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УНИВЕРСИТЕТА

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G.B. Admanova<sup>1\*</sup>, Zh.I. Kuanbay<sup>1</sup>, R. Izimova<sup>1</sup>, G.O. Keubassova<sup>1</sup>, L.S. Kozhamzharova<sup>2</sup>

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## Some enzymatic properties of lactic acid bacteria isolated from dairy products

This article presents data on the study of physiological and biochemical properties, antagonistic and enzymatic activity of lactic acid bacteria isolated from dairy products. 9 types of lactic acid bacteria were studied: *Lactobacillus bulgaricus* GM – 08, *Lactobacillus bulgaricus* KZh – 01, *Lactobacillus bulgaricus* GS – 03, *Lactococcus cremoris* – 6, *Lactococcus cremoris* – 17, *Lactococcus cremoris* – 26, *Lactococcus lactis* – 1, *Lactococcus lactis* – 15, *Lactococcus lactis* – 23. These strains were found to have resistance to 2% and 4% -vertical NaCl concentrations, bile and phenol. In addition, the antagonistic activity of Gram-positive and Gram-negative microorganisms in relation to test cultures of *Staphylococcus aureus*, *Salmonella dublin*, *Escherichia coli*, *Bacillus subtilis*, *Sarcina flava* was studied. All studied lactic acid bacteria showed activity in Test cultures with different inhibition zones. The *Lactococcus lactis* – 23 strains showed high activity for all cultures, with an inhibition zone of 17-25 mm. Further, 5 strains were selected from these strains and their aroma-forming properties, the formation of diacetyl and ammonia from arginine, hemolytic and lecithinase activity were studied. Compositions were compiled from these strains to make yeast. The compatibility of strains of lactic acid bacteria was checked with each strain individually and with the duration of milk clotting according to organoleptic indicators compared to the duration of milk clotting. Thus, the most active clot formation was obtained by the *Lactococcus lactis* – 23 strain of the selected combinations.

**Keywords:** microorganisms, strain, antagonistic activity, enzymatic activity, aroma formation, acid formation, yeast, *Lactococcus sgemogis*, *Lactobacillus bulgaricus*.

### Introduction

There is a need for compact and efficient processing of raw materials produced in agriculture for special food purposes in order to maximize the use of the capabilities of Biotechnology in many countries as well as in Kazakhstan. To achieve the goals such tasks were implemented as the importance of metabolites of microorganisms with a wide range of areas of influence on the body and prevention of various diseases is growing in the preparation and implementation of lactic acid products.

Currently, the production of new types of lactic acid products with the maximum use of probiotics and biologically active microorganisms has become the main directions of Biotechnology Science [1, 2].

One of the priority areas in agricultural production is the effective and rational use of the gene pool of microorganisms. In this context, research on the extraction of new strains of microorganisms and the preservation of existing and used strains of microorganisms in the practice of agricultural biotechnology and the creation of biopreparations based on them in the agro-industrial complex is interesting.

A special place in the gene pool of microorganisms is occupied by prokaryotes including lactic acid bacteria [3].

The ability of lactic acid bacteria to form antibiotic substances and to have a bactericidal, bacteriostatic effect on other microflora is widely used in agricultural biotechnology.



When sorting industrial strains of lactic acid bacteria, many of their biological properties are taken into account. Previously, we studied antagonistic activity; pH range capable of increasing strains; resistance to bile, phenol, increased concentration of NaCl, antibiotic resistance; adhesive activity [4, 5].

### *Experimental*

New strains of lactic acid bacteria were isolated from home-made dairy products: kefir, kumys, shubat, cow's and camel's milk. For inoculation and long-term storage of newly extracted lactic acid bacteria, the following nutrient media were used:

1. MRS nutrient medium: yeast autolysate – 5 ml, peptone – 10 g, glucose – 20 g, ammonium citric acid – 2 g, sodium acetic acid – 5 g,  $MgSO_4 \times 7H_2O$  – 200 g,  $MnSO_4 \times H_2O$  – 50 mg,  $K_2PO_4$  – 2 g, twin-1 ml, agar – agar – 20 g, water – 1000 mL, pH medium – 6.2-6.6.

2. Hydrolyzed milk nutrient medium according to Bogdanov: 1 L of boiled, cooled, sterilized milk without fat, the pH medium was 7.4 – 7.6; pancreatin – 1 g, chloroform – 5 ml. The container is tightly closed and placed on the thermostat at 40°C, 72 hours. The resulting hydrolyzes were filtered, the pH medium was set to 7.0-7.2, decontaminated for 10 minutes in 1 atmosphere [6].

3. Hydrolyzed milk containing agar-agar. To prepare agar medium, 2 times water and 2% agar-agar were added to hydrolyzed milk.

4. Wort-Agar: toxic wort – 500 ml, agar-agar – 20 g, water – 1000 ml, ph medium – 6.5-6.7 should be. It is disinfected at 116°C for 30 minutes.

5. The common diagnostic media used by everyone are: ammonia formation medium from arginine, 2% and 4%-vertical NaCl milk hydrolyzate.

Physiological and biochemical properties of lactic acid bacteria resistance to table salt, bile, phenol, ability to form flavoring substances from the composition, acid formation energy, milk clotting time, ammonia formation properties from arginine, diacetyl formation abilities were evaluated according to the generally accepted methods.

By titration with 0.1 N NaOH, the toxicity was checked and measured in Turner degrees.

Pre-decontaminated milk is added at a certain temperature, one of the strains under study, or yeast grown for 18 hours. The milk is simply mixed and kept in the thermostat at a temperature typical of this strain until thick. Later, the paste is left at room temperature for about 1-2 hours, setting the duration of its appearance. Then it was placed to the refrigerator from +3 till +5°C. A day later, during the tasting, an assessment of its taste, smell and consistency, thickening time was made.

The ability of cultures to form flavoring substances from their composition was determined by a qualitative reaction. The property of acetoin formation is established by an alkaline sample. To do this, two drops of the studied cultures are applied to a white porcelain plate, then 40% Kon and 0.04% creatine solutions are added and mixed well. The time is set by tracing until the pink color appears within 20-25 minutes. The volume of formed flavoring substances was measured based on the speed and intensity of color formation.

The ability to form diacetyl was determined by the method of A.G. Grinevich. The test cultures were sprayed on a nutrient agar medium (composition: hydrolyzed milk – 1000 mL, potassium citric acid – 10 g, glucose – 10 g, agar – 25 g, ph medium – 6.8-7.0). Cultures sprinkled with milk hydrolyzate with beveled Agar were kept in a thermostat for 24 hours at a temperature of 30°C, after which a mixture of the following reagents was added: 5 mL of 20%-vertical hydrochloric acid hydroxyl amine and 1 mL of 10%-vertical chlorine nickel. All reagents were prepared before the experiment. The prepared test tubes were placed in a horizontal position on the thermostat for about 2-4 hours. The diacetyl reaction was determined by the formation of red colored crystals of nickel dimethylglyoxima. The amount of formed diacetyl was indicated according to the degree of speed of the dye [7-9].

The antagonistic activity of lactic acid bacteria has been studied due to the diffusion of Agar. Gram-negative bacteria are *Escherichia coli*, *Salmonella dublin*, Gram-positive bacteria are *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina flava* were used as test cultures [10].

### *Results and Discussion*

This paper examines the enzymatic activity of lactic acid bacteria; aroma formation, diacetyl and ammonia formation from arginine, proteolytic, hemolytic and lecithinase activity.

The formation of aroma is the result of the vital activity of a special group of lactic acid bacteria, which is in addition to lactic acid, form diacetyl, acetoin, volatile acids, carbon dioxide, alcohols and esters. Among

these compounds, diacetyl plays a leading role in the formation of aromas. Aromatic lactic acid bacteria are a necessary component of primary yeast for the production of a number of dairy products.

The ability of lactic acid bacteria to proteolysis is widely used in cheese making based on the processes of fermentation of milk components: lactose, protein and fat. In addition, the quality of cheese is greatly influenced by the proteolytic processes present in it, which lead to the formation of flavoring compounds (peptides, free aminoacids, amides, etc.) [8].

In this regard, we studied the properties of lactic acid bacteria, a selection of the most active and compatible strains was carried out.

Previously, we studied some physiological and biochemical properties and antagonistic activity of the studied strains of lactic acid bacteria (Table 1, 2).

Table 1

### Physiological and biochemical properties of lactic acid bacteria

Types of strains	Stability to NaCl		Bile resistance		Resistance to Phenol
	2%	4%	30%	40%	
<i>Lactobacillus bulgaricus</i> GM – 08	+	-	+	-	-
<i>Lactobacillus bulgaricus</i> KZh – 01	+	-	+	-	-
<i>Lactobacillus bulgaricus</i> GS – 03	+	+-	+	+	+-
<i>Lactococcus cremoris</i> - 6	+	-	+-	-	-
<i>Lactococcus cremoris</i> - 17	+-	-	+	+-	-
<i>Lactococcus cremoris</i> – 26	+	-	+	+	+-
<i>Lactococcus lactis</i> – 1	+	-	+	-	-
<i>Lactococcus lactis</i> – 15	+	+-	+	+-	-
<i>Lactococcus lactis</i> – 23	+	+	+	+	+

Note: "+" – the presence of growth; "-" – the absence of growth; "+ -" – weak growth

Table 2

### Antagonistic activity of lactic acid bacteria

Types of microorganisms	<i>Lactobacillus bulgaricus</i> GM – 08	<i>Lactobacillus bulgaricus</i> KZh-01	<i>Lactobacillus bulgaricus</i> GS – 03	<i>Lactococcus cremoris</i> - 6	<i>Lactococcus cremoris</i> - 17	<i>Lactococcus cremoris</i> - 26	<i>Lactococcus lactis</i> – 1	<i>Lactococcus lactis</i> – 15	<i>Lactococcus lactis</i> – 23
<i>Staphylococcus aureus</i>	19	21	11	13	13	15	13	11	23
<i>Salmonella dublin</i>	21	13	11	7	0	19	0	7	19
<i>Escherichia coli</i>	19	17	21	3	0	17	0	11	17
<i>Bacillus subtilis</i>	21	13	19	9	9	21	13	9	25
<i>Sarcina flava</i>	17	19	17	7	3	19	0	0	21

Note: numbers – test-areas of inhibition of the growth of microorganisms (mm)

As a result of the study, the following active strains were selected to determine enzymatic activity based on these physiological and biochemical properties: *Lactobacillus bulgaricus* – strain 3, *Lactococcus lactis* – strain 1, *Lactococcus cremoris* – strain 1.

From Table 2, it can be seen that the study significantly inhibited the growth of *Lactococcus cremoris* – 26, *Bacillus subtilis*, *Sarcina flava*, *Salmonella dublin*, and less inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*. Strains of *Lactococcus cremoris* – 17, *Lactococcus lactis* – 1 showed little activity on all test cultures (3-13 mm). The *Lactococcus lactis* – 23 strain has a high inhibition zone for all cultures (17-25 mm). In addition, the activity range of strains of *Lactobacillus bulgaricus* GM – 08, *Lactobacillus bulgaricus* KZh-01, and *Lactobacillus bulgaricus* GS – 03 was around 11-21 mm.

Further, 5 strains selected from these lactic acid bacteria are examined for flavoring properties, the formation of diacetyl and ammonia from arginine, hemolytic and lecithinase activity, and the indicators are given in Table 3.

Table 3

Enzymatic activity of lactic acid bacteria

Enzymatic properties	Strains				
	<i>Lactobacillus bulgaricus</i> GM – 08	<i>Lactobacillus bulgaricus</i> GS – 03	<i>Lactobacillus bulgaricus</i> KZh – 01	<i>Lactococcus lactis</i> – 23	<i>Lactococcus cremoris</i> – 26
Diacetyl formation	+	+	+	+	+
Aroma formation	+-	+	+	+	+
Ammonia formation from arginine	-	-	+-	+	-
Hemolytic activity	+	-	-	+	-
Lecithinase activity	-	-	-	-	-
Proteolytic activity	+	+	-	+	-

As a result of the study, it was found that all strains of aromatic substances, diacetyl and two strains of *Lactobacillus bulgaricus* CJ – 01 and *Lactococcus lactis* – 23 form ammonia from arginine; hemolytic activity was shown by *Lactobacillus bulgaricus* GM – 08 and *Lactococcus lactis* – 23; no single strain showed lecithinase activity.

Hemolysis can be of three types: B – hemolysis (a transparent, colorless area is formed around the colonies, the width of which depends on the hemolytic activity of the microorganism); B–hemolysis (a greenish area is formed around the colonies due to the formation of methemoglobin from the hemoglobin of partially lysed red blood cells) and  $\gamma$ -hemolysis (near the colony there is a narrow cloudy area of partial hemolysis, and then a transparent area of full hemolysis) [11, 12].

To make yeast, it is necessary to study the compatibility of strains. For this, compositions of different strains were selected:

Option 1: *Lactobacillus bulgaricus* GM-08 + *Lactobacillus bulgaricus* KZh-01 + *Lactobacillus bulgaricus* GS-03;

Option 2: *Lactococcus cremoris* – 26+ *Lactococcus lactis* – 23;

Option 3: each strain is separate, it is part of the above compositions.

The compatibility of strains of lactic acid bacteria is checked with each strain individually and with the duration of milk clotting according to organoleptic indicators compared to the duration of milk clotting. When yeast is selected, strains with similar acid formation activity are combined.

Combinations are selected that coagulate milk at the level of the most active strain or even faster, as well as with a good sour-milk taste and aroma [9].

As a result, the following indicators were obtained:

1<sup>st</sup> composition: sour milk taste, pleasant, aromatic, without external taste, dense in consistency, milky in color, smooth throughout the mass.

2<sup>nd</sup> composition: the taste is delicious, without external taste, fragrant, the consistency is compacting, with small grains.

3<sup>rd</sup> composition:

3.1. *Lactobacillus bulgaricus* GM-08 – the taste is pleasant, aromatic, without external taste, the consistency is dense, the color is milky, smooth throughout the mass;

3.2. *Lactobacillus bulgaricus* GS-03 – the taste is sour-milky, pleasant, the consistency is dense, the color is milky, with small grains;

3.4. *Lactococcus lactis*-23 – the taste is sour milk and pleasant, the consistency is viscous, the color is caramelized;

3.5. *Lactococcus cremoris* – 26 is fermented milk with a bitter taste, dense in consistency with small granules, milky in color.

As follows from Table 4, as a result of the study, it was found that the selected combinations have a high activity of acid formation and clot formation at the level of the most active *Lactococcus lactis* – 23 strain (Table 4).

Acid formation and clotting activity of lactic acid bacteria

Properties	Types of strains						
	<i>Lactobacillus bulgaricus</i> GM - 08	<i>Lactobacillus bulgaricus</i> GS - 03	<i>Lactobacillus bulgaricus</i> KZh - 01	<i>Lactococcus cremoris</i> - 26	<i>Lactococcus lactis</i> - 23	1 composition	2 composition
Formation of clots (hours)	6	6,5	8	9	5,5	5,5	6
Acid formation °T	230	280	265	200	280	280	220

### Conclusion

Worldwide, the use of dairy and sour milk products has increased significantly in recent years. These are often used for the treatment of various diseases, for therapeutic purposes. This is primarily due to the fact that enzymatic processes in milk, which have long been known, occur with the help of microorganisms, which are characteristic for a long time. Modern technology has its own specifics for this purpose, using dairy microorganisms. This makes it possible to carry out fermentation work which has its own specifics under certain agreed conditions. As a result, it opened the way for the preparation of fermented milk products with very high nutritional quality, physical and chemical, sanitary and healing properties. And lactic acid bacteria significantly affect the absorption of such food in the body [13].

The physiological, biochemical properties of isolated lactic acid bacteria, including acid-forming, antagonistic activity and enzymatic activity were studied. Lactic acid bacteria were not the same in that they showed antagonistic activity to the test cultures tested, depending on the cultivation conditions. When grown in the environment of MRS, their suppression of cultures of lactococci and lactobacilli was of the highest degree. In particular, the *Lactococcus lactis* - 23 strain showed test culture with a inhibition zone of 17-25 mm.

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### Сүт өнімдерінен бөлініп алынған сүтқышқылы бактерияларының кейбір ферментативті қасиеттері

Мақалада сүт өнімдерінен бөлініп алынған сүт қышқылы бактериялардың физиологиялық және биохимиялық қасиеттері, антагонистік және ферментативтік белсенділіктері зерттелген мәліметтер көрсетілген. Зерттеуге 9 түрлі сүт қышқылы бактериялары алынды. Олар: *Lactobacillus bulgaricus* ГМ — 08, *Lactobacillus bulgaricus* КЖ — 01, *Lactobacillus bulgaricus* ГС — 03, *Lactococcus cremoris* — 6, *Lactococcus cremoris* — 17, *Lactococcus cremoris* — 26, *Lactococcus lactis* — 1, *Lactococcus lactis* — 15, *Lactococcus lactis* — 23. Осы штамдардың 2% және 4%-дық NaCl концентрациясына, өтке және фенолға төзімділігі анықталды. Сонымен қатар, грамаң және грамтеріс микроорганизмдердің *Staphylococcus aureus*, *Salmonella dublin*, *Escherichia coli*, *Bacillus subtilis*, *Sarcina flava* өсіндісінің сынамаға қатысты антагонистік белсенділігі зерттелді. Зерттеліп отырған барлық сүт қышқылы бактерияларының сынама-өсінділері тежелу аймағында әртүрлі мөлшерде белсенділік танытты. Соның ішінде *Lactococcus lactis* — 23 штамы барлық өсіндіде жоғары белсенділік көрсетті, тежелу аймағы 17-25 мм. Ары қарай осы штамдардың ішінен 5 штамм таңдалып, олардың хош иіс түзу қасиеттері, аргининнен диацетил мен аммиактың түзілуі, гемолитикалық және лецитиназдық белсенділігі зерттелді. Ашытқы жасау үшін осы штамдардан композициялар құрастырылды. Сүт қышқылы бактериялары штамдарының үйлесімділігі сүттің ұю ұзақтығымен салыстырғанда әрбір штаммен жеке-жеке және органолептикалық көрсеткіштер бойынша сүттің ұю ұзақтығымен тексерілді. Осылайша, таңдалған комбинациялардың ішінен ең белсенді ұйытқы түзуге *Lactococcus lactis* — 23 штамы ие болды.

*Кілт сөздер:* микроорганизмдер, штамм, антагонистік белсенділік, ферментативтік белсенділік, хош иіс түзу, қышқыл түзу, ашытқы, *Lactococcus cremoris*, *Lactobacillus bulgaricus*.

Г.Б. Адманова, Ж.И. Куанбай, Р. Изимова, Г.О. Кеубасова, Л.С. Кожамжарова

### Некоторые ферментативные свойства молочнокислых бактерий, выделенных из молочных продуктов

В статье представлены данные об изучении физиологических и биохимических свойств, антагонистической и ферментативной активности молочнокислых бактерий, выделяемых из молочных продуктов. Для исследования были взяты 9 различных молочнокислых бактерий: *Lactobacillus bulgaricus* ГМ-08, *Lactobacillus bulgaricus* КЖ-01, *Lactobacillus bulgaricus* ГС-03, *Lactococcus cremoris* — 6, *Lactococcus cremoris* — 17, *Lactococcus cremoris* — 26, *Lactococcus lactis* — 1, *Lactococcus lactis* — 15, *Lactococcus lactis* — 23. Установлено, что эти штаммы устойчивы ко 2 и 4%-ной концентрациям NaCl, желчи и фенолу. Кроме того, изучена антагонистическая активность грамположительных и грамотрицательных микроорганизмов в отношении тестовых культур *Staphylococcus aureus*, *Salmonella dublin*, *Escherichia coli*, *Bacillus subtilis*, *Sarcina flava*. Все исследуемые молочнокислые бактерии проявляли активность в различных количествах в зоне ингибирования тест-культур. Среди них *штамм 23* показал высокую активность на всех культурах, зона роста 17–25 мм. В дальнейшем из этих штаммов было выбрано 5, изучены их ароматические свойства, образование диацетила и аммиака из аргинина, гемолитическая и лецитиназная активность, а также были составлены композиции для изготовления заквасок. Сочетаемость штаммов молочнокислых бактерий была проверена по продолжительности свертывания молока по сравнению с продолжительностью свертывания молока каждым штаммом в отдельности и по органолептическим показателям. Таким образом, из выбранных комбинаций наиболее активным образованием закваски обладал штамм *Lactococcus lactis* — 23.

*Ключевые слова:* микроорганизмы, штамм, антагонистическая активность, ферментативная активность, ароматообразование, кислотообразование, закваска, *Lactococcus cremoris*, *Lactobacillus bulgaricus*.

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## Enhancing Pathogen Detection Methods through a Novel Molecular Diagnostic Approach with CRISPR/Cas Technology

Molecular diagnostics is widely recognized as one of the most efficient approaches for detecting and characterizing microorganisms. This method relies on the identification of specific nucleic acid sequences and enables the quick and precise determination of pathogen presence in various samples. Conventional methods for pathogen detection rely on the culture and identification of the pathogen in a laboratory setting, which can be time-consuming and expensive. Molecular diagnostic methods, such as PCR and DNA sequencing, have emerged as powerful alternatives to culture-based methods, offering greater sensitivity and specificity. However, these methods are still limited by their reliance on costly equipment and specialized expertise. In recent years, the CRISPR/Cas technology has emerged as a powerful tool for genome editing and manipulation, as well as for molecular diagnostics. This review presents a novel approach for improving pathogen detection methods through the utilization of CRISPR/Cas technology. The proposed method offers several distinct advantages over existing molecular diagnostic techniques. Notably, it provides enhanced specificity and accuracy, thereby minimizing the occurrence of false-positive results. Additionally, this method can rapidly and effectively detect pathogens, making it particularly attractive for use in clinical and laboratory settings. Therefore, molecular diagnostics using CRISPR diagnostics based on Cas12a is a powerful tool for pathogen detection and improving the accuracy and speed of diagnosis. Its prospects for future use are wide-ranging and may encompass many areas of life sciences.

*Keywords:* CRISPR, Cas12a, diagnostics, bacteria, viruses.

### Introduction

CRISPR-based diagnostics: CRISPR (short palindromic repeats regularly spaced in clusters) is a revolutionary technology that allows precise editing of DNA sequences. In recent times, scientists have made significant progress in the development of CRISPR-based diagnostic methods that enable rapid and specific detection of particular nucleic acid sequences within a sample. These methods are very accurate and can potentially be very cost effective.

### CRISPR/Cas

CRISPR/Cas is an innate adaptive immune system employed by prokaryotes, including bacteria and archaea, as a defense mechanism against viral and plasmid invasions. Upon initial infection by a virus, a small segment of the viral genetic material is integrated into the genome of the bacterium. Subsequently, if the same bacterium is exposed to the same virus again, it transcribes these integrated viral sequences into CRISPR RNA. The CRISPR RNA then combines with tracrRNA and a CRISPR-associated (Cas) protein, forming a complex. This complex recognizes and binds to complementary sequences present in the invading viral DNA or RNA. By doing so, it initiates the cleavage of the viral DNA or RNA, creating double-strand breaks (DSBs). The introduction of DSBs ultimately leads to the destruction of the virus, effectively neutralizing the viral infection.

CRISPR-Cas systems function through a series of three main steps: adaptation, expression, and interference. Adjacent to the CRISPR array are genes that encode Cas proteins responsible for governing these phases of immunity: adaptation, CRISPR RNA (crRNA) biogenesis, and interference. During adaptation, foreign genetic material is selected, processed, and integrated into the CRISPR array, creating a memory of the infection. This memory is retrieved when the CRISPR array is transcribed, resulting in the production of crRNA which is further processed within the repetitive sequences to generate mature crRNA molecules. In the event of subsequent infection, the interference mechanism is activated, wherein the crRNA guides Cas

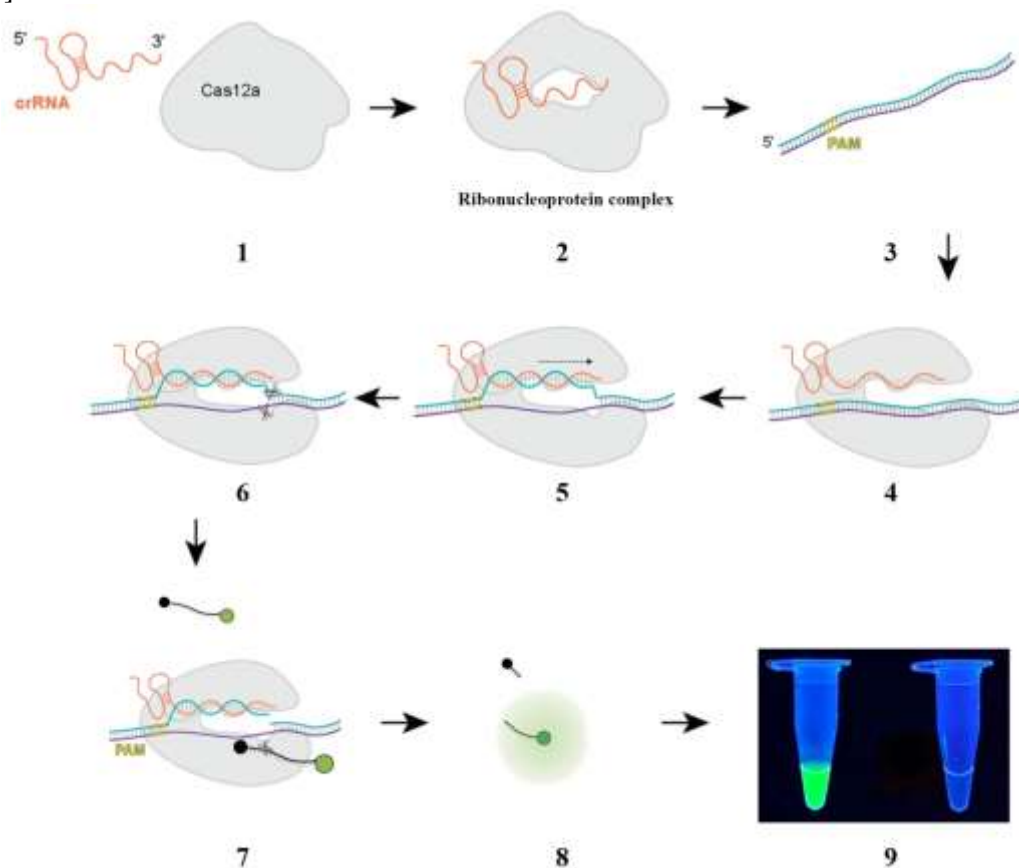


proteins to cleave complementary sequences known as protospacers within the foreign genetic material, effectively neutralizing the invader.

With the help of modern bioinformatics algorithms, in a fairly short time, it was possible to describe and classify the CRISPR-Cas system. The most studied Cas effector is Cas9. Quite quickly, the use of this enzyme led to preclinical studies in genome editing. Doudna and Charpentier received the 2020 Nobel Prize in Chemistry for their significant contributions to the advancement of CRISPR-Cas9 genome editing technology.

In 2015, a new endonuclease Cas12a was discovered [1]. This enzyme has a number of differences in the mechanism of action. First, Cas12a uses only one guide RNA. Second, Cas12a utilizes a specific region known as the protospacer adjacent motif (PAM), which is a TTTN sequence, to bind the target sequence to the guide RNA. The PAM motif is necessary for the CRISPR-Cas system to be able to distinguish its own nucleotide sequence from a foreign one. Third, Cas12a forms sticky-end double-strand hydrolysis of DNA, while Cas9 forms blunt ends. All of the above features of the enzyme's mechanism of action make it an alternative to the well-studied Cas9 in certain cases.

In 2018, it was discovered that Cas12a possesses an additional function that, after binding to the target sequence, the enzyme undergoes conformational changes and begins to cleave any single-stranded DNA [2]. Furthermore, this additional activity exhibited by Cas12a is commonly referred to as collateral or trans-cleavage activity, which will be further used for diagnostic purposes by adding fluorescently labeled single-stranded DNA (reporter with a quencher) to the reaction, the cleavage of which leads to fluorescence. Most homologues exhibit this activity (Fig. 1). Swarts described the mechanism of cis- and trans-cleaving DNase activity [3].



1 – crRNA design; 2 – Cas12a forms a ribonucleoprotein complex with crRNA; 3 – adding a substrate; 4 – Cas12a-crRNA searches for sequences of the PAM region; 5 – Upon recognition of the PAM site, a complex is formed with the target; 6 – Cas12a cleaves the substrate, creating a double-strand break; 7 – Target recognition/cleavage is followed by non-specific reporter cleavage; 8 – Cleavage of the reporter releases the fluorophore; 9 – Fluorescence is detectable with the naked eye

Figure 1. CRISPR/Cas12a detection mechanism

Cas effectors from types V (Cas12a) and VI (Cas13a) can be effectively utilized in the development of biosensor systems. Since their discovery, these enzymes have received immediate attention due to their differences from Cas9. These Cas effectors have the side activity of non-selectively cleaving single-stranded oligonucleotide sequences upon recognition of a specific target. If the activity of the first enzyme is a single turnover, then the trigger activity exhibits a multi-turn mechanism, which is very useful in the development of biosensor systems. Indeed, the Cas enzyme can recognize its target nucleic acid, and then its trigger activity can be used to report this event in an enhanced way.

To enhance the sensitivity of CRISPR diagnostics, pre-amplification methods such as (LAMP) loop-mediated isothermal amplification and (RPA) recombinase polymerase amplification are utilized.

#### ***Loop-mediated isothermal amplification (LAMP)***

Loop-mediated isothermal amplification (LAMP) represents a straightforward and rapid DNA amplification technique, suitable for deployment as a preliminary amplification approach in CRISPR/Cas-centered diagnostic procedures. This approach employs a group of four to six primers designed to precisely focus on numerous segments of the intended DNA sequence, ultimately leading to the precise and heightened amplification of the desired sequence [4].

Loop-mediated isothermal amplification (LAMP) is based on the activity of the Bst DNA polymerase enzyme from *Bacillus stearothermophilus* at 60-65 °C. The formation of specific structures occurs through the transformation into a “stem-loop” structure, facilitated by the use of four pairs of primers (forward outer primer (F3), reverse outer primer (B3), forward inner primer (FIP), and reverse inner primer (BIP)). Subsequently, the “stem-loop” structure, containing multiple initiation sites, serves as the reaction matrix for cyclic amplification in the LAMP process, ultimately generating multiple nucleotide chains of varying lengths.

Utilizing LAMP as a preliminary amplification technique in Cas12a-based diagnostics offers numerous benefits. LAMP stands out as a rapid and uncomplicated procedure, devoid of the necessity for specialized apparatus or specialized knowledge. Furthermore, LAMP exhibits the capability to magnify extremely minute amounts of the intended DNA, underscoring its exceptional sensitivity. Integrating LAMP with Cas12a-based diagnostics has the potential to heighten the assay's sensitivity, thereby diminishing the potential for incorrect negative results [5-9].

#### ***Recombinase polymerase amplification (RPA)***

RPA, or recombinase polymerase amplification, is an innovative DNA amplification technique used to amplify targeted regions of genetic material. This method has been developed to provide high sensitivity and specificity, as well as a fast amplification process, making it suitable for various applications such as infection diagnosis, genetic research and biological research.

The basic idea of RPA is that specific recombinases, such as the UvsX protein from the T4 virus, are used to separate two complementary target DNA sequences. After that, short single-stranded starting sequences, known as primers, are attached to them. Primers have the property of directing recombinases to the target sequence, and then the polymerase enzymes begin to synthesize a new DNA strand using one of the separated strands as a template.

RPA occurs under isothermal conditions, that is, at a constant temperature, and this provides a fast amplification reaction without the need for thermal cycling, which is required for PCR. This method is specific and sensitive, since the use of specific primers allows you to select only those DNA regions that correspond to the desired target sequence. The result is an amplification of only the DNA that is really of interest to the researcher, which significantly increases the efficiency of diagnostics and analysis [10-12].

#### ***CRISPR-based SHERLOK and DETECTR platforms***

Several publications have reported the use of Cas enzymes in combination with various reading strategies such as fluorescent, colorimetric and electrochemical methods. In 2017, Zhang and his collaborators first discovered the side activity of Cas13a and demonstrated its possible use for biosensing through a highly sensitive enzymatic reporter (SHERLOK) system. In this system, a target RNA or DNA gene was amplified and transcribed with RT-RPA + T7 (or RPA-T7) and then recognized with a specific Cas13a/crRNA complex. This triggered side activity and cleavage of the quenched reporter RNA resulting in an increase in fluorescence that was recorded in real time. System sensitivity was similar to digital drop polymerase chain reaction (ddPCR) and quantitative PCR (qPCR). The Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOK) platform is a molecular diagnostics system that leverages the enzymology of CRISPR-Cas to selectively identify specific DNA or RNA targets [13]. Viruses such as Zika, Dengue Fever and African Classical Swine Fever have been detected using SHERLOK.

In 2018, Doudna and her colleagues made a significant discovery regarding the side activity of Cas12a, which they subsequently harnessed for biosensor research. They pioneered the development of the DETECTR (DNA Endonuclease-Targeted CRISPR Trans Reporter) system, utilizing the CRISPR-Cas12a technology (Fig. 2). The Cas12a/crRNA complex was able to detect the target DNA amplified by RPA and the side activity was used to cleave the single-stranded fluorescence quencher DNA reporter [14]. The reaction proceeds quickly (~30 min), the method is inexpensive and accurate for the detection of viral infections as well [2].

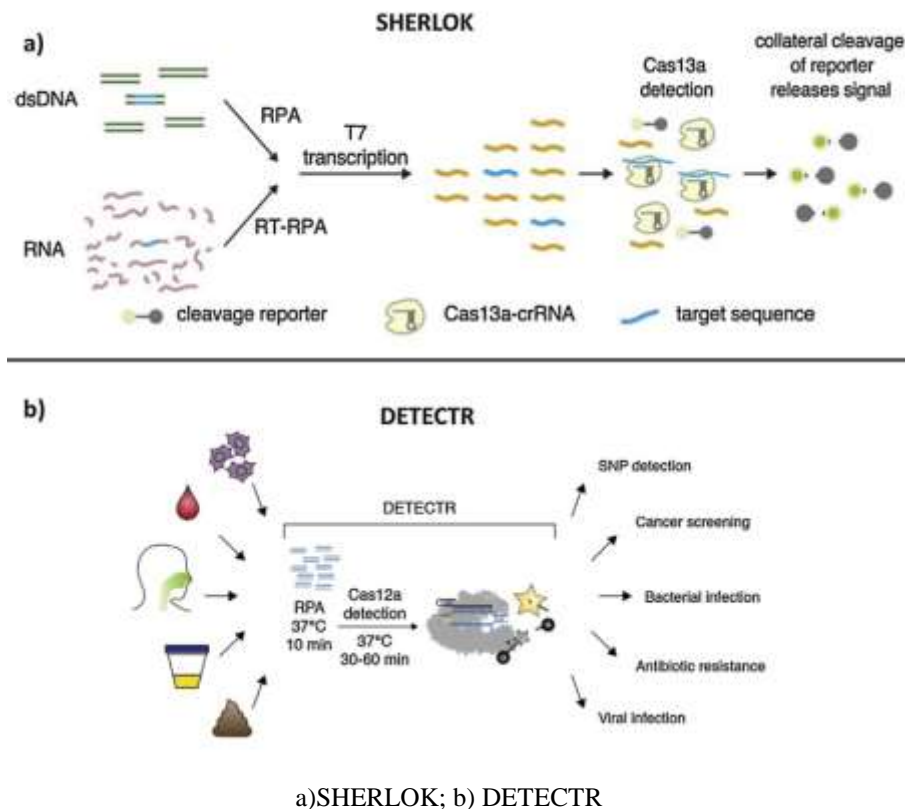


Figure 2. Diagnostic platforms: a) SHERLOK; b) DETECTR [15]

After the discovery of trans-cleavage activity, the scope of Cas12a for diagnostic purposes began to expand rapidly.

#### ***Virus diagnostics based on CRISPR-Cas12a***

The global COVID-19 pandemic has prompted the rapid advancement of cost-effective and efficient diagnostic solutions. A huge number of Cas12a-based methods with different isothermal amplification methods, different targets, different reaction time, detection limit and method of reading the result have been proposed in publications, for example, methods iSCAN, DETECTR, AIOD-CRISPR, CASdetec, CRISPR-FDS, ITP-CRISPR, CRISPR/Cas12a-NER, Lyo-CRISPR, dWS-CRISPR, PGMs-CRISPR, symRNA-Cas12a, VaNGuard, deCOViD, CASCADE, opvCRISPR and CRISPR-ENHANCE.

The attractiveness of methods using isothermal amplification (isothermal loop amplification, recombinase polymerase amplification) is that a portable thermoblock or water bath is enough to perform them.

#### ***Bacterial diagnostics based on CRISPR-Cas12a***

In addition to the detection of viruses, a significant number of publications are devoted to the detection of bacterial pathogens. Publications began to appear on the diagnosis of such bacterial infections as tuberculosis [16] and *Yersinia pestis* [17]. There are also articles on the detection of such important pathogens as *Salmonella typhimurium* (salmonellosis) [18], *Bacillus anthracis* (anthrax) [19], *Francisella tularensis* (tularemia) [10], *Escherichia coli* O157: H7 (acute diarrhea) [8], *Helicobacter pylori* (gastritis, peptic ulcer, stomach cancer) [11].

In addition to determining the pathogen, using CRISPR-Cas12a it is possible to determine the antibiotic resistance of pathogens, which will allow for the correct antibiotic therapy. Articles on the diagnosis of antibiotic resistant *Staphylococcus aureus* (MRSA strain) [20] and *Klebsiella pneumoniae* [9] have been published.

#### ***The implementation of the CRISPR-Cas12a technique in the agriculture***

Researchers and analysts pin hopes that field diagnostics in agriculture will serve as a revolutionary moment in the use of the Cas12a enzyme.

Jiao et al. published results on diagnosing apple tree viruses [21]. Luo et al. described the RPA-Cas12a system for the identification of *Xanthomonas arboricola*, a bacterial pathogen of peach [22]. Fungal diseases of citrus fruits lead to significant yield losses. In a study by Shin et al, an RPA-Cas12a diagnostic for citrus scab was established. A feature of the work is the use of immunochromatographic test strips and the possibility of analysis within 1 hour [12].

In addition to the ability to determine the presence of a pathogen, work is underway to determine GMOs [23]. A highly sensitive fluorescent analysis has been successfully developed for the detection of organophosphate pesticides [24]. The latest research focuses on the development of the platform, which aims to enable early detection of phytopathogens.

Lin et al. have presented a novel method for the detection of pathogenic *Aeromonas hydrophila* using CRISPR technology. In this study, the researchers devised a detection method that is rapid, reliable, sensitive, and could be applied without any specialized equipment. The method includes recombinase amplification and Cas12a technology to identify pathogen. Method exhibits high sensitivity, enabling the rapid detection in less than 1 hour with a LoD of 2 copies of target. Moreover, the method demonstrates excellent specificity [25].

Wang et al used a similar method to accurately identify and detect *Staphylococcus aureus* in clinical specimens [26]. *Staphylococcus aureus* is a significant contributor to hospital-acquired infections. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) result in higher mortality rates compared to those caused by methicillin-susceptible *Staphylococcus aureus*, posing a serious global concern. Consequently, there is a critical need for the prompt and highly sensitive identification of patients with clinical staphylococcal infections, as well as timely implementation of infection control measures. One promising approach lies in utilizing CRISPR and CRISPR-associated proteins (Cas) for nucleic acid detection methods, known for their exceptional diagnostic features. In this regard, a powerful method has been introduced, which combines CRISPR with RPA and employs a fluorescent detection for precise clinical identification of *Staphylococcus aureus* in samples. The results have demonstrated that technology can detect as low as ten copies within a 1-hour time. Furthermore, specificity analyses have confirmed the technology's ability to differentiate *Staphylococcus aureus* from other relevant pathogens in clinical settings. Notably, the results have exhibited strong agreement with antimicrobial susceptibility testing and PCR testing. These findings highlight the technology's exceptional diagnostic parameters, making it an indispensable tool for fast identification of *Staphylococcus aureus*.

Chen et al. conducted a comprehensive review of the current techniques employed for different viruses [27]. The prompt and accurate diagnosis of these viruses is crucial for implementing preventive measures to contain the spread of these diseases. While reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR are established and robust methods, there's a need in specific equipment. In recent years, LAMP and RPA, have emerged as rapid, and equipment-free alternatives for POC diagnostics.

#### ***Detection of foodborne pathogens***

Based on statistics provided by the World Health Organization (WHO), it is estimated that approximately 600 million individuals globally become ill due to the consumption of contaminated food each year. This leads to approximately 420,000 deaths annually, in addition to significant economic losses. These figures highlight the significant impact of foodborne illnesses on public health, underscoring the importance of ensuring food safety and implementing effective preventive measures throughout the food supply chain. Bacterial food and environmental contamination, *Escherichia coli* (*E.coli*), *Listeria monocytogens* (*L. monocytogens*), *Staphylococcus aureus* (*S. aureus*), *Salmonella* species (spp.) [28], poses a constant threat to food safety products, which is a global public health problem. The application of fluorescent sensing technology, utilizing the CRISPR-Cas12a system, has extended beyond disease diagnosis and ventured into the realm of food safety monitoring. This advancement has introduced a novel approach to food safety testing, offering a new strategy in ensuring the quality and safety of food products. By leveraging the CRISPR-Cas12a system

in combination with fluorescent sensing, rapid and accurate detection of potential contaminants or pathogens in food samples can be achieved, thereby enhancing food safety standards and safeguarding public health.

Zhang et al. constructed the corresponding cRNA by selecting the *Vibrio parahaemolyticus* specific heat-labile hemolysin gene as the target sequence, pre-installed the CRISPR-Cas12a system in the cap of the tube, mounted it on a microthermocycler, and then performed PCR amplification. After mixing the reagents, they were subjected to centrifugation and incubation. The minimum concentration achieved by this method was determined to be several copies [29].

Chen et al. developed the CRISPR-Cas12 system for rapid identification of bacterial genotypes in urinary tract infections, which detected concentrations of ampicillin-resistant (AmpR) *E. coli* in urine samples up to  $10^3$  CFU/mL within 1 hour, allowing accurate decisions on antibiotic treatment for 1 hour [30].

### Conclusion

This review provides an up-to-date assessment of the recent advancements in the field of molecular diagnostics for pathogen detection. Infectious diseases are highly prevalent and often result in severe conditions that pose significant risks to human life and well-being. Effective detection methods are critical for accurate diagnosis and timely treatment. Therefore, the development of detection technologies plays a crucial role in achieving rapid and precise pathogen detection. Conventional methods employed for pathogen detection typically involve microbiology, microscopy, enzyme immunoassays, PCR-based detection methods, and others. However, these methods suffer from limitations such as lengthy reaction times and weak sensitivity. Hence, there is a growing necessity to explore and develop new approaches for pathogen detection.

CRISPR diagnostics utilize isothermal amplification methods, such as LAMP and RPA, as initial-amplification techniques for the targets. Compared with conventional PCR, LAMP has the advantages of higher sensitivity, shorter reaction time and easy operation. The RPA reaction temperature ranges from 37°C to 42°C for 5–60 min, depending on the initial concentration of nucleotides. The LAMP reaction temperature is 65°C, the reaction time is 15–60 min. The combination of Cas12a, LAMP and RPA can provide ultra-sensitive nucleic acid detection. Pathogenic microorganisms, mycotoxins and genetically modified crops are key food safety concerns. The development of new detection technologies is essential to achieve and respond to potential food safety. CRISPR-Cas12a, a biosensor analysis technology, has a huge advantage in combating food biosafety agents and needs to be further developed.

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## **CRISPR/Cas технологиясын қолдану арқылы молекулярлық диагностикаға жаңа көзқараспен патогенді анықтау әдістерін жетілдіру**

Молекулярлық диагностика микроорганизмдерді анықтау мен сипаттаудың ең тиімді әдістерінің бірі ретінде кеңінен танылды. Бұл әдіс нуклеин қышқылының белгілі бір реттілігін анықтауға негізделген және әртүрлі үлгілерде патогеннің болуын тез және дәл анықтауға мүмкіндік береді. Патогенді анықтаудың дәстүрлі әдістері зертханада қоздырғышты өсіруге және анықтауға негізделген, бұл уақыт пен шығынды қажет етеді. ПТР және ДНК секвенциясы сияқты молекулярлық диагностикалық әдістер жоғары сезімталдық пен ерекшелікті ұсына отырып, тарату әдістеріне қуатты балама болады. Дегенмен, бұл әдістер қымбат жабдыққа және арнайы білімге тәуелді болғандықтан әлі де шектеулі. Соңғы

жылдары CRISPR/Cas технологиясы геномды өңдеу мен манипуляциялаудың, сондай-ақ молекулалық диагностиканың қуатты құралына айналды. Шолуда CRISPR/Cas технологиясын қолдану арқылы патогенді анықтау әдістерін жақсартудың жаңа тәсілі ұсынылған, бұл әдістің қолданыстағы молекулалық диагностика әдістеріне қарағанда бірнеше айқын артықшылықтары бар. Атап айтқанда, ол жоғары нақтылық пен дәлдікті қамтамасыз етеді, осылайша жалған оң нәтижелердің пайда болуын азайтады. Сонымен қатар, бұл әдіс патогендерді тез және тиімді анықтай алады, бұл оны клиникалық және зертханалық жағдайларда қолдануға ерекше тартымды етеді. Осылайша, Cas12a негізіндегі CRISPR диагностикасын қолданатын молекулалық диагностика патогендерді анықтауға және диагностиканың дәлдігі мен жылдамдығын арттыруға арналған қуатты құрал болып саналады. Оның болашақта пайдалану перспективалары өте кең және өмір туралы ғылымның көптеген салаларын қамтуы мүмкін.

*Кілт сөздер:* CRISPR, Cas12a, диагностика, бактериялар, вирустар, молекулалық диагностика, қоздырғышты анықтау.

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## Совершенствование методов обнаружения патогенов с помощью нового подхода к молекулярной диагностике с использованием технологии CRISPR/Cas

Молекулярная диагностика широко признана одним из наиболее эффективных подходов к обнаружению и характеристике микроорганизмов. Данный метод основан на идентификации специфических последовательностей нуклеиновых кислот и позволяет быстро и точно определить присутствие патогена в различных образцах. Традиционные методы обнаружения возбудителя основаны на культивировании и идентификации возбудителя в лабораторных условиях, что может потребовать много времени и средств. Методы молекулярной диагностики, такие как ПЦР и секвенирование ДНК, стали мощной альтернативой культуральным методам, предлагая более высокую чувствительность и специфичность. Однако они по-прежнему ограничены из-за их зависимости от дорогостоящего оборудования и специальных знаний. В последние годы технология CRISPR/Cas стала мощным инструментом для редактирования и манипулирования геномом, а также молекулярной диагностики. В настоящем обзоре представлен новый подход к совершенствованию методов обнаружения патогенов за счет использования технологии CRISPR/Cas, который имеет ряд явных преимуществ по сравнению с существующими методами молекулярной диагностики. В частности, он обеспечивает повышенную специфичность и точность, тем самым сводя к минимуму возникновение ложноположительных результатов. Кроме того, этот метод может быстро и эффективно выявлять патогены, что делает его особенно привлекательным для использования в клинических и лабораторных условиях. Таким образом, молекулярная диагностика с применением CRISPR-диагностики на основе Cas12a является мощным инструментом для выявления возбудителей и повышения точности и скорости диагностики. Его перспективы для использования весьма обширны и могут охватывать многие области наук о жизни.

*Ключевые слова:* CRISPR, Cas12a, диагностика, бактерии, вирусы, молекулярная диагностика, выявление возбудителей.



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## **Emotional burnout syndrome influence on the functional state of the teacher**

Nowadays, the role of psychosocial work-related factors is increasing both in the production and non-production areas, which are predisposing to the development of psycho-emotional overstrain, professional stress and burnout. Emotional burnout is associated with psychological discomfort of events, tension, anxiety, loss of life meaning and a tendency to suicide. It manifests itself in the deformation of professional development, personality destruction, in a decrease in the duration and quality of life and the productivity of professional activity. As it is known, professional activity, including the professional environment, affects the state and emotions of the worker, causing a change in the assessment of the situation and in the actions of a person in accordance with the requirements of the activity and their capabilities. Socio-economic changes, taking place in society, cause new requirements for specialists in almost all professions, including teachers. Teachers should possess not only professional knowledge, skills, and abilities, moreover they should have special abilities and personality characteristics that provide flexibility and dynamism of professional behavior. High levels of neuropsychic and emotional stress are related to teachers' occupations, and as a result, health problems develop. The issue of teachers developing emotional burnout syndrome is addressed in this article. Teachers who are gradually losing energy show signs of emotional debilitation, personal dispassion, physical fatigue, and a decrease of job satisfaction. The paper also presents a theoretical and practical research of emotional burnout syndrome among teachers, the peculiarities of the presentation and factors of emotional burnout are indicated.

*Keywords:* burnout syndrome, factors, symptoms, professional activity, teachers, stress, prevention.

### *Introduction*

It can have unfavorable impacts on workers that not as it doesn't ameliorate their dignity, but too deplete their mental assets within the situation where the work and proficient environment is ineffectively organized and managed. The emotional burnout disorder can be the one of these unfavorable impacts, which, as a rule, creates among individuals of "helping" professions. Emotional burnout has ended up one of the foremost critical psychosocial work-related dangers in advanced society, producing significant costs for both people and organizations [1-4]. Burnout was once believed to be a trait of caregivers [5], but more recent research has shown that the disorder can affect people in various kinds of professions and groups associated with the workplace. Nonetheless, the value of the burnout index is estimated to a large extent in accordance with the applied definition of burnout [6, 7].

The modern intensification of professional activity, the rise in informational and emotional loads combined with a decline in physical activity, a breach in interpersonal relationships, and a harmonious way of life all lead to emotional burnout. Due to the requirement for ongoing interpersonal contact, there is a number of professions where an individual may start to feel an inner emotional emptiness. Despite the nature of the work performed, social workers fall under a set of professions with a stronger moral responsibility to ensure the prosperity of individuals, groups of individuals, and society as a whole. A social specialist's wellbeing is negatively impacted by ongoing stressful situations that they run into while preparing for complex social interactions with clients, consistent insight into the root of the client's social problems, individual frailty, and other moral and mental considerations [8]. This phenomenon is widespread among specialists and "man-to-man" professional groups, which include the multifunctional education system [9].

In the last two decades, the educational environment has been negatively affected by a huge number of factors. Such changes as the educational modernization, the demographic decay, the move to modern educational standards and others required extra psycho-emotional endeavors from teachers. Modern requirements for the teachers' professional activities are put forward by these variables, which influence their emotional and personal well-being [10].

*Review*

According to the WHO data, a nonliving object of professional action, for instance, does not lead to critical and socially perilous phenomena of professional deformity within the field of “man-technology”, impacting the awareness of the subject. No destructive impact on the psyche of a specialist has been found in either several professions where the subject of activity is an animal. Consideration is centered only on the positive, praising effect on the arrangement of the worldview of the person, the character, and the ethical attitude to nature. Within the teaching profession the situation is quite diverse, as in numerous professions of the socio-economic type: where, firstly, within the process of activity it is essential to require into consideration the independence, uniqueness, uniqueness of each of the subjects of activity. Besides, teachers must have certain professionally vital qualities, which are subject to exceptionally high requirements [11]. The approach and outcome of pedagogical practice are negatively impacted by the emotional burnout disorder that educational specialists are presented with [12]. Hence, the issue of strategies of avoidance, conclusion and psycho-correction of emotional burnout of teachers is acute.

Generally, burnout disorder is a person’s reaction to constant stress at work that creates steadily, it also can inevitably end up persistent, causing changes in wellbeing status of the individual [13]. From a psychological way of thinking, negative behavior toward coworkers, clients, and the professional field itself is evidence that the disorder impacts cognitive, emotional, and attitudinal performance [14]. However, it is a result of certain highlights of work activity, typically not an individual issue [15].

Emotional burnout is considered as a condition and is defined as mental and physical exhaustion due to prolonged emotional stress, and on the other side, as a process and is understood as a professional crisis associated with work in general [16].

The professional work of a teacher is a high emotional load. With an increase in experience, teachers experience a “pedagogical crisis”, “exhaustion”, “burnout”. Some researchers, such as G.S. Abramova, N.A. Aminov, A.K. Markova, A.O. Prokhorov and others claim that as a result of teaching experience increasing, indicators of mental and physical health of teachers began to decline sharply. The maintenance of the educator's psychological wellbeing and his satisfaction with the procedure and work outcomes started to be considered success within the educational field, along with to the efficiency of teaching and the level of children raising [17].

There is no coincidence that teachers are a group of increased risk of realization of emotional burnout. Communicative factors are the primary group of factors that make concrete the emotional burnout of teachers. Building a long and in-depth communication with the subjects of educational relations are included in pedagogical activity. This communication is sincerely wealthy and includes the presence of numerous conflict situations. Experts who have experienced “burned out” use dominance-based interactions with other people and have a propensity to be unable to recognize the demands of the opposing side throughout a conflict [18].

Personal factors are the second group of factors. Teachers who regularly engage in intrapersonal conflict related to their jobs are at greater risk to suffer from emotional burnout syndrome. A conflict between work and family is mentioned most frequently, in which work interferes with family life because of its high demands. As a result of the social evaluation of the outcomes of the teacher's activities, it is also taken into account that one must constantly demonstrate their own competence. Teachers, who exhibit strong introversion and those who have a variety of character accents, especially those who are pedantic and stuck, are the ones who burn out the fastest. This is a result of this group of teachers' rigidity, mental inertia, propensity to experience traumatic events for extended periods of time, excessive persistence of affect, and propensity to form overvalued ideas. Teachers who have a sense of uncertainty in their job prospects and an overwhelming anxiety of quitting their jobs are more probable to experience professional burnout. In our country, teachers over 50 who express persistent anxiety associated with demonstrably tough times finding new jobs if their present positions are lost fall into this category [19].

According to L.V. Novikova's analysis of the psychological factors that contribute to the development of emotional burnout, the locus of control, type A behavior, and preferred problem-solving method are the most important elements. The ability of an individual to assign oneself as well as external factors as the cause of an action is referred to as locus of control. Professional burnout can be avoided by having an internal locus of control, which involves accepting responsibility for the results of one's own actions. Especially facing stress, those who exhibit type A behavior express sentiments of sadness, despair, and disillusionment. Such a response in stressful situations aids in the development of psychophysical burnout. The use of pas-

sive, threatening, or asocial ways to cope in situations of stress makes people more probable to get professionally burned out [20].

Environmental factors are the third group of factors. When a teacher finds himself in a scenario in which he must perform at a high level of efficiency while experiencing constant stress, environmental factors become active. The extended duration of the day, the intensity of the work, the necessity of completing a substantial amount of work in a limited period of time, and the social assessment of performance outcomes should also be noted as environmental factors influencing the development of emotional burnout in teachers [19].

A model of professional stressors for teachers was developed by T.V. Zajchikovoi, S.D. Maksimenka and L.M. Karamushki. Each of the three factors that contribute to the development of professional burnout are combined by these authors into three blocks [21].

1. Socio-psychological and socio-economic prerequisites:

- the stage of social interactions' development and the availability of socio psychological support services;

- the level of education system development;

- socio-economic level of country development.

2. Content of labor:

- workload;

- aspirations of a specialist;

- particulars regarding interactions with coworkers, management, and students;

- compliance of the position of a specialist with his professional level;

- the intensity of the process of professional improvement of the teacher.

3. Working conditions:

- socio-psychological climate in the workplace;

- type of organizational structure;

- type of managerial activity in the organization;

- the specifics of organizational processes.

Extreme emotional exhaustion, depersonalization of the subjects of communication in job-related tasks, loss of motivation for work, as well as an emotional and value attitude toward the profession are all indicators of the emotional burnout syndrome, which develops during the performance of professional activities as a deformation of professional development and the appearance of negative qualities that change the personality [22-25].

The neuro-emotional load of teachers, which has increased as a result of reforming the education system, is associated with the risk of somatic and mental disorders [26-29]. At the same time, psychosocial production factors, especially those related to the organization of work, affect less on physical health but more on mental one [30]. Approximately 60% of teachers have neurotic diseases caused by their work, according to statistics [31]. 60% of teachers demonstrate the signs of burnout syndrome, which include emotional fatigue, depersonalization, and a decrease in emotional intelligence [32, 33].

During analysis of the likelihood of developing a burnout syndrome in teachers, more than half (53%) of teachers are at risk for emotional burnout, according to some research, and a significant number (28.0%) of them demonstrate symptoms [34]. The claim that the teaching profession, regardless of working conditions, is connected with a higher probability of stress and burnout is further supported by comparison with the current status of German teachers. In a research of 100 music teachers, a neuro-psychic adaptation questionnaire revealed a greater incidence of mental discomfort (69.7%), which is a manifestation of the nonspecific asthenic influence of unfavorable labor process elements that increase the level of neuroticism overall [35, 36].

Teachers were observed to engage in passive, asocial, and aggressive kinds of coping behavior more frequently while they are experiencing the more severe condition of the emotional burnout syndrome [37-40]. It is stated that professors only consider students as "carriers of certain grades" or "marks for academic performance" in 72% of situations [41]. 40% of students report feeling unsafe at school. Among the 30% of students who report experiencing violence is violence from teachers [42]. It was discovered that 63% of teachers engage in verbal, 34% in physical, and 15% in concealed aggressiveness [43].

The prevalence of a certain degree of emotional burnout among teachers was also discovered, according to a survey of teachers in Romania from various educational levels and specializations. The results gathered also showed a correlation between personality qualities and the positive or negative nature of changes in

each of the three indicators of the burnout syndrome [44]. In a study of 689 secondary school teachers in Portugal, the levels of occupational stress (40%), burnout (including 10% in the exhaustion measure), and dissatisfaction with work (20%) all increased statistically significantly. A survey of 54 higher education educators in several European nations using questionnaires that included a working environment evaluation, emotional burnout syndrome criteria, and a scale of stressors revealed that Slovenia had a lower level of burnout than other nations [45].

According to a study conducted in India, 86% of university professors displayed a high level of professional burnout and a strong association between burnout and organizational stress [46]. The emotionality of work, emotional tiredness, and job satisfaction were all positively correlated, according to an online survey of US higher education teachers (n=598, which includes 71 teachers, 177 senior lecturers, 168 associate professors and 182 professors) [47]. For Russian teachers of higher education, characteristics of the burnout syndrome can be represented as follows: stress:  $63.37 \pm 0.69\%$ ; resistance:  $73.53 \pm 0.56\%$ ; wasting:  $62.01 \pm 0.42\%$  [48].

Teachers with between 10 and 20 years of experience as well as the ages of 41 and 55 are most probable to demonstrate the most emotional burnout syndrome symptoms in combination. Whenever teachers have more than 10 years of professional experience and are over the age of 30, specific signs such as “reduction of professional duties” and “driven into a cage” begin to demonstrate by themselves. A mid-life crisis could be the cause of the drop-in teachers' professional activities between the ages of 30 and 40. A person takes a first-time look back, assesses the past, reflects on his accomplishments, and summarizes certain results [49].

Thus, the teacher is most impacted negatively by prolonged excessive workload, lack of proper conditions for full strength recovery, social exclusion, strengths and shortcomings, and other factors, which can sometimes completely disrupt his work activity and functional state. One can expect the emergence of a number of pronounced functional as well as mental changes, deterioration in performance, a drop in the quality of work, overload with a long-term combined influence of a complex of such factors. The most characteristic mental state that develops in adverse conditions is stress. This state, complex in nature, has psychophysiological, personal and social aspects of consideration. Researchers also point out that burnout syndrome has detrimental impacts on mental and physical health, the social system, the effectiveness of professional activity, the development of negative attitudes toward coworkers and students, etc. [50].

A teacher's activities and well-being are negatively impacted by emotional burnout, but so are people who are close to them. They include close friends and family members as well as students who are compelled to remain near them and therefore become captives of the syndrome. The activity of the teacher includes the management of the mental development of the student's personality. It can be successfully implemented only by a teacher who is able to consciously regulate the manifestations of his emotions: open to his own and other people's thoughts, feelings, experiences, trusting himself and his students, able to overcome difficulties. In order to prevent the syndrome of emotional burnout, it is advised that: 1) make an effort to strategically calculate and spread your task; 2) develop the ability to switch between activities; 3) handle conflicts on work is simpler; 4) realize that work is just one aspect of life and don't attempt to be the greatest at everything [51].

### *Conclusion*

The issues of preserving the health of teachers, preventing emotional burnout were and are being dealt with many scientists, such as E.V. Kotova, I.F. Yatskovskaya, L.G. Yudina, E.S. Starchenkova, O.I. Babich, N.E. Vodopyanova, S.V. Zhulyeva, S.A. Tinkov, E.V. Tinkova and others. Despite the available scientific and practical approaches to solving this problem, there is still a high probability of its occurrence. Therefore, it is important to timely carry out the prevention of teachers' emotional burnout even before the first signs of occurrence. This paper should include informational education for teachers, knowledge about the signs and factors of emotional burnout, teaching self-regulation techniques and a responsible attitude to one's emotional and physical health, and essentially, to select such types of preventive activities that would help reduce emotional stress, remove signs of mental fatigue, increased efficiency, relieved stress, i.e., perform a compensatory function.

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## Эмоционалды күйзеліс синдромының оқытушылардың функционалдык жағдайына әсері

Қазіргі уақытта өндірістік және өндірістік емес салаларда психоземotionalды стрестің, кәсіби стрестің және күйзелістің дамуына бейім психоэлеуметтік өндірістік факторлардың рөлі артып келеді. Эмоционалды күйзеліс оқиғалардың субъективті ыңғайсыздығымен, шиеленіспен, мазасыздықпен, өмірдің мағынасын жоғалтумен және суицидке бейімділікпен байланысты, кәсіби қалыптасудың деформациясында, тұлғаның бұзылуында, өмір сүру ұзақтығы мен сапасының төмендеуінде және кәсіби қызметтің өнімділігінде көрінеді. Өздеріңіз білетіндей, кәсіби қызмет оның ішінде, кәсіби орта, еңбек субъектісінің жағдайы мен эмоцияларына әсер етеді, жағдайды бағалауда және адамның іс-әрекетінде қызметтің талаптары мен мүмкіндіктеріне сәйкес өзгерісті тудырады. Қоғамда болып жатқан элеуметтік-экономикалық өзгерістер барлық дерлік мамандықтардың, соның ішінде оқытушыларға да жаңа талаптарды тудырды. Оқытушылар тек кәсіби білімді, дағдыларды ғана емес, сонымен қатар кәсіби мінез-құлықтың икемділігі мен динамизмін қамтамасыз ететін арнайы қабілеттер мен жеке қасиеттерге ие болуы керек. Оқытушылардың еңбегі жоғары жүйке-психикалық және эмоционалдык шиеленіспен байланысты, нәтижесінде олардың денсаулық жағдайында ауытқулар пайда болады. Мақалада оқытушылардың эмоционалды күйзеліс синдромын қалыптастыру мәселесі туралы айтылады. Біртіндеп энергияны жоғалту процесі эмоционалды сарқылу, физикалық шаршау, оқытушылардың жеке бөлінуі, жұмыстан қанағаттанудың төмендеуінде көрінеді. Сонымен қатар авторлар оқытушылардың эмоционалды күйзеліс синдромы туралы теориялық және практикалық зерттеулерін ұсынған, эмоционалды күйзелістің көріну ерекшеліктері мен факторларын көрсеткен.

*Кілт сөздер:* эмоционалды күйзеліс синдромы, факторлар, белгілер, кәсіби қызмет, оқытушылар, стресс, алдын-алу.

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## Влияние синдрома эмоционального выгорания на функциональное состояние педагога

В наше время в производственной и в непроизводственной области труда увеличивается роль психосоциальных производственных факторов и условий, предрасполагающих к формированию и развитию психоземotionalного перенапряжения, профессионального стресса, а также выгорания. Эмоциональное выгорание сопряжено вместе с индивидуальной дискомфортом событий, напряженностью, тревожностью, утратой смысла жизни, а также направленностью к суициду, выражается в деформации профессионального становления, деструкция личности, в уменьшении продолжительности качества жизни и продуктивности профессиональной деятельности. Несомненно, что профессиональная деятельность, в том числе профессиональная среда, оказывает большое влияние на состояние и эмоции субъекта труда, вызывает изменение в оценке ситуации и в действиях человека в соответствии с условиями работы, а также собственными способностями. Социально-экономические перемены, происходящие в мире, вызывают новейшие условия к профессионалам почти абсолютно всех специальностей, в том числе и педагогов. Преподаватели обязаны владеть не только профессиональными знаниями, умениями и навыками, а также владеть специальными способностями и свойствами личности, обеспечивающими гибкость и динамизм профессионального действия. Труд педагогов связан с высоким нервно-психическим и эмоциональным напряжением, вследствие чего у них формируются отклонения в состоянии здоровья. В статье говорится о проблеме формирования и развития синдрома эмоционального выгорания педагогов. Процесс постепенной утраты энергии выражается в симптомах эмоционального истощения, физического утомления, личной отстраненности преподавателей, в уменьшении удовлетворенности от работы. Кроме того, авторами показано теоретическое и практическое исследование синдрома эмоционального выгорания педагогов, отмечены характерные черты проявления и факторы эмоционального выгорания.

*Ключевые слова:* синдром эмоционального выгорания, факторы, симптомы, профессиональная деятельность, педагоги, стресс, профилактика.

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## Population study of *Allium altaicum* Pall. in Kazakhstan Altai

The purpose of this work was population studies of *Allium altaicum* Pall. on the territory of the Kazakhstan Altai in 3 territorially isolated geographical regions: Southern, South-Western Kalba Altai. As a result of the survey of the territory by the route-reconnaissance method on the ridges of Sarymsacty, Lineysky and Kalba, a study was made of populations that are characterized by different environmental conditions and altitudinal limits. Ecological and phytocenotic screening revealed that on the Sarymsacty ridge the population is located in the alpine belt in the altitudinal limit of 1870 m a.s.l., on the Lineysky ridge – in the mountain-forest, 1361 m.a.s.l. Both populations are characterized by meso-psychro-petrophytic growing conditions. On the ridge Kalba, the population was examined in the mountain-steppe xero-meso-petrophytic conditions, 832 m a.s.l. Soils are mountain chernozems, slightly acidic, pH 5.6–5.9, with a high humus content from 13.5% to 14.9%, the ground cover is formed, 210–410 g/m<sup>2</sup>. In all populations, the vegetation cover is based on herbaceous perennials; layering is not pronounced. Participation of *A. altaicum* in the formation of phytocenosis on the ridge Sarymsacty is 3.2%, Lineysky – 2.2%; Kalba – 1.8%. The florocomplex of the surveyed populations is formed from 91 species of vascular plants. The similarity of the floristic compositions of the Sarymsacty population in alpine meso-psychro-petrophytic conditions and Lineysky in mountain-forest meso-psychro-petrophytic conditions at the level of 65.0% in relation to the entire cenoflora was established, and Kalba in mountain-steppe xero-meso-petrophytic conditions this figure was 42.1%. Variability of morphometric parameters of aboveground organs of *A. altaicum* in all examined populations has an average and high level of variability from 15.0 to 26.09%, which indicates the ecological plasticity of plants. It has been established that in nature vegetative reproduction occurs only in virginal and generative plants, and nests are formed. The biomorphological parameters of the bulbs in the nest have a clearly expressed age character and vary within the same coenopopulation.

*Keywords:* *Allium altaicum* Pall., biometric parameters, Kazakhstan Altai, population, vegetation cover, floristic composition.

### Introduction

Currently, the conservation of biological diversity is one of the most important problems of the modern plant world. Rare and endangered plant species have less genetic diversity than widespread ones, so they are more susceptible to extinction when environmental conditions change and the influence of the anthropogenic factor [1].

The priority task of modern botany is the development of approaches to the conservation of biodiversity, which require, first of all, a comprehensive study of the population biology of species. The most important criteria for assessing the state of populations of rare species are the morphometric characteristics of individuals and the structure of populations: abundance, age composition, spatial distribution of individuals [2].

One of the species requiring conservation in the flora of Kazakhstan is *Allium altaicum* Pall. (Altai onion, stone, wild batun) is a relic of the Ice Age, which has a mountainous Siberian-North Tien Shan area [3, 4]. In modern flora, it belongs to the family Amaryllidaceae J. St.-Hil, genus *Allium* L., names of which are adopted according to Plants of the World Online [5]. According to ecological and phytocenotic indicators, it is a cryo-xerophyitized psychrophyte, which is part of scree and glacial-morainic plant groups, as well as unformed pioneer phytocenoses from the middle to upper mountain belts [6].

*A. altaicum* is known for many useful properties – food, medicinal, decorative and melliferous. It can be used in landscaping rocky and alpine hills, as well as to create mountain landscapes [7, 8]. It is readily used by the population for food, it is often bred in vegetable gardens called “batun”. Animals eat poorly. Honey plant. The plant produces abundant nectar [9]. At the present stage, the research is also relevant in connection with the species belonging to the wild relatives of cultivated plants [10].

Altai onion attracts the attention of scientists as a source of biologically active substances. The revealed high antioxidant activity, the ability to accumulate selenium allows us to attribute *A. altaicum* to

plants with pronounced antioxidant properties, the species is also in demand for resolving controversial issues in the field of taxonomy and phylogeny of cultivated and wild onions in Kazakhstan [11, 12].

The species needs to be protected, because due to the massive collection as a food plant, it is reducing its numbers everywhere. Currently in Russia and Mongolia it is subject to state protection with category and status 3, in Kazakhstan it is still not officially protected [13-17].

The purpose of this work was population studies of *A. altaicum* on the territory of the Kazakhstan Altai in 3 territorially isolated geographical regions: Southern, South-Western and Kalba Altai.

To achieve this goal, we solved the following tasks: the geographic distribution of the species in the region was identified; Sarymsacty, Lineysky, Kalba, the floristic composition of communities with the participation of *A. altaicum* was studied, morphometric indicators of the aboveground parts of individuals were identified, their variability was determined, some parameters of underground organs were studied depending on the location of populations and the age state of individuals.

### *Experimental*

Population studies were carried out on the territory of the Kazakhstan Altai, the climate of which was formed under the influence of the proximity of the deserts of Mongolia and Central Asia, location in the center of the Eurasian continent, remote from the oceans, as well as under the influence of continental-oceanic air mass transfer [18]. The soils are light chestnut, which constitute the main background, against which the vertical range of soils characteristic of the region is manifested: dark chestnut, steppe chernozems and forest-steppe, gray forest soils, mountain taiga acid soils and mountain meadow soils [19]. Population studies were carried out in the Katon-Karagai, Kurchum, Glubokovsky administrative districts, as well as in the West Altai State Nature Reserve (near the town of Ridder). The route and semi-stationary studies covered the mountainous regions of the Kazakhstan Altai. Habitats of *A. altaicum* in the Southern, Southwestern and Kalba Altai were studied within the natural range.

The study of morphometric and quantitative indicators of individuals in the surveyed populations was carried out according to the methods recommended for the study of rare species [20; 21]. At the same time, micro- and macrorelief, exposure, mechanical composition of soils, total projective cover of vegetation, and floristic composition were taken into account. Due to the uneven distribution of *A. altaicum* individuals, geobotanical sites ranging in size from 10 to 15 m<sup>2</sup> were established. Morphometric and weight indices of aerial parts and bulbs were studied at each site according to the classification of O.V. Smirnova et al. [22], developed on the basis of the generally accepted scheme of T.A. Rabotnov [22]. In the flora and population study, the Latin names of plants are given according to the international platform POWO [5]. When analyzing quantitative indicators, statistical data processing was carried out according to the method of N.L. Udolskaya [23]. The level of variability of biometric indicators was calculated from the value of the coefficient of variation Cv: less than 12% – the level of variability is low, 13-20% – medium, 21-40% – high, more than 40% – very high [24].

### *Results and discussions*

The field work carried out on the territory of the Kazakhstan Altai made it possible to establish specific locations of *A. altaicum*: Southern Altai – the Azutau, Sarymsacty, Narym, South Altai Tarbagatai ridges; Southwestern Altai – Lineysky, Ubinsky, Ivanovsky, Ulbinsky, Listvyaga ridges; Kalba Altai – the eastern periphery of the Kalba ridge.

In the Southern Altai, the population was examined in the Alpine belt under meso-psychro-petrophytic conditions, on the northwestern rocky slope of the ridge Sarymsacty (Fig. 1) in the area of the Burkhat pass, conditionally designated by its location as Sarymsacty. Location coordinates: N 49° 05' 35" E 86° 01' 42", 1870 m a.s.l. The analyzes showed that the soil where the population is located is slightly acidic, pH 5.7, the content of total humus is 14.9%. The ground cover is formed by litter, mainly from ground mosses and lichens, 410 g/m<sup>2</sup>.

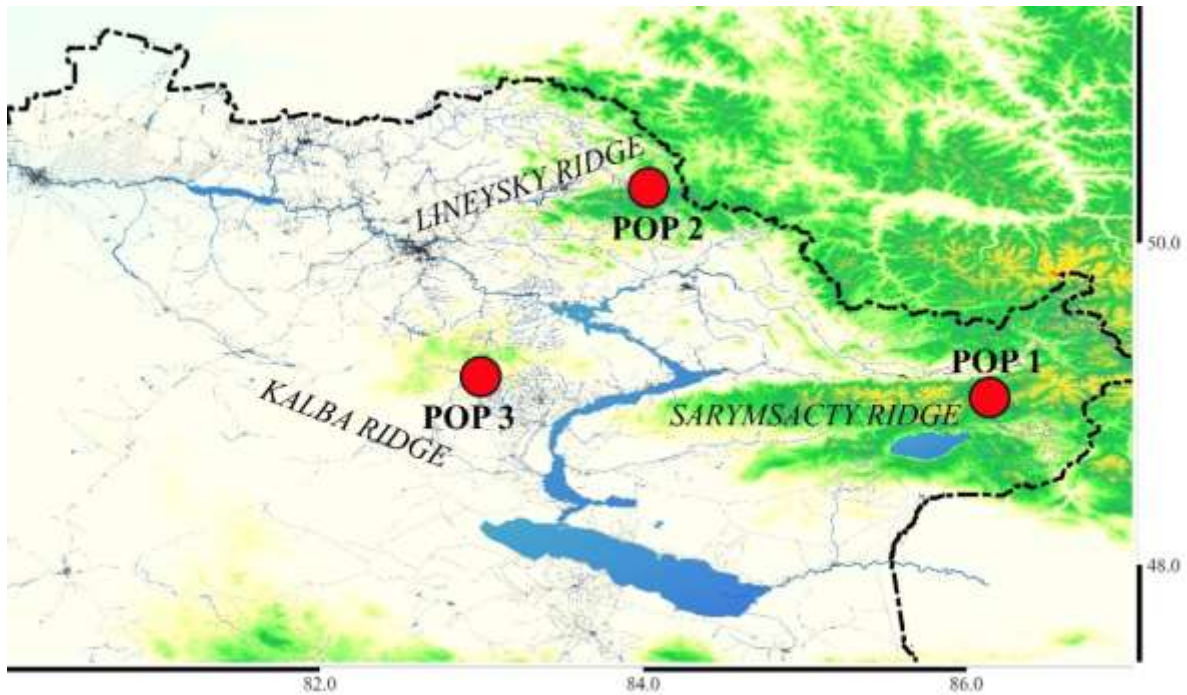


Figure 1. Location of investigated populations of *A. altaicum* in Kazakhstan Altai

The vegetation cover is formed by 66 species of vascular plants with a projective cover 55–85% (Table 1).

Table 1

**Floristic composition of the surveyed populations of *Allium altaicum* in the Kazakhstan Altai**

№	Species name	Population name		
		Sarymsacty	Lineysky	Kalba
1	<i>Abies sibirica</i> Ledeb.	+	+	-
2	<i>Adenophora lilifolia</i> Ledeb.	+	+	+
3	<i>Allium amphibolum</i> Ledeb.	+	+	-
4	<i>A. flavescens</i> Bess.	-	-	+
5	<i>A. flavidum</i> Ledeb.	+	+	-
6	<i>A. schoenoprasum</i> L.	+	+	+
7	<i>A. lineare</i> L.	+	+	-
8	<i>A. nutans</i> L.	+	+	+
9	<i>A. rubens</i> Schrad. ex Wild	+	+	+
10	<i>Aconitum altaicum</i> Steinb.	+	-	-
11	<i>A. anthoroideum</i> DC.	+	+	+
12	<i>Aquilegia glandulosa</i> Fisch. et Link.	+	+	-
13	<i>Amygdalis ledebouriana</i> Schlecht.	-	-	+
14	<i>Artemisia campestris</i> L.	+	-	-
15	<i>Aster alpines</i> L.	+	+	+
16	<i>Anemonastrum narcissiflorum</i> (L.) Holub	+	+	-
17	<i>Clematis sibirica</i> (L.) Mill.L.	+	+	-
18	<i>Bupleurum multinerve</i> DC.	-	+	-
19	<i>Betula pendula</i> Roth	-	-	+
20	<i>Berberis sibirica</i> Pall.	+	+	-
21	<i>Bergenia crassifolia</i> (L.) Fritsch	+	+	-
22	<i>Woodsia ilvensis</i> (L.) R. Br.	+	+	+
23	<i>Calamagrostis epigejos</i> (L.) Roth	-	+	+
24	<i>Campanula glomerata</i> L.	+	+	+

25	<i>Carex rupestris</i> All.	+	+	-
26	<i>C. pediformis</i> C. A. Mey.	+	+	+
27	<i>Cerastium arvense</i> L.	-	+	+
28	<i>Corydalis nobilis</i> (L.) Pers.	-	-	+
29	<i>Cotoneaster uniflorus</i> Bunge	+	+	-
30	<i>C. melanocarpus</i> Fisch. ex Blytt	+	+	+
31	<i>C. oliganthus</i> Pojark.	-	-	+
32	<i>Chamaenerion angustifolium</i> (L.) Scop.	+	+	-
33	<i>Dianthus versicolor</i> Fisch. ex Link	+	+	+
34	<i>Elymus debtatus</i> (Hook.f) Tzvelev subsp. <i>elongatiformis</i>	-	-	+
35	<i>Eremogone Formosa</i> (Fisch. ex Ser.) Fenzl	+	+	-
36	<i>Erythronium sibiricum</i> (Fisch. et Mey.) Kryl.	+	+	-
37	<i>Euphorbia macrorhiza</i> C. A. Mey. ex Ledeb.	-	+	+
38	<i>E. alpine</i> C. A. Mey.	+	+	-
39	<i>Festuca kryloviana</i> Reverd.	+	+	-
40	<i>F. borissii</i> Reverd.	+	+	-
41	<i>Fritillaria verticillata</i> Willd.	-	-	+
42	<i>Galium verum</i> L.	+	+	-
43	<i>G. boreale</i> L.	+	+	-
44	<i>Hedysarum alpinum</i> L.	+	+	-
45	<i>Hieracium umbellatum</i> L.	-	+	+
46	<i>Iris ruthenica</i> Ker Gawl.	+	+	+
47	<i>Larix sibirica</i> Ledeb.	+	+	-
48	<i>Lonicera caerulea</i> subsp. <i>altaica</i> (Pall.) Gladkova	+	+	-
49	<i>Juniperus sibirica</i> Burgsd.	+	+	+
50	<i>J. sabina</i> L.	+	-	+
51	<i>Oxytropis sulphurea</i> (Fisch. ex DC.) Ledeb.	+	+	-
52	<i>Orostachys spinosa</i> (L.) C. A. Mey.	+	-	-
53	<i>Paeonia intermedia</i> subsp. <i>intermedia</i> Pall.	-	-	+
54	<i>Pachypleurum alpinum</i> Ledeb.	+	+	-
55	<i>Patrinia intermedia</i> (Hornem.) Roem. et Schult.	-	-	+
56	<i>P. sibirica</i> (L.) Juss.	+	+	-
57	<i>Poa attenuate</i> Trin.	+	+	+
58	<i>Pedicularis elata</i> Willd.	+	-	-
59	<i>Pentaphylloides fruticosus</i> (L.) O.Schwarz	+	-	-
60	<i>Pinus sylvestris</i> L.	-	+	+
61	<i>P. Sibirica</i> Du Tour	+	+	-
62	<i>Phlomooides alpina</i> Pall. Adjlov. Kamelin et Makhm.	+	+	-
63	<i>Ph. tuberosa</i> (L.) Moench.	-	-	+
64	<i>Polemonium caeruleum</i> L.	+	-	-
65	<i>Bistorta elliptica</i> (Willd. ex Spreng.) Kom.	+	+	-
66	<i>Populus tremula</i> L.	-	-	+
67	<i>P. laurifolia</i> Ledeb.	+	+	+
68	<i>Potentilla argentea</i> L.	+	+	-
69	<i>Ranunculus polyrhizos</i> Steph. ex Willd.	-	-	+
70	<i>Rheum altaicum</i> Losinsk.	+	+	-
71	<i>Rosa spinosissima</i> L.	-	+	+
72	<i>Rhodiola rosea</i> L.	+	+	-
73	<i>R. algida</i> (Ledeb.) Fisch. et Mey.	+	+	-
74	<i>Ribes nigrum</i> L.	-	+	-
75	<i>Rubus sachalinensis</i> H.Lev.	+	+	-
76	<i>Sedum ewersii</i> Ledeb.	+	+	-
77	<i>S. hybridum</i> L.	-	+	+
78	<i>Seseli buchtormense</i> (Fisch.) W.D.J. Koch	+	+	+
79	<i>Silene graminifolia</i> Otth	+	+	-
80	<i>Spiraea chamaedryfolia</i> L.	-	-	+
81	<i>S. trilobata</i> L.	-	-	+

82	<i>S. media</i> Schmidt	+	+	+
83	<i>Solidago dahurica</i> (Kitag.) Kitag.exJuz.	+	+	-
84	<i>Sorbus aucuparia</i> subsp. <i>glabrata</i> (Wimm.et Grab.) Hedl.	+	+	-
85	<i>Thalictrum foetidum</i> L.	+	+	+
86	<i>Thesium repens</i> Ledeb.	+	-	-
87	<i>Thymus serpyllum</i> L.	-	+	+
88	<i>Trifolium lupinaster</i> L.	+	+	-
89	<i>Vaccinium myrtillus</i> L.	+	+	-
90	<i>Veronica spicata</i> L.	-	-	+
91	<i>Viola dissecta</i> Ledeb.	+	-	-
	Total	66	67	42

Its tiering is not expressed. Solitary specimens of *Populus laurifolia* Ledeb., *Pinus sibirica* Du Tour, *Abies sibirica* Ledeb. were noted from trees and *Sorbus aucuparia* subsp. *glabrata* (Wimm. et Grab.) Hedl. The shrub layer is dominated by *Spiraea media* Franz Schmidt – sp.; *Cotoneaster uniflorus* Bunge – sol and *Dasiphora fruticosa* (L.) Rydb – sol, *Juniperus sibirica* Burgsd. – s *Berberis sibirica* Pall. – s. Its occlusion does not exceed 01, with a coverage of no more than 1.0%. The herbaceous cover is represented by species typical of high-mountain regions: *Aquilegia glandulosa* Fisch. et Link. – sp, *Festuca borissii* Reverd. – sp, *Aconitum thoroidum* DC. – sol, *Thalictrum foetidum* L., *Galium boreale* L. – sol, *Allium altaicum* Pall. – sp., *Orostachys spinosa* (L.) C.A. May. – sol, *Campanula glomerata* L. – sol, *A. rubens* Schrad. ex Wild. – sol, *Potentilla argentea* L. – sol, *Patrinia sibirica* (L.) Juss. – sol, etc. Rare species are also found in the herbaceous cover: *Woodsia ilvensis* (L.) R.Br., *Rheum altaicum* Losinsk., *Rhodiola rosea* L., *R. algida* (Ledeb.) Fisch. et Mey., *Hedysarum alpinum* L. Individuals of the Altai onion in the area are diffusely distributed in small spots. Participation share of *A. altaicum* in the formation of phytocenosis was no more than 3.2%.

In the Southwestern Altai (southwestern periphery), the Altai onion population was surveyed in the Western Altai State Nature Reserve in the mountain forest belt under meso-psycho-petrophytic conditions on the northwestern slope of the ridge Lineysky in the area of the Chernoubinsky cordon between the mountain ranges, named by us as Lineysky. Location coordinates: N 50° 23' 08" E 84° 01' 14", 1361 m a.s.l. The total area occupied by the view is no more than 1500 m<sup>2</sup>. Soil layer is medium thickness. The soil is rich in humus – 13.6% with a fraction of sand due to collapsing rocks, slightly acidic, pH 5.8–5.9. The litter is well expressed, 210 g/m<sup>2</sup>. The total projective cover is heterogeneous, depending on the phytocenotic features, it ranges from 7 to 65%. In the places of growth of the Altai onion, areas with a well-developed vegetation cover were identified, in the florocomplex of which up to 67 species of vascular plants were recorded (Table 1). Layering is not expressed. The plot with the Altai onion is surrounded by shrubs *Rosa spinosissima* and *Juniperus sibirica*, *Spiraea media*, *Dasiphora fruticosa* are less common. Single bushes of *Ribes nigrum* are noted, *Abis sibirica*, *Laryx sibirica*, *Pinus sylvestris*, *P. sibirica*, *Sorbus aucuparia* subsp. *glabrata* are noted from the trees. Herbaceous plants are represented by dominants *Chamaenerion angustifolium*, *Aquilegia glandulosa*, *A. rubens*, related species: *Carex pediformis*, *Euphorbia macrorhiza*, *Festuca borissii*, *Poa attenuata*, *Galium verum*, *Solidago gebleri*, *Vaccinium myrtillus*, *Silene graminifolia*. Onions occur sparsely and unevenly over the site. The share of its participation in the formation of phytocenosis was 2.2%.

On the territory of the Kalba Altai, the population of *A. altaicum* was described in mountain-steppe xero-meso-petrophytic conditions in the eastern part of the Kalba Ridge on the northeastern rocky slope. Location coordinates: N 49° 29' 37" E 82° 36' 19", 832 m a.s.l. m. The soil layer is formed only in places where plants grow up to 35 cm, refers to mountain podzolized chernozems, with a total humus content of 13.5%, slightly acidic, pH 5.6-5.8. In the mechanical composition in the horizon from 0 to 25 cm, small cartilages predominate – 34.4% and coarse sand – 35.9%. Altai onions grow locally along the cracks of granite outcrops filled with soil or among sparse thickets of shrubs – *Spiraea trilobata* L., *Cotoneaster oliganthus* Pojark., forming small micropopulations. Vegetation cover is poorly developed, represented by forb groups. There are 42 plant species here (Table 1). The average projective cover is no more than 20%, the share of Altai onion in the formation of phytocenosis is at the level of 1.8%. The phytobiota of the population is dominated by *Allium nutans* – sp, *A. rubens* – sp. *Thymus serpyllum* – sol. In addition to the dominant species in the group, *Elymus nevskii* Tzvel., *Allium flavescens* Bess., *Paeonia hybrida* Pall., *Corydalis nobilis* (L.) Pers., *Ranunculus polyrhizos* Steph., *Phlomidoides tuberosa* L. are noted. Rare specimens are found in the herbage of *Patrinia intermedia* (Hornem.) Roem. et Schult., *Fritillaria verticillata* Willd., from shrubs – a



relic species of Ledebour almond. A moss-lichen cover is developed on the rocks. Among the trees there are singly growing *Pinus sylvestris* L., *Populus laurifolia*, *Betula pendula* Roth.

91 species of vascular plants are involved in the formation of the coenoflora of the surveyed populations of *A. altaicum* on the Sarymsacty, Lineysky and Kalba ridges. At the same time, the similarity of the floristic compositions of the Sarymsacty population in alpine meso-psychro-petrophytic conditions and the Lineysky population in mountain-forest meso-psychro-petrophytic conditions at the level of 65.0% in relation to the entire coenoflora was established, and the Kalba population in the mountain-steppe xero-meso-under petrophytic conditions, this figure was 42.1%.

Given that important indicators in population studies include morphometric parameters of individuals, measurements of the aboveground organs of *A. altaicum* were carried out with the determination of their variation in the examined 3 populations (Table 2).

Table 2

**Indicators of morphometric parameters of *Allium altaicum* in the nature of the Kazakhstan Altai (Sarymsacty, Lineysky, Kalba populations)**

Indicators	Population name					
	Sarymsacty		Lineysky		Kalba	
	M±m	C <sub>v</sub> , %	M±m	C <sub>v</sub> , %	M±m	C <sub>v</sub> , %
Height of generative shoots, cm	58.33±3.91	21.52	49.08±2.2	15.0	50.73±2.00	23.01
Leaf length, cm	38.73±2.18	17.82	34.23±2.18	14.82	32.33±2.2	18.80
Leaf width at the base, cm	2.51±0.29	21.09	2.46±0.22	26.09	2.03±0.14	21.97
Inflorescence diameter, cm	2.77±0.78	4.6	3.73±2.18	5.9	3.59±0.68	4.3
Seed length, mm	3.26±0.28	8.7	2.9±0.13	4.4	3.30±0.3	9.1
Seed width, mm	1.9±0.17	8.9	1.9±0.12	6.3	2.75±0.04	10.5

The study of average metric values showed that the length and width of seeds have a low level of variability in all populations, regardless of origin. The variability of such indicators as plant height, leaf length and width, as well as the diameter of the inflorescence have a medium and high level of variability, which indicates the ecological plasticity of *A. altaicum* individuals in the surveyed populations and indicates the prospects for their use in the breeding process.

Due to the ability to reproduce vegetatively, the Altai onion forms nests in the Kazakhstan Altai. They consist of a different number of bulbs depending on the age condition, conditionally ranked in the course of research into four groups of nests: young forming a nest, a formed nest, an aging nest and a senile (old nest). Schematic representations of the formed nests of the Altai onion on the ridge. Sarymsacty in alpine conditions, Lineysky ridge – in the mountain-forest and ridge Kalba – in the mountain-steppe are shown in Figures 2-4. An analysis of morphometric and counting indicators revealed similar and distinctive features for each age state of the nest in the surveyed populations, a brief description of which is given below.

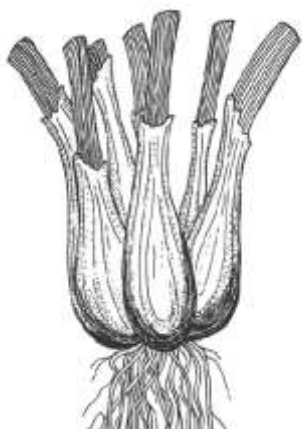


Figure 2. Formed nest of *Allium altaicum* on the Sarymsacty ridge in alpine conditions, 1760 m a.s.l.



Figure 3. The formed nest of *Allium altaicum* on the Lineysky ridge in mountain-forest conditions, 1361 m a.s.l.



Figure 4. Formed nest of *Allium altaicum* on the Kalba ridge in mountain-steppe conditions, 832 m a.s.l.

✓ The young developing nest is dense, compact, consists mainly of adult vegetative shoots, young generative ones are represented singly. The bulbs in the nest are tightly pressed to each other, there are no dead remains.

✓ The formed nest consists mainly of generative shoots, vegetative shoots are found in separate specimens. In this age state, particulation of the nest begins, which leads to loosening and formation of the central part. The amount of dead remains increases.

✓ An aging nest. It consists mainly of adult shoots that are in a vegetative state, generative ones are found singly. Dead remains accumulate in large quantities; the central part is clearly expressed. An aging nest is characterized by a cessation of weight gain, which is easily established by the ratio of the sizes of cover scales and bulbs, which often predominate in volume over living ones. Rhizomes and roots of other species easily penetrate into an aging nest. The aging of vegetative shoots occurs simultaneously with the aging of the clone; there is no increase in the number of shoots.

✓ Senile (old) nest. Represented only by vegetative underdeveloped shoots. The central part of the nest is empty, filled with dead remains of bulbs. No growth.

According to scientific publications, the potential ability for vegetative propagation in Altai onion already exists in immature plants, but according to our observations, vegetative propagation occurs only in virginal and generative plants.

Based on the analysis of the results of the biometric parameters of the bulbs, it was found that their morphometric and weight indicators are individual, depending on the age state of the nest and the ecological and phytocenotic conditions for the growth of individuals (Table 3).

Table 3

**Biometric parameters of *Allium altaicum* bulbs depending on the age state of the nest**

Origin of material	Age condition of the nest	Indicators of bulbs in the nest			
		Quantity, pcs	Weight, g	Height, cm	Diameter, cm
Southern Altai, Sarymsacty ridge, 1760 m a.s.l., alpine sample	young emerging	3.70±0.2	12.6±0.7	7.06±0.3	3.08±0.05
	formed	5.80±3.10	13.1±0.3	10.16±0.03	3.08±0.05
	aging	8.00±2.50	10.44±1.12	6.10±0.77	3.4±0.03
	senile	5.62±0.67	7.82±2.41	5.34±1.65	2.24±0.75
Southwestern Altai, Lineysky ridge, 1361 m a.s.l., mountain-forest sample	young emerging	5.00±0.2	16.9±0.75	10.0±0.83	3.5±0.08
	formed	8.50±4.42	16.3±1.83	10.30±0.83	4.25±0.23
	aging	18.50±6.50	12.85±0.67	6.4±2.18	3.79±0.21
	senile	8.5±4.42	7.38±1.83	5.87±2.53	2.87±2.13
Kalba Altai, Kalba ridge, 832 m a.s.l., mountain-steppe sample	young emerging	6.50±0.25	10.6 ± 0.6	9.25±0.72	1.7±0.04
	formed	12.33±5.67	12.7±1.9	9.4±0.72	1.8±0.09
	aging	25.63±7.37	8.1±0.87	5.3±0.21	2.1±0.13
	senile	8.62±0.63	5.82±2.41	4.84±1.65	1.84±0.25

For use as a donor plant for introduction into culture, *A. altaicum* individuals in the population on the Lineysky Ridge, characterized by large bulbs in weight, height and diameter, are promising. This genetic potential can be recommended for hybridization, development of new varieties and improvement of existing ones.

### Conclusion

The growth of *A. altaicum* in the Kazakhstan Altai on the Sarymsacty, Lineysky and Kalba ridges is established in a wide range of altitudes in the mountains from 832 to 1870 m above sea level and growing conditions from mountain-steppe to alpine. In phytocenotic terms, the vegetation cover is formed heterogeneously with a projective cover from 20 to 85%. Altai onion occurs sparsely and unevenly over the site. The share of its participation in the formation of phytocenosis ranged from 1.8 to 3.2%. The flora of *A. altaicum* populations on the Sarymsacty, Lineysky and Kalba ridges is formed by 91 species of vascular plants. At the same time, the similarity of the floristic compositions of the Sarymsacty population in alpine meso-psychro-petrophytic conditions and the Lineysky population in mountain-forest meso-psychro-petrophytic conditions at the level of 65.0% in relation to the entire cenoflora was established, and the Kalba population in the mountain-steppe xero-meso- under petrophytic conditions, this figure was 42.1%.

Depending on the geographical location of the populations in the region and the ecological conditions of existence, individuals of *A. altaicum* in the surveyed populations are characterized by individual variant and biomorphological features of the ground mass and bulbs, among which the genetic potential of the Altai onion in the mountain forest conditions of the Southwestern Altai is promising for breeding.

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## Қазақстандық Алтайдағы *Allium altaicum* Pall популяциясын зерттеу

Жұмыстың мақсаты қазақстандық Алтай аумағындағы 3 оқшауланған географиялық аймақтан, яғни: Оңтүстік, Оңтүстік-Батыс және Алтай Қалбасы аймақтарынан *Allium altaicum* Pall. популяциясын зерттеу. Сарымсақты, Линейский және Қалба жоталарында маршруттық-барлау әдісімен аумақты зерттеу нәтижесінде тіршілік ету ортасының әртүрлі жағдайларымен және биіктік шектерімен сипатталатын популяциялар зерттелді. Экологиялық-фитоценоздық скрининг анықтағандай, Сарымсақты жотасында популяция альпі белдеуінен, яғни теңіз деңгейінен 1870 м биіктік шегінде, ал Линейский жотасында популяция — таулы-орманда, яғни теңіз деңгейінен 1361 м биіктікте орналасқаны анықталды. Екі популяцияда мезо-психро-петрофиттік өсу жағдайларымен сипатталады. Қалба жотасында популяция таулы-дала ксеро-мезо-петрофиттік жағдайда, теңіз деңгейінен 832 м биіктікте зерттелді. Зерттелген популяцияларда, яғни *A. altaicum* өсетін жерлердегі таудағы топырақ күлгінделген қара топырақты, аздап қышқылды, рН 5,6–5,9, қарашірік мөлшері 13,5%-дан 14,9%-ға дейін жоғары, жер жамылғысы қалыптасқан, шамамен 210–410 г/м<sup>2</sup>. Барлық популяцияларда өсімдік жамылғысының негізін шөпті көпжылдықтар құрайды және жікқабаттылығы айқын емес. Фитоценозды қалыптастыруға *A. altaicum* қатысуы Сарымсақты жотасында — 3,2 %, Линейскийде — 2,2%, Қалбада — 1,8% құрайды. Зерттелген популяциялардың флорокешені тамырлы өсімдіктердің 91 түрінен құралған. Сарымсақты популяциясының альпілік мезо-психро-петрофиттік жағдайдағы флористикалық құрамдарының және таулы-орманды мезо-психро-петрофиттік жағдайдағы Линейский құрамының барлық ценофлораға қатысты 65,0% деңгейінде ұқсастығы анықталды, ал Қалба таулы-далалық ксеро-мезо-петрофиттік жағдайдағы бұл құрамның көрсеткіші 42,1 % құрады. *A. altaicum* жерүсті мүшелерінің морфометриялық параметрлерінің өзгермелілігі барлық зерттелген популяцияларда 15,0-ден 26,09%-ға дейін орташа және жоғары өзгергіштік деңгейіне ие, бұл өсімдіктердің экологиялық икемділігін көрсетеді. Табиғатта вегетативті көбею тек виргинилді және генеративті өсімдіктерде жүретіні анықталды. Бұл жағдайда ұялар пайда болады. Ұядағы баданалардың биоморфологиялық көрсеткіштері жас ерекшеліктеріне байланысты және бір ценопопуляция шегінде өзгереді.

*Кілт сөздер:* *Allium altaicum* Pall., биометриялық параметрлер, қазақстандық Алтай, популяция, өсімдік жамылғысы, флористикалық құрамы.

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## Популяционное изучение *Allium altaicum* Pall. в Казахском Алтае

Целью данной работы явились популяционные исследования *Allium altaicum* Pall. на территории Казахского Алтая в трех территориально изолированных географических районах: Южный, Юго-Западный, Калбинский Алтай. В результате обследования территории маршрутно-рекогносцировочным методом на хр. Сарымсақты, Линейский и Калбинский проведено изучение популяций, которые характеризуются разными условиями среды обитания и высотными пределами. Эколого-фитоценотический скрининг выявил, что на хр. Сарымсақты популяция находится в альпийском поясе в высотном пределе 1870 м над ур.м., на хр. Линейский — в горно-лесном, 1361 над ур.м. Обе популяции характеризуются мезо-психро-петрофитными условиями произрастания. На хр. Калбинский популяция обследована в горно-степных ксеро-мезо-петрофитных условиях, 832 м над ур. м. Почвы в местах произрастания *A. altaicum* в обследованных популяциях горные оподзоленные черноземы, слабокислые, рН 5,6–5,9, с высоким содержанием гумуса от 13,5 до 14,9 %, напочвенный покров сформирован, 210–410 г/м<sup>2</sup>. Во всех популяциях основу растительного покрова составляют травянистые многолетники, ярусность не выражена. Участие *A. altaicum* в формировании фитоценоза на хр. Сарымсақты составляет 3,2 %, Линейский — 2,2, Калбинский — 1,8 %. Флорокомплекс обследованных популяций сформирован из 91 вида сосудистых растений. Установлено сходство флористических составов сарымсақтинской популяции в альпийских мезо-психро-петрофитных условиях и линейской

в горно-лесных мезо-психро-петрофитных условиях на уровне 65,0 % по отношению ко всей ценофлоре, а калбинской в горно-степных ксеро-мезо-петрофитных условиях этот показатель составил 42,1 %. Вариативность морфометрических параметров надземных органов *A. altaicum* во всех обследованных популяциях имеет средний и высокий уровни изменчивости от 15,0 до 26,09 %, что указывает на экологическую пластичность растений. Установлено, что в природе вегетативное размножение происходит только у виргинильных и генеративных растений. При этом формируются гнезда. Биоморфологические показатели луковиц в гнезде носят четко выраженный возрастной характер и варьируют в пределах одной и той же ценопопуляции.

*Ключевые слова:* *Allium altaicum* Pall., биометрические параметры, Казахский Алтай, популяция, растительный покров, флористический состав.

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## **Study of the influence of some physical factors on the viability of seeds of *Echinaceae pallida* variety “Lebedushka”**

Application of physical factors allows activating seed germination and increasing plant yield. The article presents data on testing of germination parameters of seeds of *Echinaceae pallida* variety “Lebedushka” under the influence of physical factors. Seeds were preliminarily immersed in liquid nitrogen for a day, and then subjected to laser irradiation, bubbling and magnetic fields for a certain time. As a result of the experiments, it was determined that exposure to a constant magnetic field promoted a reliable increase in germination rates by 10.4 % compared to the control. The best germination was determined in seeds exposed to a constant magnetic field with an induction of 75  $\mu$ Tesla for 3 days, without prior cryopreservation (90.0 %). In the experiments with bubbling, the best germination was determined in seeds without cryopreservation – 75.25 %, and in the variant of the experiment with He-Ne laser irradiation for 2 minutes without preliminary immersion in liquid nitrogen vapor – 95.0 %.

*Keywords:* seeds, *Echinacea pallida*, magnetic fields, bubbling, radiation by He-Ne laser, cryogenic storage, germination.

### *Introduction*

Consideration of the influence of magnetic fields and laser irradiation on the preservation of viability of seed material of medicinal plant species is currently of great theoretical and applied importance. Valuable medicinal plants provide pharmaceutical plants of Kazakhstan with medicinal plant raw materials.

According to some literature data [1], a positive effect of various physical factors on the viability of germinating seeds and the passage of seedlings through the phases of ontogenesis of economically useful plants has been established.

Laser irradiation acts as a bio stimulator [2], as it triggers mechanisms of growth and development of plant organism, but this physical factor is poorly studied in relation to herbs. In the scientific environment there are data of laboratory studies proving the positive effect on the germination of seed material. With laser bio stimulation of seeds, an increase in the yield of various crops is observed. Due to ionizing radiation there is an energy exchange between endosperm and embryo, and absorption of rays occurs by certain structural components of the seed coat.

It is advisable to use the bubbling method for seeds with germination of less than 70 % [1]. Germination and germinative energy increase as a result of better permeability of the protective layer of the seed coat, as well as washing out of various pathogenic floras by air bubbles. Due to bubbling, seeds come out of deep dormancy faster; as a result they germinate 1-2 days earlier than in control variants. Seed material is placed in a vessel with water for one day, where as a result of compressor operation, oxygen bubbles are formed. Advantageous features of this method are the rapid passage of ontogenesis phases, friendly germination of seeds, and high biological productivity of plants.

Under the influence of magnetic field [3], the biological clock of plants, the main processes of synthesis of various organic compounds are started. The publications of research scientists contain data on the positive effect of magnetic fields on the viability of seed material of various plant species.

Currently, the number of most plant species, including medicinal plants, growing in Kazakhstan is decreasing due to the impact of anthropogenic factor [4, 5].

There is a need to preserve the gene pool of plants, which is based on the data of scientific research conducted in various countries, and one of the promising methods is cryogenic storage of plant germplasm in liquid nitrogen [6, 7].

To date, there is a small number of scientific works devoted to the study of the influence of various physical factors on the viability of seeds and biology of germination of seedlings of medicinal plants. The

main source of medicinal plant raw materials for the pharmaceutical industry is medicinal plants growing in natural habitats or cultivating species. One of the promising medicinal plants is *Echinacea pallida*, which has antioxidant, antiradical, anti-mutogenic, radioprotective and immunostimulating properties, antimicrobial activity due to the presence of water-soluble polysaccharide complex relative to *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Candida albicans* [5, 8].

### Experimental

Seed material of *Echinacea pallida* variety “Lebedushka” was used in the experiments. Laboratory experiments were conducted to study the effect of physical factors on germination and germination biology of seeds of *Echinacea pallida* variety Swan.

To study the effect of bubbling, seeds were packed in cloth bags and immersed in a plastic cylinder filled 2/3 with water, compressed air was pumped by an aquarium compressor. Several variants of the experiment were laid down: 1) with preliminary cryogenic storage for a day in a Dewar vessel and subsequent bubbling process for 24 hours; 2) seed bubbling for a day.

In the magnetic field experiments, seeds were packed in unsealed plastic tubes that were placed in a magnet setup. A constant magnetic field of 75  $\mu$ Tesla or 150  $\mu$ Tesla induction was continuously applied to the test seeds in the magnetic setup. The direction of the magnetic field of the setup coincided with the direction of the Earth's magnetic field. Two variations of experiments were applied: with pre-storage for a day in liquid nitrogen vapor and without cryopreservation. Seeds were irradiated for a day and 3 days.

Irradiation with He-Ne laser with a wavelength of 632.8 nm and intensity of 5mW/cm<sup>2</sup> was carried out for a certain time: 30 seconds, 1, 2 and 4 minutes. Experiments with cryogenic storage and subsequent exposure to laser irradiation and without cryopreservation were also conducted with the seeds.

Seeds were frozen in cryobiological tubes at -196 °C for a day in Dewar vessels, followed by slow thawing at room temperature [9].

Seeds of the studied species were sown immediately after the manipulations. Seeds were germinated in Petri dishes on 2 layers of filter paper moistened with water in 4-fold repetition. Seeds were disinfected with 0.5 % potassium permanganate for 5-6 minutes. Petri dishes with planted seeds were placed at a temperature of +24°C in a climatic chamber.

Seed germination parameters were studied according to the methodological instructions of M.S. Zorina and S.P. Kabanov [10], M.V. Maltseva [11]. The development of seedlings was monitored for 14 days. Statistical processing of the obtained data was performed according to the method of N.L. Udolskaia [12] and using the site medstatistic.ru [13].

### Results and Discussion

The comparative analysis of the effect of permanent magnetic field on the viability of seeds of *Echinacea pallida* variety “Lebedushka” showed that the best germination was demonstrated by seeds without preliminary cryopreservation and subjected to continuous exposure to a permanent magnetic field with an induction of 75 $\mu$ Tesla for 3 days – 90.0 %; similar germination was shown by seeds with preliminary cryopreservation for a day and subsequent exposure to a permanent magnetic field with an induction of 150 $\mu$ Tesla for 3 days. The control group showed germination of 75.0 $\pm$ 5.77 %. The best indicators of germination without preliminary cryopreservation were observed in seeds exposed to a constant magnetic field induction of 75 $\mu$ Tesla for 3 days – 90.0 %. Compared to the control group, the average germination increased by 11.7 % with cryopreservation, and by 8.2 % without cryopreservation. In the experiment with preliminary cryogenic storage for a day and subsequent exposure to a magnetic field, the best germination was observed in seeds exposed to a constant magnetic field with an induction of 150  $\mu$  Tesla for 3 days – 90.0 % (Table 1).



Table 1

**Parameters of germination of *Echinacea pallida* variety “Lebedushka” seed materials in different experiments**

Parameters of germination, %	Control	Without cryopreservation				After cryopreservation			
		Magnet field 75 $\mu$ Tesla		Magnet field 150 $\mu$ Tesla		Magnet field 75 $\mu$ Tesla		Magnet field 150 $\mu$ Tesla	
		Period of treatment		Period of treatment		Period of treatment		Period of treatment	
		1 day	3 days	1 day	3 days	1 day	3 days	1 day	3 days
Germinative energy, %	65.0 $\pm$ 6.5	75.5 $\pm$ 3.21	86.25 $\pm$ 2.76*	60.5 $\pm$ 6.35	54.25 $\pm$ 2.51	78.0 $\pm$ 3.13	65.5 $\pm$ 9.68	75.0 $\pm$ 10	75.0 $\pm$ 3.33
Seed germination, %	75.0 $\pm$ 5.77	85.25 $\pm$ 3.18	90.0*	85.0 $\pm$ 3.33	75.0 $\pm$ 3.33	80.5 $\pm$ 0.58	77.5 $\pm$ 8.66	85.0 $\pm$ 3.33	90.0*

\*reliable difference between the results of the experiment and the control at  $P \leq 0.05$

As a result of the analysis of the obtained data of laboratory studies, the positive effect of exposure to a constant magnetic field with induction from 75  $\mu$ Tesla to 150  $\mu$ Tesla on the viability of seed material and the passage of ontogenesis phases was determined. Germination improved on 15 % compared to the control data.

Thus, having considered the effect of a constant magnetic field with induction from 75  $\mu$ Tesla to 150  $\mu$ Tesla on the germination parameters of seeds of *Echinacea pallida* variety “Lebedushka”, it can be noted that the viability of seed material improved compared to control values by 9.75 %. In all variants of the experiment the seedling passes all phases of ontogenesis, in some variants of the experiment there was a delay in germination of seed material for 1-2 days.

For long-term storage of seeds it is recommended to apply preliminary cryogenic storage for a day with subsequent activation in a permanent magnetic field with an induction of 150  $\mu$ Tesla for 3 days.

At the next stage, a series of experiments on babbling of seed material was carried out. The germination comparable to control values was established. In the experiment with pre-cryopreservation and subsequent babbling, the germination decreased to 65.0 $\pm$ 3.33 % compared to the control. In the experiments with babbling, germination was 75.25 $\pm$ 3.51 %, which is 0.25 % higher than the values in the control group (Table 2)

Table 2

**Parameters of viability of *Echinacea pallida* variety “Lebedushka” seeds after babbling treatment**

Variant of experience	Germinative energy, %	Seed germination, %
Control	65.0 $\pm$ 6.5	75.0 $\pm$ 5.77
Babbling	60.0 $\pm$ 6.34	75.25 $\pm$ 3.51
Cryopreservation + babbling	60.0 $\pm$ 6.67	65.0 $\pm$ 3.33

Analyzing the dynamics of seed germination it was found that seeds germinate faster on the day in the experiment variants with barbotage – on the 2nd day, in the control group on the 3rd day (Fig. 1).

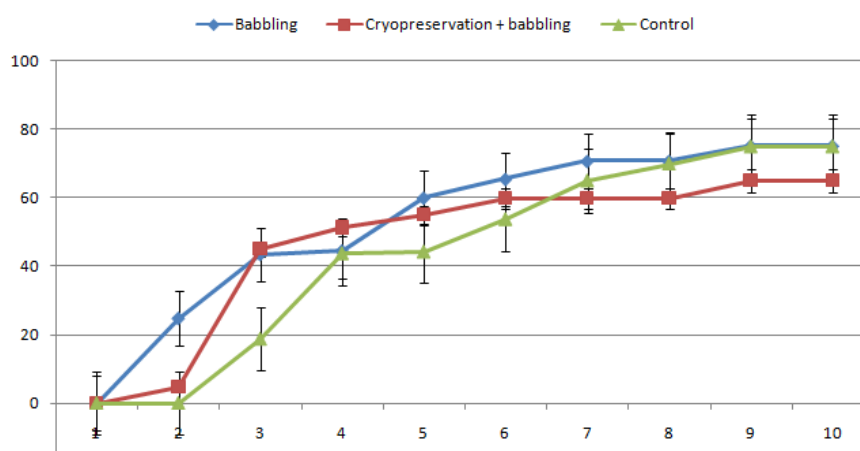


Figure 1. Dynamics of germination of seed material of *Echinacea pallida* variety “Lebedushka” after babbling treatment

The effects of laser radiation on the biology and dynamics of seed germination were examined in laboratory experiments. Seed material of *Echinacea pallida* was exposed to He-Ne laser for a duration of 30 seconds to 4 minutes. The best germination was demonstrated by seeds without preliminary cryogenic storage, irradiated for 2 minutes –  $95.0 \pm 3.33$  %, which is 20 % higher than the control values (Table 3).

Table 3

**Influence of laser radiation on seed viability of *Echinacea pallida* variety “Lebedushka”**

Parameters of germination	Control	Without cryopreservation				With cryopreservation			
		Period of treatment		Period of treatment		Period of treatment		Period of treatment	
		30 sec	1 min	2 min	4 min	30 sec	1 min	2 min	4 min
Germinative energy, %	65.0± 6.5	60.0± 6.67	64.5± 16.34	85.0± 3.33	60.0±0	55.0± 7.01	90.0± 6.67*	50.0± 6.67	40.0±0*
Seed germination, %	75.0± 5.77	70.0± 6.67	70.5± 1238	95.0± 3.33*	85.0± 3.33	66.75± 6.84	90.0± 6.67	60.0±0*	50.0± 6.67

\* reliable difference between the results of the experiment and the control at  $P \leq 0.05$

By conducting a comparative analysis of the conducted studies with and without preliminary storage of seeds for a day in liquid nitrogen vapor, followed by exposure to laser radiation, it was found that the best viability was demonstrated by seeds without cryopreservation. Thus, in experiments with seeds without cryopreservation, the best germination rates were observed in seeds irradiated with laser beam for 2 minutes –  $95.0 \pm 3.33$  %. In experiments with pre-cryopreservation, the best germination was determined in seeds irradiated for 1 minute –  $90.0 \pm 6.67$  %.

Having studied the dynamics of germination of seeds of *Echinacea pallida* variety “Lebedushka” in different variations of the experiment, it was determined that seeds irradiated for 30 seconds without preliminary cryopreservation begin to germinate on the 3rd day from the moment of sowing, on the 4th day – exposed to laser radiation for 2 and 4 minutes without cryopreservation and with preliminary cryogenic storage and subsequent irradiation of 30 seconds. In the remaining experiments, germination was observed on the 5th day.

The effect of physical factors on germination parameters of *Echinacea pallida* variety “Lebedushka” was considered and analyzed, it was found that to obtain viable seed material and well-developed seedlings should be exposed to laser beam for 2 minutes, germination in this variant of the experiment increases by 20 % compared to the control (Table 4).

Table 4

**Effect of different physical factors on seed viability parameters of *Echinacea pallida* variety “Lebedushka”**

Parameters of germination	Control	Permanent magnetic field B=75mTesla (3 days) without cryopreservation	Babbling	Without cryopreservation, He-Ne laser irradiation for 2 minutes
Germinative energy, %	65.0±6.6	86.25±2.76*	60.0±6.34	85.0±3.33*
Seed germination, %	75.0±5.77	90.0±0*	75.25±3.51	95.0±3.33*

\* reliable difference between the results of the experiment and the control at  $P \leq 0.05$

Comparing the effect of physical factors on the dynamics of germination it was found that seeds begin to germinate on the 2nd day using the method of babbling, on the 4th day – with laser beam irradiation for 2 minutes, on the 5th day – with exposure to a constant magnetic field with an induction of 75  $\mu$ Tesla for 3 days (Fig. 2).

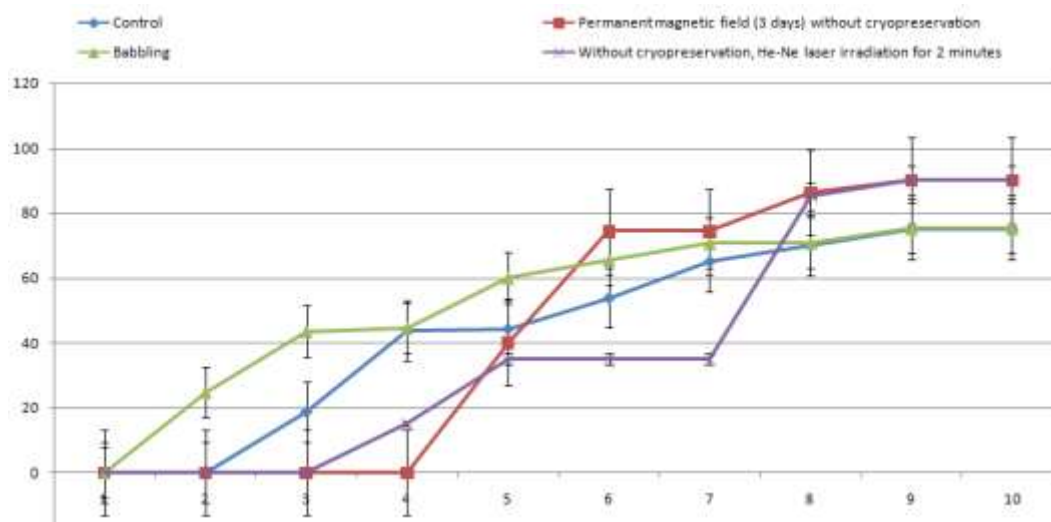


Figure 2. Effects of physical factors on the dynamics of seed germination of the test species *Echinacea pallida* variety “Lebedushka”

### Conclusion

According to the analyzed literature review, it was determined that physical factors have a positive effect on the viability of seeds of the studied species. Permanent magnetic field with induction from 75  $\mu$ Tesla and up to 150  $\mu$ Tesla has a favorable effect on germination indices of seeds of the studied species: germination increased by 10.4 % compared to the control. The best germination was observed in seeds exposed to a permanent magnetic field of 75  $\mu$ Tesla induction for 3 days, without preliminary cryogenic storage – 90.0 %. In experiments with bubbling, the best germination was found in the variant without cryogenic storage – 75.25 $\pm$ 3.51 %. In the experiment with laser irradiation, the best results were obtained in the variant of irradiation for 2 minutes without preliminary cryopreservation – 95.0 $\pm$ 3.33 %. Thus, the use of He-Ne laser for 2 minutes to activate germination can be recommended for the seed material of *Echinacea pallida* variety “Lebedushka”.

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### ***Echinaceae pallida* сортының «Лебедушка» тұқымдарының өміршеңдігіне физикалық факторлардың әсерін зерттеу**

Мақалада физикалық факторларға ұшыраған *Echinaceae pallida* сортының «Лебедушка» тұқымдарының өнуі туралы ақпарат берілген. Тұқымдар алдын-ала бір тәулік бойы сұйытылған азотқа батырылды, содан кейін белгілі бір уақыт бойы лазерлік сәулеленуге және магнит өрісіне ұшырады. Сонымен қатар зертханалық зерттеулерде көпіршік әдісі қолданылды. Тәжірибелердің нәтижесінде магнит өрісінің әсері бакылаумен салыстырғанда өну жылдамдығының 10,4 %-ға артуына ықпал ететіні анықталды. Ең жақсы өнгіштік алдын ала криосақтаусыз 3 күн бойы бір магнит өрісінің әсеріне ұшыраған тұқымдарда табылды, ол — 90,0 %. Көпіршіктенумен жүргізілген тәжірибелерде ең жақсы өну криоконсервациясыз тұқымдарда — 75,25 %, ал He-Ne лазерімен сәулелендіру тәжірибесінде алдын-ала сұйық азотқа батырылмағандықтан 2 минут ішінде — 95,0 % болды.

*Кілт сөздер:* тұқымдар, *Echinaceae pallida*, магнит өрісі, барботирлеу, He-Ne лазермен сәулелену, криогенді сақтау, өнімділік.

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### **Изучение влияния физических факторов на сохранение жизнеспособности семян *Echinaceae pallida* сорта «Лебедушка»**

В статье представлены сведения о прорастании семян *Echinaceae pallida* сорта «Лебедушка», подвергшихся воздействию физических факторов. Семена предварительно погружались в жидкий азот на сутки, затем подвергались лазерному облучению и воздействию магнитных полей в течение определенного времени. Также в лабораторных исследованиях был использован метод барботирования. В результате проведенных экспериментов было определено, что воздействие постоянного магнитного поля способствует улучшению показателей прорастания на 10,4 % по сравнению с контролем. Наилучшая всхожесть была определена у семян, подвергшихся воздействию постоянного магнитного поля индукцией 75 мкТесла в течение 3 суток, без предварительного криохранения, — 90,0 %. В экспериментах с барботированием лучшая всхожесть была установлена у семян без криоконсервации — 75,25 %, а в варианте эксперимента с облучением He–Ne лазером в течение 2 мин без предварительного погружения в пары жидкого азота — 95,0 %.

*Ключевые слова:* семена, *Echinacea pallida*, магнитное поле, барботирование, облучение He–Ne лазером, криогенное хранение, всхожесть.

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## About floristic records of the Mangystau flora

This article presents the geographical novelties of the Mangystau flora discovered during the botanical expedition in 2023. One of the oasis territories of the Mangistau desert is the mountainous and foothill strips of the mountainous Karatau. For the first time, three species of woody plants, *Prunus spinosa* L., *Rosa iliensis* Chrshan, *Armeniaca vulgaris* L. from the family *Rosaceae* Juss, were found in the gorges of Kogez, Akmysh, Samal of the Mountain Karatau in the valleys of streams and along the slopes of the mountains. Thickets of bushes *Prunus spinosa* L. and endemic species of South Kazakhstan *Rosa iliensis* Chrshan occupy an area from 100 to 200 m<sup>2</sup> and there are several single specimens of a rare and endemic species with a decreasing range of *Armeniaca vulgaris* L several specimens. All species are in good condition, plants have a high vitality, and no signs of damage were noted. *Prunus spinosa* and *Rosa iliensis* also showed abundant flowering and fruiting.

**Keywords:** tree species, rare and endemic species, Mangistau deserts, mountainous Karatau, gorges, flora of Kazakhstan.

### Introduction

The Mangystau desert is located on lowlands and plains. The height ranges from -23 m below sea level on the coast of the Caspian Sea, up to -132 m in the Karagiye depression, and up to +300+550 m above sea level on the remnant mountain elevations. Four large geomorphological regions are distinguished on the territory of Mangystau: Mountain Mangyshlak, plain Mangyshlak, lowland plain Bozashi, and Ustyurt plateau [1]. The flora of the Mangystau region belongs to a typical desert type formed under the influence of characteristic arid areas.

In 2004-2006, during the implementation of the project "State Plant Cadastre of Mangystau region", the floral composition of plants was clarified. A monographic work on vascular plants of the region was published, which included 675 species from 300 genera and 69 families. At the same time, the list of rare and in need of plant protection, numbering 40 species, was clarified and expanded [2].

In 2009, two geographically new species of the Mangystau flora were collected and described: *Corydalis schanginii* (Pall.) B. Fedtsch. and *Gagea ova* Stapf. Both species were collected on the Western Chinka of the Ustyurt plateau [3].

As a result of floristic field studies for 2010-2021, additional new geographical novelties from 14 species for the flora of Mangystau were discovered: *Artemisia sieversiana* Willd., *Ribes aureum* Pursh, *Tulipa gerneriana* L. (*T. schrenkii* Regel), *Malus siversii* (Ledeb.) M. Roem., *Urtica dioica* L., *Epilobium hirsutum* L., *Rosa canina* L., *Portulaca oleracea* L., *Hordeum murinum* L., *Hordeum murinum* L., *Atriplex verrucifera* M. Bieb. One plant (*Cakile maritima* subsp. *euxina* (Pobed.) Nyar.) was a new species for the Flora of Kazakhstan. These species were mainly collected on the Mangyshlak peninsula in the Mountainous Karatau, one – on the coast of the Caspian Sea, and 3 species – on the Western Chink of the Ustyurt plateau [4-5].

In this paper, in addition to previous research results, we presented the geographical novelties for the Mangystau flora discovered during the botanical expedition in 2023.

### Experimental

During the botanical expeditions in 2023, three geographical new species for the Mangystau flora from the *Rosaceae* Juss. family were collected: *Prunus spinosa* L., *Rosa iliensis* Chrshan., and *Armeniaca vulgaris* L. Field research was carried out by the route-reconnaissance method. Herbarium samples of the collected species are stored in the Mangyshlak Experimental Botanical Garden (MEBG) herbarium. The species' general habitat and the taxa's Latin names are given by POWO (Plants of the World Online) data [6].

The species were determined according to the summary “Flora of Kazakhstan” [7], the Latin names of plants according to the summary by S.K. Cherepanov [8]. The floristic zoning proposed according the “Flora of Kazakhstan” summary.

### Results and Discussion

One of the oasis territories of the Mangystau desert is the mountainous and foothill bands of the mountainous Karatau. Karatau is divided into Western and Eastern mountains, represented by elevations elongated in the sub-latitudinal direction. Karatau's total length does not exceed 90 km; each hill is 45 km long, with average absolute heights of 300-400 m; the highest points are 532 and 556 m (Otpan and Beshoky mountains). A characteristic feature of the Western and Eastern Karatau ridges is the presence of a well-defined leveling surface, composed of stable metamorphic rocks of the Permian-Triassic. The mountains of the Western and Eastern Karatau gorges are caused by the presence of territories with a close occurrence of groundwater self-draining springs of fresh water, which allow the formation of a rich vegetation cover.

On the slopes of the mountains between the rocks along the banks of a flowing spring stream, trees grow on a single specimen – *Elaeagnus angustifolia* is widely distributed by thickets of *Crataegus ambigua*, in places *Rubus caesius*, in deep-cut gorges with rocky slopes there are individual bushes of *Rhamnus sinensis*.

In the summer of 2023, during fieldwork in the Kogez, Akmysh, and Samal gorges in the ridges of the mountainous Western Karatau, we first discovered thickets of bushes of *Prunus spinosa* L. and the endemic species *Rosa iliensis* Chrshan, several specimens of a rare and endemic species with a shrinking range of *Armeniaca vulgaris* L. on the slopes of the mountains and the valley of streams.

*Prunus spinosa* is a highly branched, very prickly shrub. Thorn thickets were found among the rising rocks in the Kogez, Samal, and Karatau gorges 10-15 km east of the Shaiyr village (Fig. 1). They grow in plots from 20 to 30 m long and 3-4 m wide, and in places, they occur in separate patches of area in sizes 5x3 m 10x5 m. The appearance of the thorn in the gorges is in good condition; the plants have high vitality, and there are no signs of damage. The height of the bush is 2.5-3.5 m, well-leafed, thick. It bears fruit abundantly, fruits of medium size, length 1.3-1.5 cm, width 1-1.3 cm. Blooms in April-May, bears fruit in July-August.

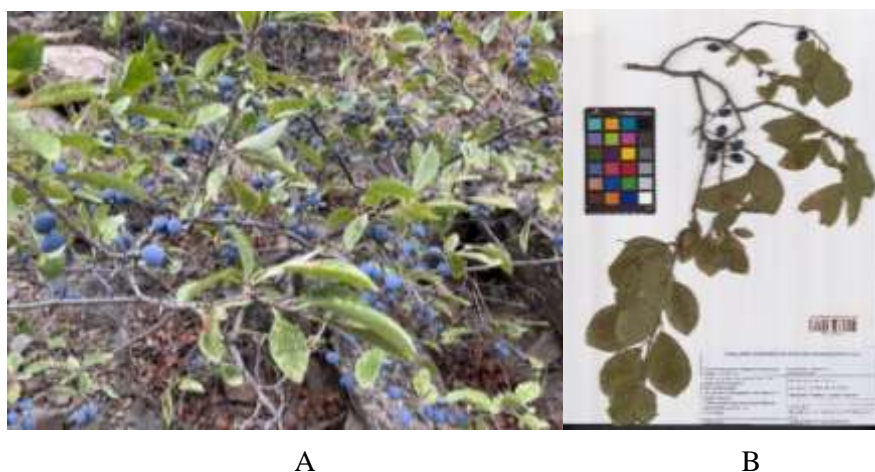


Figure 1. *Prunus spinosa*: A – sample in nature; B – herbarium specimen

The range covers Europe, North Africa, Western Asia [9] and Western Siberia [10]. In the “Flora of the south-east of the European part of the USSR”; the areas of the Volga River delta, the Astrakhan steppe; the Orenburg and Ural counties are given [11], the northern border runs from 68° on the Scandinavian Peninsula through southwestern Finland [12]. According to V.D. Gorodetskii [13], as well as according to the “Flora of Turkmenistan” [14], blackthorn is not found in Central Asia; Asia Minor and Iran are given in the “Flora of Kazakhstan”. In Kazakhstan, it is located in the floristic areas of the spurs of the Common Syrt, Mugodzhara, and the Caspian along the Ural River [7]. Earlier, it was noted about the growth of blackthorn in Western Karatau [15], but it is not included in the list of flora of Mangystau [2].



New location: Mangyshlak floral district: Mangystau region, Mangystau district, Western Karatau Mountains, Kogez Gorge. N 44°12'155", E 52°01'174", 257 m above sea level. 15.VII.2023. A.A. Imanbayeva, N.I. Duissenova (determined by A.A. Imanbayeva); Mangistau region, Mangistau district, Western Karatau Mountains, Samal gorge; N 44°12'57.1", E 51°59'37,5", 249 m above sea level. 15.VII.2023. A.A. Imanbayeva, N.I. Duissenova (determined by A.A. Imanbayeva)

There is an assumption that the blackthorn was brought to Mangyshlak by birds from the Northern Caspian Sea since the main routes of their seasonal migrations pass along the eastern shores of the Caspian Sea [15].

*Rosa iliensis* is a dense shrub with semi-branching branches. Thickets of bushes grow in the stream valley and on rocky slopes in the Kogez of Western Karatau gorges (Fig. 2).



Figure 2. *Rosa iliensis* Chrshan.: A – sample in nature; B – herbarium specimen

The shrub's height is 2.5 m, and the branches climbing along the nearby trees of the narrow-leaved loch reach up to 7 m. Its area is from 5 m to 8 m long, 3-4 m wide in several sections. In the thickets, the number of adult plants is 12-20 pcs, an average of 7-15 pcs, and more than 30 pcs of young root seedlings. The plants are well-leafy dense. It bears fruit abundantly; the fruit is smooth, spherical, black, 5-6 mm in diameter. Blooms in May-October, fruits ripen in August-October. The plant was discovered during flowering and fruit ripening. This species was first described in 1947 by V.G. Hrzhanovsky from the Ili River valley of the Almaty region [16-17]. *Rosa iliensis* Chrshan. It grows along the banks of desert rivers on the sands in the Muyunkum and Balkhash-Alakul floristic areas [7]. The species is one of the endemic plant species of the flora of Kazakhstan, which is under threat of extinction since the size of its distribution has sharply decreased in the last 45-50 years due to anthropogenic influences [18].

New location: Mangyshlak floral district: Mangystau region, Mangystau district, Western Karatau Mountains, Kogez Gorge. N 44°12'155", E 52°01'174", 257 m above sea level. 15.VII.2023. A.A. Imanbayeva, N.I. Duissenova (determined by A.A. Imanbayeva, G.Zh. Dosshchieva).

*Armeniaca vulgaris* L. is a 5-7 m tall tree. It grows in a single specimen in the valley of streams and on the rocky slopes of the gorge in Kogiz and Kamysh of Western Karatau. Plant height 5-7 m, crown diameter 6 m. According to the appearance assessment, the apricot trees are in good condition, not damaged by diseases and pests.

A rare and endemic species of the Tien Shan apricot is expected in the Flora of Kazakhstan, the distribution area is the floral areas of the Dzungarian Alatau, Zailiysky Alatau, Ketmen-Tersken Alatau, Western Tien Shan [7].

New location: Mangyshlak floral district: Mangystau region, Mangystau district, Western Karatau Mountains, Kogez Gorge. N 44°12'155", E 52°01'174", 257 m above sea level. 15.VII.2023. A.A. Imanbayeva, N.I. Duissenova (determined by A.A. Imanbayeva); Mangistau region, Mangistau district,



Western Karatau Mountains, Akmysh gorge: N 44°12155., E 52°01174, 253 m above sea level. 05.VIII.2023. A.A. Imanbayeva, N.I. Duissenova (determined by A.A. Imanbayeva) (Fig. 3).



Figure 3. *Armeniaca vulgaris* L.: A – sample in nature; B – herbarium specimen

### Conclusion

During the botanical expeditions in 2023, three species of woody plants, *Prunus spinosa* L., *Rosa iliensis* Chrshan, and *Armeniaca vulgaris* L. from the family *Rosaceae* Juss. as geographical novelties for the flora of Mangystau were discovered for the first time. The growth of this species was noted in the Kogez, Akmysh, and Samal gorges of the Mountain Karatau in the valleys of streams and along the slopes of mountains. Thickets of *Prunus spinosa* bushes and the endemic species *Rosa iliensis* cover an area of 100 to 200 m<sup>2</sup>. The plants are well-leaved, dense, and abundantly fruited. A rare and endemic species of *Armeniaca vulgaris* was found in single specimens in the gorge in Kogez and Akmysh of Western Karatau. The plants are in good condition and not damaged by diseases and pests.

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### Маңғыстау флорасындағы географиялық жаңалықтар туралы

Мақалада Маңғыстау флорасы үшін географиялық жаңалықтарды ашу бойынша 2023 жылғы ботаникалық экспедициялардың нәтижелері берілген. Шөлді Маңғыстаудың оазистік аумақтарының бірі — Қаратау тауының таулы және тау бөктеріндегі белдеулері. Көгез, Ақмыш, Самал шатқалдарында, Қаратаудың таулы, бұлақ аңғарларынан және тау бөктерінен алғаш рет *Rosaceae* Juss тұқымдасының ағаш өсімдікті үш түрі — *Prunus spinosa* L., *Rosa iliensis* Chrshan, *Armeniaca vulgaris* L. табылды. Қалың бұталы өскен *Prunus spinosa* L. және Оңтүстік Қазақстанның эндемикалық түрі *Rosa iliensis* Chrshan тау шатқалдарында 100-ден 200 м<sup>2</sup>-ге дейінгі аумақты алып жатыр. Сонымен қатар *Armeniaca vulgaris* L. таралу аймағы азайып бара жатқан сирек және эндемикалық түрлердің жалғыз үлгілері бар. Барлық түрлер жақсы жағдайда, өсімдіктердің өміршеңдігі жоғары, зақымдану белгілері жоқ. *Prunus spinosa* және *Rosa iliensis* түрінің жақсы гүлденгені және жеміс беруі байқалды.

*Кілт сөздер:* ағаш түрлері, сирек және эндемикалық түр, шөлді Маңғыстау, Қаратау таулы аймағы, шатқалдар, Қазақстан флорасы.

А.А. Иманбаева, Н.И. Дуйсенова, А.Б. Лукманов, Г.Ж. Досшиева, Г.Г. Гасанова

### О географических новинках во флоре Мангистау

В статье приведены результаты ботанических экспедиций 2023 года о находке географических новинок для флоры Мангистау. Одним из оазисных территорий пустыни Мангистау является горная и предгорная полосы горного Каратау. Впервые обнаружены три вида древесных растений: *Prunus spinosa* L., *Rosa iliensis* Chrshan, *Armeniaca vulgaris* L. из семейства *Rosaceae* Juss. в ущельях Когез, Ақмыш, Самал, горного Каратау, в долинах ручьев и по склонам гор. Заросли кустов *Prunus spinosa* L. и эндемичный вид Южного Казахстана *Rosa iliensis* Chrshan занимают площадь от 100 до 200 м<sup>2</sup>, и встречаются единичные экземпляры редкого и эндемичного вида с сокращающимся ареалом *Armeniaca vulgaris* L. Все виды в хорошем состоянии, растения имеют высокую жизнеспособность, признаков повреждений не отмечено. Наблюдалось обильное цветение и плодоношение у *Prunus spinosa* и *Rosa iliensis*.

*Ключевые слова:* древесные виды, редкий и эндемичный вид, пустыни Мангистау, горный Каратау, ущелья, флора Казахстана.

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## Effect of LSDV008 gene knockout on the cultural properties of recombinant lumpy skin disease virus

Lumpy skin disease virus (LSDV) is a poxvirus that causes a severe systemic disease in cattle and is rapidly expanding its geographic boundaries. Lumpy dermatitis (LD) is characterized by fever, nodules on the skin, mucous membranes, and internal organs. The disease can cause emaciation with swollen lymph nodes and sometimes death. In recent years, the disease has become endemic in various parts of Asia, causing significant economic damage to livestock production. Since there are no specific treatments for LSD, vaccination is the most effective way to control and eradicate the disease. The most complete immunity can be obtained by vaccination with live attenuated vaccines. Attenuation through long passages is associated with random mutations in the genome, and the mechanism of attenuation remains unclear. Targeted removal of virulence genes in the viral genome by genetic engineering is the most promising direction in the creation of attenuated poxviruses. The LSDV008 gene encodes a protein similar to the  $\gamma$ -interferon receptor and is a potential virulence gene for the LSD virus. In these studies, we studied the effect of deletion of the LSDV008 gene on the cultural properties of the recombinant Atyrau-B virus. The parental LSD virus Dermatitits nodulares/2016/Atyrau/KZ (Atyrau-KZ) was used as a control. As a result of the studies, it was found that the gene knockout did not affect the replication activity of the recombinant Atyrau-B virus *in vitro*. The recombinant virus accumulated in cell cultures in the same titers as the parent virus. The most sensitive cell systems for the reproduction of Atyrau-KZ and Atyrau-B LSD viruses are lamb testicle (LT), bull kidney (MDBK) and saiga kidney (SK) cell cultures, which can be used to obtain viral mass in further scientific research.

**Keywords:** lumpy skin disease virus, cell culture, cultivation, virus titer, replication, infectious activity, gene knockout, attenuation.

### Introduction

Lumpy skin disease is an acute, subacute, chronic, less often latent disease of cattle characterized by fever, development of nodular skin lesions, skin necrosis, generalized lymphadenitis, and edema of the ventral parts of the body and extremities. The disease can be seen in buffaloes, cattle, giraffes and impalas.

The causative agent of the disease is the bovine lumpy skin disease virus (LSDV), which has an antigenic relationship with strains of viruses that cause pox in sheep and goats, which differ at the genetic level, and together with it form an independent genus *Capripoxvirus*, family *Poxviridae*.

LSDV is one of the largest known human and animal viruses, virions have a spherical shape, the diameter of the virion is 300-450 nm. LSDV genomic DNA has a size about 150 thousand bp.

In order to prevent lumpy skin disease, active attenuated vaccines are considered effective [1]. They have been used in veterinary practice for many years and have also shown their safety, reliability and the ability to guarantee long-term protection. The development of attenuated lumpy skin disease vaccines follows a traditional approach. In the process of prolonged passaging of the virus in the cells of permissive and non-permissive hosts, mutations appear in the genome, which lead to a decrease in virulence. The mechanism of attenuation remains unclear. In this regard, there is a threat of reversion to virulence [2-4].

An alternative method for attenuating viruses has become site-directed mutagenesis. In recent years, the creation of attenuated poxviruses has increasingly begun to use genetic engineering methods, namely, the method of inactivation of virulence genes [5-8]. Potential virulence genes that confer increased pathogenicity and increase the replication activity of capripoxviruses in immunocompetent hosts have been identified by genome sequencing and annotation [9]. Only a few genes of capripox viruses have received experimental confirmation of their functions. Pilot studies were conducted in which two putative

immunomodulatory genes (ORF005 and ORF008) were deleted separately from the genome of a virulent LSDV field isolate. As a result of the study, it was determined that the deletion of the genes resulted in the manifestation of avirulent LSDV phenotype in cattle, but was safe in sheep and goats. Also, other authors obtained a strain of sheeppox virus (SPPV) in which the ORF019 gene was deleted. As a result of infection of lambs with the virus, data were obtained that indicate that the ORF019 gene is an important determinant of SPPV virulence in sheep [10-12].

Previously, we obtained a recombinant LSD virus Atyrau-B with a deletion of the LSDV008 gene. This gene encodes an interferon gamma receptor-like protein (IFN- $\gamma$ ) and is a potential virulence gene. Using the myxoma virus as an example, it was shown that this gene is expressed early after infection and remains in the supernatants of infected cells until late postinfection periods, inhibits the binding of IFN- $\gamma$  to its cellular receptor, thereby eliminating its antiviral activity [13]. Deletion of genes in the viral genome should not affect their replication activity in vitro. In these studies, we studied the effect of deletion of the LSDV008 gene on the cultural properties of the recombinant Atyrau-B virus.

### Experimental

LSDV viruses were used in the study: virulent strain Dermatitis nodulares/2016/Atyrau/KZ (Atyrau-KZ), recombinant Atyrau-B with LSDV008 gene knockout; and the following cell cultures: primary trypsinized lambs testicle cells (LT), transplantable bovine kidney cell line (MDBK), transplantable African green monkey kidney epithelial cell line (Vero), transplantable saiga kidney cell line (SK), transplantable calf testicular cell line (CT).

The sensitivity of cell cultures to viruses was determined by the method of successive passages. A monolayer cell culture was infected with Atyrau-KZ and Atyrau-B viruses and cultivated until a 100% cytopathic lesion of the monolayer appeared. Infected cells were lysed by double freeze-thaw. The infectious activity of viruses was determined by microtitrating in LT cell culture. The calculation of infectious activity was carried out according to the method of Reed and Mench and expressed in lg TCD<sub>50</sub>/cm<sup>3</sup>. Accounting for the results of microtitrating was carried out on the 10th day of incubation.

### Results and Discussion

To study the cultural properties of the Atyrau-B virus, five successive passages were carried out in various cell cultures. The parent Atyrau-KZ virus was used for comparison. The research results are presented in Figure 1 and Table.

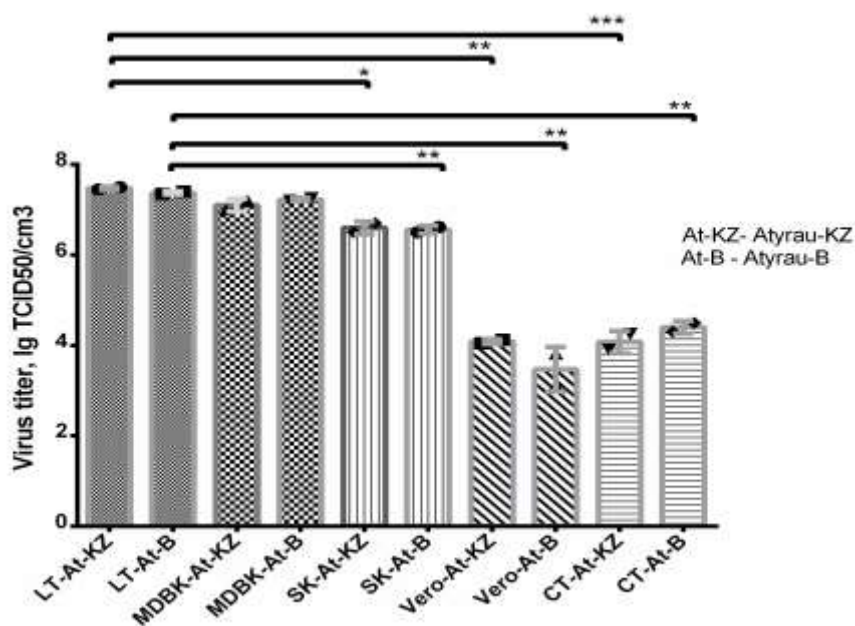


Figure 1. Infectious activity of Atyrau-KZ and Atyrau-B viruses in different cell cultures after the fifth successive passage



Reproduction of LSD viruses Atyrau-KZ and Atyrau-B in cell cultures (n=3)

Cell culture	Atyrau-KZ			Atyrau-B		
	Passage number	Cultivation time, days	Infectious activity titer, lg TCD50/cm <sup>3</sup>	Passage number	Cultivation time, days	Infectious activity titer, lg TCD50/cm <sup>3</sup>
LT	1	5	7,45±0,25	1	4	7,20±0,10
	2	4	7,20±0,10	2	4	7,35±0,05
	3	4	7,30±0,20	3	4	7,20±0,30
	4	4	7,80±0,10	4	4	7,00±0,10
	5	4	7,50±0,00	5	4	7,40±0,10
MDBK	1	7	7,20±0,10	1	7	7,00±0,30
	2	5	7,40±0,10	2	5	7,50±0,00
	3	5	7,30±0,40	3	5	7,10±0,00
	4	5	6,80±0,10	4	5	6,50±0,00
	5	5	7,00±0,25	5	5	6,75±0,25
SK	1	6	6,70±0,20	1	6	6,50±0,00
	2	6	6,37±0,12	2	6	6,50±0,25
	3	6	6,30±0,00	3	6	6,10±0,20
	4	6	6,75±0,00	4	6	6,50±0,00
	5	6	6,50±0,00	5	6	6,62±0,12
Vero	1	7	5,15±0,25	1	7	5,00±0,10
	2	10	4,75±0,50	2	10	4,20±0,10
	3	10	4,05±0,05	3	10	3,82±0,07
	4	10	2,60±0,30	4	10	2,40±0,10
	5	10	4,12±0,87	5	10	3,12±0,37
CT	1	7	5,30±0,00	1	7	5,00±0,30
	2	7	5,20±0,10	2	7	4,70±0,20
	3	8	4,60±0,10	3	8	3,90±0,00
	4	8	4,30±0,00	4	8	3,80±0,30
	5	8	4,50±0,00	5	8	4,25±0,50

From the data presented in Table, it can be seen that the accumulation of lumpy dermatitis viruses Atyrau-B and Atyrau-KZ is not the same in the used cell cultures.

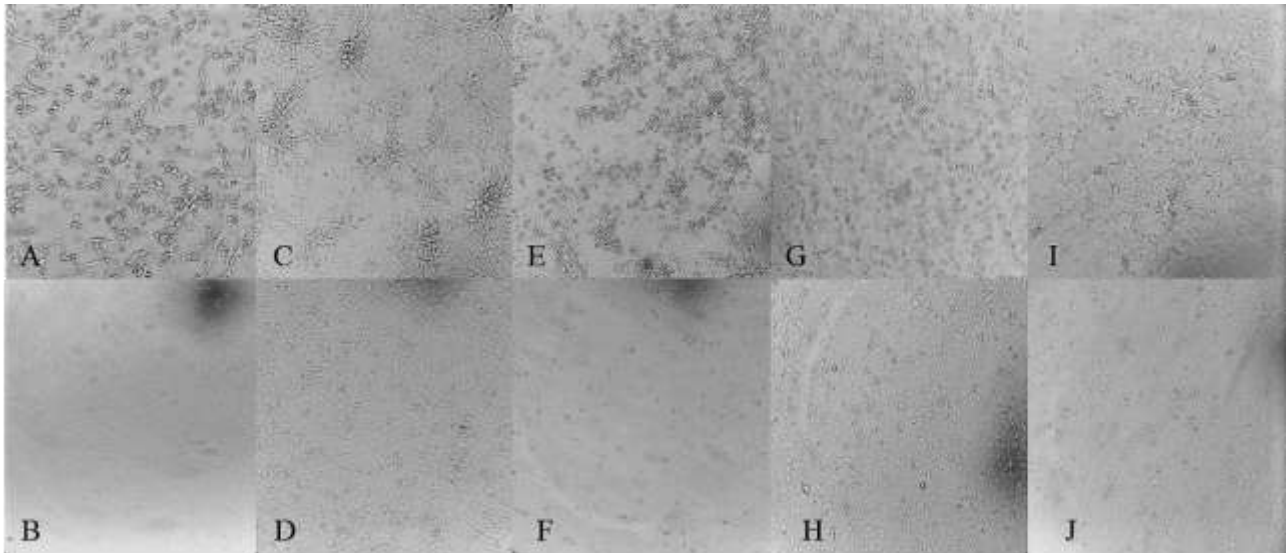
Both viruses replicated stably for five consecutive passages (observation period) in LT, MDBK, and SK cell cultures. There were no significant differences in terms of cultivation and infectious activity for Atyrau-B and Atyrau-KZ viruses. Complete damage to the monolayer of LT cells was noted after 4 days of cultivation, infectious activity reached 7.0-7.5 lg TCD50/cm<sup>3</sup>. In MDBK and SK cells, the virus accumulated up to 6.5-7.5 and 6.0-6.5 lg TCD50/cm<sup>3</sup>, respectively. The duration of incubation was 5 and 6 days, respectively.

In cultures of Vero and CT cells, even with an increase in the cultivation time, the activity of both viruses in the process of passaging significantly decreased compared to the culture of LT cells ( $p < 0.001$ ) and ( $p < 0.001$ ), respectively (Fig. 1). The titer of Atyrau-KZ and Atyrau-B viruses in Vero cells was within 2.60±0.30-5.15±0.25, 2.40±0.10-5.00±0.10 lg TCID50/cm<sup>3</sup>, in CT cells 4.30±0.00-5.30±0.00, 3.80±0.30-5.00±0.30 lg TCID50/cm<sup>3</sup>, respectively.

Lumpy skin dermatitis viruses Atyrau-KZ and Atyrau-B exhibited a similar cytopathic effect in the monolayer of used cell cultures (Fig. 2). At the same time, the morphological manifestation of the cytopathic action of viruses differed in different cultures.

In the culture of LT and SK cells, 72 hours after infection, the cells begin to stretch and separate, by 3-4 days, fusion of cell membranes and rupture of the monolayer were observed (Fig. 2A and 2G). In MDBK cells, 72 hours after infection, the formation of pronounced conglomerates was observed, and by day 4, the monolayer was broken and fragmented (Fig. 2C).

Visible changes in Vero and CT cell cultures were noted on days 5-6 of cultivation in the form of deformation of individual cells, by days 7-8, the formation of pronounced localized foci of cell damage with the formation of conglomerates (Fig. 2E and 2I).



A - Monolayer of LT cell culture 72 h after infection with the Atyrau-B virus; B - Monolayer of uninfected LT cell culture; C - Monolayer of MDBK cell culture 72 h after infection with Atyrau-B virus; D - Monolayer of uninfected MDBK cell culture; (E) Vero cell culture monolayer 72 h after infection with the Atyrau-B virus; F, Monolayer of uninfected Vero cell culture; G, Monolayer of SK cell culture 72 h after infection with the Atyrau-B virus; H, monolayer of uninfected SK cell culture; I – Monolayer of CT cell culture 72 h after infection with the Atyrau-B virus; J – Monolayer of uninfected CT cell culture

Figure 2. Cytopathic effect of lumpy skin disease virus Atyrau-B in the studied cell cultures (magnification 10×)

### Conclusions

Currently, a large number of poxvirus genes encoding virulence factors have been identified. It has been experimentally confirmed that knockout of some of them leads to attenuation of viruses in vivo. The LSDV008 gene is a homologue of the B8R gene of the vaccinia virus. Poxvirus interferon- $\gamma$  receptor-like proteins generally show limited affinity to the extracellular domains of the mammalian interferon- $\gamma$  receptor and likely competitively prevent interferons from binding to their native receptors. Knocking out virulence genes to produce attenuated viruses should not affect the reproduction of viruses in vitro, since the production of vaccines requires the production of highly active viral suspensions. As a result of our studies, it was established that knockout of the LSDV008 gene did not affect the replication activity of the recombinant Atyrau-B virus in vitro. The most sensitive cell systems for reproduction of both parental Atyrau-KZ and recombinant Atyrau-B viruses are LT, MDBK, and SK cell cultures. The resulting recombinant will be used to develop a new generation of vaccines against lumpy dermatitis in cattle. The possibility of its use as a vaccine vector for the creation of polyvalent vaccines against infectious diseases of animals will also be evaluated.

### Acknowledgments

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### **LSDV008 генінің нокаутының рекомбинантты нодулярлы дерматит вирусының өсу қасиеттеріне әсері**

Нодулярлы дерматит вирусы (LSDV) — ірі қара малда ауыр жүйелі ауруды тудыратын және географиялық шекарасын жылдам кеңейтетін поксвирус. Нодулярлы дерматит (НД) безгегімен, теріде, шырышты қабаттарда және ішкі мүшелерде түйіндер пайда болуымен сипатталады. Аурудың лимфа түйіндері ісінгенде малдың арықтауына немесе өлімге әкелуі мүмкін. Соңғы жылдары бұл ауру Азияның әртүрлі бөліктерінде эндемиялық сипатқа ие болып, мал шаруашылығына айтарлықтай экономикалық зиян келтірді. НД үшін арнайы емдеу құралдары болмағандықтан, вакцинация ауруды бақылау және жоюдың ең тиімді әдісі. Толық жарамды иммунитетті тірі әлсіретілген вакциналармен вакцинациялау арқылы алуға болады. Ұзақ жүргізілген пассаждар арқылы пайда болған аттенуация геномдағы кездейсоқ мутациялармен байланысты және әлсіреу механизмі анық емес. Вирустық геномдағы вируленттілік гендерін гендік инженерия әдістері арқылы жою — жоғары әлсіреген поксвирустарды құрудағы ең перспективалы бағыт. LSDV008 гені  $\gamma$ -интерферон рецепторына ұқсас ақуызды кодтайды және НД вирусы үшін потенциалды вирулентті ген болып саналады. Бұл зерттеулерде біз LSDV008 генінің жойылуының Атырау-В рекомбинантты вирусының өсу қасиеттеріне әсерін зерттедік. Бақылау ретінде ата-аналық НД вирусы *Dermatitis nodulares/2016/Atyrau/KZ* (Атырау-KZ) қолданылды. Зерттеулер нәтижесінде ген нокаутының *in vitro* рекомбинантты Атырау-В вирусының репликация белсенділігіне әсер етпейтіні анықталды. Рекомбинантты вирус жасушаларда ата-аналық вируспен бірдей титрлерде жинақталған. Атырау-KZ және Атырау-В НД вирустарының көбеюі үшін ең сезімтал жасушалық жүйелер — қозының аталық безі (LT), бұқа бүйрегі (MDBK) және ақбөкен бүйрегі (SK) жасушалары және де оларды әрі қарай ғылыми зерттеулерде вирустық массаны алу үшін пайдалануға болады.

*Кілт сөздер:* нодулярлы дерматит вирусы, жасуша өсіндісі, өсіру, вирус титрі, репликация, инфекциялық белсенділік, ген нокауты, аттенуация.

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### **Влияние нокаута гена LSDV008 на культуральные свойства рекомбинантного вируса нодулярного дерматита**

Вирус нодулярного дерматита (LSDV) представляет собой поксвирус, который вызывает тяжелое системное заболевание крупного рогатого скота и быстро расширяет свои географические границы. Но-



дулярный дерматит (НД) характеризуется лихорадкой, образованием узелков на коже, слизистых оболочках и внутренних органах. Заболевание может вызвать исхудание с увеличением лимфатических узлов, а иногда и гибель. В последние годы данное заболевание имеет эндемичное значение в различных частях Азии, нанося значительный экономический ущерб животноводству. Так как отсутствуют специфические методы лечения НД, вакцинация является наиболее эффективным способом контроля и искоренения болезни. Наиболее полноценный иммунитет можно получить при вакцинации живыми аттенуированными вакцинами. Аттенуация посредством длительных пассажей связана со случайными мутациями в геноме, и механизм аттенуации остается не ясен. Направленное удаление генов вирулентности в вирусном геноме методами генной инженерии является наиболее перспективным направлением в создании аттенуированных поксвирусов. Ген LSDV008 кодирует белок, подобный рецептору  $\gamma$ -интерферона, и является потенциальным геном вирулентности вируса НД. В настоящей работе мы изучили влияние делеции гена *LSDV008* на культуральные свойства рекомбинантного вируса *Atyrau-B*. В качестве контроля использовали родительский вирус НД *Dermatitis nodulares/2016/Atyrau-KZ (Atyrau-KZ)*. В результате проведенных исследований установлено, что нокаут гена не повлиял на репликационную активность рекомбинантного вируса *Atyrau-B in vitro*. Рекомбинантный вирус накапливался в культурах клеток в таких же титрах, как и родительский вирус. Наиболее чувствительными клеточными системами для репродукции вирусов НД *Atyrau-KZ* и *Atyrau-B* являются культуры клеток тестикул ягненка (LT), почки быка (MDBK) и почки сайги (SK), которые могут быть использованы для получения вирусной массы в дальнейших научных исследованиях.

*Ключевые слова:* вирус нодулярного дерматита, культура клеток, культивирование, титр вируса, репликация, инфекционная активность, нокаут генов, аттенуация.

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## The first lumpy skin disease outbreak in cattle in Kazakhstan

Lumpy skin disease (LSD) is an emerging trans-boundary viral disease of cattle originating from the African continent. Here, we describe the first LSD outbreak reported in the Republic of Kazakhstan in July 2016, as well as associated clinical manifestations of the disease, diagnostic methods, and control measures taken to combat further spread of the pathogen. To determine the cause of the disease, samples were taken from sick and dead animals, as well as from insects and ticks. LSDV DNA was detected by PCR in all samples from dead animals and ticks (*Dermacentor marginatus* and *Hyalomma asiaticum*), in 14.29% of samples from horseflies (*Tabanus bromius*), and in one of the samples from two *Stomoxys calcitrans* flies. The reproductive LSD virus was isolated from organs of dead cattle and insects in the culture of LT and MDBK cells. The virus accumulated in cell cultures of LT and MDBK at the level of the third passage with titers in the range of 5.5–5.75 log<sub>10</sub> TCID<sub>50</sub>/cm<sup>3</sup>. During the outbreak, the number of affected cattle within an epidemiological unit reached 459 cattle out of 3557 registered susceptible cattle, with 12.90% morbidity and 0.96% mortality. This manuscript presents the epidemiological situation; the diagnosis; the control measures, including mass vaccination; and the stamping out campaign.

*Keywords:* control measures, diagnosis, epidemiological data, lumpy skin disease, *Stomoxys calcitrans*, *Hematobia irritans*, outbreak.

### Introduction

Lumpy skin disease virus (LSDV) belongs to the *Capripoxvirus* genus of the, *Poxviridae* family, is a highly contagious infectious disease of cattle. It is characterized by fever, skin nodules, enlargement of superficial lymph nodes, salivation, lacrimation and nasal discharge as well as oedema and swellings of the joints and the dewlap [1]. The World Organization for Animal Health classifies Lumpy Skin Disease (LSD) as a noticeable disease due to its significant economic impact [2].

LSDV was first discovered in Zambia, where it was recorded in 1929. Subsequently, LSDV has become endemic almost the whole African continent and in the Middle East, Turkey, Azerbaijan continuing to spread to the North posing a threat to Europe and the Central Asian region. In 2015, LSD outbreaks were documented in Greece [3], from where it spread to the Balkan region. Similarly, in 2015, the disease was clinically confirmed in North Caucasus of Russia where it becomes epidemic and spread throughout the country [4, 5]. In 2016, LSD re-emerged in several regions of Southern Russia, including Astrakhan oblast bordering with Atyrau region in West Kazakhstan.

The paper aims to report on the first occurrence of LSD in the Republic of Kazakhstan and to describe the associated clinical features of the disease, diagnostic methods as well, as control measures taken to eliminate further dissemination of the pathogen.

According to Statistic Bureau of Agro-industrial complex of Ministry of Agriculture of the Republic of Kazakhstan the total cattle population of the country is estimated to be about 7.161 million heads, mostly local breeds (87.1%); the remaining are hybrids and exotic breeds (data not available). The livestock system practiced in the country is mixed farming, including intensive, small-scale beef and dairy management. Live animals are not exported from the country; meanwhile, the export share of animal products in 2017 accounted for 20 thousand tons. In rural areas, cattle are the primary source of income and mainly kept for milk and meat production. The commercial smallholding dairy and beef farms are mostly market-oriented and located around urban areas practicing intensive management.

### Experimental

#### *Animal Ethics*

The protocol was approved by the Committee on the Ethics of Animal Experiments of the Research Institute for Biological Safety Problems (RIBSP) of the Science Committee of Ministry of Education and Science of the Republic of Kazakhstan (permit number: 1205/106).

Place on investigation are noted in Figure 1.

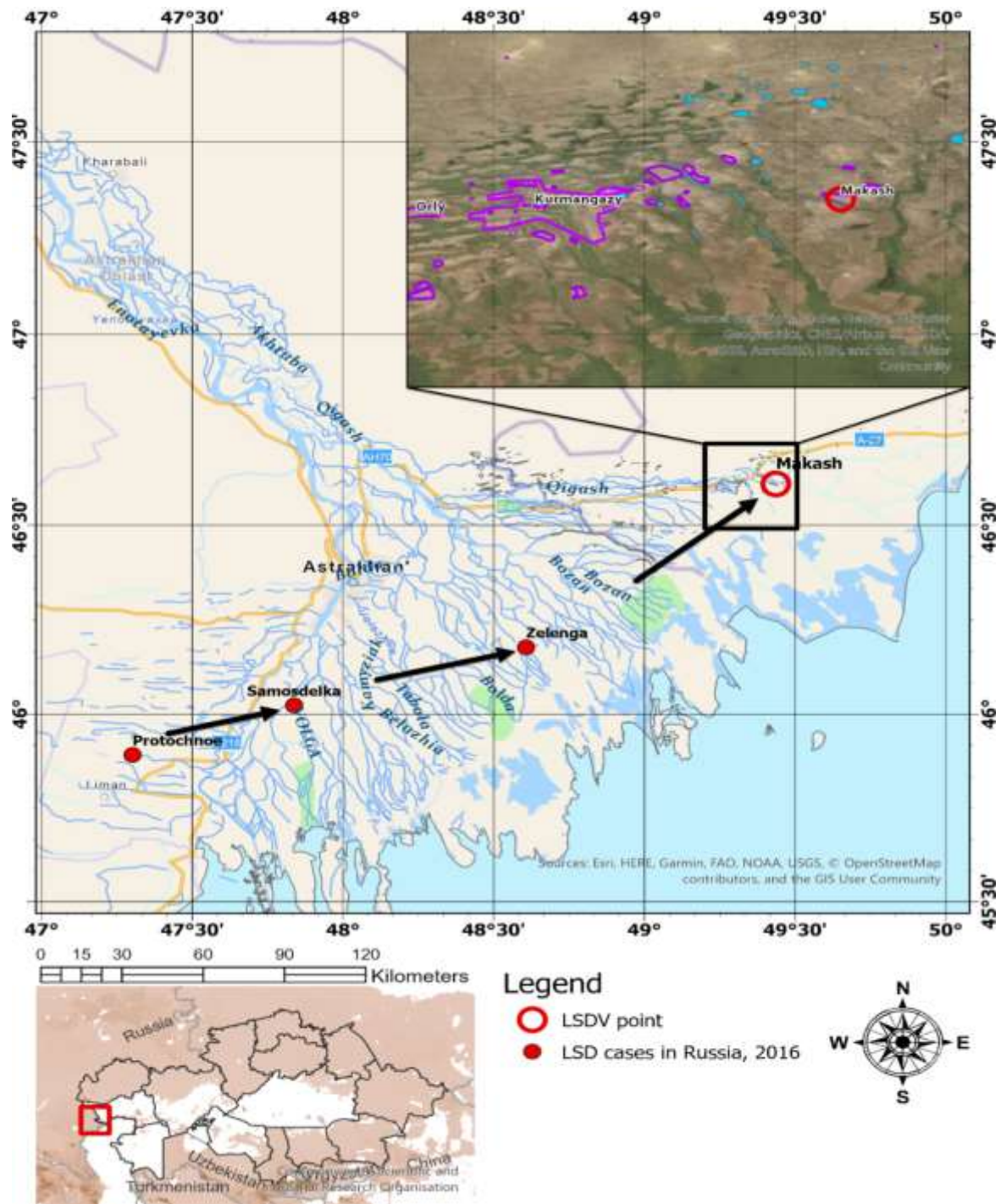


Figure 1. The location of the LSD outbreak in Atyrau region. Areas drawn in pink – indicate seasonal communal grazing lands (Food and Agriculture Organization (FAO))

Blood samples and skin lesions (Fig. 2) were collected for testing to the Virology section of the BSL-3 laboratory of the RIBSP and to OIE Reference Laboratory, All-Russian Research Institute for Animal Health (ARRIAH).



Figure 2. Cattle exhibiting LSD characteristic clinical signs in the outbreak foci in Republic of Kazakhstan in 2016. The body surface of infected animals exhibited extensive circumscribed and convex skin nodules (a-d) with ulceration of the scrotum and the teats (e-f)

#### *Control Measures*

In the first affected farm, a total stamping out as well as incineration of carcasses were undertaken to prevent the spread of the disease locally. Quarantine and cattle movement controls were initiated within the Kurmangazy District, as well as strict restrictions on vehicles commuting to and from the affected zones. In addition, ring vaccination were conducted in a radius of 30 km. Such a significant coverage explained by the high density of livestock population and use of common grazing lands on South and West from the initial foci. In Kazakhstan, vaccination campaign was launched immediately after notification was sent to OIE, more than 70.000 cattle in the affected areas and neighboring regions (Makhambet, Isatay, Makat) were vaccinated during the vaccination campaign. A total of one million doses of LSD vaccine (LUMPIVAX®,



Neethling-type, Kenya) were purchased before the outbreak and used in cattle against LSD. In Makash, veterinary personnel that were involved in LSD control and eradication campaign wore personal protective equipment (PPE) when visiting affected farms. Moreover, animal premises (walls, ceilings and floor) were disinfected, utilizing Lysoformin 3000. Farmers were instructed to apply the disinfectants every day.

In response to the LSD outbreak in 2016 on the Russian side of the border, the veterinary authorities culled only those cattle showing typical clinical signs (partial stamping out) and implemented movement restrictions. Susceptible cattle were treated with insect repellents and vaccinated with a heterologous live sheeppox virus vaccine at a dose of 10<sup>-4</sup> TCID<sub>50</sub>, produced locally by ARRIAH [5-7]. An eradication program was enacted according to the State Contingency Plan (Directive N 339-2) after field samples provided positive results using conventional PCR.

Until 21 July 2016, in the Kurmangazy District, among the officially registered 3557 cattle, the number of affected cattle reached 459 with morbidity and mortality rates accounting for 12.90% and 0.96% respectively. The case fatality rate was 7.41% [8, 9]. Kazakhstan veterinary services carried out a total stamping out measure at this first affected farm.

#### *Sample collection*

Samples were taken from 96 cattle of different ages and sexes with clinical signs characteristic of FMD. In severe cases, there was an increase in body temperature up to 42°C followed by severe salivation, nasal discharge and inflammation of the mucous membranes. The body surfaces of infected animals were completely covered with circumscribed and convex nodules, which were hard and rough when palpated. Animals exhibiting mild symptoms of FMD showed enlargement of superficial lymph nodes and edema of the limbs and brisket. A total of 74 blood samples, 47 skin lesions, 4 samples of internal organs (2 lymph nodes, 2 lung tissue samples), were taken from diseased and dead animals by official field veterinarians and dispatched to the RIBSP. In addition, 14 hard ticks attached to the diseased host were collected during the clinical examination of infected animals. Moreover, 21 horn flies (*Hematobia irritans*) and 25 stable flies (*Stomoxys calcitrans*) were caught within livestock premises using a commercial fly catching unit "Miniature CDC light trap with UV light" (USA) to investigate a possible insect vector involvement in the transmission of LSD in the field. The light trap was hung from the ceiling of the barn and checked every two hours for insects. The time of insect collection was determined as follows: 12 hours during the night.

#### *Virus isolation*

Virus isolation (VI) was conducted according to Standard Operational Procedures of the BSL-3 laboratory of the RIBSP. The tests were carried out as described by OIE (2018). Briefly, 1 ml buffy coat or supernatant were administered on to lamb testes cells in 25 cm<sup>2</sup> cell culture flasks and allowed to incubate at 37°C for 1 hour. Following incubation cell culture growth media was removed and cell monolayer was rinsed with PBS and overlaid with Glasgow's Minimal Essential Medium containing 0.1% penicillin, 0.2% gentamycin and 2% fetal calf serum (Thermo Fisher Scientific). The cell monolayer was examined daily for characteristic cytopathic effect (CPE). In the case no CPE was observed, the cell culture was freeze-thawed three times and second or third blind passages were carried out. Cell culture flasks showing CPE were tested with gel-based PCR to confirm that the CPE change was induced by LSDV.

#### *Virus detection by PCR*

A PCR assay was performed using the protocol published by Tuppurainen, Venter, and Coetzer [10, 11]. For DNA extraction, a QIAamp DNA Kit (QIAGEN, USA) was used according to manufacturer's instructions.

For PCR assay, to produce 192 bp of amplified nucleotides reactions the forward 5'-TCC-GAG-CTC-TTT-CCT-GAT-TTT-TCT-TAC-TAT-3' and reverse 5'-TAT-GGT-ACC-TAA-ATT-ATA-TAC-GTA-AAT-AAC-3' primers were used [12]. The conditions for DNA amplification in a Thermal Cycler (Eppendorf Mastercycler) were as follows: 95°C for 2 min, 95°C for 45 s, 50°C for 50 s, 72°C for 1 min (34 cycles) and 72°C for 2 min. Obtained PCR products were loaded in 1.5% agarose-gel electrophoresis and the results visualized using Bio-imaging systems MiniBIS Pro (Israel).

Complete genome sequencing of LSDV field strain was performed in collaboration with Kazakh Scientific-Research Veterinary Institute LLP (Kazakhstan) and Sciensano, Unit Exotic Viruses and Particular Diseases (Belgium) and has been deposited in GenBank under accession number MN642592 (LSDV isolate Kubash/KAZ/16) [13].

## Results

### PCR and Virus isolation

From 7th July until the end of November 2016, three outbreaks were confirmed within Makash village. A total number of 425 cattle were disposed of in the eradication program. A total of 185 samples were tested by PCR and VI. The presence of viral nucleic acid was laboratory-confirmed in a total of 102 samples, whereas 52 samples tested positive for VI. All skin lesions tested positive by PCR and VI. Viral DNA was detected in 24 of 74 blood samples and whereas virus isolation revealed a LSDV characteristic CPE in 3 out of 74 blood samples. Internal organs were tested positive by PCR, while it was not possible to isolate a live virus in cell culture infected from lymph nodes or lungs (Table). In addition, LSDV DNA was recovered from 6 out of 14 ticks, 8 out of 21 horn flies and 14 out of 25 stable flies' samples while live virus was isolated only from 2 out of 25 *Stomoxys calcitrans* samples.

Table

Summary of PCR and Virus isolation testing results [14]

Type of Sample	PCR (Positive result)	Virus isolation (Positive result)	Mean Ct value
Skin lesions	47/47	47/47	16.7
Blood	24/74	3/74	27.1
Lung	1/2	0/2	11.3
Lymph nodes	2/2	0/2	15.8
Dermacentor	6/14	0/14	16.4
<i>Stomoxys calcitrans</i>	14/25	2/25	24.3
Hematobia irritants	8/21	0/21	22.9

## Discussion

### Epidemiological investigation

To date, the source of infection and the mode of transmission of the virus to Kazakhstan remain unclear. This issue is especially urgent for trans-boundary infections. Most researchers believe that spread of the causative agent of LSD outside the epizootic focus region to a new area happens due to unauthorized movements of infected animals in the presence of insect vector [15]. These assumptions could be supported by the presence of river delta along border, which is thought to be auspicious habitat for reproduction of the insect vectors. Transmission of LSDV within the herd occurs by aerosols when a sick animal exhales, via direct contact between animals, through contaminated water and feed or by blood-feeding insect [16, 17]. It has been suggested that the spread of LSD into countries such as Iran, Azerbaijan, Republic of Dagestan, Georgia and Russia Federation was associated with the direct and indirect animal contacts when the farmers were using shared pasture lands between the bordering states [18]. Thus, practicing communal grazing and illegal animal trading between trans-boundary farms can serve as source of LSDV introduction into new area. Azerbaijan scientist suggested that also the role of human factors could be involved in mechanical transmission of the pathogen via direct contact with infected animals and their environment, farm workers may transport and spread virus to healthy herd [4]. In addition, Annandale et al. [19] reported that cattle insemination with infectious semen lead to disease development.

Despite assumption in the transmission of LSD mentioned above, it is generally accepted that a variety of blood-feeding insects play a significant role in LSDV transmission by acting as mechanical vectors. According to the epizootic investigation outcomes of LSD outbreaks in Egypt, it was considered highly likely that the pathogen was transferred by stable flies (*Stomoxys calcitrans*) [20]. This assumption was based on the seasonality of outbreaks of LSD, occurring during hot and wet summer seasons [6, 16, 21]. In recent studies, LSDV transmission from diseased to susceptible cattle by *Stomoxys* species have been demonstrated successfully under laboratory condition [15, 22].

A mathematical model of synoptic system used in recent study to calculate air long-distance dispersal (LDD) of LSDV in Israel revealed that LDD transmission by air is a feasible way of dissemination of vector borne diseases in the Middle East and should be taken into consideration when evaluating risk for new outbreaks [23]. In other studies, mathematical modeling revealed that under natural conditions the blood-feeding insects range rarely exceeds 5 km [24]. Moreover, wind has a direct impact on insect distribution [25]. Such a significant coverage range and vector capability of stable flies to carry pathogen may lead to

LSDV escape from the initial outbreak foci and rapid dissemination over neighboring farms. In relatively recent clinical experiments, the potential of ticks as a mechanical vector has been successfully demonstrated. Ticks in different molting stages have carried LSDV following feeding to repletion on artificially infected animals [26, 27]. In addition, LSDV has been detected in the saliva of mature ticks making them capable of virus transmission.

In the Kazakhstani scenario of disease development, LSD was recorded mostly among emaciated animals, lactating cows, and calves. During the current LSD epidemic in Kazakhstan, the morbidity and mortality rates constituted 12.90% and 0.96% respectively. Due to rapid response of State Veterinary Service in combination of strict quarantine, stamping out and mass vaccination campaign allowed limiting LSD outbreak within the initial foci.

In our study, several arthropod species including ixodid ticks (*Dermacentor marginatus* and *Hyalomma asiaticum*), horseflies (*Tabanus bromius*), and other biting flies (*Stomoxys calcitrans*) collected in the disease focus were assayed as potential transmitters. All tick samples were positive, and a proportion of horse flies and *Stomoxys* flies. The first LSDV isolate in cell culture was obtained from sampled horseflies (*Tabanus bromius*) collected during the outbreak of the disease. These results support the studies of Sohier et al. (2019), which showed experimentally that horseflies can mechanically transmit LSDV. We have shown that all individuals sampled, of both species of ticks collected from the region of the outbreak of the disease, were PCR positive for LSDV, and the virus was isolated from the pool of ticks of both species, through cell culture. Until 21 July 2016, in the Kurmangazy District, among the officially registered 3557 cattle, the number of affected cattle reached 459 with morbidity and mortality rates accounting for 12.90% and 0.96% respectively. The case fatality rate was 7.41% (OIE 2016). Kazakhstan veterinary services carried out a total stamping out measure at this first affected farm.

### Conclusion

Given the fact that there is a significant density of livestock in the West Kazakhstan oblast and unauthorized trade in animals occurs, it is likely that LSD will continue to spread, leading to serious social and economic consequences for the whole country and posing a real threat to animal husbandry of developing countries of the Central Asia.

Studies have shown that a new disease in cattle in the Atyrau region of Kazakhstan in 2015 was caused by FMD infection. The virus was also detected among *Tabanus bromius* and *Stomoxys calcitrans*, indicating the possibility of these species as vectors of FMD in this region.

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## Қазақстанда ірі қара малдың сүйелді дерматит ауруының бірінші ошақтарының өршуі

Ірі қара малдың кесек тері ауруы (КТА) — Африка континентінен шыққан ірі қара малдың трансшекаралық вирустық ауруы. Мұнда біз Қазақстан Республикасында 2016 жылдың шілдесінде тіркелген бірінші КТА індетін, сондай-ақ осы аурудың клиникалық көріністерін, диагностикалық әдістерді және қоздырғыштың одан әрі таралуымен күресу үшін қабылданған бақылау шараларын сипаттаймыз. Аурудың себебін анықтау үшін ауру және өлі жануарлардан, сонымен қатар жәндіктер мен кенелерден



үлгілер алынды. КТА ДНҚ-сы *Dermacentor marginatus* және *Hyalomma asiaticum* сынамаларының барлығында, *Tabanus bromius* сынамаларының 14,29%-да және *Stomoxys calcitrans* сынамаларының бірінде ПТР әдісімен анықталды. Эпидемиологиялық бірлік шегінде тіркелген 3557 бас ірі қара малдың ішінен 459 ірі қара мал ауру кезінде зардап шекті, оның ішінде ауырған мал 12,90 % және өлім-жітімі 0,96 % құрады. Бұл жұмыста эпидемиологиялық жағдай, диагноз, жаппай вакцинациялауды қоса алғанда, бақылау шаралары және ауру ошағын жою науқаны ұсынылған.

*Кілт сөздер:* бақылау шаралары, диагноз, эпидемиологиялық деректер, сүйелді дерматит, *Stomoxys calcitrans*, *Hematobia irritans*, ауру ошағы.

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## Первая вспышка нодулярного дерматита крупного рогатого скота в Казахстане

Нодулярный дерматит (НД) — это новая трансграничная вирусная болезнь крупного рогатого скота, происходящая из африканского континента. Здесь мы описываем первую вспышку НД, зарегистрированную в Республике Казахстан в июле 2016 г., а также связанные с ней клинические проявления заболевания, методы диагностики и меры борьбы с дальнейшим распространением возбудителя. Для определения причины заболевания были взяты образцы от больных и мертвых животных, а также от насекомых и клещей. ДНК НД была обнаружена методом ПЦР во всех пробах от *Dermacentor marginatus* и *Hyalomma asiaticum*, в 14,29 % проб от *Tabanus bromius* и в одной из проб от *Stomoxys calcitrans*. Репродуктивный вирус НД был выделен из органов мертвого крупного рогатого скота и насекомых в культуре клеток LT и MDBK. Вирус накапливался в культурах клеток LT и MDBK на уровне третьего пассажа с титрами в диапазоне 5,5–5,75 log<sub>10</sub> TCID<sub>50</sub>/см<sup>3</sup>. Во время вспышки количество пораженного крупного рогатого скота в пределах эпидемиологической единицы достигло 459 голов из 3557 зарегистрированных восприимчивых голов крупного рогатого скота с заболеваемостью 12,90 % и смертностью 0,96 %. В настоящей работе представлены эпидемиологическая ситуация, диагноз, меры борьбы, включая массовую вакцинацию и кампанию по искоренению очага болезни.

*Ключевые слова:* меры контроля, диагностика, эпидемиологические данные, нодулярный дерматит, *Stomoxys calcitrans*, *Hematobia irritans*, вспышка.

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## Identification on SNP polymorphisms associated with meat productivity in the local Kazakh horse breed

The aim of our study was to genotype local horses of the Kazakh breed, including the Zhabe u types from the Pavlodar and Zhetysu regions, as well as the Adai type from the Mangystau region, using PCR-RFLP methods for the LCORL and PRKAG3 gene loci. A total of 41 horses were included in the study. The results showed that the frequency of the homozygous genotype TT at the LCORL locus was 81.25% in Zhabe Pavlodar horses, with a heterozygous genotype TC of 18.75%. In Kazakh horses of the Adai type from Mangystau and Zhabe type from Zhetysu, no genetic polymorphism was detected, and all individuals had a homozygous genotype – TT. For the PRKAG3 locus, genetic polymorphism was only found in Adai type horses, with one mare out of 17 tested horses having a heterozygous genotype – CT (5.89%). Horses of the Zhabe type in Pavlodar and Zhetysu regions had a single homozygous CC genotype. The level of homozygosity for the LCORL gene locus in the three studied groups was: 81.25, 100.0 100.0. For the PRKAG3 gene is 100.0, 94.11, 100.0. The level of heterozygosity for the LCORL gene locus in horses of Pavlodar region was 18.75%, for the PRKAG3 gene locus in horses of Mangystau region 5.89%. Thus, LCORL and PRKAG3 gene polymorphisms play a significant role in determining the development of muscle mass, skeletal structure, chest circumference, and growth rate in horses. Therefore, these SNP polymorphisms can potentially serve as DNA markers for evaluating meat production in horses.

*Keywords:* horse genotyping, Kazakh horses, Zhabe, Adai, LCORL gene, PRKAG3 gene, PCR-RFLP analysis, DNA markers of productivity.

### Introduction

Increasing meat production is currently the most relevant task facing livestock breeders in Kazakhstan. In solving this problem, in addition to cattle breeding and sheep farming, there is an opportunity to develop productive horse breeding. In breeding work aimed at improving the productive qualities of stud horses, Kazakh horses of the Zhabe type have exceptional importance. They differ from the main mass of other breeds of herd horses in Kazakhstan by a higher live weight and slightly larger size. An important link in breeding work with Kazakh Zhabe horses is the development of selection methods to improve their breeding and productive qualities. The successful solution of this task largely depends on increasing the efficiency of breeding work through the wide implementation of achievements in population genetics and the maximum realization of the genetic potential of horse productivity [1].

Analysis of foreign literature indicates that the study of polymorphisms in the LCORL and PRKAG3 genes has practical significance, as the alleles of these genes have an associative impact on the development of muscles in horses.

The LCORL (ligand-dependent nuclear receptor corepressor-like) gene has been identified as a candidate gene associated with various traits, including body size, height, and skeletal growth in horses. LCORL is located on chromosome 3 in horses and is highly conserved across species, suggesting a conserved function in growth regulation [2].

One of the earliest studies on LCORL in horses was conducted by Makvandi-Nejad et al. who investigated the association of LCORL with height in Thoroughbred racehorses. The study found that the T allele of LCORL was significantly associated with height in Thoroughbreds, with the T allele carriers being taller than the CC homozygotes [2].

Another study conducted by Tetens et al. identified genetic loci associated with withers height in German Warmblood horses, and the findings indicated that the LCORL/NCAPG region on chromosome 3 plays an important role in determining withers height. The identification of this genetic variant may have important

implications for selective breeding programs aimed at producing horses of specific sizes for different purposes [3].

The results of the study Metzger et al. showed that there was a significant association between LCORL expression and body size in horses. Specifically, larger horses had higher levels of LCORL expression compared to smaller horses. Furthermore, the study identified two single nucleotide polymorphisms (SNPs) in the LCORL gene that were significantly associated with height and weight in horses [4].

Researchers Staiger et al. found a significant association between skeletal variation in the forelimbs and hind limbs of Tennessee Walking Horses and a region on chromosome 3 that includes the LCORL/NCAPG genes. This region had previously been identified as a candidate locus for withers height in horses, and this study provides further evidence of its role in determining skeletal size and morphology [5].

Tozaki et al. (2016) reported that certain sequence variants of the BIEC2-808543 locus, which was located near the LCORL gene, are associated with body composition in Thoroughbred racehorses undergoing training. The study found that specific sequence variants near the LCORL gene, particularly BIEC2-808543, were significantly associated with body composition traits such as body weight, body condition score, and subcutaneous fat thickness. These findings suggest that the LCORL gene may play a role in determining body composition and could potentially be used in breeding programs to select for desired body composition traits in Thoroughbred horses [6].

The PRKAG3 gene, also known as the AMP-activated protein kinase gamma 3 subunits, plays an important role in regulating energy metabolism in skeletal muscle. This gene has been the focus of several studies in horses, particularly in relation to athletic performance and muscle function [7].

A study was conducted by Park et al., to identify genetic variations, specifically for PRKAG3 gene SNPs, which may affect muscle development and intended performance in horses. The study included multiple horse breeds with varying phenotypes. Seven SNPs were identified, five of which resulted in amino acid substitutions. These genetic variations may have an impact on the traits of interest, including muscle development and performance, in horses [7] (The results of the study showed that certain variations in the PRKAG3 gene were associated with differences in muscle glycogen content and exercise performance in horses).

Armeiro et al. investigated the influence of specific genetic variations, or polymorphisms, on muscle performance-related traits associated with the PRKAG3 gene and male fertility-associated traits associated with the SPATA1 gene in Mangalarga horses. The study involved the analysis of DNA samples collected from a group of Mangalarga horses, followed by a series of molecular biology and statistical analyses to examine the relationships between genetic variations and performance traits [8].

In some breeds of livestock, including pigs [9], cattle [10] and goats [11], certain genetic variations in the PRKAG3 gene have been associated with traits related to meat quality, such as tenderness and juiciness.

According to the information provided above the LCORL and PRKAG3 genes has been shown to play a significant role in determining muscle development in horses.

Thus, investigating SNP polymorphisms at the loci of the LCORL and PRKAG3 genes in local horse breeds of the Kazakh population is relevant, as these markers are associated with the meat productivity of horses. The aim of this study was to investigate the distribution of genetic variants at the LCORL and PRKAG3 gene loci, determine the levels of heterozygosity and homozygosity, assess the genetic equilibrium at the studied gene loci, in local horse breeds such as Zhabe and Adai.

This study was conducted to determine the polymorphism of the LCORL and PRKAG3 genes and their association with body size in local Kazakh Zhabe and Adai horses using the PCR-RFLP method.

### *Experimental*

For the study, 41 blood samples from horses with EDTA were used, including 16 samples of the local Zhabe breed (LLP "Akshiman-Agro" of Pavlodar region), 17 samples of the local Adai breed (Peasant farm "Kozhyr-Ata" of Mangystau region) and 8 samples of the local Zhabe breed (Peasant farm "Akimbekov" of Zhetysu region). DNA extraction from blood samples was performed in the laboratory of the Department of Obstetrics, Surgery and Biotechnology of Reproduction of Kazakh National Agrarian Research University using the following method. Firstly, blood samples were thawed at room temperature for an hour. Then, 2.0 ml of thawed blood were placed in numbered tubes, which were centrifuged at 14500 rpm for 8 minutes. The upper phase was removed, leaving the sediment in the tube, to which 500 µl of TES lysis buffer was added and mixed using a vortex. The tube with its contents was centrifuged again at 14500 rpm for 8 minutes. The top part was removed, and 500 µl of TES buffer was

added again to the sediment. After mixing on a vortex until the mixture became homogeneous, the tube was centrifuged at 14500 rpm for 8 minutes. Then, 50 µl of 10% SDS and 5 µl of proteinase K were added to the tube and mixed on a vortex. After that, the tubes were shaken for 15 minutes and left overnight in a thermostat at 37°C. The samples were then removed from the thermostat, and 500 µl of 5M NaCl solution was added to each sample, mixed using a vortex, and centrifuged at 14500 rpm for 5 minutes. Using a pipette, the top layer (supernatant) was transferred to another tube in an amount of 500 µl, and isopropanol was added in a 1:1 ratio. The tube was mixed, centrifuged for 2-3 minutes at 10,000 rpm, and the top part was removed. The remaining DNA in the tube was washed twice with 70% ethanol, and the obtained DNA was dissolved in 1X TE buffer with a volume of 50 µl. The obtained DNA was evaluated using two methods: horizontal electrophoresis in a 0.8% agarose gel and measurement of DNA concentration by microspectrophotometric analysis (NanoDrop™ 2000).

The DNA samples of horses were genotyped for the LCORL gene locus using the following primers: forward F-5' - TGGAGTCAGTTGGGTTTAATG - 3' and reverse R - 5' - GACCGGATAGCATAGAGAGAG - 3'. The resulting amplicon length was 284 bp, and for allele identification, the AluI restriction enzyme with the recognition site AG/CT was used. After restriction, fragments of 284 bp, 169 bp, and 115 bp were formed depending on the animal's genotype. The PCR conditions for genotyping the DNA samples at the LCORL gene locus were as follows: initial denaturation at 94 °C for 5 min, 33 cycles of denaturation at 94 °C for 45 sec, primer annealing at 56.6 °C for 45 sec, extension at 72 °C for 45 sec, and final synthesis at 72 °C for 5 min.

Allele identification in the 8th exon of the PRKAG3 gene was performed using the following primers: forward F-5' - GAGGTGGGACAGTCTGGGGGCT-3' and reverse R - 5' - ACTGAAGGGCTGGGAAGGGACT -3'. The PCR product length was 182 bp, and to determine the genetic variants, the AluI restriction enzyme was used. After hydrolysis of the amplicon, fragments of 182 bp, 118 bp, and 45 bp were obtained depending on the animal's genotype. The PCR conditions for the PRKAG3 gene were as follows: initial denaturation at 94 °C for 5 min, 34 cycles of denaturation at 94 °C for 30 sec, primer annealing at 66 °C for 30 sec, extension at 72 °C for 30 sec, and final synthesis at 72 °C for 5 min.

### *Results and Discussion*

The work on genotyping DNA samples of horses was carried out at the laboratory of “Green Biotechnology and Cell Engineering” at the Kazakh-Japanese Innovation Center of Kazakh National Agrarian Research University. The average concentration of DNA samples was 289.24 ng/µl, with a minimum value of 1.2 ng/µl and a maximum concentration of 1344.7 ng/µl. Another important quality indicator of isolated DNA is the degree of sample purification, i.e., the ratio of DNA concentration values at A260/A280 wavelength. 85% of DNA samples had values greater than 1.70 and 15% of samples had lower values, less than 1.70. Amplification of the desired fragment of the corresponding genes was carried out according to the temperature regime, and the composition of the reaction mixture was: 2.5 µl of 10X PCR buffer with KCL, 1.0 µl each of forward and reverse primers, 2.0 µl of a mixture of four dNTPs, 0.2 µl of Taq DNA Polymerase (recombinant) 5U/µl, 1.5 µl of 25 mM MgCl<sub>2</sub>, bidistilled water in the amount of 15.8 µl, and 3.0 µl of DNA samples. The results of the polymerase chain reaction were verified using a 4.0% agarose gel stained with ethidium bromide.

To amplify the necessary regions of the LCORL and PRKAG3 genes, primer sequences and a PCR temperature regime described in the work of foreign scientists were used. The results of the amplification were checked using horizontal electrophoresis on a 4.0% agarose gel (Fig. 1, 2). The identification of alleles of the LCORL and PRKAG3 genes was carried out by hydrolyzing the PCR product with the AluI endonuclease. Thus, for the LCORL gene locus, individuals with the homozygous TT and heterozygous TC genotypes were identified (Fig. 3). However, for the PRKAG3 gene locus, out of the 41 tested samples, only one mare was found to have a heterozygous CT genotype, where fragments of 182 bp, 118 bp, 45 bp, and 19 bp were detected on the electropherogram (Fig. 4). It should be noted that samples 7 and 8 contain an additional fragment with an approximate size of 130 bp, which should not be present on the electropherogram. This may indicate that the observed pattern is the result of an artifact.

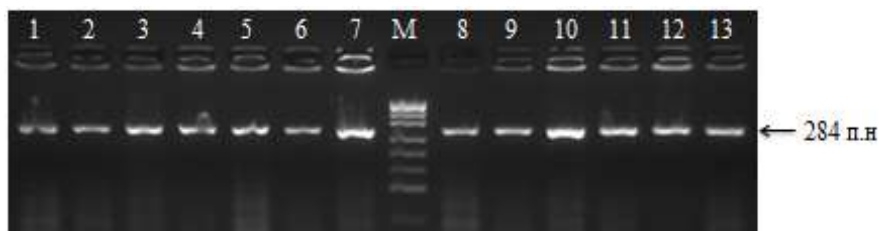


Figure 1. Electropherogram of the PCR product of the LCORL gene, lanes 1-13, with an amplicon size of 284 bp, M - DNA marker pUC19/MspI

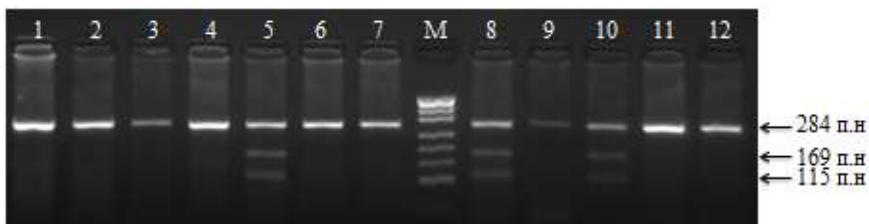


Figure 2. Electropherogram of LCORL gene amplicon after restriction digestion with AluI, lanes 5, 8, 10 - TC heterozygous genotype, fragments of 284 bp, 169 bp, and 115 bp, lanes 1-4, 6-7, 9, 11-12 - TT homozygous individuals with 284 bp fragments, M - DNA marker pUC19/MspI

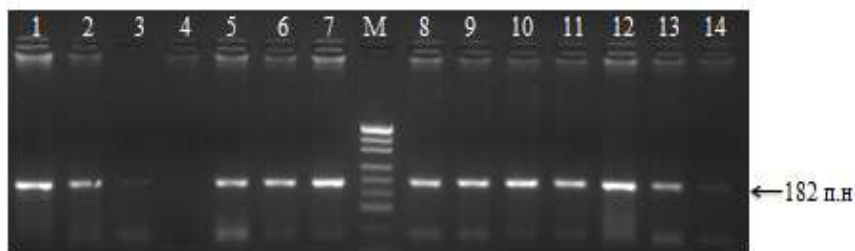


Figure 3. Electropherogram of the PCR product of the PRKAG3 gene. Lane 4 shows negative results, while lanes 1-3, 5-7 and 8-14 show a PCR product with a size of 182 bp., M - DNA marker pUC19/MspI

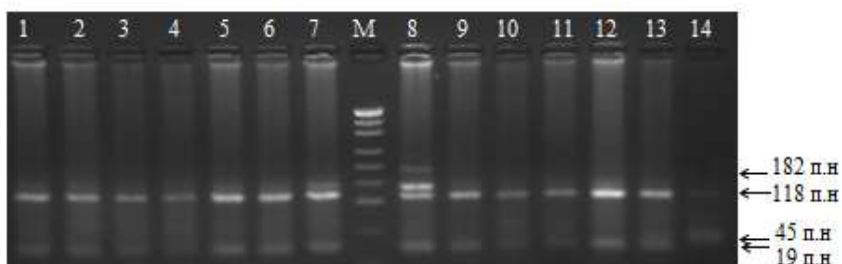


Figure 4. Electropherogram of the amplified product of the PRKAG3 gene after AluI endonuclease restriction, lane 8 - CT heterozygous genotype, fragments 182 bp, 118 bp, 45 bp, lanes 1-7 and 9-14 CChomozygous individuals with 118 bp, 45 bp fragments, M - DNA marker pUC19/MspI

To assess the level of genetic polymorphism, an analysis of the genotyping results was carried out for the LCORL gene locus. The actual occurrence of the homozygous TT genotype in horses of the Zhabe Pavlodar breed was 81.25%, the heterozygous TC genotype was 18.75%, and the other homozygous CC genotype was not detected in the studied animals. For this gene locus, no genetic polymorphism was detected in the DNA of tested horses of the Mangystau Adai type (n=17) and the local Zhabe breed of the Zhetysu region (n=8), with all individuals having a homozygous TT genotype. Genetic polymorphism was detected only in horses of the Mangystau Adai type for the second gene locus, PRKAG3, where one mare out of 17

tested horses had a heterozygous CT genotype (5.89%). According to the genotyping results, horses of the Pavlodar and Zhetysu regions had only one homozygous CC genotype for both gene loci (Table).

Table

**Distribution of genetic variants and allele frequencies, level of homozygosity and heterozygosity of individuals for the LCORL and PRKAG3 gene loci in the studied population of horses**

Locus name	LLP "Akshiman-Agro" of Pavlodar region				
	Genotype	Genotype frequency	Allele frequency	Ho	He
LCORL	TT (n=13)	81,25%	T=-0,91	81,25	18,75
	TC (n=3)	18,75%	G=0,09		
	CC (n=0)	0,0%			
Peasant farm "Kozhyr-Ata" of Mangystau region					
LCORL	TT (n=17)	100,0%	T=-1,0	100,0	0,0
	TC (n=0)	0,0%	G=0,0		
	CC (n=0)	0,0%			
Peasant farm "Akimbekov" of Zhetysu region					
LCORL	TT (n=7)	100,0%	T=-1,0	100,0	0,0
	TC (n=0)	0,0%	G=0,0		
	CC (n=0)	0,0%			
LLP "Akshiman-Agro" of Pavlodar region					
PRKAG3	CC (n=16)	100,0 %	C=1,0	100,0	0,0
	CT (n=0)	0,0%	T=0,0		
	TT (n=0)	0,0%			
Peasant farm "Kozhyr-Ata" of Mangystau region					
PRKAG3	CC (n=16)	94,11%	C=0,97	94,11	5,89
	CT (n=1)	5,89%	T=0,03		
	TT (n=0)	0,0%			
Peasant farm "Akimbekov" of Zhetysu region					
PRKAG3	CC (n=8)	100,0%	C=1,0	100,0	0,0
	CT (n=0)	0,0%	T=0,0		
	TT (n=0)	0,0%			

Gene equilibrium was violated in both gene loci, with a much greater prevalence of the T and C alleles observed, with values of 0.91, 1.0, 1.0, and 1.0, 0.97, and 1.0, respectively, in horses from Pavlodar, Mangystau, and Zhetysu regions. Another criterion characterizing the level of genetic diversity is the determination of the level of homozygosity and heterozygosity in the studied population. For the LCORL gene locus, the level of homozygosity in the three studied groups was 81.25%, 100.0%, and 100.0%, and for the PRKAG3 gene, it was 100.0%, 94.11%, and 100.0%. The level of heterozygosity was 18.75% for the LCORL gene locus in horses from the Pavlodar region and 5.89% for the PRKAG3 gene locus in horses from the Mangystau region. No individuals with homozygous CC and TT genotypes were detected for both studied gene loci.

### Conclusion

It is known that the LCORL gene locus in horses is associated with growth parameters, muscle mass formation, chest circumference, and the AY\_376689:c.773C>T SNP polymorphism in the PRKAG3 gene influences the growth rate of young animals. The study results indicate a disruption of genetic equilibrium at the LCORL and PRKAG3 gene loci, which is likely due to prolonged selection of the horse population for economically useful traits such as live weight, growth rate, carcass weight, animal endurance, disease resistance, and fertility of stallions. We believe that the preservation of the allele pool of local animal breeds is an important criterion for breeding livestock. The reduction of polymorphism level and genetic diversity in the studied horses is indirectly caused by inbreeding resulting from the use of a single method, natural mating of mares with stud stallions in a particular area for reproductive work. Thus, the LCORL gene determines the formation of muscle mass, skeleton, chest circumference, growth rate, and PRKAG3 gene alleles are associated with the growth rates of young animals. In the future, the indicated SNP polymorphisms can be used as DNA markers of meat productivity in horses.

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### Жергілікті қазақ жылқы тұқымындағы ет өнімділігімен байланысты SNP полиморфизмдері бойынша идентификация

Біздің зерттеуіміздің мақсаты — жергілікті қазақ тұқымды жылқылардың генотиптерін анықтау, сонымен қатар Павлодар және Жетісу облыстарындағы Жабы тұқымын, сондай-ақ Маңғыстау облысындағы Адай тұқымын, яғни 41 бас жылқыны LCORL және PRKAG3 гендерінің локустары бойынша ПТР-РФҰП әдістерін қолдана отырып зерттеу. Зерттеу нәтижелері бойынша Павлодар облысының Жабы тұқымының жылқысында LCORL генінің локусы бойынша ТТ гомозиготалы генотипінің кездесу жиілігі — 81,25 %, ТС гетерозиготалы генотипі — 18,75 % құрайтынын көрсетті. Маңғыстау облысының Адай жылқысы мен Жетісу облысының Жабы тұқымындағы қазақ жылқыларында генетикалық полиморфизм анықталмады, барлық гомозиготалы генотип — ТТ болды. PRKAG3 генінің екінші локусында генетикалық полиморфизм тек Адай жылқысында кездесті, сыналған 17 жылқының ішінде бір бие гетерозиготалды генотипке ие болды, яғни СТ (5,89 %). Павлодар және Жетісу облыстарының Жабы тұқымды жылқыларында СС жалғыз гомозиготалы генотипі болған. Зерттелген үш топтағы LCORL генінің локусы бойынша гомозиготалық деңгейі: 81,25; 100,0; 100,0. PRKAG3 гені

бойынша — 100,0; 94,11; 100,0. Павлодар облысының жылқыларында LCORL генінің локусы бойынша гетерозиготалық деңгей — 18,75 %, Маңғыстау облысының жылқыларында PRKAG3 генінің локусы бойынша — 5,89 % құрады. Осылайша, LCORL және PRKAG3 генінің полиморфизмдері жылқылардың бұлшықет массасының, қаңқа құрылымының, кеуде шеңберінің және өсу жылдамдығының дамуын анықтауда маңызды рөл атқарады. Сондықтан бұл SNP полиморфизмдері жылқылардағы ет өндірісін бағалау үшін ДНҚ-маркерлері ретінде қызмет ете алатыны зерттелді.

*Кілт сөздер:* жылқыны генотиптеу, қазақ жылқылары, Жабы, Адай, LCORL гені, PRKAG3 гені, ПТР-РФҰП анализі, өнімділіктің ДНҚ-маркерлері.

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## Выявление SNP полиморфизмов, ассоциированных с мясной продуктивностью, у местной казахской породы лошадей

Целью нашего исследования было генотипирование местных лошадей казахской породы: тип Жабе Павлодарской и Жетысуской областей, а также Адайский тип Мангистауской области в количестве 41 голова по локусам генов LCORL и PRKAG3 методом ПЦР–ПДРФ. По результатам исследования встречаемость гомозиготного генотипа ТТ по локусу гена LCORL у лошадей породы Жабе Павлодарской области составила 81,25 %, гетерозиготного генотипа ТС — 18,75 %. У казахских лошадей Адайского типа Мангистауской области и типа Жабе Жетысуской области генетический полиморфизм не выявлен, все особи имели гомозиготный генотип — ТТ. По второму локусу гена PRKAG3 генетический полиморфизм обнаружен только у лошадей Адайского типа, из 17 протестированных лошадей одна кобыла оказалась с гетерозиготным генотипом — СТ (5,89 %). Лошади типа Жабе Павлодарской и Жетысуской областей имели единственный гомозиготный генотип СС. Уровень гомозиготности по локусу гена LCORL у трех исследуемых групп составил: 81,25; 100,0; 100,0. По гену PRKAG3 — 100,0; 94,11; 100,0. Уровень гетерозиготности по локусу гена LCORL у лошадей Павлодарской области составил 18,75 %, по локусу гена PRKAG3 у лошадей Мангистауской области — 5,89 %. Таким образом, полиморфизм генов LCORL и PRKAG3 играют значительную роль в определении развития мышечной массы, строения скелета, окружности грудной клетки, скорости роста лошадей. Следовательно, эти SNP полиморфизмы потенциально могут служить ДНК-маркерами для оценки мясной продуктивности лошадей.

*Ключевые слова:* генотипирование лошадей, казахские лошади, Жабе, Адай, ген LCORL, ген PRKAG3, ПЦР-ПДРФ-анализ, ДНК-маркеры продуктивности.

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## Results of reconnaissance surveys of arboretum of A.N. Bukeikhan Kazakh research institute of forestry

The article presents the results of the inventory of trees and shrubs of arboretum of A.N. Bukeikhan Kazakh research institute of forestry. The paper presents the taxonomic composition of 23 quarters with the identification of dominant plant forms and an assessment of the sanitary condition of plants by category. The taxonomic composition of tree and shrub plantations is represented by 42 genera, 22 families of 14 orders from the departments – *Gymnospermae*, *Angiospermae*. Of the identified 22,489 trees and shrubs, 89.6% are deciduous and 10.4% coniferous. The greatest taxonomic diversity was noted in the families *Rosaceae* and *Pinaceae*. It has been established that most plants belong to the first category. Quarters 18, 19.2, 22, 23 turned out to have the largest number of plants belonging to 5, 6 categories, which are recommended for removal. The arboretum has a large resource of trees and shrubs, acclimatized and adapted to the climatic features of Northern Kazakhstan, and can serve as a key object for the concentration of genetic diversity.

*Keywords:* inventory, trees, shrubs, flora, sanitary condition category.

### Introduction

Green spaces are one of the main indicators of living comfort in settlements. Trees and shrubs maintain the gas composition of the atmosphere, form a microclimate, emit phytoncides, deposit suspended particles on the surface of the leaves, performing a sanitary and hygienic function, and the noise-absorbing role of green spaces is also important. The role of green spaces is also invaluable in improving the aesthetic qualities of the environment. In this regard, tree and shrub plantations require a comprehensive study and systematization of information about them for scientifically based landscaping of settlements.

Biological species diversity is an important factor in the development and conditions for the existence of any biocenosis. Recently, population growth and the consequences of active economic activity have led to irreversible changes in the nature of the planet, which is a threat to the existence of certain species and ecosystems.

The Global Strategy for Plant Conservation obliges the member countries that have ratified the Convention on Biological Diversity to develop National Plant Conservation Strategies that ensure the implementation of the Strategic Plan for Biodiversity Conservation [1-5].

Arboretums are an effective, and sometimes the only possible method of preserving plant biodiversity, both in a given area and on the planet as a whole. Arboretums are a way to increase the total number of a particular taxon, to expand its cultural area. Cultivated rare species can then be used to return to the natural environment, thereby reducing the level of anthropogenic pressure on this species. The concept of the arboretum is to create a collection of rare and endangered plants in order to familiarize children and the population of the city and region with them.

In the state dendrological parks, scientific research is carried out on the introduction and selection of natural, cultural, domestic and world flora, as well as on the study, conservation and effective use of the flora of Kazakhstan.

One of the priority activities of arboretums is the inventory of plants, as a basis for assessing the diversity of species and communities, and developing scientific foundations for the conservation and sustainability of forest ecosystems in Kazakhstan.

The purpose of the study: an inventory of the species composition and sanitary condition of tree and shrub vegetation on the territory of the dendrological park of A.N. Bukeikhan Kazakh research institute of forestry – KazRIF (Shchuchinsk, Akmola region).

### Experimental

The material for the research was tree and shrub plants of the dendrological park of KazRIF (Fig. 1).



Figure 1. General view of the studied quarters of the arboretum of A.N. Bukeikhan Kazakh research institute of forestry

The inventory of plantings in the study area was based on our own research. In 2021-2022 a detailed inventory of green spaces was carried out using the method of a complete enumeration of all trees and shrubs growing in the arboretum with a characteristic of each specimen [1].

A total of 23 quarters of woody and shrubby plants were surveyed. A visual tree-by-tree inventory of trees and shrubs was carried out. The description was carried out by continuous recalculation of trees and shrubs with the definition of species and condition. The description was carried out by the route method. Taxonomic identification was carried out using determinants [6-10]. The condition of tree and shrub plantations was established according to generally accepted methods [11]. The studies used the method of reconnaissance survey, including a preliminary visual assessment of the state of plantations, the degree of drying of trees [12].

The assessment of the category of the sanitary condition of plantations (on the scale of the sanitary condition of trees) was carried out according to the method [12], which consists of the following categories:

I – without signs of weakening – the crown is dense; the foliage is green, drying out, no damage;

II – weakened – the crown is slightly openwork; foliage falls early or eaten up to 25 percent; drying of individual branches; local damage to the trunk and root paws; single water shoots;

III – strongly weakened – the crown is strongly openwork; the foliage is very small, light, turns yellow early and falls off, growth is very weak or absent; dries up to 2/3 of the crown damage to the trunk and root paws on 2/3 of their circumference; sap flow on trunks and skeletal branches; settlement attempts or local settlements of stem pests; abundant water shoots; fruit bodies of mushrooms on the trunk;

IV – drying out – more than 2/3 of the crown has dried up or dries out; damage to more than 2/3 of the circumference of the trunk and root paws; signs of colonization by stem pests drying out water shoots;

V – fresh dead wood (current year) – foliage has withered, withered or is absent; partial fall of the bark; signs of colonization or emergence of stem pests;

VI – old deadwood (previous years) – no foliage; bark and small twigs crumbled partially or completely; exit holes of stem pests; under the bark is a mycelium of wood-destroying fungi.

### Results and Discussion

The dendrological park of KazRIF was founded in 1961 on an area of 44.3 hectares under the guidance of Professor Savich V.M.

Geographically, the arboretum is located on the Kokshetau Upland, located on three naturally formed terraces on the shore of Lake Schchuchye in the northeastern part of the city of Shchuchinsk. The territory of the arboretum is currently 31.69 hectares; it has scientific, historical and cultural significance.

The climate is sharply continental, with hot summers and harsh winters with little snow, softened by the influence of hills, reservoirs and forests. The annual precipitation is 250-295 mm in the flat part, up to 400 mm in the elevated part. In the warm season (April-September), an average of 212-254 mm falls as rain, which is 70-85% of the annual precipitation. Winter precipitation is 83-137 mm, which determines a small snow cover height (30 cm), decreasing towards the east [5].

The unique collection of introduced species on the territory of the arboretum is of great importance for this region, they are the most important source of enrichment of the gene pool of woody plants and shrubs.

The territory of the arboretum is divided into quarters (Fig. 1). Plants in quarters are arranged by families, placed in groups. In terms of species composition, the exposition groups of tree species and shrubs are represented by one species or have a wider species composition.

#### *The species composition of dendrological park*

The species composition of tree and shrub vegetation in Northern Kazakhstan is poor; there are only about 120 species. Trees make up – 17%, shrubs – 72%; other species are represented by semi-shrubs, shrubs and lianas. In the dendrological park, long-term introduction tests of ornamental trees and shrubs, as well as mother plantations of woody plants, which are unique introducers and are of great importance, are being carried out.

According to [13], more than 2 thousand species, forms and varieties of trees and shrubs from Siberia, Europe, North America, the Far East, Central Asia, Japan, China, and other countries were tested and studied in the arboretum. To date, out of the original 2 thousand valuable and ornamental species, about 500 have survived, belonging to 27 families and 89 genera. Here grows as widespread zonal plants: pines, poplars, birches, as rare species listed in the Red Book of Kazakhstan: *Quercus robur* L. (*Q. pedunculata* Ehrh.), *Corylus colurna* L., *Tilia cordata* Mill., *Malus niedzwetzkyana* Dieck, *Juglans sieboldiana* Max., syn. *J. ailantifolia* Carr.), *Cotoneaster acutifolius* var. *lucidus* L.T.Lu, *Juniperus sabina* L., *Ribes petraeum* Wulfen, *Rhodiola rosea* L., *Rhaponticum carthamoides* (Willd.) Iljin, *Paeonia anomala* L., and others.

Employees conducted long-term trials of tree species, shrubs, lianas, shrubs and other perennial plants [14]. As a result of introduction works, the best 360 species characterized by high productivity, yield, economic value and ornamental value were selected. These species were recommended for production planting in forestry, landscaping of cities and settlements of different regions of Northern Kazakhstan.

For the period from 2021 to 2022 inventory of tree and shrub plants we conducted on 23 quarters according to the map-scheme of the territory of the arboretum (Fig. 1, Table).

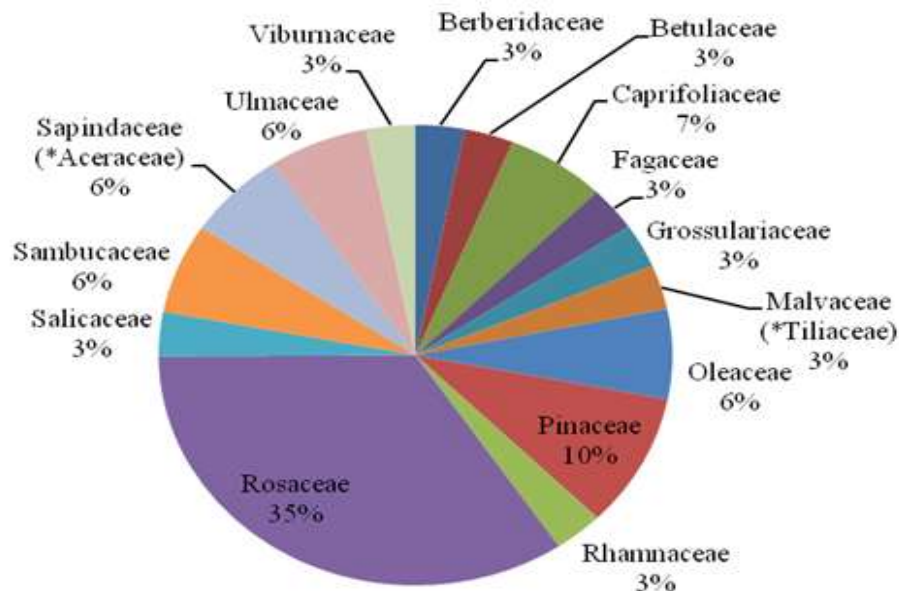


Figure 2. Percentage participation rate of tree and shrub species by family

Summary table of the studied plants state by quarters and categories

№	Quarter	Specific name	Quantity by status of the category, samples						Total:
			1	2	3	4	5	6	
1	18	<i>Symphoricarpos albus</i> (L.) S.F. Blake	80						80
2		<i>Malus baccata</i> (L.) Borkh.	22				9		31
3		<i>Padus avium</i> Mill.	28					4	32
5		<i>Picea obovata</i> Ledeb.	81	16			11		108
6		<i>Rubus idaeus</i> L.	10						10
7		<i>Rosa</i> L. sp.	8						8
8		<i>Syringa vulgaris</i> L.		2					2
9		<i>Betula pendula</i> Roth	7					2	9
10		<i>Populus italica</i> (Du Roi) Moench						1	1
11		<i>Crataegus laevigata</i> (Poir.) DC.		2					2
12		<i>Tilia cordata</i> Mill.	18					1	19
13		<i>Cupressus sempervirens</i> L.	1						1
14		<i>Larix sibirica</i> Ledeb.	11					2	13
15		<i>Sambucus racemosa</i> L.	7						7
16		<i>Caragana arborescens</i> Lam.	4						4
17		<i>Ribes alpinum</i> L.	100						100
18		<i>Rhamnus cathartica</i> L.	12						12
19		<i>Swida alba</i> (L.) Opiz	1						1
20		<i>Salix</i> × <i>fragilis</i> L.	3						3
21		<i>Sorbus aucuparia</i> L.	9						9
22		<i>Amelanchier ovalis</i> Medikus	50						50
23		<i>Ulmus pumila</i> L.	1		3			28	32
24		<i>Berberis vulgaris</i> L.	4						4
25		<i>Pinus sylvestris</i> L.	2				1		3
26		<i>Physocarpus opulifolius</i> (L.) Maxim.	10						10
27		<i>Quercus robur</i> L.	4						4
			<b>27 (26)</b>	<b>473</b>	<b>20</b>	<b>3</b>	<b>0</b>	<b>21</b>	<b>38</b>
1	19	<i>Acer platanoides</i> L.	53						53
2		<i>Acer negundo</i> L.	636						636
3		<i>Amelanchier ovalis</i> Medikus	338						338
4		<i>Berberis vulgaris</i> L.	5						5
5		<i>Betula pendula</i> Roth	94				1	1	96
6		<i>Cotoneaster lucidus</i> Schtdl.	5						5
7		<i>Crataegus laevigata</i> (Poir.) DC.	24		4	1	5		34
8		<i>Crataegus arnoldiana</i> Sarg.	1						1
9		<i>Larix sibirica</i> Ledeb.	7		20				27
10		<i>Lonicera tatarica</i> L.	1						1
11		<i>Malus baccata</i> (L.) Borkh.	24			2	2		28
12		<i>Padus avium</i> Mill.	136	9			5		150
13		<i>Padus maackii</i> (Rupr.) Kom.	4		1		1		6
14		<i>Picea pungens</i> f. <i>glauca</i> Beissn.		2		1			3
15		<i>Picea obovata</i> Ledeb.	65	6				1	72
16		<i>Quercus robur</i> L.	10						10
17		<i>Rhamnus cathartica</i> L.	35						35
18		<i>Ribes aureum</i> Pursh							0
19		<i>Rosa spinosissima</i> L.							0
20		<i>Sambucus nigra</i> L.	1						1
21		<i>Sambucus racemosa</i> L.	3						3
22		<i>Sorbaria sorbifolia</i> (L.) A. Braun	6						6
23		<i>Sorbus aucuparia</i> L.	171					1	172
24		<i>Symphoricarpos albus</i> (L.) S.F. Blake	10						10
25		<i>Syringa vulgaris</i> L.	3						3
26		<i>Tilia cordata</i> Mill.	3		1				4
27		<i>Ulmus glabra</i> Huds.	540				2	1	543
28		<i>Viburnum lantana</i> L.	20						20
		<b>28 (23)</b>	<b>2195</b>	<b>17</b>	<b>26</b>	<b>4</b>	<b>16</b>	<b>4</b>	<b>2262</b>
1	20	<i>Ulmus glabra</i> Huds.	153		1				154
2		<i>Ulmus parvifolia</i> Jacq.	3						3
3		<i>Larix sibirica</i> Ledeb.	19						19

Continuation of Table 1

№	Quarter	Specific name	Quantity by status of the category, samples						Total:
			1	2	3	4	5	6	
4		<i>Rhamnus cathartica</i> L.							0
5		<i>Acer negundo</i> L.							0
6		<i>Padus avium</i> Mill.	185						185
7		<i>Sorbus aucuparia</i> L.	16						16
8		<i>Betula pendula</i> Roth	43						43
9		<i>Acer platanoides</i> L.	12						12
10		<i>Populus italica</i> (Du Roi) Moench	1						1
11		<i>Fraxinus excelsior</i> L.	11						11
12		<i>Tilia cordata</i> Mill.	1						1
13		<i>Berberis vulgaris</i> L.	4						4
14		<i>Ribes aureum</i> Pursh	5						5
		<b>14 (13)</b>	<b>453</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>454</b>
1	21	<i>Rhamnus cathartica</i> L.	88						88
2		<i>Betula pendula</i> Roth		41				1	42
3		<i>Acer negundo</i> L.	36						36
4		<i>Caragana arborescens</i> Lam.	9						9
5		<i>Malus baccata</i> (L.) Borkh.	3						3
6		<i>Lonicera tatarica</i> L.	2						2
7		<i>Lonicera xylosteum</i> L.	152						152
8		<i>Ulmus laevis</i> Pall.	3					1	4
9		<i>Crataegus sanguinea</i> Pall.	2						2
10		<i>Ulmus pumila</i> L.	2				1		3
11		<i>Sorbaria sorbifolia</i> (L.) A. Braun	1						1
12		<i>Sambucus racemosa</i> L.	1						1
13		<i>Acer tataricum</i> L.	2					1	3
14		<i>Acer platanoides</i> L.	5			1		2	8
		<b>14 (10)</b>	<b>306</b>	<b>41</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>5</b>	<b>354</b>
1	22	<i>Amelanchier ovalis</i> Medikus	4						4
2		<i>Betula pendula</i> Roth	32					4	36
3		<i>Cotoneaster lucidus</i> Schldtl.	1						1
4		<i>Juniperus horizontalis</i> Moench	30						30
5		<i>Larix sibirica</i> Ledeb.	78	1				20	99
6		<i>Lonicera tatarica</i> L.	453						453
7		<i>Malus baccata</i> (L.) Borkh.	1						1
8		<i>Padus avium</i> Mill.	7						7
9		<i>Picea abies</i> (L.) H. Karst.	1						1
10		<i>Pinus sylvestris</i> L.	105						105
11		<i>Prunus spinosa</i> L.	15						15
12		<i>Rhamnus cathartica</i> L.	6						6
13		<i>Rosa canina</i> L.	102						102
14		<i>Rosa spinosissima</i> L.	3						3
15		<i>Sorbus aucuparia</i> L.	18						18
16		<i>Symphoricarpos albus</i> (L.) S.F. Blake	13						13
17		<i>Syringa vulgaris</i> L.	3						3
18		<i>Ulmus glabra</i> Huds.	9					25	34
19		<i>Ulmus laevis</i> Pall.						4	4
		<b>19 (17)</b>	<b>881</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>53</b>	<b>935</b>
1		<i>Amelanchier ovalis</i> Medikus	1						1
2		<i>Berberis vulgaris</i> L.	1						1
3		<i>Betula pendula</i> Roth	15	6	4				25
4		<i>Caragana arborescens</i> Lam.	1						1
5		<i>Crataegus laevigata</i> (Poir.) DC.	3						3
6		<i>Crataegus laevigata</i> (Poir.) DC.	1						1
7		<i>Larix sibirica</i> Ledeb.	2	5				4	11
8		<i>Lonicera xylosteum</i> L.	2						2
9		<i>Lonicera tatarica</i> L.	35						35
10		<i>Malus baccata</i> (L.) Borkh.	12						12
11		<i>Padus avium</i> Mill.	4						4
12		<i>Picea abies</i> (L.) H. Karst.	145					5	150
13		<i>Pinus sylvestris</i> L.	10						10
14		<i>Populus italica</i> (Du Roi) Moench	29						29

Continuation of Table 1

№	Quarter	Specific name	Quantity by status of the category, samples						Total:
			1	2	3	4	5	6	
15		<i>Prunus spinosa</i> L.	10						10
16		<i>Quercus robur</i> L.	1						1
17		<i>Rhamnus cathartica</i> L.	30						30
18		<i>Ribes alpinum</i> L.	1						1
19		<i>Rosa canina</i> L.	8						8
20		<i>Rubus idaeus</i> L.	17						17
21		<i>Sorbus aucuparia</i> L.	12						12
22		<i>Swida alba</i> (L.) Opiz	43						43
23		<i>Symphoricarpos albus</i> (L.) S.F. Blake	207						207
24		<i>Syringa vulgaris</i> L.	73						73
25		<i>Tilia cordata</i> Mill.	3						3
26		<i>Ulmus glabra</i> Huds.	40	15				44	99
27		<i>Viburnum lantana</i> L.	4						4
		<b>28 (26)</b>	<b>713</b>	<b>26</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>53</b>	<b>796</b>
1		<i>Lonicera caerulea</i> L.	9						9
2		<i>Cotoneaster lucidus</i> Schldtl.	6						6
3		<i>Malus baccata</i> (L.) Borkh.	10						10
4		<i>Crataegus sanguinea</i> Pall.	42						42
5		<i>Ulmus glabra</i> Huds.	23						23
6		<i>Malus baccata</i> (L.) Borkh.	70						70
7		<i>Acer tataricum</i> L.	65						65
8		<i>Rhamnus cathartica</i> L.	48						48
9		<i>Betula nigra</i> L.	2						2
10		<i>Picea pungens</i> f. <i>glauca</i> Beissn.	9						9
11		<i>Pinus sylvestris</i> L.	10						10
12		<i>Lonicera tatarica</i> L.	114						114
13		<i>Tilia cordata</i> Mill.	17						17
14		<i>Hippophae rhamnoides</i> L.	7	10	23	3		23	66
15		<i>Acer negundo</i> L.	42						42
16		<i>Berberis vulgaris</i> L.	4						4
17		<i>Rosa rugosa</i> Thunb.	47						47
18		<i>Caragana arborescens</i> Lam.	3						3
19		<i>Padus avium</i> Mill.	53						53
20		<i>Betula pendula</i> Roth	154						154
21		<i>Prunus spinosa</i> L.	20						20
22		<i>Amelanchier ovalis</i> Medikus	33						33
23		<i>Elaeagnus angustifolia</i> L.	34						34
24		<i>Padus virginiana</i> (L.) Mill.	15						15
25		<i>Salix alba</i> L.	4			10			14
26		<i>Populus alba</i> L.	47						47
27		<i>Cotoneaster integerrimus</i> Medik.	6						6
28		<i>Swida alba</i> (L.) Opiz	5						5
29		<i>Viburnum lantana</i> L.	2						2
30		<i>Ulmus pumila</i> L.	1						1
31		<i>Spiraea hypericifolia</i> L.	3						3
32		<i>Syringa vulgaris</i> L.	23						23
33		<i>Sorbus aucuparia</i> L.	3						3
34		<i>Symphoricarpos albus</i> (L.) S.F. Blake	21						21
35		<i>Lonicera xylosteum</i> L.	3						3
36		<i>Sambucus racemosa</i> L.	2						2
37		<i>Viburnum opulus</i> L.	1						1
		<b>37 (29)</b>	<b>958</b>	<b>10</b>	<b>23</b>	<b>13</b>	<b>0</b>	<b>23</b>	<b>1027</b>

According to preliminary data identified plant species belong to 42 genera, 22 families of 14 orders and two classes (Pinopsida, Magnoliopsida), which belong to two divisions – *Gymnospermae*, *Angiospermae*. Conifers are represented mainly by species: *Juniperus horizontalis* Moench, *Larix sibirica* Ledeb., *Picea asperata* Mast., (*P. pungens* Engelm. form blue, *P. excelsa* Link., *P. obovata* Ledeb., *P. omorica* (Panc.) Purk.), *P. pungens* Engelm., *Pinus contorta* Dougl., *P. sibirica* (Rupr.) Mayr., *P. sylvestris* L.



Deciduous species also include representatives of woody and shrub life forms. According to preliminary data, a total of 22,489 specimens of woody and shrubby plants grow on the surveyed blocks, of which 2,347 (10.4%) are conifers and 20,142 (89.6%) are deciduous. Based on the results of inventories, the highest taxonomic diversity was found in the family *Rosaceae*, followed by *Pinaceae*.

*Sanitary condition of tree and shrub plantations of the arboretum*

According to the scale of sanitary condition, trees and shrubs growing on the territory of the arboretum according to their quantitative ratio can be divided into the following categories: category 1 – 20,558 (91.41%), category 2 – 1,038 (4.62%), category 3 – 285 (1.27%), category 4 – 267 (1.19%), category 5 – 81 (0.36%), category 6 – 260 (1.16%) (Fig. 3, Table 1).

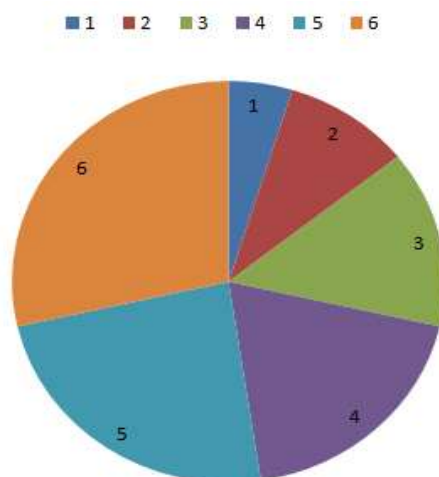


Figure 3. Percentage ratio of tree and shrub plants by their sanitary condition according to a 6-point scale

According to the condition categories, most plants belong to the first category – without signs of weakening, with a dense crown, green foliage, without drying out and damage (Fig. 4). Quarters 18, 19.2, 22, 23 turned out to have the largest number of plants belonging to 5, 6 categories.

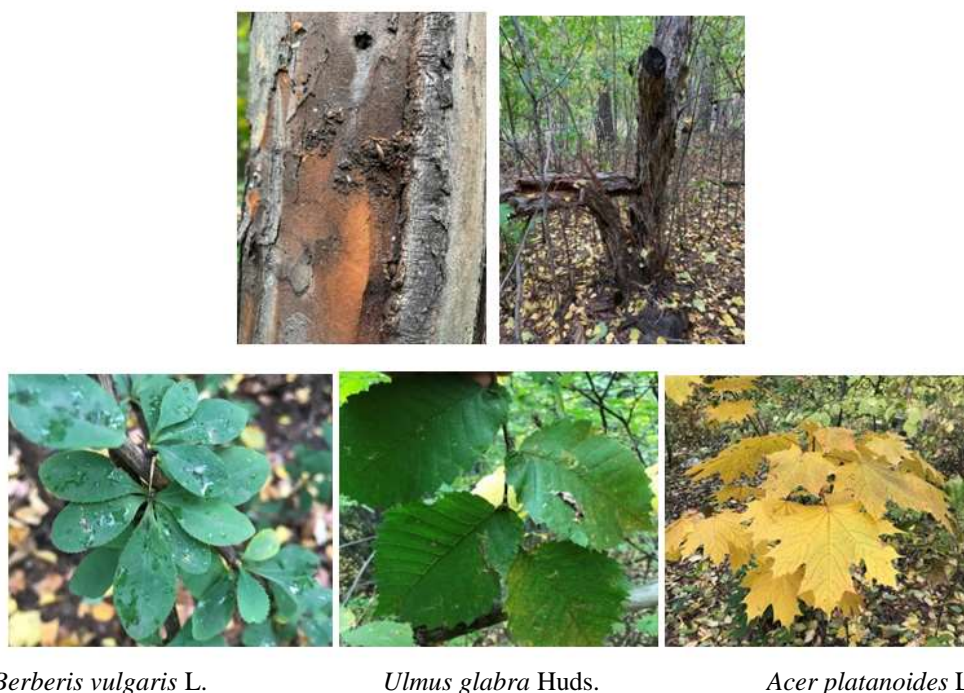


Figure 4. Woody plants of the first category: A – *Picea pungens*, B – *Tilia cordata*

It should be noted that trees and shrubs of the 5th category are fresh deadwood (of the current year), their foliage is shrunken, withered or absent, partial fall of the bark, and have signs of pest settlement or departure.

Trees and shrubs of the 6th category have the following features: lack of foliage, bark and small branches crumbled partially or completely, there exit holes of stem pests, under the bark a mycelium of wood-destroying fungi. Trees and shrubs of the 5th-6th category are in a depressed and emergency state, which is why they are fire hazardous, they need to be removed and cleared of thickets (Fig. 5, 6).





*Berberis vulgaris* L.

*Ulmus glabra* Huds.

*Acer platanoides* L.

Figure 5. Plants in emergency, fire and disease-prone condition



Figure 6. Overgrowth of self-sown oyster maple in quarter 15

In the 18th quarter, Siberian spruce (11 specimens) and Squat elm (28 specimens) turned out to be “unfavorable”. In quarter 22, all plants of the sixth category constitute a threat. These are: Rough elm (25 specimens), Siberian larch (20 specimens), Silver birch (4 specimens), and Smooth elm (4 specimens). In the 23rd quarter, also, plants of an unfavorable state belong to the 6th category: Siberian larch (4), European spruce (5), Rough elm (44 specimens). These tables also indicate the ecological vulnerability, mainly of species of the genus Elm and Spruce. A slightly worse condition of trees is observed in Quarter 19 (2), where all larch specimens are in weakened condition. All larch specimens are characterized as Category 3, i.e. weakened. The trees have foliage that is heavily trailing and very small, light-colored, yellowing and falling off early, very weak or no growth; desiccation of up to 2/3 of the crown damage to the trunk and root paws on 2/3 of their circumference; attempted settlement or local settlement of trunk pests; abundant water shoots; fruiting bodies of fungi on the trunk. Five specimens of common hawthorn were categorized as Category 5 – fresh dry wood (current year). Trees are characterized by wilted foliage, partial bark fall with signs of stem pest infestation.

Thus, the plants of the surveyed neighborhoods are represented by woody and shrub forms. According to the condition categories, most of the plants belong to the first category. Plants classified in the 5th and 6th categories are in an emergency condition and require removal.

It should be noted that the surveyed squares are often overgrown and require thinning. Root-propagating plants produce a lot of shoots, occupying the free space around them or choking out nearby plants of other species. By now, many shrubs, due to their short life cycle, have become old and naturally die off. In dense plantings, thinning, removal of damaged and stunted plants, cutting down or digging up self-seeded plants should be carried out. Old plantings should be cut down and replaced with young plantings. For this purpose, breeding material (seeds, seedlings, cuttings, etc.) shall be sought and the collection shall be replenished with new species.

In addition to the threat of extinction of rare species, there is a general degradation of introduced objects, weed infestation of the territory, their overgrowing with less valuable species, deterioration of sanitary condition, reduction of protective functions of plantations and loss of their aesthetic visibility, increasing cases of fires.

### *Conclusion*

In the course of the research work an inventory of existing plantations was carried out. The predominant number of plants in the arboretum of LLP “KAZSRIFA named after A.N. Bukeykhan” is in satisfactory condition, a significant part of them – in good condition.

It is necessary to perform shaping and sanitary pruning of preserved trees, as well as related phytosanitary measures to improve their condition.

Preservation of the planning structure, reconstruction of existing plantings, landscaping, development of the necessary infrastructure for the full utilization of the recreational potential of the garden – a way out to the modern level, today should be brought to the forefront in the strategy of its further development. This will give an opportunity to improve the recreational quality of plantings and confirm the status as a nature conservation site.

Plant introduction is the main way to enrich the northern region with new valuable plants. Especially in the conditions of increasing anthropogenic activity, it is impossible to solve the problem of preserving existing forests and artificial plantations of various purposes (forest crops, protective and landscaping plantations, fruit orchards and others) and increasing their productivity at the expense of local, naturally growing species. Dendropark activities should be aimed at attracting from other regions various species of trees and shrubs that are most sustainable for the region.

In addition to the threat of extinction of rare species, there is a general degradation of introduced objects, weed infestation of the territory, their overgrowing with less valuable species, deterioration of sanitary condition, reduction of protective functions of plantations and loss of their aesthetic visibility, increasing cases of fires.

The arboretum has a large resource of trees and shrubs acclimatized and adapted to climatic features of Northern Kazakhstan and can serve as a key site for concentration of genetic diversity.

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### «Ә.Н. Бөкейхан атындағы ҚазОШАҒЗИ» ЖШС Дендросаябағында жүргізілген барлау жұмыстарының нәтижелері

Мақалада «Ә.Н. Бөкейхан атындағы ҚазОШАҒЗИ» ЖШС Дендросаябағындағы ағаштар мен бұталарды есепке алу нәтижелері берілген. Авторлар өсімдіктердің басым формаларын анықтай отырып, 23 тоқсанның таксономиялық құрамын ұсынған және санаттар бойынша өсімдіктердің санитарлық жағдайына баға берген. Ағашты-бұталы екпелердің таксономиялық құрамы *Gymnospermae*, *Angiospermae* бөлімдеріне жататын 14 қатар, 22 тұқымдас, 42 туыспен берілген. Анықталған ағашты-бұталы өсімдіктердің 22 489 данасының 89,6%-ы жапырақты және 10,4%-ы қылқан жапырақты. Ең үлкен таксономиялық әртүрлілік *Rosaceae* және *Pinaceae* тұқымдастарында байқалды. Өсімдіктердің басым көпшілігі бірінші санатқа жататыны анықталды. 18, 19.2, 22, 23 кварталдар өсімдіктердің ең көп санына ие болды, ал 5 және 6 санатқа жататын өсімдіктерді алып тастау ұсынылды. Дендропарк Солтүстік Қазақстанның климаттық ерекшеліктеріне бейімделген және жерсіндірілген ағаштар мен бұталардың үлкен ресурсына ие және генетикалық әртүрлілік шоғырлануының негізгі объектісі бола алады.

*Кілт сөздер:* есепке алу, ағаштар, бұталар, флора, санитарлық жағдай категориясы, өсімдіктердің басым формалары.

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### Результаты рекогносцировочных обследований Дендропарка ТОО «КазНИИЛХА имени А.Н. Букейхана»

В статье приведены результаты инвентаризации древесно-кустарниковых растений Дендропарка ТОО «КазНИИЛХА имени А.Н. Букейхана». Авторами представлен таксономический состав 23 кварталов с выявлением доминирующих форм растений, и дана оценка санитарного состояния растений по категориям. Таксономический состав древесно-кустарниковых насаждений представлен 42 родами, 22 семействами из 14 порядков из отделов — *Gymnospermae*, *Angiospermae*. Из выявленных 22 489 экземпляров древесно-кустарниковых растений 89,6 % представлены лиственными и 10,4 % хвойными породами. Наибольшее таксономическое разнообразие отмечено в семействах *Rosaceae* и *Pinaceae*. Установлено, что большинство растений относятся к первой категории. Кварталы 18, 19.2, 22, 23 оказались с наибольшим количеством растений, относящихся к 5, 6 категориям, которые рекомендуются к удалению. Дендропарк располагает большим ресурсом деревьев и кустарников, акклиматизированных и адаптированных к климатическим особенностям Северного Казахстана, и может служить ключевым объектом концентрации генетического разнообразия.

*Ключевые слова:* инвентаризация, деревья, кустарники, флора, категория санитарное состояние, доминирующие формы растений.

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## The study of the biological age of university teachers in the period of urgent transition to distance education as a result of pandemic

In the article, the biological age of university teachers was studied (using the express method of V.P. Voytenko), a total of 127 people (64 women, 63 men) were examined, they were divided into three groups according to their age (1st group – 23-39 years old, 2nd group – 40-59 years old, 3rd group – 60 years old and older). A special health self-assessment questionnaire and a number of physiological indicators and mathematical formulas were used to calculate the value of biological age (BA); the appropriate biological age value (ABA) was calculated; he obtained biological age and the corresponding biological age were compared, it was determined how many years the subjects' aging rate was ahead or behind their peers. This approach makes it possible to divide people of the same age into several degrees according to the degree of “age wear”, as well as according to the “reserve” of health (degrees I and II – slow aging, III – BA corresponds to the population standard, IV and V – accelerated aging). The research was conducted during the transition of universities to distance education during the pandemic (October-November, 2020). More than half of the teachers (55.90%) were found to be experiencing accelerated aging (grade IV-V of BA), and this was more common among men than among women. Among those examined, the rate of aging is evident in the youngest group of teachers. Due to the pandemic, in the period of urgent transition from the usual traditional educational format to distance learning, the accelerated rate of aging according to biological age indicators was observed in almost all groups of teachers: 23%-80%. And there is a conclusion that the gender and age of the subjects affect the biological age.

*Keywords:* health, stress, distance education, pandemic, age, biological age, university teachers, gender.

### Introduction

The human's age is a concept that interests every person. In general, the appearance of a person corresponds to the previous years of life. But sometimes a person's appearance does not correspond to his age. People can look older or younger than their age. Calendar or passport age means how many years a person has lived. In addition, there is also a concept of biological age [1].

Biological age is the age of the human body (health), not the number of years of life. People themselves are interested in knowing their real age. For that, it is necessary to determine the biological age of a person. This allows not only a true assessment of the state of health, but also to detect the beginning of functional deterioration as early as possible and take measures before the first signs of health problems appear.

Russian scientist P. N. Sokolov (1935) published a study of biological age. He described the method of calculating the biological age indicators by dividing the table of age transitions in order to divide the informative signs (skin wrinkles) into their intensity levels using the ranks of middle-aged groups [2]. In 1975, Scientists such as T.L. Dubina, A.N. Razumovich [3] first reviewed the concept of biological age in periodicals. In addition, T.L. Dubina can be called a Russian pioneer in the development of methods for determining the biological age of humans and laboratory animals [4]. It is known that the employees of the Institute of Gerontology of the Academy of Sciences in USSR, conducted in the 80s years under the leadership of V.P. Voytenko, intensively developed new methods in the study of biological age. A detailed description of the biological age determination method, which can be used by other researchers, was published by V.P. Voytenko and co-authors in 1984 [5]. At present time, there are many methods for determining BA, which are analyzed in detail in a review article by S.G. Abramovich [6].

The formation of modern higher education aimed at the transition to the global educational space, multifaceted changes in pedagogical practice are accompanied by a radical violation of stereotypes in the minds, actions and lifestyles of subjects of higher professional education. In this regard, the study and analysis of conditions related to the health of university teachers is of particular relevance.



Currently, methodologies are being developed that combine physical and psychological-social components of the human race with other fields and consider human health as a whole system. Health is one of the necessary conditions for the effective professional activity of a modern person and is the main and relatively non-specific basis of the productivity of all aspects of work and the general well-being of a person. At the same time, the modern teacher's work, which requires a high demand for mental stability, the lack of objective criteria for evaluating work results, the large number of daily processes at work and its high temp, and the constant reforms in the field of education and the decrease in the social status of the teacher lead to a long-term stress on his nerves and it can lead to health problems. [7] This is also related to the urgent transition of higher education institutions to distance learning due to the pandemic [8]. That's why; the purpose of this study is to assess the status of personal health of university teachers by determining their biological age.

### *Experimental*

127 teachers of Karaganda University named after E.A. Buketov participated in the experiment (64 women, 63 men). We divided teachers into 3 groups according to age: 23-39 years old, 40-59 years old; 60 years old and older.

To determine the biological age of teachers, we chose the express method of V.P. Voytenko [5]. The logical scheme of assessment of BA includes the following stages: calculation of the value of BA for a given person (a mathematical formula using a series of physiological indicators and self-assessment of health according to a special questionnaire); calculate the appropriate biological age (ABA) value for this individual; comparison of obtained (BA) and appropriate value (ABA): to determine how many years the subject is ahead or behind his peers. The obtained value is relative: the calculation point is the population standard – the average value of the degree of aging (ABA) at this calendar age (BA) for the population. This method makes it possible to classify people of the same age according to the degree of “age wear”, and therefore according to the “reserve” of health (grades I and II – slow aging, III – BA corresponds to the population standard, IV and V – accelerated aging).

In this version, the following parameters were determined for the calculation of BA: body mass (kg), arterial blood pressure (BAP and pulse pressure calculation – PP), static balance (SB, sec), as well as self-assessment of health according to a special questionnaire (SAH, etc.).

### *Results and Discussion*

Examination of basic physiological indicators, analysis of self-assessment of university teachers and calculation of individual numbers of BA and ABA according to A.P. Voytenko's express method made it possible to classify the results shown in Table 1. Biological age also indicates the degree of physiological wear of the body and shows the slowing down of the aging of the body compared to the calendar age.

Table 1 shows that 21.26% of teachers belong to the slow aging group. Based on the data in the table, teachers are aging according to their population (22.83%). In addition, the analysis showed that more than half of the teachers (55.90%) are experiencing a rapid aging rate and these indicators are a stress load during the pandemic period.

Table 1

**Average biological age of university teachers (%)**

n-127	BAranks				
BA-47,07	I	II	III	IV	V
	6.30 (8)	14.96 (19)	22.83 (29)	20.47 (26)	35.43 (45)

The biological age describes the physiological status of the human body, in particular, the status of the cardiovascular, respiratory and nervous systems, it would be a matter of attention to determine BA indicators separately in men and women. Because the ability of men's and women's bodies to work has significant differences due to their hormonal characteristics [7].

It can be seen from the table that 11.11% of the examined teachers were in the group of men of I and II degrees. People in this category have a slower rate of aging and this indicates that they have a good reserve of health. The rate of aging of 9.52% of the examined (grade III) corresponds to the population standard, and

a significant group of male teachers (79.37%) has an accelerated rate of aging, which means that they are at the limit of health. And the data obtained from women turned out to be different. A third (31.25%) of the examined female teachers have a slow aging rate. 35.94% met the population standard and female teachers with a rapid aging rate were also in this range (32.82%) (Table 2).

Table 2

**Average biological age of male and female university teachers**

n-63	BA ranks of men				
	I	II	III	IV	V
	1.59 (1)	9.52 (6)	9.52 (6)	19.05 (12)	60.32 (38)
n-64	BA ranks of woman				
	10.94 (7)	20.31 (13)	35.94 (23)	21.88 (14)	10.94 (7)

Thus, our studies have shown that there's the existence of gender differences in the leading morpho-functional systems of the organism. Male teachers were more exposed to chronic stress in the context of the urgent transition to distance education [9].

It is known that the reaction to stress is manifested in different ways depending on the age characteristics of the organism [10]. In this regard, we considered BA indicators in three groups of teachers according to their age: 1st group 23-39 years old: 2nd group 40-59 years old: 3rd group 60 years old and older. Indicators of men and women, divided by age, were considered separately.

As can be seen from Table 3, 2.86% of male university lecturers aged 23-39 years were assigned to the 1st and 2nd degrees, and the same result was recorded in the 3rd degree, and 94.29% of the university teachers have a fast aging rate. 5.6% of 18 examined male teachers in group II were assigned to grades 1 and 2, that is, slow aging of these teachers can be observed. 22.2% correspond to the population standard, and 72.2% have rapid aging. According to our data, half of the male adult group is aging slowly, 10% of the teachers correspond to the population standard for BA, and 40% were found to be aging faster than the population standard.

Table 3

**BA indicator of teachers in terms of age ( % ), KarU**

Ranks	Tested groups (men) KarU		
	1-group (23-39 years old) n-35	2-group (40-59 years old) n-18	3-group (60 years old and older) n-10
I	0	0	10
II	2.86	5.6	40
III	2.86	22.2	10
IV	14.29	27.8	20
V	80	44.4	20

The examination of the features of the BA in women showed the following data (Table 4). Among the group of young teachers, 21.21% of those examined were assigned to the category I and II, and the rate of aging of these categories is slowly passing. In 36.36% of the studied female teachers, the rate of aging corresponds to the population standard, that is normal. And 42.42% of women have an accelerated rate of aging, that is, they are in a state of extreme health.

Table 4

**BA indicator of teachers in terms of age(%), KarU**

Ranks	Tested groups (women) KarU		
	1-group (23-39 years old)	2-group (40-59 years old)	3-group (60 years old and older)
I	3.03	18.52	25
II	18.18	22.22	25
III	36.36	37.03	25
IV	27.27	14.81	25
V	15.15	7.41	-

In the second age group, the number of female teachers with a slow aging rate is increasing, they constitute 40.74% of the examined and correspond to the population standard of 37.3%. In addition, 22.22% of the examined middle-aged female teachers have an accelerated rate of aging (Table 4).

Among female teachers in the oldest third group, those with a slow aging rate were 50%, and among those tested, 25% corresponds to the population standard, and a quarter of these female teachers appeared in the category of fast aging.

### Conclusion

During the determining the biological age according to the method of V.P. Voytenko, the research showed that more than half of the teachers (55.90%) felt an accelerated rate of aging (grades IV-V of BA), through this research we can see that the stress pressure during the pandemic period had a significant impact.

The received BA indicators showed gender differences in the work of leading morphological and functional systems of the body. The health of male teachers is chronically stressed due to the urgent transition to distance education.

BA indicators vary depending on the age of the examinees. The highest rate of aging is observed in the studied group (23-39 years old), and this process is more prevalent among male teachers.

An urgent transition from the usual traditional education format (during the pandemic) to distance education showed that there is an accelerated aging rate in a significant group of teachers (23-80) according to the BA indicators. At the same time, the biological age is affected by the gender and age of the person who was tested.

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## Пандемия салдарынан қашықтықтан білім алуға шұғыл көшу кезеңінде университет оқытушыларының биологиялық жасын зерттеу

Мақалада университет оқытушыларының биологиялық жасы (БЖ) (В.П. Войтенконың экспресс-әдісі бойынша) қарастырылған, барлығы 127 адам (64 әйел, 63 ер) зерттелді, олар жастарына қарай үш топқа бөлінді (1-топ — 23-39 жас; 2-топ — 40-59 жас; 3-топ — 60 жас және одан жоғары). Биологиялық жастың (БЖ) мәнін есептеу үшін арнайы денсаулықты өзін-өзі бағалау сауалнамасы және бірқатар физиологиялық көрсеткіштер мен математикалық формулалар қолданылды; тиісті биологиялық жастың мәні (ТБЖ) есептелді; алынған биологиялық жас және тиісті биологиялық жас салыстырылды, зерттелушілердің картаю жылдамдығы құрдастарынан қанша жыл бұрын немесе артта қалғаны анықталды. Мұндай тәсіл жастары бірдей адамдарды «жасына қарай қаусауы» дәрежесі бойынша, сонымен



қатар денсаулығының «қоры» бойынша бірнеше дәрежеге бөлуге мүмкіндік береді (I және II дәрежелер — баяу қартаю, III — БЖ популяциялық стандартқа сәйкес келеді, IV және V — жеделдетілген қартаю). Зерттеу жұмыстары пандемия кезінде жоғары оқу орнының қашықтықтан оқытуға көшуі кезінде жүргізілді (2020 жылғы қазан-қараша). Оқытушылардың жартысынан көбі (55,90%) тез қартаю қарқынын (БЖ IV-V дәрежесі) бастан кешіргені белгілі болды, бұл ретте әйелдерге қарағанда ерлер арасында осындай жағдай анағұрлым көп. Тексерілгендердің ішінде қартаю қарқыны ең жас оқытушылар тобында айқын көрінді. Пандемияға байланысты әдеттегі дәстүрлі оқу форматынан қашықтықтан оқытуға шұғыл ауысу кезеңінде биологиялық жас көрсеткіштері бойынша қартаюдың жедел қарқыны оқытушылардың барлық топтарында дерлік байқалды: яғни 23%-80%. Ал биологиялық жастың шамасына зерттелушілердің жынысы мен жасы әсер етеді деген тұжырым бар.

*Кілт сөздер:* денсаулық, стресс, қашықтықтан білім беру, пандемия, жас, биологиялық жас, жоғары оқу орнының оқытушылары, гендер.

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## Исследование показателя «биологический возраст у преподавателей университета» в период срочного перехода на дистанционное образование в результате пандемии

В статье изучен биологический возраст (БВ) (экспресс-метод В.П. Войтенко) преподавателей университета. Всего обследовано 127 человек (64 женщины, 63 мужчины), которых разделили на три возрастные группы (1 группа — 23–39 лет; 2 — 40–59 лет, 3 группа — 60 лет и старше). Расчет значения БВ включал математическую формулу с использованием ряда физиологических показателей и самооценки здоровья по специальной анкете; расчет должного значения биологического возраста (ДБВ); сопоставление полученной (БВ) и должной величины (ДБВ) вычисляли, на сколько лет обследуемый опережает или отстает от сверстников по темпам постарения. Такой подход позволяет ранжировать лиц одного КВ по степени «возрастного износа» и, следовательно, по «запасу» здоровья (I и II ранги — замедленное постарение, III — БВ соответствует популяционному стандарту, IV и V — ускоренное постарение). Исследование проведено в период пандемии при переходе вуза на дистанционное обучение (октябрь–ноябрь 2020 г). Оказалось, что более половины преподавателей (55,90 %) испытывают ускоренный темп постарения (IV–V ранги БВ), при этом среди мужчин таковых больше, чем женщин. Темпы постарения наиболее выражены у молодой группы обследованных (23–39 лет) и в возрастном аспекте у преподавателей-мужчин. Срочный переход на дистанционное образование с обычного традиционного (в период пандемии) проявился в регистрации значительной группы преподавателей (23–80 %) с ускоренным темпом постарения по показателям БВ. При этом на величину биологического возраста влияют пол и возраст обследованного.

*Ключевые слова:* здоровье, стресс, дистанционное образование, пандемия, возраст, биологический возраст, преподаватели вуза, гендер.

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## Flora synopsis of the “Kamenny lake tract” natural monument (Kostanay region)

The article presents the results of the floristic diversity study of the state natural monument of local significance – “Kamenny lake tract” with a total area of 2.5 hectares. According to natural conditions, this territory is included in the subzone of arid forb-feather grass steppes on low-humus southern chernozems and dark chestnut soils, plowed up to 80%. Under the birch forest soils are dark gray, loamy, and sandy; on slopes they are leached chernozem; near-shore soils are meadow-marsh soils. The studies were carried out in 2019-2022. On the basis of field studies, five main ecotopes were identified: shrub thickets on slopes, birch forest on steep slopes, upland meadows with shrub thickets, meadow-marsh communities on coasts, and meadow-steppe communities. A synopsis containing species' habitat information within the specially protected natural area was compiled. In the study area, the total list of higher vascular plants includes 177 species from 48 families and 132 genera. The leading families are *Asteraceae*, *Rosaceae*, *Poaceae*, *Fabaceae*, *Lamiaceae*, and *Caryophyllaceae*. The basis of the flora is herbaceous plants, followed by woody, aquatic plants. The steppe ecological-coenotic group is the richest by the volume of species. In the flora of the “Kamenny lake tract” a large proportion of species has significant extent areas: cosmopolitan, holarctic, and palearctic. The holarctic and palearctic types are the largest number of species. The Red Book of Kazakhstan species in the study area are *Adonis wolgensis*, *Stipa pennata*, and *Tulipa biebersteiniana*.

*Keywords:* flora, specially protected natural area, natural monument “Kamenny lake tract”, higher vascular plants, rare and endangered plants.

### Introduction

The study of flora of specially protected natural areas plays an important role in the identification and conservation of biological diversity [1]. In the valley of the Tobol River there is a unique natural monument – “Kamenny lake tract”, which in 1992 received its conservation status. The natural monument itself is a remnant of the ancient old town of Tobol, the flora of which includes a large number of boreal elements.

However, to date, a flora summary has not been compiled for this object, which allows planning monitoring and environmental measures.

The purpose of this work is to compile a summary of the flora of the natural monument “Kamenny lake tract”.

### Experimental

The object of the study is the botanical natural monument “Kamenny lake tract”, located 3 km from the village of Zarechnoye. According to natural conditions, this territory is included in the subzone of arid forb-feather grass steppes on low-humus southern chernozems and dark chestnut soils, plowed up to 80% [1, 2].

The soils under the birch forest are dark gray, loamy, and sandy, on the slopes they are formed by leached chernozem, near the shore they are meadow-marsh soils. The studies were carried out in 2019-2022. During the floristic study, the following ecotopes were identified: birch forest on a steep slope, shrub thickets on a slope, upland meadows with shrub thickets, meadow-marsh communities on the shore, meadow-steppe communities.

The collections of the authors are stored in the funds of the Kostanay Regional University named after A. Baitursynov. The collected herbarium material was supplemented with literature data [3-6].

The synopsis contains a list of wild (native and adventitious) species of vascular plants of the natural monument “Kamenny lake tract”. Families of angiosperms are located according to the system of A.L. Takhtadzhyan (2009) [7], and genera within families and species within genera alphabetically in Latin names. The Latin and Russian names of the species are given according to S.A. Abdulina (1999) [8], taking

into account modern data. Phytoprotective status was assessed on the basis of the Red Book of Kazakhstan [9].

### Results and Discussion

On the territory of 2.5 hectares, there 177 species concentrated belonging to 132 genera and 48 families. Description includes the following data: taxa names, habitat, life form, ecological group, period of reproduction, areal type, and useful properties.

#### Division Equisetophyta, Class Equisetopsida

##### Family Equisetaceae Rich. ex DC.

*Equisetum arvense* L. Northern slope to the lake basin. Meadow-marsh communities on the shore. Long-rhizome herbaceous horsetail. Cosmopolitan. Mesophyte. Weedy. V. Medicinal, food, dyeing.

*Equisetum hyemale* L. Northern slope to the lake basin. Birch forest on a steep slope; meadow-marsh communities on the shore. Long-rhizome herbaceous horsetail. Holarctic. Xeromesophyte. Forest. VII–VIII. medicinal, technical.

*Equisetum pratense* Ehrh. Meadow-marsh communities on the shore. Long-rhizome herbaceous horsetail. Holarctic. Mesophyte. Swamp. V–VI.

#### Division Polypodiophyta, Class Polypodiopsida

##### Family Athyriaceae Ching

*Athyrium filix-femina* (L.) Roth. Birch forest. Short rhizome herbaceous fern. Holarctic. Mesophyte. Forest. VI–IX. Decorative, poisonous.

*Cystopteris fragilis* (L.) Bernh. Wet ravine. Short rhizome herbaceous fern. Cosmopolitan. Hygromesophyte. Petrophytic. VII–IX. Decorative.

##### Family Onocleaceae Pichi Sermolli

*Matteuccia struthiopteris* (L.) Tod. Shore of the lake. Short rhizome herbaceous fern. Holarctic. Hygromesophyte. Forest. VII–IX. Decorative, insecticidal, poisonous.

#### Division Pinophyta, Class Ephedropsida

##### Family Ephedraceae Dumert.

*Ephedra distachya* L. 1753, Shore of the lake. Evergreen shrub. Pannono-Black Sea-Kazakhstan. Xerophyte. Steppe. VI. Medicinal, food, poisonous.

#### Division Magnoliophyta, Class Magnoliopsida, Subclass Magnoliidae, Order Nymphaeales

##### Family Nymphaeaceae Salisb.

*Nuphar lutea* (L.) Smith. Meadow-marsh communities on the shore. Floating and underwater long-rhizome polycarp. South Mediterranean. Hydrophyte. Aqueous. VI–VIII. Medicinal, food, dyeing, tanning, insecticidal, decorative.

#### Order Ceratophyllales

##### Family Ceratophyllaceae S.F. Gray

*Ceratophyllum demersum* L. Meadow-marsh communities on the shore. Floating and underwater long shoot polycarp. Plerctic. Hydrophyte. Aqueous. VII–VIII.

#### Subclass Ranunculidae, Superorder Ranunculanae, Order Ranunculales

##### Family Ranunculaceae Juss.

*Adonis wolgensis* Steven. Birch forest on a steep slope. Short rhizome polycarp. Pannono-Black Sea-Kazakhstan. Mesophyte. Steppe. V–VI. Medicinal, decorative, poisonous.

*Ranunculus lingua* L. Coast of the lake. Amphibious long-rhizome polycarp. Eastern Mediterranean. Hygrophyte. Coastal water. VI–VIII. Paginate, ornamental, poisonous.

*Ranunculus repens* L. Northern slope to the lake basin. Birch forest on a steep slope; meadow-marsh communities on the shore. Long-rhizome polycarp. Holarctic. Hygrophyte. Coastal water. V–VIII. Medicinal, melliferous, poisonous.

*Ranunculus sceleratus* L. Northern slope to the lake basin. Meadow-marsh communities on the shore. Long vegetative annuals. Holarctic. Hygrophyte. Coastal water. VI–IX. Medicinal, melliferous, poisonous.

*Thalictrum minus* L. Dry meadows with shrubs. Kistekornevy polycarpic. Holarctic. Mesophyte. Forest. VI–VII. Medicinal, dyeing, perganate, poisonous.

*Thalictrum simplex* L. Meadow-steppe communities. Kistekornevy polycarpic. Palearctic. Mesophyte. Forest. VI–VIII. Medicinal, perganic, poisonous.

#### Subclass Caryophyllidae, Superorder Caryophyllanae, Order Caryophyllales

##### Family Caryophyllaceae Juss.

*Elisanthe viscosa* (L.) Rupr. Shrubs on the slope. Biennial, perennial monocarpic. Kazakh-Turano-Central Asian. Xeromesophyte. Steppe. VI–VIII. Melliferous.

*Eremogone koriniana* (Fischer ex Fenzl) Ikonn. Dry meadows with shrubs. Semi-shrub. Zavolzhsko-Kazakhstan. Xeromesophyte. Steppe. VI–VII. Fodder.

*Eremogone longifolia* (M.Bieb.) Fenzl. Northern slope to the lake basin. Birch forest on a steep slope; meadow-steppe communities. Taprooted polycarpic. Trans-Volga-Kazakhstan-Mongolian. Xeromesophyte. Steppe. VI–VII. Fodder.

*Gypsophila paniculata* L. Northern slope to the lake basin. Shrubs on the slope; meadow-marsh communities on the shore. Taprooted polycarpic. East Palearctic. Xeromesophyte. steppe. VI–VII. Medicinal, decorative, poisonous.

*Melandrium album* (Mill.) Garcke, Northern slope to the lake basin. Birch forest on a steep slope. Taproot polycarpic, perennial monocarpic, annual. Holarctic. Mesophyte. Weedy. VI–VIII. Fodder.

*Otites wolgensis* (Hornem.) Bess. ex Spreng. Dry meadows with shrubs. Perennial, biennial monocarpic. South Palearctic. Xeromesophyte. Steppe. VI–VII.

*Silene nutans* L. Shrubs on the slope. Long-rhizome polycarp. Euro-Siberian. Mesophyte. Forest. VI–VIII. Melliferous, food, fodder.

*Stellaria graminea* L. Northeast slope to the lake basin. Dry meadows with shrubs; meadow-steppe communities. Long-rhizome polycarp. Palearctic. Mesophyte. Meadow. V–VIII. Melliferous, poisonous.

#### **Family Chenopodiaceae Vent.**

*Chenopodium album* L. Northern slope to the lake basin. Meadow-steppe communities. Long vegetative annual. Cosmopolitan. Xeromesophyte. Weedy. VI–IX. Food, fodder, perganic, dyeing, technical.

#### **Superorder Polygonanae, Order Polygonales**

##### **Family Polygonaceae Juss.**

*Persicaria lapathifolia* (L.) Gray. Northern slope to the lake basin. Meadow-steppe communities. Long vegetative annual. Palearctic. Hygrophyte. Coastal water. VII–IX. medicinal, food, fodder, melliferous.

*Polygonum aviculare* L. Northern slope to the lake basin. Birch forest on a steep slope. Long vegetative annual. Palearctic. Mesophyte. Weedy. VII–IX. Medicinal, fodder, dyeing, tanning.

*Rumex confertus* Willd. Northern slope to the lake basin. Meadow-marsh communities on the shore. Short rhizome polycarp. Palearctic. Meadow. VI–VII. Medicinal, fodder, dyeing, tanning.

*Rumex crispus* L. Meadow-steppe communities. Short rhizome polycarp. Holarctic. Hygrophyte. Meadow. VI–VIII. Food, medicinal, melliferous, fodder, dyeing, tanning.

#### **Order Plumbaginales**

##### **Family Limoniaceae Ser.**

*Limonium gmelinii* (Willd.) Kuntze. Dry meadows with shrubs. Taprooted polycarpic. Common Mediterranean. Xeromesophyte. Saline. VII–VIII. Tanning, dyeing, melliferous, decorative.

#### **Subclass Hamamelidae, Superorder Hamamelidanae, Order Betulales**

##### **Family Betulaceae S.F. Gray**

*Betula pendula* Roth. Northern slope to the lake basin. Shrub thickets on a slope, birch forest on a steep slope, meadow-marsh communities on the shore, meadow-steppe communities. Tree. Western Palearctic. Mesophyte. Forest. V. Wood, tannic, medicinal, sugar-bearing, essential oil, ornamental, perganic, dyeing, fodder, decorative.

#### **Superorder Primulanae, Order Primulales**

##### **Family Primulaceae Batsch ex Borkh.**

*Androsace filiformis* Retz. Meadow-steppe communities. Ephemera. Palearctic. Hygrophyte. Steppe. V–VIII.

*Lysimachia vulgaris* L. Northeast slope to the lake basin. Birch forest on a steep slope: meadow-marsh communities on the shore. Long-rhizome polycarp. Western Palearctic. Mesophyte. Forest. VI–VII. Medicinal, melliferous, dyeing.

*Naumburgia thyrsoflora* (L.) Rchb. Meadow-marsh communities on the shore. Amphibious long-rhizome polycarp. Holarctic. Hygrophyte. Swamp. VI–VII.

#### **Superorder Violanae, Order Violales**

##### **Family Salicaceae Mirb.**

*Populus tremula* L. Shrub thickets on a slope, birch forest on a steep slope, meadow-marsh communities on the shore, meadow-steppe communities. Tree. Palearctic. Mesophyte. Forest. V. Wood, medicinal, tannic, perganic, dyeing, fodder.

*Salix caprea* L. Northern slope to the lake basin. Birch forest on a steep slope. Bush. Palearctic. Mesophyte. Forest. V. Medicinal, woody, ornamental, melliferous, perganic, fodder, tannic, dyeing.

*Salix triandra* L. Northern slope to the lake basin. Birch forest on a steep slope, meadow-steppe communities. Bush. Palearctic. Mesophyte. Forest. V. Melliferous, perganate, ornamental, fodder, tannic, dyeing.

**Family Brassicaceae Burnett**

*Alyssum tortuosum* Waldst. & Kit. ex Willd. Northern slope to the lake basin. Dry meadows with shrubs. Semi-shrub. Western Mediterranean. Xerophyte. steppe. V-VI. Melliferous, decorative.

*Capsella orientalis* Klokov. Northern slope to the lake basin. Shrubs on the slope. Long vegetative annual. Black Sea-Central Kazakh. Xeromesophyte. Weedy. V-IX. Medicinal, food, fatty oil.

*Lepidium ruderalis* L. Shrubs on the slope. Long vegetative annual. Western Palearctic. Xeromesophyte. Weedy. V-VIII. Fatty oil, insecticidal.

*Sisymbrium loeselii* L. Northeast slope to the lake basin. Dry meadows with shrubs. Long vegetative annual. Palearctic. Xeromesophyte. Weedy. V-VIII. Fodder, melliferous, fatty oil.

**Family Cannabaceae Martinov**

*Humulus lupulus* L. Northern slope to the lake basin. Meadow-steppe communities. Lianoid polycarpic. Holarctic. Mesophyte. Forest. VI-VIII. Medicinal, food, spicy, fibrous, perganic, decorative.

**Family Urticaceae Juss.**

*Urtica dioica* L. Northern slope to the lake basin. Shrub thickets on a slope, birch forest on a steep slope, upland meadows with shrub thickets, meadow-marsh communities on the shore. Long-rhizome polycarp. Western Palearctic. Mesophyte. Weedy. VI-VIII. Medicinal, food, fodder, fiber, dyeing.

**Superorder Euphorbianaes, Order Euphorbiales**

**Family Euphorbiaceae Juss.**

*Euphorbia subcordata* C.A.Mey. Meadow-steppe communities. Long-rhizome polycarp. Transvolgo-central-Kazakhstan. Xeromesophyte. steppe. V-VI. poisonous.

*Euphorbia virgata* Waldst. & Kit. Northern slope to the lake basin. Birch forest on a steep slope, meadow-marsh communities on the shore, meadow-steppe communities. Long-rhizome polycarp. Panno-Black Sea-Kazakhstan. Xeromesophyte. Weedy. VI-VIII. Medicinal, dyeing, melliferous, insecticidal, poisonous.

**Subclass Rosidae, Superorder Rosanaes, Order Saxifragales**

**Family Crassulaceae J. St.-Hil.**

*Hylotelephium triphyllum* (Haw.) Holub (*Sedum telephium* L.). Northern slope to the lake basin. Shrubs on a slope birch forest on a steep slope, upland meadows with shrubs, shrubs. Succulents. Eurosiberian. Mesophyte. Forest. VII-VIII. Medicinal, melliferous, perganic, decorative.

**Family Grossulariaceae DC.**

*Ribes nigrum* L. Northern slope to the lake basin. Birch forest on a steep slope. Bush. Eurosiberian. Hygrophyte. Forest. V-VI. Food, medicinal, spicy, tea substitute, melliferous.

**Order Rosales**

**Family Rosaceae Juss.**

*Amygdalus nana* L. Birch forest on a steep slope. Bush. Pannono-Black Sea-Kazakhstan. Xeromesophyte. steppe. V. Medicinal, melliferous, essential and fatty oil, ornamental, poisonous.

*Cerasus fruticosa* (Pall.) G. Woron. Northeast slope to the lake basin. Dry meadows with shrubs. Bush. Pannono-Black Sea-Kazakhstan. Xeromesophyte. Forest. V-VI. Food, melliferous, decorative.

*Cotoneaster melanocarpus* Fisch. Ex Blytt. Northeast slope to the lake basin. Shrubs on the slope. Bush. Paleoarctic. Xeromesophyte. Forest. V-VI. Food, melliferous, decorative.

*Crataegus sanguinea* Pall. Northeast slope to the lake basin. Shrubs on the slope. Tree. Trans-Volga-Kazakhstan-Mongolian. Xeromesophyte. Forest. V-VI. Medicinal, food, melliferous, dyeing, decorative.

*Filipendula vulgaris* Moench. Northeast slope to the lake basin. Shrubs on the slope; upland meadows with shrubs. Tuber-forming polycarpic. South Palearctic. Xeromesophyte. steppe. VI-VII. Food, Fodder, melliferous, tea substitute, decorative.

*Fragaria viridis* (Duchesne) Weston. Northeast slope to the lake basin. Birch forest on a steep slope. Stolon-forming polycarpic. Western Palearctic. Xeromesophyte. steppe. V-VI. Food (berry), medicinal, tea substitute, melliferous.

*Malus domestica* Borkh. Meadow-steppe communities. Tree. Originated in culture. Mesophyte. Cultivated and wild. V. Food (fruit), melliferous.

*Padus avium* Mill. Northern slope to the lake basin. Birch forest on a steep slope; meadow-marsh communities on the shore. Bush. Western Palearctic. Mesophyte. Forest. V-VI. Medicinal, food, melliferous, ornamental, decorative.

*Potentilla anserine* L. Northern slope to the lake basin. Birch forest on a steep slope; meadow-steppe communities. Stolone-forming and creeping polycarp. Cosmopolitan. Mesophyte. Coastal water. VI-IX. Medicinal, food, melliferous, dyeing.

*Potentilla argentea* L. Northeast slope to the lake basin. Dry meadows with shrubs. Stolone-forming polycarpic. Eurosiberian. Xerophyte. Meadow. VI-IX. Medicinal, melliferous, fodder, tannic, dyeing.

*Potentilla bifurca* L. Northern slope to the lake basin. Birch forest on a steep slope, shrub thickets on the slope, upland meadows with shrub thickets. Long-rhizome polycarp. Trans-Volga-Kazakhstan-Mongolian. Xeromesophyte. Steppe. VI-VIII. Fodder, melliferous.

*Potentilla canescens* Besser. North slope. Meadow-steppe communities. Short rhizome herbs. Western Palearctic. xeromesophyte. Steppe. VI-VII.

*Potentilla humifusa* Willd. Ex Schltl. Northeast slope to the lake basin. Dry meadows with shrubs. Short rhizome herbs. Black Sea-Kazakhstan. Xeromesophyte. Steppe. V-VI.

*Rosa acicularis* Lindl. Shrubs on the slope. Bush. Holarctic. Mesophyte. Forest. VI-VII. Medicinal, food, melliferous, essential and fatty oil, decorative.

*Rosa majalis* Herrm. Northern slope to the lake basin. Shrub thickets on the slope, meadow-marsh communities on the shore, meadow-steppe communities. Bush. Eurosiberian. Xeromesophyte. Forest. VI-VII. Medicinal, food, melliferous, perganic, essential and fatty oil, dyeing, decorative.

*Rosa spinosissima* L. Shrubs on the slope. Bush. Western Palearctic. Xeromesophyte. steppe. V-VI. food, melliferous.

*Rubus caesius* L. Northern slope to the lake basin. Meadow-marsh communities on the shore. Creeping shrub. Western Palearctic. Mesophyte. Forest. VI-VIII. Medicinal, food, fodder, melliferous, perganate, dyeing, decorative.

*Rubus idaeus* L. In the undergrowth of a birch forest. Bush. Eurosiberian. Mesophyte. Forest. VI-VII. Food, medicinal, tea substitute, melliferous.

*Sanguisorba officinalis* L. Northern slope to the lake basin. Birch forest on a steep slope, meadow-marsh communities on the shore, meadow-steppe communities. Long-rhizome polycarp. Holarctic. Mesophyte. Meadow. VI-VII. Medicinal, food, melliferous, fodder, dyeing, tanning.

*Spiraea hypericifolia* L. Northern and northeastern slope to the lake basin. Shrub thickets on a slope, birch forest on a steep slope, meadow-steppe communities. Bush. Kazakh-Turano-Central Asian. Xeromesophyte. steppe. V-VI. Ornamental, melliferous, decorative.

#### **Family Onagraceae Juss.**

*Chamaenerion angustifolium* (L.) Scop. Northern slope to the lake basin. Birch forest on a steep slope. Long-rhizome polycarp. Holarctic. Mesophyte. Forest. VII-VIII. Medicinal, Melliferous, Food, Fodder, Tea Surrogate, Tannic, Fibrous, Fatty Oil, Decorative.

*Epilobium montanum* L. Meadow-marsh communities on the shore. Stolone-forming and creeping polycarp. Holarctic. Mesophyte. Coastal water. VI-VII. Fodder.

#### **Superorder Fabanae, Order Fabales**

##### **Family Fabaceae Lindl.**

*Amoria repens* (L.) C. Presl. Northern slope to the lake basin. Birch forest on a steep slope; meadow-steppe communities. Stolone-forming and creeping polycarp. Cosmopolitan. Mesophyte. Weedy. VI-IX. Fodder, melliferous, medicinal, dyeing.

*Astragalus danicus* Retz. North slope. Meadow-steppe communities. Long-rhizome polycarp. Eurosiberian. Mesophyte. meadow. VI-VII. Fodder, melliferous.

*Astragalus cornutus* Pall. Lug, June 29, 2004, collections of KSPI students. Semi-shrub. Black Sea-Kazakhstan. xeromesophyte. Steppe. V-VI.

*Astragalus onobrychis* L. North slope. Meadow-steppe communities. Taprooted polycarpic. Western Palearctic. Xeromesophyte. Steppe. VI-VII. Fodder, melliferous.

*Astragalus varius* S.G. Gmel. North slope. Meadow-steppe communities. Semi-shrub. Black Sea-Kazakhstan. Xeromesophyte. Steppe. VI-VII. Fodder.

*Caragana frutex* (L.) K. Koch. Northern slope to the lake basin. Shrub thickets on the slope, upland meadows with shrub thickets. Bush. Black Sea-Kazakhstan-Mongolian. Xeromesophyte. steppe. V-VI. Ornamental, fodder, melliferous, dyeing, decorative.

*Genista tinctoria* L. Northern slope to the lake basin. Shrubs on the slope. Bush. Pannono-Black Sea-Kazakhstan. Xeromesophyte. steppe. VI–VIII. Medicinal, dyeing, decorative, poisonous.

*Medicago falcata* L. Northeast slope to the lake basin. Dry meadows with shrubs, meadow-marsh communities on the coast, meadow-steppe communities. Taprooted polycarpic. Palearctic. Xeromesophyte. Steppe. VI–VIII. Fodder, melliferous.

*Melilotus officinalis* (L.) Pall. Northern slope to the lake basin. Shrubs on the slope. Taprooted polycarpic. Western Palearctic. Xeromesophyte. Weedy. VI–IX. Medicinal, Fodder, spicy, melliferous, insecticidal, dyeing.

*Oxytropis pilosa* (L.) DC. Dry meadows with shrubs. Taprooted polycarpic. South Palearctic. Xerophyte. steppe. VI–VIII. Melliferous, ornamental, poisonous.

**Superorder Rhamnanae, Order Rhamnales**

**Family Rhamnaceae Juss.**

*Rhamnus cathartica* L. Meadow-marsh communities on the shore. Bush. Western Palearctic. Mesophyte. Forest. V–VI. Medicinal, dyeing, tannic, melliferous, ornamental, decorative.

**Order Geraniales**

**Family Geraniaceae Juss.**

*Geranium collinum* Stephan ex Willd. Northern slope to the lake basin. Meadow-marsh communities on the shore. Long-rhizome polycarp. South Palearctic. Hygromesophyte. Meadow. VI–VII. Tanning, dyeing

*Geranium pratense* L. Northern slope to the lake basin. Meadow-steppe communities. Short rhizome polycarp. Palearctic. Mesophyte. Forest. VI–VIII. Medicinal, fodder, melliferous, dyeing, decorative.

**Order Apiales**

**Family Apiaceae Lindl**

*Falcaria vulgaris* M. Bernh. Meadow-steppe communities. Taprooted polycarpic. Western Palearctic. xeromesophyte. Steppe. VII–VIII.

*Heraclium sibiricum* L. Northern slope to the lake basin. Birch forest on a steep slope, meadow-marsh communities on the shore. Taprooted polycarpic. Eurosiberian. Mesophyte. Forest. VI–VII. Medicinal, food, fodder, dyeing, melliferous.

*Kadenia dubia* (Schkuhr) Lavrova & V.N.Tikhom. Northern slope to the lake basin. Birch forest on a steep slope, meadow-steppe communities. Short rhizome polycarp. Western Palearctic. Mesophyte. Forest. VII–VIII.

*Seseli ledebourii* G. Don. Dry meadows with shrubs. Taprooted polycarpic. Zavolzhsko-Kazakhstan. xeromesophyte. Steppe. V–VI.

*Seseli strictum* Ledeb. Birch forest on a steep slope. Taprooted polycarpic. Kazakh. Mesophyte. Meadow. VII–VIII.

**Family Caprifoliaceae Juss.**

*Lonicera tatarica* L. Northern and northeastern slope to the lake basin. Birch forest on a steep slope, upland meadows with shrubs, meadow-marsh communities on the shore. Bush. Zavolzhsko-Kazakhstan. Mesophyte. Forest. VI. Melliferous, decorative, poisonous.

**Family Valerianaceae Batsch**

*Valeriana tuberosa* L. Northeast slope to the lake basin. Dry meadows with shrubs. Tuber-forming polycarpic. Western Mediterranean. Mesophyte. Steppe. V–VI.

**Subclass Lamiidae, Superorder Lamianae, Order Rubiales**

**Family Rubiaceae Juss.**

*Galium boreale* L. Shrubs on the slope. Long-rhizome polycarp. Holarctic. Mesophyte. Forest. VI–VIII. Dyeing, melliferous.

*Galium palustre* L. Meadow. Long-rhizome polycarp. Holarctic. Hygromesophyte. Swamp. VI–VIII.

*Galium verum* L. Northeast slope to the lake basin. Dry meadows with shrubs meadow-steppe communities. Short rhizome polycarp. Holarctic. Xeromesophyte. steppe. VI–VIII. Dyeing, melliferous, fodder.

**Order Solanales**

**Family Solanaceae Juss.**

*Hyoscyamus niger* L. Meadow-steppe communities. Biennial monocarpic. Palearctic. Xeromesophyte. Weedy. VI–VIII. Medicinal, melliferous, insecticidal, poisonous.

**Family Convolvulaceae Juss.**

*Calystegia sepium* (L.) R. Br. Meadow-marsh communities on the shore. Lianoid polycarpic. Cosmopolitan. Mesophyte. Forest. VI–VIII. Medicinal, melliferous, decorative.



*Convolvulus arvensis* L. Shrub thickets on the slope meadow-steppe communities. Long-rhizome polycarp. Cosmopolitan. Mesophyte. Weedy. VI–IX. Medicinal, melliferous, fodder.

**Family Boraginaceae Juss.**

*Cynoglossum officinale* L. Meadow-marsh communities on the shore. Biennial. Western Palearctic. Xeromesophyte. Weedy. VI–VIII. Melliferous, insecticidal, poisonous.

*Nonea rossica* Steven. Meadow-steppe communities. Taprooted polycarpic. Eurosiberian. Xeromesophyte. Weedy. V–VIII. Melliferous.

*Onosma simplicissima* L. Northern slope to the lake basin. Shrub thickets on the slope, upland meadows with shrub thickets. Long-rhizome polycarp. Eastern Black Sea-Kazakhstan. Xeromesophyte. Steppe. V–VIII. Dyeing, melliferous, decorative.

**Family Scrophulariaceae Juss.**

*Veronica longifolia* L. Meadow-steppe communities. Long-rhizome polycarp. Palearctic. Mesophyte. Lugovoi. VI–VIII. Melliferous, fodder, decorative.

*Veronica prostrata* L. Shrubs on the slope. Long-rhizome polycarp. Western Palearctic. Xeromesophyte. Steppe. V–VI.

*Veronica spicata* L. Northern slope to the lake basin. Shrub thickets on the slope, meadow-steppe communities. Long-rhizome polycarp. Western Palearctic. Xeromesophyte. Forest. VI–VIII. Perganate, fodder, decorative.

*Veronica spuria* L. Northern and northeastern slope to the lake basin. Shrub thickets on the slope dry meadows with shrub thickets. Long-rhizome polycarp. Eurosiberian. Xeromesophyte. Steppe. VI–VIII. Decorative.

**Family Plantaginaceae Juss.**

*Plantago major* L. Meadow-marsh communities on the shore. Kistekornevy polycarpic. Holarctic. Mesophyte. Weedy. V–IX. medicinal, food, fodder.

*Plantago media* L. Northeast slope to the lake basin. Dry meadows with shrubs. Kistekornevy polycarpic. Holarctic. Xeromesophyte. Weedy. V–IX. Medicinal, fodder.

*Plantago urvillei* Opiz. Steppe. Kistekornevy polycarpic. Pannono-Black Sea-Kazakhstan. Mesophyte. Lugovoi. V–VIII. Medicinal, fodder.

**Family Lamiaceae Lindl.**

*Dracocephalum thymiflorum* L. Northeast slope to the lake basin. Dry meadows with shrubs. Long-term vegetative one-biennial plants. Kazakh-Turano-Central Asian. Xeromesophyte. Weedy. VI–VIII. Melliferous.

*Glechoma hederacea* L. Northern slope to the lake basin. Shrub thickets on the slope, upland meadows with shrub thickets, meadow-marsh communities on the shore. Stolon-forming polycarpic. Palearctic. Mesophyte. Forest. V–VI. Medicinal, essential oil, food, melliferous.

*Hyssopus ambiguus* (Trautv.) Iljin. Mesophilic meadow-forest community (Pugachev, 1994). Semi-shrub. Trans-Volga Kazakh-Mongolian. Xeromesophyte. Steppe. VI–VIII.

*Leonurus glaucescens* Bunge. Dry meadows with shrubs meadow-steppe communities North-eastern slope to the lake basin, shrubs; meadow community northern slope. Short rhizome polycarp. Common Mediterranean. Xeromesophyte. Weedy. VI–VIII.

*Phlomis tuberosa* L. Northeast slope to the lake basin. Dry meadows with shrubs, meadow-steppe communities. Tuber-forming polycarpic. Palearctic. Xeromesophyte. Steppe. V–VIII.

*Salvia stepposa* Des.-Shost. Dry meadows with shrubs. Short rhizome polycarp. Common Mediterranean. Xerophyte. steppe. VI–VIII. Medicinal, essential oil, melliferous.

*Scutellaria dubia* Taliev et Sirj. Meadow-forest community. Long-rhizome polycarp. Eurosiberian. Hygromesophyte. Forest. VI–IX. Medicinal, dyeing, melliferous.

*Stachys palustris* L. Meadow-steppe communities. Long-rhizome polycarp. Western Palearctic. Hygromesophyte. Coastal water. VI–IX. food, melliferous.

*Thymus marschallianus* Willd. Northeast slope to the lake basin. Dry meadows with shrubs. Semi-shrub. South Palearctic. Xeromesophyte. steppe. VI–VIII. medicinal, essential oil, melliferous, spicy, decorative.

**Superorder Asteranae, Order Campanulales**

**Family Campanulaceae Juss.**

*Campanula wolgensis* P.A. Smirn. Northern slope to the lake basin. Birch forest on a steep slope. Short rhizome polycarp. Eurosiberian. Mesophyte. Steppe. VI–VII. Melliferous, decorative.

**Order Asterales****Family Asteraceae Berht. et J. Presl**

*Achillea millefolium* L. Northern and northeastern slopes to the lake basin. Birch forest on a steep slope, upland meadows with shrubs, meadow-marsh communities on the shore. Long-rhizome polycarp. Holarctic. Mesophyte. VI–IX. medicinal, essential oil, spicy, melliferous, dyeing, insecticidal.

*Achillea setacea* Waldst. & Kit. Dry meadows with shrubs. Long-rhizome polycarp. Western Palearctic. Xeromesophyte. Forest. VI–VIII. medicinal, essential oil, spicy, melliferous, dyeing, insecticidal.

*Arctium tomentosum* Mill. Northern slope to the lake basin. Birch forest on a steep slope. Meadow-marsh communities on the shore. Biennial monocarpic. Palearctic. Xeromesophyte. Weedy. VII–VIII. Medicinal, melliferous, food, fodder.

*Artemisia austriaca* Jacq. Meadow-steppe communities. Long-rhizome polycarp. Western Palearctic. Mesoxerophyte. steppe. VII–IX. Essential oil.

*Artemisia commutata* Besser. Meadow community northern slope. Short rhizome polycarp. Central Kazakh-Mongolian. Xeromesophyte. Steppe. VII–VIII

*Artemisia dracuncululus* L. Northern slope to the lake basin. Shrub thickets on the slope meadow-steppe communities. Short rhizome polycarp. Holarctic. Xeromesophyte. steppe. VII–IX. Spicy, essential oil, fodder.

*Artemisia proceraeformis* Krasch. Meadow-marsh communities on the shore. Semi-shrub. Western Palearctic. Mesophyte. Steppe. VII–IX.

*Artemisia vulgaris* L. Northern slope to lake. Birch forest on a steep slope, upland meadows with shrubs, meadow-marsh communities on the shore. Short rhizome polycarp. Holarctic. Mesophyte. Weedy. VII–IX. Medicinal, spicy, insecticidal

*Centaurea scabiosa* L. Northeast slope to the lake basin. Dry meadows with shrubs. Taprooted polycarpic. Western Palearctic. Xeromesophyte. Forest. VII–VIII. Melliferous, fodder.

*Centaurea sibirica* L. Dry meadows with shrubs. Taprooted polycarpic. Zavolzhsko-Kazakhstan. Xerophyte. steppe. V–VII. Melliferous, decorative.

*Centaurea stoebe* L. Dry meadows with shrubs. Biennial. Black Sea-Central Kazakh. Mesophyte. Forest. VII–VIII. Melliferous, decorative.

*Cichorium intybus* L. Shrubs on the slope. Taprooted polycarpic. Western Palearctic. Xeromesophyte. Weedy. VII–IX. Medicinal, food, coffee substitute, fodder, melliferous.

*Cirsium setosum* (Willd.) Besser. Meadow-marsh communities on the shore. Long-rhizome polycarp. Palearctic. Xeromesophyte. Weedy. VII–IX. Melliferous, medicinal.

*Echinops ritro* L. Northern slope to the lake basin. Shrub thickets on the slope, meadow-steppe communities. Taprooted polycarpic. Common Mediterranean. Xeromesophyte. steppe. VII–VIII. Medicinal, melliferous, ornamental, poisonous.

*Helichrysum arenarium* (L.) Moench. Northeast slope to the lake basin. Dry meadows with shrubs. Long-rhizome polycarp. South Palearctic. Xerophyte. Steppe. VII–VIII. Medicinal, decorative.

*Lactuca serriola* Torner. Meadow-marsh communities on the shore. Long vegetative annuals. Palearctic. Xeromesophyte. Weedy. VI–IX. Medicinal, fodder.

*Ptarmica salicifolia* (Bess.) Serg. Meadow-marsh communities on the shore. Long-rhizome polycarp. Palearctic. Mesophyte. Meadow. VII–IX. Melliferous.

*Scorzonera austriaca* Willd. Meadow-steppe communities. Long-rhizome polycarp. South Palearctic. Mesoxerophyte. steppe. V–VI. Fodder, decorative.

*Scorzonera parviflora* Jacq. Meadow-marsh communities on the shore. Long-rhizome polycarp. Common Mediterranean. Hygrophyte. Meadow. VI–VIII.

*Scorzonera purpurea* L. Shrubs on the slope. Long-rhizome polycarp. South Palearctic. Xeromesophyte. Steppe. V–VII. decorative.

*Senecio jacobaea* L. Meadow-steppe communities. Long vegetative annual. Palearctic. Mesophyte. Forest. VII–VIII. Poisonous.

*Solidago virgaurea* L. Northern slope to the lake basin. Birch forest on a steep slope. Long-rhizome polycarp. Western Palearctic. Mesophyte. Forest. VII–IX. Melliferous, dyeing.

*Tanacetum vulgare* L. Northeast slope to the lake basin. Meadow-steppe communities. Long-rhizome polycarp. Holarctic. Mesophyte. Forest. VII–IX. Medicinal, essential oil, spicy, insecticidal, poisonous.

*Taraxacum officinale* F.H. Wigg. Northern and northeastern slopes to the lake basin. bushes on the slope, birch forest on a steep slope, upland meadows with shrubs. Long vegetative annual. Cosmopolitan. Mesophyte. Weedy. V-VI. Medicinal, melliferous.

*Tragopogon pratensis* L. Shrubs on the slope. Perennial, biennial. West Palearctic. Xeromesophyte. Meadow. V-VI.

*Tripleurospermum inodorum* (L.) Sch.Bip. Shrubs on the slope, meadow-marsh communities on the shore. Perennial, biennial. Pannono-Black Sea-Kazakhstan. Mesophyte. Weedy. VI-IX. Insecticidal.

*Trommsdorffia maculata* (L.) Bernh. Northeast slope to the lake basin. Dry meadows with shrubs, meadow-steppe communities. Short rhizome polycarp. South Palearctic. Xeromesophyte. Forest. VI-VIII.

**Class Liliopsida, Subclass Alismatidae, Superorder Alismatanae, Order Butomales**

**Family Butomaceae Mirb.**

*Butomus umbellatus* L. Meadow-marsh communities on the shore. Amphibian short-rhizome polycarp. Palearctic. Hydrohydrophyte. Coastal water. VI-VII. Food, fodder, melliferous.

**Family Hydrocharitaceae Juss.**

*Hydrocharis morsus-ranae* L. Meadow-marsh communities on the shore. Floating and underwater grasses. West Palearctic. Hydrophyte. Aqueous. VII-VIII.

*Stratios aloides* L. Meadow-marsh communities on the shore. Floating and underwater grasses. Western Palearctic. Hydrophyte. Aqueous. VII-VIII. Fodder

**Family Potamogetonaceae Dumort**

*Potamogeton lucens* L. Meadow-marsh communities on the shore. Floating and underwater grasses. Holarctic. Hydrophyte. Aqueous. VII.

**Order Liliales**

**Family Liliaceae Juss.**

*Fritillaria meleagroides* Patrin ex Schult. et Schult. fil. Northeast slope to the lake basin. Dry meadows with shrubs. Bulbous polycarp. Black Sea-Kazakhstan. Hygromesophyte. Meadow. V. Decorative.

*Fritillaria ruthenica* Wikstr. Northern slope to the lake basin. Shrubs on the slope, birch forest on the steep slope. Bulbous polycarp. Black Sea-Kazakhstan. Xeromesophyte. Steppe. V. Decorative.

*Tulipa biebersteiniana* Schult. et Schult. f. Northern and northeastern slope to the lake basin. N53.28597°, W63.77815°, h=141 m a.s.l. Shrubs on the slope, upland meadows with shrubs. Bulbous polycarp. Black Sea-Kazakhstan. Mesophyte. Steppe. IV-V. Decorative.

**Family Alliaceae Agardh**

*Allium lineare* L. Northern and northeastern slope to the lake basin. Shrub thickets on the slope, upland meadows with shrub thickets. Bulbous polycarp. Black Sea-Kazakh-Mongolian. Xeromesophyte. Steppe. VI-VII. Food.

**Order Asparagales**

**Family Convallariaceae Horan.**

*Polygonatum odoratum* (Mill.) Druce. Northern slope to the lake basin. Birch forest on a steep slope. Long-rhizome polycarp. Palearctic. Mesophyte. Forest. V-VI. Medicinal, decorative, poisonous.

**Family Asparagaceae Juss.**

*Asparagus officinalis* L. Northeast slope to the lake basin. Dry meadows with shrubs. Short rhizome polycarp. Eurosiberian. Mesophyte. Forest. VI-VII. Medicinal, food, perganic, decorative.

**Family Cyperaceae Juss.**

*Carex riparia* Curtis. Meadow-marsh communities on the shore. Long-rhizome polycarp. Western Palearctic. Hygrophyte. Coastal water. V-VI. Fodder, pulp, technical.

*Carex supina* Willd. ex Wahlenb. Northeast slope to the lake basin. Dry meadows with shrubs. Long-rhizome polycarp. Pannono-Black Sea-Kazakhstan. xerophyte. Steppe. IV-V. Fodder.

**Superorder Poanae, Order Typhales**

**Family Typhaceae Juss.**

*Typha angustifolia* L. Meadow community on the shore. Amphibious long-rhizome polycarp. Cosmopolitan. Hydrohydrophyte. Coastal water. VI-VII. Technical, cellulose, ornamental, food.

*Typha latifolia* L. Meadow-marsh communities on the shore. Amphibious long-rhizome polycarp. Cosmopolitan. Hydrohydrophyte. Coastal water. VI-VII. Technical, cellulose, ornamental, food.

**Order Poales****Family Poaceae Barnhart**

*Achnatherum splendens* (Trin.) Nevski. Northern slope to the lake basin. Dry meadows with shrubs. Short rhizome polycarp. South Palearctic. Xeromesophyte. steppe. VI–VII. Cellulosic, technical.

*Agropyron cristatum* (L.) Gaertn. North slope. Meadow-steppe communities. Short rhizome polycarp. Western Mediterranean. Xeromesophyte. Steppe. VI–VII.

*Agrostis gigantea* Roth. Northern slope to the lake basin. Birch forest on a steep slope. Soddy loose bushy polycarp. Palearctic. Mesophyte. Lugovoi. VI–VIII. Fodder, decorative.

*Agrostis stolonifera* L. Northern slope to the lake basin. Meadow-marsh communities on the shore. Stolniforming friable bush polycarpic. Eurosiberian. Hygrophyte. Meadow. VI–VIII. Fodder, decorative.

*Bromopsis inermis* (Leyss.) Holub. Northeast slope to the lake basin. Meadow-marsh communities on the shore, meadow-steppe communities, upland meadows with shrubs. Long-rhizome polycarp. Palearctic. Mesophyte. Weedy. VI–VII. Fodder.

*Calamagrostis epigeios* (L.) Roth. Meadow-steppe communities. Long-rhizome polycarp. Palearctic. Xeromesophyte. Meadow. VII. Fodder, technical.

*Elytrigia repens* (L.) Nevski. Northern slope to the lake basin. Birch forest on a steep slope. Long-rhizome polycarp. Palearctic. Mesophyte. Meadow. VI–VIII. medicinal, food, fodder.

*Festuca valesiaca* Gaudin. Northern and northeastern slopes to the lake basin. Shrub thickets on a slope, birch forest on a steep slope, meadow-steppe communities. Sod dense bush polycarpic. Black Sea-Kazakhstan. Xerophyte. Steppe. VI–VII. Fodder, decorative (lawn).

*Hierochloe odorata* (L.) Beauv. Northern slope to the lake basin. Birch forest on a steep slope. Long-rhizome polycarp. Holarctic. Mesophyte. Forest. V–VI. Medicinal, technical, fodder.

*Leymus angustus* (Trin.) Pilg. North slope. Meadow-steppe communities. Long-rhizome polycarp. Turano-Central Asian. xerophyte. Steppe. VI–VII. Fodder.

*Melica altissima* L. Birch forest on a steep slope. Long-rhizome polycarp. Western Palearctic. Mesophyte. Forest. VI–VII. Decorative, poisonous.

*Poa angustifolia* L. Northern and northeastern slope to the lake basin. Birch forest on a steep slope, upland meadows with shrubs, meadow-marsh communities on the shore. Long-rhizome polycarp. Palearctic. Xeromesophyte. Meadow. VI–VII. Fodder.

*Poa pratensis* L. Northern and northeastern slope to the lake basin. Birch forest on a steep slope, upland meadows with shrubs, meadow-marsh communities on the shore. Long-rhizome polycarp. Palearctic. Xeromesophyte. Meadow. VI–VII. Fodder.

*Puccinellia distans* (Jacq.) Parl. Northeast slope to the lake basin. Meadow-steppe communities. Turf loose polycarpic. Eurosiberian. Mesophyte. Weedy. VI–VIII. Fodder.

*Stipa capillata* L. Northern slope to the lake basin. Shrubs on the slope. Sod dense bush polycarpic. Western Mediterranean. Xerophyte. Steppe. VI–VII. Fodder.

*Stipa lessingiana* Trin. et Rupr. Northeast slope to the lake basin. Dry meadows with shrubs meadow-steppe communities. Sod dense bush polycarpic. Eastern Black Sea-Kazakhstan. Xerophyte. Steppe. V–VI. Fodder, decorative.

*Stipa pennata* L. Northern and northeastern slopes to the lake basin. Shrubs on the slope. Sod dense-bush polycarpic West Palearctic. Xerophyte. Steppe. V–VI. Fodder, decorative.

**Superorder Aranae, Order Arales****Family Lemnaceae Martinov**

*Lemna minor* L. In the water of stagnant and slow-flowing water bodies. Floating and underwater monocarpic. Holarctic. Hydrophyte. Aqueous. VI–VII. Fodder.

*Lemna trisulca* L. Meadow-marsh communities on the shore. Floating and underwater monocarpic. Holarctic. Hydrophyte. Aqueous. VI–VII. Fodder.

*Conclusion*

In the flora of "Kamenny lake tract" 177 species of vascular plants belonging to 132 genera and 48 families have been registered. The leading families are *Asteraceae*, *Rosaceae*, *Poaceae*, *Fabaceae*, *Lamiaceae*, *Caryophyllaceae*. The richest genera are: *Artemisia*, *Astragalus*, *Potentilla*, *Centaurea*, *Galium*, *Plantago*, *Ranunculus*, *Veronica*.

The basis of the flora is herbaceous plants, followed by woody and aquatic plants. The steppe ecological-coenotic group is the richest in terms of the volume of species.

In the flora of the “Kamenny lake tract” a large proportion of species has a significant area of distribution – Cosmopolitan, Holarctic, Palearctic. The Holarctic and Palearctic types are the largest in terms of the number of species.

Three species of plants *Stipa pennata*, *Tulipa biebersteiniana*, and *Adonis wolgensis* listed in the Red Book of Kazakhstan were found on the territory of the natural monument [9].

The presented summary of the “Kamenny lake tract” flora is the basis for further work on plant monitoring of this unique territory.

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## Г.Ж. Султангазина, А.Н. Куприянов, Д.К. Ермакова, А.У. Утебасова «Каменное озеро» табиғи ескерткіші флорасының конспектісі (Костанай облысы)

Мақалада жалпы ауданы 2,5 га құрайтын, жергілікті маңызы бар Мемлекеттік табиғи ескерткіш «Каменное озеро» шатқалының флористикалық алуантүрлілігін зерттеудің нәтижелері берілген. Табиғат жағдайларына сәйкес бұл аумақ 80%-ға дейін жыртылған, қарашірігі аз оңтүстік қара топырақты және қоңыр-қызғылт топырақты, құрғақ түрлі шөпті ақ селеулі далалардың аймағына кіреді. Қайыңды орманының топырағы қара-сұр, сазды және құмды, беткейлерде олар сілтісізденген қара топырақпен, жағалауының маңы шалғынды-батпақты топырақтармен қалыптасқан. Зерттеулер 2019-2022 жылдары жүргізілді. Далалық зерттеулер негізінде бес негізгі экотоптар анықталды: беткейдегі бұталы өсімдіктер, тік беткейдегі қайың орманы, құрғақ шалғындардағы бұталы тоғай, жағалаудағы шалғынды-батпақты қауымдастықтар, шалғынды-дала қауымдастықтары. Далалық зерттеулер негізінде ерекше қорғалатын табиғи аймақ шегіндегі түрлердің мекен ортасы туралы мәліметтер бар конспект жасалды. Зерттелетін аумақта 48 тұқымдастан және 132 туыстан жоғары сатылы өсімдіктердің 177 түрі өседі. Олардың ішінде *Asteraceae*, *Rosaceae*, *Poaceae*, *Fabaceae*, *Lamiaceae*, *Caryophyllaceae* жетекші орын алады. Флораның негізін шөптесін өсімдіктер, одан кейін ағаш және су өсімдіктері құрайды. Дала экологиялық-ценоздық тобы түр көлемі жағынан ең бай түр болып саналады. «Каменное озеро» шатқалының флорасында таралу аумағы бойынша космополиттік, голарктикалық, палеарктикалық сияқты түрлердің үлесі едәуір үлкен. Оның ішінде саны жағынан ең үлкені — голарктикалық және палеарктикалық түрлер. Зерттелген аймақта Қазақстанның Қызыл кітабына енгізілген *Adonis wolgensis*, *Stipa pennata* және *Tulipa biebersteiniana* деген түрлер өседі.

*Кілт сөздер:* флора, ерекше қорғалатын табиғи аймақ, «Каменное озеро» шатқалы табиғи ескерткіші, жоғары сатылы өсімдіктер, сирек кездесетін және жойылып бара жатқан өсімдіктер.

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## Конспект флоры памятника природы «Каменное озеро» (Костанайская область)

В статье представлены результаты изучения флористического разнообразия Государственного памятника природы местного значения урочища «Каменное озеро», общей площадью 2,5 га. По природным условиям эта территория входит в подзону засушливых разнотравно-ковыльных степей на малогумусных южных черноземах и темно-каштановых почв, распаханых до 80 %. Почвы под березовым лесом темно-серые, суглинистые и песчаные, на склонах сформированы черноземом выщелоченным, возле берега — лугово-болотные почвы. Исследования проводились в 2019–2022 гг. На основе полевых исследований были выделены пять основных экотопов: кустарниковые заросли на склоне, березовый лес на крутом склоне, суходольные луга с кустарниковыми зарослями, лугово-болотные сообщества на берегу, лугово-степные сообщества. Составлен конспект, содержащий сведения о местобитании видов, в пределах особо охраняемой природной территории. На изучаемой территории установлено произрастание 177 видов высших сосудистых растений из 48 семейств и 132 родов. Среди них ведущее значение занимают *Asteraceae*, *Rosaceae*, *Poaceae*, *Fabaceae*, *Lamiaceae*, *Caryophyllaceae*. Основу флоры составляют травянистые растения, далее следуют древесные и водные. Степная эколого-ценотическая группа по объему видов наиболее богата. Во флоре урочища «Каменное озеро» большая доля видов имеет значительные по протяженности ареалы: космополитный, голарктический, палеарктический. Наиболее крупными по числу видов являются голарктический и палеарктический типы. На исследуемой территории произрастают виды, включенные в Красную книгу Казахстана: *Adonis wolgensis*, *Stipa pennata* и *Tulipa biebersteiniana*.

**Ключевые слова:** флора, особо охраняемая природная территория, памятник природы урочище «Каменное озеро», высшие сосудистые растения, редкие и исчезающие растения.

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## **Comparative evaluation of preparations' fungicidal activity and their effect on pathogenic micromycetes of spring wheat**

The growing season of 2022 was characterised by a variety of weather conditions, some of which were unfavourable to spring wheat growth and development. Plants were stressed by daytime temperatures, soil and air moisture, and fungal disease pathogenic impacts. Spring wheat was infected with powdery mildew (*Erysiphe graminis*), leaf brown rust (*Puccinia triticina*), and septoria leaf blotch (*Septoria* sp.). The main fertilizer, foliar dressing, and compounds with regulatory properties in relation to the crop are used to boost yield and improve grain quality. Fungicides such as Varro, Rex Duo and Kolosal Pro are effective for managing spring wheat diseases. The study established the impact of Varro, Rex Duo, and Kolosal Pro fungicides on the reduction of the main spring wheat pathogens. This had an effect on grain quality and yield characteristics. The fungicides (tested under stressful conditions produced by phytopathogens and unfavourable meteorological conditions) enhanced the growth of spring wheat plants and positively influenced yield morphometric parameters, productivity, and grain quality parameters. The most effective agent for controlling diseases of spring wheat is Rex Duo fungicide. The use of fungicidal treatments in combination with fertilizers and plant growth regulators increased yield structure indicators, which resulted in an even greater impact on yield and quality indexes. It was discovered that the use of fertilizers and growth stimulants to crops can be combined with the application of fungicides.

*Keywords:* spring wheat, phytopathogens, micromycetes, fungicides, fertilizers, growth stimulator, powdery mildew, brown rust, septoria leaf blotch.

### *Introduction*

Plant diseases are one of the main factors leading to the loss of about 20% of the crop. Seed infection occupies a special place among the pathogenic microflora [1]. It is estimated that 60% of all grain crops' pathogens (*alternaria*, *fusarium*, *smut*, and others) are seed-transmitted [2]. The spread of fungal diseases of plants occurs in the form of epiphytosis, which causes the loss of an extremely significant amount of agricultural crops, and is also expressed in the colonization of the soil with fungi that form toxins which affect the quality and productivity of cultivated crops [3]. The fight against pests and diseases of crops should be based on the seed material's phytosanitary state. These findings are justified by the increased level of nutritional components contained in the seeds, which are the optimal nutrient medium for the development of microorganisms that produce mycotoxins [4]. Approximately 50 different microorganisms can be found on seeds. Seed infection with pathogenic microflora occurs at any stage of plant development, both during the growing season and when grain storage is disturbed [5].

In order to reduce plant disease incidences, it is necessary to conduct a phytosanitary examination of seeds [6]. Phytoexamination of seed material makes it possible to assess the level of phytopathogenic load exerted on seeds (as the main source of infection), and to give a quantitative and qualitative assessment of their general condition, as well as their suitability for cultivation [7]. Crops must be treated in order to protect them from pathogenic microorganisms [8]. This fundamental component of crop cultivation technology allows for the protection of the plant against phytopathogens even at the seed stage as well as the eradication of the infection's cause [9].

A wide range of different disinfectants, including one or more active substances, which make it possible to obtain a healthy crop, even taking into account the increased incidence of seed infections, is present on the modern market [10]. At the same time, the degree of effectiveness of certain drugs depends on the nature of the diseases in general as well as their specificity. As a result, the effectiveness of the outcome depends on the selection of the disinfectant, which is made in light of the seeds' phytoexamination [11].

Currently, fungicides, which can both combat infections of phytopathogens and enhance the qualitative attributes of seeds (growth activity, stress resistance to sharp fluctuations in abiotic environmental factors, development of nonspecific immunity), are of significant interest [12]. These drugs are modern highly effec-

tive chemical means of protection against phytopathogenic microorganisms. A suitable fungicide should be chosen for each seed lot based on the degree of infection of the seed material and the pathogen composition. The impact of fungal infections is manifested in significant damage to the studied crops at each stage of plant development and production [13]. The evaluation of fungicide effects on seed material enables the identification and control of the safety and quality of food and feed resources, reducing and thereby preventing significant economic losses.

The scientific study's goal is to compare the fungicidal activity of chemical protection agents and investigate their impact on pathogenic micromycetes of spring wheat.

### *Experimental*

In the field conditions, the effect of fungicides on protection against a variety of fungal infections was investigated. The field experiment was conducted in the 2022 growing season on the experimental spring wheat fields of the Zhumabek Agro seed farm, which is located in the steppe zone of North-Eastern Kazakhstan (Pavlodar region).

“Omskaya 35” is a spring wheat variety of foreign selection that was used in the study. The breeder of the “Omskaya 35” variety is FGBNU “Omsk Agrarian Scientific Center” (Russia). The variety is lutescens. Its vegetation period is 87–90 days. Resistant to lodging is moderately drought tolerant. Moderately susceptible to brown rust, susceptible to dusty mildew, strongly susceptible to hard knotweed, stem rust, powdery mildew, root rot. The “Omskaya 35” variety has a high potential yield and forms high-quality heavy grains. Thanks to the high productivity in combination with resistance to diseases and lodging, this variety can successfully compete with varieties of similar ripeness groups.

Considering the spread of disease, we determined the systematic position of the pathogen, the intensity of the plants damage, and the time at which the disease began (according to phenological and calendar indicators). Observations were made at stationary sites during the plant growing season (at least every 10 days). They were used to identify the time of disease occurrence. As a result, the dynamics of the disease's development and the timing of its initial manifestations were defined. The route technique was used to account for affected plants along the diagonals of the field by looking at 10 plants in 10 areas. Two parameters were used to measure the phytopathological status of the crops: prevalence and degree or intensity of disease development [14].

Prevalence calculation formula:

$$P = \frac{n}{N} * 100,$$

n – number of affected plants (organs) involved in the samples, pcs;

N – total number (diseased and healthy) plants (organs) involved in the samples, pcs.

Disease progression (R) calculation formula:

$$R = \frac{\Sigma(n*b)}{\Sigma n},$$

$\Sigma(nb)$  – sum of the products of affected plants multiplied by the degree of damage;

$\Sigma n$  – total number of damaged plants or similar organs in the samples, pcs.

The severity of the damage was determined by conditional grading [15].

The severity of disease development was expressed in percentages. We used the formula to convert the score to a percentage:

$$R = \left[ \frac{\Sigma(n*b)}{A*\Sigma n} \right] * 100,$$

$\Sigma(nb)$  – sum of the products of affected plants multiplied by the disease progression score;

$\Sigma n$  – total number of damaged plants or similar organs in the samples, pcs;

A – the highest score on the chart.

The calculation formula for the biological effectiveness of fungicides:

$$C = \left[ \frac{(Y-y)}{y} \right] * 100,$$

Y – rate of disease progression (control);

y – indicator of disease progression in the treated area.



Laboratory tests were performed in accordance with generally accepted State Standards and methods: state of crops and crop productivity according to the phenological stages of plant development; nature of the seeds according to State Standard PO42-80; yield was recalculated for standard (14%) moisture and 100% purity; crop structure – with the method of individual analysis of plants in a sheaf. Plant sampling was carried out on the days of counting. The analysis was conducted with the help of special equipment at the Biological Research Laboratory of Toraighyrov University. The mass of the roots and plants' underground parts was determined by using an analytical balance. Mathematical processing was conducted according to B.A. Dospekhov [16]. Variance and correlation analyses were performed on an IBMPC using the Excel program.

### Results and Discussion

The following climatic conditions characterized the growing season of 2022. The temperature regime in May 2022 was quite mild. The average monthly temperature during the day was 17.5°C, which is 4.3°C higher than the long-term average temperature. The first and third 10 days of the month were marked by relative temperature stability, while the second decade was defined by temperature indicator variations. The lack of rainfall during the month was remarkable; the average amount of rainfall in May – 12 mm. The relative humidity (RH) was about 38%.

June was characterized by a gradual temperature rise. The first and second 10 days of June are marked by temperature reductions, although the average temperature for the whole month was almost 18.5°C. The temperature indicators were relatively stable in the third decade. The average June rainfall increased by up to 30 mm, causing the RH to rise by 50%.

The average monthly temperature in July was 21.5°C, which formed a moderate temperature regime that was 0.8°C higher than the long-term average. The monthly rainfall indicators showed uneven fluctuations up to 33 mm (39% less than normal) and relative humidity of about 55%. August was characterized by an absence of significant temperature variations: plus 20°C (average temperature) and a RH of 61%. Over the previous months, precipitation indicators increased by up to 52.1 mm (Table 1).

Table 1

**Meteorological conditions (2022 growing season)**

Month	Average temperature, °C		
	Long-time average	2022	Deviation
May	13.2	17.5	+4.3
June	19.7	18.5	-1.2
July	20.7	22.5	+1.8
August	17.8	20.0	+2.2
Month	Precipitation, mm		
	Long-time average	2022	Deviation
May	24.4	12.0	-12.4
June	39.3	30.0	-9.3
July	54.0	33.0	-21.0
August	37.4	52.1	+14.7

The growing season of 2022 was characterized by a variety of weather conditions, some of which resulted in a negative impact on plant development and growth. A prolonged moisture deficit affected air temperatures, resulting in indicators that were mostly above long-term average values. The climate created favorable conditions for the development of many fungal diseases and their significant manifestation in spring wheat crops. Plants were affected by air and soil humidity, daytime temperatures, and fungal diseases.

Spring wheat was infected with powdery mildew (*Erysiphe graminis*), leaf brown rust (*Puccinia tritici-na*), and septoria leaf blotch (*Septoria sp.*). Powdery mildew intensity of manifestation was around 30%, rust intensity – 15%, and septoria leaf blotch intensity – 25% prior to the use of fungicides (during the stage of flag leaf emergence). The intensity of disease manifestation was considerably reduced in 10 days after fungicide application: powdery mildew by 75.1-90.1%, rust by 55.2-61.7%, and septoria leaf blotch by 35.1-48.6%. At the same time, Rex Duo fungicide was the most effective against the disease complex. After 20 days, Rex Duo completely suppressed powdery mildew, reduced leaf rust manifestation by 78.7% and septoria leaf blotch by 71.4%. By this time, the intensity of powdery mildew manifestation had dropped to

17.0% due to the action of abiotic factors (untreated region), but the same indicators of leaf rust and septoria leaf blotch had reached their maximum values (44,1% and 89,4%) (Table 2, Fig. 1-2).

Table 2

**Biological efficiency of fungicides (flag leaf emergence)**

Option	Biological efficiency against disease, %					
	Powdery mildew		Brown rust		Septoria leaf blotch	
	10 days	20 days	10 days	20 days	10 days	20 days
Kolosal Pro, 0.6 L/ha	75.1	91.1	55.2	67.2	35.1	61.6
Rex Duo, 0.5 L/ha	90.1	100.0	61.7	78.7	48.6	71.4
Varro, CS, 0.25 L/ha	85.4	98.8	58.3	70.0	44.4	68.2
Control	27.5	17.0	17.7	44.1	28.5	89.4
LSD <sub>0,5</sub>	3.2	4.9	4.0	2.7	5.4	2.8

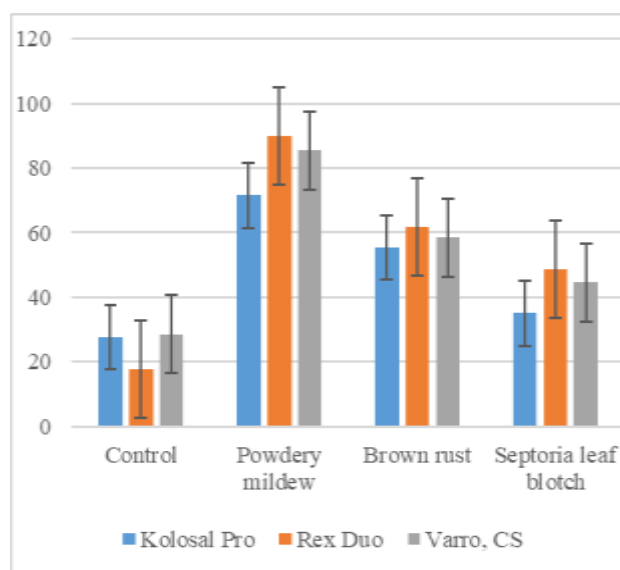


Figure 1. Biological efficiency of fungicide application after 10 days

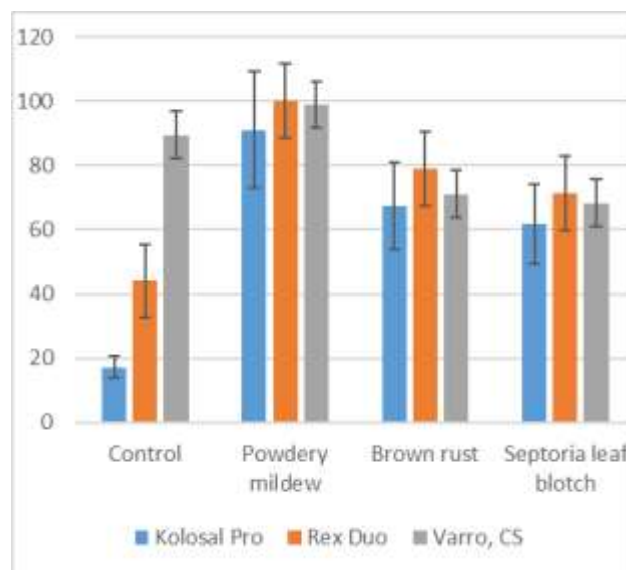


Figure 2. Biological efficiency of fungicide application after 20 days

Fungicides used during the growing season to control fungal diseases of spring wheat contribute to an increase in plant growth, the number of productive stems, ear height, grain number per ear, weight of 1000 grains, and the crop's biological yield (Table 3).

Table 3

**Spring wheat indicators as a result of fungicide treatment (flag leaf emergence (37-39))**

Option	Indicators					
	Productive stems, pcs/m <sup>2</sup>	Plant height, cm	Grain number per ear, pcs	Weight of grains per ear, g	Weight of 1000 grains, g	Biological yield, centner/ha
Kolosal Pro, 0.6 L/ha	393	97.0	25.2	0.932	36.6	36.5
Rex Duo, 0.5 L/ha	394	98.3	27.8	1.092	39.5	42.8
Varro, CS, 0.25 L/ha	391	98.3	25.8	0.985	38.1	38.5
Control	392	95.3	25.1	0.883	35.0	34.6
LSD <sub>0,5</sub>	4.9	2.1	1.6	0.13	1.2	1.3

The use of foliar dressings with Ammonium nitrate  $N_{34}$  and Rex Duo fungicide significantly improved the condition of spring wheat plants. As a result, the number of productive stems, height of plants, grain number per ear, and weight of 1000 grains have increased. These two dressings are especially effective in the tillering and heading stages. If the biological yield in the variant without fertilizers and fungicide was 38.9 centers/ha and 43.4 centers/ha in the variant with only fungicide, then a single dressing with ammonium nitrate (tillering stage) followed by a fungicide (flag leaf stage) increased the indicator to 52.7 centers/ha, while two dressings (tillering and heading stages) allowed for the maximum biological yield of 60.9 centers/ha (Table 4).

Table 4

**Some indicators of spring wheat plants as a result of fungicide application  
(Nitrogen fertilizers background)**

Option	Indicators					
	Productive stems, pcs/m <sup>2</sup>	Plant height, cm	Grain number per ear, pcs	Weight of grains per ear, g	Weight of 1000 grains, g	Biological yield, centner/ha
Ammonium nitrate $N_{34}$ (tillering stage) + Rex Duo 0.5 l/ha (flag leaf stage)	413	107.4	29.1	1.275	44.1	52.7
Ammonium nitrate $N_{34}$ (tillering stage) + $N_{34}$ + Rex Duo 0.5 l/ha (ending of stem elongation stage) + $N_{34}$ (heading stage)	421	112.8	31.2	1.448	46.8	60.9
Rex Duo 0.5 l/ha (ending of stem elongation stage)	389	104.2	27.8	1.115	40.1	43.4
Control	390	99.1	26.8	0.997	36.9	38.9
LSD <sub>0.5</sub>	6.5	4.9	2.2	0.22	2.1	2.9

The Rex Duo fungicide treatment (at a rate of 0.5 L/ha) led to a reduction in powdery mildew prevalence. As a result, on the 12<sup>th</sup> day, plant damage dropped from 100% to 18-20% and the severity of manifestation decreased from 45 to 5%. In view of the degree of powdery mildew occurrence, the drug's biological efficacy reached 89% (Table 5).

On the 22<sup>nd</sup> day, the powdery mildew prevalence decreased to 3-5% (100% for the control) and the severity of manifestation dropped from 60% (control) to 3% (treated plants). The biological effectiveness after treatment against powdery mildew was 92-95%.

Table 5

**Some indicators of spring wheat plants as a result of fungicide application  
(Nitrogen fertilizers and growth regulator Binoram background)**

Disease manifestation	Options								
	Rex Duo 0.5 L/ha + Binoram			Rex Duo 0.5 L/ha			Control		
	Before treatment	After 12 days	After 22 days	Before treatment	After 12 days	After 22 days	Before treatment	After 12 days	After 22 days
Powdery mildew									
Disease prevalence, %	100	18	4	100	20	5	100	100	100
Degree of development, %	30	5	3	30	5	3	30	45	60
Biological efficiency, %	-	89	95	-	88	92	-	-	-

Brown rust									
Disease prevalence, %	50	13	12	60	14	13	50	60	70
Degree of development, %	15	10	8	15	10	8	15	25	45
Biological efficiency, %	-	64	84	-	60	80	-	-	-
Septoria leaf blotch									
Disease prevalence, %	45	28	20	45	30	23	45	60	90
Degree of development, %	25	20	15	25	20	15	25	35	50
Biological efficiency, %	-	45	60	-	40	50	-	-	-

The drug's fungicidal activity on spring wheat was observed to be moderate in respect to leaf rust. After 12 days, the prevalence in the treated variations was 13-14%, compared to 60% in the control, and the degree of disease manifestation was 10%, compared to 25% in the control. According to the level of development, biological efficiency for this time period was 60-64%. After 22 days, the prevalence of brown rust in the control plot climbed to 70%, whereas it was substantially lower in the treated variations – up to 12-13%. After 22 days, the degree of disease development in the control area reached 45%, while in the treated variants it was 8%. In 2022, the biological efficacy of Rex Duo against spring wheat brown rust was 80-84% (after 22 days).

The drug's fungicidal effect on spring wheat was less pronounced with regard to septoria leaf blotch. After 12 days, the prevalence in the treated variations was 28-30%, compared to 60% in the control, and the degree of disease manifestation was 20%, compared to 35% in the control. According to the level of development, biological efficiency for this time period was 40-45%. After 22 days, the prevalence of septoria leaf blotch in the control plot increased to 90%, whereas it was substantially lower in the treated variations – up to 20-23%. After 22 days, the degree of disease development in the control area reached 60%, while in the treated variants it was 15%. In 2022, the biological efficacy of Rex Duo against spring wheat septoria leaf blotch was 50-60% (after 22 days).

The efficacy of Binoram was marginally improved by additional application to growing plants.

#### Conclusion

The adverse effect of fungal infections on cereal plants rose in the 2022 growing season. It was discovered that if modern fungicides were not applied, disease spread, intensity of manifestation, and rate of development reached maximum levels.

The fungicides (tested under stressful conditions produced by phytopathogens and unfavorable meteorological conditions) enhanced the growth of spring wheat plants and positively influenced yield morphometric parameters, productivity, and grain quality parameters.

The research demonstrated an inhibiting effect of the fungicides (Varro, Rex Duo, and Kolosal Pro) on pathogenic microorganisms. This had an effect on grain quality and yield characteristics. The most effective agent for controlling diseases of spring wheat in the Pavlodar region is the fungicide named Rex Duo.

The use of fungicidal treatments in combination with fertilizers and plant growth regulators increased yield structure indicators, which resulted in an even greater impact on yield and quality indexes. Thus, it was discovered that the use of fertilizers and growth stimulants to crops can be combined with the application of fungicides.

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## **Препараттардың фунгицидтік белсенділігін және олардың жаздық бидайдың патогенді микромицеттеріне әсерін салыстырмалы бағалау**

2022 жылдың вегетациялық маусымы ауа райының әртүрлі жағдайларымен ерекшеленді және жаздық бидайдың дамуының белгілі бір кезеңдерінде дақылдың өсуі мен даму көрсеткіштеріне қолайсыз болды. Өсімдіктер күндізгі температураға, ауа ылғалдылығына және жер жамылғысына, саңырауқұлақ ауруларының деструктивті әсеріне байланысты күйзеліске ұшырады. Жаздық бидайда ақұнтақ (*Erysiphe graminis*), қоңыр тат (*Puccinia triticina*) және септориоз (*Septoria* sp.) сияқты аурулар дамыды. Өнімділікті арттыру және астық сапасын жақсарту үшін негізгі тыңайтқыш, тамырдан тыс үстеп коректендіргіш, дақылға қатысты реттеуші қасиеттері бар заттар қолданылады. Жаздық бидай ауруларын бақылаудың тиімді саңырауқұлақжойғылары — Варро, Рекс Дуо, Колосал Про. Зерттеу нәтижесінде осы саңырауқұлақжойғыларының жаздық бидайдың негізгі қоздырғыштарын басуға және соның салдарынан астықтың өнімділігі мен сапасына әсері анықталды. Қоздырғыштар мен ауа райының қолайсыздығынан туындаған күйзеліс жағдайында саңырауқұлақжойғылар жаздық бидай өсімдіктерінің өсуіне, дақыл құрылымының көрсеткіштеріне, өнімділік және астық сапасының көрсеткіштеріне оң әсер етті. Жаздық бидай ауруларын бақылаудың ең тиімді заты — Рекс Дуо фунгициді. Тыңайтқыштар мен өсімдіктердің өсуін реттегіштерді қолдана отырып, фунгицидтік препараттарды біріктіргенде жаздық бидайдың өгін жинау құрылымының көрсеткіштері жақсарғаны анықталды, бұл дақылдың өнімділігі мен астық сапасының артуына әсер етті. Тыңайтқыштар мен өсу стимуляторларын пайдалану дақылдарды фунгицидтік препараттармен өңдеумен үйлесімді екендігі айқындалды.

*Кілт сөздер:* жаздық бидай, фитопатогендер, микромицеттер, фунгицидтер, тыңайтқыштар, өсу стимуляторы, ақұнтақ, қоңыр тат, септориоз.

Р.М. Уалиева

## Сравнительная оценка фунгицидной активности препаратов и их воздействие на патогенные микромицеты яровой пшеницы

Вегетационный сезон 2022 г. отличался различными условиями погоды, и в определенные фазы развития яровой пшеницы был неблагоприятен для показателей роста и развития культуры. Растения испытывали стресс по отношению к дневным температурам, влажности воздуха и почвенного покрова, деструктивному влиянию грибных болезней. На яровой пшенице получили развитие мучнистая роса (*Erysiphe graminis*), листовая бурая ржавчина (*Puccinia triticina*) и септориоз (*Septoria* sp.). С целью повышения урожайности культуры и улучшения качества зерна используют удобрения, внекорневые подкормки, также вещества, которые обладают регулятивными свойствами по отношению к культуре. Одними из эффективных средств по контролю болезней яровой пшеницы являются фунгициды Варро, Рекс Дуо, Колосаль Про. В результате проведенного исследования установлено влияние указанных выше фунгицидов на подавление основных фитопатогенов яровой пшеницы, и, как следствие, на урожайность, и качество зерна. В стрессовых условиях, вызванных фитопатогенами и неблагоприятными условиями погоды, исследуемые фунгициды способствовали росту растений яровой пшеницы, положительно влияли на морфометрические показатели урожая, урожайность культуры и показатели качества зерна. Наиболее эффективное действие при борьбе с болезнями яровой пшеницы, такими как мучнистая роса, бурая ржавчина, септориоз, оказывает фунгицид Рекс Дуо. Установлено, что в сочетании фунгицидных препаратов с применением удобрений и регуляторов роста растений улучшились показатели структуры урожая яровой пшеницы, что сказалось на повышении урожайности и качества зерна культуры. Выявлено, что внесение удобрений и стимуляторов роста совместимо с обработкой посевов фунгицидными препаратами.

**Ключевые слова:** яровая пшеница, фитопатогены, микромицеты, фунгициды, удобрения, стимулятор роста, мучнистая роса, бурая ржавчина, септориоз.

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## Floristic composition of sand massifs in the western part of the Sub Ural plateau

Currently, on the territory of Kazakhstan, one of the topical issues is the study of the current state of ecosystems. The aim of the study is a biomorphological, geographical and ecological analysis of the sand massifs flora in the western part of the Sub Ural plateau. 137 species of vascular plants belonging to 99 genera and 35 families were registered in the Karagash and Akkum sand massifs. Most of the flora (70; 50.4%) is occupied by species belonging to the *Asteraceae*, *Poaceae*, *Fabaceae* and *Chenopodiaceae* families. The predominant in the flora are rhizomatous species: *Calamagrostis epigeios*, *Carex colchica*, *Helichrysum arenarium*, *Leymus racemosus*, *Scirpoides holoschoenus*, etc. The presence of adventitious species in the flora indicates high anthropogenic pressure, unfavorable growing conditions and habitat disturbance. The predominance of xerophytes is associated with the adaptation of psammophytes and steppe species to growing in arid climates. The chorological analysis showed the predominance of the wide-ranging Eurasian and Ancient Mediterranean species. There is a need to protect meadow and psammophytic mixed-grass-stipa grass and juniper communities. They belong to plant species distribution area of which is currently being intensively reduced, so it is necessary to organize comprehensive measures to protect their growing environment. Information about the species composition of plants, their biological and ecological features, which in the future will become the basis for the organization of monitoring work, are of great practical importance.

**Keywords:** West Kazakhstan region, Sub Ural plateau, sand massif, psammophytes, floristic analysis, vegetation cover, Akkum, Karagash.

### Introduction

The United Nations Convention on combating desertification, adopted in Paris on June 17, 1994, is considered one of the most important international documents aimed at ensuring sustainable development. Currently, the intensification of the desertification process leads to the formation of ecological disaster zones. More than 66% of the territory of the Republic of Kazakhstan is affected by this phenomenon. This process continues from year to year, and in this regard, one of the most urgent issues is the study of ecosystems of sand massifs found in the dry steppe region. In this regard, the Republic of Kazakhstan is carrying out a set of measures aimed at fulfilling the requirements mentioned in this convention [1].

The West Kazakhstan region is located in the north-west of Kazakhstan. The relief of the region is formed by spurs of the Obshii Syrt, the Sub Ural plateau, the Presyrt slope, the Peri-Caspian depression, and the Zhayik river valley [2].

The Sub Ural plateau is characterized by a high denudation step-slope hilly and ridged topography with a height of 250-400 m, divided by the Presyrt slope, which stretches from the northwest to the southeast from the Peri-Caspian depression in the west. There are salt domes, large and small chalk hills and individual chalk outcrop, sand massifs, lakes, salt flats, river valleys, ravines and gullies. In the western part of the Sub Ural plateau there are sandy massifs formed from surface alluvial-delta marine sediments. Their appearance is due to the action of rivers found in the Sub Ural plateau. During the multi-phase transgression and regression of the ancient Caspian Sea, observed in the Pleistocene period, it was formed in the place of the deltas of abundant water rivers flowing into the sea.

The well-known scientist, researcher of desert regions A.G. Gael and his colleagues believe that the formation of these sand massifs was influenced by the alluvial sediments transported due to active erosion processes during the Ice Age [3].

At the edge of the sand massifs, there are small sand dunes, and towards the central part, they change to medium-height sand dunes and rolling sand ridges. Sand dunes vary in height from 2 to 17 meters. They are alternated with large dune depressions, the depth of which is between 1-8 m, and the area reaches 1-2 sq.km in some places.



Currently, due to the rapid development of the processes of desertification and degradation of natural ecosystems, the information about the species composition of plants growing in the sand massifs of the western part of the Sub Ural plateau, their biological and ecological features is of great practical importance.

### Experimental

Research work on the flora composition of sand massifs in the western part of the Sub Ural plateau was carried out in 2020-2022.

The purpose of the work: to conduct a comprehensive analysis of the floral composition of sand massifs in the western part of the Sub Ural plateau. In the course of the study, the following tasks were set: to clarify the systematical groups of plants growing in the sand massifs of the western part of the Sub Ural plateau, to analyze the biomorphological, geographical and ecological composition and to justify protection measures.

The Karagash and Akkum sand massifs in the western part of the Sub Ural plateau were taken as the research area. They are located in the eastern part of the West Kazakhstan region, which, according to geobotanical zoning, is included in the Black Sea region – Kazakhstan region of the Eurasian steppe zone [4]. Doctor of biological sciences, professor V.V. Ivanov, who has studied the West Kazakhstan region for many years, refers these sand massifs to the psammophytic southern steppes with feather grasses vegetation [5]. The Karagash sand massif is located between the Buldyrty and Tamdy rivers, and Akkum is located at the confluence of the Kaldygayty and Kuagash rivers (Fig. 1).

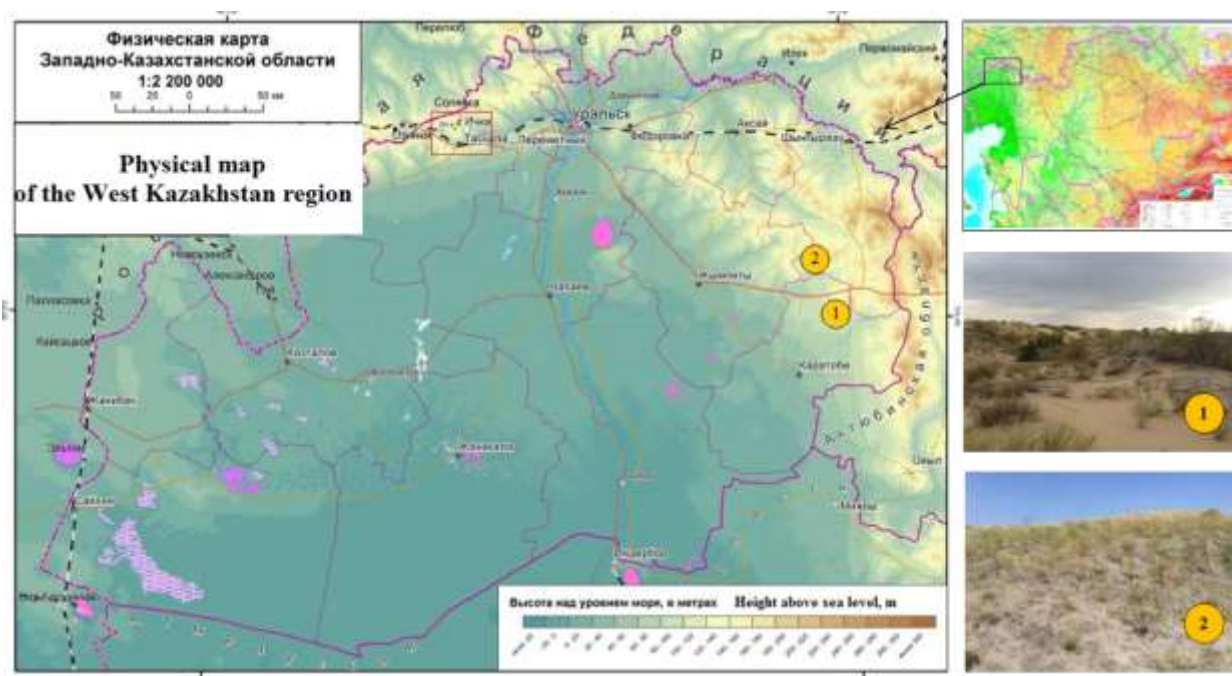


Figure 1. Research area in the West Kazakhstan Region: Point 1 – Akkum sand massif; Point 2 – Karagash sand massif

Geobotanical studies were conducted on a standard area of 100 square meters using existing methods [6]. The floristic composition and projective cover of vascular plants were determined in the test areas. To identify the plants, “Flora of the USSR” (1934-1960) [7], “Flora of Kazakhstan” (1956-1966) [8], “Illustrated determinant of plants of Kazakhstan” (1969-1972) [9], according to V.V. Ivanov “The determinant of the families of the Northern Caspian” (1966-1974) [10] and other handbooks were used. The flora list was clarified according to the literary sources [11] and the data of the Herbarium fund of M. Utemisov West Kazakhstan University (PPIU). Biomorphological analysis was carried out according to Serebryakov [12]. Cenoctic groups according to the data of R.V. Kamelin [13], geographical groups according to A.L. Takhtajyan [14] and ecological groups were determined based on the relation of plants to moisture. The names of vascular plants are given taking into account the summary of S.K. Cherepanov [15].

The Jaccard’s coefficient, which indicates the floristic similarity between the sites, is calculated as:

$$K_J = (c / a + b - c) * 100\%$$

with:  $K_J$  = Jaccard's coefficient; a – the number of species in the first site; b – the number of species in the second site; c – the number of common species for these two sites [16].

Microsoft Excel statistical analysis package was used for statistical processing of the received research materials.

### Results and Discussion

Studies of the flora and vegetation of sand massifs in the western part of the Sub Ural plateau were carried out by Shtromberg Ch. (1894), V.M. Savich (1908), I.V. Larin (1929), A.P. Shimanyuk (1941), A.G. Gael et al. (1932, 1949), S.A. Nikitin (1954), V.V. Ivanov (1958), A.Z. Petrenko (1968), A.Yu. Bogdanov (1971), S.K. Ramazanov (2001), T.E. Darbaeva (2002), T.E. Darbaeva et al. (2021), etc.

The Karagash sand massif is located at the high source of the Buldurty river [17]. On an area of 7 thousand hectares, there are moderately high and low sand dunes formed as a result of Aeolian processes. The dominant psammophyte plants of sand dunes include *Artemisia marschalliana* Spreng, *Carex colchica* J. Gay, *Calligonum aphyllum* (Pall.) Gürke, *Helichrysum arenarium* (L.) Moench, *Leymus racemosus* (Lam.) Tzvel., etc. In the inter-sand depressions lying near the groundwater grows *Betula pendula* Roth., *B. pubescens* Ehrh., *Carex colchica* J. Gay, *C. praecox* Schreb., *Populus tremula* L., *Salix caspica* Pall., *S. rosmarinifolia* L., etc. In their shrub tier includes *Frangula alnus* Mill., *Rhamnus cathartica* L., *Rosa cinnamomea* L., etc., and the herbaceous tier consists of mesophilic grasses and mixed-grass. On the slopes and tops of individual sand dunes can be found juniper communities (*Juniperus sabina* L.) (Fig. 2).

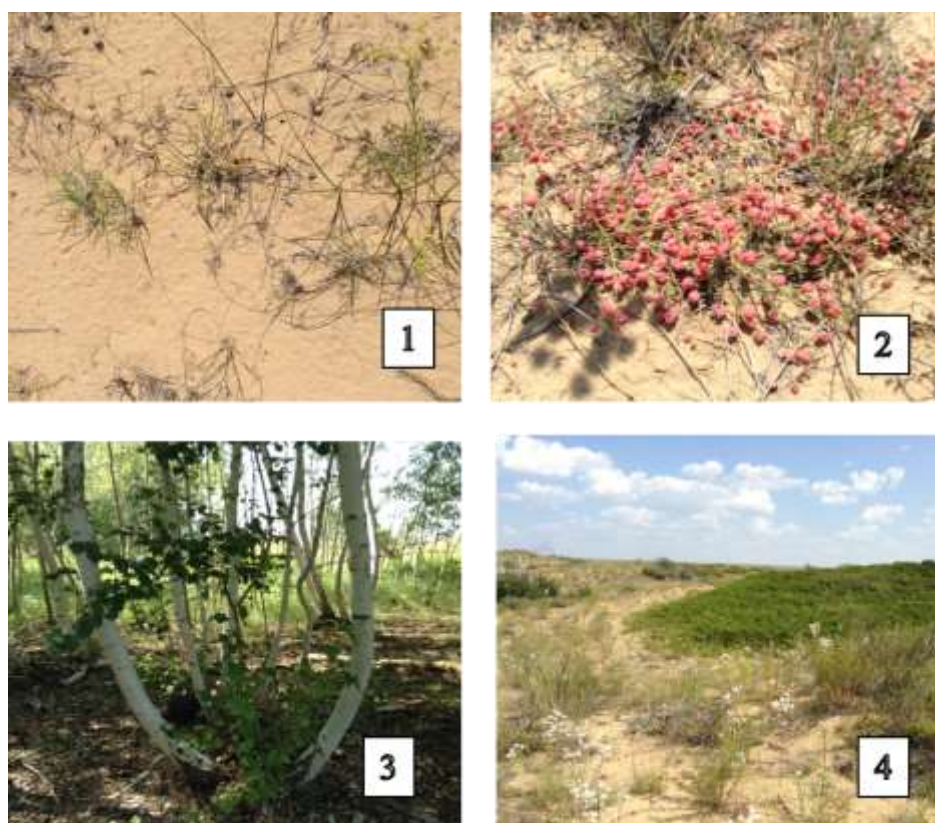


Figure 2. Karagash sand massif: 1 – The sparse psammophilous communities; 2 – Communities with *Ephedra distachya* L.; 3 – Birch-aspen groves; 4 – Communities with *Juniperus sabina* L.

In the central part of the region there are loose sand dunes that are not supported by vegetation. Sandy massifs in the north, north-east and east side gradually change to fescue-feather-grass steppes (*Festuca valesiaca* Gaud. + *Stipa* sp.).

Akkum sand massif is located on the plain terrace of the Kuagash and Kaldygayty rivers with an area of 7.5 thousand hectares. It is believed to have formed in the Quaternary period on the site of the abundant warty river deltas which flowed into the Khvalyn sea. The central part consists of rolling medium-height sand dunes, the Windward slope of which is elongated and flat, and the slope side is steep towards inter-sand de-



pressions in the form of horseshoe or crescent. On the outskirts of sand massifs grow psammophyte plants (*Artemisia marschalliana* Spreng, *Carex colchica* J. Gay, *Calligonum aphyllum* (Pall.) Gürke, *Leymus ramosus*, *Juniperus sabina* L., etc.).

In the areas close to the shores of the Akkum sand massif, tree-shrub plants grow in the hollows between high sand dunes and on their grassy slopes. Groundwater is unmineralized fresh (0.3-0.7 g/l) and is located close to the surface (0.2-1.0 m). The vertical structure of birch communities consists of 3 tiers. The first tree tier is birch (*Betula pendula* Roth., *B. pubescens* Ehrh.), which is considered an edifier, its height is 7-10 m, and its diameter is 15 cm.

The height of the second tier is 1.5-2.1 m., consisting of shrubs: *Calligonum aphyllum* (Pall.) Gürke, *Chamaecytisus ruthenicus* (Fisch. ex Woloszcz.) Klásk, *Salix caspica* Pall., *Spiraea hypericifolia* L. The height of the third grassy tier is 0.2-0.9 m., consists of herbaceous plants (*Asperula danilewskiana* Basiner, *Calamagrostis pseudophragmites* (Haller f.) Koeler, *Carex colchica* J. Gay., etc.). Projective cover reaches 20-40%, saturation of species – 4-5, number of species – 9-11. Hand-planted *Populus alba* L., *P. nigra* L., *Pinus sylvestris* L. can be found in some depressions between dunes (Fig. 3).

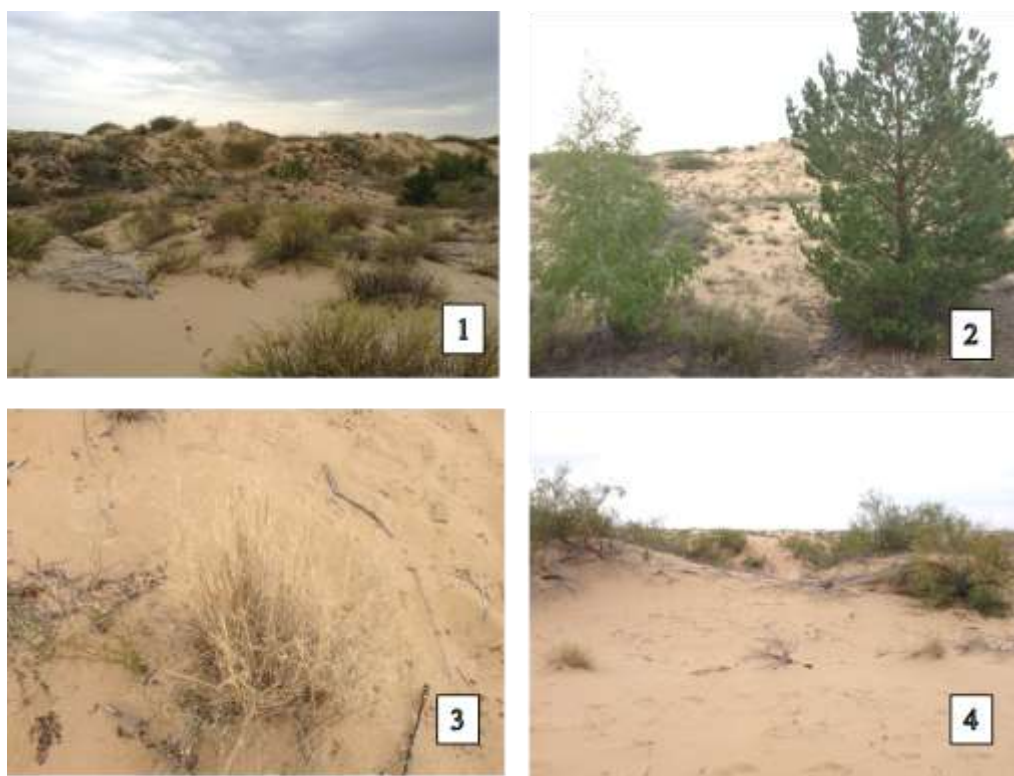


Figure 3. Akkum sand massif: 1 – General view, 2 – Pine plantings in depressions between sandy dunes, 3 – The sparse psammophytic communities, 4 – The willow communities

On the slopes of moderately high sand dunes and ridges, and sometimes even at elevations, there are shrubs (*Salix caspica* Pall., *S. rosmarinifolia* L., *S. daphnoides* Vill., *Chamaecytisus ruthenicus* (Fisch. ex Woloszcz.) Klásk., *Ch. zingeri* (Nenukow ex Litv.) Klask, *Spiraea hypericifolia* L.), semishrubs (*Artemisia marschalliana* Spreng.) and communities of psammophytic herbaceous vegetation (*Achillea micrantha* Willd., *Carex colchica* J. Gay, *Festuca beckeri* (Hack.) Trautv., *Polygonum arenarium* Waldst. et Kit. etc.).

The first tier with a height of 0.8-1.2 m. is overgrown with shrubs; the second tier has a height of 0.4-0.7 m. forms herbaceous plants, and semi-shrubs that can be: *Artemisia marschalliana* Spreng, *Chondrilla ambigua* Fisch. ex Kar. et Kir., *Helichrysum arenarium* (L.) Moench., *Jurinea arachnoidea* Bunge, in the third tier there are low (0.15-0.35 m) herbaceous plants: *Agropyron desertorum* (Fisch. ex Link) Schult., *Festuca beckeri* (Hack.) Trautv., *Poa bulbosa* L. Projective cover in these communities does not exceed 30-50%, the saturation of species is 3-5, the number of species is 11-13.

Towards the central part, on the slopes of sand dunes and ridges, communities with *Calligonum aphyllum* (Pall.) Gürke, are formed. In these communities, 3 tiers are also distinguished. The first tier is dominated by *Calligonum aphyllum* with a height of up to 1.5 m. The second tier is formed by *Agropyron fragile* (Roth)

*P. Candargy*, *Aristida pennata* Trin., *Artemisia marschalliana* Spreng, *Gypsophila paniculata* L., *Leymus racemosus* (Lam.) Tzvel. with a height of 0.4-0.7 m. and 3 tiers are formed by low plants with a height of 0.15-0.3 m: *Anisantha tectorum* (L.) Nevski, *Poa bulbosa* L. Projective cover does not exceed 40-50%, species saturation – 7-9, number of species – 14-18.

Towards the southern part of the massif, monocenosis communities of Juniper (*Juniperus sabina* L.) are observed on the slopes and higher sides of the sand dune.

As a result of the conducted research, it was revealed that 137 species of vascular plants belonging to 99 genera and 35 families grow on the sand massifs of the western part of the Sub Ural plateau. 94 species were recorded in the Karagash sand massif, and 121 species in the Akkum massif (Table 1).

Table 1

#### Taxonomic diversity of the sand massif flora in the western part of the Sub-Ural plateau

Regions	Number of			Average number of		
	species	genera	families	species in family	genera in family	species in genera
West Kazakhstan region	1256	487	117	10.7	4.16	2.57
Sand massifs of the western part of the Sub Ural plateau	137	99	35	3.91	2.83	1.38
Karagash sand massif	94	74	29	3.24	2.55	1.27
Akkum sand massif	121	86	32	3.78	2.69	1.41

This is 10.9 percent of the 1256 species found in the region, according to the scientist O.T. Kolchenko, who studied the vegetation cover of the West Kazakhstan region for many years [2].

A comparative analysis of the species composition of these two arrays was performed by calculating the Jaccard's similarity coefficient,  $K_j = 56.9\%$ . The high coefficient of similarity indicates the similarity of their species composition, that is, it can be concluded that the similarity of environmental conditions plays an important role in the formation of flora.

Among them, the basis of the flora is made up of *Magnoliophyta* (132 species; 96.4%), and the share of *Equisetophyta* (2; 1.4%) and *Pinophyta* plants (3; 2.2%) is insignificant. The *Magnoliopsida* are dominant with 102 species (74.5%). The *Liliopsida* include 30 species (21.9%). The ratio of monocotyledonous and dicotyledonous plants is 1: 3.4.

The taxonomic analysis of the composition of plants found in the region showed the predominance of the *Asteraceae* family, which includes 25 species, representing 18.2% of the plants found in the study area. The dominant families include *Poaceae* (20 species; 14.6%), *Fabaceae* (16; 11.7%), and *Chenopodiaceae* (9; 6.5%). This 4 family makes up half of the species found in the region (50.4%). Most of the dominants that make up the main vegetation cover also belong to this family (*Agropyron fragile* (Roth) P. Candargy, *Aristida pennata* Trin., *Artemisia lerchiana* Web., *A. marschalliana* Spreng, *Leymus racemosus* (Lam.) Tzvel. etc)

The average species richness per family is 3.91. The level of species wealth above the average is 8 families; the total number of them is 94 species (68.6%). The rest of the families are limited to 2-3 species. The 16 families are limited to only 1 species.

The largest genera are *Carex* (5 species), *Astragalus* (4), *Achillea* (4), *Artemisia* (4). Results of the analysis show a small number of large polymorphic genera; most genera are limited to only 2-3 species. 73 genera are limited to only 1 species.

Life forms of plants are considered an integral indicator of their relationship with the environment formed in the course of adaptive evolution. The flora of sand massifs in the western part of the Sub Ural plateau contains 7 species of trees and 17 shrubs. They are often found in inter-sand depressions where fresh groundwater lies close to the surface (Table 2).

**Life forms of the sand massif flora in the western part of the Sub-Ural plateau, according to I.G. Serebryakov's classification**

Life forms	Number of species	Percentage of the total number of species
Tree and woody plants 32 species (23.36%)		
Trees	7	5.11
Shrubs	17	12.41
Semishrubs	4	2.92
Dwarf semishrubs	4	2.92
Perennial (polycarpic) herbaceous plants 80 (58.39%)		
Grassy horsetails	3	2.19
Long-rhizomatous	15	10.95
Rhizomatous	10	7.30
Short-rhizomatous	8	5.84
Tuberiferous-rhizomatous	1	0.73
Taproot	26	18.98
Bulbiferous	2	1.46
Loose-tussock	1	0.73
Dense-tussock	7	5.11
Tussock	4	2.92
Soboliferous	3	2.19
Annual (monocarpic) herbaceous plants 25 (18.25%)		
Biennials	12	8.76
Annuals	13	9.49
Total	137	100

The results of the analysis of the flora composition of the research area showed that the dominant life form is perennial herbs (80 species, 58.39%). Polycarpics are dominated by vegetatively mobile biomorphs (37 species, 27.01%). The vast majority are long rhizomes, as they are well adapted to fixing sand: *Calamagrostis epigeios* (L.) Roth., *C. pseudophragmites* (Haller f.) Koeler., *Carex colchica* J. Gay., *Helichrysum arenarium* (L.) Moench., *Leymus racemosus* (Lam.) Tzvel., *Scirpoides holoschoenus* (L.) Soják., etc. There are 25 species of monocarpics, which make up 18.25 percent of the flora.

Since the degradation processes are quickly observed in sandy areas, on the species composition of plants also has a significant influence. For example, the degraded steppes are mainly dominated by annual plants but the protected steppes contain more perennials [18].

The flora of sandy massifs is characterized by its diversity in its cenotic composition (Table 3).

Table 3

**Cenotic analysis of the sand massif flora in the western part of the Sub-Ural plateau**

Florocenotic element	Number of species	Percentage of the total number of species
Forest 12 species (8.76%)		
Forest	11	8.03
Meadow-forest	1	0.73
Forest-steppe 17 (12.41%)		
Steppe 67 (48.91%)		
Steppe	48	35.04
Desert steppe	7	5.11
Mountain steppe	4	2.92
Meadowsteppe	8	5.84
Desert 8 (5.84%)		
Desert	6	4.38
Steppe desert	1	0.73

Sandydesert	1	0.73
Meadow 23 (16.79%)		
Meadow	18	13.14
Meadow-swamp	1	0.73
Field-Meadow	3	2.19
Forest-meadow	1	0.73
Waterside 3 (2.19%)		
Weed 7 (5.11%)		
Total	137	100

7 major florocenotypes consisting of 16 florocenoelements were distinguished. The dominant florocenotype is the steppe florocenotype, which occupies 48.91% of the flora of the region. It should be noted that the steppe type of vegetation includes communities of xerophilous, for the most part, herbaceous plants [19]. Abundant moisture and proximity of unmineralized groundwater in the depressions between the sand dunes contribute to the increase in the share of meadow florocenotypes (23; 16.79%).

The flora also contains adventive species (weeds) (7; 5.1%), which is associated with high anthropogenic pressure on the region and unfavorable growth environment, and is also an indicator of ecosystem destruction. Deterioration of the habitat leads to the reduction of the range of local natural species, the reduction of nutritious fodder plants, and the increasing effect of poisonous, harmful and poor forage plants adapted to grow in unfavorable conditions [20].

In the composition of the flora, there was a predominance of plants adapted to growth in drought, especially with an increased proportion of intermediate groups – mesoxerophytes and xeromesophytes (Table 4).

Table 4

#### Ecological groups in relation to the humidity of the sand massif flora in the western part of the Sub-Ural plateau

Ecological groups according to humidity	Number of species	Percentage of the total number of species
Mesohygrophytes	3	2.19
Hygromesophytes	2	1.46
Mesophytes	35	25.55
Xeromesophytes	31	22.63
Mesoxerophytes	31	22.63
Xerophytes	35	25.55
<b>Total</b>	137	100

Chorological or geographical analysis shows the physico-geographic features of the region, nature and climate conditions within the range of plants and the nature of migration. The results of the analysis showed that there are 7 major areal types and 13 geoelements (Table 5).

Chorological or geographical analysis shows the physical and geographical features of the region, the climatic conditions of nature and the nature of migration within the range of plant distribution. The results of the analysis showed that 7 large area types and 13 geoelements occur (Table 5).

Table 5

#### Chorological analysis of the sand massif flora in the western part of the Sub-Ural plateau

Name of types of area (geoelements)	Number of species	Percentage of the total number of species
Eurasian areal type 48 species (35.04%)		
Eurasian	34	24.82
Euro-Siberian	13	9.49
East European-Kazakhstan	1	0.73
European areal type 24 (17.52%)		
European	5	3.65
Pontic	19	13.87

Holarctic areal type 11 (8.03%)		
Ancient Mediterranean area type 29 (21.17%)		
Ancient Mediterranean	26	18.98
Asiatic	3	2.19
Mediterranean area type 9 (6.57%)		
Turanian areal type 10 (7.30%)		
Aral-Caspian	6	4.38
Pre-Caspian	2	1.46
Sarmatian	2	1.46
Pluriregional areal type 6 (4.38%)		
Total	137	100

The analysis of the composition of the flora showed a predominance of wide-area Eurasian (48 species, 35.04%) and Ancient Mediterranean (29 species, 21.17%) species. The meeting of Holarctic, European, Eurasian and Turanian species is explained by migration processes.

The vegetation cover of sand massifs fixes loose sands and has a favorable effect on the ecological condition of the adjacent territory. Both sand massifs are surrounded by riverine forests with unique forest and meadow vegetation, denser forests and large lake basins in depressions near large ravines and groundwater. Sand massifs are used in animal husbandry, therefore it is necessary to systematically carry out measures to improve the variety and quality composition of vegetation and maintain ecological stability. Degraded areas are dominated by milkweed communities (*Euphorbia seguieriana* + *Leymus racemosus*).

Both sand massifs are included as geological objects in the list of subsurface areas of special ecological, scientific and cultural value [21]. *Stipa pennata* L. is listed in the "List of Rare and Endangered species of plants and animals of the Republic of Kazakhstan" (2006) [22], and 8 rare and endangered species are listed in the Green Book of the West Kazakhstan region [23]: *Achnatherum splendens* (Trin.) Nevski, *Betula pendula* Roth, *B. pubescens* Ehrh., *Chamaecytisus borysthenticus* (Gruner) Klask., *Ch. zingeri* (Nenukow ex Litv.) Klask., *Helichrysum arenarium* (L.) Moench., *Juniperus sabina* L., *Rhaponticoides kasakorum* (Iljin) M.V. Agab. & Greuter ex C. Shih & L. Martins. They need to status correction by preparation of new issue of the Red data book.

Forest groves, meadows, mixed-grass-stipa grass communities and juniper-grown areas are now rare due to the influence of natural and anthropogenic factors (wildfires, excessive livestock grazing, non-observance of mowing regime, unsystematic collection of medicinal plants, etc.), therefore their protection it is necessary to organize measures. In order to organize effective protection measures for individual plants, their populations and communities, there is a need to comprehensively protect their growing environment first.

"Karagash" and "Akkum" psammophyte massifs should be organized not only as specially protected natural areas at the local level, but also as a complex ecosystem at the republican level with large-scale biosphere significance.

### Conclusions

As a result of the conducted research, it was revealed that 137 species of vascular plants belonging to 99 genera and 35 families grow on the sand massifs of the western part of the Sub Ural plateau.

The flora composition of sand massifs in the western part of the Sub Ural plateau was determined. In the study area, 137 species belonging to 35 families are found. Most of the flora composition (70 species; 50.4%) is occupied by the families *Asteraceae*, *Poaceae*, *Fabaceae* and *Chenopodiaceae*. The results showed that in the flora composition of the study area is dominated by long-rhizomatous species that adapted to fixing sand: *Calamagrostis epigeios* (L.) Roth., *Carex colchica* J. Gay., *Helichrysum arenarium* (L.) Moench, *Leymus racemosus* (Lam.) Tzvel., *Scirpoides holoschoenus* (L.) Soják., etc. The meadow vegetation is dominated in the blowing depressions and in sites with closely located non-mineralized fresh groundwater (23; 16.79%). The flora showed a predominance of xerophytes and intermediate groups (mesoxerophytes and xeromesophytes). Their predominance is associated with the adaptation of psammophytes and steppe species to growing in arid climates. The chorological analysis showed the predominance of the wide-ranging Eurasian (48 species, 35.04%) and Ancient Mediterranean (29 species, 21.17%) species. The pres-

ence of adventitious species in the flora (7; 5.1%) indicates high anthropogenic pressure, unfavorable growing conditions and habitat disturbance. The deterioration of the ecological state of the distribution area affects the communities of rare plants both directly and indirectly. In this regard, it is advisable to organize reference areas of biodiversity or key botanical territories in meadows, and psammophytic mixed-grass-stipa grass and juniper communities. It is necessary to carry out measures to reduce anthropogenic pressure on the ecosystems of this territory, taking into account the biodiversity of plants and the possibilities of their effective use in the economy.

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## Подурал үстіртінің батыс бөлігіндегі құмды массивтердің флоралық құрамы

Бүгінгі күні Қазақстан аумағында экожүйелердің қазіргі жай-күйін зерделеу өзекті мәселелердің бірі. Зерттеудің мақсаты — Подурал үстіртінің батыс бөлігіндегі құмды алқаптардың флорасына биоморфологиялық, географиялық және экологиялық талдау жасау. Аққұм және Қараағаш құмды массивтерінде 99 туыс пен 35 тұқымдасқа жататын түтікті өсімдіктердің 137 түрі тіркелді. Флора құрамының басым бөлігін (70 түр; 50,4%) *Asteraceae*, *Poaceae*, *Fabaceae* және *Chenopodiaceae* тұқымдасына жататын түрлер алады. Талдау нәтижелерінде тамырсабақты түрлер басымдығын көрсетті. Олар: *Calamagrostis epigeios*, *Carex colchica*, *Helichrysum arenarium*, *Leymus racemosus*, *Scirpoides holoschoenus* және т.б. Флора құрамында адвентивті түрлердің кездесуі антропогендік қысымның жоғарылығын, өсу ортасының қолайсыздығын және мекен ету ортасының бұзылғандығын көрсетеді. Ксерофиттердің басымдығы псаммофиттер мен далалық түрлердің құрғақ климат жағдайында өсуге бейімделуімен байланысты. Хорологиялық талдау нәтижелері кең аралды еуразиятық және ежелгі жерортатеңіздік түрлердің басым екендігін көрсетті. Шалғынды және псаммофитті әр түрлі шөпті-селеулі, сондай-ақ арша қауымдастықтарын қорғау қажеттілігі туындайды. Бұл қауымдастықтар қазіргі уақытта таралу аймағы қарқынды түрде азайып бара жатқан өсімдік түрлеріне жатады, сондықтан олардың өсу ортасын тұтас қорғау арқылы кешенді шараларды ұйымдастыру қажет. Өсімдіктердің түрлік құрамы, олардың биологиялық және экологиялық ерекшеліктері туралы мағлұматтардың практикалық маңызы зор, олар болашақта мониторинг жұмыстарын ұйымдастыруға негіз болып саналады.

*Кілт сөздер:* Батыс Қазақстан облысы, Подурал үстірті, құмды массив, псаммофиттер, флоралық талдау, өсімдік жамылғысы, Аққұм, Қараағаш.

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## Флористический состав песчаных массивов западной части Подуральского плато

В настоящее время на территории Казахстана одним из актуальных вопросов является изучение современного состояния экосистем. Целью исследования является биоморфологический, географический и экологический анализ флоры песчаных массивов западной части Подуральского плато. В песчаных массивах Карагач и Аккум зарегистрировано 137 видов сосудистых растений, относящихся к 99 родам и 35 семействам. Большую часть состава флоры (70; 50,4 %) занимают виды, принадлежащие к семействам *Asteraceae*, *Poaceae*, *Fabaceae* и *Chenopodiaceae*. Преобладающими в составе флоры являются корневищные виды: *Calamagrostis epigeios*, *Carex colchica*, *Helichrysum arenarium*, *Leymus racemosus*, *Scirpoides holoschoenus* и др. Наличие адвентивных видов в составе флоры свидетельствует о высоком антропогенном давлении, неблагоприятных условиях произрастания и нарушения среды обитания. Преобладание ксерофитов связано с адаптацией псаммофитов и степных видов к произрастанию в условиях засушливого климата. Результаты хорологического анализа показали преобладание широкоареальных евразийских и древнесредиземноморских видов. Возникает необходимость охраны луговых и псаммофитных разнотравных-ковылных и можжевелевых сообществ. Они относятся к видам растений, ареал распространения которых в настоящее время интенсивно сокращается, поэтому необходимо организовать комплексные меры защиты среды их произрастания. Важное практическое значение имеют сведения о видовом составе растений, об их биологических и экологических особенностях, которые в будущем станут основой для организации мониторинговых работ.

*Ключевые слова:* Западно-Казахстанская область, Подуральское плато, песчаный массив, псаммофиты, флористический анализ, растительный покров, Аккум, Карагач.

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## To the methodology for the study of intraspecific variability and selection of wild forms of *Viburnum opulus* L.

The methodological recommendation for the study of intraspecific variability and the selection of wild-growing promising for breeding forms of common *Viburnum* written on the basis of a large amount of factual material obtained from the study of this species in the East Kazakhstan region. It includes an assessment of the current state of *Viburnum opulus* L. populations, with range mapping in ArcMap and ArcGis programs. The geobotanical, phytocenotic description is given. First, the forms are described according to 25 quantitative and qualitative features. Then, on the basis of this, the selection of promising forms, the collection of seeds and cloning with green cuttings is carried out. The distribution of *Viburnum* plants occurred according to the degree of flowering, fruiting, fruit length and diameter. The data were divided into classes with the smallest, average and largest values into five groups, by weight and number of fruits into four groups. The scoring system was applied to seven characteristics: the general condition of plants, winter hardiness, the degree of flowering and fruiting, color, taste, and the nature of the detachment of fruits. The study of endogenous variability of morphological and economic-biological characters is carried out in the crown of one individual. After studying the average indicator of the trait and the amplitude of its variation for different individuals, they proceed to the study of intra-population variability. After mathematical processing, a questionnaire is filled out for each plant according to the selected form, which will make it possible to better represent the studied form to study genotypic and phenotypic variability in the future. The use of this recommendation in research work will allow assessing the genetic potential of the species, the level of its intraspecific variability, and selecting promising forms.

*Keywords:* distribution, quantitative characteristics, degree of flowering, fruits, color, variability.

### Introduction

A comprehensive study of fruit and berry plants in natural habitats is essential for solving issues of ecology, phytocenology, floristics, population biology, and nature conservation.

In the programs for breeding and variety study of fruit and berry crops, the main attention is paid to such leading fruit and berry crops as apple, pear, plum, cherry, apricot, black currant, strawberry (1980). There are several methods for studying rare fruit and berry plants: sea buckthorn [1, 2], blueberries, cranberries [3, 4].

Common viburnum – *Viburnum opulus* L. is the most interesting in terms of studying variability and introducing into cultivation among wild-growing species of fruit and berry plants growing on the territory of the East Kazakhstan region. The advantages of *viburnum vulgare* include high winter hardiness, productivity and usefulness of fruits. Due to its medicinal properties of all parts the plant is widely used in medicine. Its fruits have the richest chemical composition. They contain up to 8% sugars, mainly glucose and fructose, up to 3% tannins. Viburnum bark, which contains up to 4% tannins, has an exceptional therapeutic effect on the human body and most importantly, viburin glycoside, which has a pronounced vaso-constrictive effect [5].

The genus *Viburnum* L., belongs to the *Adoxaceae* family, order *Dipsacales*, includes about 140 species. Common viburnum (*Viburnum opulus* L.) is a multi-stemmed shrub, 1.9 – 4.5 m high with brown-gray bark and smooth light young branches. Buds with two fused outer scales, ovoid, slightly pointed, reddish green. The bushes are multi-stemmed, their number reaches 35 pcs. The leaves are broadly ovate, rounded, 6–13 cm long, 5–11 cm wide, with 3, rarely 5 large-toothed lobes. The middle lobe is larger, with parallel, often elongated lateral margins, shortly pointed at the very apex or with a more or less elongated point. The upper side of the leaves is bare; the lower side is bare or pubescent. *Viburnum vulgare* is a cross-pollinated, anemophilous plant that blooms in late May – early June. White and pink-white flowers are collected in large inflorescences – umbellate corymbs up to 8 cm in diameter, along the edges of the inflorescence there are larger sterile flowers (1.8-2.5 cm) in diameter with white petals. The flowers are heteromorphic, with a double perianth, collected in flat umbellate 6-8- ray panicles, 4.9-7.5 cm in diameter, on a peduncle 2.5-5.0 cm long, marginal flowers on pedicels 1-2 cm long, barren, flat, white, 4-5 times larger than internal, median –

bisexual, sessile or almost sessile, white or pinkish-white, shortly bell-shaped, about 5 mm in diameter. Inflorescences are located at the tops of young branches. The fruit of the viburnum is a bright red spherical drupe 8.3-10.3 mm long and wide, with one large flat stone inside, ripens in September – early October.

*V. opulus* is undemanding to growing conditions, easily tolerates drought and frost, and is most common in the temperate climate of Europe and Asia. It grows in the undergrowth and along the edges of moist deciduous and mixed forests, in tree and shrub thickets along the banks of springs, rivers, lakes, swamps, ravines, gorges and mountain slopes. It grows everywhere in the European part of Russia (except the north and southeast) in Eastern and Western Siberia, the Caucasus, Central Asia, Western Europe, Asia Minor, North Africa.

In Kazakhstan it grows in the following floristic regions: 2. Tob. Ishim. (north), 3. Irtysh, 4. Semipalatinsk forest, 5. Kokshetau, 7. Aktyubinsk, 10a. Ulutau, 11. Eastern Uplands, 12. Zaisan, 22. Altai, 23. Tarbagatai, 24. Dzhungarskiy Alatau (north), 25. Zailiskiy Alatau [6].

It is widely distributed on the territory of the East Kazakhstan region: the Saur ridge, Tarbagatai, in the floodplain of the Kenderlyk river, in the foothills of the Ubinsky ridge, near the village of Cheremshanka, Zimovye, the village of Sharavka, Mount Kozlushka, as well as on the floodplain terraces of the river Bolshaya Talovka, Malaya Talovka, on southeastern slope of Mount Barkhot near the village of Livino, at the foot of the Ivanovsky ridge in the area of the Gornyyak rest house [7].

### *Experimental*

Surveys of natural habitats are carried out by expedition trips in September-October, during the period of harvesting fruit ripeness.

To determine the distribution of common viburnum in one area, we recommend using the Brown-Blanque scale in points:

1 – grows singly, 2 – in groups, 3 – in small spots, 4 – in small colonies or large spots, 5 – in large thickets.

In the description of phytocenoses the concept of layering is used, based on the life forms of the species included in its composition [8]. Latin names of plants are given according to S.K. Cherepanov [9].

At each trial plot, a detailed geobotanical description is carried out, the collection of herbarium, seeds, cloning of selected forms with green cuttings, measurements and description of morphological features in even-aged plants with a continuous count of trees.

### *Results and Discussions*

The selection and description of forms of *viburnum vulgare* in wild populations is carried out in the following order.

Specify the date of the examination.

Assign a number to the plant.

Specify the location through range mapping in ArcMap and ArcGis.

Brief description of the habitat (relief, soil, plant community).

Do a soil analysis.

The description of wild-growing forms of viburnum is carried out according to the phenotype, which takes into account quantitative and qualitative indicators of traits.

Determine the life form: tree, shrub.

Crown shape: round, oval, conical, etc.

Indicate the age state of the described plant (approximate number of years), according to the size of the bush, the diameter of the stems and the general condition.

Shrub height, m.

Stem diameter, cm.

In determining the general condition of the bushes, the main attention should be paid to the ratio of the processes of neogenesis (the number of zero shoots) and the death of old stems in the crown, the diameter of which is higher than 6–8 cm, as well as broken stems. The condition of the plant is assessed as follows: excellent – 3 points, completely healthy, not damaged by pests and diseases, height more than 2.2 m, number of stems from 8 to 16 pieces, no broken and dry stems, growth 18-35 cm, healthy leaves; average – 2 points; not damaged by pests and diseases, height more than 1.8 m, number of stems 6-12 pieces, broken and dry stems up to 10%, growth 12-25 cm, leaf apparatus without damage; bad – 1 point; up to 25% of the leaf apparatus

on the plant is damaged by pests and diseases, the height is more than 1.8 m, the number of stems is 6–12, broken and dry stems are up to 15%, the growth is 8–20 cm.

Winter hardiness: 0 – no damage, 1 – very weak damage, 2 – weak (up to 10% of fruit-bearing branches), 3 – medium (up to 30%), 4 – strong (up to 40%), 5 – very strong (more than 50%).

The color of the bark of skeletal branches (gray, light gray).

The length of annual shoots (with an accuracy of 0.5 cm, the average of 30–40 measurements).

Blade length and width, cm (average of 30 measurements).

The color of the upper side of the leaves is established according to the A.S. Bondartseva (light green, green, dark green), while indicating the intensity of the shade.

The degree of flowering of plants (in points) according to the following scale:

1 – very weak (single brushes, up to 3 pcs. per branch, the number of flowers in a brush up to 35 pcs.); 2 – weak (the number of brushes per branch, up to 6 pcs., The number of flowers in the brush 36–65 pcs.); 3 – medium (number of brushes per branch, up to 9 pcs., number of flowers in a brush 66–95 pcs.); 4 – good (the number of brushes per branch, up to 12 pieces, the number of fruits in the brush is more than 95 pieces); 5 – plentiful (the number of brushes per branch, up to 15 pieces and more, the number of fruits in the brush is more than 95 pieces).

Fruit set: below 30% – low, 40 – 50% – medium, above 60% – high, determined fourteen days after flowering, when an increase in the size of the ovary is noticeable.

The degree of fruiting plants (in points) according to the following scale:

1 – very weak (single brushes, up to 3 pieces per branch, the number of fruits in the brush up to 15 pieces); 2 – weak (the number of brushes on a branch, up to 6 pcs., The number of fruits in a brush 16–30 pcs.); 3 – medium (the number of brushes per branch, up to 9 pieces, the number of fruits in the brush 31–45 pieces); 4 – good (the number of brushes per branch, up to 12 pieces, the number of fruits in the brush is more than 45 pieces); 5 – plentiful (the number of brushes per branch, up to 15 pieces and more, the number of fruits in the brush is more than 45 pieces).

To determine the weight of 100 fruits, with an accuracy of 0.01 g, fruits from different parts of the crown are collected from each plant in triplicate, 25–30 pieces each. Taking into account the population parameters by weight in fruit-bearing plants of *viburnum vulgare* from 0.30 g to 0.79 g, an interval of 0.15 g was adopted in the gradation, four groups: small – below 0.45 g; medium – from 0.46 to 0.60 g; large – from 0.61 to 0.75 g; very large – above 76 g.

In determining the size of fruits with a caliper, with an accuracy of 0.1 mm, measure the length and diameter of 25–30 fruits from different parts of the crown, then determine statistical indicators that will characterize not only this sample (25–30 fruits), but the entire set fruits of a given bush in a particular area of growth.

Describing the shape of the fruit, their index is established – the ratio of the length of the fruit to its diameter, given the index (0.95; 1.01–1.06; 1.07–1.2), the shape of the fruit is described as (round, spherical, oval).

In determining the color of *viburnum* fruits, the colors of the solar spectrum are taken, they are described as (orange, orange-red, reddish-orange, red, cherry). Fruit color: cherry – 15 points, dark red and red – 10 points, orange – 5 points.

The evaluation of fruit taste is determined by the organoleptic method (excellent, good, mediocre, poor). The nature of the taste of fruits (sweet, sour, sour-sweet, sweet-sour, indicating the presence of bitterness. Taste of fruits: dessert – 20 points, pleasant with a slight aftertaste of bitterness – 15 points, with a significant bitter aftertaste – 10 points, bitter – 5 points.

The nature of the detachment of fruits from the hand is dry – the fruit is torn off without juice release (10 points), wet – with juice release (5 points).

Taking into account the population parameters in terms of the number of fruits in the cluster of fruit-bearing plants of *viburnum vulgare*, from 9.7 to 50.9 pieces, an interval of 15 pieces was adopted in the gradation, four groups: few – less than 15 pieces; average – 16–30 pieces; high – 31–45 pieces; very high – more than 45 pcs.

Seeds: a) Color (light brown, brown); b) shape (ovoid, elliptical).

The percentage of seed weight from the weight of fresh fruits is determined within a week.

In terms of ripening, we distinguish early forms, fruit ripening in the third decade of September and late ones in the first decade of October.

Pests and diseases of *viburnum vulgare*, nature of damage.

The study of intraspecific variability of a species (endogenous and individual) is carried out by studying the most common populations within its range, on temporary trial plots.

Variability is one of the basic biological concepts along with the concepts of metabolism, reproduction, and heredity. All changes are divided into two groups: hereditary and non-hereditary [10].

Variability is the main property of all living organisms, therefore in nature there are no individuals absolutely identical in all signs and properties. I.I. Schmalhausen [11] defined variability as the property of living organisms to reproduce similar but not identical. “Variability”, he wrote, “is an expression of the continuity of organic forms in the process of reproduction, in which dissimilar structures and functions appear (i.e. develop) in the offspring”.

In individual variability, a change characterizes only an individual, and such changes are always caused by the influence of external conditions, i.e. arise as a result of modifications. This kind of variability corresponds more or less to non-hereditary variability.

With group (intrapopulation) variability, the change no longer characterizes an individual, but a whole group of them within the species. Such changes always arise as a result of mutations or combinations, due to which this kind of variability more or less corresponds to hereditary variability.

Baur, on the other hand, suggested that the word “variation” should be given a broader meaning and understood by variations as all three types of possible changes in organisms, i.e. modifications, mutations and combinations. The first group covers a change of a non-hereditary nature, the second and third – hereditary; modifications and mutations arise without any participation of crossing, combinations – as a result of the latter, but all these are different types of variability, as a process or variations.

Thus, the division of variability, in fact, into individual and group is as basic as the division of variations into modifications, mutations and combinations. In one case we are dealing with a known state, in the other with a process.

Crossings are constantly taking place in natural populations, as a result of which new forms appear due to the formation of new combinations from old combinations of hereditary factors. New combinative changes are always of a group nature. Meanwhile, most combinations are split as well as the forms that produced them, due to the fact that each such new phenotype usually includes several different genotypes. In nature, mutational and combinative variability act together, intertwining in the most intimate way with each other. The variability of organisms in a population under the pressure of natural selection leads to the formation of new species and morphogenesis processes.

Studies of the intraspecific variability of common viburnum are carried out on the basis of a population-genetic approach to species assessment, where a species is understood as a system of populations [12-14].

The study of intraspecific variability of common viburnum is carried out according to the method [15] in three stages:

1. The study of endogenous variability of morphological and economic-biological traits in the crown of one individual. In order to determine the endogenous variability, the sample size is 20-50 measurements of each trait, depending on the variability, by crown tiers, exposure sides, etc. (Table).

T a b l e

**Accuracy and number of measurements of signs of *Viburnum opulus***

Feature	Measurement accuracy	Number of measurements
Plant height	10 cm	30-40
The number of trunks and the presence of zero shoots, pcs.	1 pcs.	40-45
Length (one-year growth)	0,5 cm	30-40
Number of flowers in inflorescence, pcs.	1 pcs.	40-50
Number of fruits in inflorescence, pcs.	1 pcs.	40-50
Fruit length	0,1 mm	20-25
Fruit diameter	0,1 mm	20-25
Weight 100 pcs. fruits, g	0,01 g	20-25
Weight 100 pcs. seeds, g	0,01 g	30-40

2. After studying the average indicator of the trait and the amplitude of its variation for individuals, they proceed to the study of intrapopulation (organism, individual) variability. The sample size (the number of

model bushes) required to obtain reliable results when characterizing the structure of populations is determined by the level of variability of different traits.

3. Study of interpopulation variability. To assess the degree of variability of traits, a unified scale of variability levels developed [16] is used. According to the scale, the amplitude of variability is estimated by the value of the coefficient of variation: less than 12% – the level of variability is low, 13%-20% – medium, 21%-40% – high, more than 40% – very high.

Phenotypic variability is a set of features and properties of an organism or a group of individuals formed under the combined influence of heredity and environmental factors.

In the study of variability, biometric research methods are used, based in whole or in part on statistical or probabilistic patterns. To find the arithmetic averages –  $M$  and the errors of the averages –  $m$  of the studied parameters, the coefficients of variation  $C\%$ , the standard deviation –  $\sigma$ , the reliability criterion –  $t$ , and the accuracy of the experiment  $P$  for each plant and for a separate population, the obtained digital data are processed using methods of mathematical statistics [17-20].

The calculation of statistical indicators is carried out separately at two levels of variability: endogenous (within the body) and individual within the population (location).

A number of other features: differences in the color of fruits, bark, the nature of taste and separation of fruits are of a qualitative nature. Let us consider an example of comparing the qualitative variability in our case by the color of viburnum fruits. Statistical data show that among the examined plants, the following percentage distribution of the total number of individuals by color was obtained: with red fruits 42.6%, with orange 37.4%, with cherry 20.0%.

With qualitative (alternative variability), no matter how many separate groups (in our case, three), they are divided into two classes: class 1 – having one or another feature and class 0, devoid of it. In cases of qualitative variability, the coefficient of variation completely replaces the quadratic deviation. The calculation of the square deviation is carried out according to the following formula:

$$\sigma = \pm(a)/(a+b)$$

and always expressed as a percentage. The first includes individuals, the second  $b$  individuals. According to the color of the fruits of each of them, it is necessary to contrast with all the others (red – not red, cherry – not cherry, etc.), and for each, the following values of the square deviation are obtained: red 42.6% versus 57.4% not red; orange 37.4% versus 62.6% non-orange; cherry 20.0% versus 80.0% non-cherry.

$$\sigma = \pm(a)/100 = 48,3\%$$

The square deviation is always expressed here as a percentage, and its value does not exceed 50%.

After mathematical processing, it is necessary to fill out a questionnaire for each studied plant in a selected form (data on 25 traits), which will make it possible to better imagine the studied form and study genotypic and phenotypic variability in the future.

### Conclusions

The ecological and biological features of the common viburnum from natural habitats, its comparative characteristics reveal its genetic potential, which is extremely important for the conservation of biological diversity and breeding work.

For each region, the plants of *viburnum vulgare* have their own ecological and biological features. This recommendation will help to study this plant according to the main morphological and economic characteristics, to conduct a comparative characteristic.

In the section objects and methods of research based on actual data, a map has been developed for describing wild-growing forms of viburnum, which is filled in according to 25 quantitative and qualitative characteristics.

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### ***Viburnum opulus* L. жабайы формаларын таңдау және түршілік өзгергіштікті зерттеу әдістемесіне**

Түршілік өзгергіштікті зерттеу және өсіруге перспективалы кәдімгі калинаның жабайы өсетін түрлерін таңдау бойынша әдістемелік ұсыныс Шығыс Қазақстан облысындағы осы түрді зерттеу нәтижесінде алынған үлкен көлемдегі фактілік материалдар негізінде жазылған. Ол ArcMap және ArcGis бағдарламаларында ауқымды карталау арқылы *Viburnum opulus* L. популяцияларының ағымдағы жағдайын бағалауды қамтиды. Геоботаникалық, фитоценоздық сипаттама берілген. 25 сандық және сапалық белгілер бойынша формалардың сипаттамасы жасалған, соның негізінде перспективалық түрлерді іріктеу, тұқым жинау және көк қалемшемен клондау жүргізілген. Калина өсімдіктерін гүлдену, жеміс беру дәрежесі, жемістердің ұзындығы мен диаметрі бойынша бөлу кезінде деректер ең кіші, орташа және ең үлкен мәндері бар (бес топ) кластарға; ал жемістердің массасы мен саны бойынша — төртке бөлінді. Балдық жүйе жеті белгі бойынша қолданылды: өсімдіктердің жалпы жағдайы, қысқа төзімділігі, гүлдену және жеміс беру дәрежесі, түсі, дәмі, жемістердің үзілу сипаты. Морфологиялық және шаруашылық-биологиялық белгілердің эндогендік өзгергіштігін зерттеу бір дарактың жапырағына жүргізілді. Белгінің орташа көрсеткішін және оның өзгеру амплитудасын жеке дарактар үшін зерттегеннен кейін популяция ішілік өзгергіштік зерттелді. Математикалық өңдеуден кейін әрбір өсімдікке таңдап алынған түр бойынша сауалнама толтырылады, бұл зерттелетін түрлерді жақсы білу-



ге және болашақта генотиптік және фенотиптік өзгергіштікті зерттеуге мүмкіндік береді. Бұл ұсынысты ғылыми-зерттеу жұмыстарында қолдану түрдің генетикалық әлеуетін, оның түршілік өзгергіштік деңгейін бағалауға және перспективалы түрлерді таңдауға мүмкіндік береді.

*Кілт сөздер:* таралуы, сандық сипаттамасы, гүлдену дәрежесі, жемістері, түсі, өзгергіштігі.

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## К методике по изучению внутривидовой изменчивости и отбору дикорастущих форм *Viburnum opulus* L.

Методическая рекомендация по изучению внутривидовой изменчивости и отбору дикорастущих форм калины обыкновенной, перспективных для селекции, написана на основе большого фактического материала, полученного при исследовании этого вида на территории Восточно-Казахстанской области. Она включает оценку современного состояния популяций *Viburnum opulus* L., с картированием ареалов в программах ArcMap и ArcGis. Дано геоботаническое, фитоценоотическое описание. Проведено описание форм по 25 количественным и качественным признакам, на основе чего были произведены отбор перспективных форм, сбор семян и клонирование зелеными черенками. При распределении растений калины по степени цветения, плодоношения, длине и диаметру плодов данные были разбиты на классы с наименьшими, средними и наибольшими значениями (пять групп), по массе и количеству плодов — на четыре. Бальная система применена к семи признакам: общее состояние растений, зимостойкость, степень цветения и плодоношения, окраска, вкус, характер отрыва плодов. Изучение эндогенной изменчивости морфологических и хозяйственно-биологических признаков проведено в кроне одной особи. После изучения среднего показателя признака и амплитуды его варьирования для отдельных особей перешли к изучению внутривидовой изменчивости. После математической обработки заполнена анкета на каждое растение по выделенной форме, что даст возможность лучше представить исследуемую форму и в будущем изучать генотипическую и фенотипическую изменчивость. Использование настоящей рекомендации в исследовательской работе позволит дать оценку генетического потенциала вида, уровня его внутривидовой изменчивости, отобрать перспективные формы.

*Ключевые слова:* распространение, количественные признаки, степень цветения, плоды, окраска, изменчивость.

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## A study of the cenopopulations and ontogenetic structure of *Adonis wolgensis* in the Akmola region

The article presents the results of study of six cenopopulations of rare medicinal plant *Adonis wolgensis* Steven ex DC. (Ranunculaceae) in Akmola region of Northern Kazakhstan. Ecological-phytocenotic characterization was given and ontogenetic structure of cenopopulations was studied. It is established that in the studied region *Adonis wolgensis* grows among steppe grasses, in bushes, occasionally on forest edges and on forest lawns. In general, the condition of the studied *Adonis wolgensis* cenopopulations is satisfactory. Analysis of age spectra showed that cenopopulations not or slightly affected by anthropogenic impact are characterized by a spectrum in which the absolute maximum falls on the age states of the pre-generative period. In the four cenopopulations, a significant proportion of individuals are in the young generative state, indicating their capacity for self-renewal. This ensures the stability of the species in the plant communities of the study areas. The condition of two cenopopulations is assessed as degrading, and urgent measures are needed to preserve them and restore their numbers. Cattle grazing in cenopopulations leads to a decrease in plant viability and suppression of seed reproduction. To preserve the species it is necessary to limit grazing, prohibit collection of raw materials, develop methods of cultivation in culture, organize reproduction of the species in industrial volumes, and strengthen protection measures. The results obtained have provided new information on the status of the species in Northern Kazakhstan and serve as a reliable basis for its further conservation.

**Keywords:** *Adonis*, Flora of Kazakhstan, rare species, biodiversity conservation, Northern Kazakhstan, state of cenopopulations.

### Introduction

Preservation of rare and endangered species has become an integral part of the general problem of plant world protection. In connection with the growth of anthropogenic impact on ecosystems, the rate of industrial development, a wide scale of economic development of new and new territories, leads to significant changes in landscapes, disruption of primary biocenoses, reduction of biodiversity reserves. Especially acute is the problem of preservation of steppe cenoses, the destruction and transformation of which leads to a decrease in species diversity, reduction in the range of typical steppe species, the formation of populations with a disturbed structure. One of them is *Adonis wolgensis* Stevex DC. (Ranunculaceae family) is a steppe Pannonian-Pontic-Transwolgian-Kazakhstan species, listed in the Red Data Book of Kazakhstan (2014) in the category of rarity III, a declining species [1]. Populations of this species are decreasing in number due to intensive action of a number of factors of anthropogenic origin: economic activity of enterprises and population, plowing of steppe areas, grazing and trampling of habitats by cattle, mowing, as well as intensive its harvesting as a medicinal raw material and collection of bouquets.

Significant imprint on the state of cenopopulations is also imposed by biological features of the species – low germination of seeds due to the short period of ontogenesis, slow development of seedlings, narrow range of ecological and cenotic conditions necessary for their germination, interspecific phytocenotic competition. The genus *Adonis* L. is represented by annual and perennial rhizomatous herbaceous plants, which belong to the tribe Adonideae T. Duncan et Keener, subfamily Ranunculoideae, family Ranunculaceae Juss. Duncan et Keener subfamily Ranunculoideae of the family Ranunculaceae Juss. [2, 3]. The genus includes 40-50 species [4], which are mainly distributed in the extratropical zones of Eurasia. About 30 species grow in Southwest Asia, Europe, North Africa, and the Mediterranean region [5]. Seeds, leaves and roots of the

genus *Adonis* are poisonous to humans. The plant has numerous stamens, linear filaments, and single-seeded ovaries with permanent stigmas [6]. Plants of this genus have historically been traditionally used to for the treatment of edema in many peoples of the former Soviet Union. Extracts were first introduced into medicine as a cardiac stimulant in 1879 by the Russian physician N.O. Buhnov. Since then, species of this genus have been of great interest to people [7]. The chemical composition of *Adonis* is similar to other species, all organs of the plant contain the same glycosides [8], carbohydrates, coumarins, cardenolides, flavonoids, fatty oil and are used in scientific medicine as a cardiogenic agent and as a potential substitute for *Adonis vernalis* [9-11], are used similarly to *A. vernalis*, but have less pronounced therapeutic properties [9].

*A. wolgensis* is mainly found in southeastern Europe, Western Siberia and the far north of Central Asia (the Aral-Caspian and Balkhash regions) habitats. This species is common in steppe communities, very rarely in shrubs and on the edges of forests. The range of *A. wolgensis* occurs in the Caucasian ecoregion and is isolated. In this region, *A. wolgensis* has a disjunctive range, and is distributed in the northeast of Turkey, in Armenia (Sevan and Aparan floristic regions), in the extreme southeast of Azerbaijan (Zuvand, Talysh), in the north and northwest of Iran. The distribution of this species is limited to the above-mentioned territories. *A. wolgensis* was found in Shida Kartli, Gori municipality [12]. On the territory of Kazakhstan it is found in the following floristic areas: 1. Spurs of the Common Syrt, 2. Tobol-Ishim, 4. Semipalatinsk boron, 6. Caspian lowland, 10. Western and 11. Eastern Shallow Soil, 11a. Kark, 22. Altai [13].

In the flora of Kazakhstan genus *Adonis* is represented by 8 taxa, of which 5 species grow on the territory of the study region [13]. A.N. Kupriyanov in his abstract "Flora of the Kazakh shallow-slope zone" [14] gives 4 species: *Adonis parviflorus* Fisch., *A. vernalis* L., *A. villosa* Ledeb., *A. wolgensis*. In the *Adonis wolgensis* Steven ex DC. conspectus it is indicated for Akmola region, vicinities of Katarkol settlement and Ereimentau Mountains.

*A. wolgensis* Steven ex DC. belongs to sect. Adonanthe W.T. Wang, subsect. Amurenses (Poschkurl.) M.H. Hoffm., is of great interest in systematic, biological and resource relations.

The aim of the work is to study the distribution and density of *A. wolgensis* populations in Akmola region of Northern Kazakhstan, floristic characteristics and ontogenetic states of individuals.

### Experimental

The work was carried out on the basis of expeditionary studies conducted in 2021-2022 in the steppe areas of Akmola region. 6 populations of *A. wolgensis* were surveyed and described (Fig. 1).

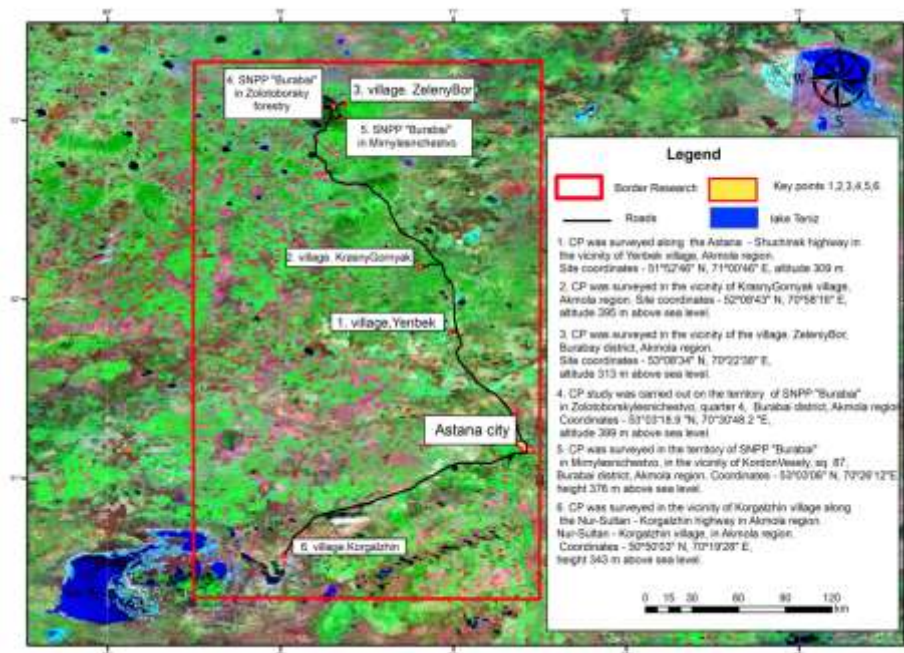


Figure 1. Map of studied population of *A. wolgensis*

The studies were carried out by detailed-route and cameral methods, geobotanical common methods were used to describe the objects. Ontogenetic age states were carried out according to the method of A. Rabotnov [15] and Smirnova [16]. The following classification of age groups was used to describe the age

groups: seedlings, juvenile, immature, virgin, young generative, mature generative, old generative, and senile individuals.

To study the ontogenetic structure and population size of *A. wolgensis*, plots with a high density of flowering individuals were selected with an area of 1 m<sup>2</sup>. In each population, 15 study plots were established: 10 × 10 m (100 m<sup>2</sup>). A total of 90 plots were counted. Characterization of age states was used according to A.P. Poshkurlat [4]. The ontogenetic spectrum was constructed according to the generally accepted methodology [15]. The type of cenopopulation was determined according to the classification of A.A. Uranov and O.V. Smirnova [16, 17].

All plant names are given according to the Plants of the World Online directory (POWO 2022) [18].

In addition to the materials of field studies, we used literature and materials from herbarium collections (AA) of the Institute of Botany and Phytointroduction and Astana Botanical Garden (Nur). Data on the distribution of *A. wolgensis* are fragmentary [19], special studies of the structure of cenopopulations (CP) of this region have not been conducted before.

Distribution map of *A. wolgensis* was obtained by ArcMap program.

### Results and discussion

The geographical, ecological and phytocenotic features of habitats, population characteristics and parameters of individuals were studied. Age composition was determined, total population size was calculated. As a result of floristic studies it was established that the cenoflora of *A. wolgensis* includes 119 species belonging to 28 families and 75 genera.

*A. wolgensis* is a perennial herbaceous, beautifully flowering plant, 5-25 cm tall, a species with a shrinking range. It grows among steppe grasses, in bushes, occasionally on forest edges and on forest lawns.

This plant has pale yellow flowers, oblong, narrow, narrowed at the ends, sometimes weakly toothed, 1-1.5 cm long and 0.5-1 cm wide; however, smaller than those of *Adonis vernalis*, 15-30 cm tall at the beginning of flowering, lengthening to 30-40 cm after flowering. The rhizome is erect, short, thick, with lace-like brownish-black roots. Stems solitary, with spreading branches; entire plant spreading-hairy, lower leaves brown, scaly, subsequent stem leaves twice pinnate, sessile, with 2 shortened lobes at the base; flowers 3.5-4.5 cm in diameter, petals 17-20 mm long and 6-7 mm wide; bracts globular or ovoid, sometimes drooping; fruits numerous, rounded, vaguely wrinkled, 3-4 mm long, ovoid, slightly wrinkled, pubescent, spout small, hooked downwardly bent. Nuts slightly wrinkled, hairy, about 4 mm long. The species blooms in late March-April, fruits in May-June [13]. It grows along the edges of birch forests and in mountain steppes (Fig. 2).



Figure 2. Period of flowering of *A. wolgensis* in the surrounding of village of Enbek, Akmola region

*A. wolgensis* is easier to cultivate than *A. vernalis*. Propagation is seed and vegetative – by rhizomes. In culture it blooms 6-8 years after sowing seeds, in separated years it gives self-seeding.

In nature grows singly or in small groups, natural reserves of the plant are quickly depleted. Obviously, in such a situation, studies of the peculiarities of biology, ecology and state of populations of this species in natural conditions are extremely important. Availability of such information is necessary for organization of works on preservation and increase of the number of its individuals and populations in the territories of their natural growth.

One of the most important features characterizing the state of a cenopopulation is ontogenetic structure [20], which is one of the existing features of a population. The side of structural organization provides the ability of a population system to self-maintenance and determines its sustainability. The analysis of the ontogenetic structure of plants provides insight into the future fate of species populations [21, 22].

Life cycle duration and reproductive characteristics are the main indicators of the ontogenetic state of a species. The ratio of the number of individuals of different age states allows determining the viability of cenopopulations. The biological peculiarities of the species under study include short period and average seed germination. Assessment of the vital state of *A. wolgensis* cenopopulations showed that plants in cenopopulations are flowering and fruiting. Adults for this species have an optimal height of the aboveground part [23, 24].

Below a characterization of *A. wolgensis* cenopopulations (CP) is given.

1 CP was surveyed along the Astana – Shuchinsk highway in the vicinity of Yenbek village, Akmola region. Site coordinates – 51°52'46" N, 71°00'46" E, altitude 309 m above sea level (Fig. 1). *A. wolgensis* is a part of steppe-grass-grass communities. The dominant species in the phytocenosis are *Festuca valesiaca*, *Stipa pennata*, *Limonium gmelinii*. Steppe xerophytic species often occur in the community: *Artemisia campestris*, *A. glauca*, *A. sericea*, *Glycyrrhiza glabra*, *Agropyron pectinatum*, *Galium verum*. Total projective coverage is 85%. Saline steppe chernozem soils.

Juvenile sprouts were absent in the cenopopulation, which is probably connected with sodden grass cover, as the vegetation cover was dominated by species of the *Poaceae* family. Species of this family prevent seed germination due to high density of rhizomes.

2 CP was surveyed in the vicinity of Krasny Gorniyak village, Akmola region. Site coordinates – 52°08'43" N, 70°58'16" E, altitude 395 m above sea level (Fig. 1). The species is a member of shrub-grass-grass communities. The dominant species in the phytocenosis are *Stipa capillata*, *Spiraea hypericifolia*, *Festuca valesiaca*, *Poa transbaicalica*. Among minor species *Achillea millefolium*, *Potentilla virgata*, *Allium lineare*, *Artemisia sericea*, *Phlomis tuberosa*, *Galium verum*, *Fragaria viridis*. Cenopopulations are subjected to anthropogenic impact in the form of cattle grazing. Adonis population is degrading, as young specimens and seedlings are damaged and die due to trampling by cattle. The total projective cover is 65%.

3 CP was surveyed in the vicinity of the village. Zeleniy Bor, Burabay district, Akmola region. Site coordinates – 53°08'34" N, 70°22'38" E, altitude 313 m above sea level (Fig. 1). The *A. wolgensis* population is a part of steppe-grass-grass communities. The community is dominated by the following species: *Stipa capillata*, *S. zaleskii*, *Festuca valesiaca*, *Pulsatilla flavescens*. Xerophytic species often occur in the community: *Carex humilis*, *Potentilla virgata*, *P. bifurca*, *Alyssum desertorum*, *Orostachys spinosa*, *Artemisia glauca*, *Thymus pulegioides* subsp. *pannonicus*, *Antennaria dioica*. The total projective coverage is 55%. Populations of *A. wolgensis* occur in the form of microphytocenoses of 500-700 m<sup>2</sup>, mainly on gentle slopes of hills or on tops. Participation of *A. wolgensis* in the herbage is 25-40%, the largest number of adult generative individuals is present, almost all of them flowered this summer and formed fruits (only 1.8 % were temporarily non-flowering), no sub-senile and senile plants were found. In 40 % of plants shoots branched up to the third order. Juvenile plants accounted for 15.5 %. No seedlings were found.

4 CP was carried out on the territory of SNPP “Burabai” in Zolotoborskylesnichestvo, quarter 4, Burabai district, Akmola region. Coordinates – 53°03'18.9 "N, 70°30'48.2 "E, altitude 399 m above sea level (Fig. 1). The species is a part of mixed grass sparse birch colony forests and intercolumnar steppe areas. The tree stand is formed by *Betula pendula* with insignificant inclusion of *Populus tremula*. The herbaceous layer is dominated by mesoxerophytic grasses: *Calamagrostis epigeios*, *Festuca valesiaca*, *Poa pratensis*, *Elymus repens*. Among herbs *Artemisia sericea*, *Filipendula vulgaris*, *Inula salicina*, *Fragaria vesca*, *Thalictrum simplex*, *Phlomis tuberosa*, *Rubus saxatilis*, *Lathyrus pratensis*, *Vicia cracca*, *Geranium pratense*, *Allium obliquum* frequently met. *A. wolgensis* is scattered over the territory of the population, renewal is satisfactory. The cenopopulation contains the largest number of adult generative and juvenile individuals.

5 CP was surveyed in the territory of SNPP “Burabai” in Mirnylesnichestvo, in the vicinity of Kordon Vesely, sq. 87, Burabai district, Akmola region. Coordinates – 53°03'06" N, 70°26'12" E, height 376 m above sea level (Fig. 1). The species is a part of pine-birch herbaceous forests. The tree stand is formed by *Betula*



pendula and *Pinus sylvestris*. The herbaceous tier is relatively species-poor, dominated by xeromesophytic vegetation – *Calamagrostis epigeios*, *Equisetum sylvaticum*, *Pyrola rotundifolia*, *Poa nemoralis*, *Rubus saxatilis*, *Geranium pratense*, *Melica nutans*, *Orthilia secunda*, *Phlomis tuberosa*. *Adonis* population of normal type, seed renewal is noted.

A high number of adult generative individuals were observed in the cenopopulation, the presence of juvenile and mature generative ontogenetic states indicates mature, established and stable populations.

6 CP was surveyed in the vicinity of Korgalzhin village along the Astana – Korgalzhin highway in Akmola region. Nur-Sultan – Korgalzhin village, in Akmola region. Coordinates – 50°50'03" N, 70°19'28" E, height 343 m above sea level (Fig. 1). The species is a part of grass-stipa-wormwood steppes (*Stipa sarillata*, *S. zaleskii*, *Festuca valesiaca*, *Artemisia glauca*, *A. frigida*, *A. commutata*). The community often contains xerophytes such as: *Achillea millefolium*, *Galium verum*, *Pulsatilla uralensis*, *Eryngium planum*, *Artemisia sericea*, *Potentilla virgata*, *Ranunculus repens*. The relief of the site is leveled, soils are steppe chernozems. The population is of normal type, good seed renewal is observed, the number of young individuals varies within 3-6 pcs/m<sup>2</sup>.

A high number of adult generative individuals were observed in the cenopopulation, which indicates the ecological plasticity of the cenopopulation. The presence of juvenile and mature generative ontogenetic states indicates maturity, formation and stability. In the floristic environment of *A. wolgensis* there are the following rare and protected plants of Akmola region: *Iris sibirica*, *Tulipa schrenkii*.

As a result of analyzing the age composition of the effective size of the studied cenopopulations of *A. wolgensis*, it was noted that most of them are full-members, as evidenced by the presence of juvenile individuals, with small deviations of CPs belong to normal. The exception is CP1 and CP2 located in a depression along the highway on saline soils, where we noted incomplete CPs associated with a small number of senile individuals. A large number of young vegetative individuals was noted. We managed to identify seedlings only in CP 2.

### Conclusion

Thus, the condition of the studied 6 cenopopulations of *A. wolgensis* was studied. They are in satisfactory condition, which is provided by vegetative and seed reproduction methods. The cenopopulations not or slightly affected by anthropogenic impact are characterized by the ontogenetic spectrum, in which the absolute maximum falls on the age states of the pre-generative period. In terms of vitality, they are medium thriving and are not located in a protected area. Grazing in cenopopulations leads to a decrease in plant vitality, suppression of seed reproduction.

Ontogenetically, CPs are close and mainly centered on young generative individuals, indicating that they are capable of self-renewal, are mature or transitional in mature. The evaluation of the gene pool status of the studied CPs of *A. wolgensis* is satisfactory. They are depressed with a medium degree of depression. The populations need urgent measures for their conservation and population recovery. It is necessary to prohibit the collection of raw materials, develop methods of cultivation in culture, organize reproduction of the species in commercial quantities, and strengthen protection measures in the places where the species grows.

In the long term, it is necessary to continue monitoring of price populations and careful counting of individuals of this species in order to develop and improve measures aimed at ensuring the conservation, protection and reproduction of this red-listed species.

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### Ақмола облысы бойынша *Adonis wolgensis* ценопопуляцияларын және онтогенетикалық құрылымын зерттеу

Мақалада Солтүстік Қазақстанда, оның ішінде Ақмола облысында сирек кездесетін дәрілік өсімдік *Adonis wolgensis* Steven ex DC. (*Ranunculaceae*) алты ценопопуляциясын зерттеу нәтижелері келтірілген. Өсімдікке экологиялық-фитоценоздық сипаттама берілген және ценопопуляциялардың онтогенетикалық құрылымы зерттелген. Зерттелетін аймақта *Adonis wolgensis* далалық шөптесін өсімдіктерде, бұталы жерлерде, кейде орман жиегінде және орман көгалдарында өсетіні анықталды. Жалпы зерттелген *Adonis wolgensis* ценопопуляцияларының жағдайы қанағаттанарлық. Жас спектрлерін талдау көрсеткендей, антропогендік әсерге ұшырамайтын немесе аз ұшыраған ценопопуляциялар спектрмен сипатталады, онда абсолютті максимум прегенеративті кезеңнің жас жағдайларына сәйкес келеді. Төрт ценопопуляцияда дарактардың едәуір бөлігі жас генеративті күйде болады, бұл олардың өзін-өзі



қалпына келтіру қабілеттілігін көрсетеді. Бұл зерттелетін аумақтардың өсімдіктер қауымдастығында түрдің тұрақтылығын қамтамасыз етеді. Екі ценопопуляцияның жағдайы деградациялық деп бағаланған, оларды сақтау және олардың санын қалпына келтіру үшін шұғыл шаралар қабылдау қажет. Ценопопуляцияларда мал жаю өсімдік тіршілігінің төмендеуіне және тұқымның көбеюінің тежелуіне әкеледі. Түрді сақтау үшін мал жаюды шектеу, шикізат жинауға тыйым салу, өсімдіні өсіру әдістерін әзірлеу, түрдің өнеркәсіптік көлемде көбеюін ұйымдастыру, қорғау шараларын күшейту қажет. Алынған нәтижелер Солтүстік Қазақстандағы түрдің жай-күйі туралы жаңа мәліметтер алуға мүмкіндік береді және оны одан әрі сақтап, іске жаратуға болады.

*Кілт сөздер:* *Adonis wolgensis*, ценопопуляция, онтогенетикалық құрылым, сирек, Ақмола облысы.

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## Изучение ценопопуляций и онтогенетической структуры *Adonis wolgensis* в Акмолинской области

В статье представлены результаты изучения шести ценопопуляций редкого лекарственного растения *Adonis wolgensis* Steven ex. DC. (Ranunculaceae) в Акмолинской области Северного Казахстана. Дана эколого-фитоценотическая характеристика, и изучена онтогенетическая структура ценопопуляций. Установлено, что в исследуемом регионе *Adonis wolgensis* произрастает среди степного разнотравья, в зарослях кустарников, изредка по опушкам лесов и на лесных лужайках. В целом, состояние изученных ценопопуляций *Adonis wolgensis* удовлетворительное. Анализ возрастных спектров показал, что ценопопуляции, не подвергающиеся или слабо подвергающиеся антропогенному воздействию, характеризуются спектром, в котором абсолютный максимум приходится на возрастные состояния прегенеративного периода. В четырех ценопопуляциях значительная часть особей находится в молодом генеративном состоянии, что указывает на их способность к самовозобновлению. Это обеспечивает стабильность вида в растительных сообществах исследуемых территорий. Состояние двух ценопопуляций оценивается как деградирующее, они нуждаются в принятии срочных мер по их сохранению и восстановлению численности. Выпас скота в ценопопуляциях приводит к снижению жизнеспособности растений и подавлению семенного размножения. Для сохранения вида необходимо ограничить выпас скота, запретить сбор сырья, разработать методы выращивания в культуре, организовать воспроизводство вида в промышленных объемах, усилить меры охраны. Полученные результаты позволили получить новые сведения о состоянии вида в Северном Казахстане и служат надежной основой для его дальнейшего сохранения.

*Ключевые слова:* *Adonis*, флора Казахстана, редкий вид, сохранение биоразнообразия, Северный Казахстан, состояние ценопопуляций.

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### Фармакогностическое исследование сырья *Nepeta pannonica*

Фармакогностический анализ лекарственного растительного сырья является важным этапом введения вида в официальное использование. Объектом исследования являлись надземные органы *Nepeta pannonica* (семейство *Lamiaceae*), для которого выполнен макро- и микроскопический анализ. Определены следующие диагностические признаки на макрокопическом уровне: лист — форма листовой пластинки, тип жилкования; стебель — тип ветвления побега, окраска, супротивное расположение листьев, слабое опушение; соцветие — расположение цветков; чашечка цветка — форма зубцов, тип опушения, мелкая ребристость; венчик цветка — форма и размеры, окраска. В качестве микроскопических признаков сырья определены форма клеток эпидермиса верхней и нижней сторон, участков над жилками; диатитные устьица, наличие мелких многоклеточных трихом и крупных эфирномасличных железок в строении листа, стебля, чашечки и венчика цветка; разделение мезофилла на столбчатую и губчатую ткани на поперечном срезе листа.

*Ключевые слова:* *Nepeta pannonica*, фармакогнозия, растительное сырье, макро- и микроскопический анализ, диагностические признаки.

#### Введение

Род котовник (*Nepeta* L.) относится к семейству *Lamiaceae* Lindl. (*Labiatae*) [1], растения которого представляют интерес в качестве источников лекарственных препаратов.

Род *Nepeta* L. насчитывает более 250 видов, из которых в Казахстане произрастает 16 наименований. Представители данного рода обладают антиоксидантными, противосудорожными, тонизирующими, жаропонижающими, противокашлевыми, седативными, противоастматическими, спазмолитическими и противовоспалительными свойствами [2, 3]. Фармакологическая активность объясняется содержанием в составе разных групп биологических соединений, как стероиды, органические кислоты, эфирное масло, дубильные вещества, флавоноиды, витамины и иридоиды [4–8]. Также в состав различных экстрактов некоторых видов котовника входят фенолпропаноиды. Например, хлорогеновая, розмариновая и хинная кислоты оказались наиболее распространенными соединениями. Кроме того, были идентифицированы кемпферол, *n*-кумаровая кислота, апигенин, лютеолин и рамнетин [9], эфирное масло [10].

Котовник венгерский (*Nepeta pannonica* L., синоним *N. nuda* L.) — многолетнее травянистое растение высотой 50–120 см. Растение достаточно распространено на территории Евразии, в том числе широко произрастает и в Центральном Казахстане.

Котовник венгерский применяется в народной медицине [10], однако имеет перспективы включения в Государственную фармакопею Республики Казахстан, что требует проведения фармакогностического анализа.

Цель нашего исследования — провести анализ макро- и микроскопических показателей котовника венгерского и выявить диагностические признаки растения.

*Материалы и методы*

Объектом исследования являлись надземные части (листья, соцветия и побеги) *N. pannonica*. Данный вид собран в фазу цветения, место сбора — Спасские сопки (Карагандинская область), дата — 2-ая декада июня 2021 г. (рис. 1).



Рисунок 1. *Nepeta pannonica* в фазе цветения (Спасские сопки)

При анализе морфологических показателей исследовали особенности роста, внешнего вида, структуры поверхности, цвета побегов, листьев, соцветий и цветков [11]. Образцы сырья рассматривали с помощью Digital Microscope Levenhuk DTX 30.

При выполнении анатомического исследования сухие образцы надземных органов размачивали в горячей воде и размягчали в смеси глицерин–спирт–вода дистиллированная в соотношении 1:1:1 (реактив Штрауса–Флеминга) [12, 13]. Изготавливали поверхностные препараты и срезы вручную. Микрофотографии выполняли на сканирующем микроскопе BioMed в программе Altami Studio, при различном увеличении. Обработку фотографий выполняли в программе Paint 10.1.

При описании морфологии и анатомического строения использовали принципы, изложенные в трудах В.Н. Вехова, Л.И. Лотовой и нормативах Фармакопеи Республики Казахстан [14–16].






*Результаты и их обсуждение*

*Морфологический анализ.* Для определения особенности строения *Nepeta pannonica* нами проанализированы показатели надземных органов и составлено соответствующее морфологическое описание (табл. 1).

Т а б л и ц а 1

**Морфологические показатели надземных органов *Nepeta pannonica***

Показатели	Описание
Побеги	Прямостоящие, в верхней ветвистые и хорошо олиственные, в нижней части — голые. Цвет светло-зеленый в нижней и средней части побегов. В верхней части с сиреневыми или фиолетовыми ребрами. Листья на стебле расположены супротивно 
Опушение побегов	Побеги голые на всем протяжении
Листья	Форма узкоэллиптическая, верхушка заостренная, основание клиновидное, край — мелкозубчатый, жилкование сетчатое, жилки хорошо просматриваются с нижней стороны

Продолжение Таблицы 1	
Структура верхней стороны листа	Верхняя сторона листа шероховатая, с неясными жилками, верхние листья зеленые, нижние — фиолетово-зеленые 
Структура нижней стороны листа	Нижняя сторона светло-зеленая, жилки хорошо выражены, тип жилкования — сетчатый. По всей поверхности равномерно разбросаны многочисленные точечные железки 
Форма соцветия	Соцветия верхушечные, узкометельчатые, цветки по 3–6 собраны в мутовки, длина от 15 до 40 (50) см. На соцветии размещены мелкие шиловидные прицветные листья 
Чашечка	Чашечка коротко опушенная, 4–6 мм длиной, радиально-симметричная, зубцы короткие, шиловидные. Поверхность — мелко-ребристая. Цвет чашечки снаружи — зеленый; ребра и зубцы чашечки — фиолетово-окрашенные. Зубцы чашечки почти голые, основание и средняя часть — густо-опушенные белыми волосками, преимущественно вдоль жилок 
Венчик	Венчик вдвое длиннее чашечки, 8–9 мм длиной, бледно-фиолетовый или розоватый 

Таким образом, диагностическими признаками на макроскопическом уровне определены следующие: форма и окраска стебля, строение листа (верхняя и нижняя стороны, тип жилкования), форма соцветия, строение и цвет чашечки и венчика цветка.

*Анатомический анализ.* Клетки верхнего и нижнего эпидермиса листа *N. rannonica* округлой или овальной формы с сильно-извилистыми стенками (рис. 2); над жилками листа — прямоугольные и удлиненные (рис. 3). Устьица мелкие, диацитного типа, встречаются с обеих сторон листовой пластины, но преимущественно с нижней стороны. По всей поверхности разбросаны многочисленные

крупные эфирно-масличные железки, округлой формы, лежат на уровне эпидермы листа. Трихомы редкие, одноклеточные, наблюдаются по краю листовых пластины.



1 — устьица; 2 — основные клетки эпидермиса  
А



1 — эфирно-масличные железки  
Б



1 — трихомы  
В

Рисунок 2. Верхний эпидермис листа *Nepeta pannonica*. Ув. 16x40: А — фрагмент с основными клетками эпидермиса и устьицы; Б — фрагмент с эфирно-масличными железками; В — фрагмент края листа с простыми трихомами

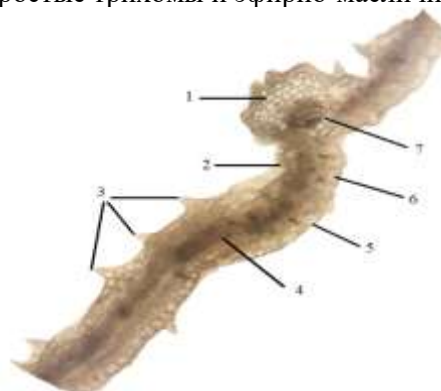


1 — эфирно-масличные железки; 2 — устьица; 3 — основные клетки эпидермиса, 4 — трихомы

Рисунок 3. Нижний эпидермис листа *Nepeta pannonica*. Ув. 16x40

На поперечном срезе лист *N. pannonica* плоский, дорзо-вентрального типа (рис. 4), с ясно выраженным делением мезофилла на палисадную и губчатую ткани. С обеих сторон лист окружен 1-слойным эпидермисом, клетки которого овальной формы с утолщенными наружными стенками. Столбчатый мезофилл состоит из 1–2 слоев клеток.

Проводящий пучок коллатерального типа, закрытый, состоит из тяжа ксилемы и тяжа флоэмы. Над главной жилкой расположен участок колленхимы (ориентирован на нижнюю сторону листа). Хорошо просматриваются редкие простые трихомы и эфирно-масличные железки.



1 — колленхима; 2 — нижний эпидермис; 3 — трихомы; 4 — губчатый мезофилл; 5 — верхний эпидермис; 6 — столбчатый мезофилл; 7 — центральный проводящий пучок

Рисунок 4. Поперечный срез листа *Nepeta pannonica*. Ув. 16x10



Стебель *N. pannonica* на поперечном срезе прямоугольно-лопастный (рис. 5), пучкового типа. По периметру стебля расположена 1-слойная эпидерма с утолщенными оболочками клеток. Под эпидермой залегают участки хлоренхимы, прерывающиеся над ребрами крупными участками уголкового колленхимой. Между проводящей зоны и хлоренхимой залегают коровая паренхима. Проводящая зона ограничена однослойной эндодермой. Пучки коллатеральные, закрытого типа. Наблюдаются пучки 2-х типов: в углах – крупные широкояйцевидные пучки, между углами – мелкие и округлые. Хорошо выражены цепочки ксилемы. В центре стебель заполнен рыхлыми клетками сердцевинной паренхимы.



А  
Б  
1 — сердцевинная паренхима; 2 — ксилема; 3,4 — флоэма; 5 — эндодерма; 6 — хлоренхима; 7 — уголковая колленхима; 8 — эпидермис

Рисунок 5. Поперечный срез стебля *Nepeta pannonica*. Ув. 16x10: А — общий поперечный срез; Б — фрагмент поперечного среза

На поверхностном препарате чашечки цветка *N. pannonica* основные клетки прямоугольной (рис. 6) вытянутой формы со слабоизвилистыми стенками. Хорошо просматриваются простые и многоклеточные трихомы и крупные эфирно-масличные железки, округлой формы.



1 — основные клетки эпидермы; 2 — трихомы; 3 — эфирномасличные железки



1 — трихомы; 2 — эфирномасличные железки

Рисунок 6. Поверхностный эпидермис чашечки цветка *Nepeta pannonica*. Ув. 16x40

Рисунок 7. Поверхностный эпидермис венчика цветка *Nepeta pannonica*. Ув. 16x40

На поверхности препарата венчика цветка *N. pannonica* основные клетки прямоугольной формы (рис. 7) с тонкими стенками. Хорошо просматриваются редкие простые трихомы и округлые эфирно-масличные железки.

Микроскопические признаки сырья котовника были обобщены в таблице 2.

Микроскопические показатели надземных органов *Nepeta pannonica*

Показатели	Описание
Верхний эпидермис листа	Округлой или овальной формы с сильно-извилистыми стенками
Нижний эпидермис листа	Округлой или овальной формы с сильно-извилистыми стенками; над жилками листа — прямоугольные и удлиненные
Тип устьиц, локализация	Мелкие, диацитного типа
Эфирно-масличные железки эпидермиса листа	По всей поверхности разбросаны многочисленные крупные эфирно-масличные железки, округлой формы, лежат на уровне эпидермы листа
Трихомы	Трихомы редкие, одноклеточные, наблюдаются по краю листовой пластины
Тип листа на поперечном срезе	Лист плоский, дорзо-вентрального типа, но с ясно-выраженным делением мезофилла на палисадную и губчатую ткани
Чашечка цветка	Клетки эпидермы вытянутые, со слабо-извилистыми стенками, по поверхности разбросаны редкие, мелкие многоклеточные трихомы и крупные эфирно-масличные железки
Венчик цветка	Клетки прямоугольные, с тонкими стенками, по поверхности отмечены редкие простые одноклеточные трихомы и округлые эфирно-масличные железки. Многоклеточные волоски размещены по краю венчика
Форма стебля на поперечном срезе	Прямоугольно-лопастной, пучкового типа
Тип проводящей системы	Коллатерального типа, закрытый, пучки двух типов — крупные, мелкие

Анализ полученных данных позволяет определить следующие микроскопические диагностические признаки: форма и строение клеток эпидермиса листа и чашелистника, степень и выраженность опушения, форма и расположение эфирно-масличных железок, строение листа и стебля на поперечном срезе.

## Заключение

По итогам исследований определены макро- и микроскопические признаки сырья *N. pannonica*:

*Для листа:* макроскопические признаки: форма листовой пластинки, верхушки, основания и края, более выраженные жилки с нижней стороны листа. Микроскопические признаки: форма клеток эпидермиса, извилистость стенок, устьица диацитного типа, крупные эфирно-масличные железки и редкие трихомы.

*Для стебля:* форма роста стебля, ветвление преимущественно в верхней части, цвет, супротивное расположение листьев. Микроскопические признаки: форма стебля на поперечном срезе, строение пучков 2-х типов.

*Для соцветия:* Макроскопические признаки: тип соцветия, особенности расположения цветков и прицветных листьев в соцветии.

*Для чашечки цветка:* макроскопические признаки: форма чашечки и зубцов чашечки, тип опушения — преимущественно по ребрам, окраска, наличие мелких ребрышек на поверхности. Цвет чашечки снаружи — зеленый; ребра и зубцы чашечки — фиолетово-окрашенные. Микроскопические признаки: клетки эпидермы вытянутые, со слабо-извилистыми стенками, по поверхности форма клеток эпидермиса, наличие мелких многоклеточных трихом и крупных эфирно-масличных железок.

*Для венчика цветка:* макроскопические признаки: форма и размеры венчика, цвет — бледно-фиолетовый или розоватый. Микроскопические признаки: форма клеток эпидермиса, наличие простых одноклеточных трихом на поверхности и многоклеточных — по краю, наличие крупных эфирно-масличных железок.

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### *Nepeta pannonica* шикізатын фармакогностикалық зерттеу

Дәрілік өсімдік шикізатын фармакогностикалық талдау түрді ресми колдануға енгізудің маңызды кезеңі. Зерттеу нысаны макро- және микроскопиялық талдау жүргізілген *Nepeta pannonica* (Lamiaceae тұқымдасы) жерүсті мүшелері. Макроскопиялық деңгейде келесі диагностикалық белгілер анықталды: жапырақ — жапырақ алақанының пішіні, жүйкелену түрі; сабақ — өркеннің тармақталу түрі, түсі, жапырақтардың қарама-қарсы орналасуы, әлсіз түктілік; гүлшоғыры — гүлдердің орналасуы; гүл тостағаншасы — тістерінің пішіні, жасуша түрі, кішкентай қырлы; гүл кестесі — пішіні мен мөлшері, түсі. Шикізаттың микроскопиялық белгілері ретінде жоғарғы және төменгі жақтардың эпидермис жасушаларының пішіні, талшық аймақтары анықталды; диацитті саңылаулар, жапырақтың, сабақтың, тостағаншаның және гүлшоғырының құрылымында ұсақ көп жасушалы трихомалар мен ірі эфир майы бездерінің болуы; жапырақтың көлденең қимасында мезофилдің бағаналы және кеуек тәрізді тіндерге бөлінуі.

*Кілт сөздер:* *Nepeta pannonica*, фармакогнозия, өсімдік шикізаты, макро-және микроскопиялық талдау, диагностикалық белгілер.

G.K. Kurmantayeva, M.Yu. Ishmuratova

**Pharmacognostic analysis of plant material of *Nepeta pannonica***

Pharmacognostic analysis of medicinal plant raw materials is an important stage of introducing the species into official use. The object of the study was the above-ground organs of *Nepeta pannonica* (family *Lamiaceae*), for which macro- and microscopic analysis was performed. The following diagnostic characters were determined at the macroscopic level: leaf — shape of leaf lamina, type of veining; stem — type of shoot branching, coloration, sub-rotative arrangement of leaves, weak pubescence; inflorescence — arrangement of flowers; flower calyx — shape of teeth, type of pubescence, fine ribbing; flower corolla — shape and size, coloration. The shape of epidermis cells of the upper and lower sides, areas above the veins; diacytic stomata, presence of small multicellular trichomes and large essential oil glands in the structure of leaf, stem, calyx and flower corolla; division of mesophyll into columnar and spongy tissues on the transverse section of the leaf were determined as microscopic features of the raw material.

**Keywords:** *Nepeta pannonica*, pharmacognosy, plant raw material, macro- and microscopic analysis, diagnostic signs.

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## Розмариновая кислота из *Salvia stepposa* и ее антирадикальная активность

Растения рода Шалфей (*Salvia* L., семейство *Lamiaceae*) включают 700 видов, которые широко применяются в народной медицине многих стран как лекарственное сырье. В настоящее время в Государственную фармакопею Республики Казахстан включен только Шалфей лекарственный (*Salvia officinalis* L.), однако в естественных условиях Центрального Казахстана этот вид не произрастает. В качестве замещающего растения нами предложен Шалфей степной (*Salvia stepposa* Des.-Shost.), широко распространенный на территории Республики Казахстан, имеющий значительные сырьевые ресурсы и обладающий сравнительно выраженными антимикробными, противовоспалительными, антиоксидантными свойствами за счет мажорного компонента в своем составе фенилпропаноида розмариновой кислоты. Экстракция сырья впервые проведена различными методами с использованием ультразвуковой кавитации, микроволновой экстракции и мацерации с использованием водно-этанольных растворов. Методом ВЭЖХ проведено определение содержания розмариновой кислоты в сухих экстрактах шалфея степного. Максимальный выход экстрактов отмечен при микроволновой экстракции 40 %-ным этиловым спиртом. Для последующей стандартизации лекарственных средств на основе шалфея степного нами был выделен и идентифицирован основной компонент — розмариновая кислота, которая обладает антирадикальной активностью.

**Ключевые слова:** шалфей, фенилпропаноид, розмариновая кислота, ВЭЖХ, антирадикальная активность.

### Введение

Фенилпропаноиды — природные ароматические соединения фенольного характера, содержащиеся в структуре один или несколько С6–С3 фрагментов. Фенилпропаноиды выделены в отдельный класс природных химических соединений сравнительно недавно. Учитывая широкое распространение фенилпропаноидных соединений в растениях, их необходимо изучить как ведущие группы соединений с позиции получения лекарственных средств. Розмариновая кислота представляет собой фенилпропаноид, распространенный во многих видах высших растений.

Несмотря на разнообразие современных принципов создания лекарственных средств, поиск перспективных соединений среди веществ растительного происхождения продолжает оставаться одним из основополагающих при разработке высокоэффективных и безопасных лечебно-профилактических препаратов. В настоящее время фенилпропаноидные соединения растений рассматриваются как новый класс низкомолекулярных веществ.

Фенилпропаноиды являются перспективным источником адаптогенных, тонизирующих, иммуномодулирующих, гепатопротекторных и антиоксидантных лекарственных средств. Фенилпропаноиды введены проф. В.А. Куркиным в фармакогнозию как самостоятельный класс биологически активных соединений с точки зрения физико-химических, химических свойств, биосинтеза, спектра фармакологической активности в 1992 году, что нашло отражение в химической классификации лекарственных растений, а также в учебнике «Фармакогнозия» [1–4]. Также В.А. Куркин — создатель 20 новых лекарственных средств на основе фенилпропаноидного сырья эхинацеи пурпурной, родиолы розовой, расторопши пятнистой, Melissa лекарственной, сирени обыкновенной и др. [5–13].

Фенилпропаноид розмариновая кислота представляет собой сложный эфир кофейной кислоты и 3,4-дигидроксифенилмолочной кислоты, которая обычно продуцируется некоторыми ароматическими и лекарственными растениями, принадлежащими к разным семействам, например, *Lamiaceae*, *Boraginaceae*, *Ariaceae* [14]. Соединение обладает множественными биологическими эффектами, среди которых антиоксидантный, мембранотропный, радиозащитный и противовоспалительный [15]. Ранее скрининг растений на содержание фенилпропаноидных соединений и их выделение в Казахстане не проводились.

Шалфеей степной является новым источником для выделения розмариновой кислоты, которая представляет интерес для фармации и медицины как вещество со сравнительно высокой противовоспалительной, антиоксидантной, противовирусной, антигерпетической, антиаллергической, противоопухолевой активностями при низкой токсичности.

#### *Материалы и методы*

Дикорастущее растение флоры Казахстана *Salvia stepposa* Des.-Schost (Шалфеей степной) собрано в экспедиционных выездах по Карагандинской области, Республика Казахстан, координаты сбора (N 49,88898; E 73,15569) в фазе бутонизации и цветения в июле–августе 2023 года.

Тонкослойная хроматография была выполнена в соответствии с ГФ РК, т. 1, 2.2.27. С использованием пластин «Сорбфил ПТСХ-АФ-А-УФ», длина волны УФ 366 нм, система растворителей хлороформ–метанол–вода, 26:14:3.

Анализ полифенольных соединений экстрактов проводили с использованием высокоэффективной жидкостной хроматографии (ВЭЖХ) в сочетании с ультрафиолетовым детектором (УФ) и тандемной масс-спектрометрией в реальном времени (ESI-MS/MS).

Анализ выполняли на жидкостном хроматографе «Agilent 1260 Infinity HPLC system» (Agilent Technologies, США), оборудованном четырехканальным насосом G1311C 1260 Pump VL, автосамплером G1329B 1260 ALS, термостатом колонки G1316A 1260 TCC; детектором с переменной длиной волны G1314C 1260 VWD VL + и масс-спектрометром G6130A Quadrupole LC-MS/MS. Использовалось программное обеспечение ChemStation с управлением Windows NT.

Спектры ЯМР записывали на спектрометрах «Bruker DRX-600» (рабочая частота — 600.30 МГц для  $^1\text{H}$ ).

Определение антирадикальной активности (далее — АРА) розмариновой кислоты проводили методом ингибирования реакции 2,2-дифенил-1-пикрилгидразилрадикала (DPPH). 0,1 мл спиртового раствора розмариновой кислоты в диапазоне концентраций 0,25; 0,5; 0,75 и 1,0 мг/мл добавляли к 3 мл  $6 \times 10^{-5}\text{M}$  раствора радикала. Одним из условий успешного проведения эксперимента является проведение реакции в темноте, в связи с выраженной АРА розмариновой кислоты. Для пролонгирования времени протекания реакции пробирки для центрифугирования помещаются в штатив, который предварительно заворачивается в черный полиэтилен для создания темноты реакционной смеси. После перемешивания растворы оставляли в темноте на 30 мин, далее измеряли оптическую плотность растворов при 520 нм. Значения АРА (%) определяли по формуле (1):

$$APA = A_0 - A_t / A_0 \times 100 (\%), \quad (1)$$

где  $A_0$  — значение оптической плотности контрольной пробы;  $A_t$  — величина оптической плотности при определенной концентрации исследуемого раствора. В качестве стандарта использовали бутилгидроксианизол (ВНА).

#### *Результаты и их обсуждение*

Ранее нами установлено, что водно-этанольные экстракты шалфея степного обладают различным спектром биологической активности и на их основе разработаны таблетки для рассасывания [16]. Экстракция сырья впервые проведена различными методами с использованием ультразвуковой кавитации, микроволновой экстракции и мацерации с использованием водно-этанольных растворов.

Методом ВЭЖХ проведено определение розмариновой кислоты в сухих экстрактах шалфея степного, полученных различными методами, и путем сравнения времени ее удерживания со стандартом розмариновой кислоты. Максимальный выход экстрактов отмечен при микроволновой экстракции 40%-ным этиловым спиртом. Результаты приведены в таблице 1.

Для максимального выделения фенилпропаноида розмариновой кислоты необходимо проводить мацерацию надземной части шалфея степного 40%-ным этиловым спиртом. Анализ ВЭЖХ данных экстрактов показал, что экстракт шалфея степного, полученный мацерацией, имеет большее содержание розмариновой кислоты среди представленных экстрактов и составляет 6,14 % в пересчете на массу экстракта.

Далее проводили выделение розмариновой кислоты аналогично способу ее получения из травы шалфея мутовчатого [17], но в данном патенте сырье предварительно экстрагировалось 70 %-ным этиловым спиртом. Нами получен готовый продукт с выходом 0,5 % от массы воздушно-сухого сы-

рья. Выделенную кристаллическую розмариновую кислоту далее идентифицируют аналитическим анализом ТСХ в системе растворителей хлороформ–метанол–вода, 26:14:3. Розмариновая кислота проявляется на хроматограмме в УФ-свете при длине волны в 366 нм в виде одного доминирующего ярко-голубого флуоресцирующего пятна, величина  $R_f$  составляет 0,5. Полученная розмариновая кислота имеет физико-химические и спектральные характеристики, соответствующие индивидуальному соединению.

Т а б л и ц а 1

**Содержание розмариновой кислоты в сухих экстрактах шалфея степного, полученных различными методами**

№ п/п	Время удерживания розмариновой кислоты	Наименование экстракта	Количественное содержание, %
1	17.070	Экстракт шалфея степного, полученного мацерацией 40%-ным этиловым спиртом	6,14±0,230
2	17.070	Экстракт шалфея степного, полученного микроволновой экстракцией 40%-ным этиловым спиртом	6,092±0,176
3	17.070	Экстракт шалфея степного, полученного ультразвуковой кавитацией 40%-ным этиловым спиртом	5,53±0,089

Выделенная розмариновая кислота представляет светло-желтое аморфное вещество состава  $C_{18}H_{16}O_8$ .  $^1H$ NMR (DMSO-d<sub>6</sub>, 600 МГц, J, Гц): 7.02 (д, 2.0 Гц, Н-2), 6.73 (д, 8.1 Гц, Н-5), 6.92 (дд, 8.2 Гц, 2.1 Гц, Н-6), 7.35 (д, 15.9 Гц, Н-7), 6.16 (д, 15.9 Гц, Н-8), 6.65 (д, 2.1 Гц, Н-2'), 6.58 (д, 8.0 Гц, Н-5'), 6.47 (дд, 8.0 Гц, 2.0 Гц, Н-6'), 2.73 (дд, 14.4 Гц, 10.1 Гц, Н-7'), 2.99 (дд, 14.4 Гц, 3.1 Гц, Н-7'), 4.81 (дд, 10.0 Гц, 3.1 Гц, Н-8').

Проведена оценка АРА выделенной розмариновой кислоты. Исследование способности розмариновой кислоты улавливать радикалы было количественно оценено путем определения реакционной способности радикала DPPH с розмариновой кислотой при 520 нм. Данные по проценту ингибирования свободных радикалов DPPH представлены в таблице 2 и на рисунке.

Т а б л и ц а 2

**Процент ингибирования свободных радикалов DPPH розмариновой кислотой и ВНА**

Концентрация раствора, мг/мл	Ингибирование розмариновой кислотой, %	Ингибирование стандарта ВНА, %
0,25	32,76	80,7
0,5	68,96	80,3
0,75	88,01	80,5
1	89,45	80,7

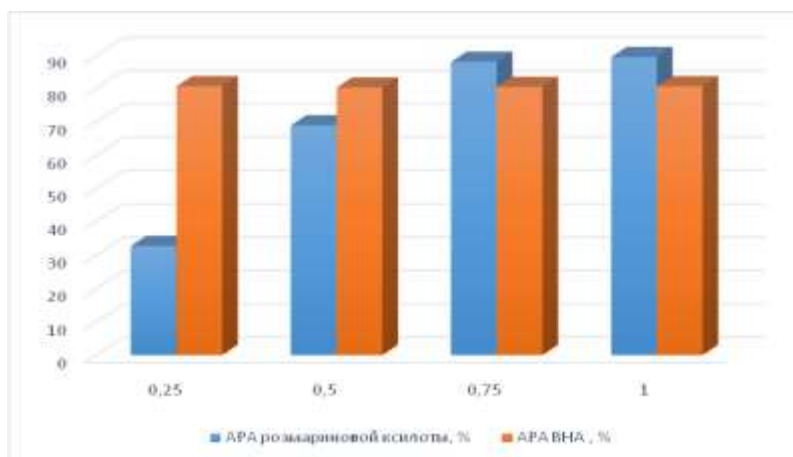


Рисунок. Антирадикальная активность розмариновой кислоты и стандарта ВНА

Ингибирование DPPH розмариновой кислотой колебалось от 32,76% до 89,45% при концентрациях от 0,25 мг/мл до 1 мг/мл. Высокой АРА обладает розмариновая кислота при концентрациях 0,75 мг/мл и 1 мг/мл, процент удаления свободных радикалов составляет 88,01 % и 89,45 % соответственно. АРА розмариновой кислоты значительно превосходит стандартный образец ВНА в тех же концентрациях, что свидетельствует о высокой антирадикальной способности.

#### Заключение

Розмариновая кислота представляет собой природное фенольное соединение, обладающее многими биологическими свойствами, такими как ингибирование ВИЧ–1, противоопухолевым и противовоспалительным действием. В данной статье предложено выделение розмариновой кислоты из *Salvia stepposa*, проведена оценка ее антирадикальной активности в отношении DPPH радикала. Исходя из литературных данных, ранее выделение розмариновой кислоты из шалфея степного, произрастающего на территории Республики Казахстан, не проводилось.

Установлено, что выделенная розмариновая кислота имеет физико-химические и спектральные характеристики, схожие с индивидуальным веществом, а также обладает высокой антирадикальной активностью в концентрациях 0,75 мг/мл и 1 мг/мл.

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### ***Salvia stepposa* розмарин қышқылы және оның антирадикалды белсенділігі**

Шалфей тұқымдасы өсімдіктерінің (*Salvia* L., *Lamiaceae* тұқымдасы) 700 түрі бар, олар көптеген елдердің халық медицинасында дәрілік шикізат ретінде кеңінен қолданылады. Қазіргі уақытта Қазақстан Республикасының Мемлекеттік фармакопеясына тек емдік шалфей (*Salvia officinalis* L.) енгізілген, бірақ бұл түр Орталық Қазақстанның табиғи жағдайында өспейді. Біз алмастыратын өсімдік ретінде Қазақстан Республикасының аумағында кеңінен таралған, айтарлықтай шикізат ресурстары бар және құрамында фенилпропаноидты розмарин қышқылының негізгі компоненті есебінен салыстырмалы түрде айқын микробқақарсы, қабынуғақарсы, антиоксиданттық қасиеттері бар дала шалфейін (*Salvia stepposa* Des.-Shost.) ұсындық. Шикізатты экстракциялау алғаш рет ультрадыбыстық кавитацияны, микротолқынды экстракцияны және сулы-этанол ерітінділерін пайдаланып, мацерацияны қолдану арқылы әртүрлі әдістермен жүргізілді. ЖӨСХ әдісімен дала шалфейінің құрғақ экстракциядағы розмарин қышқылының құрамы анықталды. Экстракттердің максималды шығымы микротолқынды экстракцияда 40% этил спиртінде байқалады. Дала шалфейінің негізіндегі дәрілік заттарды кейіннен стандарттау үшін біз антирадикалды белсенділікке ие негізгі компонент — розмарин қышқылын бөліп, анықтадық.

*Кілт сөздер:* шалфей, фенилпропаноид, розмарин қышқылы, ЖӨСХ, антирадикалды белсенділік.

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### **Rosmarinic acid from *Salvia stepposa* and its antiradical activity**

Plants of the genus Sage (*Salvia* L., family *Lamiaceae*) include 700 species, which are widely used in folk medicine in many countries as medicinal raw materials. Currently, only medicinal sage (*Salvia officinalis* L.) is included in the State Pharmacopoeia of the Republic of Kazakhstan, but this species does not grow in the natural conditions of Central Kazakhstan. As a replacement plant, we have proposed steppe sage (*Salvia stepposa* Des.-Shost.), which is widespread in the Republic of Kazakhstan, has significant raw material resources and has relatively pronounced antimicrobial, anti-inflammatory, antioxidant properties due to the major component in its composition, the phenylpropanoid rosmarinic acid. Extraction of raw materials was carried out for the first time using various methods using ultrasonic cavitation, microwave extraction and maceration using water-ethanol solutions. The HPLC method was used to determine the content of rosmarinic acid in dry extracts of steppe sage. The maximum yield of extracts was observed during microwave extraction with 40% ethyl alcohol. For the subsequent standardization of medicines based on steppe sage, we isolated and identified the main component — rosmarinic acid, which has antiradical activity.

*Keywords:* sage, phenylpropanoid, rosmarinic acid, HPLC, antiradical activity.

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## Overview on remogliflozin SGLT-2 inhibitor in the management of type-2 diabetic mellitus in human beings

Sodium-glucose transport protein 2 inhibitors have become a significant category of oral medications in order to manage type 2 diabetic mellitus, particularly in people with heart disease or kidney disease, and have been highly advised overall current studies advice on how to treat. They can reduce blood pressure and help people lose weight, but they also come with drawbacks like genitourinary infections. The newly approved remogliflozin combinations are the product study of the pharmaceutical business for a new pharmaceutical class and novel drug combination with a view to managing diabetes. Remogliflozin etabonate is the term for the remogliflozin ester form. This study examines the clinical effectiveness, safety profile, pharmacokinetics, and pharmacodynamics of remogliflozin etabonate. However, its analytical profiling using RP-HPLC, UV, RP-UPLC, HPTLC, and LC/MS. this review addresses the individual experiments conducted during the development of analytical method for remogliflozin etabonate.

*Keywords:* Remogliflozin Etabonate, SGLT-2 Inhibitor, Type-2 Diabetic Mellitus, Analytical Method.

### Introduction

Type 2 diabetes is a complicated metabolic condition that frequently needs several Therapy approaches to be effectively treated. The 2 sodium-dependent glucose transporteris inhibited (SGLT-2) gives a unique therapeutic strategy for type 2 diabetes using enhanced renal glucose excretion, it brings down blood sugar and results in losing weight [1]. Numerous specific sglT2 inhibitors are being developed or are already in use [2]. Selective SGLT2 inhibitors are being created or are already in use in numerous applications: [3].

The goal of this trial was to assess the effectiveness, security, and tolerability of daily one remogliflozin (RE) given to type 2 diabetic patients for a period of 12 weeks as a monotherapy, oral drug class of the new generation (t2dm). They work by increasing blood sugar levels are lowered by urinating glucose out. They benefit patient in the presence of insulin resistance or decreased pancreatic function due to their distinct insulin-independent mode of action.

They inhibitors of SGLT-2 are a common type 2 diabetes mellitus are known to enhance renal function, lower the especially beneficial in patients are SGLT-2 with hypertension and high risk of hypoglycaemia [4-6].

Worldwide, kind 2 diabetic mellitus incidence and prevalence are growing, along with 90% of adults having diabetes mellitus and one in eleven having t2dm. China and India are the diabetes epidemic's epicentres in Asia [7]. Today, 8.9% of India's population, or 77 million people, have diabetes [8]. Guidelines for t2dm management are offered in accordance with the American organization of clinical endocrinologists. They incorporate lifestyle counselling, weight loss with medical assistance, and personalized targets for reaching a haemoglobin a1c (hba1c) level of 6.5%.selection of antidiabetic agents is based on the traits of the patient, including lifestyle, co-morbidities, weight, glycemic index, and unfavourable side effects of pharmacotherapy. Digestive problems, hepato-renal damage, hyperinsulinemia-related weight gain, and hypoglycemia are the adverse consequences of oral medicines that treat diabetes that are frequently reported [9, 10]. A safer anti-diabetic drug is necessary given the rise in adverse effects, long-term negative effects, possibility for weight gain with this medication, and hypoglycemia are the critical effects to be consider [11].

Review

**History and Development of Remogliflozin Regimen**

Japan's Kissei pharmaceutical company made the initial discovery of remogliflozin; and was later created by BHV pharma, a joint venture between Glaxosmithkline and Glenmark [12]. Remogliflozin has been tested on 2500 people from different ethnic backgrounds in 26 registered trials worldwide [13]. A phase 3 study and two pharmacokinetic (PK) studies, and a clinical trial for this medication were all carried out in India (CTRI/2017/06/0088887, 2017/07/009121, and 2017/10/010043) [14-16]. Glenmark pharmaceuticals gained regulatory authorization for 100 mg pills to administered twice daily for t2dm in April 2019 following the conclusion of In a phase-3 clinical investigation, remogliflozin etabonate showed its Comparing efficacy and safety to dapagliflozin [17, 18]. Glenmark then introduced remogliflozin on the market in India, and under a sub-licensing deal, an Indian company named torrent pharmaceuticals took over marketing [19, 20]. After Glenmark released remogliflozin onto the Indian market, torrent pharmaceuticals, an Indian business, took over marketing as part of a sub-licensing agreement [21]. Remogliflozin and remogliflozin-metformin combination are safe and effective for treating type 2 diabetes in a real-world context, according with regard to a potential active post-marketing surveillance research called the reform India trials, which was started in November 2019 [22, 23]. A Phase II study of remogliflozin for non-alcoholic steato-hepatitis etabonate in rash In the US, a patient (rein) research is now in progress in addition to these Indian investigations. A Phase-I study of the obesity drug remogliflozin and a Phase-I experiment for people with type 1 diabetes were both stopped in us in May 2019 for unknown reasons [24].

**Mechanism of Action (Fig. 1, 2):**

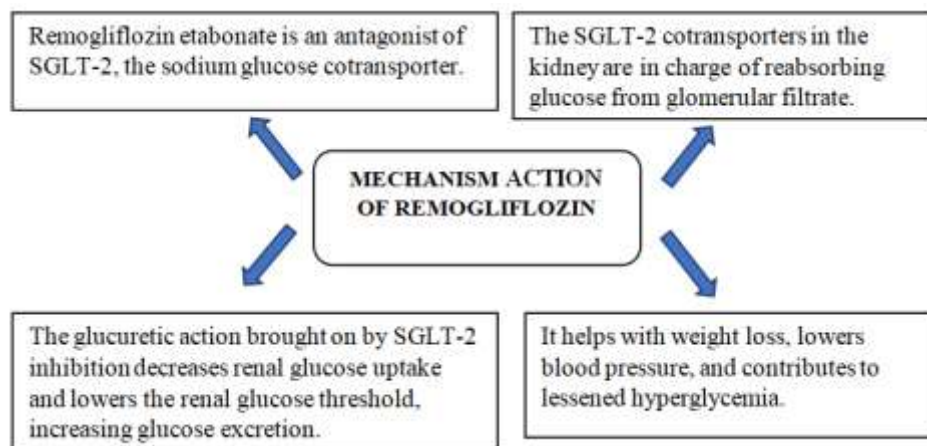


Figure 1. Mechanism of action of remogliflorzin

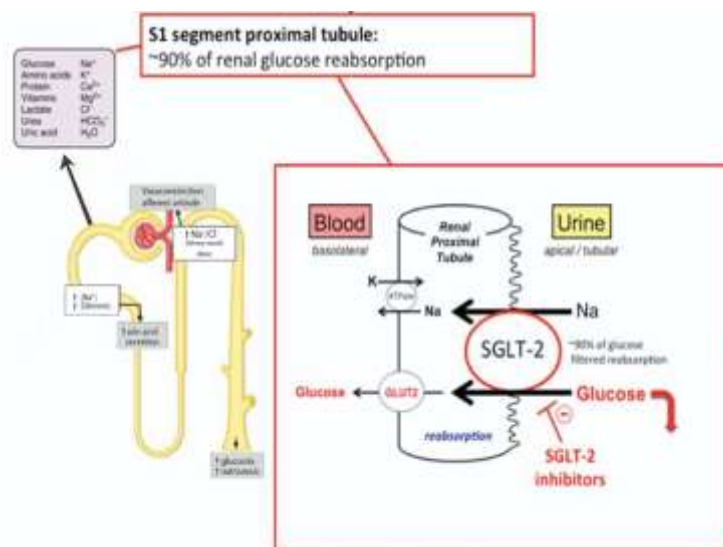


Figure 2. Mechanism of action of SGLT-2 inhibitor

## Pharmacological Profile

### Pharmacodynamics

Remogliflozin having *in vitro*  $k_i$  values for human  $slgt1$  and 2 of 4520 and 12.4 nm, respectively [25]. Remogliflozin etabonate, when given orally in standard rats and mice, an amount-dependent rise in urine insulin excretion and a fall in plasma insulin concentrations *in vivo*. In normal and streptozotocin-induced diabetic rats, delivering a single oral dosage decreased the rise as a result of glucose loading in plasma glucose in a dose-dependent manner, with the latter group showing a noticeably improved effect [25]. Remogliflozin etabonate was given orally in a single dosage to db/db mice. remogliflozin etabonate was also found to lower fasting blood sugar, glycated haemoglobin (hba1c), and insulinand urine expelling glucose in db/db mice when given orally once daily for six weeks. Remogliflozin etabonate, when taken orally for eight weeks, reduced hyperglycaemia, hyperinsulinemia, hypertriglyceridemia, and having a high-fat diet causes insulin resistance in goto-kakizaki rats [25]. The drug remogliflozin etabonate was given orally and decreased plasma lipids by 40%, hepatic lipid content by 42%, and liver weight by 42%. 76 and 48% decreases in the levels of alanine and aspartate aminotransferases, respectively when compared to a mouse model of non-alcoholic fatty liver disease [26]. Remogliflozin etabonate was given to healthy volunteers (50–1000 mg) and t2dm patients (50 and 500 mg) in a phase I trial. This led to a rise in total urine glucose excretion that is dose-dependent from 0–24 h. the proportion of Filtered glucose load was comparable amongst the groups. When urine glucose excretion was adjusted in accordance with Plasma glucose levels and creatinine clearance in circulation (to calculate the proportion of filtered glucose load) [27, 28].

### Pharmacokinetics

Pharmacokinetics after oral treatment, Ester prodrug remogliflozin etabonate is quickly riveted before being thoroughly gastrointestinal mucosa, where it is de-esterified to create the crucial element, which manifests most noticeably in the plasma. The metabolism of remogliflozin primarily by cytochrome p450 (CYP) 3a4 to GSK 279782 (the energetic metabolite) and GSK 333081 before being glucuronidase to form inactive glucuronide conjugates. Remogliflozin etabonate is taken orally after oral administration is quickly absorbed, and the gastrointestinal mucosa significantly de-esters it to the active molecule remogliflozin, that seems most prominently in plasma over 93% of [14] remogliflozin etabonate was absorbed, according to a mass balance investigation with a single dosage on healthy participants. remogliflozin is metabolised primarily by cytochrome p450 (CYP) 3a4 to GSK 279782 and GSK 333081, which then undergo glucuronidation to create [30]. Remogliflozin C max and AUC at constant state and in Indian individuals with t2dm was 559 ng/ml and 1861 ng/h/ml, respectively [29] about 65% of remogliflozin remained tethered to plasma protein. Remogliflozin neither differently transported toward plasma cells nor have they been found to selectively associate with tissues that contain melanin [29]. Cyp3a4 and CYP2C19 both significantly contribute to the metabolism of drug ketoconazole, a powerful cyp3a4 antagonist, demonstrating a modest hazard of medication interactions with drugs that suppress CYP [29] individuals with mildly implies some degree of renal impairment showed similar pharmacokinetic profiles for remogliflozin etabonate, indicating that dosage modifications are not required in these patients [31] when metformin is co-administered with 500 mg twice per day of remogliflozin etabonate in individuals with t2dm (n=13) there is no change in the steady-state pharmacokinetic profile [32, 33].

### Clinical Efficacy and Safety

During phase III study, remogliflozin 100 mg twice daily for 24 weeks was given to those with uncontrolled type 2 diabetes hyperglycaemia who were receiving metformin alone. These participating patients noticed an early and steady decline in hba1c, with a decline of 0.72% at 24 weeks. At 24 weeks, a decrease with postprandial plasma glucose values of 39.2 mg/dl and 17.86 mg/dl, respectively, while fasting, was seen. After 24 weeks, a 2.7 kg decrease in body weight was seen. Systolic pressure decreased by 2.6 mmhg, and diastolic pressure decreased by 2 mmhg [34, 35] having a low prevalence of urinary tract infections (3.1%), mycotic infections in the vagina (1.7%), and hypoglycemia (1.3%) the whole unfavourable reactions occurring mid-treatment occurred 8.5% of the time. 100 mg bid of remogliflozin treatment was therefore determined must be efficient, secure, and well-tolerated [35, 36].

### Adverse effect

During phase III trial mentioned above (ctri2017-07-009121), among the most commonly reported side effects were urinary tract infections, pyrexia, headaches, bacteriuria, constipation, diarrhoea, reduced glo-

merular filtration rate, ketonuria, cough, dyslipidaemia, asthenia, and viral infections [37] in the phase iii trial, individuals taking dapagliflozin or remogliflozin as a supplement using metformin reported hypoglycaemia in roughly equal amounts (2% in each treatment arm). There were 1.8%, 1.2%, and 2.7% of recipients 100 and 250 mg, respectively, of the medication remogliflozin etabonate, and recipients from dapagliflozin who reported having vulvovaginitis, balanitis, and other similar genital diseases, all of mild to moderate severity, all of which responded to a first round of conventional treatment. Urinary tract infections had been seen in 3.1% and 6.6% respectively. By people who took 100 and 250 mg of remogliflozin etabonate dapagliflozin patients in 2.1% of cases. Remogliflozin or Those who take dapagliflozin did not experience hypovolemia [37].

#### Advantages and Limitations of Remogliflozin Etabonate Based on Available Data

Advantages effectiveness that is acceptable, with an average hba1c reduction of 0.5% to 1.0% mild weight loss lower the risk of hypoglycaemia low possibility of contact with medications that block the p450 enzyme system overall, it was positively received. Limitations to obtain optimal efficacy, twice-daily dose could be recommended. Genital mycotic infections, urinary tract infections, and light-headedness are typical side events (3–12% versus 0% placebo), must be examined as a supplement therapy, in the elderly, and in people with chronic renal disease lack of long-term safety and effectiveness data not published in another trial [38]. Additionally, urinary tract infections (UTI) were reported by 3–11% more frequently with re use compared to 0% with placebo [39, 40]. The higher prevalence of hereditary mycotic infections and urinarytract infection is most likely connected to the rise in urine glucose excretion caused by sgl2 inhibitors, whilst dizziness is most likely connected to dehydration as a result of their diuretic impact [41, 42] increased genital mycotic infection prevalence was linked to higher re dosages, but other negative side effects were not clearly associated with this trend [39, 40]. It was interesting and comforting that none of the two biggest re trials had hypoglycaemia documented [39, 40]. In fact, SGLT-2 inhibitors generally have a low risk of causing hypoglycaemia, with the exception of when they are used with sulfonylureas or insulin [41-43].

**Pharmacoeconomic consideration** (Table 1, 2).

Table 1

Comparison of the prices of sgl-2 inhibitors on the Indian market

Sr.no.	Drug	Manufacture	Company name	Dose (in mg)	Cost per tables
1	Remogliflozin	Glenmark pharmaceuticals	Remo	100	12.5(\$0.17)
			Remozen	100	12.5(\$0.17)
		Mankind pharma ltd	SGLTR	100	12.6 (\$ 0.17)
			Zucator 100	100	12.6 (\$ 0.17)
2	Empagliflozin	Lupin pharmaceuticals	Gibtulio	25	57 (\$ 0.76)
				10	47 (\$ 0.63)
		Boehringer Ingelheim	Jardiance	25	57 (\$ 0.76)
				10	47 (\$ 0.63)
3	Dapagliflozin	Sun pharmaceutical industries ltd	Oxra	10	52 (\$ 0.69)
				5	50 (\$ 0.67)
		Astrazeneca plc	Forxigo	10	52 (\$ 0.69)
				5	50 (\$ 0.67)
		Abbott	Gledepa	10	52 (\$ 0.69)
				5	50 (\$ 0.67)
4	Canagliflozin	Johnson & Johnson Ltd	Invokana	100	54.5 (\$ 0.73)
		USV ltd	Sulisent	100	55 (\$ 0.74)
		Janssen pharmaceuticals	Motivyst	300	120 (\$ 1.6)
5	Ertugliflozin	Merck Sharp &Dohme Corp	Steglatro	5	300(\$3.75)
				15	310(\$3.89)

Comparative costs of SGLT-2 inhibitors with dpp4 available in combination form

Sr.no.	Drug	Manufacture	Brand name	Dose (in mg)	Price per tablet
1	Dapagliflozin+Metformin	Sun pharmaceutical industries ltd	OxrametXR Udapa-M	10\500	15
		USVLTD	500XR	500	12.5
2	Empagliflozin+Metformin	Boehringer Ingelheim	Jardiance met 12.5mg/500mg	500 1000	40 42
		Lupin ltd	Gibtulio met 12.5mg/500mg	500 1000	40 42
3	Empagliflozin+ Linagliptin	Cipla ltd	Tiptengio	10/5 25/5	75 82
		Boehringer Ingelheim	Glyxambi	10/5 25/5	75 82
4	Dapagliflozin +Saxagliptin	Astrazeneca	Qtern	5/10	46
5	Ertugliflozin +Sitagliptin	Merck Sharp & Dohme Corp.	Steglujan	5/100 15/100	1500 1500
6	Remogliflozin etabonate + Vildagliptin + Metformin	Glenmark pharmaceuticals ltd	Remo-zen mv	500	16.5
			Remo mv	1000	17.5

### Analytical Method

Analytical processes are created to compare certain chemical properties to predetermined acceptability criteria for those qualities. As a result, choosing the most accurate assay techniques to assess a drug's composition is a part of developing analytical methods. In order to obtain clean extract with good quality, sample preparation is crucial in bioanalysis. Effectiveness of extraction additionally, the analyte influences the detector choice. Conscientiousness span choosing an appropriate internal standard (ISTD) is also crucial. Bio-analytical method has development problem. The function of internal standard is to make up for getting precise findings and avoiding matrix effects.

### HPLC and RP-HPLC Method

One of the most vital methods for separating proteins and the technique of choice for separating peptides is reversed-phase HPLC (RP-HPLC). In addition to being used on a large industrial scale for preparative purifications, RP-HPLC has also been used on a nano, micro, and analytical size. RP-HPLC is a crucial instrument in proteomic research because it works well with mass spectrometry. Complex protein-peptide combinations can be separated at attomolar levels for subsequent analysis using contemporary apparatus and columns.

### Nandeesh I tigmitha et. al in 2020

By the HPLC method, a 4.6 mm x 250 mm x 5 mm c18 kromasil column was used as the stationary phase, even if the mobility phase was containing a 0.02 M ammonium acetate buffer, acetonitrile, and tetrahydrofuran in that order (v/v), with the pH being corrected to 4.0 by 1.0 Orthophosphoric Acid (m-OPA). With a 2.0 millilitres per minute flow rate, 101 sizes of the sample injection, and a 228 nm detection wavelength, this experiment was completed. In the spectral UV approach pure ethanol was used to dilute the RMZ. At 228 nm, the remogliflozin had the highest absorption. Consequently, 228 nm was employed throughout the investigation to determine during the analysis for the purpose of determining RMZ, 228 nm was employed. The RTS for atorvastatin (ATST), an internal benchmark, and RMZ they were 6.2 and 7.0 minutes, respectively. It was discovered that the resolution between the peaks was greater than 2.0. There were 10 minutes allotted for the run. At a fixed concentration of std, it was discovered that the RP-HPLC method's linearity window was between 10 g ml<sup>-1</sup> to 50 g ml<sup>-1</sup>. The use of the UV spectroscopic method was demonstrated to have a between 100 and 250 g linearity ml<sup>-1</sup>. Both strategies had regression coefficients (r<sup>2</sup>) that were higher than 0.999 it was discovered that the thresholds for quantification and detection of RMZ were 1.0 g ml<sup>-1</sup> and 3.5 g ml<sup>-1</sup>, respectively. Remogliflozin etabonate (RMZ) has been determined using straightforward, unique, further more to selective RP-HPLC, ultraviolet (UV), and liquid chromatography spectroscopy procedures that have been created and improved. The primary peak and internal standard peak were individually eluted using various retention durations (RT) in the HPLC technique [44].

**Swapnil Suresh Mankar et al. (2021)**

Tests for the system's applicability, reproducibility, precision (day and night/interval), linearity and calibration, robustness, force degradation, specificity, and drug recovery and accuracy studies are used to test the method's suitability and validate it for solid dosage form estimation of diabetes medications using RP-HPLC results: in accordance with each criteria, remogliflozin's performance met all standards for system appropriateness, including those for factors for tailings (t), separations, theoretical plates (n), capacity (k'), resolution (r), and RSD (%); the verified stress breakdown investigations for remogliflozin under heat, oxidative, alkaline, and acidic circumstances in a few breakdown products (rem) [45].

**Dr. Srinivasan et al. (2020)**

Remogliflozin and ertugliflozin are estimated using UV, RP-UPLC, RP-HPLC, and LC-MS methods. There are numerous previously published articles that describe analytical methodologies and similar approach validation, the established levels of remogliflozin and ertugliflozin in its pharmaceutical preparations and biological matrices are accounted for by the disclosed analytical procedures that are detailed in the current review. The most popular approaches, including spectrometric and liquid chromatographic procedures, are outlined in this article. Remogliflozin and ertugliflozin HPLC procedures take into account factors like the matrix, composition of the stationary and mobile phases, detection wavelength, and others for remogliflozin and ertugliflozin both separately and together, spectrometric methods incorporate variables like max, solvent, matrix, etc., variables used in HPTLC procedures include combinations of rf, stationary phase, and mobile phase. Additionally, this review gives thorough details on how remogliflozin and ertugliflozin separate when used separately, in combination with other medications, and when their breakdown products are present. There have been reported Remogliflozin and Ertugliflozin determination techniques [46].

**Vashi Dhara et al. (2022)**

Isocratic mode methodology was used to perform the RP-HPLC process on a column of reversed-phase Cosmosil C18 (250 mm, 4.6 mm, 5 i.d.). Acetonitrile made up 60:40% of the mobile phase by volume and water, with a 1 ml/min flow rate, 210 nm detector wavelength was employed. Vildagliptin and remogliflozin etabonate had average retention durations of 3.29 and 5.64 minutes, respectively. The calibration curves for vildagliptin and remogliflozin etabonate were linear ( $r^2 > 0.999$ ) for between 5-80 g/ml and 10-80 g/ml in terms of concentration, respectively vildagliptin and remogliflozin etabonate both had LODs of 0.010 g/ml and 0.029 g/ml and, correspondingly, LOQs of 0.031 g/ml and 0.088 g/ml. The measurement of Pharmaceutical dose type of Vildagliptin with Remogliflozin Etabonate, an easy, sensitive, verified, specific, and accurate Chromatography RP-HPLC method has been developed [47].

**Amit Chaudhary et al. (2022)**

With the use of a C18 (250 mm 4.6 mm, i.d. 2.5 m) column and isocratic elution, the separation was accomplished. The separation of the analytes employed acetonitrile and 20 mm ammonium formate buffer (PH 3.5) combined at a 60:40 ratio with a flow rate of 1 ml/minute as the stage of mobility. At a wavelength of 243 nm, a diode array detector was used to monitor the segregated effluents. For remogliflozin and met, respectively, the results revealed satisfactory linearity. Additionally, the typical % assessment of commercially available compositions of met as well as remogliflozin turned out to be 100.52% and 100.30%. LOD and LOQ for remogliflozin 0.42 and 1.28 g ml<sup>-1</sup>, respectively, while these values are 1.97 gml<sup>-1</sup> and 5.96 g ml<sup>-1</sup> for met. The suggested approach's selectivity, precision, linearity, and accuracy were all verified. There were no validation parameters outside of the permitted range [48].

**Mahesh Attimarad et al. (2020)**

Utilizing a monolithic c18 column and full factorial box-Behnken design model, under ideal chromatographic circumstances, MFH and RGE were separated by chromatography. The basis for the spectroscopic method was the peak UV spectral intensity of the second-order derivative at zero crossings. Additionally; this is an attempt used for the simultaneous estimate in laboratory-based mixed solutions and formulations of MFH and RGE. Out of 47 possibilities, the final chromatographic condition was selected. Recommended Plots of perturbations, response surface models, and the desirability function demonstrated the impact of the Chromatographic specifications. Additionally, the RGE and MFH HPLC methods both shown high linearity in the range by using spectroscopic and HPLC methods. Average percent assay for MFH and RGE, respectively, yielded results of 99.51% and 99.80% and 99.60% and 100.07%. Remogliflozin as well as metformin in dosage form was effectively determined using this approach, with acceptable recoveries [49].

**Mahesh Attimarad et al 2022**

Three variables, acetonitrile, ethanol, and water, interact and have a quadratic impact. Percentage, movable stage, pH, and the resolution of the flow rate between the peaks were optimised using the box-Behnken

approach and response surface design. The desirable function design space was utilised to identify the ideal chromatographic environment in order to predict the Resolution of the three anti-diabetic medications' peaks (2.7 and 6.5) the isocratic elution method, which employed Acetonitrile and phosphate buffer (20 mm  $\text{KH}_2\text{PO}_4$ , pH adjusted to 4.9 with orthophosphoric acid) were applied over a Zorbax C18 HPLC column at a ratio of 58:42, successfully separated all three analytes in 2.5 minutes. Additionally, recommendations were used to validate the optimised HPLC process. The low percent relative standard deviation (0.60-1.65%), good percentage recovery (98.18-101.50%), and low percent (0.20-1.82%) relative errors provided evidence for the precision and accuracy of the developed HPLC method. By gently changing the five various parameters, the resilience of the method was also shown [50].

#### **Shivani v. Trivedi et al. (2021)**

In order to measure metformin HCL and remogliflozin etabonate simultaneously are in their synthetic mixture. There was developed a reverse phase high performance liquid chromatographic technique created by using a Cosmosil C18 column (250 mm x 4.6 mm, 5 m) methanol (60:40) as the mobile phase, flowing at a rate of 1 ml/min, and buffer (pH 4.0), the separation was accomplished. The detection wavelength was 241 nm. It was discovered that metformin HCL and remogliflozin etabonate have retention times of 5.493 minutes and 3.183 minutes, respectively. Linearity, accuracy, and precision have all been verified for the approach. 5–15 g/ml of remogliflozin etabonate and 20–60 g/ml of metformin HCL both showed linearity. This approach was created with the goal of simultaneously estimating the remogliflozin and metformin in bulk and commercial dose forms [51].

#### **UV -Spectroscopic**

Spectrophotometry, another name for UV-vis spectroscopy, is a quantitative technique for determining how much light each particular molecule absorbs. In order to do this, the amount of light passing through a sample is compared to the amount of light passing through a reference sample or a blank. Glass, liquids, solids, thin films, and other sample types can all be analysed using this method.

#### **Attimarad Mahesh et al. (2022)**

In order to establish two processed UV spectrophotometric approaches, the peak amplitude at zero-crossing of second derivative spectra of analytes. The second approach is creating zero-order spectra from a mixture of analyte spectra by multiplying and dividing them by the spectra of the pure analyte in order to cancel out the influence of one of the analytes. Results: for RGF and VGT, respectively, both methods demonstrated linearity concentrations in the range of 2-75 g/ml and 2-50 g/ml. Low LOD and LOQ values discovered for RGF and VGT by both approaches demonstrated the methods' high sensitivity. For RGF and VGT, respectively, the mean percentage recovery was 98.60% and 100.78%, and both had low relative error percentages of 98.81% and 99.15%. The results were compared to the reported techniques for the assay of the VGT and RGF from the drug [52].

#### **HPTLC method**

HPTLC (high-performance thin-layer chromatography), a development of thin layer chromatography (TLC), is a reliable, easy-to-use, quick, and effective technique for quantitative analysis of substances. An analytical method called HPTLC is based on traditional liquid chromatography (TLC), but it has been improved to allow for quantitative analysis of the compounds and to improve the separation of the compounds' resolution. Some of the improvements allow for better resolution, such as when higher-quality TLC plates are used, which have finer stationary phase particle sizes. Using a multiple development apparatus, the plate can be developed again to further increase the separation. Consequently, HPTLC delivers higher resolution and a lower limit of detection (LOD's) [53].

#### **Reema Jaiswal et al. (2022)**

On a precoated silica gel aluminium plate 60F254 (20 x 10) 100 m thick, the medicines were separated by chromatography, with the mobile phase being a mixture of methanol, ethyl acetate, and acetic acid (6:3.5:0.5v/v). The TLC scanner was tuned at 245 nm. 0.23 R<sub>F</sub> values for met and 0.83 for remogliflozin, respectively, indicate that the two medicines were satisfactorily resolved. Remogliflozin etabonate and metformin hydrochloride had polynomial calibration curves in the 200–1200 ng/band and 1000–6000 ng/band concentration ranges, respectively. The values for the Metformin has correlation values (r) of 0.9999 and 0.9999 etabonate and hydrochloride, respectively, of remogliflozin. The suggested method is accurate and resilient, as evidenced by the both with a modest relative standard deviation (2%) the studies on precision and robustness. The approach obtained a 98.82% accuracy rate for metformin hydrochloride and 98.42% for remogliflozin etabonate were approved in accordance with regulatory regulations. They came to the conclusion this experiment that the newly created approach for simultaneously estimating the remogliflozin and

metformin hydrochloride discovered towards straightforward, accurate, exact, high resolution, with time-efficient which made this technique extra palatable and economical [54].

#### **DimalA. Shah et al. (2021)**

The stationary phase was a mixture of methanol, ethyl acetate, toluene, and  $\text{nh}_3$  (2:4:4:0.1, v/v/v) on HPTLC plates coated with silica gel 60 f254 was utilized as the mobile phase, and densitometry was employed to quantify the drug's estimate. The suggested approach was tested prior to usage, for durability, linearity, accuracy, and precision estimate the dosage of a medicine in tablet form Remogliflozin etabonate was found to have a RF value of 0.61 at 229 nm, the reflectance mode was used to carry out the densitometric estimation. For remogliflozin etabonate, the technique was discovered to be linear in the 500–8000 ng/band range by conducting tests on forced degradation, the potential degradation pathway was estimated. With respect to their rf value, The deteriorating summits were satisfactorily separated since the drug peak. The newly created HPLC technique was easy, quick, accurate, and precise. Therefore, the technique can be effectively applied to the study of the pharmaceutical industry use of remogliflozin etabonate [55].

#### **UPLC and RP-UPLC**

UPLC is a novel class of separation technology that makes use of sub-2  $\mu\text{m}$  particles for the stationary phase and is based on established liquid chromatography concepts. The clarity and sensitivity, and analysis rapidity of these particles have dramatically increased due to their high mobile phase linear velocities. This approach has received a lot of interest recently for pharmaceutical and biomedical analysis due to its speed and sensitivity [56].

#### **Ali, s. m. et al. (2021)**

A new kind of separation is known as UPLC method that makes use for the stationary phase, of sub-2  $\mu\text{m}$  particles and is based on well-known liquid chromatography principles 0.1  $\mu\text{m}$  acetate buffer at PH 5.7 and a ratio of 25:75 (v/v) methanol at a flow rate of 0.3 ml/min with a PDA detector operating at 215 nm under these circumstances, the compounds' it was resolved measured at 12.57, with remogliflozin's retention duration being 2.67 minutes, compared to 3.84 minutes for vildagliptin. The technique's applicability for the system, scope of analysis, accuracy, specificity, stability, and robustness were all confirmed. The analytes were subjected to five distinct stress conditions throughout the forced degradation investigation, and the overall degradation condition. The approach can separate and measure the amounts of vildagliptin and remogliflozin in pharmaceutical formulations, and the percentage of degradation was very low [57].

#### **V.A. Patel et al. (2021)**

The goal of this project is to simultaneously create quick and easy RP-UHPLC methods for quantifying remogliflozin. Remogliflozin and met were separated by chromatography using acetonitrile: phosphate buffer (PH: 3) (60:40%, v/v) on a zorbax eclipse plus c18 (1504.6 mm, 5 m) column. Mobile phase, a diode array detector, and a flow rate of 1.0 ml/min at 230 nm. Remogliflozin had an average percent assay of 99.51%, whereas met had an average percent assay of 99.60%. The outcomes of the retrieval studies conducted validate the projected process's high degree of accuracy. The devised chromatographic approach can therefore be effectively used for estimate of metformin and remogliflozin in their formulation and bulk. It is accurate, precise, and selective [58].

#### **LC-MS method**

In the LC-MS analytical procedure, target chemicals (or analytes) are physically separated before being detected by mass spectrometry despite being a relatively new technology, it has quickly gained popularity for its ability to detect various analytes in microgram or even nanogram concentrations, such as insecticides, food additives, medication metabolites, and natural product extracts.

Application of LC-MS /MS:

1. The measurement of genotoxic contaminants in pharmaceutical active ingredients [59].
2. Measurement of drug metabolites in bodily fluids.
3. Bacterial cell quantitation of nucleotides and their derivatives [60].

#### **Sai Prudhvi n. et al. (2021)**

In the therapy of type II diabetes in human plasma, remogliflozin and vildagliptin are utilized. Liquid-liquid extraction was used to separate the analytes from the spiked plasma matrix and choose the medication analogliptin as the internal standard and the extracts underwent chromatographic analysis on an in ertsilods (4.6 mm100 mm, 5 m) C18 column. The mobile phase contains methanol, acetonitrile, and 0.1% formic acid in a ratio of 40:50:10 (v/v) at a flow rate of 0.5 ml/min. The analysis was finished in 6 minutes. The technique generates peaks at 2.6 min for remogliflozin etabonate, 2.7 min for vildagliptin, and 1.2 min for alogliptin with adequate symmetry and resolution with satisfactory system compatibility (internal standard).



The characteristic fragment ion transitions for remogliflozin and vildagliptin at  $m/z$  523 to  $m/z$  247 and 304 to  $m/z$  180, respectively, are confirmed by mass spectral analyses. The technique has a lower LOQ of 5 ng/ml and can identify analytes upto 1.5 ng/ml. It has a wide calibration range of LOQ to 300 ng/ml & HQC (high quality control concentration) levels and generate findings that are suitable. Remogliflozin and vildagliptin multiplexed quantification techniques have been designed and validated using sensitive, specific, and dynamic LC-MS/MS assay. The presented techniques can handle significant research trials with enough throughput.

#### **Dr. Satyadev et al. (2022)**

This article reviews current developments in bioanalytical LC-MS/MS techniques for utilising water-soluble C<sub>18</sub> column (150x4.6 mm, 3.5) and 70:30 ratio's organic mobile phase, combine acetonitrile and sodium dihydrogen phosphate. In the case of remogliflozin, the calibration curve was linear at 350 ng/ml. Results for stability, matrix effect, accuracy, recovery, and precision were determined to be within acceptable bounds. In pharmacokinetic investigations, a quick and effective method for observing the examined analyte and was created.

#### *Conclusion*

This review article discusses the remogliflozin's physiological characteristics, drug profile, and clinical studies including safety and efficacy and adverse effect of a drug. The review that is being presented provides on the many approaches that have been used to identify remogliflozin and its ester form. This review concludes that many analytical techniques, including RP-HPLC, UV, HPTLC, RP-UPLC, LC-MS method have been described for estimating remogliflozin alone and its combinations. Thus, these techniques were discovered to be straightforward, precise, economical, and repeatable in nature because RP-HPLC and RP-UPLC offered the best available reliability, repeatability, analysis time, and sensitivity, these techniques were used for the majority of procedures. The developed RP-HPLC and RP-UPLC chromatographic conditions are used in a method for the simultaneous measurement of singles and their combination is quick, accurate, precise, and simple, and it may be used in laboratories for standard quality control tests on both formulations.

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## Адамдардағы 2-типті қант диабетін емдеуде SGLT-2 ремоглифлозин ингибиторына шолу

Натрий-глюкоза тасымалдау ақуызы 2 ингибиторлары, әсіресе жүрек немесе бүйрек аурулары бар адамдарда, 2-типті қант диабетін емдеуге арналған пероральды дәрі-дәрмектердің маңызды санатына айналды және жалпы заманауи зерттеулердің жоғары бағасына ие болды. Олар қан қысымын төмендетіп, адамдарға салмақ жоғалтуға көмектеседі, бірақ олардың кемшілігі бар, атап айтсақ, несепжыныс жүйесінің инфекциясын тудырады. Ремоглифлозиннің жаңадан мақұлданған комбинациясы — бұл жаңа фармацевтикалық класс пен қант диабетін емдеуге арналған препараттардың жаңа комбинациясын зерттеу. Ремоглифлозин этабонаты — ремоглифлозиннің эфирлік түріне арналған термин. Бұл шолуда ремоглифлозин этабонатының клиникалық тиімділігі, қауіпсіздік профилі, фармакокинетикасы және фармакодинамикасы зерттелген. Алайда, оны RP-HPLC, UV, RP-UPLC, HPTLC және LC/MS көмегімен аналитикалық профильдеу қажет. Авторлар ремоглифлозин этабонатының аналитикалық әдісін әзірлеу кезінде жүргізілген жеке эксперименттерді қарастырған.

*Кілт сөздер:* ремоглифлозин этабонаты, SGLT-2 ингибиторы, 2-типті қант диабеті, аналитикалық әдіс.

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## Обзор ингибитора SGLT–2 ремоглифлозина в лечении сахарного диабета 2-го типа у человека

Ингибиторы натрий-глюкозного транспортного белка 2 стали значительной категорией пероральных препаратов для лечения сахарного диабета 2-го типа, особенно у людей с заболеваниями сердца или почек, и, в целом, получили высокую оценку современных исследователей. Они могут снижать артериальное давление и помогают людям в процессе снижения веса, однако у них есть и недостатки, например, инфекции мочеполовой системы. Недавно одобренная комбинация ремоглифлозина — это исследование нового фармацевтического класса и новой комбинации препаратов для лечения диабета. «Ремоглифлозин этабонат» — термин, обозначающий эфирную форму ремоглифлозина. В настоящем обзоре изучены клиническая эффективность, профиль безопасности, фармакокинетика и фармакодинамика ремоглифлозина этабоната, но его аналитическое профилирование производится с использованием RP-HPLC, UV, RP-UPLC, HPTLC и LC/MS. Авторами рассмотрены отдельные эксперименты, проведенные при разработке аналитического метода для ремоглифлозина этабоната.

*Ключевые слова:* ремоглифлозин этабонат, ингибитор SGLT–2, сахарный диабет 2-го типа, аналитический метод.

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## **Анализ зависимости между погодными условиями и качеством атмосферного воздуха в городах Казахстана за 11-летний период (2010–2020 гг.) как основа эпидемиологических рисков**

В статье был проведен ретроспективный анализ зависимости между погодными условиями и присутствием поллютантов в воздушном пространстве города Караганды и Петропавловска за 11-летний период (2010–2020 гг.). Динамика кода загрязненности атмосферы исследуемых городов свидетельствовала о более высоком уровне загрязнения воздуха в г. Караганде на протяжении всех сезонов, по сравнению с показателями г. Петропавловска. В газообразных выбросах по г. Караганде достоверно преобладали вещества, относящиеся к первой категории опасности: диоксид азота в зимний период, озон весной и летом. Фенолы и формальдегид присутствовали в воздухе г. Караганды на протяжении всех сезонов, достоверно превышая ПДК. Анализ корреляционных зависимостей между погодными и экологическими факторами позволил обнаружить средние и слабые прямые и обратные корреляции между загрязнением атмосферы, погодными условиями и сезонами. Было обнаружено, что в зимний сезон синергизм мог проявляться вследствие одновременного присутствия диоксида азота и формальдегидов, содержание которых в атмосфере достоверно превышало ПДК. В летний сезон синергизм можно было ожидать вследствие одновременного присутствия в воздухе больших концентраций озона и формальдегида. Комплексное воздействие погодных условий, уровня и характера загрязненности атмосферного воздуха в Карагандинском регионе по сравнению с г. Петропавловском свидетельствует о вероятности значительного усиления или видоизменения негативных последствий для здоровья человека.

*Ключевые слова:* погода, загрязнение воздуха, код загрязненности, комплексный индекс загрязненности, стандартный индекс, биоклиматические индексы, твердые и газообразные вещества.

### *Введение*

Изменение климата широко признано серьезной угрозой общественному здоровью [1] с широким спектром последствий. Недавние исследования показали, что следует тщательно учитывать двустороннее взаимодействие между погодными переменными (например, температурой) и загрязнением воздуха, чтобы охарактеризовать синергетическое воздействие на здоровье [2, 3], особенно в контексте изменения климата [4, 5].

Влияние метеорологических переменных варьирует в зависимости от загрязняющего вещества. Показано, что влияние погодных изменений зависит как от явлений переноса, таких как адвекция или диффузия, так и химических реакций в атмосфере, управляющих образованием вторичных загрязнителей. При этом температура играет фундаментальную роль в химическом составе атмосферы NO<sub>2</sub> [6]. Более высокие температуры увеличат скорость окисления NO, а более слабые ветры ограничат рассеивание. Изменения влажности также могут играть роль, поскольку они влияют на относительное содержание гидроксильного радикала (ОН), который, в свою очередь, оказывает фундаментальное влияние на химические процессы, контролирующие концентрацию газообразных загрязнителей, включая NO<sub>2</sub> [7].

Обычно оценки риска на основе эпидемиологических исследований моделируют температуру как искажающий фактор, в то время как в нескольких исследованиях изучалась роль температуры как модификатора эффекта кратковременного воздействия загрязняющих веществ, таких как озон, динамика которого также сильно зависит от температуры.

Керол и другие [8] изучили тенденции качества воздуха в Испании за период 2001–2012 гг. и предположили, что, хотя сокращение загрязнения воздуха в основном было обусловлено сокращением выбросов, метеорологические изменения также могли повлиять на тенденции качества воздуха в течение этого периода. Другие исследования загрязнения воздуха во временных рядах также рас-

сма тривали изменения погоды как в лиятельный фактор в Европе [9–11]. В то время как количество регулируемых загрязняющих веществ ( $\text{SO}_2$ ,  $\text{NO}$ ,  $\text{CO}$ ,  $\text{PM}_{10}$  и  $\text{PM}_{2,5}$ ) существенно сократилось за последние годы, уровни  $\text{O}_3$  остались постоянными или в некоторых случаях увеличились. Усилия по сокращению выбросов в Испании оказались успешными в снижении смертности, связанной с загрязнением воздуха. Обнаружено, что снижение концентрации  $\text{PM}_{10}$  в окружающей среде уменьшило количество смертей, связанных с загрязнением воздуха, за 25-летний период исследования.

Изучена и роль температуры как модификатора эффекта кратковременного воздействия озона. Джун и другие [12] пришли к выводу, что тепловое воздействие может усугубить неблагоприятное действие озона на здоровье, при этом взаимодействие между загрязнением воздуха и температурой носит нелинейный характер. Изменения погоды способствовали более высоким концентрациям  $\text{O}_3$  в теплое время года, что изучено предыдущими исследованиями, в которых определялась чувствительность  $\text{O}_3$  к погодным параметрам [13–15].

Тем не менее, поскольку климат и качество воздуха тесно взаимосвязаны, их следует рассматривать комплексно в оценке экологической ситуации. Актуальная информация о масштабах последствий необходима для разработки эффективных мер по снижению вредных выбросов в атмосферу с целью защиты здоровья населения. В этой статье нами был проведен ретроспективный анализ загрязненности воздуха и корреляционной зависимости между метеорологическими условиями и экологией за последние 11 лет (2010–2020 гг.) в городах Казахстана.

#### *Материалы и методы*

Материалы для анализа загрязнения атмосферного воздуха были скопированы из открытых источников («Информационные бюллетени о состоянии окружающей среды по Республике Казахстан» за 2010–2021 гг.). Наблюдения за состоянием окружающей среды, согласно ст. 144 Экологического кодекса РК, осуществляют аккредитованные аналитические лаборатории областных филиалов РГП «Казгидромет». Наблюдения за состоянием атмосферного воздуха в г. Караганде велись на 4, а с 2014 г. на 5 ручных постах. Материалы по эмиссии загрязняющих веществ взяты из Департамента статистики Карагандинской области по охране окружающей среды (16 серия) — Бюллетень «О состоянии атмосферного воздуха в Карагандинской области» за 2021 г.

Наблюдения за состоянием атмосферного воздуха в г. Петропавловске велись на 2 ручных и 1 автоматическом постах. В атмосферном воздухе определялись 27 параметров, среди которых в непрерывном режиме регистрировалось содержание пыли  $\text{PM}_{10}$ ,  $\text{PM}_{2,5}$ , диоксида серы, оксида углерода, оксида и диоксид азота, сероводорода, озона, аммиака, суммы углеводородов, метана.

Для оценки качества загрязнения атмосферного воздуха за месяц мы использовали три показателя: 1) ИЗА — комплексный индекс загрязнения атмосферы, параметр определяет хроническое (длительное) загрязнение воздуха; 2) СИ — стандартный индекс, рассчитывается делением разовой концентрации примеси на ПДК; 3) НП — наибольшая повторяемость (%) превышения максимально разовой ПДК за время.

Вариационный и корреляционный анализы проводились с помощью пакета Statistica 10 (использовались только значимые значения коэффициентов корреляции при  $p \leq 0,05$ ).

#### *Результаты и обсуждение*

Анализ годовой динамики кода загрязненности атмосферы исследуемых городов за 11-летний период позволил обнаружить в целом более высокие уровни загрязнения воздуха в г. Караганде на протяжении всех сезонов, по сравнению с показателями г. Петропавловска (см. рис.).

Код загрязненности в зимний сезон составил — 2 в декабре и январе месяцах (повышенный уровень) и 3 — в феврале, что оценивается как высокий уровень загрязненности. В тоже время в г. Петропавловске код загрязненности не превышал 2 все зимние месяцы и оценивался как низкий. В весенние месяцы в г. Караганде уровень загрязненности был высоким, а в г. Петропавловске код составил 2,27 в марте месяце. В летне-осенний период загрязненность воздуха была повышенной в г. Караганде, в Петропавловске же наблюдалось повышение в июле и августе месяцах, в остальные месяцы загрязненность была низкой.

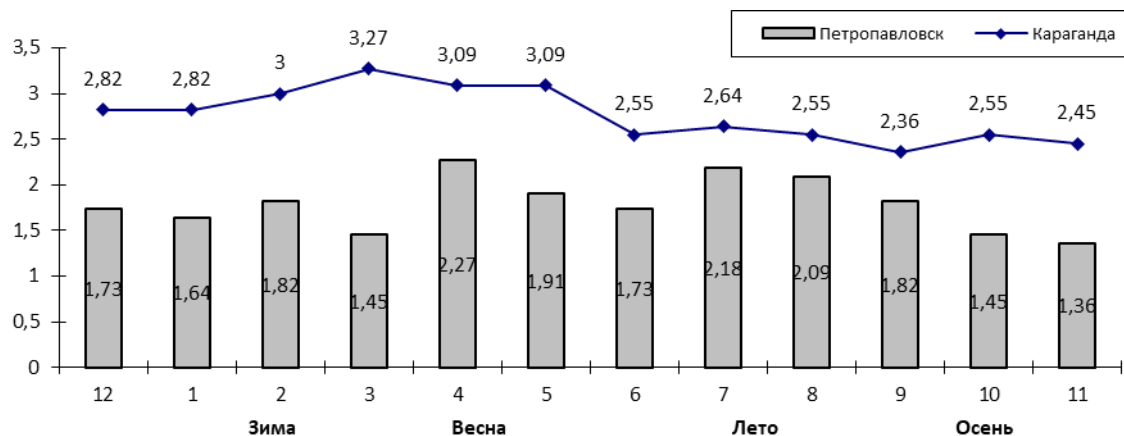


Рисунок. Годовая динамика кода загрязненности атмосферы гг. Петропавловска и Караганды за 2010–2020 гг.

Комплексный индекс загрязнения атмосферы, характеризующий хроническое (длительное) загрязнение воздуха все сезоны, не превышал низкий уровень в исследуемых регионах, однако показатели г. Караганды были выше в 1,5–2 раза аналогичных результатов по г. Петропавловску. Для оценки качества загрязнения атмосферного воздуха мы использовали два показателя: СИ (стандартный индекс) — наибольшая измеренная концентрация примеси, деленная на ПДК м. р., и НП — наибольшая повторяемость превышения разовыми концентрациями примеси ПДК (в %). По г. Караганде стандартный индекс в зимние месяцы и в весенне-летний период был высоким. По г. Петропавловску СИ был повышенным. Наибольшая повторяемость превышения разовыми концентрациями примеси ПДК составила  $11,91 \pm 4,068$  % зимой и  $11,4 \pm 2,644$  % осенью в г. Караганде, что является признаком повышенной повторяемости, в то время как по г. Петропавловску повышение НП было отмечено только в летний период (табл. 1).

Т а б л и ц а 1

**Показатели ИЗА, СИ, НП и кода загрязненности по городам**

Караганда	Зима	Весна	Лето	Осень
ИЗА 5	$3,06 \pm 0,685$	$2,91 \pm 0,646$	$2,62 \pm 0,622$	$2,45 \pm 0,621$
СИ	$7,1 \pm 1,413$	$5,02 \pm 0,977$	$3,17 \pm 0,528$	$5,2 \pm 0,972$
НП, %	$11,91 \pm 4,068$	$6,59 \pm 1,526$	$6,39 \pm 1,292$	$11,4 \pm 2,644$
Код загрязненности	$3,09 \pm 0,133$	$2,75 \pm 0,123$	$2,48 \pm 0,098$	$2,69 \pm 0,119$
Петропавловск	Зима	Весна	Лето	Осень
ИЗА 5	$1,6 \pm 0,35$	$1,5 \pm 0,33$	$1,4 \pm 0,34$	$1,44 \pm 0,34$
СИ	$1,4 \pm 0,28$	$4,1 \pm 1,27$	$3,8 \pm 1,18$	$1,26 \pm 0,258$
НП, %	$5,4 \pm 2,03$	$4,8 \pm 1,85$	$10,1 \pm 3,86$	$4,42 \pm 1,846$
Код загрязненности	$1,66 \pm 0,13$	$2,33 \pm 0,196$	$2,12 \pm 0,193$	$1,54 \pm 0,134$

Масса выброшенных в атмосферный воздух г. Караганды твердых частиц диаметром 2,5 мкм за исследуемый период достоверно превышала ПДК в зимний ( $0,13 \pm 0,065$ ) и весенний периоды ( $0,04 \pm 0,007$ ). Твердых частиц диаметром 10 мкм было достоверно больше ПДК в весенний период ( $0,07 \pm 0,038$ ) (табл. 2).

Т а б л и ц а 2

**Показатели загрязненности атмосферного воздуха твердыми и газообразными веществами (мг/м<sup>3</sup>) г. Караганды**

Караганда	ПДК	Зима	Весна	Лето	Осень
Взвешанные вещества (ВВ)	0,150	$0,084 \pm 0,01217$	$0,0778 \pm 0,00884$	$0,0761 \pm 0,00878$	$0,0769 \pm 0,01227$
Взвешенные частицы PM <sub>2,5</sub>	0,035	$0,13 \pm 0,065^{**}$	$0,04 \pm 0,007^{**}$	$0,01 \pm 0,005$	$0,04 \pm 0,009$
Взвешенные частицы PM <sub>10</sub>	0,060	$0,06 \pm 0,012$	$0,07 \pm 0,038^{*}$	$0,01 \pm 0,003$	$0,03 \pm 0,007$
SO <sub>2</sub> (диоксид серы)	0,050	$0,0217 \pm 0,00236$	$0,0121 \pm 0,00177$	$0,0117 \pm 0,0017$	$0,0143 \pm 0,00213$
Сульфаты		$0,0028 \pm 0,00061$	$0,0025 \pm 0,00058$	$0,0328 \pm 0,03023$	$0,0044 \pm 0,00156$

СО (оксид углерода)	3,0	1,65 ±0,207	1,08 ±0,115	1,36 ±0,163	1,25 ±0,115
NO <sub>2</sub> (диоксид азота)	0,050	0,07 ±0,017*	0,05 ±0,006	0,05 ±0,007	0,05 ±0,005
NO (оксид азота)	0,060	0,0155 ±0,00268	0,0133 ±0,00265	0,0108 ±0,00236	0,0136 ±0,0025
Озон	0,030	0,03 ±0,005	0,05 ±0,003*	0,04 ±0,003*	0,01 ±0,001
Сероводород	0,002	0,0008 ±0,00006	0,0008 ±0,00007	0,0015 ±0,00121	0,002 ±0,00062
Фенол	0,003	0,05 ±0,02**	0,06 ±0,022**	0,05 ±0,02**	0,04 ±0,017**
Формальдегид	0,010	0,08 ±0,027**	0,08 ±0,028**	0,07 ±0,024**	0,07 ±0,023**
Аммиак	0,040	0,0043 ±0,00091	0,0046 ±0,00093	0,008 ±0,00301	0,0044 ±0,00092
СН (сумма углеводородов)		0,24 ±0,067	0,27 ±0,071	0,32 ±0,079	0,34 ±0,085
СН <sub>4</sub> (метан)		0,73 ±0,1	0,61 ±0,094	0,63 ±0,088	0,52 ±0,093

Примечание. \* P≤0,05; \*\* P≤0,01.

В газообразных выбросах достоверно преобладали диоксид азота в зимний период (0,07 + 0,017) (p≤0,05), озон весной (0,05+0,003) и летом (0,04+0,003) соответственно. Из многочисленных ингредиентов, загрязняющих воздушный бассейн города, эти вещества относятся к первой категории опасности. Вещества второй категории опасности — фенол и формальдегид присутствовали в воздухе г. Караганды на протяжении всех сезонов, достоверно превышая ПДК (p≤0,01).

По г. Петропавловску отмечено достоверное превышение ПДК только по озону и сероводороду в весенне-летний период. По остальным показателям превышения ПДК не обнаружено (табл. 3).

Т а б л и ц а 3

**Показатели загрязненности атмосферного воздуха твердыми и газообразными веществами  
г. Петропавловска**

Петропавловск	ПДК	Зима	Весна	Лето	Осень
Взвешанные вещества (ВВ)	0,150	0,072 ± 0,0076	0,075 ± 0,0073	0,079 ± 0,0056	0,07 ± 0,0067
Взвешенные частицы РМ <sub>2,5</sub>	0,035	0,007 ± 0,0018	0,009 ± 0,0012	0,005 ± 0,0011	0,004 ± 0,0008
Взвешенные частицы РМ <sub>10</sub>	0,060	0,008 ± 0,0024	0,009 ± 0,001	0,013 ± 0,0049	0,007 ± 0,0014
SO <sub>2</sub> (диоксид серы)	0,050	0,007 ± 0,0004	0,02 ± 0,0099	0,015 ± 0,0051	0,006 ± 0,0004
Сульфаты		0,008 ± 0,0004	0,008 ± 0,0003	0,008 ± 0,0006	0,007 ± 0,0003
СО (оксид углерода)	3,000	0,95 ± 0,088	0,78 ± 0,081	0,78 ± 0,071	0,86 ± 0,066
NO <sub>2</sub> (диоксид азота)	0,050	0,026 ± 0,003	0,021 ± 0,0021	0,02 ± 0,0015	0,019 ± 0,0021
NO (оксид азота)	0,060	0,009 ± 0,0034	0,003 ± 0,001	0,002 ± 0,0008	0,004 ± 0,0013
O <sub>3</sub> (озон)	0,030	0,028 ± 0,0059	0,049 ± 0,0055*	0,036 ± 0,0046*	0,03 ± 0,0049
H <sub>2</sub> S (сероводород)	0,002	0,001 ± 0,0002	0,004 ± 0,0023*	0,006 ± 0,0026*	0,001 ± 0,0002
Фенол	0,003	0,001 ± 0,0001	0,001 ± 0,0001	0,001 ± 0,0001	0,001 ± 0
НСОН (формальдегид)	0,010	0,006 ± 0,0005	0,006 ± 0,0006	0,006 ± 0,0007	0,006 ± 0,0007
NH <sub>3</sub> (аммиак)	0,040	0,0044 ± 0,00026	0,003 ± 0,0009	0,004 ± 0,0011	0,003 ± 0,0009
СН (сумма углеводородов)		1,39 ± 0,435	1,55 ± 0,026	1,12 ± 0,278	1,91 ± 0,192
СН <sub>4</sub> (метан)		1,74 ± 0,56	1,98 ± 0,055	1,39 ± 0,344	1,89 ± 0,232

Примечание. \* P≤0,05; \*\* P≤0,01.

Анализ корреляционных зависимостей между погодными и экологическими факторами городов за исследуемый период позволил обнаружить прямые и обратные корреляции между загрязнением атмосферы, погодными условиями и сезонами.

Так, в г. Караганде прослеживалась средняя положительная корреляция между наличием ветра и присутствием в воздухе таких загрязнителей, как оксид и диоксид азота, фенолы и формальдегиды (табл. 4).



**Корреляционные зависимости между метео- и экологическими факторами г. Караганды**

Караганда	Код загрязненности	ВВ	ВЧ РМ-2,5	ВЧ РМ-10	SO <sub>2</sub> (диоксид серы)	СО (оксид углерода)	NO <sub>2</sub> (диоксид азота)	NO (оксид азота)	Озон	Фенол	Формальдегид	СН <sub>4</sub> (метан)
Сезоны	-0,30	-	-0,19	-	-0,26	-	-	-	-	-	-	-
Ветер	-	-0,40	-	-	-0,34	-0,49	0,36	0,55	-	0,69	0,59	-0,33
Т (С)	-0,31	-	-0,18	-	-0,27	-	-	-	0,21	-	-	-
f влажность (%)	0,24	-	-	-	0,27	-	-	-	-0,29	-	-	-
Р (гПа)	0,18	-	-	-	-	0,23	-	-	-0,29	-0,21	-0,17	-
Осадки (мм)	-0,17	-	-	-	-	-	-	-	0,18	-	-	-
Снег (см)	0,24	-	0,28	-	0,25	-	-	-	-	-	-	-

Примечание. В таблице указаны только достоверные значения коэффициентов корреляции ( $p < 0,05$ ).

Наряду с этим наблюдалась слабая отрицательная корреляция между ветром и взвешенными частицами, диоксидом серы, оксидом углерода и метаном.

Кроме того, присутствие озона в атмосфере положительно коррелировало с температурой и осадками, отрицательно — с влажностью и давлением водяных паров. Код загрязненности слабо коррелировал с сезоном и практически со всеми погодными параметрами, за исключением ветра.

Слабая отрицательная корреляция, отмеченная между концентрациями ВВ, диоксида азота, оксида углерода и ветром, отражает влияние застойных явлений на рост приземных концентраций.

По г. Петропавловску обнаружены слабые корреляционные зависимости между погодными условиями и загрязнением воздуха, вместе с тем, наблюдалась связь между ветром и положительная корреляция с ВВ, оксидом углерода и отрицательная корреляция с такими загрязнителями воздуха, как диоксид серы и озон (табл. 5).

**Корреляционные зависимости между метео- и экологическими факторами г. Петропавловска**

Петропавловск	Код загрязненности	ВВ	ВЧ РМ-2,5	ВЧ РМ-10	SO <sub>2</sub> (диоксид серы)	СО (оксид углерода)	О <sub>3</sub> (озон)	Фенол	НСОН (формальдегид)	СН <sub>4</sub> (метан)
Сезоны	-	-	-	-	-	-	-	-	-0,21	-
Ветер	-	0,31	-	-	-0,27	0,27	-0,32	-	-	-
Т (С)	-	-	-	-	-	-	-	-	0,20	-
f влажность (%)	-	-	-0,20	-0,20	-	-	-	-	-	-
Р (гПа)	-	0,26	0,27	-	-	0,25	-	-	-	-
Р осадки (мм)	-0,21	-0,43	-0,30	-	-	-0,32	-	-	-	-
S снег (см)	-	0,21	-	-	-	0,21	-	-	-	-0,20

Анализ статистических связей между концентрациями загрязняющих веществ и метеорологическими параметрами, такими как скорость ветра, температура и влажность воздуха, позволил выявить лишь слабую корреляцию, что связано, на наш взгляд, с тем, что на уровень загрязнения влияет не отдельная метеорологическая характеристика, а комплекс этих характеристик, определяющих синоптическую ситуацию.

Биоклиматические индексы в физическом отношении характеризуют особенности тепловой структуры среды и являются косвенным индикатором состояния теплового поля окружающего человека. При расчете корреляционных связей между комплексными показателями погоды и экологическими факторами г. Караганды слабые положительные и отрицательные связи выявлены в основном

между кодами загрязнения и биоклиматическими показателями. Эквивалентно-эффективная температура (ЭЭТ) — показатель тепловой чувствительности с учетом влияния ветра. Как видно из табличных данных, этот показатель имел слабую отрицательную корреляцию с диоксидом серы, мелкими взвешенными частицами и кодом загрязненности и положительно коррелировал с озоном (табл. 6). Показатели г. Петропавловска не обнаружили подобных корреляций.

Т а б л и ц а 6

**Корреляционные зависимости между комплексными показателями погоды и экологическими факторами г. Караганды**

Караганда	Код загрязненности	ВВ	ВЧ PM <sub>2,5</sub>	ВЧ PM <sub>10</sub>	SO <sub>2</sub> (диоксид серы)	CO (оксид углерода)	NO <sub>2</sub> (диоксид азота)	NO (оксид азота)	O <sub>3</sub> (озон)	Фенол	НСОН (формальдегид)	СН <sub>4</sub> (метан)
ЭЭТ	-0,32	-	-0,18	-	-0,26	-	-	-	0,22	-	-	-
S суrowsть погоды Бодмана	0,31	-	-	-	0,19	-	0,21	0,25	-0,19	-	-	-
W ветро-холодовой индекс Сайпла	0,31	-	-	-	-	-	0,23	0,29	-0,18	0,18	0,18	-
AT эффект t	-0,32	-	-0,18	-	-0,26	-	-	-	0,25	-	-	-

Таким образом, как видно из наших исследований, вещества, загрязняющие воздушное пространство, могут влиять на погодные показатели, в частности, температуру воздуха, наряду с этим, погодные условия в свою очередь могут способствовать концентрации загрязнителей воздуха и усилению их воздействия на организм, о чем свидетельствуют положительные и отрицательные корреляционные связи между исследуемыми показателями.

*Заключение*

Ретроспективный анализ загрязненности атмосферы в исследуемых регионах позволил обнаружить высокий уровень загрязненности воздуха на протяжении всех сезонов в г. Караганде по сравнению с г. Петропавловском. Из многочисленных ингредиентов, загрязняющих воздушный бассейн города, в воздухе присутствовали вещества первой категории опасности — диоксид азота и озон, а также вещества второй категории опасности — фенол и формальдегид, содержание которых значительно превышало ПДК. Прослеживалась средняя положительная корреляция между наличием ветра и присутствием в воздухе названных загрязнителей. Наряду с этим, наблюдалась слабая отрицательная корреляция между ветром и взвешенными частицами, диоксидом серы, оксидом углерода и метаном. Кроме того, присутствие озона в атмосфере положительно коррелировало с температурой и осадками, отрицательно — с влажностью и давлением водяных паров, что согласуется с исследованиями и других авторов [13–15].

Следует отметить, что ситуация могла усугубляться в результате синергизма некоторых веществ, сходных по химическому строению и способных при совместном присутствии в воздухе усиливать негативное влияние на здоровье населения. В наших исследованиях было обнаружено, что в зимний сезон такой синергизм мог проявляться при совместном действии диоксида азота и формальдегидов, содержание которых в атмосфере достоверно превышало ПДК. В летний сезон аналогичные эффекты могли быть проявлены в результате синергизма озона и формальдегида. Таким образом, можно предполагать, что комплексное воздействие климатических условий и уровень загрязненности атмосферного воздуха в Карагандинском регионе могли значительно усиливать или видоизменять те возможные негативные последствия для здоровья человека, которые можно было ожидать при воздействии этих факторов в отдельности.

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### **11 жылдық кезеңдегі (2010–2020 жж.) Қазақстан қалаларындағы ауа райы жағдайы мен ауа сапасы арасындағы байланысты талдау**

Мақалада 11 жылдық кезеңдегі (2010–2020 жж.) Қарағанды және Петропавл қалаларының ауа кеңістігінде ауа райы жағдайлары мен ластанушы заттардың болуы арасындағы өзара байланысқа ретроспективті талдау жүргізілген. Зерттелетін қалалар атмосферасының ластану кодының динамикасы Петропавл қаласының көрсеткіштерімен салыстырғанда барлық маусымдар бойы Қарағанды қаласында ауаның ластануының неғұрлым жоғары деңгейін көрсетті. Қарағанды қаласының газтәріздес шығарындыларында бірінші қауіпті санатқа жататын заттар айтарлықтай басым болды: қыста азот диокси-

ді, көктемде және жазда озон. Қарағандының ауасында фенолдар мен формальдегидтер барлық маусымда ШРК-дан едәуір асып түсті. Ауа райы мен қоршаған орта факторларының арасындағы корреляцияны талдау атмосфераның ластануы, ауа райы жағдайлары мен жыл мезгілдері арасындағы орташа және әлсіз тікелей және кері корреляцияны табуға мүмкіндік берді. Қысқы маусымда синергизм азот диоксиді мен формальдегидтердің бір мезгілде болуына байланысты көрінуі мүмкін екендігі анықталды, олардың атмосферадағы мөлшері ШРК-дан айтарлықтай асып кетті. Жазғы маусымда ауада озон мен формальдегидтің жоғары концентрациясының бір мезгілде болуына байланысты синергияны күтуге болады. Петропавл қаласымен салыстырғанда Қарағанды облысындағы ауа райы жағдайларының, атмосфералық ауаның ластану деңгейі мен сипатының кешенді әсері адам денсаулығына жағымсыз салдарлардың айтарлықтай артуы немесе модификациялану ықтималдығын көрсетеді.

*Кілт сөздер:* ауа райы, ауаның ластануы, ластану коды, ластанудың кешенді индексі, стандартты индекс, биоклиматтық көрсеткіштер, қатты және газтәрізді заттар.

N.K. Smagulov, A.E. Konkabayeva, G.M. Tykezhanova, A.Zh. Sadykova,  
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## **Analysis of the relationship between weather conditions and air quality in the cities of Kazakhstan over an 11-year period (2010-2020) as the basis of epidemiological risks**

In this study, a retrospective analysis of the relationship between weather conditions and the presence of pollutants in the airspace of the city of Karaganda and Petropavlovsk for an 11-year period (2010-2020) was carried out. The dynamics of the air pollution code of the studied cities indicated a higher level of air pollution in the city of Karaganda throughout all seasons, compared with the indicators of the city of Petropavlovsk. Substances belonging to the first hazard category significantly prevailed in gaseous emissions in the city of Karaganda: nitrogen dioxide in winter, ozone in spring and summer. Phenols and formaldehyde were present in the air of Karaganda during all seasons, significantly exceeding the MPC. An analysis of the correlations between weather and environmental factors made it possible to find medium and weak direct and inverse correlations between atmospheric pollution, weather conditions and seasons. It was found that in the winter season, synergism could manifest itself due to the simultaneous presence of nitrogen dioxide and formaldehydes, the content of which in the atmosphere significantly exceeded the MPC. In the summer season, synergy could be expected due to the simultaneous presence of high concentrations of ozone and formaldehyde in the air. The complex impact of weather conditions, the level and nature of air pollution in the Karaganda region compared to the city of Petropavlovsk indicates the likelihood of a significant increase or modification of negative consequences for human health.

*Keywords:* weather, air pollution, pollution code, complex pollution index, standard index, bioclimatic indices, solid and gaseous substances.

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