species and taxonomic groups. In recent years, special attention has been paid to the impact of HMs on plants and the processes of their absorption and transportation by plant organisms [16]. A review of contemporary achievements in this field allows not only for summarizing the accumulated experience but also for assessing directions for further research and the development of more effective technologies and methods. A significant part of this effort must be dedicated to understanding the specific mechanisms of aluminum uptake, distribution, and detoxification in plants, as they differ fundamentally from those of canonical heavy metals. Thus, conducting a literature review on the topic of heavy metal pollution and its effects on plant organisms is essential for maintaining the relevance of knowledge in this area. Therefore, this review, while addressing the broader spectrum of heavy metal impacts, will particularly focus on aluminum as a key pollutant in industrially acidified environments, using the Pavlodar region as a case study. This focus aims to synthesize the available information on Al's specific phytotoxicity, analytical methods for its detection in environmental samples, and the ecological implications for similar industrial regions [7]. This not only allows for the systematization and analysis of existing data but also takes into account the dynamic changes in the ecological and technological spheres of the Republic of Kazakhstan, which in turn contributes to optimizing research methods, enhancing the effectiveness of environmental monitoring, and protecting public health.

Experimental

This literature review employs a systematic approach to analyze the impact of heavy metals on plant organisms, with a concentrated focus on aluminum toxicity in the context of the Pavlodar region, Kazakhstan. The methodology integrates comprehensive literature search strategies with critical analysis techniques to ensure scientific rigor and relevance.

The research methodology included four key stages:

1. Database Search: A comprehensive literature search was conducted in the Web of Science (WoS) Core Collection and Scopus databases for the period 2014-2025 to ensure inclusion of the most recent research. Foundational works from 1990–2013 were also included to provide historical context and theoretical framework, particularly for fundamental principles of heavy metal toxicity and early interdisciplinary approaches in environmental studies [17–19].

2. Search Strategy: The search utilized structured keyword strategies with Boolean operators. Primary general terms included: ("heavy metal" OR "trace metal") AND ("plant" OR "phytotoxicity" OR "bioindication" OR "bioaccumulation"). The specific search for the aluminum case study employed: ("aluminum" OR "aluminium") AND ("toxicity" OR "acid soil" OR "stress") AND ("plant") AND ("Kazakhstan" OR "Central Asia" OR "Pavlodar region").

3. Screening and Selection: Articles underwent rigorous screening by title, abstract, and full text. Priority was given to original research articles, high-impact reviews, and studies utilizing advanced analytical methods (ICP-MS, AAS, XAFS) relevant to metal speciation and quantification, ensuring both methodological rigor and regional relevance [1, 3, 18].

4. Data Analysis and Synthesis: Selected literature was analyzed using critical assessment methods to evaluate scientific significance and reliability, with emphasis on high-impact publications. Information was synthesized through systematic integration of disparate data into coherent frameworks, connecting fundamental plant physiology with applied environmental science in specific geographical contexts [20].

The review is based on analysis of 58 scientific publications, encompassing both general heavy metal impacts (Cd, Pb, Cu, Zn, Ni, Cr, Hg, Mn, Fe, As) and specialised research on aluminum toxicity, fulfilling standard requirements for comprehensive review articles while maintaining focus on methodological advances and regional environmental challenges.

Sources of plant contamination by HMs

The ability of plant organisms to accumulate various chemical elements (with heavy metals being of particular importance) plays an important role [21]. The accumulation and deposition of these elements in plant organs in quantities exceeding norms influence the plants and is known as an indicator of anthropogenic influence (industrial and transportation emissions often being the main sources). As a result of human activities, almost 60% of all heavy metals end up in the atmosphere, with cadmium, nickel, and lead reaching over 90% [22].

Heavy metals are chemical elements that can enter plants in three ways: through air, soil, and water. Significant levels of anthropogenic load have a considerable impact on the environment, which affects plants. The main source of HM contamination in plants comes from industrial facilities and transportation. The annual development of industry and increased work volumes at such facilities lead to higher emissions of xenobiotics into the atmosphere. Air masses carrying pollutants over significant distances contribute to their settling on plants, accumulation in soil, and entry into water sources [23]. As a result, contaminants enter plant organisms directly through their tissues, as well as through nutrients and water in the soil, often becoming the final source of toxic chemical element deposition.

Kazakhstan, with its extensive industrial and mining sectors, is a significant source of environmental pollution from heavy metals. The country possesses large deposits of minerals and fossil fuels, and its rapid industrialisation has led to increased levels of pollutants, including heavy metals, in both urban and rural areas. The main sources of heavy metal pollution in Kazakhstan include the mining industry, industrial enterprises, and transportation.

Sources of Heavy Metal Pollution in Kazakhstan

Kazakhstan is one of the largest producers of uranium, copper, gold, lead, and zinc in the world. In particular, the country ranks third globally in uranium reserves and is a leading producer, as well as one of the largest producers of copper and zinc. Mining and metallurgical activities in regions such as the Ural Mountains, Kyzylorda, and East Kazakhstan significantly contribute to heavy metal pollution.

Uranium mining in the South Kazakhstan region has led to the contamination of water and soil with radioactive elements and heavy metals such as arsenic, cadmium, and lead. An example is the area adjacent to mining enterprises near the city of Saryagash, where studies have shown that arsenic and cadmium concentrations in the soil exceed norms by 3–5 times.

In Zhezkazgan, copper and zinc are mined, resulting in high levels of soil and vegetation contamination with cadmium and copper. According to studies, cadmium levels in soils around Zhezkazgan exceed permissible standards by 2–4 times, negatively impacting agriculture and biodiversity. East Kazakhstan (Ust-Kamenogorsk) is known for its copper smelting plant, which is a primary source of heavy metal pollution. Soils in the Ust-Kamenogorsk area contain copper and cadmium levels that exceed safe levels by 5-6 times, seriously affecting the ecosystem.

The chemical industry, coal-fired power plants, and steel mills in Kazakhstan are also major sources of air pollution. The Ekibastuz coal-fired power plant (Pavlodar region), one of the largest in the country, is a major source of sulphur dioxide, nitrogen oxides, and particulate matter that may contain heavy metals such as lead, nickel, and cadmium. Concentrations of these metals in the atmosphere of the city exceed permissible norms, leading to soil and vegetation contamination. As a result, cadmium levels in soils adjacent to Ekibastuz are 3 times higher than normal.

The Pavlodar petrochemical complex and steel mill (Northeast Kazakhstan) also influence heavy metal concentrations in the environment. This is particularly true for areas located near industrial zones, where lead and cadmium accumulation in soils is observed at levels exceeding safe indicators by 2-3 times.

Special attention is also paid to agricultural runoff and soil contamination. Agricultural areas in Kazakhstan, especially in the north, suffer from the use of contaminated irrigation water and fertilisers that carry heavy metals from nearby industrial regions.

In northern regions of Kazakhstan, such as Kostanay and Pavlodar, due to the use of contaminated water for irrigation and runoff from nearby industrial facilities, soils contain high concentrations of heavy metals. Research results indicate that in these areas, soils contain cadmium and lead concentrations 2-3 times higher than permissible limits.

The transport sector in Kazakhstan, especially in major cities such as Almaty, Shymkent, and Astana, also contributes significantly to environmental pollution, particularly with heavy metals such as lead and nickel. Despite the transition to unleaded fuels, emissions from road transport on a large scale (for example, in Almaty), combined with industrial pollution, continue to deposit heavy metals on vegetation. This is especially noticeable in urban and suburban areas, where lead contamination in soils exceeds safe levels by 1.5-2 times [4, 11, 12, 24].

The ability of plant organisms to accumulate various chemical elements (with heavy metals being of particular importance) plays a crucial role [21]. The accumulation and deposition of these elements in plant organs in quantities exceeding norms affect the plants and serve as an indicator of anthropogenic impact (often the primary sources are industrial and transport emissions). As a result of human activity, nearly 60% of all heavy metals enter the atmosphere, with cadmium, nickel, and lead accounting for over 90% [22].

Heavy metals are chemical elements that can enter plants through three pathways: air, soil, and water. Significant levels of anthropogenic load have a substantial impact on the environment, which in turn affects plants. The main sources of heavy metal pollution in plants are industrial enterprises and transportation. The annual development of industry and increased work volumes at such enterprises lead to an increase in xenobiotic emissions into the atmosphere. Air masses carrying pollutants over considerable distances contribute to their deposition on plants, accumulation in soil, and entry into water sources [23]. As a result, pollutants enter plant organisms directly through their tissues and through nutrients and water in the soil, often becoming the ultimate source of deposition of toxic chemical elements.

Accumulation of heavy metals in plants in Kazakhstan

Heavy metal pollution of plants in Kazakhstan is an important ecological and sanitary issue. Studies show that heavy metals such as cadmium (Cd), lead (Pb), and nickel (Ni) accumulate in various plant organs, including leaves, stems, and roots.

Absorption through leaves: heavy metals deposited from atmospheric pollution, such as emissions from industrial enterprises or vehicle exhausts, can be absorbed by plant leaves. Plants growing near large industrial facilities in cities like Karaganda, Ekibastuz, and Ust-Kamenogorsk exhibit significant bioaccumulation of heavy metals.

Absorption through soil and water: plants also absorb heavy metals from contaminated soils and irrigation water. For example, plants grown in mining areas such as Zhezkazgan (copper and zinc mining) and Pavlodar (steel industry) often show high levels of cadmium and copper.

Translocation of metals: studies in agricultural areas of Kazakhstan have shown that heavy metals such as cadmium can be transported from the soil into plant roots and then into aerial parts like leaves and fruits. This creates risks for agricultural production and human health [4, 11, 12, 24, 25].

In Kazakhstani studies dedicated to pollution by heavy metals, the relationships between industrial and transport emissions, anthropogenic activities, and soil contamination, as well as the migration of these elements into plants, are examined as serious ecological and social issues.

A study conducted by L.M. Kalimoldina, G.S. Sultangazieva, and M.Sh. Suleimenova focused on the urban area of Almaty. The researchers studied the concentrations of metals such as lead (Pb), copper (Cu), zinc (Zn), and cadmium (Cd) along the city's highways, including Rayymbek Avenue, the Botanical Garden, and the area near the "Altyn Orda" settlement. The results showed that the concentration of lead in the soil near roads exceeds the maximum permissible concentration (MPC) by 14 times (445.72 mg/kg), while copper exceeds it by more than 3 times (136.45 mg/kg). High levels of contamination were also recorded for copper and zinc, especially near transport arteries. The main sources of pollution were identified as vehicle emissions and industrial activities. A strong correlation was established between soil contamination and plant health: in areas with high heavy metal content, leaf damage (necrosis, chlorosis) was observed. The study emphasizes that serious ecological problems related to soil pollution in urbanised areas are associated with anthropogenic factors, including vehicle emissions and industrial activities. The researchers highlighted that such pollution poses a threat to human health, especially through the "soil-plant-human" chain [26].

Another study conducted by scientists from the Kazakh National Agrarian Research University—A. Zhyrgalova, S. Yelemessova, B. Ablaikhana, G. Aitkhozhayeva, and A. Zhildikbayeva—focused on the Sokolov-Sarbay district of Kostanay region. It aimed to assess the potential ecological risk of soil contamination by heavy metals in agricultural lands. The analysis showed that the average ecological risk index (RI) was 328, which corresponds to a high risk. Concentrations of arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) significantly exceeded national standards, especially in areas affected by mining activities. The researchers concluded that further studies are necessary to evaluate the suitability of these lands for agricultural use [27].

Research conducted by I.V. Matveyeva, O.I. Ponomarenko, N.B. Soltangaziyev, N.A. Nursapina, Sh. N. Nazarkulova, and A.N. Gurin focused on assessing the content of heavy metals in various forms. Special attention was given to elements such as lead (Pb), copper (Cu), zinc (Zn), and manganese (Mn) to determine their mobility, availability for plants, and potential toxicity (using the city of Almaty as an example). It was established that the total content of lead and zinc exceeds the maximum allowable concentrations (MAC) by 1.55 and 3.28 times, respectively. The mobile forms of zinc also exceed the MAC by 1.44 times, indicating a possible ecological threat. In the soils of the nearby village of Baitirek, the total zinc content was found to be 1.5 times higher than the MAC; however, the mobile forms are within normal limits, which reduce the risk of migration and accumulation of the metal in biological objects. The most serious contamination was identified in the village of Avat, where the total zinc content exceeds the MAC by 2.7 times, and the mobile forms exceed it by 2.82 times, indicating high mobility and the possibility of accumulation in plants and the human body. These pollution indicators are linked to several factors: industrial activity (the presence of TPP-1 near Almaty, whose emissions may have contributed to the accumulation of heavy metals such as lead and zinc in the surrounding soil); transportation infrastructure (a significant portion of pollution is due to emissions from road traffic; lead, copper, and zinc often accumulate in soils near roads due to fuel use, tire wear, brake pads, and road surfaces); agricultural and domestic activities (in the villages of Baitirek and Avat, pollution may be associated with the use of fertilisers and pesticides that contain heavy metals, as well as household waste). Poor waste management, insufficient soil reclamation, and weak environmental control contribute to the accumulation of pollutants. Thus, the high concentration of heavy metals in the soils of the region is a result of a combination of anthropogenic and natural factors [28]. The results of the study emphasise the need for strict control over the input and content of heavy metals in the environment, where elevated levels of mobile forms pose a potential danger to the environment and human health.

Studies conducted by R.M. Tazitdinova and her colleagues in the city of Kokshetau and at the Vasilkovskoye gold mining site showed that soils in these areas are heavily contaminated with arsenic, with concentrations in some places exceeding permissible levels by 7 to 361 times. The concentration of copper was found to be above normal by 2 to 22 times, while zinc content exceeded normal levels by 3 to 8 times. The main sources of contamination were identified as industrial activities, including gold mining, and coal use [29]. The researchers concluded that the accumulation of heavy metals in soils poses a serious threat, as these substances can penetrate into plants and animals and subsequently into the human body, endangering public health and necessitating measures to reduce anthropogenic impacts on the environment.

Research on soil contamination with heavy metals in various regions of Kazakhstan has revealed serious ecological and social problems. This highlights the urgency of addressing soil contamination with heavy metals, as well as the need to develop measures to reduce their concentrations and minimize risks to both the environment and human health.

The paradigm of aluminum pollution: the Pavlodar case study

Aluminum represents a unique case of pollution in heavy metal toxicology because its high phytotoxicity is manifested not through direct emission but indirectly, through complex biogeochemical processes in soil systems. Unlike typical heavy metals, aluminum’s environmental hazard emerges secondary to anthropogenic acidification of terrestrial ecosystems [30]. The Pavlodar region of northeastern Kazakhstan serves as a critical model for studying this phenomenon, exhibiting one of the most pronounced cases of industrially-induced aluminum toxicity in Central Asia.

The formation of technogenic aluminum anomalies in this region follows a well-defined causal chain: SO_2/NO_x emissions (Ekibastuz GRES-1, GRES-2, Pavlodar Aluminum Plant) → Acid deposition (pH 4.2-4.8) → Soil acidification (pH drop to 3.8-4.5) → Dissolution of aluminosilicates → Mobilization of Al^{3+} ions → Toxic effects on biota.

Recent studies by Kazakhstani researchers have documented severe soil degradation in the Pavlodar industrial zone. Beisekova et al. (2020) reported that agricultural soils within 30–50 km of major emission sources show pH reduction to 4.0-4.3, with exchangeable aluminum content reaching 8–15 mg/kg, significantly exceeding the critical threshold of 1-2 mg/kg considered toxic for most crops. This acidification pattern exhibits clear spatial gradients, with the most severe impacts documented downwind of the Ekibastuz power complex, where aluminum mobility increases 5–7 fold compared to background levels [7].

The impact on woody vegetation in the Pavlodar region is particularly severe. Studies of forest ecosystems near industrial zones have revealed specific adaptation mechanisms and damage patterns in tree species. Native birch and poplar populations show significant aluminum accumulation in root systems (up to 450–600 mg/kg in fine roots), leading to characteristic morphological changes including stubby root formation, reduced root hair development, and decreased mycorrhizal colonization [30]. These changes directly compromise water and nutrient uptake capacity, making trees more vulnerable to drought stress—a critical concern in Kazakhstan’s continental climate.

Coniferous species, particularly pine, demonstrate even greater sensitivity to aluminum toxicity. Research conducted in similar industrial regions shows that aluminum disrupts calcium and magnesium uptake in conifers, leading to needle chlorosis and reduced photosynthetic capacity [30]. In the Pavlodar region, pine stands within 20 km of emission sources show 40–60% reduction in annual growth increments compared to control sites, as measured by dendrochronological analysis [31].

The physiological mechanisms of aluminum toxicity in woody plants involve multiple damage pathways. Aluminum ions (Al^{3+}) preferentially target root apex meristems, disrupting cell division and elongation through interactions with the plasma membrane and cell wall components [32]. This results in immediate inhibition of root growth, typically observable within hours of exposure. Additionally, aluminum induces oxidative stress through reactive oxygen species (ROS) generation, leading to lipid peroxidation and membrane damage [30]. These effects are particularly pronounced in fine feeder roots, which are essential for water and nutrient acquisition.

Urban landscaping species in Pavlodar city face similar challenges. A study by Kochian et al. (2015) examined aluminum accumulation in common urban trees [32]. Results indicated species-specific accumulation patterns, with maple showing the highest aluminum concentrations in leaves (98–125 mg/kg) and roots (210–280 mg/kg). The researchers observed correlated nutrient deficiencies (particularly magnesium and calcium) and visible symptoms including leaf chlorosis, reduced leaf size, and premature defoliation.

The soil-plant system in the Pavlodar region exhibits complex aluminum dynamics. Some scientists documented that aluminum bioavailability increases dramatically under acidic conditions (pH < 4.5), with the proportion of phytotoxic Al^{3+} species rising from <10% at pH 5.5 to >60% at pH 4.2. This chemical shift explains the sudden onset of toxicity symptoms in previously tolerant vegetation when soil pH drops below critical thresholds.

Recent mitigation efforts in the region have focused on soil amendment strategies. Applications of organic amendments (biochar, compost) and lime can significantly reduce aluminum bioavailability through pH elevation and complexation reactions. Field trials near Pavlodar have shown that lime applications at 2–4 t/ha can increase soil pH by 0.8-1.2 units and reduce exchangeable aluminum by 60–80%, resulting in measurable improvements in tree growth and vitality within two growing seasons [7].

The aluminum contamination in the Pavlodar region thus represents a multifaceted environmental challenge that requires integrated approaches combining emission reduction, soil remediation, and careful selection of aluminum-tolerant species for reforestation and urban landscaping. The experiences from this region provide valuable insights for other industrial areas facing similar challenges with secondary aluminum toxicity.

Impact of HMs on plants

Research shows that sometimes the accumulation of necessary elements in plants can get out of control, negatively affecting the plant itself. Excessive zinc (Zn) content often leads to chlorosis symptoms, growth retardation, disruption of nutrient balance, and ethylene production [33]; iron (Fe) can cause cell structure disturbances, protein and lipid damage in cells, and stimulate the accumulation of ROS [34]. Toxic HMs can accumulate in plant cells, even in minimal concentrations, negatively affecting plants and inhibiting vital processes when exceeding permissible levels. Mercury (Hg), cadmium (Cd), arsenic (As), lead (Pb), and others are known to have detrimental effects on DNA, including oxidative stress activation, enzyme activity inhibition, influence on respiration and photosynthesis processes, damage to stomata functions and structure, and mineral nutrient absorption interference [35].

Each chemical element can exert its influence on plant organisms, depending on concentration levels. Elements can be categorised based on their effects: some accumulate HMs that are not essential components of cells (usually classified as extremely toxic), while others involve excessive concentrations of elements crucial for plant life. Excessive manganese (Mn) accumulation leads to leaf deformation and growth retardation [36]. Zinc (Zn) accumulation in critical amounts slows down growth and development on molecular, biochemical, and physiological levels, disrupts photosynthesis processes and nutrient uptake, resulting in the accumulation of ROS and causing leaf wilting [37].

Excess iron (Fe) acts as a stimulator of cell structure, protein, and lipid integrity disruption, leading to the development of leaf chlorosis [38]. Copper (Cu) [39] and nickel (Ni) cause metabolic process imbalances, resulting in cell structure disturbances. Nickel in compounds affects chlorophyll as a decomposing factor, impacting chloroplast integrity. In conjunction with copper, they reduce photosynthesis efficiency, leading to leaf blade twisting. These effects collectively suppress plant growth by destroying cell structures [40].

Molecular and physiological mechanisms of aluminum toxicity

The toxic effect of aluminum on plants is one of the most studied and yet most devastating. Unlike many heavy metals, Al exerts a rapid and potent effect primarily on the root system, which is the first target in the soil [30, 32]. The mechanisms of Al toxicity are particularly relevant for industrial regions like Pavlodar, where soil acidification from industrial emissions mobilizes Al^{3+} ions into biologically available forms.

Key mechanisms of toxicity:

1) Inhibition of root growth: Al^{3+} ions bind with pectin matrices and plasma membranes of root apex cells, disrupting their elasticity and division, leading to rapid shortening and thickening of roots, thereby im-

pairing their function [30, 32]. Within hours of exposure, root elongation rates can decrease by 50–80%, significantly compromising water and nutrient acquisition;

2) Dysfunction of the plasma membrane: Al disrupts the operation of ion channels (Ca^{2+}, K^{+}), blocks H^{+}-ATPase, which is critically important for maintaining gradients and nutrient uptake [1]. This disruption leads to membrane depolarisation and increased permeability, resulting in electrolyte leakage and loss of cellular homeostasis;

3) Disruption of mineral nutrition: Al competes with Mg^{2+}, Ca^{2+} and P ions, disrupting their absorption and transport, leading to deficiency symptoms of these elements even when they are present in sufficient quantities in the soil [1]. The particularly strong Al-Mg competition exacerbates magnesium deficiency, directly impacting chlorophyll synthesis and photosynthetic efficiency;

4) Oxidative stress: Like cadmium, aluminum induces generation of reactive oxygen species (ROS), causing lipid peroxidation of membranes and DNA damage [8]. The oxidative burst primarily involves increased production of superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH), overwhelming the plant's antioxidant defense systems;

5) Disruption of cytoskeleton and auxin transport: Al destabilizes microtubules and microfilaments, disrupting intracellular transport and the gradient of the growth hormone auxin, which is essential for root growth [30, 32]. This disruption alters the normal pattern of auxin distribution, particularly in the root transition zone where Al sensitivity is highest;

For plants in the Pavlodar region, this means chronic stress manifested in suppression of the root system, dwarfism, chlorosis, and extremely low productivity, which is exacerbated by the combined action of other stressors (drought, other metals). Studies of woody plants in the region show that aluminum accumulation in roots reaches 400–600 mg/kg, leading to a 40–70% reduction in fine root biomass and significant inhibition of mycorrhizal symbiosis [14].

Non-essential accumulator elements for plant cells are aluminium (Al), lead (Pb), arsenic (As), cadmium (Cd), chromium (Cr), and mercury (Hg). Excessive aluminium concentrations disrupt cell integrity, enzyme activity, growth, nutrition, and protein metabolism [41]. Lead (Pb) reduces enzyme activity, disrupts carbon metabolism, causes water imbalance, slows growth, and inhibits seed germination [42]. Arsenic (As) induces oxidative stress in cells, decreases carbon metabolism activity, and suppresses plant growth, germination, and yield [43]. Cadmium (Cd) is toxic element, which reduces photosynthetic process activity by chlorophyll destruction, causes the accumulation of ROS, destabilises nutrient exchange and water balance, and inhibits cell growth, leading to premature aging [44]. Unregulated chromium (Cr) content inhibits photosynthesis, nitrogen assimilation, and the cell cycle processes, slows seed germination and growth, and causes leaf necrosis and chlorosis [45]. Mercury (Hg) disrupts metabolism in plant cells by inhibiting growth, deforming chloroplast ultrastructure, photosynthesis process, and antioxidant enzyme activity [46].

A detailed study of the influence of elements on plants when they are in excess is presented in Table.

T a b l e

The influence of elements on plants when they are in excess

Element	**Effect on plants**	**Mechanism of action**	**Consequences for the plant**	**Specifics for acidified soils (e.g., Pavlodar region)**
Zinc (Zn) [33, 37]	Chlorosis, growth retardation	Disruption of photosynthesis and nutrient balance, accumulation of ROS	Leaf wilting, growth disturbance	Secondary concern compared to Al toxicity in acidified soils
Iron (Fe) [34, 38]	Chlorosis, cell damage	Destruction of cellular structure, damage to proteins and lipids, stimulation of ROS	Disruption of physiological processes, cell destruction	Reduced availability in acid soils despite high total content
Manganese (Mn) [36]	Deformation of leaves	Disruption of normal growth	Growth retardation, plant deformation	Can reach toxic levels in highly acidified conditions (pH < 4.5)
Copper (Cu) [39]	Metabolism disorders	Destruction of cellular structures, disruption of photosynthesis	Growth reduction, leaf curling	Enhanced mobility and toxicity in acidic environments
Nickel (Ni) [40]	Photosynthesis disorders	Destruction of chloroplasts, impact on chlorophyll	Decreased efficiency of photosynthesis, leaf curling	Increased bioavailability in acid soils near industrial sites

Continuation of Table

Element	Effect on plants	Mechanism of action	Consequences for the plant	Specifics for acidified soils (e.g., Pavlodar region)
Aluminium (Al) [41]	Cellular integrity disorders	Destruction of enzymes, proteins, metabolism	Growth retardation, nutritional deterioration	Dominant toxicant in industrially acidified soils. Inhibits root development, leading to nutrient and water deficiency. Requiress oil pH management (liming) rather than direct phytoextraction
Lead (Pb) [42]	Carbon metabolism disorders	Decrease in enzyme activity, water imbalance	Growth retardation, delayed germination	Reduced mobility in acid soils but enhanced plant uptake
Arsenic (As) [43]	Oxidative stress, growth inhibition	Disruption of carbon metabolism, suppression of yield	Decreased growth, germination, yield	Increased availability and toxicity in acidified conditions
Cadmium (Cd) [44]	Reduction in photosynthesis activity	Destruction of chlorophyll, accumulation of ROS	Growth retardation, premature aging	Enhanced mobility and plant availability in acidic soils
Chromium (Cr) [45]	Inhibition of photosynthesis	Disruption of nitrogen metabolism and cell cycle	Growth retardation, necrosis, chlorosis of leaves	Variable toxicity depending on oxidation state (Cr^{3+} vs Cr^{6+})
Mercury (Hg) [46]	Metabolism disorders	Destruction of chloroplasts, reduction in photosynthesis	Growth retardation, cell deformation, metabolic disturbance	Complex behavior with both organic and inorganic forms in acidic soils

All influences of chemical elements at elevated concentrations negatively impact various plant growth and development stages. Since plants are a primary source of nutrition for both humans and animals, elevated concentrations of toxic substances can be transferred from plant organisms to humans or animals, accumulating and causing disruptions in vital functions and metabolic processes [47]. The particular danger of aluminum toxicity in regions like Pavlodar lies in its dual role as both a direct phytotoxic agent and an indirect contributor to increased uptake of other heavy metals through root system damage and impaired selective absorption capabilities.

HMs in plants and their phytotoxicity

HMs accumulate in plant organisms even when there is no need for them. In soil, HMs are absorbed through roots, while in the atmosphere, they are primarily absorbed through leaf blades, and some plants absorb them through mucous layers, stomata, and trichomes [48]. Normally, plants absorb certain chemical elements they require. However, HMs can retain their toxic effects for a long time. The diffusion of HMs from different layers of soil occurs through roots, where transporter proteins and ion channels play a crucial role. For example, cadmium absorption can be facilitated through transporters (YSL, ZIP, NRAMP, HMA, and IRT), as well as through Ca^2+ and K+ channels. Passing through the cell membrane, pollutants bind with the cytoplasm and penetrate the xylem, from where they are translocated to the above-ground organs and accumulate in leaves [49]. Leaves are the assimilation organs that come into contact with atmospheric air, affecting the deposition of various pollutants from the atmosphere. Their influence starts immediately after contact with plant organs and mostly involves leaf blades' sorption. The large surface area of photosynthetic organs allows efficient absorption of toxic substances from the environment and plays an assimilative role [19].

Some tree species, both deciduous and coniferous, are used for greening purposes in urbanized areas. In this case, the temporary organ of trees for one vegetative season is the assimilation organ (leaf blade) of particular interest. Researchers from the Forest Institute and Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences have found that the highest levels of HM content are reached by the time the growing season ends. Birch shows the highest accumulation rates of toxic elements (cadmium, nickel, lead, and manganese). Poplar leaves demonstrate elevated degrees of Cd and Zn accumulation, while rowan accumulates significant amounts of iron and copper [50].

Aluminum: accumulation vs. toxicity in situ

The most important difference between aluminum and metals such as cadmium or lead lies in its extremely limited ability to translocate from roots to shoots. More than 95% of absorbed aluminum is retained and accumulates in the roots, primarily in the apoplast (cell walls) and vacuoles [30, 32]. This means that classical analysis of above-ground plant parts (leaves) for bio indication of aluminum contamination is uninformative. The main objects of monitoring should be root systems and, even more importantly, soil samples with determination of pH and content of mobile Al forms (extracted, for example, with KCl solution).

Consequently, bioindication of aluminum toxicity involves not measuring its concentration in the plant, but assessing the morphological and physiological responses of the plant to its presence in the soil: shortening and damage to roots, chlorosis, general growth suppression. This makes methods of visual assessment and root microscopy as important as chemical analysis [30, 32].

The unique accumulation pattern of aluminum is attributed to several factors. First, Al^{3+} ions rapidly form complexes with cell wall pectins and phospholipids in the root apex, creating a strong barrier to further translocation. Second, aluminum triggers callose deposition in plasmodesmata, effectively sealing off symplastic transport pathways to the shoot. Third, the high reactivity of Al^{3+} with phosphate groups leads to precipitation in root tissues, further limiting mobility.

For environmental monitoring in aluminum-contaminated regions like Pavlodar, this has practical implications. Soil analysis for exchangeable aluminum (extracted with 1M KCl) and pH measurement provide more reliable contamination indicators than plant tissue analysis [14]. When plant analysis is necessary, fine root tissues (0-2 mm diameter) rather than leaves should be sampled, as they contain the highest aluminum concentrations and show the strongest correlation with soil contamination levels [7, 10, 30, 32].

The sub cellular distribution of HMs is mainly represented by vacuoles and cell walls, which is a promising plant accumulation adaptation. Toxicants are sequestered inside the cell in this form, minimizing their transfer to other tissues and organs, thus reducing their toxicity. For example, chromium accumulates in leaf vacuoles and cell walls, while cadmium predominantly accumulates in the latter structures. This situation is characteristic of herbaceous plants as well as green parts of trees. Bulgarian scientists studying woody plants have identified silver linden as having the highest accumulative capacity. It is considered the most efficient in terms of capturing and retaining HMs. Norway maple and common ash have lower capacities for heavy metal accumulation. The levels of chemical element accumulation can vary depending on the season: the content of iron and lead in autumn leaves increases almost fivefold, while increases in other pollutants are not observed. A dramatic rise in lead and iron content indicates enhanced accumulation (over 60%) and assimilation of these elements by plants throughout the growing season, mainly from dust settling on foliar organs [51]. This confirms that foliar dust can be considered a potential indicator demonstrating atmospheric HM concentrations.

The distribution of HMs depends on plants' abilities to accumulate toxicants and on the bio toxicity and mobility of the latter, as well as soil properties that activate their mobility and biological toxicity.

Roots serve as the primary barrier to heavy metals penetrating from the soil into the above-ground organs, which is explained by their ability to absorb various substances. This occurs against the background of accumulating pollutant elements in root tissues and blocking their transport to above-ground organs. This suggests that roots are important in detoxifying plants. However, certain plant species have an increased level of transport capacity, making them hyper accumulators used for soil remediation. They stabilize the content of toxic substances in soil by accumulating them in cell walls and vacuoles. Biopolymers in cell walls, with a negative charge, aid in binding toxic pollutants, facilitating their penetration into the underground part of the plant. Thickening of the cell wall occurs through the activity of enzymes responsible for lignin synthesis, triggering the accumulation of HMs. Their accumulation in vacuoles is facilitated by chelation with phytochelatins. Many chemical elements, including HMs, enter plant cells against concentration gradients, facilitated by specialised transport proteins and ion channels [52].

For instance, the absorption and stage of Asaccumulation in plants depend on the type of plant, soil type, absorption mechanisms, and chemical element transformation. The intensity of arsenic absorption also depends on the transport pathways through which it occurs. Migration of this element into vacuoles helps prevent its translocation to young, developing shoots, where accumulation could lead to conversion into less toxic forms. Studies have shown that arsenic can exist in various valence states (III and V), with the III form being the most toxic, according to research conducted by Souri Z., Karimi N., and Sandalio L. M. Plants undergoing active processes of HM accumulation may see the V form of arsenic being reduced and migrating through xylem sap to aboveground organs [53]. Chromium content in leaves and roots can be assessed using

XANES. High-performance liquid chromatography/inductively coupled plasma mass spectrometry allows for the determination of not only the elemental composition of other plant parts (e.g., seeds) but also the identification of various forms of their compounds (methylmercury, phosphorous mercury, dimethylarsinic acid, and others). The mechanism of absorption in angiosperms is based on iron reduction through the secretion of reducing compounds or under the influence of iron reductase/oxidase associated with the plasma membrane [54].

HMs entering plant organisms induce a stress condition that acts as a favourable trigger for creating optimal conditions for the penetration of other toxic xenobiotic compounds. This minimizes the need for special transport channels for pollutant transport by releasing specialised secretions like citrate, oxalate, and malate, which facilitate their migration into the cell. Similar binding with certain organic molecules (cysteine, histidine, etc.) helps alleviate the toxic effects of excess zinc concentrations [47].

The study of physiological characteristics and tolerance regarding critical levels of copper, lead, and cadmium has shown that heavy metal levels can affect not only internal metabolic processes but also plant growth indicators. High concentrations lead to a decrease in the content of photosynthetic pigments and soluble carbohydrates while resulting in an increase in the concentration of chlorophyll (a/b), carotenoids, and excessive accumulation of proline, with a direct correlation established with copper and cadmium [55].

Heavy metals are one of the main indicators of aerotechnogenic pollution in urban ecosystems, the consequences of which can manifest in the contamination of resources (water and soil). Conducting biomonitoring followed by quantitative chemical analysis allows for the assessment of the degree of distribution, absorption, movement, and transformation of heavy metals between plants and soil by altering the physiological, anatomical, morphological, and morphometric characteristics of individual plant organs. Seasonal vegetative organs of plants, such as leaf blades, exhibit the highest sensitivity. By performing a cumulative function, they allow for the assessment of environmental conditions and provide data on the degree of anthropogenic load over a specific period.

Methods and techniques of minimizing and detecting HMs in plants

Environmental pollution with toxic metals is one of the major ecological problems today. Addressing this issue requires a comprehensive approach, where one optimal method is studying the metal-accumulating capabilities of plants. Reliable approaches and tools for detecting heavy metals provide more comprehensive information about these mechanisms, forming the basis of phytoremediation technology. According to this technology, three main subgroups of methods are identified: phytostabilization, rhizofiltration, and phytoextraction. Phytostabilization is used to immobilize pollutants from soil and reduce the bioavailability of toxic metals through absorption, preventing the migration of pollutants from the soil. Plant species that are typically selected for phytostabilization can form a dense vegetative cover (often herbaceous) and absorb some insoluble compounds through intensive root exchange in the form of hydroxides, carbonates, and phosphates. Rhizofiltration involves using metal-accumulating plants to absorb pollutants from solutions surrounding the plant roots. For example, *Elodea* is used for organic pollutants, while *Azolla sp.* and *Lemna sp.*, being good metal accumulators, are used for inorganic pollutants. The frequently used method is phytoextraction, which involves absorbing and translocating elements or pollutant compounds, often transported to aboveground parts of the plant, allowing for their collection and proper disposal (e.g., incineration) [56]. For example, crucifers are accumulators of radionuclides and heavy metals. Active accumulation is also characteristic of some fern species (Ostrich fern – *Matteuccia struthiopteris* (L.) Todaro). Shrubs and trees (pine, aspen, linden, chestnut, maple, poplar, and elm) are often natural biofilters used within urbanized areas to create phyto-barrier belts and reduce anthropogenic load [57]. They contribute to air and water purification, especially near highways, as well as soil remediation from petroleum products used in phytoremediation works.

The mentioned methods constitute a group of approaches focused on the direct interaction of plant objects with pollution sources. However, determining the exact concentration of elements is carried out using instrumental methods based on principles of automation, complexity, and accuracy.

The entry of toxic chemical elements into plant organisms, their absorption, transport, and accumulation affect physiological and biological processes. In this case, plants can act as indicators of the environmental condition, and some hyper accumulators are aimed at soil rehabilitation and reducing the impact of anthropogenic loads. A rational assessment of these mechanisms and determining the degree of anthropogenic impact is based on specialized methods and tools for obtaining information on the absorption, distribution, and translocation pathways of toxicants. Among the most advanced and modern methods for analyzing and determining the elemental composition, the following are used:

- the content of HMs is determined by AAS and ICP-MS;
- LA-ICP-MS and XRF methods are used to assess the distribution of elements in space;
- XAS and AFS are aimed at analyzing chemical forms;
- transport and absorption dynamics are recorded using non-invasive micro testing technology (NMT).

Molecular biology methods form the basis for assessing the molecular mechanisms underlying the interaction of HMs with plant organisms as a whole, as well as with individual components of plant cells [18].

ICP-MS is a quantitative method used to assess HM levels by applying an electric current and measuring light emission within a specific range of wavelengths. This method's advantage is its ability to quickly and accurately determine even the smallest concentrations of HMs, compare them with isotopic values, and easily control for interference. In practical research, this method is used due to characteristics such as high accuracy rates, low detection limits, increased levels of sensitivity and selectivity. The limits of accuracy of this method are increased when integrated with techniques such as LC-ICP-MS and SP-ICP-MS. ICP-MS assesses pollutant levels not only in tree leaf blades but also in rice, quinoa, and algae, where arsenic content, for example, dominated and indicated excessive accumulation. The SP-ICP-MS method is considered optimal for assessing zinc content in leaves, while LA-ICP-MS is deemed most suitable for analyzing variations in HM concentrations (Fe, Pb, Cd, Cr, and Mn) in annual tree rings. Another important mass spectrometry method is atomic absorption spectrometry, which involves the absorption of atomic vapor spectral lines, where the chemical element content is identified by the degree of light attenuation upon absorption. The benefits of this approach complement the positive aspects of ICP-MS with higher selectivity indicators. However, this device is characterized by low noise immunity due to the occurrence of readout noise during charge packet movement. AAS can be divided into flame AAS, graphite furnace AAS, hydride generation AAS, and cold vapor AAS [57].

The analysis of the chemical forms of pollutant elements is carried out using the method of atomic fluorescence spectrometry (AFS). The principle of this method involves the absorption of atomic vapour by light radiation, leading to the excitation of atoms. Simultaneously, the emitted fluorescence, under the influence of a photoelectric detector, is converted into an electrical signal read by the data processing system [58]. Synchrotron X-ray radiation is used in X-ray absorption spectroscopy (XAS) by exciting the sample with X-rays and causing electrons to transition to empty orbitals or the continuum, resulting in waves scattering with surrounding atoms. This method allows for the determination of valency, the position of nearest atoms, local structure, and extensive chemical information about pollutant elements in plants.

Specifics of aluminum analysis and speciation

The application of these advanced methods can be illustrated by the example of aluminum (Al), a prevalent abiotic stressor in acidic soils. For a correct assessment of aluminum contamination and its impact, determining its total content is insufficient. The analysis of mobile forms (Al^{3+}) in the soil and studying its distribution in root tissues is critically important.

1. Soil analysis: The gold standard for determining exchangeable aluminum is soil extraction with a 1M KCl solution, followed by the analysis of the extract using AAS or ICP-MS [57]. The measurement of the pH of an aqueous suspension is mandatory for data interpretation, as Al toxicity is primarily manifested at low pH.

2. Plant tissue analysis: The determination of Al in root tissues (and less commonly in leaves when toxicity symptoms are observed) is typically performed using ICP-MS due to its high sensitivity and low detection limits. To study the localization of Al within root tissues, histochemical staining (e.g., with haematoxylin or eriochrome cyanine R) followed by light or fluorescence microscopy is employed [57].

3. Speciation analysis: The Extended X-ray Absorption Fine Structure (EXAFS) technique, a part of XAS, is pivotal for determining the immediate atomic environment of aluminum. It identifies the ligands (e.g., OH, PO_4, organic acids) to which Al is bound in the soil or plant matrix. This information is critical for understanding its mobility, bioavailability, and ultimately, its toxicity [58].

Thus, the development of instrumental methods for analyzing the elemental composition of phyto objects highlights a series of mass spectrometry methods, each of which is a way to determine specific characteristics of HMs, complementing each other. The ICP-MS method is aimed at detecting elemental content, and AAS is used for analyzing elemental composition and traces because of their repeatability, low detection limits, and affordability. The XAS method reveals the local forms of the studied HMs, enabling the assessment of the transport and absorption mechanisms of these chemical elements by plants. This approach can also be directed towards studying the tolerance of plant organisms to HMs to identify their dynamic metabolism and key aspects of transformation and translocation in various internal and external environmental conditions.

Conclusions

Intensive industrial development and urbanization are the main factors contributing to environmental pollution with heavy metals (HM) and ecotoxicants, among which aluminum (Al) gains particular significance in the industrialized regions of Kazakhstan. Unlike canonical heavy metals, the toxic effects of aluminum are manifested indirectly—through large-scale soil acidification caused by acid rain, which results from sulfur dioxide (SO_2) and nitrogen oxides (NO_x) emissions by energy and metallurgical enterprises. The Pavlodar region, with its concentration of facilities such as the Ekibastuz thermal power plants and the Pavlodar Aluminum Plant, serves as a vivid example of a region where aluminum mobilized in the form of Al^{3+} ions becomes a dominant stress factor for plants, leading to root system suppression, disruption of mineral nutrition, and a decline in ecosystem productivity.

An analysis of 58 scientific sources selected from the Web of Science and Scopus databases for the period 2014-2025 (with reference to foundational works since 1990) has shown that the issue of HM pollution in Kazakhstan remains critical. High levels of lead, cadmium, arsenic, and copper contamination are recorded in industrial centers (Karaganda, Zhezkazgan, Ust-Kamenogorsk), yet it is the secondary aluminum contamination in acidic soils ($pH < 4.5$) that requires special attention and the adaptation of monitoring methods.

Standard methods for analyzing the aerial parts of plants are not informative for assessing aluminum’s impact, since more than 95% of Al is accumulated in the root system. The most effective approaches include:

- soil monitoring: determining exchangeable aluminum (extraction with 1M KCl) and soil pH;
- fine root analysis: using highly sensitive methods such as ICP-MS to determine Al content;
- speciation analysis: applying XAFS (X-ray Absorption Fine Structure) methods to identify aluminum’s chemical forms and bioavailability.

The unique toxicity mechanism of aluminum—root growth inhibition, cytoskeleton disruption, oxidative stress induction, and impaired uptake of Ca^{2+} and Mg^{2+}—necessitates the development of specific mitigation measures tailored to Kazakhstan:

- application of soil amendments: liming (2-4 t/ha) and addition of organic amendments (biochar, compost) to increase pH and immobilize Al^{3+};
- selection of tolerant species: for landscaping and reforestation in industrial regions, it is essential to use species tolerant to aluminum and acidic soils;
- improved monitoring systems: integrating modern analytical techniques (ICP-MS, XAFS) with traditional biological assessment methods (visual evaluation of root damage, chlorosis).

Thus, a comprehensive approach that combines the reduction of primary SO_2/NO_x emissions, active remediation of acidic soils, and the implementation of an adapted phytomonitoring system is essential to reduce environmental stress and maintain the productivity of agro- and natural ecosystems in industrial regions of Kazakhstan such as the Pavlodar region. Future research should focus on a detailed study of the tolerance mechanisms of local plant species to aluminum, the development of regional standards for mobile forms of Al in soils, and the assessment of the effectiveness of various reclamation practices under the conditions of specific industrial hubs in the country.

Conflict of Interest

Authors declare no conflict of interest.

Author contribution

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Kaverina M.M. —** investigation, methodology, writing-review & editing; **Ualiyeva R.M. —** conceptualization, data curation; **Syso A.I. —** investigation, literature review, data collection.

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М.М. Каверина, Р.М. Уалиева, А.И. Сысо

Ауыр металдардың өсімдік организмдеріне әсері және оларды талдау әдістері: шолу

Шолу Қазақстанның өнеркәсіптік өңірлеріндегі алюминий уыттылығының бірегей мәселесіне ерекше назар аудара отырып, ауыр металдардың өсімдіктерге әсерін кешенді талдауға арналған. Тау-кен және металлургия салаларының қарқынды дамуы ластаушы заттардың едәуір көлемде шығарылуына әкелуде, олардың ішінде алюминий айрықша экологиялық қауіп тудырады. Басқа металдардан айырмашылығы, оның жоғары фитоуыттылығы жанама түрде — қышқыл жаңбырлардың (SO_2 және NO_x шығарындыларының) топырақты қышқылдандыруы арқылы байқалады. Бұл өз кезегінде табиғи алюмосиликаттардан жоғары уытты Al^{3+} иондарының босап шығуына себеп болады. Павлодар облысының мысалында қайталама алюминиймен ластану парадигмасы мен оның өсімдіктердің тамыр жүйесіне әсер етуінің негізгі механизмдері жан-жақты талданған. Оларға өсу процесінің тежелуі, цитоскелеттің бұзылуы, минералдық қоректенудің бұзылуы және тотығу стресінің индукциясы жатады. Шолуда Қазақстанның түрлі өңірлеріндегі өсімдіктерде ауыр металдардың жинақталуы бойынша заманауи деректер жүйеленіп, алюминийдің биожетімділігі мен уыттылығын дәл бағалау үшін қажетті озық аналитикалық әдістерге (ICP-MS, XAS, EXAFS) сыни талдау жасалған. Өсімдіктердің биоиндикатор ретіндегі рөліне және фиторемедиация әдістерін қолдану мүмкіндіктеріне ерекше назар аударылды. Әдебиеттерге шолу негізінде Қазақстан үшін нақты бейімделу шараларын әзірлеу қажеттігі айқындалады. Оларға топырақты әктеу, көгалдандыру үшін алюминийге төзімді өсімдік түрлерін іріктеу және экологиялық тәуекелдерді азайту мен экожүйелердің өнімділігін сақтау мақсатында заманауи экологиялық мониторинг әдістерін енгізу жатады.

Кілт сөздер: ауыр металдар, алюминий, фитоуыттылық, өнеркәсіптік ластану, қышқыл топырақ, экологиялық бақылау, биожетімділігі, фиторемедиация

М.М. Каверина, Р.М. Уалиева, А.И. Сысо

Влияние тяжелых металлов на растительные организмы и методы их анализа: обзор

Данный обзор посвящен комплексному анализу воздействия тяжелых металлов на растительные организмы, с особым фокусом на уникальную проблему токсичности алюминия в промышленных регионах Казахстана. Интенсивное развитие горнодобывающей и металлургической отраслей приводит к значительным выбросам загрязняющих веществ, среди которых особую экологическую угрозу представляет именно алюминий. В отличие от других металлов, его высокая фитотоксичность проявляется опосредованно через подкисление почв кислотными дождями (обусловленными выбросами SO_2 и NO_x), что приводит к мобилизации высокотоксичных ионов Al^{3+} из природных алюмосиликатов. На примере Павлодарской области детально разбирается парадигма вторичного алюминиевого загрязнения, его ключевые механизмы воздействия на корневые системы растений, включая ингибирование роста, разрушение цитоскелета, нарушение минерального питания и индукцию окислительного стресса. В обзоре систематизированы современные данные о накоплении тяжелых металлов в растениях различных регионов Казахстана и критически оценены передовые аналитические методы (ICP-MS, XAS, EXAFS), подчеркивается необходимость специализированного анализа для точной оценки биодоступности и токсичности алюминия. Особое внимание уделено роли растений как биоиндикаторов и перспективам применения методов фиторемедиации. На основе анализа литературы делается вывод о необходимости разработки целевых адаптивных мер для Казахстана, включая известкование почв, подбор алюминий-толерантных видов для озеленения и интеграцию современных методов экологического мониторинга для снижения экологических рисков и сохранения продуктивности экосистем.

Ключевые слова: тяжелые металлы, алюминий, фитотоксичность, промышленное загрязнение, кислотные почвы, экологический мониторинг, биодоступность, фиторемедиация

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Microscopic analysis of *Tilia cordata*

The raw material base for medicinal plant resources can be expanded both by introducing new plant species into practical use and by utilizing additional plant organs of species already in use. The flowers of *Tilia cordata* are a valuable type of medicinal raw material; however, their collection is constrained by a short flowering period. The leaves of this species also exhibit pharmacological activity, making them a promising independent source of medicinal plant material. This article presents the results of a microscopic analysis of *Tilia cordata* leaves. Anatomical examination was performed on dried leaves, with an assessment of the structural features observed in surface preparations and cross sections. The leaf was found to be hypostomatic, with the main epidermal cells having straight or wavy walls. On the abaxial surface, four types of trichomes were identified: glandular, forked, capitate, and stellate. A crystalline coating was observed along the leaf veins. In cross section, the leaf is dorsiventral, with a single-layered palisade mesophyll, a 2-3-layered spongy mesophyll, mucilage channels, and druses of calcium oxalate. Diagnostic microscopic features have been identified that may be used in the preparation of a pharmacopoeial monograph.

Keywords: *Tilia cordata*, plant raw material, microscopic analysis, leaf, micro-diagnostic signs

Introduction

The introduction of new types of plant medicinal raw materials is a pressing task for botany and pharmacy. Not all types of raw materials included in the Pharmacopoeia [1] are available in sufficient quantities to meet the needs of the pharmaceutical industry in the production of drugs.

One of the most sought-after types of raw materials is the flowers of the heart-shaped linden tree [1, 2]. In official medicine, heart-shaped linden flowers (*Tiliae flores)* are used as a diaphoretic, expectorant, and anti-inflammatory agent [3–6]. However, the flowering period of this plant species is short (late June to early July, about two weeks), which limits the time available for harvesting the flowers (Fig. 1). The plant itself has a limited habitat: the Caucasus, Karelia, Arkhangelsk Region, Komi Republic, Tomsk Region, and Khanty-Mansi Autonomous Okrug. Extensive areas are occupied by forests in the Urals [7].

A B C

Figure 1. Leaves (A), tree (B), and flowers (C) of *Tilia cordata*

Recently, the leaves of *Tilia cordata* Mill. (family Malvaceae) have attracted the attention of scientists. Experiments have shown that total extracts obtained from *Tilia cordata* leaves exhibit a wide range of biological activity, including general strengthening, anti-inflammatory, wound-healing, and antimicrobial effects [8–10].

In this regard, *Tilia cordata* leaves are of scientific and practical interest for detailed study, and their introduction as medicinal raw materials will allow for the partial replacement of the scarce flowers of this species.

Based on the above, the aim of this study was to examine the anatomical structure of *Tilia cordata* leaves and identify the diagnostic features of the raw material.

Experimental

The objects of the study were *Tilia cordata* leaves (Fig. 2) collected at different times of the growing season (early May to late September 2023) in the forest park area of the State Humanitarian-Technical University (Orekhovo-Zuyevo).

Figure 2. Dried samples of *Tilia cordata* leaves

The raw material was soaked in hot water and fixed in a mixture of 50 % aqueous glycerin, distilled water, and 90 % ethyl alcohol [11]. Copper salts were added to the fixative to preserve the color of the cells. Surface preparations and cross sections were made manually using a razor. For cross sections, fragments of leaf plates along the midrib were selected.

Cross sections were stained with a 5 % alcohol solution of Lugol's iodine for a clearer picture.

The studies were conducted using a MICMED-6 microscope with a UCMOS05100 digital camera at magnifications of 10x4, 10x10, and 10x40. The micro-preparations were described in accordance with the recommendations of R. Crang [12], P.J. Rudall [13], and the State Pharmacopoeia of the Russian Federation [14].

Results and Discussion

The leaf *of Tilia cordata* is simple, on a long petiole, with a heart-shaped or broadly ovate base, a pointed tip, and a finely serrated margin [15, 16]. The upper side of the leaf blade is dark green and smooth, while the lower side is light green, almost grayish, with characteristic colored tufts of trichomes along the veins. The leaves are 3–10 cm long and 4–12 cm wide.

A leaf fragment taken from the upper side of the leaf (Fig. 3) shows the absence of stomata, but prismatic crystals are visible through the epidermis. The main epidermal cells have straight anticlinal walls or wavy walls.

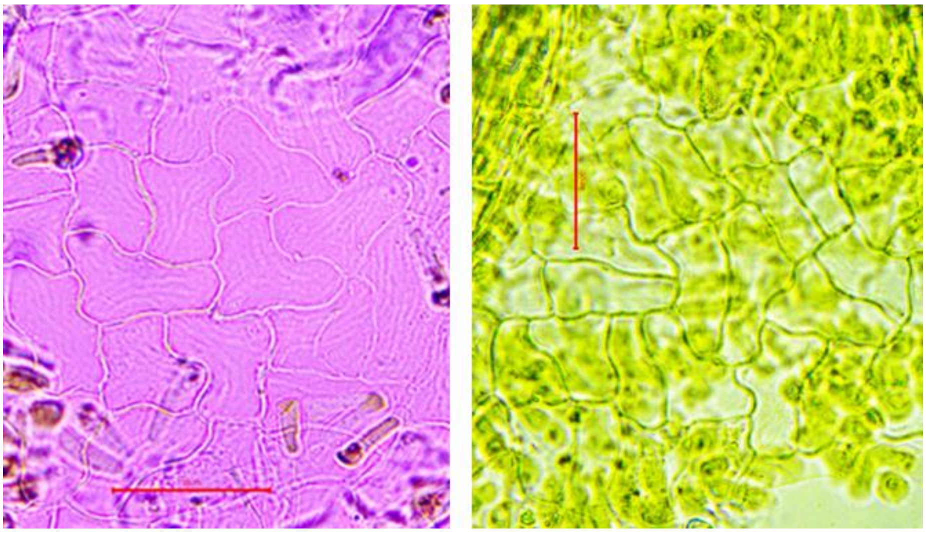

Figure 3. Upper epidermis of a *Tilia cordata* leaf

A superficial preparation of the underside of the leaf revealed the presence of stomatal cells of the anomocytic type (Fig. 4). The stomata have an average size of 14.6 × 23.5 μm and are surrounded by 3–4–5 epidermal cells. Thus, the leaf *of Tilia cordata* is defined as hypostomate, since the stomata are located on the lower surface of the leaf blade.

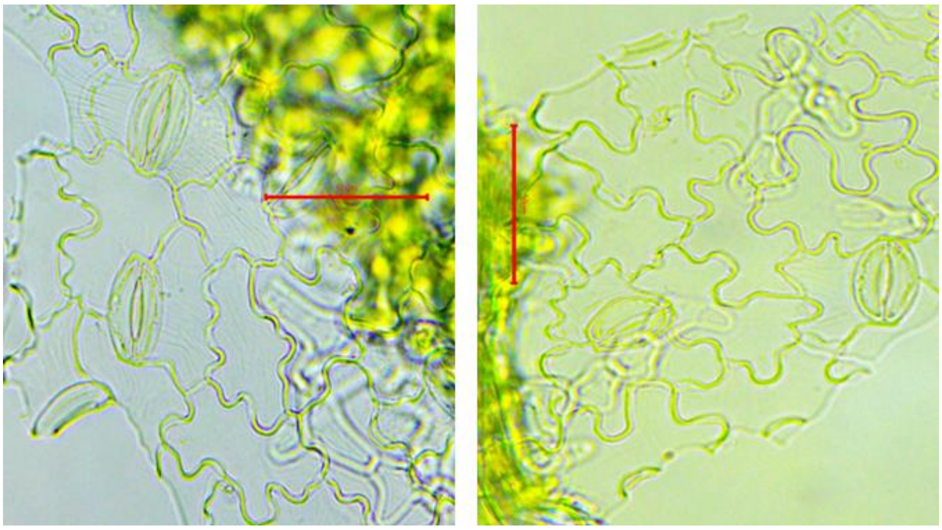

Figure 4. Lower epidermis of *Tilia cordata* leaf

There are different types of trichomes [17]. For example, glandular trichomes are confined to the sinuses of secondary or tertiary veins (Fig. 5). They are usually bulbous or bottle-shaped.

Along the main veins are forked trichomes (Fig. 6), which have a short stalk and are pressed against the surface of the axis. In the axils of the veins, there are capitate multicellular (Fig. 7) and stellate trichomes (Fig. 8). The latter vary in the number of branches, from 4 to 8.

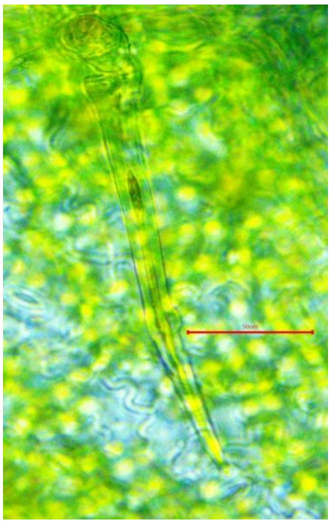

Figure 5. Glandular trichomes on the leaves of *Tilia cordata*

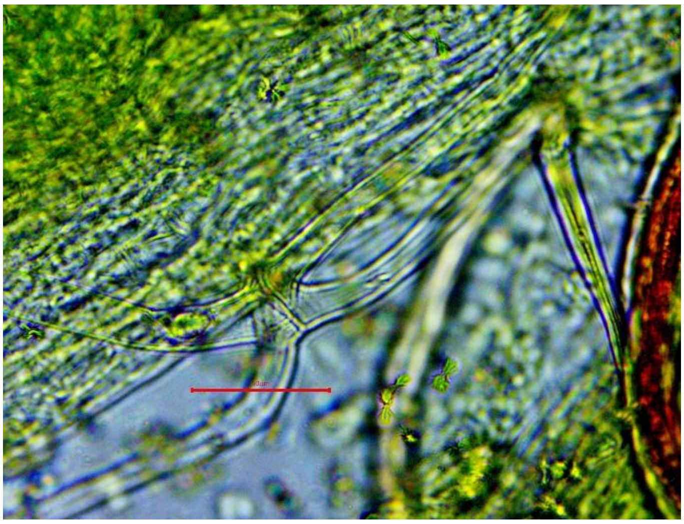

Figure 6. Forked trichomes on the leaves of *Tilia cordata*

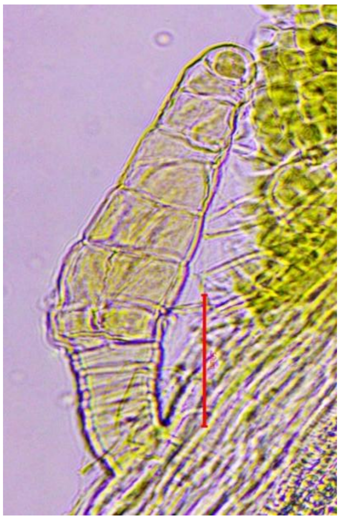

Figure 7. Capitate trichomes on the leaves of *Tilia cordata*

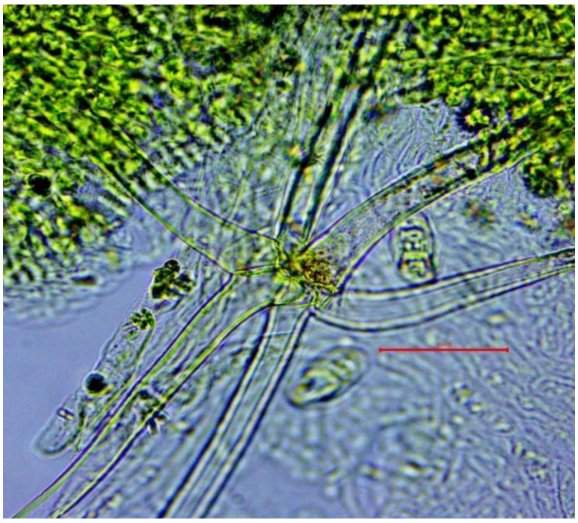

Figure 8. Stellate trichomes on the leaves of *Tilia cordata*

Crystalline inclusions are visible along the veins of the leaves on both sides (Fig. 9). The crystals are prismatic in shape.

The leaf cross-section (Fig. 10) is identified as dorsoventral, containing palisade parenchyma on one side and spongy parenchyma on the other. The palisade mesophyll is single-layered, while the spongy mesophyll contains 2-3 layers.

On the cross section of the midrib (Fig. 10a) and lateral fragments (Fig. 10b), a single-layered epidermis is located around the perimeter. Its cells are elongated, almost rectangular. In the area of the midrib, there are 1-2 layers of collenchyma, and the parenchyma and sclerenchyma consist of 3-4 layers. In the midrib, mucous channels containing calcium oxalate crystals are visible. The conducting bundle is collateral, closed, and broadly ovoid in shape.

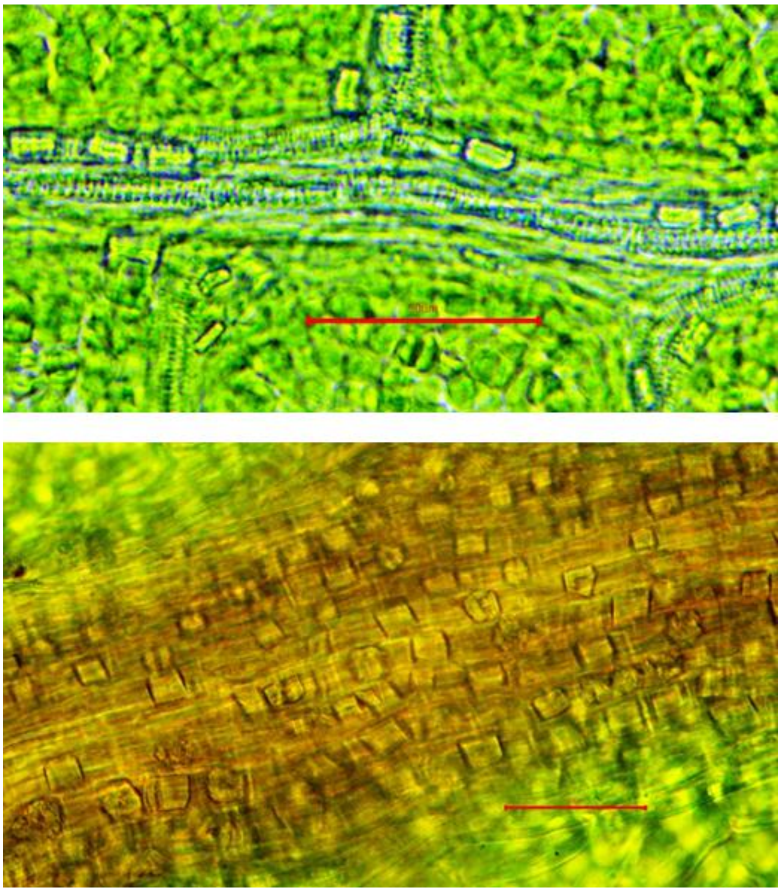

Figure 9. Crystalline coating along the veins of a *Tilia cordata* leaf

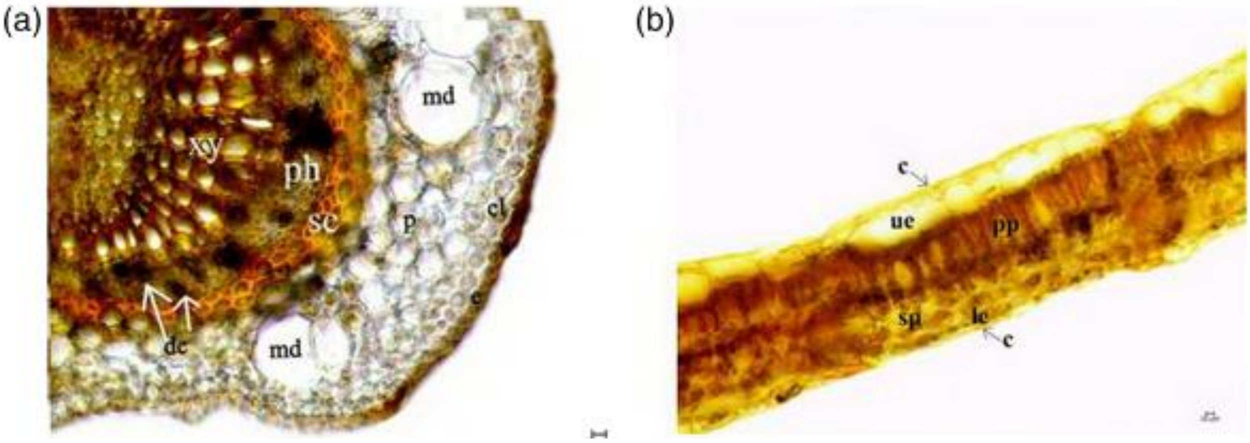

Figure 10. Cross section of a *Tilia cordata* leaf
a — midrib; b — lateral fragment; c — cuticle; cl — collenchyma; dc — calcium oxalate druses;
le — lower epidermis; md — mucous canal; p — cortical parenchyma; ph — phloem; pp — palisade mesophyll;
sc — sclerenchyma; sp — spongy mesophyll; ue — upper epidermis; xy — xylem

The structure of a true *Tilia cordata* leaf has similar features to the leaves of other species [18, 19]. However, there are distinctive features, consisting of the peculiarities of the structure of the epidermis cells, the types of hairs, and the presence of mucous channels and crystal druses in the cross section.

The identification of structural features can serve as diagnostic criteria for the identification of plant raw materials at the microscopic level.

Conclusion

Thus, a study of the anatomical structure of the *Tilia cordata* leaf was carried out, including analysis of surface preparations and cross sections. The structural features of the leaf epidermis, the type of leaf based on the arrangement of veins, and the presence of four types of trichomes were established.

For the transverse section, a dorsoventral type of leaf plate, the presence of a single-layer palisade and multi-layer spongy tissue, the presence of mucous channels and calcium oxalate druses were identified. The bundle is collateral, closed type, surrounded by 2-3 layers of sclerenchyma.

The diagnostic features identified can help in determining the species and identifying medicinal plant raw materials.

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Conflict of Interest

The author declares no conflict of interest.

Author contribution

Babeshina L.G. — investigation, visualization, manuscript writing.

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Л.Г. Бабешина

Tilia cordata микроскопиялық талдауы

Дәрілік шикізаттың шикізат базасын кеңейтуді өсімдіктердің жаңа түрлерін іс жүзінде пайдалануға енгізу, сондай-ақ бұрын іс жүзінде пайдалануға енгізілген жаңа түрлерді қолдану есебінен жүргізуге болады. *Tilia cordata* гүлдері шикізаттың сұранысқа ие түрі, алайда оларды жинау қысқа гүлдену кезеңімен шектеледі. Осы түрдің жапырақтары да фармакологиялық белсенділікті көрсетеді, бұл оларды дербес дәрілік өсімдік шикізаты ретінде перспективалы етеді. Мақалада *Tilia cordata* жапырақтарын микроскопиялық талдау нәтижелері келтірілген. Кептірілген жапырақтардың анатомиялық талдауы жүргізілді, оның ішінде беткі препараттар мен көлденең қималардың құрылымдық ерекшеліктерін талдау жүргізілді. Жапырақтың гипостоматикалық типке жататыны анықталды, эпидермистің негізгі жасушалары түзу немесе толқынды қабырғаларға ие; төменгі жағында трихомалардың 4 түрі бар, олар: безді, айырлы, бас тәрізді және жұлдыз тәрізді. Жапырақ тамырларының бойында кристалды жабын бар. Көлденең қимада жапырақтың дорсовентральды құрылым түрі бар: палисадты мезофилл бір қабатты, кеуек тәрізді — 2-3 қабатты, шырышты арналар мен кальций оксалат кристалдарының друздары бар. Фармакопеялық мақаланы дайындау үшін қолдануға болатын микроскопиялық деңгейде диагностикалық белгілер анықталды.

Кілт сөздер: *Tilia cordata*, өсімдік шикізаты, микроскопиялық талдау, жапырақ, микро диагностикалық белгілер.

Л.Г. Бабешина

Микроскопический анализ *Tilia cordata*

Расширение сырьевой базы лекарственного сырья можно производить как за счет введения в практическое использование новых видов растений, так и за счет применения новых органов, ранее включенных в практическое использование. Цветки *Tilia cordata* являются востребованным видом сырья, однако их сбор ограничен коротким периодом цветения. Листья данного вида также проявляют фармакологическую активность, что делает их перспективными в качестве самостоятельного лекарственного растительного сырья. В настоящей статье приводятся результаты микроскопического анализа листьев *Tilia cordata*. Анатомирование проведено для высушенных листьев с анализом особенностей строения поверхностных препаратов и поперечных срезов. Установлено, что лист относится к гипостоматическому типу, основные клетки эпидермиса имеют прямые или волнистые стенки; с нижней стороны присутствуют 4 типа трихом: железистые, вильчатые, головчатые и звездчатые. Вдоль жилок листьев присутствует кристаллоносная обкладка. На поперечном срезе лист имеет дорзовентральный тип строения: палисадный мезофилл однослойный, губчатый — 2-3-слойный, присутствуют слизистые каналы и друзы кристаллов оксалата кальция. Установлены диагностические признаки на микроскопическом уровне, которые могут использоваться для подготовки фармакопейной статьи.

Ключевые слова: *Tilia cordata*, растительное сырье, микроскопический анализ, лист, микро диагностические признаки

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Dendroclimatic analysis of common pine (*Pinus sylvestris L.*) on the SNNP "Burabay"territory

The study aims to obtain new data on the chronology of *Pinus sylvestris* L. under arid conditions in the forest-steppe zone of northern Kazakhstan, specifically in the northern part of the SNNP "Burabay" territory. Two sites were selected to compare how different factors influence wood growth. Analysis of the generalised tree-ring chronologies revealed a substantial number of trees in the study area that are between 170 and 220 years old. First-order autocorrelation values ranged from 0.68 to 0.78. The sensitivity coefficient indicated the presence of a weak climatic signal. Short-term climate projections suggest a slight increase in temperature and a decrease in warm-season precipitation over the next 10 years.

Keywords: *Pinus sylvestris* L., climate prediction, Burabay, precipitation, air temperature

Introduction

Climate change cycles and increased human activity have had a significant impact on the world's forest area. Since the conclusion of the last ice age (approximately ten thousand years ago), the area of forestry has decreased from six billion hectares to four billion hectares, which equates to 31 % of the total land surface [1]. The structure and functions of forest communities in climate-sensitive regions are subject to uncertain changes in response to global warming. In particular, the reaction of pine growth to climate change in northern Central Asia remains uncertain [2]. The long-term utilization of pine forests in Kazakhstan for recreational purposes, in conjunction with the trend of climate change towards aridification and the occurrence of catastrophic fires in this region, has resulted in substantial detrimental alterations to the condition and sustainability of these plantations [3]. In this regard, monitoring of forest ecosystems is carried out on the territory of the SNNP "Burabay".

The first dendroclimatic studies on the territory of the southern part of the SNPP were published in 2017, where the impact of climate change on the width of annual rings was observed. The variability and sensitivity of annual ring widths increased after the 1940s. The observed trend of increasing precipitation, temperature and annual ring width is of particular interest. In conclusion, it can be posited that the annual rings of common pine in Burabay exhibit heightened sensitivity to prevailing growing conditions and are susceptible to the effects of climate change [4]. A classical dendroclimatic study was conducted for six coniferous forest areas near their semi-arid borders throughout Kazakhstan, including Pinus sylvestris L., a species found in temperate forest-steppes, and Picea schrenkiana Fisch. & C.A. Mey, a species found in foothills in the south-eastern region of the Western Tien Shan; Juniperus seravschanica Kom., a species found in the mountain zone in the south-eastern region of the Western Tien Shan, in the southern subtropics. In the context of Kazakhstan, it has been determined that the primary factor impeding the growth of conifers is heat stress. To address this issue, it is recommended that experiments be conducted to develop heat-protection measures for plantations and urban trees. Additionally, it is suggested that the dendroclimatic network be expanded to encompass the influence of habitat conditions and climate-driven dynamics of perennial growth [5].

Experimental

The study area is the Burabay SNNP, located in northern Kazakhstan, specifically in the Akmola region, within the Burabay district (53°05′00″N 70°18′00″E). The topography of the Burabay tract is characterized by undulating terrain, which is part of the Kazakh Shallow Soil. In the spring of 2024, two plots were established within the SNNP to collect cores of common pine (*Pinus sylvestris* L.). One of these plots was designated as Bur, representing natural growing conditions, while the second plot was designated as BurA, representing conditions influenced by anthropogenic factors. These plots were situated in the vicinity of Zhukey village, located in the Yenbekshilder district (see Figure 1 and Table 1 for further details). The sites were selected in order to compare how different factors affect wood growth.

The vegetation of the area is closely related to the landscape features of the Kokshetau upland. The presence of a forest-steppe landscape within the steppe zone is attributable to several factors, including its elevated position and strong ruggedness. Furthermore, the forest-steppe landscape is distinguished by its slightly higher precipitation levels (300–350 mm) compared to the surrounding areas, resulting in increased water availability. The topsoil is characterized by a brown earth, loessy, sandy loam composition. Common pine (Pinus sylvestris) constitutes 65 % of the total tree vegetation of the park.

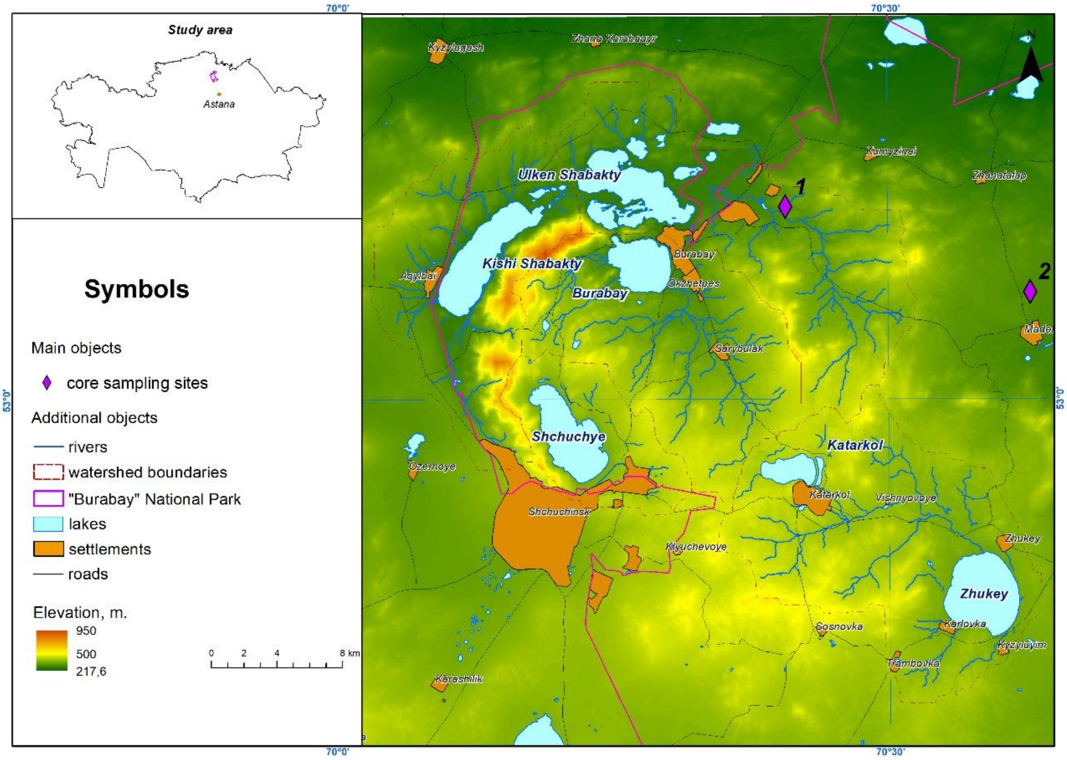


Figure 1. Sampling points in Burabay SNNP
No.1 — Bur site (natural growing conditions), No.2 — BurA site (under the influence of anthropogenic factors)

Table 1

Location of sampling sites and sources of climatic data

Sampling location	Species	Geographical coordinates			Climatic grid points
Bur	PISY	53°00'38.2	70°17'44.5	388	53.25 70.25
BurA	PISY	52°54'57.1	70°36'10.6	426	52.75 70.75
*PISY — Pinus sylvestris					

The winter season is characterized by its severity and protracted nature, and is marked by a scarcity of precipitation, a consequence of the continental climate. Conditions during the winter months are considered to be within normal parameters, except on days when there is a significant increase in wind speed. However, such winds are not particularly characteristic of the northern region during the winter months. Should these occur primarily during the first two months of winter, a sharp decline in air temperature can be expected. It has been demonstrated that, on occasion, the indicators can reach a value of -45 °C. During the winter months, the presence of warm winds can result in an increase in air temperature to approximately

+5 °C. Furthermore, it has been observed that snow cover persists for duration of approximately four months. The mean annual temperature is 1.3 °C. The mean temperature in January is -16.8 °C, while the mean temperature in July is 19.1 °C. The mean duration of the frost-free period is a mere 140 days. The annual average amount of precipitation is 332.3 millimeters, with a maximum of 257.9 millimeters recorded from April to October (Fig. 2).

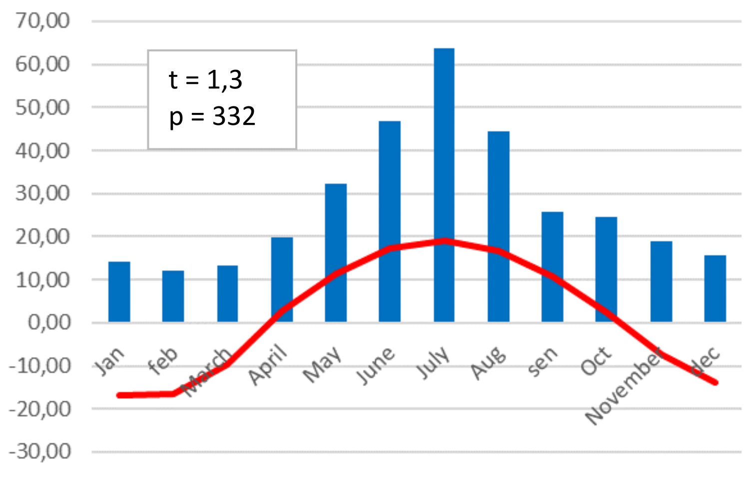


Figure 2. Average annual temperature and precipitation on the territory of SNNP "Burabay".

A distinctive feature of closed stands is the presence of a substantial layer of forest litter. The thickness of the layer varies between 3–5 cm, with some areas reaching 10–15 cm in hollows. The litter is predominantly composed of fallen needles and small pine branches, with a paucity of grass cover in certain areas.

Samples of wood (cores) were obtained for dendrochronological analysis. These samples were taken from a variety of common pine trees of different ages. Trees for core drilling were selected in sparse forests in the areas of their most significant accumulation, in stands—within the sample areas. Cores were extracted at a height of 1.3 m from the root neck. The cores were collected and prepared for dating according to the methods adopted in dendrochronology [6–8]. The samples were meticulously cleaned using a combination of blades and a clerical knife. The presence of boundaries within annual rings was determined by applying tooth powder to the cleaned surface of the core. The width of annual rings was measured with an accuracy of 0.01 mm on a semi-automatic LINTAB VI instrument. The calendar year of each ring was determined using the CrossDating method in the TsapWin computer program [9, 10]. The accuracy of cross-dating was checked using cross-correlation analysis in the specialized computer program COFEÑHA [11-12].

The quality of the dendrochronological material was assessed using the ARSTAN program by the following indicators: Pearson correlation coefficient, standard deviation, skewness, average sensitivity coefficient, first-order autocorrelation, and total population signal of the chronology EPS (expressed population signal). A value of 0.85 is taken as the threshold value of EPS, where a total variance below this threshold indicates an unacceptable amount of noise in the chronologies [13].

Climate data (temperature and precipitation) were utilized to project climate change, which was then interpolated for the corresponding geographic coordinates of the spatially distributed CRU TS field (1901–2023). This data is publicly available in the KNMI Climate Explorer database (https://climexp.knmi.nl/start.cgi).

Results and Discussion

The indicators of the qualitative evaluation of the obtained dendrochronological material are summarized in Table 2. The obtained individual chronologies can be considered to be relatively insensitive to external environmental factors, as the sensitivity coefficient does not exceed the threshold value of 0.3. However, the site of BcrA shows a small signal, which amounts to 0.25. This phenomenon is likely attributable to anthropogenic influences, particularly the establishment of plantations in exposed locations. These plantations are susceptible to strong winds, which can result in lopsided crowns and severely bent trunks in affected trees. It is frequently observed that substantial roots, which are scarcely concealed by soil, extend across the surface of granite, particularly along crevices where diminutive branches of roots penetrate. The process of crack expansion is often accompanied by the deposition and subsequent lifting of substantial granite blocks by skeletal roots, a phenomenon that is particularly evident during the process of root growth. A high value of first-order autocorrelation (0.68-0.72) was observed, indicating that past climatic conditions are related to current annual pine growth. As demonstrated by the EPS calculations, the generalized chronology has a sufficient supply of dendrochronological data for the period from 1876–2023 (Bur) and from 1918–2023 (BurA). Generally, statistical indicators suggest the reliability of the constructed dendrochronological series and the significant impact of abiotic factors on the radial growth of common pine.

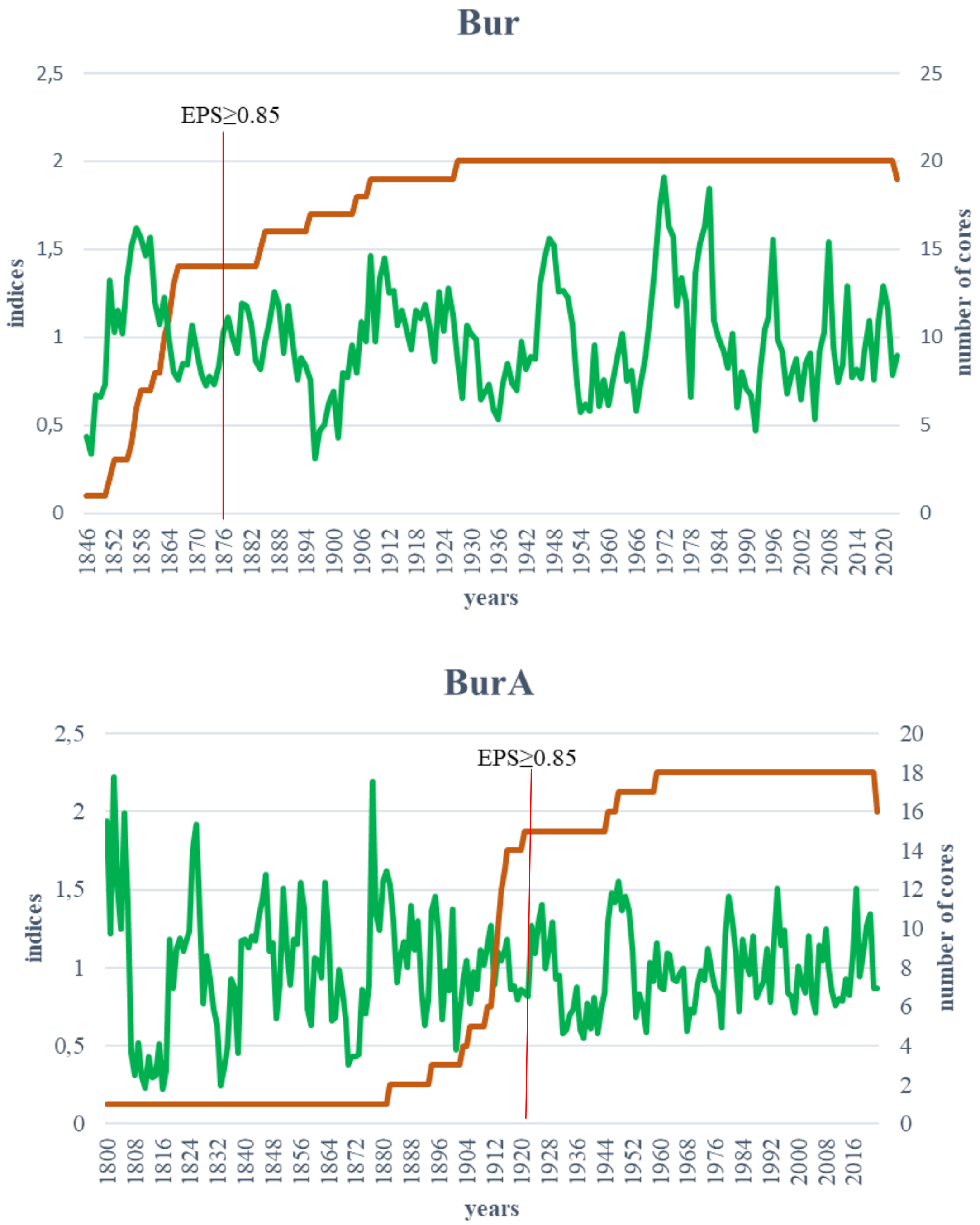


Figure 3. Tree-ring chronologies (TRC) of Bur and BurA.
The green line represents the standard chronology; the brown line represents sampling depth (number of cores for each year); the vertical red line shows the first year of EPS ≥ 0.85, i.e., the beginning of the period suitable for dendroclimatic analysis.

The total sample volume by site was 38 cores; the generalized chronologies of Burabay are shown in Figure 3, the maximum age was 223 years, and the period suitable for dendroclimatic analysis (EPS>0.85) Bur — 147 years, BurA — 105 years.

Table 2

Annual ring width chronology statistics

Plot	Number cores	Period chronologies	Maximum length (years)	Sensitivity	Autocorrelation of 1 order
Bur	20	1849-2023	178	0.20	0.68
BcrA	18	1800-2023	223	0.25	0.72

The correlation analysis demonstrated that Bur and BurA chronologies exhibited a satisfactory correlation relationship (Table 3). The correlation between the sites is (R=0.415).

Table 3

Correlation coefficients of tree-ring chronologies

Site name	BurA
Bur	0,415344

The climate was predicted using trends in air temperature and precipitation. The monthly mean climate data set utilized in this study spans from 1901 to 2023. The trends evident throughout the 20th century demonstrate that, since 1950, the region has experienced a marked increase in winter warming and precipitation. To achieve a higher degree of accuracy in the prediction, the trend calculation was utilized since 1950 (Fig. 4).

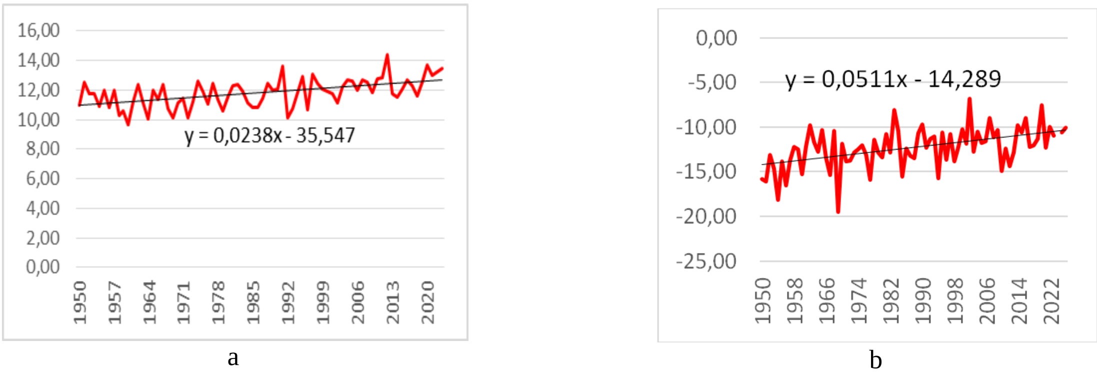
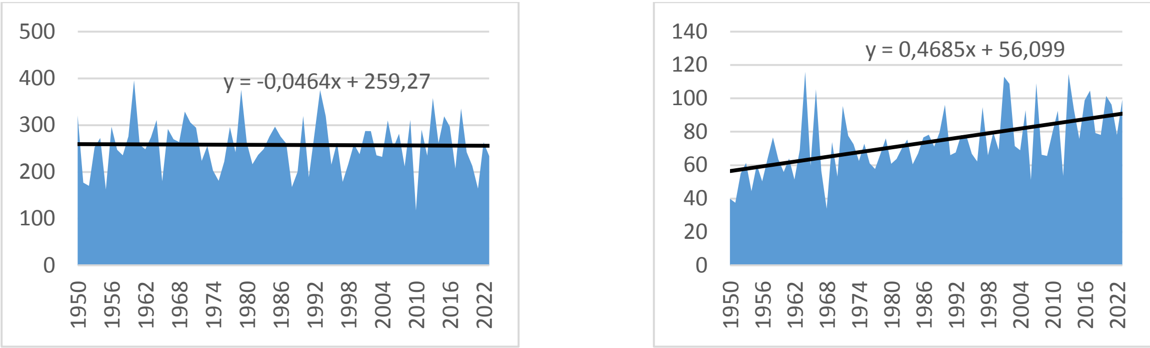


Figure 4. Air temperature trends: a) temperatures of warm months (April-October);
b) temperatures of cold months (November–March)

During the final decade of observation, from 2014 to 2023, the mean temperatures recorded were +12.60 °C and -10.57 °C for the respective warm and cold seasons. Projections indicate that, for the 2024–2033 period, the long-term trends observed during the 20th century are expected to continue, with an anticipated increase in average warm-season temperatures of +12.75 °C and a decrease in average cold-season temperatures of -10.31 °C.

During the final decade of observation, 2014–2023, the mean precipitation levels were recorded as 253.46 mm for the warm season and 90.46 mm for the cold season (Fig. 5).



a b

Figure 5. Precipitation trends: a) precipitation of warm months (April-October);
b) precipitation of cold months (November-March)

Projections for 2024–2033, under the assumption that long-term trends from the 20th century persist, anticipate a modest decline in warm-season precipitation, with an expected average of 253.41 mm. Conversely, there is a projected increase in cold-season rainfall, estimated at an average of 90.93 mm.

Conclusions

A comprehensive analysis of the generalized tree-ring chronologies revealed that a substantial number of trees, with an age exceeding 170–220 years, are present within the designated study area. The first-order autocorrelation values range from 0.68 to 0.78. The highest value is observed for the chronology BurA.

All chronologies exhibit a low sensitivity coefficient, indicative of an absence of a substantial climatic signal. This is further substantiated by the observation of a minimal signal (0.25) at the BurA site. This phenomenon is likely attributable to anthropogenic and biotic factors, as the trees are situated near the village and are susceptible to soil erosion and wind. This fact also confirms the EPS calculation, which indicates the amount suitable for dendrochronological work, including the construction of tree-ring chronologies and comparison with climatic parameters. The core samples obtained from this site exhibited signs of damage, indicating the onset of processes related to decomposition and the softening of plant tissues, collectively referred to as “rot”. These processes were initiated from the core of the trunk.

Statistically significant correlation values between the chronologies (0.415) indicate the presence of a standard climatic signal, as they are located in the same study area. The primary reasons for the negligible correlation between plots may be multifaceted, including the geographical separation between plots, the impact of distinct and identifiable factors, the heterogeneity of the local soil landscape, and the climatic conditions of the habitat (particularly in mountain ecosystems), which regulate the dynamics of tree growth even within the same species and climatic zone [14–16].

Short-term climate projections indicate a marginal increase in temperature and a decrease in warm-season precipitation over the ensuing decade.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Mapitov N.B.** — conceptualization, data curation, investigation,

methodology, editing; **Kassanova A.Zh**. — data curation, formal analysis, supervision; **Ibraeva K.T.** — data curation, methodology; **Yestayeva M.T.** — data curation, methodology; **Daulet Z.K.** — methodology.

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«Бурабай» МҰТП аумағында кәдімгі қарағайдың (*Pinus sylvestris L.*) дендроклиматтық талдауы

Зерттеу солтүстік Қазақстанның орманды даласында, дәлірек айтқанда «Бурабай» Мемлекеттік ұлттық паркі аумағының солтүстік бөлігіндегі құрғақ жағдайда қарағайдың (Pinus sylvestris L.) хронологиясы туралы жаңа деректер алуға бағытталған. Ағаштың өсуіне әртүрлі факторлардың қалай әсер ететінін салыстыру үшін 2 учаске таңдалды. Жалпыланған ағаш-сақина хронологияларын талдау зерттеу аймағында 170-220 жастан асқан көптеген ағаштар өсетінін көрсетті. Бірінші ретті автокорреляция мәндері 0,68-ден 0,78-ге дейін ауытқиды. Сезімталдық коэффициенті шағын климаттық сигналдың болуын көрсетті. Қысқа мерзімді климаттық болжам алдағы 10 жылда температураның шамалы жоғарылауын және жылы мезгілде жауын-шашынның төмендеуі күтілетінін көрсетеді.

Кілт сөздер: Pinus sylvestris L., климаттық болжам, Бурабай, жауын-шашын, ауа температурасы

Н.Б. Мапитов, А.Ж. Касанова, К.Т. Ибраева, М.Т. Естаева, З.Қ. Дәулет

Дендроклиматический анализ сосны обыкновенной (*Pinus sylvestris L.*) на территории ГНПП «Бурабай»

Исследование направлено на получение новых данных хронологий сосны обыкновенной (*Pinus sylvestris L.*) в засушливых условиях в лесостепи северного Казахстана, а именно в северной части на территории ГНПП «Бурабай». Были выбраны 2 участка, чтобы сравнить, как влияют различные факторы на прирост древесины. Анализ обобщенных древесно-кольцевых хронологий показал, что в пределах района исследования произрастает большое количество деревьев возрастом более 170–220 лет. Значения автокорреляции 1-го порядка варьируются от 0,68 до 0,78. Коэффициент чувствительности показал присутствие небольшого климатического сигнала. Краткосрочный прогноз климата показывает, что в ближайшие 10 лет ожидается небольшое повышение температуры и понижение количества осадков теплого сезона.

Ключевые слова: Pinus sylvestris L., прогноз климата, Бурабай, осадки, температура воздуха

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