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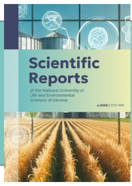

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Development of soybean yield and seed productivity elements depending on the variety

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Abstract. In the context of global climate change and the food crisis, soybeans (*Glycine max*) requires optimisation of the genetic potential to ensure stable and high productivity through the identification of genotypes with high adaptability in specific soil and climatic conditions, in particular the forest-steppe of Ukraine. The purpose of the study was to investigate the influence of varietal characteristics on the development of key morphological and productive features of soybeans and assess its compensatory ability during two years of research. The study was conducted during 2024-2025 using field, biometric, and correlation analyses to estimate plant height, the number of pods per plant, the number of branches, and the weight of 1,000 seeds. A high varietal dependence of all studied traits has been established, which emphasises the decisive importance of the genotype. A strong negative correlation was found between plant height and the number of pods per plant ($r = -0.897$), which indicates a negative effect of excessive vegetative growth on generative productivity due to a violation of the balance of sources and drains. It was confirmed that the intensity of branching had only a weak effect on crop development. The mechanism of compensatory ability during the years of research (phenotypic plasticity) was revealed: a decrease in the number of pods under unfavourable conditions led to a compensatory increase in the weight of 1,000 seeds. Among the samples studied, the varieties 'ES Mentor', 'Antracit' and 'Muza' had the most balanced combination of high seed productivity, seed quality, and satisfactory attachment height

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of the lower pods. The results of the study provide important information for optimising the varietal composition in the region, and also serve as a basis for breeding work aimed at adapting soybeans and improving the efficiency of fruit setting in the forest-steppe zone

Keywords: genotype; morphological features; compensatory ability; vegetative growth; correlation analysis

Introduction

Global demand for soybeans as one of the leading oilseed and protein crops necessitates not only an increase in yield, but also greater stability in the face of climate change and growing anthropogenic pressure on agroecosystems. The development of the soybean crop is a complex biological process determined by the interaction of the genetic characteristics of the variety with the growing conditions and is implemented through a system of morphological and productive characteristics of the plant. It is the structure of the crop, which reflects the relationship between the elements of vegetative and generative development that plays a key role in realising the potential of the crop and ensuring its adaptability in specific soil and climatic conditions.

R. Rani *et al.* (2023a) analysed genotype-environment interactions for soybean agronomic traits, specifically seed number and yield. Using associative mapping, the researchers found out how different environmental conditions affect the genetic characteristics and productivity of soybeans, which is important for breeding programmes and optimising the cultivation of this crop in changing climatic conditions. R. Rani *et al.* (2023b) focused on the study of genetic variations in soybeans through genome-wide association analysis (GWAS), aimed at identifying candidate genes that are responsible for crop yield characteristics. The results showed the importance of certain genetic markers that can be used to improve soybean varieties, in particular, to increase yield and stress resistance. The need to create stable and highly productive varieties emphasises the critical importance of detailed analysis of varietal characteristics. Although the use of biostimulants, as shown by B. Mazurenko *et al.* (2025), can significantly increase key yield components (number and weight of seeds), the authors themselves emphasise that these techniques only help to realise the genetic potential inherent in the variety, and their effectiveness largely depends on the biological characteristics of the crop, the initial level of genotype productivity, and the specific soil and climatic conditions of cultivation.

The key varietal trait is the plant architecture, which determines the efficiency of light use and resistance to lodging. N. Li *et al.* (2025) confirmed that varietal characteristics such as plant height, degree of branching, and height of attachment of lower pods are critical determinants, since their targeted genetic modification allows optimising the spatial organisation of shoots and increasing the outflow of assimilants to generative organs. The height of plants with high heritability serves as the basis for the development of nodes – potential places for the development of pods, which was analysed in detail by Q. Yang *et al.* (2021), who showed that this indicator is controlled by both stable genetic mechanisms and the response of plants to environmental conditions, in particular, temperature and water regime. Contemporary soybean breeding, according to research by S. Srivastava *et al.* (2025), is aimed at optimising the architecture (internode length, branching) to increase photosynthetic efficiency, since multivariate analysis of traits proved their direct and indirect effect on yield development through improved light interception and balance between vegetative and generative growth. Current research shows that soybean plant architecture is the result of the interaction of regulatory genes, hormonal signals, and environmental factors that control plant height, internode length, and branching (Li *et al*., 2024). J. Chandler (2025) found a paradox: although more nodes correlate with potentially more branches and pods, the total number of nodes can have a significant negative association with final yield. This is conditioned by the fact that varieties that over-invest resources in vegetative growth show a decrease in the efficiency of assimilation transfer to seeds.

A.T. Assfaw *et al*. (2025) found that the attachment height of lower pods is a highly inherited trait, giving breeders the opportunity to purposefully increase it. However, breeders' efforts to maximise the attachment height of the top pods face some challenges. The researchers have established a negative correlation between this indicator and the overall yield. This means that the energy resources spent on excessive elongation of the lower internodes (to increase the height of the pod attachment) can be diverted from the development of the crop. Thus, according to A.T. Assfaw *et al*., indeterminate varieties that continue vegetative growth after the beginning of flowering usually form a higher attachment height of the lower pods, compared to determinant varieties. Agricultural techniques (for example, narrow row spacing) can also be used to indirectly control this indicator, especially for early sowing. A.S. Karyawati *et al*. (2025), using path analysis, determined that the key traits with the highest direct positive impact on yield are the number of seeds in pods and the number of lower internodes.

The purpose of the study was to investigate the influence of varietal characteristics on the development of key morphological and productive features of soybeans and assess its compensatory ability during two years of research.

Materials and Methods

The objects of the study were 8 soybean varieties: 'Siverka', 'Arnika', 'Muza', 'Adamos', 'Aleksandrit', 'Antracit', 'ES Mentor', 'ES Vizitor' (Table 1). The study was carried out in accordance with the ethical standards provided for by the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973). The provisions of these documents aimed at protecting biological diversity and preventing threats to the species under study were observed.

Table 1. *List of soybean varieties*

No.	Variety	Type of development	Growing season, days	Originator
1	'Siverka'	semi-determinate	83-85	National Research Centre "Institute of Agriculture of the Ukrainian Academy of Agrarian Sciences of Ukraine"
2	'Arnika'	semi-determinate	83-85	National Research Centre "Institute of Agriculture of the Ukrainian Academy of Agrarian Sciences of Ukraine"
3	'Muza'	determinate	100-102	National Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine"
4	'Adamos'	indeterminate	95-100	Liudmyla Biliavska
5	'Aleksandrit'	semi-determinate	95-100	Liudmyla Biliavska
6	'Antracit'	determinate	100-105	Poltava State Agrarian Academy, Liudmyla Biliavska
7	'ES Mentor'	semi-determinate	105-115	Euralis Semences LLC
8	'ES Vizitor'	indeterminate	105-110	Euralis Semences LLC

Source: compiled by the authors

The research was conducted during 2024-2025 in the fields of the "Agronomic Research Station", a separate division of the National University of Life and Environmental Sciences of Ukraine, located in the forest-steppe soil and climate zone of Ukraine. To ensure the reliability and complexity of the assessment of economically valuable characteristics of soybean varieties, the study was carried out using field and statistical and analytical methods that provide relevant results in real agroecological conditions.

The field method was the main method for obtaining primary empirical data, including direct field observations and biometric measurements. It was used to evaluate the main indicators of soybean productivity elements, namely: plant height, number of branches on the plant, number of pods on the plant, number of seeds in the pod, number of seeds from the plant, height of attachment of the lower pod, weight of 1,000 seeds, duration of the growing season, and type of growth. Field observations were used to determine the phenophases and duration of vegetation, and to determine the type of growth. Biometric measurements allowed quantifying the structural elements of the crop. The assessment was carried out in accordance with generally accepted methods, which allowed comparing varieties by the main indicators of productivity elements. The weight of 1,000 seeds was determined by laboratory field analysis at a base humidity of 12%. The experimental plots had an area of 5.6 m^2 for each variety, where the predecessor in all years of research was corn. Pre-sowing tillage included the introduction of a compensatory rate of fertilisers at the rate of $N_{90}P_{60}K_{90}$. A single treatment with the fungicide Amistar Gold (Syngenta) was carried out during the growing season at a rate of 0.8 l/ha.

The obtained data was processed using the statistical and analytical method, using Microsoft Excel software and the ANOVA online calculator. Basic statistical calculations were performed using Excel, in particular, sums, averages, standard deviations, variation coefficients, and the least significant difference (LSD) between the studied indicators were determined. To perform dispersion analysis (ANOVA) for the purpose of comparing group averages and establishing the statistical significance of differences between varieties, ANOVA Calculator was used, namely, comparisons were made using the Tukey criterion (HSD). The use of statistical methods, in particular ANOVA, was justified because it allowed testing the influence of the "variety" factor on performance indicators and ensures the reliability of conclusions by separating random fluctuations from actual varietal differences. The difference between the indicators was considered statistically significant at a significance level of $p < 0.05$.

Results and Discussion

As a result of the conducted studies, a significant variability in the main morphological characteristics of soybean plants was established depending on the variety and year of cultivation. In 2024, the average plant height was 90.0 cm, ranging from 73.3 cm ('Antracit') to 104.7 cm ('ES Vizitor') (Table 2). The highest number of branches per plant was observed in varieties 'Adamos' (3.1 units) and 'ES Vizitor' (2.6 units). The number of pods on the plant varied from 27.3 units ('Siverka' up to 52.3 units ('Adamos'), and the number of seeds per plant – from 51.8 to 127.3 units, which indicates a significant productivity of the varieties 'ES Mentor' and 'Adamos'.

Table 2. *Parameters of valuable economic characteristics of soybean varieties in 2024*

Variety	Plant height, cm	Number of branches per plant, units	Number of pods per plant, units	Number of seeds per plant, units	Number of seeds per pod, unit	Height of lower pod attachment, cm	Weight of 1,000 seeds, g
'Siverka'	**103.0**	1.4	27.3	51.8	1.8	**21.1**	161.0
'Arnika'	78.8	2.5	44.7	115.9	2.6	15.6	122.0
'Muza'	100.2	1.6	29.8	74.4	**2.8**	13.8	**193.0**
'Adamos'	87.6	**3.1**	**52.3**	104.6	2.0	17.1	167.5
'Aleksandrit'	97.2	1.9	28.6	67.1	2.4	17.8	157.5
'Antracit'	73.3	2.2	**48.4**	114.8	2.4	13.2	**185.5**
'ES Mentor'	74.8	1.5	43.3	**127.3**	**2.9**	13.6	175.0

Table 2. *Continued*

Variety	Plant height, cm	Number of branches per plant, units	Number of pods per plant, units	Number of seeds per plant, units	Number of seeds per pod, unit	Height of lower pod attachment, cm	Weight of 1,000 seeds, g
'ES Vizitor'	104.7	**2.6**	38.7	116.1	**3.0**	13.7	132.5
Average	90.0	2.1	39.1	96.5	2.5	15.7	161.8

Note: values in columns that are significantly higher than the average at a 5% significance level are highlighted in bold

Source: developed by the authors based on own research

The number of seeds per pod averaged 2.5 units, the highest statistically significant values were observed in the varieties 'ES Vizitor' (3.0 units), 'ES Mentor' (2.9 units) and 'Muza' (2.8 units). The height of attachment of the lower pods ranged from 13.2-21.1 cm, which indicates different suitability of varieties for mechanised harvesting. The highest attachment height was observed in the variety 'Siverka', which is a positive sign for reducing losses during harvesting. The weight of 1,000 seeds varied from 122.0 g ('Arnika') to 193.0 g ('Muza'), averaging 161.8 g. This indicates the development of full-fledged, well-filled seeds under favourable growing conditions in 2024. In 2025, the average values of most attributes were lower compared to the previous year (Table 3). The average plant height was 75.3 cm, which is 14.7 cm less than in 2024. The highest were plants of the varieties 'Siverka' and 'Aleksandrit' (87.2 cm).

Table 3. *Parameters of valuable economic characteristics of soybean varieties in 2025*

Variety	Plant height, cm	Number of branches per plant, units	Number of pods per plant, units	Number of seeds per plant, units	Number of seeds per pod, unit	Height of lower pod attachment, cm	Weight of 1,000 seeds, g
'Siverka'	**87.2**	1.3	32.4	65.7	2.0	9.6	187.9
'Arnika'	65.4	**2.3**	38.0	73.9	1.9	7.3	186.4
'Muza'	80.0	1.7	35.5	86.8	**2.4**	8.1	110.1
'Adamos'	64.4	2.0	37.3	72.1	1.9	10.4	192.6
'Aleksandrit'	**87.2**	1.6	28.6	54.1	1.9	**14.0**	184.3
'Antracit'	79.5	1.5	30.4	63.2	2.1	7.4	**194.0**
'ES Mentor'	58.2	1.4	**52.6**	**126.2**	**2.4**	8.6	149.2
'ES Vizitor'	80.8	**2.5**	36.3	96.9	**2.7**	**12.8**	151.1
Average	75.3	1.8	36.4	79.9	2.2	9.8	169.5

Note: values in columns that are significantly higher than the average at a 5% significance level are highlighted in bold

Source: developed by the authors based on own research

The number of branches on average decreased to 1.8 units, and the maximum values were typical for varieties 'Arnica' (2.3 units) and 'EU Vizitor' (2.5 units). The number of pods on the plant was 36.4 units, the highest value was noted for the 'ES Mentor' variety (52.6 units). The average number of seeds per plant was 79.9 units, with a range from 54.1 ('Aleksandrit') to 126.2 units ('ES Mentor'). The number of seeds per pod was stable – 2.2 units on average, with the highest indicators in the varieties 'Muza', 'ES Mentor' (2.4 units) and 'EU Vizitor' (2.7 units). The attachment height of the lower pods ranged from 7.3 cm ('Arnika') to 14.0 cm ('Aleksandrit'), which also indicates a varietal feature. The weight of 1,000 seeds in 2025 ranged from 110.1 g ('Muza') to 194.0 g ('Antracit'), the average value was 169.5 g, which was higher than in the previous year.

According to N. Li *et al.* (2025), the number of seeds per pod is usually in the range of 2.5 units, which coincides with the results of current studies, considering the natural error and varietal differences. In 2024, the development of generative organs and overall plant productivity were higher due to optimal year conditions. The availability of resources at critical stages of growth contributed to the development of the maximum number of nodes and pods. This observation is consistent with the findings of H. Berhanu *et al.* (2021), who established a positive and significant correlation between plant height and the number of commercial pods per plant (r = 0.821), and between the number of commercial pods per plant and commercial pod yield (r = 0.822). Thus, the high growth rates that were observed in 2024 (in particular, higher plant heights) could directly contribute to the development of more pods, which became a key factor in overall productivity. H. Berhanu *et al.* also noted that the number of commercial pods per plant, the weight of green beans, and the height of plants are important selection criteria for increasing yield, which confirms the significance of the indicators evaluated in the current study. In 2025, despite a decrease in the height of plants and the number of pods, which, according to the correlations described by H. Berhanu *et al.*, led to a general decrease in yield), an increase in the weight of 1,000 seeds was observed. This result indicates a redistribution of assimilants by the plant. A limited number of developed pods received more nutrients, which improved the quality of individual seeds. The observation of the current study that high overall productivity in 2024 was accompanied by favourable generative organ development and high growth rates is consistent with results by other researchers, including H. Berhanu *et al.*, that the height of plants indirectly affects the yield due to the number of pods. This highlights that in both years of research – both under favourable and less favourable conditions – the number of pods per plant remains the dominant element of productivity. According to J. Chinnarat *et al.* (2025), it is not optimal to maximise the attachment height of the lower pods, but to reach the minimum allowable threshold (more than 10 cm), which ensures mechanised harvesting while maintaining the maximum yield potential. Three varieties corresponded to this parameter: 'Adamos' (10.4 cm), 'Aleksandrit' (14.0 cm), 'ES Vizitor' (12.8 cm).

The results of the study confirmed the high phenotypic plasticity of soybeans, which is a key adaptive feature of the crop. In particular, the data for 2025 clearly illustrate the mechanism of compensatory response: a decrease in the number of pods on the plant led to a statistically significant increase in the weight of 1,000 seeds. This fact confirms the existence of a classical negative correlation between quantitative and qualitative indicators of the crop structure. In conditions of limiting the number of nodes (for example, under the influence of stress), the plant redistributes assimilants, directing them to the remaining seed rudiments. This ensures better seed production and increases its seed value, serving as a mechanism for partially compensating for potential crop losses. The revealed compensatory ability of soybeans is consistent with the conclusions of a number of researchers who also studied the crop plasticity. X. Ran *et al.* (2023), examining the effect of density and seeding patterns, found that as row spacing increased, the yield and number of pods per plant decreased, but the weight of 1,000 seeds gradually increased. This confirms that in conditions of reduced competition (or a decrease in the number of elements), soybeans effectively move to an increase in individual seed weight as a compensatory element. A.A.B. Junior *et al.* (2018) emphasised that the most defining change associated with yield plasticity per plant is the number of pods on the side shoots, not the weight of 1,000 seeds. This suggests that compensatory mechanisms may be different: either due to morphogenesis (increased branching, as in A.A.B. Junior *et al.*), or due to physiological processes (an increase in the weight of 1,000 seeds, as in the current study and by X. Ran *et al.*). These differences are conditioned by varietal characteristics, the phase in which stress occurred, and the type of agrotechnical factors. The ability of varieties to adapt effectively, as shown by O. Mazur *et al.* (2024) (classification of varieties 'Artemida', 'Hoverla', and 'Ametist' as highly plastic), emphasises that the compensatory potential is a variety-specific feature. The weight of 1,000 seeds is the most sensitive element of the crop structure to adverse conditions that occur in the late stages of development, especially during the grain filling phase. D.A. Kiriziy & O.O. Stasik, (2022) claimed that off-season stress (drought, high temperatures) can dramatically reduce seed size, which requires the development of more seeds to achieve the same weight per unit area.

According to the results of two years of research, the varieties 'ES Mentor', 'Antracit', and 'Muza' demonstrated the most balanced complex of morphological and productive characteristics. They combine satisfactory plant height, high seed productivity and the weight of 1,000 seeds, which indicates their high efficiency in regulating assimilates, ensuring reliable realisation of yield potential in an unstable environment. Evaluating seed productivity, it should be noted that the varieties 'ES Mentor', 'Antracit', and 'Muza' were characterised by the highest indicators of the total weight of seeds per plant and the weight of 1,000 seeds, which indicates the effective use of photosynthetic products for grain filling. The high weight of 1,000 seeds, with a relatively moderate number of pods, is evidence of stable reproductive capacity, that is, high seed productivity. This combination of characteristics is desirable for varieties intended for seed production, since it ensures the development of homogeneous, aligned seeds with high germination.

To establish a significant difference between the average values of morphological features of soybean plants, multiple comparisons were performed using the Tukey criterion (HSD) (Table 4). The larger the numerical value of the difference, the more significantly the corresponding features differ from each other. Close relationships between individual traits and significant discrepancies between others were found.

Table 4. *Results of multiple comparison of soybean morphological traits (average for 2024-2025) according to the Tukey criterion (HSD)*

Pairs of indicators	Average difference between the compared groups	Standard error, SE	Tukey criterion, Q	Confidence interval limits		Critical average	p-value
x1-x2	80.66	4.10	19.66	62.83	98.50	17.84	1.327e-11
x1-x3	44.86	4.10	10.93	27.03	62.70	17.84	1.018e-8
x1-x4	5.55	4.10	1.35	-12.29	23.39	17.84	0.96
x1-x5	80.33	4.10	19.58	62.49	98.16	17.84	1.327e-11
x1-x6	69.90	4.10	17.04	52.06	87.74	17.84	1.329e-11
x1-x7	82.98	4.10	20.22	65.14	100.81	17.84	1.327e-11
x2-x3	35.80	4.10	8.73	17.96	53.64	17.84	0.00
x2-x4	86.21	4.10	21.01	68.38	104.05	17.84	1.327e-11
x2-x5	0.34	4.10	0.08	-17.50	18.17	17.84	1.00
x2-x6	10.76	4.10	2.62	-7.07	28.60	17.84	0.52
x2-x7	163.64	4.10	39.88	145.80	181.47	17.84	1.327e-11
x3-x4	50.41	4.10	12.29	32.58	68.25	17.84	3.719e-10
x3-x5	35.46	4.10	8.64	17.63	53.30	17.84	0.00
x3-x6	25.04	4.10	6.10	7.20	42.87	17.84	0.00
x3-x7	127.84	4.10	31.16	110.00	145.67	17.84	1.327e-11
x4-x5	85.88	4.10	20.93	68.04	103.71	17.84	1.327e-11
x4-x6	75.45	4.10	18.39	57.61	93.29	17.84	1.328e-11
x4-x7	77.43	4.10	18.87	59.59	95.26	17.84	1.328e-11
x5-x6	10.43	4.10	2.54	-7.41	28.26	17.84	0.56

Table 4. *Continued*

Pairs of indicators	Average difference between the compared groups	Standard error, SE	Tukey criterion, Q	Confidence interval limits		Critical average	p-value
x5-x7	163.30	4.10	39.80	145.46	181.14	17.84	1.327e-11
x6-x7	152.88	4.10	37.26	135.04	170.71	17.84	1.327e-11

Note: x1 – plant height; x2 – number of branches on the plant; x3 – number of pods on the plant; x4 – number of seeds per plant; x5 – number of seeds in the pod; x6 – height of attachment of the lower pods; x7 – weight of 1,000 seeds

Source: developed by the authors based on own research

According to the results of multiple comparison of soybean morphological features, most of the pairs compared had statistically significant differences ($p < 0.001$), which indicates significant variability of morphological features among the study groups. The largest differences were observed between the number of branches (x2) and the weight of 1,000 seeds (x7) (difference 163.64; $p < 0.001$), and between the number of seeds in a pod (x5) and the weight of 1,000 seeds (x7) (difference 163.30; $p < 0.001$). High values of differences were also found between the attachment height of the lower pods (x6) and the weight of 1,000 seeds (x7) (152.88; $p < 0.001$). That is, over the years of research, the weight of seeds changed regardless of the number of seeds per pod or the number of branches. Significant differences were also found between the number of seeds per plant (x4) and the number of pods (x3) (difference 50.41; $p < 0.001$), which logically reflects the structural relationship between these elements of productivity. The smallest differences, which have no statistical significance ($p > 0.05$), were observed between pairs x2-x5 (difference 0.34) and x5-x6 (difference 10.43), which indicates a close relationship between the number of branches, the number of seeds in the pod and the height of attachment of the lower pods – these traits are relatively stable within the variety.

Based on the analysis of the Tukey test, the most variable indicators in the soybean plants under study were the weight of 1,000 seeds and the height of attachment of the lower pods, while the number of branches and the number of seeds per pod were relatively constant. During the analysis of the correlation matrix data, both direct and inverse relationships between the main morphological and productive characteristics of soybean plants were identified (Table 5).

Table 5. *Nature of the relationship between the main biometric indicators and structural elements of the soybean crop*

	Plant height, cm	Number of branches per plant, units	Number of pods per plant, units	Number of seeds per plant, units	Number of seeds per pod, unit	Height of lower pod attachment, cm	Weight of 1,000 seeds, g
Plant height, cm	1						
Number of branches per plant, units	-0.148	1					
Number of pods per plant, units	-0.897	0.357	1				
Number of seeds per plant, units	-0.688	0.262	0.852	1			

***Table 5.** Continued*

	Plant height, cm	Number of branches per plant, units	Number of pods per plant, units	Number of seeds per plant, units	Number of seeds per pod, unit	Height of lower pod attachment, cm	Weight of 1,000 seeds, g
Number of seeds per pod, unit	-0.054	0.090	0.230	0.688	1		
Height of lower pod attachment, cm	0.625	-0.037	-0.566	-0.642	-0.513	1	
Weight of 1,000 seeds, g	-0.204	-0.247	0.031	-0.366	-0.735	0.124	1

Source: compiled by the authors

Plant height had a high negative correlation with the number of pods per plant (r = -0.897) and an average negative correlation with the number of seeds per plant (r = -0.688). This indicates a clear tendency to reduce the level of productivity with excessive vegetative growth. The relationship between plant height and the number of branches (r = -0.148) and the weight of 1,000 seeds (r = -0.204) was very weak and negative. Moreover, an average positive dependence (r = 0.625) was noted with the attachment height of the lower pods, which indicates the tendency of higher plants to form lower fruits at a higher height from the soil.

The results obtained contradict the data by J. Chandler (2025), who pointed out a significant positive correlation between plant height, number of nodes, and number of branches with the potential number of pods per plant. In particular, J. Chandler obtained a correlation coefficient of r = 0.697 between plant height and the number of pods per plant, which shows a direct relationship, while in the current study there is a strong inverse relationship (r = -0.897). The number of branches on the plant was weakly positively correlated with the number of pods (r = 0.357) and the number of seeds from the plant (r = 0.262), which indicates a weak direct relationship between the intensity of branching and overall productivity. The relationships with the weight of 1,000 seeds (r = -0.247) and the attachment height of the lower pods (r = -0.037) were weakly negative, which indicates that there was no significant influence of these indicators in the sample of the current study. Genetic research by J. Chandler revealed an important paradox: despite the fact that a larger number of nodes is positively correlated with a larger number of branches (r = 0.923) and pods (r = 0.899), the total number of nodes in a plant can have a significant negative association with the final yield (r = -0.611). This conclusion confirms the importance of a balance between the growth of vegetative mass (number of nodes) and the efficiency of its use for generative production, which partly explains the negative relationship in the current study between the height and number of pods.

A high positive correlation was found between the number of pods per plant and the number of seeds per plant (r = 0.852), which confirms their close interdependence as the main structural elements of yield. The correlation with the number of seeds per pod (r = 0.230) was weakly positive, and with the attachment height of the lower pods – an average negative (r = -0.566), which indicates a tendency to decrease the number of pods as the node point increases. The number of seeds from the plant had an average positive association with the number of seeds in the pod (r = 0.688). A weak negative correlation was found between the number of seeds and the weight of 1,000 seeds (r = -0.366), reflecting a typical feedback loop between the number and size of seeds (compensatory mechanism). The number of seeds per pod had a high negative correlation with the weight of 1,000 seeds (r = -0.735). This pattern is explained by the fact that when more seeds compete for assimilants within a single pod, their individual weight inevitably decreases.

The results obtained are consistent with modern ideas about soybean seed productivity as an integral indicator of the realisation of varietal potential. By definition of J.S.P.C. Evangelista *et al.* (2021), seed productivity, which combines quantitative and qualitative parameters of seeds, is a more stable feature compared to overall yield, since it is less susceptible to short-term fluctuations in environmental conditions. A similar opinion was expressed by Y. Gai *et al.* (2025), who in their review study emphasised that the ability to form full, well-filled seeds reflects the genetically determined effectiveness of the crop's reproductive system and can serve as a reliable criterion for variety adaptability. In conditions of high variability of agroecological factors, the stability of key elements of the crop structure in different years of cultivation becomes particularly important. This opinion is confirmed by the results of three-year studies by I. Havryliuk & H. Kovalyshyna (2024), who, using variance and correlation analysis, established significant genotypic differences in the weight of 1,000 seeds, the number of grains from the main reproductive unit, and their weight. The researchers concluded that it is the stability of these indicators that determines the adaptability of the variety, which is fully consistent with the differences between varieties in seed productivity parameters identified in the current study. According to A.M. Rybalchenko & Ye.V. Chub (2021), the assessment of soybean varieties cannot be limited to the analysis of individual elements of the crop structure, since such an assessment does not reflect the effectiveness of reproductive processes in general. The researchers emphasise the feasibility of an integrated approach, which includes the number of seeds per plant, the weight of seeds per plant, and the weight of 1,000 seeds as interrelated indicators of the realisation of varietal potential. Thus, the results of the current study, based on the simultaneous analysis of structural and seed indicators, confirm the feasibility of using seed productivity as a key criterion for comparative evaluation of soybean varieties. Therefore, the generalisation of the results of the observations in combination with the literature data shows that seed productivity is not only an indicator of the level of yield, but also an indicator of genetic stability and adaptive potential of the variety, which is of fundamental importance for the selection of promising genotypes in an unstable environment.

As a result of the study of varietal characteristics of soybeans in the conditions of the forest-steppe of Ukraine, it was found that the genotype has a significant impact on the development of elements of the crop structure and seed productivity. Studies have shown that the number of pods per plant is the main factor determining productivity, and the height of the plant has a negative impact on this indicator due to excessive vegetative growth. In addition, it was found that soybeans can adapt to unfavourable conditions through compensatory ability, redistributing assimilates to ensure greater seed weight.

Conclusions

During a two-year analysis of varietal characteristics of soybean yield structure development in the forest-steppe soil and climate zone of Ukraine, it was established that the development of yield structure elements and seed productivity is a highly varietal-dependent process. This is confirmed by the significant variability of the morphological and productive traits under study, which is statistically significant for most of the compared pairs of varieties, which emphasises the critical role of the genotype in the realisation of yield potential. A number of key internal relationships that regulate performance were identified. It was found that the number of pods per plant is the dominant element of the crop structure, showing a high positive correlation with the number of seeds per plant ($r = 0.852$). Simultaneously, a strong negative correlation was found between plant height and the number of pods per plant ($r = -0.897$), which indicates a negative effect of excessive vegetative growth on generative productivity. It should be noted that higher plants form lower pods at a higher altitude (r = 0.625), which is favourable for minimising losses during mechanised harvesting.

It was proved that soybean productivity is regulated by the mechanism of compensatory ability (phenotypic plasticity). In particular, in 2025, the activation of this mechanism was recorded, when a decrease in the number of pods led to a significant increase in the weight of 1,000 seeds. This reflects a fundamental negative correlation between quantitative and qualitative indicators, where limiting the number of nodes ensures better seed filling by redistributing assimilates. A similar high negative correlation was found between the number of seeds per pod and the weight of 1,000 seeds (r = -0.735), which confirms competition for resources between seeds in a pod. It was noted that the number of branches on the plant had only a weak positive correlation with the number of pods (r = 0.357) and the number of seeds from the plant (r = 0.262). This indicates that in the studied varieties under these conditions, the intensity of branching was not a determining factor in the development of seed productivity. The leading role in ensuring the final yield remains with the productivity of the main stem and the efficiency of fruit setting on it. According to the results of research, the varieties 'ES Mentor', 'Antracit', and 'Muza' demonstrated the most balanced development of elements of the crop structure and high seed productivity. They combine high seed productivity with a weight of 1,000 seeds, which indicates their effective regulation of the distribution of assimilates, high adaptive potential, and makes them promising for introduction into production in the forest-steppe of Ukraine.

Prospects for further research consist in an in-depth study of the interaction between genotype and environmental conditions using long-term observations and an expanded set of soybean varieties to clarify the mechanisms of seed productivity and adaptive stability of the crop.

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Conflict of Interest

None.

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Формування елементів структури врожаю та насіннєвої продуктивності сої залежно від сорту

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Анотація. У контексті глобальних кліматичних змін та продовольчої кризи, соя (*Glycine max*) потребує оптимізації генетичного потенціалу для забезпечення стабільної та високої продуктивності через ідентифікацію генотипів з високою адаптивністю в конкретних ґрунтово-кліматичних умовах, зокрема Лісостепу України. Мета дослідження полягала у вивченні впливу сортових особливостей на формування ключових морфологічних і продуктивних ознак сої та оцінці її компенсаційної здатності протягом двох років досліджень. Дослідження проводили протягом 2024-2025 років із застосуванням польового, біометричного та кореляційного аналізів для оцінки висоти рослин, кількості бобів на рослині, кількості гілок та маси 1000 насінин. Встановлено високу сортову залежність усіх досліджуваних ознак, що підкреслює визначальне значення генотипу. Виявлено сильну від'ємну кореляцію між висотою рослини та кількістю бобів на рослину (r = -0,897), що свідчить про негативний вплив надмірного вегетативного росту на генеративну продуктивність через порушення балансу джерел і стоків. Підтверджено, що інтенсивність гілкування мала лише слабкий вплив на формування врожаю. Виявлено механізм компенсаційної здатності в роки досліджень (фенотипової пластичності): зниження кількості бобів у несприятливих умовах призводило до компенсаторного збільшення маси 1000 насінин. Серед досліджених зразків сорти ‘ЕС Ментор’, ‘Антрацит’ і ‘Муза’ мали найбільш збалансоване поєднання високої насіннєвої продуктивності, якості насіння та задовільної висоти прикріплення нижніх бобів. Результати дослідження надають важливу інформацію для оптимізації сортового складу в регіоні, а також слугують основою для селекційної роботи, спрямованої на адаптацію сої та підвищення ефективності зав'язування плодів у зоні Лісостепу

Ключові слова: генотип; морфологічні ознаки; компенсаційна здатність; вегетативний ріст; кореляційний аналіз

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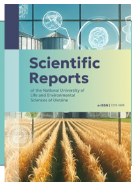

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Vermiculture cultivation on a substrate containing composted wheat straw using an accelerated method

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Abstract. The relevance of the study is conditioned by the need to improve the technology of manufacturing substrates for vermiculture growing, which is important for obtaining high-quality organic fertiliser and improving the efficiency of biotechnological processes for organic waste disposal. The goal was to determine the optimal dose of fermented wheat straw as part of the substrate to increase the growth, development and reproductive capacity of vermiculture. The experiment was conducted in the vivarium of the Bila Tserkva National Agrarian University on seven groups of microbeds formed based on bovine cattle manure with varying contents of composted wheat straw. The Control Group contained cattle manure and only fresh crushed straw, and the substrate from the experimental microbeds included 2.0-6.0% fermented wheat straw. One hundred sexually mature worms were placed in each microbed. For 125 days, the number and weight of sexually mature and non-sexually mature individuals, the number and weight of their cocoons were monitored. The results were processed using variational statistics methods. The best indicators were found with the introduction of 3.0-4.0% of composted straw in the substrate. In these groups, a significant increase in the number and biomass of worms, and an increase in the number of cocoons were recorded. It was recorded that the optimal level of composted straw contributed to improving the adaptation of the population, increasing the number and weight of sexually mature worms in the experimental microbeds. Exceeding the content of composted wheat straw to 5.0-6.0% did not provide an increase in the number of worms in microbeds. This indicates the presence of an upper limit of the population's tolerance to an increase in the carbon-to-nitrogen ratio in the substrate. It was determined that the optimal dose is 3.0% composted wheat straw, which provides a statistically significant increase in the number and weight of worms compared to the Control. The use of this dose helped to maintain a biologically balanced ratio of nutrients and created conditions for effective reproduction and development of vermiculture. The results obtained can be used to develop effective methods for growing vermiculture based on composted wheat straw, which will help to improve soil fertility and increase the efficiency of organic farming

Keywords: hybrid Red California worms; worm cocoons; microbed; sexually mature individuals; fermentation

Introduction

Growing vermiculture on a substrate with composted wheat straw by the accelerated method is a promising area in the field of organic farming, which contributes to the sustainable use of natural resources and reduces the negative impact on the environment. Composting wheat straw together with other organic materials allows creating a high-quality substrate, which is an ideal environment for the growth and reproduction of worms. The use of vermiculture products not only improves the structure of the soil and increases its fertility, but also contributes to the preservation of moisture, which is especially important in the context of climate change and insufficient water resources. In addition, vermicompost is an environmentally friendly, organic fertiliser that provides plants with the necessary macro- and microelements and other essential nutrition factors that contribute to the realisation of their genetic potential.

The transition from growing vermiculture on a substrate with composted wheat straw to using it in animal feed rations opens up new opportunities for sustainable management of agricultural resources. Analysing the effect of wheat straw chopping length, the study by C.D. Havekes *et al.* (2020a) showed how the physical properties of straw can affect feed consumption and animal health, which, in turn, increases the efficiency of using organic materials in agriculture. This study adds to the understanding of how innovative straw processing techniques can be applied to improve feed quality and optimise animal nutrition. L.V. Mitiohlo *et al.* (2023) investigated the physical and chemical parameters of wheat straw fermented using a Ukrainian-made biodestructor. The results showed that fermentation of straw significantly improves its organic composition and increases its suitability for use as a feed or substrate for growing vermiculture. This confirms the effectiveness of using biotechnological approaches in the treatment of agricultural waste to improve its quality and environmental safety.

F.A.M. Tuyttens (2005), C.D. Havekes *et al.* (2020b) considered that livestock production technologies involve the use of various types of straw, in particular wheat straw, which is used as coarse feed and as bedding. In the conditions of Ukraine in recent years, there has been a decrease in the number of ruminants, which contributes to the accumulation of significant volumes of straw, which is often stored for a long time. This leads to its spoilage, microbiological contamination, and loss of nutritional value. According to Q. Sun *et al.* (2025), accumulated straw poses an environmental burden, since it releases greenhouse gases during rotting and can be a source of pathogens.

In modern environmental approaches, the development of efficient and safe technologies for its disposal is relevant. One of these solutions was the use of accelerated microbiological composting. I.S. Osipenko & S.V. Merzlov (2023) developed a method for biodegradation of spoiled wheat straw using the BTU-CENTR biological product. Under the action of microorganisms, intensive hydrolysis of the hemicellulose complex and partial destruction of lignin occurred, which significantly accelerated composting. It was found that a dose of biodestructor of 28 cm^3/t reduced the loss of nitrogen and phosphorus by 26.7% and 37.2%, respectively, which is important for reducing the negative impact on the environment. I.S. Osipenko & S.V. Merzlov paid special attention to the interaction between microbial consortia and vermiculture in combined straw processing technologies. The researcher noted that fermentation not only enriches the substrate with available nutrient compounds, but also plays the role of a "detoxification" stage: at excessive concentrations of ammonia, worms die or are suppressed, while enzymatic processes allow stabilising nitrogen compounds in a form that is safe for their development. Thus, the interaction of bacteria and vermiculture has a synergistic character: microorganisms create optimal conditions for worms, and worms, in turn, structure the substrate and form the final product with high agrochemical value.

Global studies confirm the effectiveness of pre-microbial treatment of straw. A. Sahu *et al.* (2025), who found that thermophilic lignocellulosolytic microorganisms significantly increase the composting efficiency of rice and wheat straw, providing a significant increase in the nutrient content of compost compared to conventional methods and offering an environmentally safe alternative to processing plant residues. One of the promising areas for further use of the resulting composted biomass is its use as a substrate for vermiculture. Vermicomposting with the participation of *Eisenia fetida* is widely recommended as an effective method of disposing of plant waste and improving its structural and nutritional properties (Alwaneen, 2016). P. Vyas *et al.* (2022) found that the combination of microbial degradation and vermicomposting increases the rate of substrate stabilisation by 30-50% and promotes an increase in the content of macronutrients (N, P, K). Similar results are presented in the paper by Y. Wang *et al.* (2024), where a study on composting corn straw and pig manure with microbial inoculants showed that thermophilic and low-temperature microbial systems significantly enhance lignocellulose degradation and humic substance development, which increases process efficiency and is essential for sustainable organic waste management in agriculture.

In general, the analysis of the literature confirms that integrated approaches to processing spoiled wheat straw are the most technologically sound and environmentally appropriate. The tendency to combine microbial destruction and vermiculture corresponds to modern principles, reduces the burden on the environment, and promotes the production of organic fertilisers that increase soil fertility. Thus, the results of numerous studies indicate a high prospects for the use of integrated biotechnologies in solving the problem of organic waste disposal in the agricultural sector. In agriculture, straw-based vermicompost has shown significant agronomic potential. Based on the results of the considered studies, it can be concluded that the use of composted wheat straw as a substrate for growing Red California worms is a promising area for environmentally oriented management of agricultural waste. Therefore, conducting research to establish the effectiveness of growing worms on a substrate of fermented bovine cattle manure with different doses of spoiled wheat straw composted by the accelerated method is of important scientific and practical importance.

The purpose of the study was to determine the optimal dose of composted (prepared by the accelerated method) wheat straw as part of the vermiculture substrate.

Materials and Methods

The study of the effect of different doses of fermented wheat straw in the substrate on the growth and development of vermiculture was conducted in the vivarium of the Institute of Animal Husbandry and Food Technologies of the Bila Tserkva National Agrarian University. A substrate for growing vermiculite for 7 groups of microbeds was prepared from bovine cattle manure composted for 8.0 months using conventional methods (without the addition of biodestructors and aeration) and shredded uncomposted wheat straw. The study was conducted according to the Convention on Biological Diversity (1992).

In the Control Group, bovine cattle manure containing 3.0% by weight of fresh wheat straw was used as a substrate for a hybrid of Red California worms. In the Experimental Group 1, the microbed substrate, in addition to the main components, contained 2.0% wheat straw composted using the BTU-CENTR biodestructor. Microbeds from the Experimental Group 2 were formed from a substrate containing 3.0% fermented wheat straw. In the Experimental Group 3, bovine manure containing 4.0% composted wheat straw was used to form microbeds. The substrate from Experimental Groups 4 and 5 contained 5.0 and 6.0% fermented wheat straw. The weight of the substrate in each microbed was 17.5 kg.

The average weight of worms older than 90 days, which were placed in the microbed at the beginning of the experiment, was 0.75 ± 0.03 g. The experiment lasted 125 days. 100 sexually mature worms were added to each microbed. At the end of the study, worms were divided into sexually mature and non-sexually mature, counted, and the weight of worms and their cocoons was determined. The air temperature in the vivarium with microbeds was maintained at the level of 23-25°C. Aeration of the substrate with vermiculture was performed every 2 days (Table 1).

Table 1. *Experiment design using worms*

Microbed group	Number of individuals in one microbed	Factor under study
Control	60.0	Substrate (main) for growing worms (97.0% fermented cattle manure + 3.0 crushed wheat straw)
Experimental 1	60.0	Main substrate containing 2.0% compostable wheat straw
Experimental 2	60.0	Main substrate containing 3.0% compostable wheat straw
Experimental 3	60.0	Main substrate containing 4.0% compostable wheat straw
Experimental 4	60.0	Main substrate containing 5.0% compostable wheat straw
Experimental 5	60.0	Main substrate containing 6.0% compostable wheat straw

Source: compiled by the authors

The weight of sexually mature and non-sexually mature worms and their cocoons was recorded using techno-chemical and analytical scales. The experimental results were statistically processed to determine the significance using Statistica software suite.

Results and Discussion

Control of the number of worms and their weight was carried out on day 125 of the experiment. In the Control Group, the number of sexually mature worms in one microbed was 405 units. When 2.0% of fermented wheat straw was included in the substrate, the number of sexually mature worms increased by 1.2%, but the difference in relation to the Control Group was not statistically significant. Cultivation of vermiculture on a substrate containing 3.0% fermented wheat straw revealed an increase in the number of sexually mature worms by 18.5% relative to the Control Group ($p < 0.001$). This confirms the rapid adaptation of vermiculture to the substrate and the increased transformation of nutrients from it into the body of worms. In the Experimental Group 3, an increase in the number of sexually mature worms by 19.2% compared to the Control Group was shown. The difference was statistically significant (Table 2).

Table 2. *Number of worms on day 125 of the study, M ± m, n = 6*

Microbed group	Sexually mature individuals per bed		Sexually mature individuals per bed	
	quantity, units	weight, g	quantity, units	weight, g
Control	405 ± 5.4	303.7 ± 7.88	4,750 ± 84.6	522.5 ± 14.6
Experimental 1	410 ± 6.8	315.7 ± 10.5	4,790 ± 89.6	526.9 ± 17.5
Experimental 2	480 ± 5.5***	369.6 ± 8.6***	5,100 ± 86.5*	663.0 ± 11.4***
Experimental 3	482 ± 6.3***	376.0 ± 9.3***	5,050 ± 83.7*	606.3 ± 12.9**
Experimental 4	415 ± 4.3	307.1 ± 7.7	4,764 ± 88.5	514.4 ± 19.5
Experimental 5	395 ± 7.5	284.4 ± 11.6	4,630 ± 97.7	457.1 ± 18.2*

Note: ** – $p < 0.01$; *** – $p < 0.001$ – relative to the Control Group

Source: compiled by the authors

With an increase in the additional content of composted wheat straw in the substrate (Experimental Group 4), the increase in the number of worms in microbeds was not statistically significant. At the highest dose of composted straw, the number of sexually mature worms in microbeds decreases by 2.4% compared to the Control Group. The difference was not statistically significant. The influence of the content of composted straw in the substrate on the body weight of sexually mature worms was proved. The average weight of one individual in the Control Group was 0.74 g. In microbeds with a substrate content of 2.0% composted wheat straw, the average weight of one worm (sexually mature) was 4.0% higher than in the Control Group. Growing vermiculture on a substrate with a compostable wheat straw content of 3.0%, it was possible to obtain 21.6% more biomass of sexually mature worms. The difference was statistically significant. A statistically significant increase in the biomass of the Red California worm hybrid was also found in the Experimental Group 3, where the substrate contained 4.0% composted wheat straw. The weight of sexually mature worms grown on a substrate with contents increased by a statistically insignificant amount. The difference with the Control Group was 1.1%. When growing vermiculture in a substrate containing 6.0% composted wheat straw, the weight of sexually mature worms decreased by 6.3%. The difference was of a trend nature.

Describing the number of individuals who did not reach sexual maturity, it was proved that in microbeds from the Control Group, the indicator was 4,750 units. When growing vermiculture on a substrate containing 2.0% composted wheat straw, the number of worms was 0.8% higher than in the Control Group. The difference was within the margin of error. The largest number of young worms was in a substrate containing 3.0% composted wheat straw. The difference with the Control Group was 7.3% and was statistically significant. An increase in the population size relative to the Control Group in microbeds where worms were grown using a substrate containing 4.0% composted wheat straw was also proved. The increase in population size was statistically significant. In terms of the number of non-sexually mature worms, the Experimental Group 4 practically did not differ from the Control Group. The increase in the number of worms relative to the Control Group was only 0.2%. At the highest dose of composted straw in the substrate, the number of non-sexually mature worms was 2.5% lower compared to the Control Group. The difference was within the margin of error.

Investigating the weight of worms that did not reach sexual maturity, it was found that the more individuals in microbeds, the greater their weight. The average weight of individuals who did not reach sexual maturity in the Control Group was at the level of 0.11 g. When growing worms on a compostable substrate (Experimental Group 1), the average weight of one individual increased by 0.8%. It was found that the weight of vermiculture from the Experimental Group 2 increased by a statistically significant amount. The difference with the Control Group was 26.8%. The use of a substrate containing 4.0% composted straw contributed to an increase in the weight of worms in the Experimental Group 3 by 16.0% ($p < 0.01$). A lower average weight of one worm (not sexually mature) was found in the Experimental Group 3. The difference with the Experimental Group 2 was at the level of 8.5%. Despite the fact that the number of non-sexually mature individuals in the Experimental Group 4 was higher than in the Control Group, however, their weight was lower than in the Control Group by 1.6%. It was found that the weight of worms grown on a substrate with the highest content of composted wheat straw decreased by a statistically significant amount. The difference with the Control Group was 12.5%.

The optimal content of composted wheat straw in the substrate composition has been experimentally established. Growing vermiculture on a substrate of bovine cattle manure with a content of 3.0% composted straw allows increasing the number of worms and their weight due to the optimal content and availability of nutrients. The number of young worms depends on the number of cocoons. At the end of the experiment, the number of cocoons in the Control Group was at the level of 640 units. When using a substrate containing 2.0% composted straw, the number of cocoons in microbeds increased by 2.0% relative to the Control Group. In the Experimental Group 2, the number of cocoons was 10.1% higher than in the Control Group. The difference was statistically significant (Figure 1).

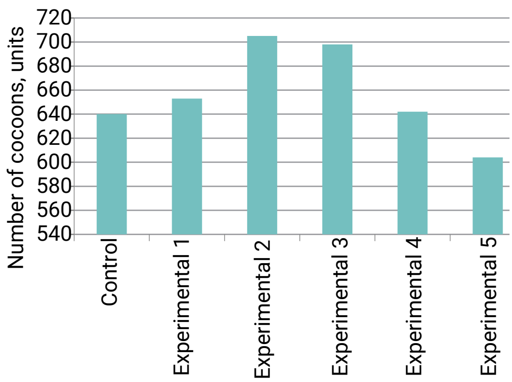

Figure 1. *Average number of cocoons in microbeds*

Source: compiled by the authors

When growing worms on a substrate containing 4.0% composted wheat straw, the number of cocoons was higher relative to the Control Group by a statistically significant amount. The difference with the Control Group was at the level of 9.0%. The indicator of the number of cocoons in the Experimental Group 4 almost did not differ from the data obtained in the Control Group. The lowest cocoon content was found in microbeds where the substrate contained 6.0%. The difference with the Control Group was of a trend nature.

Not all groups increased the number of cocoons in proportion to the number of worms that reached sexual maturity. A study was also conducted to establish the weight of cocoons before the young worms left them. In the Control Group, this indicator was 640 units. The weight of cocoons depends mainly on the number of worms that develop inside. In the Control Group, this indicator was at the level of 15.2 mg. When growing worms on a substrate containing 2.0% composted straw, the weight of cocoons deposited by them was 3.2% higher compared to the Control Group. The difference was of a trend nature (Fig. 2).

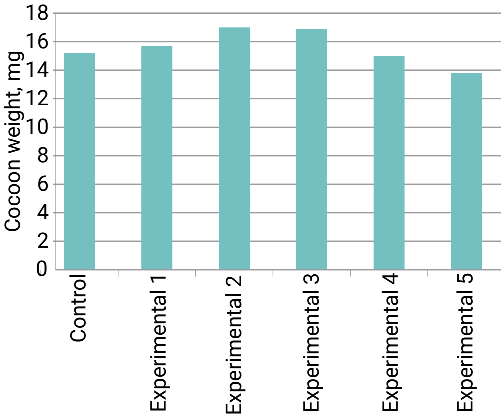

Figure 2. *Average weight of cocoons in microbeds*

Source: compiled by the authors

In the Experimental Group 2 of microbeds, the average weight of cocoons was higher by 11.8% compared to the Control Group. The difference was statistically significant. It was found that cocoons from the group where the substrate contained an additional 4.0% of composted wheat straw had a higher weight compared to cocoons from the Control Group by 11.2 %. The increase in the indicator was at the level of statistical significance. An increase in the content of composted straw in the substrate to 5.0% does not lead to an increase in the weight of worm cocoons in microbeds from the Experimental Group 4. An increase in the available carbon content due to the content of 6.0% composted wheat straw in the substrate affected the decrease in the average weight of cocoons. The difference with the Control Group was 9.2% and was statistically significant.

The concentration of fermented wheat straw in the substrate affects the reproduction and growth of worms, and the number of cocoons they form. Growing vermiculture in a substrate containing 3.0% composted wheat straw leads to an increase in the number of sexually mature worms in one microbed compared to the option where the substrate contained only fresh crushed straw (Control Group). According to their biological characteristics, worms reach sexual maturity by day 90 of their life, and young worms develop in a cocoon for up to 15 days under optimal growing conditions (Herasymenko, 2006). Based on this, an increase in the number of sexually mature individuals in microbeds in the presence of 3.0% composted straw contributes to accelerated adaptation of worms and the creation of optimal conditions for mating. The rationale for this phenomenon is that with the optimal content of composted wheat straw in the substrate, worms do not experience habitat discomfort. In addition, this indicates the absence of harmful compounds in composted straw. According to Y. Cao *et al.* (2021) straw mainly improves the productivity of earthworms during vermicomposting.

The results obtained in the current study show that the introduction of an optimal dose of composted wheat straw into the substrate for growing Red California worms provides a significant increase in both the number of sexually mature individuals and the total weight of worm biomass. This pattern is consistent with the logic of biochemical processes that occur during microbial destruction of straw, and is confirmed by a number of scientific sources. Composting using a biodestructor containing a complex of cellulolytic, amylolytic, and nitrogen-fixing microorganisms significantly increases the bioavailability of organic nitrogen and carbon, which is a key factor in earthworm nutrition. As noted by V.H. Herasymenko (2006), the optimal ratio of carbon to nitrogen in vermiculture substrates should be approximately 20:1. The results of the current study show that adding exactly 3.0% by weight of composted straw allows bringing the value of this indicator closer to the biologically optimal one. In addition, the increased availability of easily accessible forms of carbon after composting allows worms to adapt more quickly to the substrate and consume organic matter more actively.

When comparing the data of the current study with the results of international scientific papers, it was found that the identified patterns were consistent with the conclusions of R. Panjgotra *et al.* (2019). In particular, these researchers have shown that the use of wheat straw, even non-composted, contributes to an increase in biomass growth of Eisenia fetida by increasing the structural looseness of the substrate and increasing its air permeability. However, R. Panjgotra *et al.* did not use preliminary microbial destruction, which is a limitation of their study, since it did not consider the potential for increasing the bioavailability of nutrients. But the data of the current study shows that it is biodegradation that accelerates the decomposition of cellulose and hemicellulose, which creates more favourable conditions for the assimilation of the substrate by worms.

The properties of the compostable straw substrate observed in the current study are also consistent with the results of A. Nigussie *et al.* (2021), who found that microbial inoculants generally have a positive effect on the composting process, increasing the total nitrogen and phosphorus content, humification levels, compost maturity, and accelerating the degradation of cellulose, hemicellulose, and lignin, although their effect on bioavailable forms of nutrients is negligible. In the case of a similar effect – the preservation of nitrogen and phosphorus – was found by I.S. Osipenko & S.V. Merzlov (2023), which is fully consistent with international data. In addition, the results of current studies are consistent with the findings of A. Vyas *et al.* (2022), who obtained an increase in vermicomposting productivity using a combined microbial-vermicomposting approach. The researchers proved that pre-destruction of plant material stimulates the reproduction of worms, increases their biomass, and accelerates the development of humic substances. Data from the current study also indicate an increase in live weight gain of *Eisenia fetida* with an optimal content of composted straw, which is a confirmation of similar trends. In addition, P. Vyas *et al.* (2022) found that the combination of microbial fermentation and vermicomposting leads to improved degradation and conversion of organic matter, which is consistent with an increased conversion rate of organic mass to vermicompost and stimulation of worm growth with optimal substrate treatment. This is consistent with the results of the current study, because the optimal dose of composted straw contributed to faster assimilation of the substrate by worms and improved their reproduction and biomass accumulation. A slightly different aspect of the problem was considered by I. Ahmad *et al.* (2022), who investigated the effects of vermicompost derived from straw. They found that composted straw is a high-quality substrate for creating nutritious vermicompost, which confirms the positive impact of such biomass on ecosystem processes. This is consistent with the findings of the current study on the positive effects of such compost on vermiculture. Therefore, the results obtained in the current study logically fit into global trends involving the integration of microbial composting and vermiculture.

Summarising, it is worth noting that the results of the current study do not contradict the data of other researchers, but on the contrary – they complement them, highlighting the scientific originality: the use of the optimal dose of composted wheat straw (3.0%), which provides the most favourable conditions for growth and development of *Eisenia fetida*. Thus, the established pattern regarding the effectiveness of this dose is a logical continuation of existing scientific developments and contributes to improving the efficiency of biotechnologies for processing agricultural waste. In scientific publications, the problem of accumulation of spoiled wheat straw was considered as an important environmental and production challenge for the agricultural sector. J.K. Leet & D.C. Volz (2013) and X. Shen *et al.* (2015) noted that unutilised straw can cause a complex of negative consequences: contamination of soil and water with decomposition products, accumulation of phytopathogenic microorganisms and harmful metabolites, and a potential increase in greenhouse gas emissions due to uncontrolled rotting. It was emphasised that straw, even in its damaged state, remains a renewable resource and contains organic compounds (carbohydrates, fibre, proteins, etc.) that are suitable for use as a substrate in biotechnological processes. This position is consistent with the approach used in the current study, where straw was considered not as inefficient waste, but as a substrate component for bio-processing.

In a number of studies, the expediency of technological schemes in which preliminary microbial treatment of organic mass was combined with subsequent vermicultivation was justified. In particular, H. Zhang *et al.* (2016) showed that controlled biotechnological composting conditions contributed to more active decomposition of organic matter and substrate stabilisation. A. Nasiru *et al.* (2013) considered vermicomposting as a tool for sustainable organic waste management and emphasised that the efficiency of the process increases with pre-preparation of the substrate, which reduces toxicity and improves nutrient availability. A similar statement was supported by I. Zekker *et al.* (2019; 2021), who noted that the use of microbial conglomerates allows controlled initiation of hydrolysis of organic components, breakdown of complex polysaccharides, reduction of substrate toxicity, and optimisation of ammonia concentrations. The results obtained in the current study were consistent with the above provisions. The effect of including composted straw in the substrate was manifested as an improvement in the conditions for the growth and reproduction of vermiculture, which was logically explained by the mechanisms described by A. Nasiru *et al.* (2013). In particular, during fermentation, as noted in these studies, microorganisms synthesised a range of hydrolytic enzymes that hydrolysed insoluble polymers (cellulose, hemicellulose, and partially lignin) into more accessible forms. The resulting intermediates (simple sugars, amino acids, mineral compounds) formed a more nutritious substrate suitable for the next stage – vermicomposting. It was this transformation of the substrate that could determine the improvement in vermiculture performance found in the study after the addition of composted straw.

Importantly, the results of the current study are also consistent with the biological prerequisites for effective vermiculture described by K.D Brown (2019). The researcher emphasised that the most effective in world practice are various species of manure worms, in particular, a hybrid of Red California worms *Eisenia fetida*, which is characterised by a high intensity of consumption of the nutrient medium in which it lives, rapid growth of biomass, and the ability to efficiently process organic substrates. It was also noted that under the condition of preliminary fermentation of the substrate, such worms show maximum growth rates and quickly transform the mass treated with microorganisms into high-quality vermicompost. Therefore, the use of a fermented (composted) component in the substrate in this study corresponded to the approaches that were considered most effective for realising the biological potential of *E. fetida*.

The results of the study show that the optimal content of composted wheat straw in the substrate for growing worms is 3.0%. This concentration provides a significant increase in both the number and biomass of worms, contributing to their efficient development. Higher doses of composted straw do not provide additional benefits and do not contribute to further population growth. Thus, the use of 3.0% composted straw is the most effective for optimising the processes of growing vermiculture. Adding composted straw to the substrate improves the conditions for the growth and development of worms, increasing their adaptation and reproductive ability. Exceeding the optimal dose of straw does not lead to improved results and may negatively affect the efficiency of the process.

Conclusions

With the introduction of an additional 3.0% composted wheat straw in the substrate (bovine cattle manure containing 3.0% by weight of fresh non-composted wheat straw), a significant increase in the total biomass of vermiculture in one microbed was established. The data obtained confirm that composted straw is an effective structural and nutritional supplement that can stimulate both the development of the substrate microbiota and the subsequent growth of vermiculture. It was also found that the use of 3.0% composted wheat straw as part of the substrate contributes to an increase in the average weight of sexually mature worms. In particular, their weight increased by 21.6% compared to the option where composted straw was not added. This indicates that composted straw has a positive effect not only on the total number and biomass of the worm population, but also on their physiological state, providing a more stable and balanced nutrient environment for their development and reproduction.

A statistically significant increase in the number and weight of worms that have not reached sexual maturity in microbeds with a substrate containing 3.0% composted wheat straw indicates an improvement in the bioavailability of hydrolysed organic compounds. The presence of available carbon and its optimal ratio to nitrogen formed favourable conditions for the growth of non-sexually mature worms, which are the first to respond to changes in the qualitative characteristics of the substrate, demonstrating an increased growth rate. The results of the study confirm that composted wheat straw obtained using Ukrainian-made biodestructors can be considered as a promising environmentally safe component for enriching substrates for vermiculture. The use of 3.0% composted straw is the optimal dose to stimulate the growth and reproduction of Red California worms.

Thus, the research is not only theoretical, but also of significant practical importance for the development of ecological technologies for bio-processing of organic raw materials and improving the efficiency of vermicompost production. A promising area of further research is the investigation of the chemical composition of vermiculture biomass grown on a substrate with different contents of composted wheat straw.

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Conflict of Interest

None.

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Вирощування вермикультури на субстраті із вмістом компостованої соломи пшениці прискореним методом

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Анотація. Актуальність дослідження зумовлена необхідністю вдосконалення технології виготовлення субстратів для вирощування вермикультури, що має важливе значення для отримання високоякісного органічного добрива та підвищення ефективності біотехнологічних процесів утилізації органічних відходів. Мета полягала у визначенні оптимальної дози ферментованої соломи пшениці у складі субстрату для підвищення росту, розвитку та відтворювальної здатності вермикультури. Експеримент проводився у віварії Білоцерківського національного аграрного університету на семи групах мікролож, сформованих на основі гною великої рогатої худоби (ВРХ) із різним вмістом компостованої соломи пшениці. Контрольна група містила гній ВРХ і лише свіжу подрібнену солому, до субстрату із дослідних мікролож включали 2,0-6,0 % ферментованої соломи пшениці. У кожне мікроложе вносили по 100 статевозрілих черв'яків. Протягом 125 діб контролювали чисельність та масу статевозрілих і нестатевозрілих особин, кількість і масу їх коконів. Обробку результатів здійснювали методами варіаційної статистики. Найкращі показники встановлено за включення 3,0-4,0 % компостованої соломи у субстрат. У цих групах зафіксовано істотне зростання чисельності та біомаси черв'яків, а також підвищення кількості коконів. Зафіксовано, що оптимальний рівень компостованої соломи сприяв покращенню адаптації популяції, підвищенню кількості та маси статевозрілих черв'яків у дослідних мікроложах. Перевищення вмісту компостованої соломи пшениці до 5,0-6,0 % не забезпечило збільшення кількості черв'яків у мікроложах. Це свідчить про наявність верхньої межі толерантності популяції до збільшення співвідношення Карбону до Нітрогену у субстраті. Визначено, що оптимальною є доза 3,0 % компостованої соломи пшениці, що забезпечує статистично достовірне збільшення кількості та маси черв'яків у порівнянні з контролем. Застосування цієї дози дозволило підтримувати біологічно збалансоване співвідношення елементів живлення та створює умови для ефективного розмноження й розвитку вермикультури. Отримані Результати можуть бути використані для розробки ефективних методів вирощування вермикультури на основі компостованої соломи пшениці, що сприятиме поліпшенню родючості ґрунтів та підвищенню ефективності органічного землеробства

Ключові слова: гібрид червоних каліфорнійських черв'яків; кокони черв'яків; мікроложе; статевозрілі особини; ферментація

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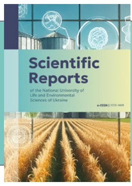

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The influence of agrotechnical factors on the formation of indicators of seed productivity in winter bread wheat

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Abstract. Optimising sowing dates and selecting suitable preceding crops is crucial for realising the productive potential of modern winter wheat varieties under changing climate conditions. The aim of the study was to determine the effects of sowing dates and preceding crops on the formation of indicators of seed productivity in winter bread wheat varieties under the conditions of the Right-Bank Forest-Steppe of Ukraine. The research material included nine winter bread wheat varieties: 'AFK Stabiliti', 'AFK Lait Hrin', 'AFK Elit Hrein', 'AFK Fentezi', 'AFK Yunion', 'AFK Premium', 'MIP Feieriia', 'MIP Roksolana', and 'Podolianka'. It was established that sowing dates, preceding crops, and varietal characteristics significantly influenced seedling formation, morphological traits, and biological yield of winter bread wheat. The optimal sowing date was determined to be September 30, which provided the fastest germination (13 days), the highest tillering coefficient (1.57), the maximum number of productive stems (5.5-5.6 million/ha), and a biological yield of up to 6.3 t/ha. The most favourable preceding crops were pea and rapeseed, which ensured dense seedling stands (4.1-4.2 million/ha) and a high tillering coefficient (1.57-1.67). After sunflower, almost all studied indicators decreased due to soil nutrient depletion. The greatest ear length (up to 13.5 cm) was recorded in the 'AFK Lait Hrin' variety sown on September 30 after rapeseed, while the tallest plants were observed after pea (82.2 cm) and in the 'AFK Elit Hrein' variety (up to 89 cm). The highest biological yields were formed

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by 'AFK Premium' (6.6-7.2 t/ha), 'AFK Elit Hrein' (6.3-6.8 t/ha), and 'AFK Fentezi' (5.8-6.4 t/ha). Analysis of variance revealed that varietal characteristics played the dominant role in determining productivity (up to 95% of variation), while preceding crops had the greatest influence on stand density, and sowing dates affected yield levels. The study demonstrated the effectiveness of applying different sowing dates after various preceding crops to differentiate winter wheat varieties by seed productivity indicators. The obtained results can be used in agricultural practice to improve the efficiency of winter wheat seed production across diverse soil and climatic conditions and to optimise crop rotation systems

Keywords: *Triticum aestivum* L.; variety; preceding crop; sowing date; germination stage

Introduction

The seed productivity of winter bread wheat plays a vital role in determining overall crop yield, influencing both the quantity and quality of seeds produced. This productivity is closely linked to a range of agronomic factors, including the timing of sowing, plant density, fertilisation practices, and pest management. As climate change continues to alter growing conditions, it becomes increasingly important to optimise sowing schedules and select appropriate preceding crops. These adjustments are necessary to ensure that modern winter wheat varieties can fully realise their productive potential under variable environmental conditions.

Winter bread wheat (*Triticum aestivum* L.) is one of the world's leading food crops. N. Rudavska *et al.* (2025) reported that in Ukraine the sown area of winter wheat in 2025 exceeded 5 million hectares. Considering global market trends, further growth in demand for wheat grain is expected, which can primarily be met by increasing yields. At the same time, climate change observed over the past decades significantly affects the state of agroecosystems and crop formation. Under these conditions, improving technological elements of cultivation that ensure crop adaptation to changing weather conditions and production stability becomes increasingly important.

Sowing date plays a key role, as it determines the growth and development rate of plants, their winter hardiness, and the realisation of the yield potential of winter wheat. O.A. Demidov *et al.* (2024) reported that the most favourable conditions for sowing occur when the average daily air temperature ranges from 14 to 17°C and sufficient productive soil moisture is available in the arable layer, ensuring seedling emergence within 6-8 days. U. Kumar *et al.* (2021), conversely, noted that excessively developed plants resulting from early sowing dates exhibit reduced resistance to adverse winter conditions, thereby increasing the risk of overwintering damage. J. Liu *et al.* (2023) investigated the effects of sowing date adjustment on winter wheat yield and established that the optimal sowing period ranged from 1 to 10 October, with no significant interannual variation.

High yields of winter wheat are mainly achieved when it follows the best preceding crops. I.M. Didur & G.V. Pantsyreva (2024) determined the productivity of winter wheat depending on the predecessors: meadow clover and white burkun. Authors noted that the choice of a preceding crop takes into account its harvest timing, ability to restore soil fertility, and the availability of soil moisture and mineral nutrients for subsequent crops. V.V. Polishchuk & Yu.M. Prytula (2025) determined the peculiarities of formation of yield and quality of winter wheat seeds depending on genotype and predecessors. Authors established that the germination energy, germination rate and weight of 1,000 seeds depended on the predecessors. Significantly lower germination energy was obtained when sowing after sunflower, and when sowing after winter rape and perennial grasses, it was the same. V. Hanhur & Y. Kotliar (2023) reported that the highest productivity of winter wheat was achieved when grown after grass pea and vetch-oat mixtures for green fodder. The least favourable preceding crops for winter wheat were sunflower and sugar beet. The authors concluded that the inclusion of leguminous crops in crop rotations serves an important ecological function by reducing the need for mineral nitrogen fertilisers and increasing the efficiency of soil nitrogen utilisation.

Achieving high and stable yields is possible only with adherence to optimal sowing dates and rational selection of preceding crops. This necessitates targeted research on the response of modern wheat varieties to variations in preceding crops and sowing time. O. Demydov *et al.* (2022) identified the features of the manifestation and variability of yield and grain quality indicators of winter wheat depending on the growing season, genotype, sowing date and preceding crop. They reported that the preceding crop had the greatest influence on yield, weight of 1,000 kernels, and protein content, whereas sowing date primarily affected yield. I. Sokolovska *et al.* (2024) reported that the preceding crop had the greatest influence on variations in yield parameters. The authors concluded that winter wheat should be sown around 15 September when following sunflower and between 15 and 30 September when following soybean. V.V. Kyrylenko *et al.* (2024) observed that the effects of preceding crops and sowing dates on germination energy and laboratory germination of winter wheat were less pronounced compared with their effect on seed sprouting activity.

Thus, numerous studies confirm the importance of an in-depth investigation of agrotechnical factors, particularly preceding crops and sowing dates, in the formation of seed productivity indicators of winter wheat under contemporary climatic conditions. At the same time, changes in the specialisation of agricultural production, a shift toward economically attractive crops, and climatic transformations necessitate their revision in modern intensive crop rotations. This substantiates the relevance of conducting the corresponding field studies.

The study aimed to evaluate the effects of different sowing dates and preceding crops on the formation of seed productivity parameters in winter bread wheat varieties grown in the Right-Bank Forest-Steppe zone of Ukraine.

Materials and Methods

The research was conducted under the conditions of "Agrofirma 'Kolos'", LLC (Pustovarivka village, Bila Tserkva district, Kyiv region), located in the Right-Bank Forest-Steppe zone of Ukraine, during 2022/23 and 2023/24. The study material included nine winter bread wheat (*Triticum aestivum* L.) varieties: 'AFK Stabiliti', 'AFK Lait Hrin', 'AFK Elit Hrein', 'AFK Fentezi', 'AFK Yunion', 'AFK Premium', 'MIP Feieriia', 'MIP Roksolana', and 'Podolianka'. The studied winter bread wheat varieties were chosen because they combine high yield potential with adaptive traits that ensure tolerance to unfavourable environmental factors, including climate variability. The above-mentioned varieties were studied under three sowing dates (I – September 20, II – September 30, III – October 10) and after four preceding crops (pea, soybean, sunflower, winter rapeseed). The study was conducted in accordance with ethical standards, adhering to the guidelines set forth by the Convention on Biological Diversity (1992), ensuring the responsible use of biological resources and respect for environmental sustainability throughout the research process.

The soil of the experimental field is a typical medium-humus, silty medium loam chernozem formed on loess. Humus content – 4.6-4.8% (by Tyurin method), easily hydrolysed nitrogen – 14.4 mg/100 g soil, available phosphorus – 9.6 mg/100 g soil, and exchangeable potassium – 15.2 mg/100 g soil. Bulk density – 1.24 g/cm^3, hydrolytic acidity – 1.14 meq/100 g soil, salt pH – 6.4. The soils are characterised by a moderate level of nutrient availability.

Crop cultivation technology followed the generally accepted practices for the Forest-Steppe zone (Poltoretskyi *et al.*, 2020; Demidov *et al.*, 2024). Sowing was carried out using a breeding seeder SN-10 at a depth of 3-4 cm with a seeding rate of 5 million viable seeds per hectare. The plots were arranged in a completely randomised design with four replications and a plot size of 10 m². Harvesting was performed using a "Sampo-130" combine harvester (Sampo-Rosenlew Ltd., Finland). Phenological observations, measurements, counts, and sample collections were conducted according to the V.V. Volkodav (2003) and other relevant methods (Tkachyk, 2016). Laboratory analysis of plants included the assessment of yield structure elements: tillering coefficient, plant density, number of productive stems, ear weight, grain yield per ear, ear length, plant height, and biological yield (Tsenov *et al.*, 2020; Demidov *et al.*, 2024). Statistical analysis of the obtained data was carried out using descriptive and variation statistics, as well as analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD) test, employing the software packages Statistica 10 and Microsoft Excel 2013. Differences between compared indicators were considered statistically significant at $p < 0.05$.

Results

The years of the study were characterised by contrasting hydrothermal conditions and uneven precipitation distribution throughout the year (Figs 1, 2). The average annual air temperature in 2022/23 and 2023/24 exceeded the long-term mean by 0.6°C and 2.5°C, respectively. The greatest fluctuations in monthly average temperatures were recorded mainly from September to February. Overall, the growing conditions in 2022/23 can be described as sufficiently moist, whereas the 2023/24 season was notably dry. The total annual precipitation amounted to 130% and 92% of the long-term norm in the respective years. A particularly pronounced precipitation deficit in 2023/24 was observed in August and September (46 mm and 47 mm below the norm), as well as in May and July (43 mm and 68 mm less than the long-term average).

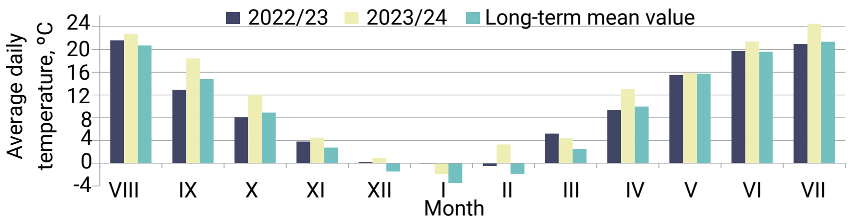

***Figure 1.** Mean daily air temperature in the study years compared to the long-term mean daily air temperature*

Source: constructed by the authors based on data from the Bila Tserkva Agrometeorological Station

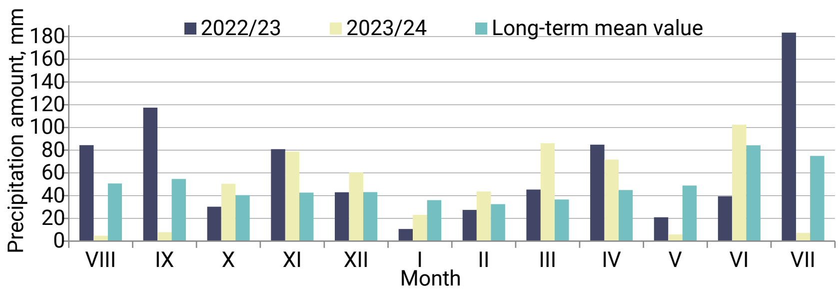

***Figure 2.** Monthly precipitation in the study years compared to the long-term mean value*

Source: constructed by the authors based on data from the Bila Tserkva Agrometeorological Station

Observations and experimental data obtained in the present study demonstrated that, at the full germination stage, the timing of seedling emergence of winter wheat varied considerably depending on the sowing date and preceding crop (Table 1).

Table 1. *Time of seedling emergence in winter bread wheat varieties depending on sowing dates and preceding crops, days*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	21	21	20	21	21	21	21	21	21	21
	Soybean	22	22	21	22	22	22	22	22	22	22
	Sunflower	24	24	23	24	24	24	24	24	24	24
	Rapeseed	21	21	20	21	21	21	21	21	21	21
	Average	22	22	21	22	22	22	22	22	22	22
30.09	Pea	12	13	11	13	13	13	13	13	12	13
	Soybean	13	14	12	14	14	14	14	14	13	14
	Sunflower	13	14	12	14	14	14	14	14	13	14
	Rapeseed	13	14	12	14	14	14	14	14	13	14
	Average	13	14	12	14	14	14	14	14	13	13
10.10	Pea	14	15	13	15	15	15	15	15	14	15
	Soybean	15	16	14	16	16	16	16	16	15	16
	Sunflower	15	16	14	16	16	16	16	16	15	16
	Rapeseed	15	16	14	16	16	16	16	16	15	16
	Average	15	16	14	16	16	16	16	16	15	15

Source: developed by the authors

Under early sowing (September 20), the emergence period averaged 22 days. The fastest emergence was observed under the September 30 sowing date, averaging 13 days. In the case of late sowing (October 10), seedling emergence lasted 15 days, which can be explained by lower temperatures and slower physiological processes in the seeds. A significant effect of the preceding crop on the emergence rate was revealed. The shortest period from sowing to emergence was observed after pea – 21, 13, and 15 days for the three sowing dates, respectively. After soybean and sunflower, emergence occurred 1-2 days later. Rapeseed as a preceding crop provided similar indicators to pea, indicating its favourable influence on the early growth of wheat plants. Differences among varieties were minor (within 1-2 days), suggesting the predominance of agronomic factors (sowing date and preceding crop) over genetic variation. Slightly faster emergence was noted for the varieties 'AFK Elit Hrein' and 'Podolianka', which exhibited higher germination energy. Thus, results of the current study confirm that the sowing date significantly affects the duration of seedling emergence, as different sowing periods create variable environmental conditions for the growth and development of winter wheat plants.

One of the key criteria for yield formation is the optimal plant density per unit area. On average, the plant density in the experiment ranged from 3.9 to 4.3 million plants per hectare (Table 2). It is noteworthy that the sowing date had no significant effect on this indicator.

Table 2. *Seedling density of winter bread wheat varieties depending on sowing dates and preceding crops, million plants/ha*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	4.1	4.2	4.3	4.3	4.3	4.3	4.2	4.2	4.2	4.2
	Soybean	3.9	4.0	4.1	4.1	4.1	4.1	4.0	4.0	4.0	4.0
	Sunflower	3.9	3.9	4.1	4.0	4.0	4.0	3.9	3.9	3.9	4.0
	Rapeseed	4.0	4.1	4.2	4.2	4.2	4.1	4.1	4.1	4.0	4.1
	Average	4.0	4.1	4.2	4.2	4.2	4.1	4.1	4.1	4.0	4.1
30.09	Pea	4.2	4.2	4.3	4.3	4.3	4.3	4.2	4.2	4.2	4.2
	Soybean	3.9	4.0	4.1	4.1	4.1	4.1	4.0	4.0	4.0	4.0
	Sunflower	3.9	4.0	4.0	4.0	4.1	4.0	3.9	4.0	3.9	4.0
	Rapeseed	4.0	4.1	4.2	4.2	4.2	4.2	4.1	4.1	4.1	4.1
	Average	4.0	4.1	4.2	4.2	4.2	4.2	4.1	4.1	4.1	4.1
10.10	Pea	4.1	4.2	4.3	4.3	4.3	4.3	4.2	4.2	4.2	4.2
	Soybean	3.9	4.0	4.0	4.0	4.1	4.0	3.9	4.0	3.9	4.0
	Sunflower	3.8	3.9	4.0	4.0	4.0	4.0	3.9	3.9	3.9	3.9
	Rapeseed	4.0	4.1	4.1	4.1	4.1	4.1	4.0	4.1	4.0	4.1
	Average	4.0	4.1	4.1	4.1	4.1	4.1	4.0	4.1	4.0	4.1
LSD 0.95		0.07									

Source: developed by the authors

Regarding preceding crops, the highest stand density was formed after pea and rapeseed – within 4.1-4.2 million plants/ha – whereas after soybean and sunflower it was lower (3.9-4.0 million plants/ha). Differences among varieties were minor (within 0.1-0.2 million plants/ha) and did not exceed the least significant difference (LSD 0.95 = 0.07), indicating no substantial genotype effect on stand density under uniform growing conditions. Slightly higher densities were recorded for the varieties 'AFK Elit Hrein', 'AFK Fentezi', and 'AFK Yunion', likely due to their greater germination energy and field emergence. Thus, the most favourable conditions for achieving optimal stand density of winter bread wheat were ensured by sowing on September 20 or 30 after pea or rapeseed, when the average plant density reached 4.2 million plants/ha. This confirms the importance of adhering to these sowing dates and selecting suitable preceding crops to ensure uniform plant development during the early stages of vegetation. The highest tillering coefficient was observed for the September 30 sowing date, with an average value of 1.57, which exceeded those of the early (September 20 – 1.52) and late (October 10 – 1.47) sowings (Table 3).

Table 3. *Tillering coefficient in winter bread wheat varieties depending on sowing dates and preceding crops*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	1.70	1.59	1.80	1.59	1.59	1.69	1.49	1.59	1.54	1.62
	Soybean	1.59	1.49	1.69	1.49	1.49	1.59	1.39	1.49	1.49	1.52
	Sunflower	1.49	1.39	1.59	1.39	1.39	1.49	1.29	1.39	1.39	1.42

Table 3. *Continued*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Rapeseed	1.59	1.49	1.69	1.49	1.49	1.59	1.39	1.49	1.49	1.52
	Average	1.59	1.49	1.69	1.49	1.49	1.59	1.39	1.49	1.48	1.52
30.09	Pea	1.75	1.64	1.85	1.64	1.64	1.74	1.54	1.64	1.59	1.67
	Soybean	1.64	1.54	1.74	1.54	1.54	1.64	1.44	1.54	1.54	1.57
	Sunflower	1.54	1.44	1.64	1.44	1.44	1.54	1.34	1.44	1.44	1.47
	Rapeseed	1.64	1.54	1.74	1.54	1.54	1.64	1.44	1.54	1.54	1.57
	Average	1.64	1.54	1.74	1.54	1.54	1.64	1.44	1.54	1.53	1.57
10.10	Pea	1.65	1.54	1.75	1.54	1.54	1.64	1.44	1.54	1.49	1.57
	Soybean	1.54	1.44	1.64	1.44	1.44	1.54	1.34	1.44	1.44	1.47
	Sunflower	1.44	1.34	1.54	1.34	1.34	1.44	1.24	1.34	1.34	1.37
	Rapeseed	1.54	1.44	1.64	1.44	1.44	1.54	1.34	1.44	1.44	1.47
	Average	1.54	1.44	1.64	1.44	1.44	1.54	1.34	1.44	1.43	1.47
LSD 0.95		0.05									

Source: developed by the authors

This indicates an optimal combination of temperature and moisture conditions during late September, when uniform seedling development and more intensive tillering were observed. The present results also showed that tillering intensity depended on the preceding crop. The highest tillering coefficients across all sowing dates were recorded after pea (1.57-1.67), confirming the beneficial effect of this predecessor on the growth and development of wheat plants. Slightly lower but comparable values were observed after rapeseed. After soybean, the coefficient decreased on average by 0.1, while the lowest values were recorded after sunflower, which can be explained by soil nutrient depletion following this crop and a reduction in available nutrients. The tillering intensity was also influenced by the biological characteristics of the varieties. The highest coefficients were recorded for 'AFK Elit Hrein' (1.80), 'AFK Stabiliti' (1.75), and 'AFK Premium' (1.74), whereas the varieties 'MIP Feieriia' and 'Podolianka' exhibited lower tillering activity. These results confirm the effectiveness of using leguminous preceding crops and maintaining a medium sowing date to ensure intensive autumn tillering in winter bread wheat. The number of productive stems is an important component of winter bread wheat yield structure because it directly reflects the realisation of the plant's tillering potential. The present research demonstrated a significant dependence of this indicator on sowing date, preceding crop, and variety characteristics (Table 4).

Table 4. *Number of productive stems in winter bread wheat varieties depending on sowing dates and preceding crops, million/ha*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	5.0	4.9	5.6	5.0	4.9	5.2	4.6	4.8	4.6	5.0
	Soybean	4.6	4.4	5.1	4.4	4.4	4.7	4.1	4.3	4.3	4.5
	Sunflower	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2

Table 4. *Continued*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Rapeseed	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7	4.7
	Average	4.6	4.6	4.9	4.6	4.6	4.7	4.4	4.5	4.5	4.6
30.09	Pea	5.7	5.4	6.2	5.6	5.5	5.9	5.1	5.5	5.2	5.6
	Soybean	5.1	4.9	5.6	4.9	5.0	5.2	4.6	4.8	4.8	5.0
	Sunflower	4.8	4.6	5.1	4.6	4.6	4.9	4.1	4.5	4.5	4.6
	Rapeseed	5.2	4.9	5.7	5.1	5.1	5.3	4.6	5.0	4.9	5.1
	Average	5.2	5.0	5.7	5.1	5.1	5.3	4.6	5.0	4.9	5.1
10.10	Pea	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
	Soybean	4.9	4.7	5.4	4.7	4.8	5.1	4.4	4.7	4.6	4.8
	Sunflower	4.6	4.3	5.0	4.4	4.4	4.7	4.0	4.3	4.3	4.4
	Rapeseed	5.1	4.8	5.5	4.9	4.9	5.1	4.4	4.8	4.8	4.9
	Average	5.0	4.8	5.4	4.9	4.9	5.1	4.6	4.8	4.8	4.9
LSD 0.95		0.15									

Source: developed by the authors

The highest number of productive stems on average across the experiment was recorded for the September 30 sowing date – 5.1 million stems/ha, exceeding the values for early sowing (September 20 – 4.6 million stems/ha) and late sowing (October 10 – 4.9 million stems/ha). This indicates that the medium sowing date provided optimal conditions for autumn tillering, promoting the formation of a greater number of productive shoots. For early sowing, excessive vegetative growth could weaken some shoots, whereas late sowing shortened the autumn vegetation period, limiting stem development. Across all sowing dates, the highest number of productive stems was observed after pea – ranging from 5.0 to 5.6 million stems/ha, highlighting the significant role of this preceding crop in improving nitrogen nutrition and soil structure. After rapeseed, the values were slightly lower (4.7-5.1 million stems/ha) The lowest values were observed after sunflower (4.2-4.6 million stems/ha), which can be attributed to soil nutrient depletion and reduced availability of essential elements following this crop. Among varieties, the highest numbers of productive stems were recorded for 'AFK Elit Hrein' (4.9-5.7 million stems/ha) and 'AFK Premium' (4.7-5.3 million stems/ha).

The present study showed that spike mass in winter bread wheat varieties depended more on preceding crops and variety traits, while no significant differences were observed between sowing dates for this trait (Table 5). The highest spike mass, regardless of sowing date, was recorded after soybean, sunflower, and rapeseed (1.5-1.6 g), whereas after pea this value was lower (1.2-1.4 g). The varieties 'AFK Premium', 'MIP Roksolana', and 'AFK Fentezi' produced the largest spike mass (up to 1.6-1.7 g), indicating their high potential productivity. Thus, the most favourable conditions for achieving high spike mass were provided by the preceding crops soybean, sunflower, and rapeseed. Among the studied varieties, the best results were obtained by 'AFK Premium', 'MIP Roksolana', and 'AFK Fentezi'. Grain weight per spike ranged from 83.0 to 92.3%, depending on sowing date, preceding crop, and variety characteristics (Table 6). On average, the highest values were recorded for the October 10 sowing date – 87.8%, exceeding the values for earlier sowings (86.9% for September 20 and 86.0% for September 30).

Table 5. *Ear weight in winter bread wheat varieties depending on sowing dates and preceding crops, g*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	1.2	1.3	1.2	1.4	1.3	1.4	1.4	1.4	1.3	1.3
	Soybean	1.4	1.5	1.4	1.6	1.5	1.7	1.6	1.6	1.4	1.5
	Sunflower	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.4	1.5
	Rapeseed	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.4	1.5
	Average	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.4	1.5
30.09	Pea	1.2	1.3	1.2	1.3	1.2	1.4	1.3	1.4	1.3	1.3
	Soybean	1.4	1.5	1.4	1.6	1.5	1.7	1.6	1.6	1.5	1.5
	Sunflower	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.5	1.5
	Rapeseed	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.5	1.5
	Average	1.4	1.5	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.5
10.10	Pea	1.2	1.3	1.2	1.3	1.3	1.4	1.3	1.4	1.3	1.3
	Soybean	1.4	1.5	1.4	1.6	1.5	1.7	1.6	1.6	1.5	1.5
	Sunflower	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.5	1.5
	Rapeseed	1.4	1.5	1.4	1.6	1.5	1.6	1.5	1.6	1.4	1.5
	Average	1.4	1.5	1.4	1.5	1.5	1.6	1.5	1.6	1.4	1.5
LSD 0.95		0.05									

Source: developed by the authors

Table 6. *Grain yield per ear in winter bread wheat varieties depending on sowing dates and preceding crops, %*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	90.4	85.4	91.4	86.4	88.4	87.4	86.4	86.4	87.4	87.7
	Soybean	88.9	83.9	89.9	84.9	86.9	85.9	84.9	84.9	85.9	86.2
	Sunflower	89.4	84.4	90.4	85.4	87.4	86.4	85.4	85.4	86.4	86.7
	Rapeseed	89.7	84.7	90.7	85.7	87.7	86.7	85.7	85.7	86.7	87.0
	Average	89.6	84.6	90.6	85.6	87.6	86.6	85.6	85.6	86.6	86.9
30.09	Pea	89.5	84.5	90.5	85.5	87.5	86.5	85.5	85.5	86.5	86.8
	Soybean	88.0	83.0	89.0	84.0	86.0	85.0	84.0	84.0	85.0	85.3
	Sunflower	88.5	83.5	89.5	84.5	86.5	85.5	84.5	84.5	85.5	85.8
	Rapeseed	88.8	83.8	89.8	84.8	86.8	85.8	84.8	84.8	85.8	86.1
	Average	88.7	83.7	89.7	84.7	86.7	85.7	84.7	84.7	85.7	86.0
10.10	Pea	91.3	86.3	92.3	87.3	89.3	88.3	87.3	87.3	88.3	88.6
	Soybean	89.8	84.8	90.8	85.8	87.8	86.8	85.8	85.8	86.8	87.1
	Sunflower	90.3	85.3	91.3	86.3	88.3	87.3	86.3	86.3	87.3	87.6
	Rapeseed	90.6	85.6	91.6	86.6	88.6	87.6	86.6	86.6	87.6	87.9
	Average	90.5	85.5	91.5	86.5	88.5	87.5	86.5	86.5	87.5	87.8
LSD 0.95		1.61									

Source: developed by the authors

Among preceding crops, the highest grain weight per spike was observed after pea (87.7-88.6%), whereas slightly lower values were recorded after soybean, sunflower, and rapeseed

(85.3-87.9%). In terms of genotypic characteristics, the varieties 'AFK Elit Hrein' (90.5-92.3%), 'AFK Stabiliti' (89.5-91.3%), and 'AFK Fentezi' (85.5-87.3%) demonstrated the highest grain weight per spike, indicating their stable ability to produce well-filled spikes. The differences among treatments were statistically significant (LSD 0.95 = 1.61%), confirming the influence of sowing date, preceding crop, and variety on grain weight per spike. On average, the best results were obtained under late sowing (October 10) after pea, with values ranging from 86.3 to 91.3%.

Spike length varied from 8.1 to 13.5 cm, depending on sowing date, preceding crop, and variety (Table 7). On average, the highest spike length was recorded for the September 30 sowing date – 9.7 cm, exceeding values for early (September 20 – 9.4 cm) and late (October 10 – 9.2 cm) sowings. This suggests that optimal conditions for growth and development during the stem elongation stage were provided by the medium sowing date, positively influencing the formation of generative organs.

Table 7. *Ear length in winter bread wheat varieties depending on sowing dates and preceding crops, cm*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	8.9	12.5	9.1	9.5	9.1	9.7	9.0	9.2	8.5	9.5
	Soybean	8.8	12.3	9.0	9.3	9.0	9.6	8.9	9.0	8.4	9.4
	Sunflower	8.7	12.2	8.9	9.2	8.8	9.5	8.8	8.9	8.3	9.3
	Rapeseed	8.8	13.0	9.0	9.3	9.6	9.6	8.9	9.7	8.4	9.6
	Average	8.8	12.5	9.0	9.3	9.1	9.6	8.9	9.2	8.4	9.4
30.09	Pea	9.1	13.0	9.6	9.6	9.1	9.7	9.1	9.4	9.1	9.7
	Soybean	9.0	12.9	9.5	9.5	8.9	9.6	8.9	9.3	9.0	9.6
	Sunflower	8.8	12.7	9.4	9.4	8.8	9.5	8.8	9.1	8.9	9.5
	Rapeseed	8.9	13.5	9.5	9.5	9.6	9.6	8.9	9.9	9.0	9.8
	Average	9.0	13.0	9.5	9.5	9.1	9.6	8.9	9.4	9.0	9.7
10.10	Pea	8.7	12.2	8.9	9.2	8.8	9.5	8.8	8.9	8.3	9.3
	Soybean	8.6	12.0	8.8	9.1	8.7	9.4	8.7	8.7	8.2	9.1
	Sunflower	8.5	11.9	8.7	9.0	8.5	9.3	8.6	8.6	8.1	9.0
	Rapeseed	8.6	12.7	8.8	9.1	9.3	9.4	8.7	9.4	8.2	9.4
	Average	8.6	12.2	8.8	9.1	8.8	9.4	8.7	8.9	8.2	9.2
LSD 0.95		0.32									

Source: developed by the authors

Among preceding crops, the longest average spikes were observed after rapeseed (9.6-9.8 cm), while the shortest spikes occurred after sunflower (9.0-9.5 cm). By variety, the longest spikes were consistently formed by 'AFK Lait Hrin' (12.0-13.5 cm) across all sowing dates and preceding crops, indicating its genetically determined advantage in spike formation. Slightly shorter but relatively stable values were observed in 'AFK Premium' (9.3-9.7 cm), 'AFK Elit Hrein' (8.8-9.6 cm), and 'AFK Fentezi' (9.0-9.6 cm), while the shortest spikes were recorded in 'Podolianka' and 'MIP Feieriia' (8.2-8.9 cm). The effects of sowing date and preceding crop on spike length were statistically significant (LSD 0.95 = 0.32 cm), emphasising the importance of these factors in shaping spike morphology. The optimal combination – sowing on September 30 after rapeseed – produced the highest spike length (up to 13.5 cm) in 'AFK Lait Hrin'. Plant height was significantly influenced by sowing date, preceding crop, and variety. The tallest plants were recorded for the September 30 sowing date, averaging 80.7 cm, which exceeded the heights for early (September 20 – 78.7 cm) and late (October 10 – 79.7 cm) sowings (Table 8).

***Table 8.** Plant height in winter bread wheat varieties depending on sowing dates and preceding crops, cm*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	83.5	78.5	88.5	83.5	83.5	58.5	78.5	78.5	88.5	80.2
	Soybean	81.5	76.5	86.5	81.5	81.5	56.5	76.5	76.5	86.5	78.2
	Sunflower	80.5	75.5	85.5	80.5	80.5	55.5	75.5	75.5	85.5	77.2
	Rapeseed	82.5	77.5	87.5	82.5	82.5	57.5	77.5	77.5	87.5	79.2
	Average	82.0	77.0	87.0	82.0	82.0	57.0	77.0	77.0	87.0	78.7
30.09	Pea	85.5	80.5	90.5	85.5	85.5	60.5	80.5	80.5	90.5	82.2
	Soybean	83.5	78.5	88.5	83.5	83.5	58.5	78.5	78.5	88.5	80.2
	Sunflower	82.5	77.5	87.5	82.5	82.5	57.5	77.5	77.5	87.5	79.2
	Rapeseed	84.5	79.5	89.5	84.5	84.5	59.5	79.5	79.5	89.5	81.2
	Average	84.0	79.0	89.0	84.0	84.0	59.0	79.0	79.0	89.0	80.7
10.10	Pea	84.5	79.5	89.5	84.5	84.5	59.5	79.5	79.5	89.5	81.2
	Soybean	82.5	77.5	87.5	82.5	82.5	57.5	77.5	77.5	87.5	79.2
	Sunflower	81.5	76.5	86.5	81.5	81.5	56.5	76.5	76.5	86.5	78.2
	Rapeseed	83.5	78.5	88.5	83.5	83.5	58.5	78.5	78.5	88.5	80.2
	Average	83.0	78.0	88.0	83.0	83.0	58.0	78.0	78.0	88.0	79.7
LSD 0.95		2.79									

Source: developed by the authors

Among preceding crops, pea provided the tallest plants, with an average height of 82.2 cm, whereas after soybean and rapeseed heights were 80.2 and 81.2 cm, respectively. The shortest plants were recorded after sunflower (79.2 cm). By genotype, the tallest plants were observed in 'AFK Elit Hrein' (88-89 cm), indicating an intensive growth type. Heights of 82-84 cm were recorded for 'AFK Fentezi', 'AFK Yunion', and 'Podolianka', whereas 'AFK Premium' exhibited a shorter-stemmed habit (57-59 cm).

Analysis of the obtained data showed that the formation of biological yield in winter wheat was significantly influenced by sowing date, preceding crop, and variety (Table 9). The highest yield was obtained for the September 30 sowing date – averaging 6.3 t/ha, exceeding the results for early sowing (September 20 – 5.7 t/ha) and late sowing (October 10 – 6.2 t/ha). Thus, the optimal sowing period fell in the third decade of September, when weather conditions favoured better crop development and a greater number of productive stems.

***Table 9.** Biological yield in winter bread wheat varieties depending on sowing dates and preceding crops, t/ha*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
20.09	Pea	5.4	5.4	6.3	5.9	5.6	6.6	5.4	6.0	5.3	5.8
	Soybean	5.8	5.5	6.4	5.9	5.7	6.7	5.5	6.0	5.4	5.9
	Sunflower	5.4	5.1	5.9	5.5	5.3	6.3	4.9	5.5	4.9	5.4
	Rapeseed	6.0	5.5	6.4	6.0	5.8	6.7	5.4	6.1	5.4	5.9
	Average	5.7	5.4	6.3	5.8	5.6	6.6	5.3	5.9	5.3	5.7

Table 9. *Continued*

Sowing date	Preceding crop	'AFK Stabiliti'	'AFK Lait Hrin'	'AFK Elit Hrein'	'AFK Fentezi'	'AFK Yunion'	'AFK Premium'	'MIP Feieriia'	'MIP Roksolana'	'Podolianka'	Average
30.09	Pea	6.2	5.8	6.7	6.4	6.1	7.1	5.8	6.4	5.8	6.3
	Soybean	6.4	6.0	7.0	6.5	6.3	7.4	6.0	6.6	6.1	6.5
	Sunflower	6.0	5.7	6.4	6.1	5.9	6.9	5.4	6.2	5.6	6.0
	Rapeseed	6.6	6.1	7.1	6.7	6.5	7.5	6.0	6.8	6.2	6.6
	Average	6.3	5.9	6.8	6.4	6.2	7.2	5.8	6.5	5.9	6.3
10.10	Pea	6.1	5.8	6.7	6.3	6.0	7.1	5.7	6.4	5.7	6.2
	Soybean	6.4	6.0	6.9	6.4	6.3	7.3	5.9	6.5	5.9	6.4
	Sunflower	5.8	5.5	6.4	6.0	5.7	6.8	5.3	6.0	5.5	5.9
	Rapeseed	6.5	6.0	7.0	6.6	6.4	7.4	5.9	6.5	5.9	6.5
	Average	6.2	5.8	6.8	6.3	6.1	7.2	5.7	6.4	5.8	6.2
LSD 0.95		0.23									

Source: developed by the authors

Among preceding crops, rapeseed provided the highest average yield (6.6 t/ha), surpassing values after pea (6.3 t/ha), soybean (6.5 t/ha), and especially sunflower (6.0 t/ha). Regarding variety characteristics, the highest biological yield was recorded for 'AFK Premium' (6.6-7.2 t/ha across sowing dates), which showed stable productivity in all experimental variants. High yields were also observed in 'AFK Elit Hrein' (6.3-6.8 t/ha) and 'AFK Fentezi' (5.8-6.4 t/ha). The results indicate that the best growth, development, and yield of winter wheat were achieved through a combination of optimal sowing date (September 30), suitable preceding crop (rapeseed or pea), and use of high-yielding varieties ('AFK Premium', 'AFK Elit Hrein', and 'AFK Fentezi'). This approach promoted the formation of well-developed plants and ensured stable yields under the climatic conditions of the study region.

In the present experiment later sowing (September 30) did not reduce productivity, which may be attributed to the regional climatic specifics (a longer warm autumn and sufficient soil moisture) and the use of modern varieties with increased adaptability to later sowing. Additionally, rapeseed and pea as preceding crops provided favourable nutrition and moisture accumulation, compensating for any potential negative effects of delayed sowing. Thus, differences in optimal sowing dates reported by various authors can be explained not only by regional cultivation conditions but also by differences in variety composition, preceding crops, and weather conditions of specific years. This emphasises the need for regional adaptation of technological recommendations and consideration of the genetic potential of varieties when planning sowing dates. Analysis of variance showed that the formation of the main elements of winter wheat seed productivity is largely determined by variety genetics, while the role of sowing date and preceding crop varied depending on the specific trait (Table 10). The greatest variety effect was observed for plant height (95.2%) and spike length (92.4%), highlighting the leading role of genetic factors in determining morphological parameters. A similar trend was observed for grain weight per spike (72.6%) and biological yield (59.7%), where variety characteristics accounted for the majority of trait variation. Meanwhile, the effect of the preceding crop was significant for plant density (66.4%), spike weight (53.1%), and number of productive stems (43.0%), indicating that preceding crops substantially influence the formation of productive tillers, particularly stand density. The sowing date had a comparatively smaller but still noticeable impact on certain traits, notably productive stems (21.4%) and biological yield (21.3%), underscoring the importance of adhering to optimal sowing dates to ensure harmonious plant development during critical organogenesis phases. Interactions among factors contributed minimally to overall variation (up to 3%), indicating stable variety responses and a minor synergistic effect of agronomic combinations. The proportion of unexplained factors ranged from 1.7 to 10.3%, confirming the high accuracy of the experiment. Thus, the study results demonstrated that variety characteristics play a decisive role in determining seed productivity of winter wheat, while agronomic factors (sowing dates and preceding crops) modulate trait expression, particularly through their effects on stand density, number of productive stems, tillering, and spike weight.

Table 10. *Contribution of factors to the formation of seed productivity indicators in winter bread wheat varieties, %*

Indicator of seed productivity	Sowing date	Preceding crop	Variety	Sowing date× Preceding crop	Sowing date × Variety	Preceding crop × Variety	Sowing date× Preceding crop × Variety	Unexplained factors
Tillering coefficient	11.8	34.3	49.0	0.0	0.0	0.4	0.0	4.4
Seedling density	2.0	66.4	20.2	0.6	0.6	0.6	2.9	6.6
Number of productive stems	21.4	43.0	31.7	0.2	0.1	0.8	0.2	2.6
Ear weight	0.0	53.1	38.0	0.4	1.0	2.9	0.9	3.6
Grain yield per ear	11.0	6.1	72.6	0.0	0.0	0.0	0.0	10.3
Ear length	2.7	1.1	92.4	0.0	0.8	1.3	0.0	1.7
Plant height	0.9	1.6	95.2	0.0	0.0	0.0	0.0	2.3
Biological yield	21.3	14.3	59.7	0.3	0.2	0.6	0.4	3.2

Source: developed by the authors

Discussion

Summarising the results of the conducted studies, it was established that crop predecessors and sowing dates are key agronomic factors that significantly affect yield formation and seed productivity indicators of winter bread wheat. Scientists from Ukraine and other countries have conducted a series of studies addressing this issue. I.I. Yarchuk & T.V. Melnyk (2018) showed that shifting sowing to later dates resulted in a reduction of all plant growth parameters. At the same time, the number of newly formed nodal roots after a stubble preceding crop was lower under both early and late sowing dates. The authors found that by the time of cessation of autumn vegetation, winter bread wheat plants reached the highest level of development when sown early (3 September) after a fallow preceding crop. At the same time, maximum yield after a fallow preceding crop was formed under a high level of mineral nutrition when sowing was carried out on 17 September, whereas after a stubble preceding crop the highest yield was obtained when sowing on 10 September. These results are consistent with the findings of I.V. Pravdziva *et al.* (2020), who noted the effectiveness of using contrasting sowing dates after different preceding crops for the evaluation of winter bread wheat genotypes. The authors established that the growing year conditions accounted for 66.2% of the variation in yield, preceding crops for 12.5%, sowing dates for 6.1%, and genotype for 1.7%. A statistically significant difference in genotype responses to sowing dates after different preceding crops was observed, with a tendency toward a decrease in mean yield when sowing dates were shifted from 26 September to 16 October, whereas for some genotypes the optimal sowing date was 5 October.

Yu.M. Priadko (2014) reported similar patterns in a study investigating the effects of preceding crops, sowing dates, and mineral fertiliser rates on the growth and development of winter wheat during the autumn growing period. The author emphasised the substantial influence of sowing dates on the growth processes of winter wheat in autumn. At the same time, under favourable hydrothermal conditions, the differences between early and optimal sowing dates decreased. However, a strong relationship was identified between sowing dates, field germination of seeds, and plant stand density of winter wheat. It was established that under an adequate level of mineral nutrition and optimal sowing dates (the third ten-day period of September), the tillering coefficient at the time of cessation of autumn vegetation ranged from 2.6 to 3.6 across all preceding crops (rapeseed, mustard, vetch, and fallow). A. Siroshtan *et al.* (2021) reported that the thousand-kernel weight of winter wheat cultivars following a green manure preceding crop was significantly higher compared with that after soybean as a preceding crop. The influence of preceding crops and sowing dates on germination energy and laboratory seed germination was insignificant. The authors concluded that in the Right-Bank Forest-Steppe zone of Ukraine, green manure is the best preceding crop for seed production, especially for the cultivation of pre-basic seed, while the optimal sowing dates were identified as 15 and 25 September. For soybean as a preceding crop, the highest yield was observed when sowing was carried out on 25 September and 5 October compared with 15 October. The researchers noted that placing winter wheat seed production fields after the best preceding crops in crop rotations and at optimal sowing dates promotes more efficient use of natural-climatic and anthropogenic factors, increases gross grain yields, and improves seed sowing quality. A.D. Gyrka *et al.* (2017) indicated that the most favourable combination of yield structure components was observed at the optimal sowing date of 20 September. Delaying sowing until 30 September or 10 October had a negative effect on plant stand density, productive tillering, spike fertility, and grain weight. This confirms that even slight shifts in sowing dates can substantially alter the morphological and yield-related traits of plants.

Studies by M. Babulicová (2014) emphasised the importance of preceding crops in crop rotation: winter wheat grown in rotations with 80% cereals produced a statistically higher yield after pea compared with winter barley, while the yield increase of 1.9 t/ha was attributed to the choice of an appropriate preceding crop and combined fertilisation. This trend is further supported by the research of M. Groeneveld *et al.* (2024), who conducted field experiments in Northern Germany. The authors found that winter wheat productivity strongly depends on the interaction between the preceding crop and sowing date. Under later sowing dates, crop yield increased following sugar beet, silage maize, and winter wheat, whereas after winter oilseed rape the effect of sowing date was negligible. Overall, sugar beet and winter oilseed rape were identified as the most favourable preceding crops; however, the need for further long-term field studies to clarify the interactions among agronomic factors was emphasised. O.L. Ulich (2018) noted that due to increasing drought intensity and the prolongation of the autumn growing period, a tendency toward a shift of optimal sowing dates to later periods has been observed. Over recent decades, sowing dates of winter wheat have shifted from early to intermediate and moderately late periods: by approximately 30 days compared with the 1950s, 20 days compared with the 1970s, 15-20 days compared with the 1980s, and about 10 days compared with the 1990s. At present, optimal sowing dates predominantly fall around 30 September and largely depend on genotype. S. Poltoretskyi *et al.* (2020) confirmed that consensus on optimal agronomic parameters has not yet been achieved, and a shift in sowing dates from early to intermediate and moderately late has been observed, accompanied by changes in cultivar choice, seeding rate, and sowing method.

Thus, an analysis of the literature and experimental data indicates that the effective formation of yield and seed productivity of winter bread wheat is largely determined by the interaction between preceding crops and sowing dates. The differences in the assessment of the effects of these agronomic factors reported by various researchers can be explained not only by regional growing conditions but also by cultivar differences and year-to-year weather variability. This highlights the need for regional adaptation of technological recommendations and for considering the genetic potential of cultivars when optimising sowing dates. Overall, the selection of agronomic practices should be based on an integrated consideration of climatic conditions, crop genotype, and the specific characteristics of preceding crops, which ensures the realisation of productive potential and high seed quality.

Conclusions

It was established that sowing dates, preceding crops, and variety characteristics significantly influenced the formation of seedlings, morphological traits, and biological yield of winter bread wheat. The optimal sowing date was determined to be September 30, which provided the fastest germination (13 days), the highest tillering coefficient (1.57), the maximum number of productive stems (5.5-5.6 million stems/ha), and the highest biological yield (6.3 t/ha). The most favourable preceding crops were pea and rapeseed, which promoted optimal stand density (4.1-4.2 million plants/ha), high tillering coefficient (1.57-1.67), and the largest number of productive stems (up to 5.6 million stems/ha). Values were reduced after sunflower due to soil nutrient depletion. Spike mass depended mainly on the preceding crop and ranged from 1.5-1.6 g after soybean, sunflower, and rapeseed.

The highest grain weight per spike (up to 91.3%) was recorded for late sowing (October 10) after pea. The maximum spike length (up to 13. cm) was observed in the 'AFK Lait Hrin' variety sown on September 30 after rapeseed. Regarding plant height, the advantage was observed for crops sown on September 30 after pea (82.2 cm), with 'AFK Elit Hrein' standing out among varieties (up to 89 cm). The highest biological yields were recorded for 'AFK Premium' (6.6-7.2 t/ha), 'AFK Elit Hrein' (6.3-6.8 t/ha), and 'AFK Fentezi' (5.8-6.4 t/ha) under September 30 sowing after rapeseed. Variance analysis showed that variety characteristics played a leading role in determining productivity, accounting for more than 50% of the variation in most traits (up to 95% for plant height and 92% for spike length). The influence of the preceding crop was most significant for stand density (66.4%) and spike mass (53.1%), while the contribution of sowing date to the variation in biological yield was about 21%.

These results indicate that a comprehensive combination of optimal sowing date (September 30), appropriate preceding crop selection (rapeseed or pea), and the use of high-yielding varieties ('AFK Premium', 'AFK Elit Hrein', 'AFK Fentezi') provides the best conditions for achieving key indicators of seed productivity in winter bread wheat under the studied regional conditions. Future research could focus on exploring additional agronomic factors, long-term climate effects, and the resilience of wheat varieties to extreme weather conditions to further enhance seed productivity under changing environmental conditions.

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Conflict of Interest

None.

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Вплив агротехнічних чинників на формування показників насіннєвої продуктивності пшениці м'якої озимої

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Анотація. Оптимізація строків сівби та добору попередників є важливою для реалізації продуктивного потенціалу сучасних сортів пшениці озимої в умовах змін клімату. Мета дослідження полягала у встановленні впливу строків сівби та попередників на формування показників насіннєвої продуктивності сортів пшениці м'якої озимої в умовах Правобережного Лісостепу України. Матеріалом дослідження були дев'ять сортів пшениці м'якої озимої: 'АФК Стабіліті', 'АФК Лайт Грін', 'АФК Еліт Грейн', 'АФК Фентезі', 'АФК Юніон', 'АФК Преміум', 'МІП Феєрія', 'МІП Роксолана' та 'Подолянка'. Встановлено, що строки сівби, попередники та сортові особливості суттєво впливали на формування сходів, морфологічні показники й біологічну врожайність пшениці м'якої озимої. Оптимальним визначено строк сівби 30 вересня, за якого відзначено найшвидше проростання (13 діб), найвищий коефіцієнт кущення (1,57), максимальну кількість продуктивних стебел (5,5-5,6 млн шт./га) та біологічну врожайність до 6,3 т/га. Найсприятливішими попередниками були горох і ріпак, що забезпечували формування густих сходів (4,1-4,2 млн шт./га) та високий коефіцієнт кущення (1,57-1,67). Після соняшнику майже всі досліджувані показники знижувалися через виснаження ґрунту. Найвищу довжину колоса (до 13,5 см) відмічено у сорту 'АФК Лайт Грін' за сівби 30 вересня після ріпаку, а за висотою рослин перевагу мали посіви після гороху (82,2 см) і сорт 'АФК Еліт Грейн' (до 89 см). Найвищу біологічну врожайність формували сорти 'АФК Преміум' (6,6-7,2 т/га), 'АФК Еліт Грейн' (6,3-6,8 т/га) та 'АФК Фентезі' (5,8-6,4 т/га). Дисперсійний аналіз засвідчив, що провідну роль у формуванні продуктивності відіграють сортові особливості (до 95 % варіації), тоді як попередники найбільше впливали на густоту посівів, а строки сівби – на рівень врожайності. Виявлено ефективність застосування різних строків сівби після різних попередників для диференціації сортів пшениці м'якої озимої за показниками насіннєвої продуктивності. Результати можуть бути використані у виробничій практиці для підвищення ефективності вирощування насіння пшениці м'якої озимої в різних ґрунтово-кліматичних умовах та оптимізації системи сівозмін

Ключові слова: *Triticum aestivum* L.; сорт; попередник; строк сівби; стадія проростання

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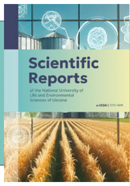

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Formation of maize grain quality indicators under the influence of seeding rates and field productivity zones

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Abstract. Maize grain quality is an important indicator of the effectiveness of agrotechnologies and is shaped by the spatial heterogeneity of soil and climatic conditions and the level of intra-crop competition. The aim of the study was to determine the effect of field productivity zones and variable seeding rates on yield and maize grain quality indicators, in particular test weight, thousand-kernel weight, starch, protein and fat content. Field experiments were conducted during 2023-025 within three field productivity zones (high, medium and low), identified on the basis of long-term yield maps and spatial analysis. The experiment was established using five seeding rates: 65, 70, 75, 80 and 85 thousand seeds per hectare. The results showed that the productivity zone was the dominant factor in the formation of yield and grain quality indicators, while the seeding rate determined the degree to which the potential was realised within each zone. The highest average yield (11.3 t/ha) was obtained in the high-productivity zone at a seeding rate of 80 thousand seeds per hectare, whereas in the low-productivity zone the maximum values did not exceed 7.7 t/ha. Grain test weight and thousand-kernel weight decreased from high to low productivity zones, indicating a deterioration of grain filling conditions under limited resource availability. Starch content was higher in high-productivity zones (up to an average of 72.5%), while medium and low zones showed an increased concentration of protein and fat, reflecting an adaptive response of the crop to stress conditions and reduced yield. The obtained results

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confirm the feasibility of applying zonally differentiated seeding rates as a tool for simultaneous optimisation of maize grain yield and quality

Keywords: *Zea mays* L.; grain quality indicators; test weight; thousand-kernel weight; starch, protein and fat content; variable-rate seeding; productivity zone

Introduction

Under current conditions of climate change and increasing spatial heterogeneity of agricultural landscapes, improving maize (*Zea mays* L.) production technologies with consideration of local soil and environmental field conditions is becoming particularly relevant. Spatial variability of the physical and chemical properties of soil, water regime and nutrient availability leads to the formation of different productivity zones within a single field, resulting not only in differences in yield but also in changes in grain quality indicators. Under such conditions, the application of fixed seeding rates does not allow full realisation of the crop's potential or efficient use of available resources.

Studies by H.M. Salem *et al*. (2024) confirmed that within-field spatial variability of soil properties can be effectively used to delineate management zones based on geostatistical and multivariate analysis methods. The authors showed that field zoning based on a combination of soil properties is a key prerequisite for the implementation of adaptive agrotechnologies aimed at increasing productivity and sustainability of agroecosystems. At the same time, such zones create different nutritional and growth conditions for plants, which can potentially affect grain quality formation. In the study by R. Łukowiak *et al*. (2024), it was demonstrated that spatial differences in soil mineral nitrogen content are closely related to crop productivity variability and nitrogen-use efficiency indicators. The authors emphasised that even within a single field there are zones with fundamentally different nitrogen supply conditions that respond differently to agrotechnical practices. Such differences may alter the balance between carbohydrate accumulation and nitrogen-containing compounds in grain, which is directly related to its quality indicators.

The physical condition of soil is also an important factor contributing to the spatial heterogeneity of agrocenoses. A. Romero-Ruiz *et al*. (2024) showed that spatially heterogeneous soil compaction significantly affects biomass growth, nutrient availability and root system functioning. These changes create local differences in the soil water-air regime and nutrient uptake, which may indirectly influence grain filling processes and its physico-chemical properties. Studies by E. Lippold *et al*. (2022) demonstrated that maize can partially compensate for soil heterogeneity through local adaptation of root system architecture. However, such adaptation does not always ensure uniform growth and productivity of plants in different field microzones. This means that under the same seeding rate, plants may form different individual productivity and potentially different grain quality depending on local soil conditions.

Physiological and molecular mechanisms of maize responses to changes in soil physico-chemical properties were described in detail by S. Zhou *et al*. (2024), who showed that uneven nitrogen distribution in soil causes significant differences in gene expression and root system development. The authors found that such differences result in asymmetric nutrient uptake and may affect the balance between vegetative growth and reproductive organ development. This creates prerequisites for variation in grain chemical composition across different field zones. In addition, S. Zhou *et al*. (2022) showed that the combination of variable nitrogen availability and intraspecific competition significantly affects maize root system development and plant biomass. As competition between plants increases, resource-use efficiency decreases, which is particularly evident at high seeding rates. This confirms the relevance of regulating plant density with consideration of field productivity zones to minimise competition and stabilise grain quality indicators.

The use of variable-rate seeding based on management zone maps is consistent with modern precision agriculture approaches. In particular, M.A. Munnaf *et al*. (2022) demonstrated that site-specific seeding, implemented based on multisensor field zoning, ensures more efficient spatial placement of plants and reduces intraspecific competition. The authors emphasised that combining productivity zones with variable seeding rates is a key factor in stabilising agronomic indicators within a field. The feasibility of optimising seeding rates with consideration of within-field heterogeneity is also supported by the findings of A.A. Anselmi *et al*. (2021), who showed that establishing maize plant density based on the potential productivity of management zones increases resource-use efficiency without yield loss. The authors noted that fixed seeding rates do not ensure realisation of the genetic potential of the crop under conditions of field spatial variability, whereas zone-adapted solutions create prerequisites for more stable formation of both yield and grain quality.

Thus, analysis of recent studies indicates that spatial heterogeneity of soil conditions and plant responses to within-field variability are key factors determining the effectiveness of agrotechnical decisions. At the same time, the influence of variable seeding rates on maize grain quality indicators under different field productivity zones remains insufficiently studied. This determines the relevance of research aimed at assessing the effects of field spatial heterogeneity and differentiated seeding rates on the physical and chemical quality indicators of maize grain.

The aim of the study was to determine the effects of field productivity zones and seeding rates on maize yield and grain quality indicators, as well as to identify optimal combinations of these factors for each zone in order to improve resource-use efficiency and yield quality stability under conditions of spatially heterogeneous agricultural landscapes.

Materials and Methods

The study was conducted during 2023-2025 on the fields of "Chernihiv Industrial Dairy Company" LLC, located in the Chernihiv district of Chernihiv region, within the transition zone between Polissia and the Forest-Steppe. The territory of the farm was characterised by a complex microrelief and high variability of soil cover, which created prerequisites for studying the response of maize to the spatial heterogeneity of agroecological conditions. The experiment was established according to a two-factor design. The first factor (A) was field productivity zones (high, medium and low), delineated on the basis of long-term maize yield maps, digital agrochemical map data and relief features, which corresponds to modern concepts of spatial heterogeneity of the soil-plant system and its influence on crop productivity (Havlin *et al*., 2013).

Spatial variability of yield was determined using yield monitoring data from a grain harvester with a spatial resolution of 5 × 5 m, which were considered a reference source of information for analysing within-field variability of productivity and crop responses to growing conditions (Deines *et al*., 2020). Productivity zones were formed using k-means cluster analysis in the QGIS environment, which enabled the grouping of field areas with similar yield characteristics and soil conditions into stable management zones recommended for differentiated agromanagement (Salem *et al*., 2024). The identification of three productivity zones ensured an optimal balance between the level of detail of the field's spatial structure and the temporal stability of zones, which was critically important for further analysis of the effects of variable seeding rates on maize grain quality indicators.

The second factor (B) was maize seeding rate (65, 70, 75, 80 and 85 thousand seeds per hectare). Seeding rate treatments were implemented within each productivity zone using prescription maps for variable-rate seeding under different agroecological conditions of the field. The maize hybrid DKC 3939 of the medium-early maturity group was used in the experiment; it is characterised by high adaptability to changing hydrothermal growing conditions. The choice of this hybrid was justified by the results of field studies by O. Barabolia & I. Kosenko (2024), which demonstrated its stable yield under different sowing dates and variable temperature and moisture regimes, as well as higher productivity compared with other medium-maturity hybrids under optimal and later sowing dates.

Winter wheat was the preceding crop. The experiment was arranged in four replications across three experimental plots with an area of approximately 6.6 ha each. After crop emergence, actual plant density was checked to ensure compliance with the prescribed seeding rates. The fertilisation and crop protection system was uniform for all experimental treatments and corresponded to the farm's production technology, which minimised the influence of confounding factors on the formation of grain quality indicators. Weather conditions during the study years differed in terms of temperature regime and precipitation amount, which made it possible to assess the stability of the effects of seeding rates and productivity zones on grain quality indicators under different hydrothermal conditions. All fieldwork was carried out in compliance with national legislation of Ukraine, the principles of good scientific practice and international standards for the responsible use of plant resources, including the provisions of the Convention on Biological Diversity (1992).

Maize grain quality was assessed using the following indicators: grain test weight (g/L), thousand-kernel weight (g), protein content (%), starch content (%) and fat content (%). Grain test weight was determined using a standard one-litre measuring cylinder with control weighing in accordance with generally accepted methods specified in DSTU 10840:2019 (2019). Protein, starch and fat content in the grain were determined by infrared spectroscopy using an Infratec 1241 Grain Analyzer (FOSS Tecator, Denmark), previously calibrated in accordance with the requirements of ISO 12099:2017 (2017). Thousand-kernel weight was determined by weighing a sample of 1,000 kernels on analytical scales with an accuracy of ±0.01 g, in accordance with DSTU 4117:2007 (2007).

For each treatment, three composite grain samples were formed after post-harvest processing. The results were statistically analysed using analysis of variance and correlation analysis methods in the Statistica software. Mean values, standard deviations and coefficients of variation were calculated, and relationships between seeding rate, productivity zone and the main grain quality indicators were determined. Experimental data were visualised using modern graphical methods (boxplot, heat map), which made it possible to clearly illustrate the variability of grain quality indicators and the patterns of their change depending on seeding rate and productivity zone. Graphs were generated using the Python software environment.

Results

During the 2023-2025 research period, the formation of maize grain yield of the hybrid DKC 3939 was analysed depending on field productivity zones and variable seeding rates. The study covered three vegetation periods that were contrasting in terms of weather conditions, which made it possible to assess the stability of the crop response to agrotechnical factors under conditions of spatial heterogeneity of the agro-background. Despite year-to-year variability in yield caused by weather conditions, similar patterns of the effect of seeding rate within individual productivity zones were observed in all years. In the high-productivity zone, the highest mean grain yield over the three years of the study was obtained at a seeding rate of 80 thousand seeds per hectare, indicating the ability of this zone to effectively support increased plant density (Fig. 1).

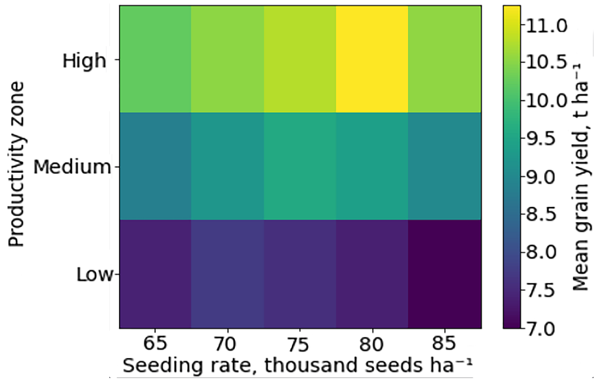

Figure 1. *Heat map of mean grain yield depending on productivity zone and seeding rate (2023-2025)*

Source: developed by the authors

At the same time, a further increase in the seeding rate to 85 thousand seeds per hectare did not provide an additional increase in yield, indicating that the optimal level of intraspecific competition had been reached. In the medium-productivity zone, the highest mean yield values were formed at a seeding rate of 75 thousand seeds per hectare, whereas an increase in plant density to 80-85 thousand seeds per hectare was accompanied by a decrease in the efficiency of resource use. In the low-productivity zone, a seeding rate of 70 thousand seeds per hectare proved to be optimal, while its further increase led to a reduction in yield, indicating the limited resource potential of this zone. The level of intraspecific competition, determined by the seeding rate and the resource potential of microzones, directly affects grain filling processes, which is reflected in test weight, thousand-kernel weight, as well as in the ratio of the main storage substances. In this regard, further analysis was focused on assessing the effect of productivity zones and variable seeding rates on starch, protein and fat content in maize grain (Table 1).

Table 1. *Test weight and thousand-kernel weight of maize depending on seeding rate and productivity zones (2023-2025)*

Productivity zone	Seeding rate, thousand seeds/ha	Test weight, g/L				Thousand-kernel weight, g			
		2023	2024	2025	Mean	2023	2024	2025	Mean
High	65	720	712	728	720	305	295	315	305
	70	722	714	730	722	308	298	318	308
	75	724	716	732	724	310	300	320	310
	80	726	718	736	727	312	302	325	313
	85	723	715	729	722	309	299	317	308
Mean value for the high-productivity zone		723	715	731	723	309	299	319	309
Medium	65	705	695	715	705	285	275	298	286
	70	708	698	718	708	288	278	302	289
	75	710	700	720	710	292	282	305	293
	80	709	699	719	709	290	280	303	291
	85	707	696	716	706	287	276	300	288
Mean value for the medium-productivity zone		708	698	718	708	288	278	302	289

***Table 1.** Continued*

Productivity zone	Seeding rate, thousand seeds/ha	Test weight, g/L				Thousand-kernel weight, g			
		2023	2024	2025	Mean	2023	2024	2025	Mean
Low	65	690	670	695	685	270	245	275	263
	70	692	672	698	687	273	248	278	266
	75	691	671	697	686	271	247	277	265
	80	689	669	694	684	269	245	274	263
	85	685	665	690	680	265	240	270	258
Mean value for the low-productivity zone		689	669	695	685	270	245	275	263

Source: developed by the authors

Analysis of the data in Table 1 indicates that maize grain test weight and thousand-kernel weight varied significantly depending on the field productivity zone, seeding rate and growing season conditions. During 2023-2025, the highest values of both indicators were consistently formed in the high-productivity zone, reflecting more favourable grain filling conditions under a high soil resource potential.

In the high-productivity zone (Fig. 2), the multi-year average values of test weight increased with an increase in seeding rate from 65 to 80 thousand seeds per hectare (from 720 to 727 g/L).

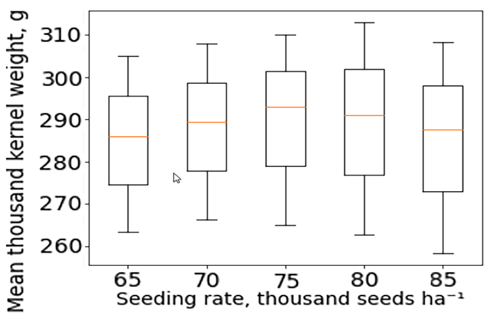

***Figure 2.** Distribution of mean maize kernel weight according to seeding rates (2023-2025)*

Source: developed by the authors

With a further increase in the seeding rate to 85 thousand/ha, a slight decrease in the indicator was observed. A similar trend was observed for the weight of 1,000 grains, the maximum average value of which (313 g) was formed at a seeding rate of 80 thousand/ha (Fig. 3). This indicates an optimal combination of plant density and resource availability at this particular seeding rate.

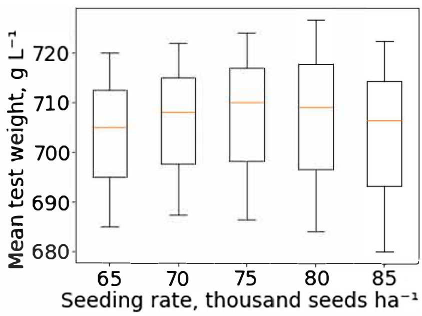

***Figure 3.** Distribution of the mean weight of 1,000 maize kernels according to sowing rates (2023-2025)*

Source: developed by the authors

In the medium-productivity zone, test weight and thousand-kernel weight were lower than in the high-productivity zone; however, they retained a clear dependence on seeding rate. The highest mean values of test weight (710 g/L) and thousand-kernel weight (293 g) were observed at a seeding rate of 75 thousand seeds per hectare, whereas a further increase in plant density to 80-85 thousand seeds per hectare did not result in an improvement of grain physical properties (Figs 2 and 3). This indicates a limited resource potential of the medium-productivity zone and increased intraspecific competition at higher seeding rates.

In the low-productivity zone, the lowest values of both test weight and thousand-kernel weight were recorded, with a consistent downward trend as the seeding rate increased. The maximum mean values of test weight (687 g/L) and thousand-kernel weight (266 g) were formed at seeding rates of 65-70 thousand seeds per hectare, while an increase in density to 85 thousand seeds per hectare led to a significant deterioration of grain physical characteristics. This suggests that under conditions of low soil productivity, increased competition between plants restricts grain filling processes.

Analysis of the indicators by years showed that in 2025, higher values of test weight and thousand-kernel weight were formed across all productivity zones compared with 2023 and especially 2024, which was associated with more favourable growing conditions. At the same time, regardless of the year of cultivation, a general pattern was maintained: the optimal seeding rate shifted towards higher values in the high-productivity zone and decreased in the medium and low zones, confirming the feasibility of a differentiated approach to regulating plant density. Analysis of the data in Table 2 indicates that the chemical composition of maize grain depended significantly on the field productivity zone, seeding rate and growing season conditions. During 2023-2025, a clear differentiation between zones was observed, reflecting different levels of resource availability for crops and, consequently, the intensity of grain filling processes.

Table 2. *Protein, fat and starch content in maize grain depending on seeding rate and productivity zones (2023-2025)*

Productivity zone	Seeding rate, thousand seeds/ha	Protein content, %				Fat content, %				Starch content, %			
		2023	2024	2025	Mean	2023	2024	2025	Mean	2023	2024	2025	Mean
High	65	8.4	8.6	8.2	8.4	4.2	4.3	4.0	4.2	71.8	71.0	72.4	71.7
	70	8.3	8.5	8.1	8.3	4.1	4.2	3.9	4.1	72.0	71.2	72.7	72.0
	75	8.2	8.4	8.0	8.2	4.1	4.2	3.9	4.1	72.3	71.4	72.9	72.2
	80	8.1	8.3	7.8	8.1	4.0	4.1	3.8	4.0	72.6	71.6	73.4	72.5
	85	8.2	8.4	8.1	8.2	4.1	4.2	3.9	4.1	72.2	71.3	72.6	72.0
Mean value for the high-productivity zone, %		8.2	8.4	8.0	8.2	4.1	4.2	3.9	4.1	72.2	71.3	72.8	72.1
Medium	65	8.8	9.0	8.5	8.8	4.4	4.5	4.2	4.4	70.5	69.8	71.2	70.5
	70	8.7	8.9	8.4	8.7	4.3	4.4	4.1	4.3	70.8	70.0	71.5	70.8
	75	8.6	8.8	8.3	8.6	4.3	4.4	4.1	4.3	71.1	70.2	71.8	71.0
	80	8.6	8.8	8.4	8.6	4.3	4.4	4.1	4.3	70.9	70.1	71.6	70.9
	85	8.7	8.9	8.5	8.7	4.4	4.5	4.2	4.4	70.7	69.9	71.3	70.6
Mean value for the medium-productivity zone, %		8.7	8.9	8.4	8.7	4.3	4.4	4.1	4.3	70.8	70.0	71.5	70.8
Low	65	9.2	9.7	9.0	9.3	4.7	4.9	4.5	4.7	69.2	67.8	69.8	68.9
	70	9.1	9.6	8.8	9.2	4.6	4.8	4.4	4.6	69.5	68.0	70.2	69.2
	75	9.1	9.6	8.9	9.2	4.6	4.8	4.4	4.6	69.4	67.9	70.0	69.1
	80	9.3	9.8	9.1	9.4	4.7	4.9	4.6	4.7	69.1	67.7	69.7	68.8
	85	9.5	10	9.2	9.6	4.8	5.0	4.7	4.8	68.7	67.3	69.4	68.5
Mean value for the low-productivity zone, %		9.2	9.7	9.0	9.3	4.7	4,9	4.5	4.7	69.2	67.7	69.8	68.9

Source: developed by the authors

In the high-productivity zone, protein content was characterised by the lowest mean values (8.1-8.4%) compared with other zones, which was due to intensive starch accumulation and the manifestation of a "dilution" effect of nitrogen-containing compounds. At the same time, this zone exhibited the highest starch content, with mean values increasing as the seeding rate rose to 80 thousand seeds per hectare (72.5%), after which a tendency towards stabilisation or a slight decrease was observed. Fat content in the high-productivity zone remained relatively low (4.0-4.2%) and showed only a weak response to changes in seeding rate, which is typical of conditions of high productivity and the dominance of carbohydrate metabolism. In the medium-productivity zone, indicators of the chemical composition of grain had intermediate values. Protein content averaged 8.6-8.8% and varied only slightly depending on seeding rate, indicating a more balanced relationship between starch accumulation and nitrogen-containing compounds. The maximum mean starch content (71.0%) was formed at a seeding rate of 75 thousand seeds per hectare, whereas an increase in plant density to 80-85 thousand seeds per hectare did not result in its further increase. Fat content in the grain of the medium-productivity zone ranged from 4.3 to 4.4% and showed a weak response to changes in plant density. In the low-productivity zone, the highest values of protein and fat content were recorded simultaneously with the lowest starch content. Mean protein content increased from 9.3% at a seeding rate of 65 thousand seeds per hectare to 9.6% at 85 thousand seeds per hectare, which was associated with a reduction in the intensity of carbohydrate synthesis and a relative increase in nitrogen concentration in the grain. A similar trend was observed for fat content, which reached maximum values (4.8%) at the highest seeding rate. Starch content in the low-productivity zone decreased with increasing sowing density and had the lowest mean values (68.5-69.2%), indicating limitations in photosynthetic productivity and deterioration of grain filling conditions.

Analysis of the indicators by years showed that in 2025, higher starch content and lower protein and fat concentrations were formed across all productivity zones compared with 2024, which is consistent with more favourable growing conditions. At the same time, regardless of the year of cultivation, a general pattern was maintained: as the productive potential of the zone decreased, the proportion of protein and fat increased and starch content decreased, confirming a close relationship between yield and grain quality indicators.

Discussion

The results of the conducted studies convincingly demonstrate that spatial heterogeneity of the field is a key factor in the formation of both yield and the physical and chemical quality indicators of maize grain. During 2023-2025, it was established that the productivity zone determines the direction and intensity of the maize plant response to seeding rate, while plant density performs a regulatory function, the effectiveness of which varies depending on the resource potential of the agro-background. The feasibility of using spatial productivity categories for assessing crop condition and yield is also supported by recent studies by E. Birinyi *et al.* (2024), which demonstrated a close relationship between yield maps, satellite vegetation indices and actual yield losses at the field level.

The obtained results are consistent with data from a multiregional study conducted in China, which showed that the spatial distribution of grain quality indicators (starch, protein, fat and test weight) does not replicate yield maps and is formed under the combined influence of soil and climatic factors. In particular, W. Liu *et al.* (2023) identified a negative relationship between starch content and protein and fat concentrations, which is fully confirmed by the results of the present study for low-productivity field zones. Similar conclusions were reported by V. Babić *et al.* (2024), who showed that environmental conditions of the year and the growing environment have a decisive influence on the physical parameters of grain and its chemical composition, even at similar yield levels. The authors emphasised the protein "dilution" effect typical of intensive conditions, which was also observed in the present study in high-productivity zones under optimal seeding rates. Studies by D.J. Ogunniyan *et al.* (2021) demonstrated that thousand-kernel weight and seed morphometric characteristics are positively related to yield; however, under excessive plant density this relationship weakens due to limited resource availability for individual plants. A similar trend was recorded in the present study in medium- and low-productivity zones.

Under the conditions of Ukraine, the zonal specificity of optimal sowing density was confirmed in the work of M. Dudka & O.P. Yakunin (2023), which showed that increasing plant density is effective only up to a certain threshold, beyond which both yield and yield structure components decline. The results of the present study complement these findings by demonstrating that exceeding the optimal plant density also negatively affects grain quality. The inverse relationship between starch content and protein and fat concentrations identified in this study is consistent with the results of long-term research by D.S. Sachan *et al.* (2023), who showed that intensification of agrotechnologies and increased yield promote starch accumulation but reduce the relative protein content in grain. Although the authors primarily analysed the effect of nutrient supply, the mechanism of assimilate redistribution is universal and also relevant to the effects of plant density. Similar patterns were also reported by E.M.S. Gheith *et al.* (2022), who found that with increasing nitrogen nutrition levels and maize biomass, the relative protein concentration in grain decreases due to the dominance of carbohydrate metabolism. In the present study, this effect was clearly expressed in high-productivity zones under optimal seeding rates. The role of adaptive selection of seeding rate and nutrition under conditions of climatic variability was confirmed in the studies of S. Kothiyal *et al.* (2023) and G.K. Tadiboina *et al.* (2024), which showed that variable-rate seeding and fertilisation technologies based on productivity zones make it possible to stabilise maize productivity under diverse agroclimatic conditions. The results of the present study indicate that even under the contrasting weather conditions of 2023-2025, zonal patterns of grain quality formation remained stable. The results obtained are also consistent with data from on-farm experiments conducted by M. Videgain *et al.* (2024), in which differentiated management of seeding and fertilisation under semi-arid conditions allowed clear identification of zonal differences in crop response. The authors noted that the effect of precise regulation of agrotechnologies is manifested not only at the yield level but also through changes in the physical and quality characteristics of grain, which confirms the feasibility of a zone-oriented approach.

Thus, the results of the study are consistent with contemporary scientific concepts of spatially oriented maize crop management and extend them with new data on grain quality formation. It was established that optimisation of seeding rates should be zone-specific and aimed not only at maximising yield but also at the targeted formation of physical and chemical grain quality indicators. This confirms the feasibility of implementing variable-rate seeding (VRS) as an effective precision agriculture tool under conditions of spatially heterogeneous agroecosystems.

Conclusions

The studies conducted during 2023-2025 confirmed that the spatial heterogeneity of the field is a decisive factor in the formation of not only yield, but also the physical and chemical quality parameters of maize grain. The productivity zone had a significant effect on grain test weight, thousand-kernel weight, and the ratio of the main storage substances, while the seeding rate played a regulatory role, the effectiveness of which varied depending on the resource potential of a particular zone.

In the high-productivity zone, optimal nutrient supply and water availability contributed to the formation of grain with the highest test weight and thousand-kernel weight, as well as the maximum starch content combined with lower concentrations of protein and fat. Seeding rates of 75-80 thousand seeds per hectare were the most effective in this zone, ensuring an optimal balance between plant density and grain filling processes. A further increase in the seeding rate did not lead to an improvement in quality indicators, indicating that the limit of efficient resource use had been reached. In the medium-productivity zone, intermediate values of grain quality indicators were formed, and the optimum seeding rate shifted towards 70-75 thousand seeds per hectare. Increasing sowing density beyond this level was accompanied by a decrease in resource-use efficiency, which was reflected in the stabilisation or slight deterioration of test weight, thousand-kernel weight and starch content. This indicates the limited resource potential of medium-productivity zones and the need for a more cautious approach to increasing sowing density. In the low-productivity zone, the lowest values of test weight and thousand-kernel weight were recorded, together with the highest concentrations of protein and fat and a reduced starch content. Lower seeding rates (65-70 thousand seeds per hectare) were optimal for this zone, whereas increasing them intensified intraspecific competition, limited grain filling processes and led to a deterioration of grain physical characteristics. These patterns indicate the limiting influence of soil and hydrological conditions in low-productivity areas.

Despite interannual variability in weather conditions, the established patterns of the effects of productivity zones and seeding rates on grain quality indicators were maintained in all years of the study. This confirms the dominant role of spatial differences in the soil resource potential of the field over seasonal climatic variability in the formation of maize grain quality. The prospects for further research lie in the scientific substantiation of the feasibility of applying precision agriculture technologies, in particular variable-rate seeding (VRS), taking into account field productivity zones, as differentiated regulation of plant density allows not only the stabilisation of yield but also targeted influence on maize grain quality parameters, increasing seed-use efficiency and the adaptability of the technology to the spatial heterogeneity of agroecosystems.

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Conflict of Interest

None.

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Формування показників якості зерна кукурудзи за впливу норм висіву та зон продуктивності поля

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Анотація. Якість зерна кукурудзи є важливим показником ефективності агротехнологій і формується під впливом просторової неоднорідності ґрунтово-кліматичних умов та рівня внутрішньоценозної конкуренції. Метою дослідження було встановити вплив зон продуктивності поля та змінних норм висіву на урожайність і показники якості зерна кукурудзи, зокрема натуру, масу 1000 зерен, вміст крохмалю, білка та жиру. Польові дослідження проводили упродовж 2023-2025 рр. у межах трьох зон продуктивності поля (високої, середньої та низької), виділених на основі багаторічних карт урожайності та просторового аналізу. Дослід закладали з використанням п'яти норм висіву – 65, 70, 75, 80 та 85 тис. насінин/га. Результати досліджень показали, що зона продуктивності була домінуючим чинником формування урожайності та якісних показників зерна, тоді як норма висіву визначала ступінь реалізації потенціалу в межах кожної зони. Найвища середня урожайність (11,3 т/га) сформувалася у високопродуктивній зоні за норми висіву 80 тис. насінин/га, тоді як у низькопродуктивній зоні максимальні показники не перевищували 7,7 т/га. Натура зерна та маса 1000 зерен знижувалися у напрямку від високих до низьких зон продуктивності, що свідчить про погіршення умов наливу зерна за обмеженого ресурсного забезпечення. Вміст крохмалю був вищим у високопродуктивних зонах (у середньому до 72,5 %), тоді як у середніх і низьких зонах спостерігалося підвищення концентрації білка та жиру, що відображає адаптивну реакцію культури на стресові умови та зниження врожайності. Отримані результати підтверджують доцільність застосування зонально диференційованих норм висіву як інструменту одночасної оптимізації урожайності та якості зерна кукурудзи

Ключові слова: *Zea mays* L.; урожайність; показники якості зерна; натура; маса 1000 зерен; вміст крохмалю; білка і жиру; диференційована сівба; зона продуктивності

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Formation of a regional system for environmental monitoring of atmospheric air in tourist destinations

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Abstract. Despite the development of environmental monitoring systems in Ukraine, existing approaches do not take into account the specifics of tourist regions, which creates significant challenges for assessing environmental risks and ensuring air quality in conditions of intense tourist flows. The aim of the study was to substantiate the concept of forming a regional system for monitoring and managing the environmental safety of tourism in the territorial communities of the Ivano-Frankivsk region, taking into account European standards for atmospheric air quality control. The methodological basis was the analysis of European and national environmental legislation, geospatial modelling based on OpenStreetMap open data, indicators of tourist capacity and overnight stays, as well as optimisation tasks of maximum coverage and p-median. It has been shown that the existing state observation system has limited spatial coverage, is focused mainly on industrial centres, does not cover resort and mountain destinations, and does not reflect peak seasonal loads associated with tourist flows. A two-level network architecture has been developed, combining three high-precision reference stations (urban, valley and industrial background) with an adaptive number of low-cost sensors (10-15 LCS) located in the centres of tourist demand and transport corridors. Weighted clustering of 3,725 tourist sites revealed the dominant role of the Polianytsia-Yaremcha resort valley and made it possible to identify a configuration of 12 sensors as a balanced option that minimises the average distance to

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monitoring points and ensures measurement redundancy in sensitive areas. The results obtained provide a basis for the phased deployment of an air quality monitoring network, the integration of environmental indicators into tourism management policy, environmental marketing of territories, and ESG reporting by tourism businesses

Keywords: environmental safety of tourism; atmospheric air quality; regional monitoring system; low-cost sensors; geospatial modelling; station placement optimisation; tourist destinationscondition of forests

Introduction

The quality of atmospheric air is now recognised as an important factor in environmental safety and sustainable development, directly affecting not only public health but also the level of tourist attractiveness. Recent studies emphasise that clean air is not only a natural resource but also an economic asset on which the competitiveness of tourist destinations depends. Tourists are increasingly taking environmental conditions into account when choosing a holiday destination, so the availability and openness of data on air quality builds trust, reduces risks for visitors and enhances the image of the area.

The problem is that the current environmental reporting system covers only a limited list of pollutants, mainly from stationary sources, while international practice and the results of modern research emphasise the need to monitor fine particulate matter ($PM_{2.5}$, PM_{10}) and other specific pollutants. The impact of these microparticles, which are produced by the combustion of fuel in transport engines, heating systems in accommodation facilities and during food preparation in catering establishments, directly affects the health of the population, tourist attractiveness and environmental safety. Research by the World Health Organisation shows that even short-term exposure to $PM_{2.5}$ causes exacerbation of respiratory and cardiovascular diseases, as well as a decrease in immunity, which is especially dangerous for children and the elderly. In its updated 2021 recommendations, the World Health Organisation has lowered the permissible concentrations of $PM_{2.5}$: the annual level from 10 to 5 μg/m^3, and the daily level from 25 to 15 μg/m^3. The decision was based on a meta-analysis of more than 500 studies that confirmed significant risks even at concentrations below the previous standards (World Health Organization, 2021). In particular, a study by P.J. Landrigan *et al*. (2018), which covered data from 41 countries, showed an increase in mortality from cardiovascular diseases by 6.2%, respiratory diseases by 3.1%, and overall mortality by 4.1% with an increase in PM2.5 by every 10 μg/m^3.

A study by the European Environment Agency (2020) found that visitors to urban and suburban tourist destinations are exposed to increased levels of fine particulate matter during peak seasons. An analysis conducted by S. Adamenko *et al*. (2024) in 25 European cities found that PM2.5 concentrations in tourist centres exceed background levels by 15-30% during peak hours, especially near popular attractions and in areas of heavy traffic. Tourists who spend most of their day outdoors are exposed to levels 1.5-2 times higher than the average for local residents, whose activities are more concentrated indoors.

A further contribution to the study of the impact of the air environment on tourists was made by A. Tobías *et al*. (2020). The study used mobile monitoring stations located in tourist areas of Barcelona. The results confirmed that even a short stay (2-3 hours) in conditions of elevated PM2.5 concentrations (>25 μg/m^3) leads to significant physiological effects, including a decrease in lung function and an increase in inflammation markers. The most vulnerable groups were children under 12 and older people, who experienced a 7-12% decrease in peak expiratory flow and an increase in C-reactive protein levels. The researchers emphasise that such changes pose a particular risk to tourists, as they often have limited access to medical care while travelling and may not identify the deterioration in their health as a result of air pollution.

A study by the European Environment Agency (2021) showed that 63% of tourists consider air quality when choosing a destination, and 78% are willing to change their travel plans if environmental conditions deteriorate. These results were confirmed by a study by I. Arbulú *et al.* (2021), which, based on data from 87 European cities, found a statistically significant correlation between air quality deterioration and a decline in tourist arrivals with a lag of 1-2 months. Family and "silver" tourism segments were particularly vulnerable to this factor, with a 17-23% drop in bookings following widespread media coverage of pollution incidents.

The air quality monitoring system is characterised by spatial and temporal limitations. Stationary monitoring stations are concentrated mainly in large cities and industrial regions, while tourist destinations in mountainous and rural areas remain outside the scope of regular observation. In addition, reports are generated on a quarterly or annual basis, which does not allow for the reflection of short-term peak loads characteristic of tourist flows. According to data from L.M. Arkhypova *et al.* (2025), tourist activity is highly seasonal (winter and summer peaks in the Carpathian region) and subject to daily fluctuations, during which pollutant concentrations significantly exceed average annual levels, particularly during peak hours, periods of mass events or unfavourable weather conditions. Such episodes have a significant impact on tourists' subjective perception of the destination and their willingness to return, but they become statistically invisible in aggregated annual data.

Another systemic limitation is the lack of differentiation of pollution sources in reporting by economic sector. Tourism generates specific pollution patterns due to the intensive use of private and bus transport, increased energy consumption in accommodation and catering establishments, and increased volumes of household waste. However, these sources often fall into different reporting categories or are not recorded when they do not exceed threshold values. In particular, a significant number of vehicles serving tourists are classified as private transport and are not included in industry statistics on tourism. Similarly, small and medium-sized accommodation establishments use individual heating systems, whose emissions are not subject to systematic monitoring. Such fragmentation of data creates "blind spots" in the analysis of the environmental impact of tourism and highlights the need to implement integrated approaches to monitoring that take into account the complex nature of the interaction between tourism and the environment.

The aim of the article was to provide a scientific justification for approaches to the formation of a regional network for monitoring air quality in tourism-oriented areas. The scientific novelty lies in the development of a comprehensive approach to the formation of a network for monitoring air quality in tourist regions, based on the integration of geospatial clustering of tourist facilities, tourism capacity indicators and European requirements for the representativeness of monitoring networks, as well as in substantiating the functional structure of such a network as a tool for environmental safety and tourism load management.

Materials and Methods

The study was conducted during 2024-2025. A set of complementary methods was applied. Formal legal and comparative legal methods were used to analyse European and national requirements for the organisation of air-quality monitoring systems, as well as to identify methodological and regulatory constraints on their implementation at the regional level in accordance with the provisions of European Union directives in the field of air quality and Ukraine's obligations under the EU–Ukraine Association

Agreement (Verkhovna Rada of Ukraine, 2014; European Union, 2008; 2024).

Spatial analytical methods were implemented in a geographic information system environment to process and integrate the coordinates of tourist facilities, administrative boundaries of territorial communities, the transport and road network, and the orographic characteristics of the area. The methodological basis of the study was the integration of geospatial data covering the spatial distribution of tourist points of interest (POIs) according to OpenStreetMap data, together with proxy indicators of tourist capacity, indicators of tourist overnight stay (TN), administrative boundaries of territorial communities, the configuration of transport infrastructure, topographic characteristics of the terrain, and, where available, information on prevailing wind directions. This corresponded to European practices of spatial analysis of environmental pressures and risks (European Environment Agency, 2020; 2021).

The algorithmic core of the optimisation was formed on the basis of geospatial methods, with priority given to the practical interpretability of the results. The methodological framework included weighted k-means clustering based on criteria of tourist capacity and intensity of use to identify the cores of tourist demand and determine candidate sensor locations; the p-median and Maximum Coverage Problem models for the optimal selection of a subset of points under given constraints on the number of sensors and the minimum distance between them; analysis of zones of responsibility through the construction of Voronoi diagrams; and mapping of coverage heat maps taking into account the effective operating radius of the measuring equipment. This approach is consistent with contemporary methods for designing air-quality monitoring networks and using low-cost sensors (Dechezleprêtre *et al.*, 2019; Neis *et al.*, 2023).

The optimality criteria for the network structure included maximising the share of tourist capacity within the coverage radius of the sensors, minimising the average distance from tourist facilities to the nearest monitoring post, and ensuring balanced representation of different environment types, including resort valleys, urban and industrial background locations, and high-altitude reference points, in accordance with the requirements of European and international environmental regulation (European Union, 2008; World Health Organization, 2021).

The proposed approach ensured compliance with European practices of scientifically grounded design of air-quality monitoring networks and created an information basis for integrating environmental data into regional policies for managing tourist pressure during peak seasons, communicating environmental conditions to visitors, and developing ESG reporting for tourism businesses in line with the strategic priorities of Ukraine's state environmental policy up to 2030 (Law of Ukraine No. 2697-VIII, 2019; European Environment Agency, 2021).

To solve the problem of optimal placement of the monitoring network in tourist regions, the study applied a methodology based on the spatial distribution of tourist infrastructure facilities (POIs from OpenStreetMap) and aimed at determining the optimal placement of p low-cost sensors (LCS) and q reference monitoring stations. The target optimisation criteria were as follows:

1. ensuring representative spatial coverage of the main tourist clusters and zones of visitor concentration;

2. minimising the average accessibility distance from tourist demand points to the nearest sensors of the monitoring network;

3. taking into account technological and methodological constraints, such as the minimum permissible distances between neighbouring sensors and the balanced placement of monitoring points covering different functional zones (resort cores, urban and industrial background areas, as well as high-altitude reference sites).

This formulation of the problem makes it possible to create a scientifically grounded spatial network of environmental monitoring adapted to the specifics of tourist land use.

Figure 1 schematically presents the stages of the study on the optimal placement of the air-quality monitoring network. The following set of spatial data was used to optimise the placement of air-quality monitoring stations (Table 1).

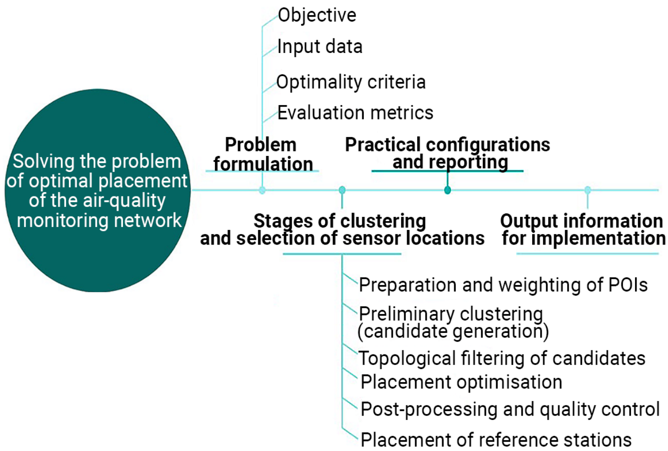

Figure 1. *Scheme for conducting the study on the optimal placement of an air-quality monitoring network*

Source: developed by the authors

Table 1. *Structure of input spatial data and optimisation parameters for the air quality monitoring network*

Data/parameter block	Designation	Content and characteristics
Set of tourist demand points	I	A set of spatially localised tourist points of interest (POIs) that generate demand for environmental monitoring
Spatial coordinates of POI	x_i, y_i	Geographical coordinates of each tourist point of interest
Capacity weighting factor	w_i	Capacity indicator of the facility, reflecting the number of beds, maximum throughput, or other relevant characteristics
Weight value in the absence of data	wi = 1	The base value used in the absence of reliable information about capacity
Territorial affiliation attribute	-	The POI's affiliation with a specific territorial community or transport corridor, determined by the spatial connection method
Set of potential locations for station placement	J	A set of candidate points for the installation of monitoring stations
Generation of candidate locations	-	Automatic generation according to specified spatial and functional criteria
Spatial coordinates of locations	(Xj, Yj)	Geographical coordinates of each potential station location
Monitoring network configuration parameters		
Effective coverage radius	r	Maximum range of a single monitoring station within which the representativeness of measurements is ensured (e.g. 2 km)
Minimum permissible distance between stations	dmin	Restriction that ensures spatial dispersion of the network and prevents excessive duplication of measurements (e.g., 2 km in valley areas)
Target number of local stations	p	Number of low-cost sensors selected from a set of alternatives {10; 12; 15} in accordance with budgetary and spatial constraints
Number of reference posts	q	Number of stationary reference stations for calibration and data verification, selected from a set {2; 3}

Table 1. *Continued*

Data/parameter block	Designation	Content and characteristics
Coverage quotas by functional zones		Minimum requirements for spatial representation of different types of territories within the monitoring network
Resort centres	-	Areas with maximum concentration of tourist demand
Transport corridors and leeward valleys	-	Areas of potential accumulation of pollutants along transport infrastructure
Urban background pollution areas	-	Urban locations without the dominant influence of individual sources
Industrial impact zones	-	Areas with local or point industrial emissions
High mountain areas	-	Reference areas for determining background concentrations of pollutants

Source: developed by the authors

To determine the optimal configuration of the monitoring network, two equivalent mathematical formulations are used: the Maximum Coverage Location Problem (MCLP) model and the *p*-median model (distance minimisation).

(A) Maximum Coverage Location Problem (MCLP). This model is aimed at maximising the weighted coverage of tourist demand points. The formalisation included:

- a proximity matrix $a_{ij} = 1[d(i,j) \leqslant r]$, where d(i,j) – the distance between demand point *i* and potential location *j*, and *r* is the coverage radius;
- binary decision variables:
  - $y_j \in 0.1$ – indicator of selecting location *j*;
  - $z_i \in 0.1$ – indicator of coverage of demand point *i*.

The objective function maximised weighted coverage:

$$max\ \sum_{i \in I} \omega_i z_i \quad (1)$$

subject to the following constraints: $\sum_{j \in J} y_j = p$ (selection of exactly *p* locations), $z_i \leqslant \sum_{j \in J} a_{ij} y_j$; $\forall i$ (a point is considered covered if it lies within the radius of at least one selected location), $y_j \in \{0,1\}$, $z_i \in \{0,1\}$ binary variables).

(B) p-median model (distance minimisation). An alternative formulation aimed at minimising the weighted distances between demand points and the nearest monitoring stations:

- assignment variables $x_{ij} \in 0,1$ – indicator that demand point *i* is served by location *j;*
- binary site selection variables $y_j \in 0,1$.

The objective function minimised the weighted distance:

$$\min \sum_{i \in I} \sum_{j \in J} \omega_i \cdot d(i,j) \cdot x_{ij} \quad (2)$$

subject to the following constraints: $\sum_{j \in J} x_{ij} = 1$; $\forall i$ (each demand point is served by exactly one location), $x_{ij} \leqslant y_j$; $\forall i$, j (a point can be served only by a selected site), $\sum_{j \in J} y_j = p$ (selection of exactly *p* locations), $y_j \in 0,1$, $x_{ij} \in 0,1$ (binary variables). To ensure the representativeness and functional balance of the network, additional constraints were introduced:

- quotas by functional zones: $\sum_{j \in G_g} y_j \geqslant L_g$ for each group g (resort cores, transport corridors, urban areas, etc.);
- minimum distance constraint: for pairs of sites (*j*,*k*) with distance d(*j*,*k*) < d_{min} the constraint $y_j + y_k \leqslant 1$, was imposed, preventing excessive concentration of stations.

For a comprehensive assessment of the quality of the obtained solutions, the following metrics are used:

1. Demand coverage ratio:

$$CR = \frac{\sum_i \omega_i \cdot 1\left[\min_j d(i,j) <= r\right]}{\sum_i \omega_i}, \quad (3)$$

which reflects the share of weighted demand located within the coverage radius of at least one monitoring station.

2. Mean nearest distance:

$$MND = \frac{\sum_i \omega_i \cdot \min_i d(i,j)}{\sum_i \omega_i}, \qquad (4)$$

which characterises the weighted average distance from demand points to the nearest stations.

3. Indicators of balance and reliability:

- variance and the Gini coefficient by the level of coverage of territorial communities;
- share of the territory with double coverage (to ensure redundancy);
- degree of compliance with quotas by functional station types;
- level of coverage of priority tourist clusters (Polianytsia, Yaremche territorial communities, etc.).

The comprehensive application of these criteria made it possible to form an optimal configuration of the monitoring network that ensures maximum coverage of tourist demand while maintaining functional balance and spatial representativeness of the system. The database of tourist facilities was formed by extracting the following categories from OpenStreetMap (OSM). Accommodation establishments included objects with the tags tourism = hotel, tourism = guest_house, tourism = apartment, tourism = hostel, tourism = camp_site, tourism = alpine_hut, tourism = chalet. Where necessary, catering establishments with the tags amenity = restaurant and amenity = cafe were added to the database. The selection was carried out using geospatial queries that ensured comprehensive coverage of the tourist infrastructure of the study area. Tourist facilities are visualised in Figure 2.

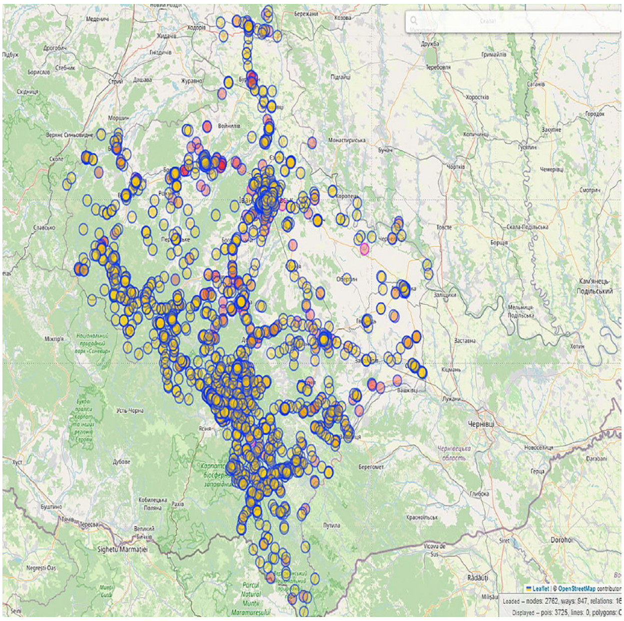

Figure 2. *List of POIs for tourism and related industries from OpenStreetMap*
Source: developed by the authors

As shown in Figure 2, 3,725 tourist facilities were selected within the territory of Ivano-Frankivsk Region that meet the conditions specified above (object categories). The process of optimising the placement of monitoring stations is based on a comprehensive analysis of the spatial distribution of tourist infrastructure. Clustering was performed using a dataset of tourist points of interest (POIs) extracted from OpenStreetMap, including accommodation facilities (hotels, guest houses, apartments) and related infrastructure (campsites, mountain huts). To ensure representativeness, differentiated weighting of objects was applied according to their capacity, using the attributes beds and capacity:persons; where these were absent, estimated values were calculated based on the number of rooms and camping pitches. To improve the model's robustness to extreme values, weight capping at the 99th percentile of the distribution was applied.

The spatial structure was determined using a weighted k-means algorithm with weighted k-means++ initialisation, which takes into account not only spatial proximity but also the significance of the facilities. The medoids of the resulting clusters were used as candidate points for the placement of monitoring stations. To prevent excessive concentration of sensors, a topological constraint of a minimum mutual distance between sites of $d_{min} = 2$ km was applied, ensuring the spatial dispersion of the network.

Three alternative network configurations with different numbers of LCS were considered: $p \in \{10, 12, 15\}$. For each option, cartographic materials were developed, including cluster maps with the boundaries of territorial communities and Voronoi diagrams illustrating the zones of responsibility of each station.

Results and Discussion

The European Union's regulatory framework forms a comprehensive and hierarchically structured system of requirements for monitoring PM_{10} and $PM_{2.5}$ concentrations, as well as for ensuring the transparency, comparability and accessibility of environmental data. For a long time, Directive 2008/50/EC (European Union, 2008) was the key basic act in this area, which established the obligation of Member States to establish representative networks of fixed observation stations in zones and agglomerations, taking into account the population, level of urbanisation and spatial structure of pollution sources. Particular attention was paid in this legal act to the average exposure to PM2.5, for the assessment of which it was envisaged to install at least one sampling point per million inhabitants, aggregated by agglomerations and additional urban areas with a population of over 100,000, which was in line with the concept of urban background monitoring and a focus on assessing the impact of pollution on public health. The further evolution of European environmental regulation took place in the context of the implementation of the European Green Deal, within which the European Commission presented a proposal in 2022 to revise the current directive on ambient air quality (European Commission, 2022). This initiative was aimed at gradually bringing the limit and target values for pollutant concentrations closer to the recommendations of the World Health Organisation, as well as strengthening the requirements for the spatial density and functional differentiation of monitoring networks, particularly in regions with high tourist traffic and seasonal peaks of anthropogenic impact.

The new approach was enshrined in law in 2024 with the adoption of Directive (EU) 2024/2881 (European Union, 2024) in its recast version, which replaces Directive 2008/50/EC (European Union, 2008) as of 12 December 2026 and introduces updated air quality standards, improved assessment methods, expanded modelling and forecasting requirements, and mandatory mechanisms for open access to environmental information for the public and public authorities. The change in the regulatory paradigm reflects the European Union's strategic course towards integrating environmental safety, public health and increasing the competitiveness of tourist destinations within the framework of sustainable development.

The implementation of European environmental monitoring standards in Ukraine is carried out in the context of fulfilling international obligations set out in the Association Agreement between Ukraine and the European Union (Verkhovna Rada of Ukraine, 2014). An important aspect of this process is the harmonisation of national legislation with the acquis communautaire in the field of environmental protection. Law of Ukraine No. 2697-VIII (2019) defines the development of an atmospheric air monitoring system as a strategic priority of state environmental policy, which directly correlates with the provisions of Directive 2008/50/EC (European Union, 2008) on the assessment and management of atmospheric air quality.

The practical mechanism for implementing these standards is enshrined in Resolution of the Cabinet of Ministers of Ukraine No. 827 (2019). The document defines methodological approaches to the creation of zones and agglomerations for monitoring, including areas with high tourist activity. It regulates the deployment of a network of automated observation posts equipped with modern devices for measuring concentrations of fine particulate matter $PM_{2.5}$ and PM_{10}, which meets the technical and procedural requirements of the European Union. The implementation of these provisions ensures the gradual approximation of the Ukrainian environmental monitoring system to European standards, forming the basis for effective air quality management in tourist-attractive regions and improving the environmental safety of the population.

However, the gap between regulatory requirements and their actual implementation remains significant, especially in tourist regions, where the lack of systematic monitoring hinders effective environmental risk management and reduces the competitiveness of destinations compared to their European counterparts. Open sources of data on atmospheric air quality are shown in Table 2.

Table 2. *Open sources of data on air quality*

Source category	**Resource name**	**Brief description**
Global aggregators and APIs	OpenAQ	The largest open data aggregator for $PM_{2.5}$, PM_{10}, NO_2, O_3, SO_2 concentrations; REST-API v3, archive on AWS; suitable for multi-country research and prototyping
	World Air Quality Index (WAQI)	Global map and API with AQI and concentration indicators based on official stations and partner networks
European platforms	EEA Air Quality e-Reporting	Official downloads of time series and statistics for EU countries and partners; station data is validated; visualisation portal available
	Copernicus Atmosphere Monitoring Service (CAMS)	Open analyses and forecasts for Europe and the world; data provided via Atmosphere Data Store (CDS API)
US sources	AirNow (US EPA)	Real-time API and forecasts for the US, Canada, and Mexico; includes AQI and station metadata
	EPA AQS API	Access to the Air Quality System database with retrospective, verified data
Ukraine (official and public initiatives)	Ukrainian Hydrometeorological Centre (UHMC)	Daily air pollution indicators by city in table format, available for download
	SaveEcoBot (SaveDnipro)	Aggregator of official and public sensors; open API and CSV files with hourly $PM_{2.5}$, NowCast, and meteorological data series; integrated into an open data portal
Public LCS networks (low-cost sensors)	Sensor.Community (Luftdaten)	Fully open network of public sensors; map and API for PM measurements; ability to connect own sensors
	openSenseMap (senseBox)	Open sensor station platform; REST API, bulk uploads; supports PM measurements
	PurpleAir	Extensive LCS network; official API and access to historical data
Model and combined services	Open-Meteo Air Quality API	Free forecasts and reanalyses based on CAMS for Europe and the world; returns concentrations of $PM_{2.5}$, PM_{10}, NO_2, O_3, SO_2, NH_3; convenient for basic assessments and gap filling

Source: developed by the authors

Compared to the European Union, which has a multi-level air quality monitoring system, Ukraine lags significantly behind in terms of network density and organisational structure. The

European model is based on regulatory reference stations of various types: urban background, traffic and industry. These stationary points form a highly accurate infrastructure framework that ensures data reliability and reproducibility. This framework in the EU is supplemented by thousands of low-cost sensors integrated into municipal networks and citizen science initiatives, which creates high spatial detail and temporal continuity of observations, allows for reanalysis and forecasting in combination with meteorological models, and ensures regular calibration of data from LCS sensors relative to reference stations. The result is a high level of trust in environmental information among the public and the scientific community, as evidenced by the integration of such data into open portals and APIs (Fig. 3). It is the combination of official and public tools that ensures the systematic and consistent quality of European environmental monitoring.

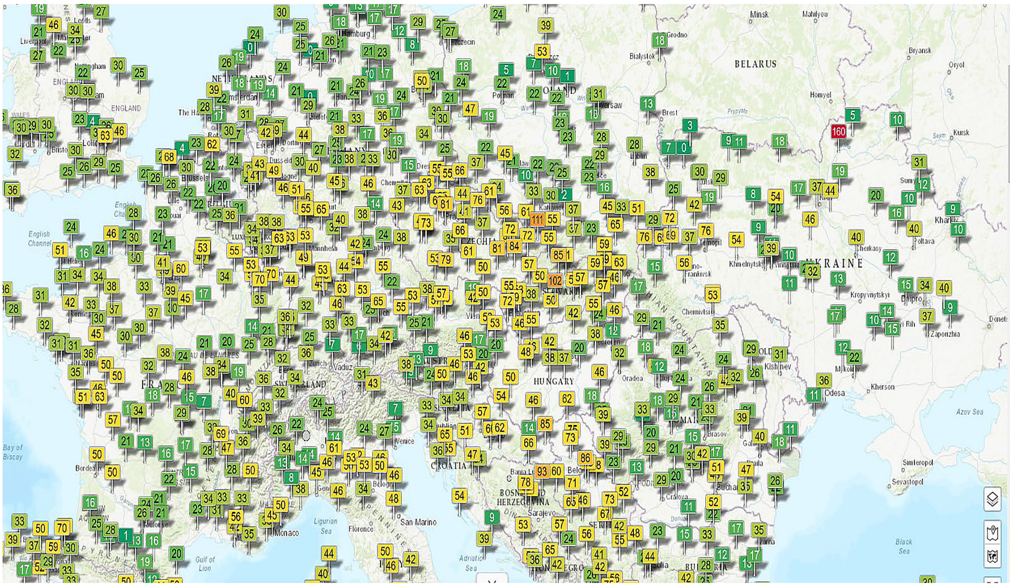

Figure 3. *Density of the air quality monitoring system in the EU*
Source: developed by the authors based on data of World's Air Pollution (n.d.)

In Ukraine, however, there has historically been limited funding, fragmentation of standards, uneven geographical coverage outside regional centres, a lack of regular LCS calibration, and a lack of methodological integration of public data with official data. Military risks and damage to infrastructure have been additional factors. Figure 4 shows that there is no information available on air quality in tourist destinations in the Carpathian region. That is why local initiatives by tourism operators – installing low-cost sensors linked to reference points, opening up data and basic analytics (seasonality, peak hours, correlation with occupancy) – have a double payoff. They improve risk management and service quality at the facility, while also filling gaps in the state network in tourist clusters (Polianytsia-Yaremche-Vorokhta, etc.), where environmental conditions change rapidly and unevenly across the region. As a result, the region receives sufficient data for forecasting, targeting environmental interventions and honest communication with guests, which is a direct investment in the sustainability and competitiveness of the tourism product. Analysis of the clustering results reveals stable spatial patterns that persist over a wide range of p parameters: The "Carpathian resort core" is steadily forming as the largest cluster in terms of total capacity along the Polianytsia-Tatariv-Vorokhta-Yaremche axis, reflecting the actual distribution of accommodation and services and at the same time demonstrating a high degree of consistency with the predefined TN indicators.

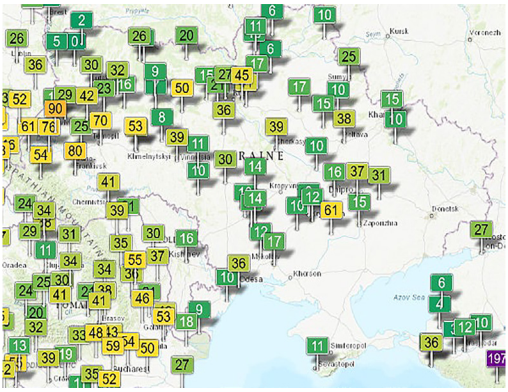

Figure 4. *Density of the air quality monitoring system in Ukraine*

Source: developed by the authors based on data of World's Air Pollution (n.d.)

Overlaying heat maps of tourist load on the configuration of spatial clusters of tourist POIs demonstrates a high level of consistency between demand intensity and clustering structure (Fig. 5-7). The maximum values of tourist load are concentrated in the resort core nodes, in particular in the Bukovel-Polianytsia agglomeration, as well as in the settlements of Tatariv, Vorokhta and Yaremche, which form a functionally linked system with a high density of tourist facilities. Within the satellite microclusters represented by Mykulychyn, Yablunytsia and Deliatyn, a downward gradient of demand is observed, while the peripheral areas are characterised by minimum values of the indicators. This spatial distribution reflects the hierarchical organisation of the tourist space, with the resort core dominating and the intensity of activity gradually decreasing towards the periphery.

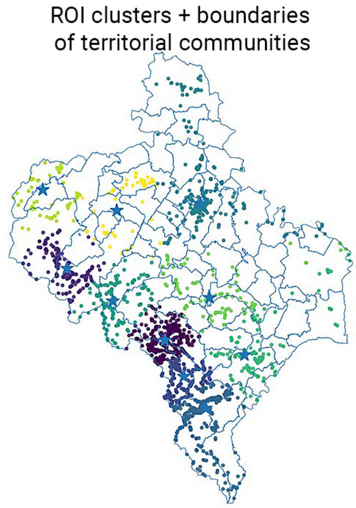

Figure 5. *Clusters of tourist POIs with territorial community boundaries*

Note: configuration p = 10; stars indicate the recommended LCS installation points (medoids of weighted clusters with a minimum mutual distance of 2 km). The spatial framework reflects the dominance of the Polianytsia–Yaremche resort core and its "gateways", with background points in urban and industrial nodes

Source: developed by the authors

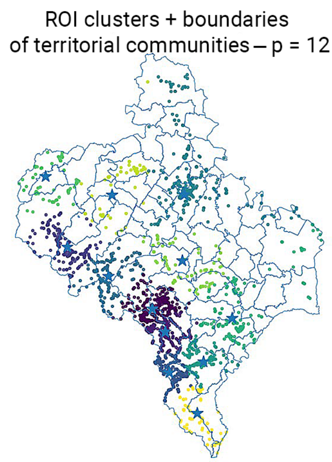

Figure 6. *Clusters of tourist POIs with territorial community boundaries*

Note: network expansion reduces gaps in corridors "downwind" from the core and improves control in the southern sector (configuration p = 12).

Source: developed by the authors

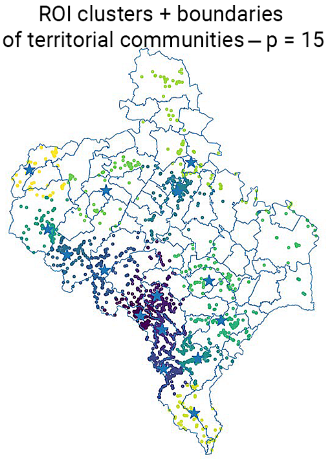

Figure 7. *Clusters of tourist POIs with territorial community boundaries*

Note: additional points ensure more even coverage of peripheral areas, while maintaining the priority of the resort valley; configuration p = 15

Source: developed by the authors

The stability of the selected core is confirmed by the preservation of stable centres of gravity of clusters and insignificant changes in the configuration of boundaries with variations in parameter p (compare Figures 5-7). Such invariance of the spatial structure indicates the presence of a pronounced agglomeration effect, caused by a combination of tourist specialisation, transport accessibility and the valley morphology of the territory. The spatial framework correlates with the linear organisation of transport infrastructure along the H-09 motorway corridor and railway line, which form the main axis of concentration of tourist flows and related facilities. A comparison of configurations with different numbers of clusters demonstrates the evolution of the spatial coverage of the network of observation points. In the p = 10 configuration, a basic spatial framework is formed with a clearly defined resort core and "gateways" to it, while background points tend to gravitate towards urban and industrial hubs (Fig. 5). Increasing the number of clusters to p = 12 reduces spatial gaps in transport and valley corridors, particularly in directions located "downwind" from the main core, and increases control in the southern sector of the study area (Fig. 6). The p = 15 configuration provides more uniform coverage of peripheral areas without losing the priority of the resort valley, which indicates the possibility of scaling the network taking into account the territorial heterogeneity of tourist load (Fig. 7). The analysis of areas of responsibility constructed using the Voronoi method details the functional logic of the spatial organisation of the monitoring network (Figs. 8-10).

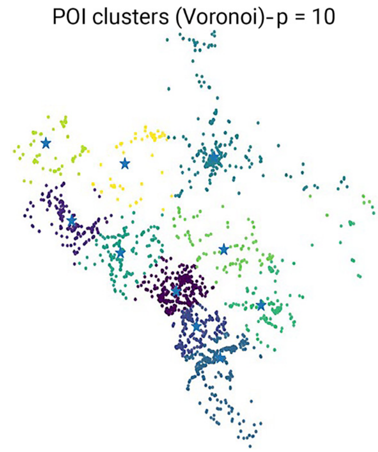

Figure 8. *Areas of responsibility (Voronoi) for p = 10*

Source: developed by the authors

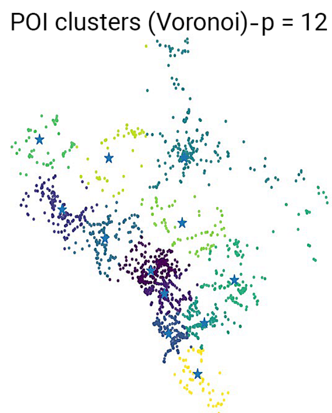

Figure 9. *Areas of responsibility (Voronoi) for p = 15*

Note: there is a finer division of polygons in the high-capacity zone (Polianytsia-Yaremcha core), which reduces the average distance from the POI to the sensor

Source: developed by the authors

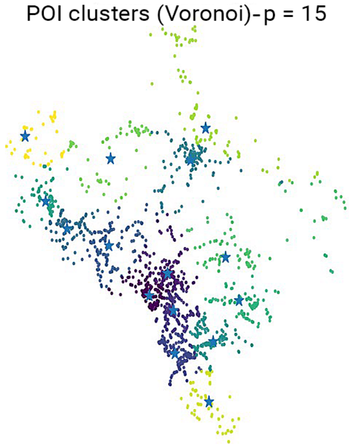

Figure 10. *Areas of responsibility (Voronoi) for p = 15*

Note: the proportion of territory with double coverage in sensitive corridors is increasing, which improves signal reliability in adverse weather conditions

Source: developed by the authors

The basic configuration is characterised by polygons elongated along valleys with minimal overlap, corresponding to the pilot observation framework (Fig. 8). With an increase in the number of points to p = 12, a finer division of polygons is observed in the high-capacity area of the Polianytsia-Yaremche resort core, resulting in a reduction in the average distance from tourist POIs to control points (Fig. 9). Further densification of the network to p = 15 leads to an increase in the proportion of areas with double coverage in the most sensitive transport and recreational corridors, which increases the reliability of signal reception in adverse weather conditions and forms a reserve of spatial stability for the monitoring system (Fig. 10). Analysis of Voronoi diagrams (Fig. 8-10) demonstrates the evolution of the "areas of responsibility" of each site as their number increases. At p = 10, the polygons have an elongated shape along river valleys, reflecting the orographic influence on the distribution of tourist infrastructure. When the number of sites increases to p = 12 and p = 15, fragmentation of polygons is observed in areas of increased tourist capacity, which leads to a decrease in the average distance from points of interest to the nearest sensor and increases the redundancy of the system due to partial overlap of monitoring areas. In the base configuration (p = 10), most locations are concentrated within the main tourist valley and on key transport approaches to it, while peripheral points provide monitoring of the urban background (Ivano-Frankivsk, Kolomyia) and industrial and transport hubs, which is necessary for calibration and control of background concentrations (Table 3).

Table 3. *Recommended LCS points (configuration p = 10)*

Number of POIs in cluster	Total capacity (cond. units)	Coordinates (lon)	Coordinates (lat)	LCS number	Role	Cluster centre	Sector
767	931,000	24.501	48.390	1	resort core	Polianytsia	southern
349	470,000	24.753	48.114	2	high-rise background	Verkhovyna	southeastern
244	425,000	23.902	48.682	3	industrial background	Kalush	western
338	424,000	24.618	48.245	4	resort core	Vorokhta	southeastern
384	406,000	24.719	48.943	5	urban background	Ivano-Frankivsk	northeastern
232	331,000	24.182	48.550	6	resort core	Polianytsia	western
156	167,000	24.989	48.332	7	corridor/valley "downwind"	Kosiv	southeastern
121	121,000	24.776	48.563	8	resort core	Yaremche	eastern
101	112,000	23.754	49.000	9	industrial background	Kalush	northwestern
71	82,000	24.208	48.916	10	industrial background	Kalush	northwestern

Source: developed by the authors

Expanding the network to p = 12 adds coverage in the southern and eastern sectors of the region, corresponding to the directions of the catchment basins from the resort core and the main transit corridors (Table 4). The maximum configuration (p = 15) provides a more uniform presence in the peripheral areas of the mountain and foothill belts and enhances control of the "high-altitude background", which is important for understanding the vertical stratification of pollutants (Table 5).

Table 4. *Recommended LCS points (configuration p = 12)*

Number of POIs in cluster	Total capacity (cond. units)	Coordinates (lon)	Coordinates (lat)	LCS number	Role	Cluster centre	Sector
618	762,000	24.482	48.403	1	resort core	Polianytsia	southern
378	452,000	24.570	48.298	2	resort core	Vorokhta	southeastern
244	425,000	23.902	48.682	3	industrial background	Kalush	western
385	407,000	24.700	48.917	4	urban background	Ivano-Frankivsk	northeastern
256	330,000	24.598	48.113	5	resort core	Vorokhta	southern
219	318,000	24.182	48.550	6	resort core	Polianytsia	western
162	243,000	24.831	48.162	7	high-altitude background	Verkhovyna	southeastern
178	178,000	25.028	48.360	8	corridor/valley "downwind"	Kosiv	eastern
101	112,000	23.754	49.000	9	industrial background	Kalush	northwestern
84	84,000	24.687	48.621	10	resort core	Yaremche	northeastern
70	81	24.20759	48.916028	11	industrial background	Kalush	northwestern
68	77,000	24.788	47.927	12	high-altitude background	Verkhovyna	southeastern

Source: developed by the authors

Table 5. *Recommended LCS points (configuration p = 15)*

Number of POIs in cluster	Total capacity (cond. units)	Coordinates (lon)	Coordinates (lat)	LCS number	Role	Cluster centre	Sector
375	455,000	24.442	48.353	1	resort core	Polianytsia	southern
351	427,000	24.553	48.445	2	resort core	Yaremche	southeastern
295	357,000	24.590	48.292	3	resort core	Vorokhta	southeastern
256	330,000	24.598	48.113	4	resort core	Vorokhta	southern
219	307,000	24.198	48.559	5	resort core	Polianytsia	western
277	299,000	24.694	48.910	6	urban background	Ivano-Frankivsk	northeast
150	297,000	23.942	48.629	7	industrial background	Kalush	western
164	245,000	24.831	48.162	8	high-altitude background	Verkhovyna	southeast
112	157,000	23.811	48.742	9	industrial background	Kalush	western
118	118,000	24.989	48.332	10	corridor/valley "downwind"	Kosiv	eastern
115	115	24.7851614	49.040794	11	urban background	Ivano-Frankivsk	northeast

***Table 5.** Continued*

Number of POIs in cluster	Total capacity (cond. units)	Coordinates (lon)	Coordinates (lat)	LCS number	Role	Cluster centre	Sector
109	109,000	24.907	48.511	12	urban background	Kolomyia	eastern
81	92	23.6862807	49.0080632	13	industrial background	Kalush	northwest
79	90,000	24.208	48.916	14	industrial background	Kalush	northwest
62	71,000	24.805	47.912	15	high-altitude background	Verkhovyna	southeast

Source: developed by the authors

The proposed methodology and optimisation results provide a scientifically grounded basis for the phased deployment of an air-quality monitoring network that corresponds to the spatial structure of tourist demand and accounts for orographic features. To ensure the metrological reliability of the regional air-quality monitoring network, it is advisable to establish a core of three permanent high-precision reference stations intended for the calibration and verification of data obtained from LCS. The spatial placement of the reference stations should correspond to different environmental types and reflect the functional diversity of the region, thereby ensuring the representativeness of measurements and enabling differentiated interpretation of monitoring results. A reference station of the "urban background" type is appropriate for placement within the city of Ivano-Frankivsk, in an area distant from intensive traffic flows and industrial facilities, for example within a residential district or on a university campus. The functional purpose of such a station is to record background concentrations of pollutants in the urban environment, provide a baseline calibration point for the LCS network, and support long-term analysis of air-quality dynamics in the regional administrative centre. The availability of stable power supply, telecommunications infrastructure, and physical security enhances the reliability of observations, while location within an educational environment creates additional opportunities for integrating monitoring into scientific and educational programmes.

A reference station of the "valley background" type should be located within the Polianytsia–Yaremche basin, at a representative point in the lower part of the valley that characterises the typical exposure conditions of the resort zone. The purpose of such a station is to monitor background concentrations of pollutants in the region's key tourist location, analyse the spatial redistribution of impurities under mountain orographic conditions, and record episodes of temperature inversion characteristic of valley environments. The resulting data form the basis for assessing seasonal fluctuations in air quality associated with tourist loads and enable the co-location and calibration of LCS during peak recreational periods.

A reference station of the "industrial background" type is advisable for placement near the town of Burshtyn, in an area situated downwind of the Burshtyn thermal power plant but at a distance sufficient to register dispersed rather than local emissions. The functional significance of such a station lies in separating the industrial component of pollution from tourism-related impacts, monitoring the region's largest stationary emission source, and providing a control baseline for analysing transboundary pollutant transport. Industrial background data enable comparative analysis with tourist zones and serve as an informational basis for evaluating the effectiveness of environmental protection measures.

The proposed configuration of reference stations forms a comprehensive calibration framework for the entire low-cost sensor network, enhances the reliability of spatio-temporal air-quality assessments, and creates the preconditions for scientifically grounded interpretation of monitoring results in the context of interactions between tourism activities and the atmospheric environment of the region.

The results of weighted clustering of tourist points of interest (POIs) extracted from OpenStreetMap indicate the spatial dominance of the Polianytsia–Yaremche resort valley, where the majority of accommodation capacity and related infrastructure is concentrated. The identified distribution of tourist demand justifies the prioritised placement of the low-cost sensor network within this zone. A comparative analysis of alternative configurations shows that the option with twelve stations ($p = 12$) provides an optimal compromise between investment costs and the quality of spatial coverage, significantly reducing the average distance from tourist sites to the nearest monitoring point in environmentally sensitive corridors located under the influence of airflows from the main centres of recreational activity. Expanding the network to fifteen stations ($p = 15$) increases system reliability through partial overlap of responsibility zones and improves monitoring of peripheral areas, which is critically important for the timely detection of pollution episodes during peak tourist seasons.

The integration of background monitoring points in urban and industrial areas is a methodologically necessary condition for calibrating low-cost sensor data and correctly differentiating tourism-related impacts from general anthropogenic loads. The coordinates of the optimal location of stations and the defined zones of their spatial responsibility are presented in a reproducible format in the form of software scripts and CSV files, which ensures the transparency of the methodology and the readiness of the results for practical validation during a micro-situational survey of potential locations. As a result, a network configuration of three permanently operating reference stations and an optimised number of LCSs is proposed, capable of ensuring the metrological reliability, analytical depth and practical applicability of the air quality monitoring system in the tourist regions of the Ivano-Frankivsk region.

A summary of the results of recent empirical studies confirms a stable and statistically significant relationship between the level of atmospheric pollution and the spatial-temporal dynamics of tourist flows. In particular, the work of Y. Wang *et al*. (2023) demonstrates that the transparency of environmental information is an independent factor in the formation of tourist mobility, influencing the redistribution of demand between destinations. The public disclosure of data by F. Neirotti *et al*. (2020) on air quality has created competitive advantages for areas with low concentrations of pollutants and, at the same time, reduces the attractiveness of regions where environmental reporting is fragmentary or unavailable. Econometric estimates indicate a direct correlation between pollutant concentrations and the sensitivity of tourist demand to information transparency, which provides a scientific basis for institutional investment in monitoring systems and digital platforms for environmental communication at the destination level.

An in-depth analysis of tourist behaviour data conducted by Y. Yang *et al*. (2022) based on millions of TripAdvisor user reviews complements macroeconomic conclusions with micro-level evidence. The negative and statistically significant relationship between PM2.5 concentrations and tourist attraction ratings confirms that air pollution directly affects the subjective perception of the tourist experience. The use of panel regression models with spatial and seasonal controls minimises the risk of biased ratings and allows the decline in tourist demand to be interpreted as a result of environmental pressure rather than confounding factors. The systematisation of results in the review by C. Eusébio *et al.* (2021) demonstrates the conceptual consistency of scientific conclusions in different regional and methodological contexts. The negative impact of air quality deterioration can be seen not only in the reduction in the number of tourist arrivals, but also in the reduction in the length of stay and the level of tourist satisfaction. In this interpretation, atmospheric air monitoring is not an auxiliary technical tool, but a component of strategic management of tourist demand and destination sustainability.

Macro-level empirical estimates by S.A. Churchill *et al*. (2022) for a sample of G20 countries for 1995-2014 confirm the systemic nature of environmental determinants of international tourism. Increases in PM2.5 and CO_2 concentrations are associated with a statistically significant reduction in international tourist arrivals, with developing countries showing greater sensitivity to environmental degradation. The results indicate an asymmetric distribution of environmental risks in the global tourism space and strengthen the argument for preventive environmental policies in regions with high dependence on tourism. Regional studies complement the overall picture by capturing the bidirectional nature of the interaction between tourism and the atmospheric environment. An analysis by M. Robaina *et al*. (2020) using European countries as an example shows that air quality degradation reduces tourist flows, while peak tourist activity can be a factor in the deterioration of local environmental indicators. This interdependence confirms the limitations of one-dimensional approaches to tourism management and highlights the need to integrate environmental constraints into spatial and seasonal planning of tourism activities. The results of Y. Tan *et al*. (2022), obtained using mobile tracking and PCSE assessment, expand the analytical framework by demonstrating the significant impact of air quality at both tourist destinations and points of departure. The review by S. Wang *et al*. (2024) added an economic dimension, demonstrating that in most cases the benefits of reducing fine particulate matter concentrations outweigh the costs of environmental interventions. For tourist destinations, these results mean not only reduced health risks, but also increased competitiveness and stability of tourist demand.

Compared to other studies, the current study emphasises the importance of integrating the public into the air quality monitoring process, as confirmed by the work of H. Relvas *et al*. (2025), which highlights the key role of involving local residents and tourists in improving the accuracy and timeliness of data collection. This is consistent with the findings of the current study, where the use of low-cost sensors in tourist areas contributes to improved monitoring and effective management of environmental risks. Also important is the work of N. Song & Y. Zhang (2025), which shows how environmental pollution affects tourist flows, particularly during peak tourist activity. The current study confirms this by applying geospatial methods to optimise the placement of monitoring stations in the most intensively visited tourist clusters, taking into account seasonal fluctuations in air pollution. Overall, the results of the current study make a significant contribution to the development of air quality monitoring methodology, taking into account both tourist activity and seasonal changes in pollution, allowing for more accurate forecasts and more effective management of environmental risks in tourist regions.

Compared to the study by I. Logothetis *et al.* (2025), which focuses on optimising air quality monitoring and waste management for sustainable island development, the current study extends this approach by applying geospatial models to create an air quality monitoring network in tourist regions. The study by I. Logothetis *et al*. focused on the integration of various aspects of environmental management, particularly in the context of small islands with limited infrastructure for air pollution control. However, in contrast to this, the current study proposes a more complex monitoring network structure that includes both stationary and low-cost sensors for broader coverage of high-traffic tourist areas. The current study also focuses on the specifics of tourist regions, where environmental pollution varies depending on seasonality and the intensity of tourist flows, which is an important aspect not covered in the work of I. Logothetis *et al*. Taking these seasonal fluctuations into account is important for ensuring a comprehensive approach to monitoring and managing environmental risks in such regions, which makes the approach of the current study particularly relevant for tourist areas.

The economic feasibility of investments in pollution control is also confirmed in the analytical work of A. Dechezleprêtre *et al*. (2019), which shows that the benefits of environmental policies in the European context are comparable to or higher than the costs, even without accounting for the full range of social effects. Together with the findings of the systematic review by I.B. Bacos *et al*. (2024), this forms an understanding of air-quality monitoring as a “soft” infrastructure factor which, without substantial capital investment, can significantly influence tourist behaviour and the long-term competitiveness of destinations. An additional argument in favour of the economic viability of investments in the control and monitoring of atmospheric air pollution is provided by the study of X. Wu *et al*. (2020), which analysed the relationship between long-term exposure to fine particulate matter $PM_{2.5}$ and COVID-19 mortality rates across the United States. Based on an ecological regression model, the authors show that even a slight increase in average annual $PM_{2.5}$ concentrations is statistically significantly associated with higher mortality, indicating substantial hidden socio-economic losses linked to chronic air pollution. The study emphasises that the effects of pollution manifest themselves through the burden on health care systems, reduced population resilience to epidemiological risks, and increased indirect economic costs. At the same time, the authors critically outline the limitations of the environmental approach, emphasising the need for high-quality, spatially detailed air quality monitoring data for the correct interpretation of results. In this context, monitoring systems are seen as part of a preventive environmental policy that can reduce long-term social and economic losses, even if the direct costs of deploying them are relatively modest. The practical dimension of implementing such approaches is detailed in the work of P. Neis *et al*. (2023), which proves that low-cost sensors provide an acceptable level of reliability provided that calibration and operating protocols are followed. In tourist regions with complex terrain, the use of LCS technologies allows the creation of dense observation networks that form a spatially detailed picture of air quality and open up new opportunities for environmentally oriented management of tourist activity.

Summarising the above results, air quality is a key factor in safety and comfort for tourists, influencing their choice of destination, travel time parameters and the nature of recreational activities. The availability of data on $PM_{2.5}$, PM_{10}, NO_2 and ozone concentrations reduces information asymmetry between tourists and market operators, builds trust in destination management practices and strengthens the case for the implementation of local, transparent and scientifically sound air quality monitoring systems.

Conclusions

The study confirmed that the formation of a regional system for monitoring and managing the environmental safety of tourism is a necessary prerequisite for ensuring the sustainable development of tourist destinations and increasing their competitiveness. The results obtained demonstrate the limitations of the existing air quality monitoring system in terms of spatial coverage, temporal resolution, and the ability to record the impact of tourist load, especially in resort and mountain areas of the Ivano-Frankivsk region. The proposed approach to designing a monitoring network, based on a combination of high-precision reference stations and a network of low-cost sensors, ensures the metrological reliability of measurements and, at the same time, high spatial detail of data. The use of geospatial clustering of tourist sites and optimisation models for station placement made it possible to justify the priority of the Polianytsia-Yaremcha resort valley and determine the optimal network configurations that minimise the average distance to monitoring points and increase the sensitivity of the system to peak seasonal loads.

The results of the study are consistent with current international scientific findings on the impact of air quality and information transparency on tourist demand and visitor behaviour. The methodology developed provides an analytical basis for integrating environmental data into regional tourism management policies, communication with tourists, and management decisions in the field of environmental safety. Further research should focus on empirical validation of the proposed network configuration based on a pilot deployment of sensors, expanding the range of monitored pollutants, taking into account the specifics of tourism activities, as well as developing models for forecasting short-term risks for tourists during periods of peak load and adverse weather conditions.

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Conflict of Interest

None.

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Формування регіональної системи екологічного моніторингу атмосферного повітря в туристичних дестинаціях

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Анотація. Незважаючи на розвиток систем екологічного моніторингу в Україні, існуючі підходи не враховують специфіку туристичних регіонів, що створює значні виклики для оцінки екологічних ризиків та забезпечення якості атмосферного повітря в умовах інтенсивних туристичних потоків. Метою дослідження було обґрунтування концепції формування регіональної системи моніторингу та управління екологічною безпекою туризму в територіальних громадах Івано-Франківської області з урахуванням європейських стандартів контролю якості атмосферного повітря. Методологічну основу становили аналіз європейського та національного екологічного законодавства, геопросторове моделювання на базі відкритих даних OpenStreetMap, індикаторів туристичної місткості та інтенсивності ночівель, а також оптимізаційні постановки задачі максимального покриття та p-медіани. Показано, що наявна державна система спостережень має обмежене просторове покриття, зорієнтована переважно на промислові центри, не охоплює курортні та гірські дестинації й не відображає пікові сезонні навантаження, пов'язані з туристичними потоками. Розроблено дворівневу архітектуру мережі, що поєднує три референсні станції високої точності (міський, долинний та промисловий фон) з адаптивною кількістю низьковартісних сенсорів (10-15 LCS), розташованих у ядрах туристичного попиту й транспортних коридорах. Зважена кластеризація 3 725 туристичних об'єктів виявила домінуючу роль курортної долини Поляниця-Яремча та дала змогу виділити конфігурацію з 12 сенсорів як збалансований варіант, що мінімізує середню відстань до пунктів моніторингу та забезпечує резервування вимірювань у чутливих зонах. Отримані результати створюють підґрунтя для поетапного розгортання мережі моніторингу якості повітря, інтеграції екологічних показників у політику управління туризмом, екологічний маркетинг територій та ESG-звітність суб'єктів туристичного бізнесу

Ключові слова: екологічна безпека туризму; якість атмосферного повітря; регіональна система моніторингу; низьковартісні сенсори; геопросторове моделювання; оптимізація розміщення станцій; туристичні дестинації

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Turnip yield in open soil conditions in Polissya under different plant protection systems

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Abstract. The aim of the study was to evaluate the effectiveness of bacterial and chemical preparations regarding their impact on turnip yield and specific quality indicators of its root crops under different plant protection systems. The research was conducted at the Volyn State Agricultural Research Station of the NAAS in 2024-2025 using the turnip varieties 'Zolota Kulya', 'Purpurova', 'Purpletop', and 'Geisha'. To protect against diseases, the chemical fungicide Magnicur Boost and the biological products Phytocide-r and Mycohelp (BTU company) were applied; for pest protection, the chemical insecticide Confidor Maxi and the biological products Bitoxibacillin-BTU and Aktoverm-formula (BTU company) were used. An untreated variant served as the control. The study was established with fourfold replication following standard vegetable-growing methodology. The results showed that the application of the biofungicide Phytocide-r increased the root crop yield of the 'Purpletop' variety to 22.2 t/ha, and for the 'Zolota Kulya', 'Purpurova', and 'Geisha' varieties (using Phytocide-r or Mycohelp) by 0.9-1.2 t/ha. Magnicur Boost and Phytocide-r increased the starch content in 'Zolota Kulya' root crops to 7.12-7.30% and vitamin C to 19.3-19.5 mg/100 g. The 'Purpletop', 'Geisha', and 'Purpurova' varieties proved to be more resistant to downy mildew; Mycohelp limited the spread of the disease to 2.0-2.7% and its development to 0.21-0.31%, while Magnicur Boost limited the spread to 0.76%. The systemic use of the bioinsecticides Bitoxibacillin-BTU and Aktoverm-formula increased the yield

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of the 'Purpletop' and 'Purpurova' varieties to 21.6-22.0 t/ha, whereas Confidor Maxi only increased it by 5%. Without insecticidal protection, the yield was at its lowest. Biological products increased the dry matter content to 16.3-16.4%, starch to 6.29-6.74%, protein to 5.4%, and vitamin C to 19.2-19.3 mg/100 g. Bitoxibacillin-BTU and Aktoverm-formula proved to be more effective against the adults of the black crucifer flea beetle

Keywords: fungicide; insecticide; turnip; yield; root crop; quality; effectiveness

Introduction

Food quality is a critical factor for the national security of Ukraine. Plant products, particularly vegetables, form the basis of the Ukrainian consumer's diet. The full-scale aggression of the Russian Federation has altered the geography of vegetable production: cultivation is shifting from the southern regions to the central and western provinces. In modern conditions, increasing attention is being paid to minor vegetable crops with a high content of biochemical components. Among these, the turnip stands out for its resistance to unfavourable climatic conditions, long shelf life, and nutritional value. At the same time, the low yield of the crop is often due to insufficient protection against diseases and pests. Traditional chemical agents, despite their effectiveness, raise concerns regarding the accumulation of toxic substances in the produce. This prompts the search for biological preparations that ensure the ecological safety of the produce while maintaining high crop productivity (Vakhovska, 2023).

In the context of searching for effective and ecologically safe turnip protection systems, the recommendations of G.I. Yarovyi & O.V. Romanov (2025) are significant. The authors systematised the principles of integrated protection for cruciferous crops against downy mildew, alternaria, black leg, and flea beetles, emphasising the advantages of combining agro-technical, biological, and, where necessary, chemical methods to ensure high yields and root crop quality in the conditions of the Ukrainian Polissya. S.V. Stankevych *et al.* (2024) provided a detailed characterisation of the main harmful organisms of cruciferous crops, particularly rapeseed. The authors developed a comprehensive integrated protection system that combined agro-technical, biological, and chemical measures to limit the spread of diseases, pests, and weeds. The work focused on ecologically justified approaches to minimising yield losses and improving product quality under Ukrainian conditions.

V. Shvets (2023) investigated the implementation of biological protection methods for cruciferous crops in modern organic production, highlighting the need for a comprehensive approach to ensuring the phytosanitary welfare of crops. The author emphasised the importance of using biological preparations based on beneficial microorganisms for protection against fungal diseases and pests as an alternative to traditional chemical means. V.P. Rud (2023) analysed the effectiveness of various application schemes for biofungicides to protect cruciferous vegetable crops. The results demonstrate that correctly selected biological products can provide a sufficient level of protection against the main pathogens when agro-technical requirements and the timeliness of treatments are observed.

Research by G. Wu *et al.* (2024) is of great importance for understanding the protection problems of cruciferous crops; they conducted a comprehensive review of the pathogenesis of the turnip mosaic virus and host plant resistance mechanisms. The authors systematised information on the molecular mechanisms of virus interaction with plants of the genus Brassica, identified candidate resistance genes, and proposed new strategies for controlling viral infection based on genetic resistance. This comprehensive approach to studying viral diseases opens new possibilities for breeding resistant varieties of cruciferous crops. P. Palukaitis & S. Kim (2021) researched the diversity of turnip mosaic virus isolates and their impact on various Brassica species. The authors established that significant variability exists in the virulence of different virus strains, which must be considered when developing protection strategies. The study confirmed the presence of different types of resistance in representatives of the genus Brassica rapa, including turnips, which can be utilised in breeding programmes.

In the context of biological plant protection, the review by R. Lahlali *et al*. (2022) is significant, as it analysed global perspectives on the biological control of phytopathogens. The researchers established that biological control agents, particularly representatives of the genera Pseudomonas, Bacillus, Burkholderia, and Trichoderma, demonstrate high effectiveness against a wide spectrum of foliar and soil-borne pathogens. The authors noted that, despite a slightly lower efficacy compared to chemical preparations, biological protection agents offer additional benefits such as plant growth stimulation and environmental safety.

C. Lago *et al*. (2024) investigated the impact of the turnip yellows virus and drought on rapeseed productivity under climate change conditions. The authors established that the combined stress of viral infection and water deficit significantly exacerbates the negative impact on plant growth and development. These results highlight the need to develop comprehensive protection strategies that account for changing climatic conditions and the spread of viral diseases. The authors paid particular attention to the necessity of using resistant varieties and optimising protection systems in the face of the predicted increase in the frequency and intensity of droughts. K. Macleod *et al*. (2023) conducted a thorough analysis of resistance sources to the turnip yellows virus in various Brassica species. The researchers identified several new quantitative trait loci (QTL) in the genomes of *B. napus*, *B. rapa*, and *B. oleracea*, which can be used to develop commercial varieties with increased resistance. The authors emphasised the importance of implementing diverse resistance sources to reduce selection pressure on the virus and prolong the effectiveness of the resistance.

C.F. Nellist *et al*. (2022), in their comprehensive review, characterised the turnip mosaic virus as a "virus for all seasons", describing its broad host range and ability to adapt to various environmental conditions. The authors analysed the phylogenetic groups of the virus, the specifics of its interaction with different cruciferous species, and the mechanisms of transmission via aphids. Particular attention was paid to the diversity of isolates adapted to Brassica and Raphanus, which is vital for understanding the epidemiology of the disease.

The aim of the study was to establish the influence of different plant protection systems (using preparations of bacterial and chemical origin) on turnip productivity and the biochemical quality indicators of its root crops

Materials and Methods

The experimental part of the research was conducted under the conditions of the Volyn State Agricultural Research Station of the Institute of Agriculture of the Carpathian Region of the National Academy of Agrarian Sciences of Ukraine during 2024-2025, using turnip varieties 'Zolota Kulya', 'Purpurova', 'Purpletop', and 'Geisha'. The seeds of these varieties were sown in the second decade of April. The soil of the experimental plot is sod-podzolic light loamy with the following agrochemical indicators: humus content 1.18%; pH of salt extract – 5.3; nitrogen content (easily hydrolysed) – 61 mg/kg, mobile phosphorus – 135.1 mg/kg, and mobile potassium – 81.33 mg/kg of air-dry soil.

To evaluate the effectiveness of fungicides, the chemical preparation Magnicur Boost was used at a dose of 800 g/ha, along with biological preparations of bacterial origin, Phytocide-r and Mycohelp, produced by the BTU company. Seed treatment with Phytocide-r was carried out at a rate of 1 l/kg of seeds, followed by spraying at 2 l/ha in two stages – at the 1-2 true leaf phase and 15 days after the first treatment. For Mycohelp, the seed treatment rate was 0.7 l/kg of seeds, with spraying at 2 l/ha according to a similar scheme. To evaluate the effectiveness of insecticides, the chemical preparation Confidor Maxi was used at a rate of 20 g/ha, as well as the biological products Bitoxibacillin-BTU and Aktoverm-formula produced by the BTU company. Spraying with Bitoxibacillin-BTU was carried out at a rate of 8 l/ha in three stages: at the cotyledon leaf phase, during the formation of vegetative mass, and 15 days after the second treatment. Aktoverm-formula was applied at a rate of 4 l/ha following the same schedule. The control variant consisted of plants that were not treated with an y preparations. The quality indicators of the produce were determined in the root crops of the 'Zolota Kulya' variety. The study was established with fourfold replication according to the methodology of experimental work in vegetable and melon growing (Bondarenko & Yakovenko, 2001; Rozhkov, 2016). The research was conducted in compliance with the principles of the Convention on Biological Diversity (1992), specifically regarding the sustainable use of plant genetic resources, the conservation of agroecosystem biodiversity, and the fair distribution of benefits from the use of biological materials. The use of bacterial-origin biological products contributed to minimising negative environmental impacts and maintaining ecological balance in agrocenoses.

Quality indicators of the root crops were determined under laboratory conditions using standard methods. In samples of the 'Zolota Kulya' variety, the dry matter content (by drying to constant weight), starch (by the iodometric method), crude protein (by the Kjeldahl method with conversion to protein using a factor of 6.25), and ascorbic acid (vitamin C) by titration with 2.6-dichlorophenolindophenol were analysed. The results of the biochemical analyses are summarised in Table 2, which shows the starch and protein content as a percentage of fresh weight, as well as the vitamin C content in mg per 100 g of fresh root crop weight. Determinations were carried out in three analytical replicates for each field replication.

Results and Discussion

The studied varieties formed typical root crops that matched their varietal characteristics in shape and colour and were not damaged by either bacterial or fungal diseases. During the analysis of plant productivity for the varieties under study, the indicator was not constant and varied depending on the growing conditions and the fungicide applied. The soil and climatic conditions of the 2024 growing season were more favourable than those of 2025 for the 'Purpurova', 'Purpletop', and 'Geisha' turnip varieties. However, on average, the root crop yield in the experiment, when sown in the second decade of April, amounted to 19.5-22.2 t/ha (Table 1).

Table 1. *Turnip yield with fungicide protection, t/ha*

Varieties	Years of cultivation	Control	Magnicur Boost	Phytocide-r	Micohelp	LSD_{05}
'Zolota Kulya'	2024	20.7	20.9	21.7	20.8	0.3
	2025	20.6	21.1	21.5	21.6	0.6
	Average	20.7	21.0	21.6	21.2	
	± to control	-	+0.3	+0.9	+0.5	
'Purpurova'	2024	21.3	21.5	21.8	21.6	0.3
	2025	20.3	21.4	21.5	21.4	0.8
	Average	20.8	21.5	21.7	21.5	
	± to control	-	+0.7	+0.9	+0.7	
'Purpletop'	2024	21.5	22.0	22.2	22.0	0.4
	2025	20.4	21.7	22.1	21.5	0.6
	Average	21.0	21.9	22.2	21.8	
	± to control	-	+0.9	+1.2	+0.8	

Table 1. *Continued*

Varieties	Years of cultivation	Control	Magnicur Boost	Phytocide-r	Micohelp	LSD_{05}
'Geisha'	2024	19.5	20.0	21.6	21.4	0.6
	2025	19.8	19.9	20.3	20.4	0.3
	Average	19.7	20.0	21.0	20.9	
	± to control	-	+0.3	+1.3	+1.2	
Yield of tops, t/ha		30.17	32.45	33.04	32,42	2.22

Source: developed by the authors

In determining the effectiveness of the fungicide according to the existing protection scheme (seed treatment at 1 l/kg + spraying at 2 l/ha in two stages: 1-2 true leaves and 15 days after the first treatment), it was established that the biofungicide Phytocide-r ensured the highest turnip root crop yield when growing the 'Purpletop' variety. In this variant, the yield indicator, averaged over the years of cultivation, was 22.2 t/ha, which exceeded the control root crop yield by 1.2 t/ha.

Simultaneously, when growing the 'Zolota Kulya' and 'Purpurova' varieties using Phytocide-r (seed treatment 1 l/kg + spraying 2 l/ha in two stages: 1-2 true leaves and 15 days after the first treatment) and the 'Geisha' variety using the biofungicide Mycohelp (seed treatment 0.7 l/kg + spraying 2 l/ha in two stages: 1-2 true leaves and 15 days after the first treatment), the root crop yield was slightly lower but still exceeded the control yield by 0.9-1.2 t/ha. The plants of these varieties responded better to the protection system compared to the control variant when using these biological products. This is likely due to the presence of natural bacteria *Bacillus subtilis* in the preparations at a concentration of at least 1.0×10^9 CFU/cm^3, which provided improved plant protection by increasing resistance to unfavourable environmental conditions, promoting better growth and development, and protecting against a wide range of fungal and bacterial pathogens, as evidenced by the yield of turnip tops. The continued use of biological preparations and their positive effect on plant productivity is consistent with key European Union documents and the conference "Modern Biotechnologies in Agriculture – Paving the Way for Responsible Innovation", organised by the European Food Safety Authority (European Commission, 2017).

The application of a fungicide of chemical origin is also effective for growing turnips in open-soil conditions. As a result of growing the 'Purpletop' variety and using the fungicide Magnicur Boost, the average root crop yield was also quite high at 21.9 t/ha, with an increase over the control of 0.9 t/ha. The use of this systemic fungicide, which contains 800 g/kg of fosetyl-aluminium, allowed for rapid penetration of the solution into the plant, which ensured the stimulation of the plant's natural immunity and a long-lasting preventive effect; the turnip plants also exhibited resistance to fungal diseases. The obtained turnip yield data do not coincide with the turnip yield data of S.A. Vdovenko (2019), who grew them in the open-soil conditions of the Right-Bank Forest-Steppe. In that author's research, turnip yields fluctuated depending on the sowing dates between 12.5 and 22.1 t/ha, whereas in the current study, the yield was close to the maximum value, indicating high productivity and the potential of the varieties studied. However, the size of the turnip root crops in current research was 4-9 cm, which coincides with the data of V.V. Khareba *et al.* (2016).

The studied disease protection products also influenced the quality of the turnip root crops. The biochemical indicators of the turnip root crops depended on the origin of the preparation. Phytocide-r (seed treatment 1 l/kg + spraying 2 l/ha in two stages: 1-2 true leaves; 15 days after the first treatment) and Mycohelp (seed treatment 0.7 l/kg + spraying 2 l/ha in two stages: 1-2 true leaves; 15 days after the first treatment) contribute to a slight decrease in the total dry matter content. In these variants, the studied indicator was at the level of 15.1-15.4% (Table 2).

Table 2. *Quality of root crops of the turnip variety 'Zolota Kulia' after fungicide application (average for 2023-2025)*

Turnip disease protection system	Content of biochemical substances in turnip root crops, %			
	Dry matter	Starch	Protein	Vitamin C, mg/100 g
1. Control	15.6 ± 0.04	6.22 ± 0.03	5.1 ± 0.03	18.4 ± 0.05
2. Magnicur Boost	15.4 ± 0.03	7.12 ± 0.03	5.3 ± 0.04	19.3 ± 0.04
3. Phytocide-r	15.1 ± 0.04	7.30 ± 0.03	5.4 ± 0.03	19.5 ± 0.03
4. Mycohelp	15.4 ± 0.04	6.65 ± 0.03	5.4 ± 0.02	18.9 ± 0.03

Source: developed by the authors

The chemical preparation Magnicur Boost and the biofungicide Phytocide-r ensured an increase in the starch content of 'Zolota Kulya' root crops up to 7.12% and 7.30% respectively, which exceeded the control variant by 15-17%. A trend towards increasing starch content relative to the control was identified with the use of the biofungicide Mycohelp, which contains a higher bacterial count. The protein content in the turnip root crops remained at a level of 5.1-5.4% regardless of the protection system; however, a trend towards its increase was observed in the variants where fungicides were applied.

One of the vital quality indicators for root crops is the vitamin C value. The highest vitamin C content was established using the biofungicide Phytocide-r and the fungicide Magnicur Boost, where the studied value could increase to 19.5 and 19.3 mg/100 g of product respectively, exceeding the control by 1.1 times. During the cultivation of the 'Zolota Kulya' variety and the application of the biofungicide Mycohelp, research established a trend towards an increase in vitamin C content relative to the control by only 0.5 mg/100 g of product. In the studies by V.I. Smolyar (2000), the physiological and hygienic properties of turnips were studied, specifically their chemical composition. According to that work, turnips contained 10.5-13.0% dry matter, 0.1% starch, up to 1.74% protein, and up to 60 mg/100 g of vitamin C. This provided baseline parameters for assessing the nutritional value of turnips. However, the current study revealed that the application of biofungicides and improved agro-techniques contributed to a significant increase in the content of starch, protein, and vitamin C. For instance, with the use of the biofungicide Phytocide-r, the starch content in 'Zolota Kulya' root crops reached 7.30%, significantly exceeding the figures cited in V.I. Smolyar's research. This indicates the possibility of significantly improving the nutritional value of turnips through the application of modern cultivation and plant protection methods. In a study by C. Chen-Tien *et al*. (2000), the purification and properties of chitinases isolated from radish root crops were examined. It was found that the chitinases contained in radishes possess antifungal activity, which adds value to the crop in the food and medical industries. In the current study, the use of biological products such as Mycohelp, which contains natural bacteria, also demonstrated effectiveness in disease control, particularly against fungal pathogens. This confirms the importance of using biological agents to improve product quality and disease protection. Research by O.V. Khareba *et al*. (2021) highlights the turnip as a rare vegetable crop containing significant amounts of minerals such as iron, potassium, and calcium. The work notes that turnips are a promising product for therapeutic nutrition due to their properties in normalising blood pressure and improving the condition of the respiratory system. The current study also identified a significant increase in the mineral content of turnip root crops grown using modern agro-technologies, confirming the importance of proper plant care for achieving high-quality indicators. The vitamin C content in the root crops was 19.5 mg/100 g, which exceeds the data reported in the literature. These values attest that the conditions for growing turnips in Polissya and the protection system can significantly improve the quality indicators of the root crop, ensuring they fully meet modern requirements.

During the cultivation of vegetable plants in open-soil conditions, both the spread and development of disease were observed. According to research by M.V. Zubets *et al*. (2010), pathogens can have a dual impact on yield and quality indicators, depending on growing conditions and the applied agro-technical measures. They note that in organic farming, a correct approach to cultivation technology is capable of minimising the impact of diseases on yield. The current study also recorded that a proper plant protection system, including biological products, ensures not only a reduction in disease levels but also improves the qualitative characteristics of turnip root crops. For example, the application of biofungicides significantly reduced the spread and development of downy mildew, confirming the ability of biological preparations to enhance product quality. According to the work of L.A. Zhurakovska (2013), pathogens can either reduce yields or, under certain conditions, even improve quality indicators. This depends on the choice of protection methods and cultivation technologies. In the current research, it was established that the application of biological products such as Phytocide-r and Mycohelp not only increased yield but also contributed to the improvement of biochemical indicators in the root crops. Thus, the starch content in turnip root crops rose to 7.30%, a significant improvement compared to the control variants. This indicates that the selection of appropriate agro-technologies and protection agents can influence product quality not only by reducing disease levels but also by improving the physiological indicators of the plants.

In the experiment conducted, the systemic application of fungicides of both bacterial and chemical nature contributed to a significant reduction in the spread and development of the disease, which fluctuated at levels of 2.0-6.41% and 0.21-2.98% respectively. Downy mildew is considered the most dangerous disease for turnip plants. Among the turnip varieties studied, 'Purpletop', 'Geisha', and 'Purpurova' proved to be more resistant to infection and the further development of downy mildew (Table 3).

Table 3. *Fungicidal protection of turnip depending on technological components (average for 2024-2025)*

Variety	Fungicidal protection							
	Control		Magnicur Boost		Phytocide-r		Mycohelp	
	downy mildew							
	disease spread, %	disease development, %	disease spread, %	disease development, %	disease spread, %	disease development, %	disease spread, %	disease development, %
'Zolota Kulya'	4.94	1.67	3.0	1.08	3.30	1.15	3.4	0.45
'Purpurova'	6.41	2.98	2.9	0.76	3.81	1.22	2.7	0.31
'Purpletop'	5.16	1.57	2.4	1.92	3.74	1.20	2.0	0.21
'Geisha'	5.85	1.82	3.0	1.24	4.52	1.63	2.3	0.27

Source: developed by the authors

Based on the data obtained, it was established that in the variant where no fungicidal preparations were applied, the rate of spread and development of downy mildew was considerably high. As a result of growing the 'Purpurova', 'Purpletop', or 'Geisha' varieties and applying Mycohelp (seed treatment at 0.7 l/kg + spraying at 2 l/ha in two stages: 1-2 true leaves and 15 days after the first treatment), the disease spread value was the lowest, amounting to only 2.0-2.7%, and the lowest rate of downy mildew development (0.21-0.31%) relative to the control was also obtained. Evidently, the bacteria that form the basis of the preparation work more effectively against harmful fungi, especially during periods of excessive rainfall, which positively influenced the overall plant yield. A significant reduction in the spread and subsequent development of the disease was ensured by the chemical fungicide Magnicur Boost at a dose of 800 g/ha during the cultivation of the 'Purpurova' variety. In this variant, the reduction in disease spread on turnip crops was 0.76%, which differed from the control variant value. However, according to S.V. Stankevych *et al.* (2024), the reduction and control of disease through chemical fungicides can show somewhat higher indicators. Simultaneously, the authors emphasised that modern protection of the Brassicaceae family must account for organisational, management, agro-technical, biological, and chemical methods: these are the most reliable and profitable, while each method individually significantly limits the spread and development of a given disease, where plant infection can fluctuate from 4% to 45%. In current research, the spread of the disease was at a significantly low level, which does not coincide with the data of S.V. Stankevych *et al.* When using the biofungicide Phytocide-r, the rate of disease spread and development decreased relative to the control by 1.1-2.4 times, yet remained high relative to the values where Mycohelp and Magnicur Boost were applied.

On turnip crops, the most common pests were the black crucifer flea beetle and aphids. Without a proper protection system, these representatives are capable of significantly reducing the overall turnip yield. Turnip productivity, depending on the insecticides applied, was not constant and depended on the origin and composition of the preparation as well as varietal characteristics. It was established that throughout the years of cultivation, turnip root crop yields remained practically at the same level. Among the varieties studied, 'Purpurova' and 'Purpletop' were characterised by higher yields, amounting to 21.3 and 21.6 t/ha respectively, exceeding the yield of the control variety 'Zolota Kulya' by 0.7-1.0 t/ha.

As a result of the systemic application of the biological products Bitoxibacillin-BTU (spraying the plant at a dose of 8 l/ha in three stages: cotyledon leaves; formation of vegetative mass; 15 days after the second treatment) or Aktoverm-formula (spraying plants at a dose of 4 l/ha in three stages: cotyledon leaves; formation of vegetative mass; 15 days after the second treatment) during the cultivation of the 'Purpletop' or 'Purpurova' varieties, the yield indicator was the highest, reaching 21.6-21.7 t/ha and 21.9-22.0 t/ha respectively. In this study, the obtained yield values significantly expand the understanding of turnip productivity compared with those recorded under the conditions of the Right-Bank Forest-Steppe (Vdovenko, 2019). Viable cells of *Bacillus thuringiensis* bacteria, through systemic application, provided better protection against insect pests compared to the preparation Confidor Maxi. This was particularly observed during steady increases in air temperature starting from 14°C. In these variants, the increase in turnip yield amounted to 0.8-1.1 t/ha (Table 4).

Table 4. *Turnip yield with insecticide protection, t/ha*

Varieties	Years	Control	Confidor Maxi	Bitoxibacillin-BTU	Aktoverm-formula	LSD_{05}
'Zolota Kulya'	2024	19.6	20.6	20.9	20.7	0.6
	2025	20.6	20.8	20.9	20.8	0.1
	Average	20.1	20.7	20.9	20.8	
	± to control	-	+0.6	+0.8	+0.7	

Table 4. *Continued*

Varieties	Years	Control	Confidor Maxi	Bitoxibacillin-BTU	Aktoverm-formula	LSD_{05}
‘Purpurova’	2024	20.3	21.0	21.4	21.2	0.6
	2025	20.8	21.7	21.9	21.8	0.8
	Average	20.6	21.4	21.7	21.6	
	± to control	-	+0.8	+1.1	+1.0	
‘Purpletop’	2024	21.4	21.7	22.0	21.9	0.4
	2025	20.8	21.7	21.9	21.8	0.8
	Average	21.1	21.7	22.0	21.9	
	± to control	-	+0.6	+0.9	+0.8	
‘Geisha’	2024	19.1	20.0	20.8	20.4	0.8
	2025	19.0	19.7	20.2	20.1	0.6
	Average	19.1	19.9	20.5	20.3	
	± to control	-	+0.8	+1.4	+1.2	
Yield of tops, t/ha		28.23	30.15	31.26	31.33	1.90

Source: developed by the authors

Somewhat lower turnip root crop yields, although still satisfactory, were observed in variants where Confidor Maxi was used during the cultivation of the ‘Purpurova’ variety, and where Bitoxibacillin-BTU or Aktoverm-formula were used for the ‘Geisha’ variety. In these variants, the total yield of the varieties increased relative to the control by only 5-7% respectively. However, this yield increase differs slightly from the research results of V.V. Khareba *et al*. (2016) and S.V. Stankevych *et al*. (2024), where the authors recorded yield increases of up to 10%. It was established that Confidor Maxi can also be applied during turnip cultivation, as it was characterised by systemic-contact action. Due to the active ingredient imidacloprid, the pest’s nervous system is blocked for 30 days, while also exerting a stimulating effect on the plant’s growth processes.

In the absence of protection (control), a significant decrease in turnip root crop yield was established, amounting to 20.1 t/ha for ‘Zolota Kulya’, 20.6 t/ha for ‘Purpurova’, 21.1 t/ha for ‘Purpletop’, and 19.1 t/ha for ‘Geisha’. Based on the plant inspections conducted, the population of adult black crucifer flea beetles in the experiment was 1-14 individuals per m^2. The biological products Bitoxibacillin-BTU and Aktoverm-formula are more effective in controlling the adults of the black crucifer flea beetle. Following systemic plant protection, the pest population decreased by 92.86% for Bitoxibacillin-BTU and Aktoverm-formula, compared to 85.72% for Confidor Maxi. The positive effect of the studied preparations was also established during the growth of the plant’s vegetative mass. Regardless of the insecticide used, the top yield significantly exceeded the leaf mass yield of the control variant, which also influenced the formation of the root crop and the overall yield.

A qualitative analysis of turnip root crops under the pest protection system showed its partial influence on biochemical indicators, namely the content of dry matter, starch, and protein. Under the formation of optimal open-soil growing conditions and nutrient processes, an optimal photosynthesis process, accumulation of vegetative mass, and an increase in organic matter content were observed. During turnip cultivation and the application of bacterial-origin preparations such as Bitoxibacillin-BTU or Aktoverm-formula, the dry matter content was higher relative to the control. The dry matter indicator reached levels of 16.4% and 16.3%, exceeding the control variant by 0.4-0.5% respectively. Evidently, the viable cells of *Bacillus thuringiensis* bacteria and endospores provide reliable plant protection against insect pests; simultaneously, a prolonged period of action was observed, which did not cause resistance in the insect pests (Table 5).

Table 5. *Quality of root crops of the 'Zolota Kula' turnip variety depending on the use of insecticide protection (average for the years of research)*

Pest protection system	Content of biochemical substances in turnip root crops, %			
	Dry matter	Starch	Protein	Vitamin C, mg/100 g
1. Control	15.9 ± 0.02	6.11 ± 0.05	5.2 ± 0.01	18.3 ± 0.03
2. Confidor Maxi	15.7 ± 0.02	6.26 ± 0.04	5.3 ± 0.02	19.1 ± 0.02
3. Bitoxibacillin-BTU	16.4 ± 0.03	6.29 ± 0.04	5.4 ± 0.03	19.3 ± 0.03
4. Aktoverm-formula	16.3 ± 0.02	6.74 ± 0.05	5.4 ± 0.01	19.2 ± 0.03

Source: developed by the authors

A similar positive influence of the biological products Bitoxibacillin-BTU or Aktoverm-formula was also established during the determination of starch, protein, and vitamin C content. The timely application of these preparations within the biological protection system for the 'Zolota Kulya' turnip variety contributed to the fact that the starch indicator in the root crops can increase to levels of 6.29% or 6.74% (an increase of 3-10%); protein can reach 5.4% (a 4% increase); and the vitamin C content can rise to 19.3% or 19.2% (a 5% increase) respectively. The obtained values for the root crop quality indicators do not coincide with the data already existing in primary sources (Khareba *et al.*, 2021). Furthermore, the application of the insecticide Confidor Maxi can ensure an increase in the biochemical indicators studied relative to the control variant; however, their magnitude depends on the scheme and recommendations for its application, as confirmed by the results of M.V. Zubets *et al.* (2010). Overall, the application of biological products during turnip cultivation for pest and disease control also ensured that they are safe for humans and warm-blooded animals, as biological protection agents do not pollute the environment. Simultaneously, biological products exhibit high selectivity, are convenient for mass application, and possess inexhaustible resources for this purpose, which aligns with the assertions of L.A. Zhurakovska (2013) and key European Union food safety documents and regulations (European Commission, 2017).

The work of Z.D. Sych *et al.* (2018) emphasised the necessity of a comprehensive approach to vegetable growing, encompassing both theoretical knowledge and practical skills. This study became an important benchmark for understanding the complexities of vegetable cultivation, and the results of the current research confirmed the need to integrate modern methodologies and technologies to increase yield and quality while simultaneously considering ecological sustainability. The research by O.Yu. Barabash *et al.* (1994) examined the biological characteristics of vegetable production and the influence of various environmental factors on crop development, which was also a significant aspect of the current study. The interaction of soil conditions, water regimes, and climatic factors mentioned in their work directly influenced the efficiency of vegetable production. The results of the current study also confirmed the importance of employing ecological methods to enhance the productivity and stability of agricultural systems. S.V. Berzina (2011) investigated the ecological aspects of the production and certification of organic food products. She focused on the implementation of environmental standards in agriculture, which became vital for improving vegetable production and its compliance with ecological certification requirements. This approach correlated harmoniously with the emphasis on ecological principles in the current study, confirming the relevance of integrating sustainable practices into modern vegetable growing to reduce negative environmental impacts and improve product quality. Broadly, the current study built upon these scientific works, highlighting the importance of both practical experience and ecological sustainability in vegetable production. The combination of modern agro-technical methods with ecological practices ensured the sustainable development of the vegetable sector, meeting both market demands and environmental standards.

Thus, the results of the current study confirmed the high efficiency of applying biological products and modern agro-technologies in turnip cultivation. The increase in yield and the improvement of biochemical indicators – such as starch, protein, and vitamin C content – evidence the positive impact of the plant protection methods used. The application of biofungicides and bioinsecticides allows for a significant reduction in disease spread and enhances plant resistance to stress conditions. Therefore, the research results confirm the effectiveness of ecologically safe protection agents for improving the quality and productivity of turnips.

Conclusions

The application of the biofungicide Phytocide-r in turnip cultivation technology increases the root crop yield for the 'Purpletop' variety to a level of 22.2 t/ha. When growing the 'Zolota Kulya' and 'Purpurova' varieties using Phytocide-r or Mycohelp, and when growing the 'Geisha' variety, the total root crop yield increases by 0.9-1.2 t/ha. Chemical protection ensures a total root crop yield of only 21.9 t/ha. Magnicur Boost and the biofungicide Phytocide-r contribute to an increase in starch content in 'Zolota Kulya' root crops to 7.12% and 7.30% respectively, and vitamin C to 19.5 and 19.3 mg/100 g, or by 1.1 times. The 'Purpletop', 'Geisha', and 'Purpurova' turnip varieties are more resistant to infection and the further development of downy mildew. With the use of Mycohelp, the spread of the disease may not exceed 2.0-2.7%, and its development is only 0.21-0.31%. The chemical fungicide Magnicur Boost helps reduce the spread of the disease in turnip crops to 0.76%.

With the systemic application of the biological products Bitoxibacillin-BTU or Aktoverm-formula, when growing the 'Purpletop' or 'Purpurova' varieties, the root crop yield can increase to 21.6-21.7 t/ha and 21.9-22.0 t/ha respectively. The yield of turnip root crops from the use of Confidor Maxi during the cultivation of the 'Purpurova' variety increases by only 5%, while the lowest root crop yield is obtained in the absence of insecticidal protection. The positive effect of the studied preparations was also established during the increase of the plant's vegetative mass. The top yield increases regardless of the insecticide used, which influences the formation of the root crop and the overall yield. During the application of Bitoxibacillin-BTU or Aktoverm-formula, the dry matter content in the root crops increases by 0.4-0.5% and can reach 16.4% and 16.3%. Timely application of the preparations ensures an increase in starch content in the root crops to 6.29-6.74%, or an increase of 3-10%; protein increases to 5.4%, and vitamin C content rises to 19.3-19.2 mg/100 g, or by 5% respectively.

The biological products Bitoxibacillin-BTU and Aktoverm-formula are more effective in controlling the adults of the black crucifer flea beetle. Following systemic spraying of the plants with bioinsecticides, the pest population is reduced when using Bitoxibacillin-BTU and Aktoverm-formula: adults by 92.86%; with the preparation Confidor Maxi, the reduction is 85.72% respectively. The use of biological products during turnip cultivation to control pests and diseases is a safe measure for humans and warm-blooded animals; biological protection agents do not pollute the environment. Biological products exhibit high selectivity, are convenient for mass application, and have inexhaustible resources. Further research regarding the long-term impact of biological products on soil microbiota and nitrate accumulation in turnip root crops under different crop rotation systems in Polissya conditions is promising.

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Conflict of Interest

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Урожайність ріпи в умовах відкритого ґрунту Полісся за різної системи захисту рослини

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Анотація. Метою дослідження було оцінити ефективність препаратів бактеріального та хімічного походження щодо впливу на врожайність ріпи та окремі якісні показники її коренеплодів за різних систем захисту рослин. Дослідження проведено в умовах Волинської державної сільськогосподарської дослідної станції НААН у 2024-2025 роках із використанням сортів ріпи ‘Золота куля’, ‘Пурпурова’, ‘Пурпулепоп’ та ‘Гейша’. Для захисту від хвороб застосовано хімічний фунгіцид Магнікур Буст та біопрепарати Фітоцид-р і Мікохелп (компанія BTU); для захисту від шкідників хімічний інсектицид Конфідор Максі та біопрепарати Бітоксибацилін-БТУ й Актоверм-формула (компанія BTU). Контролем був варіант без обробки препаратами. Дослідження закладено в чотириразовій повторності за стандартною методикою овочівництва. Результати показали, що застосування біофунгіциду Фітоцид-р підвищило врожайність коренеплодів сорту ‘Пурпулепоп’ до 22,2 т/га, а сортів ‘Золота куля’, ‘Пурпурова’ та ‘Гейша’ (з Фітоцид-р або Мікохелп) на 0,9-1,2 т/га. Магнікур Буст і Фітоцид-р збільшили вміст крохмалю в коренеплодах сорту ‘Золота куля’ до 7,12-7,30 % та вітаміну С до 19,3-19,5 мг/100 г. Сорти ‘Пурпулепоп’, ‘Гейша’ та ‘Пурпурова’ виявилися стійкішими до пероноспорозу; Мікохелп обмежив поширення хвороби до 2,0-2,7 % і розвиток до 0,21-0,31 %, а Магнікур Буст поширення до 0,76 %. Системне використання біоінсектицидів Бітоксибацилін-БТУ та Актоверм-формула підвищило врожайність сортів ‘Пурпулепоп’ і ‘Пурпурова’ до 21,6-22,0 т/га, тоді як Конфідор Максі лише на 5 %. Без інсектицидного захисту врожайність була найнижчою. Біопрепарати збільшили вміст сухої речовини до 16,3-16,4 %, крохмалю до 6,29-6,74 %, білка до 5,4 % та вітаміну С до 19,2-19,3 мг/100 г. Бітоксибацилін-БТУ та Актоверм-формула виявилися ефективнішими проти імаго чорної хрестоцвітої блішки

Ключові слова: фунгіцид; інсектицид; ріпа; врожайність; коренеплід; якість; ефективність

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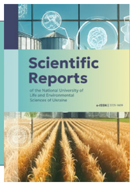

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High-permeability selective polymer membranes for pyrocondensate separation

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Abstract. The aim of the work was to determine the optimal technological parameters for obtaining pure hydrocarbon fractions and extracting pyrocarbon and heavy pyrolysis resin. The work used a method of membrane separation of pyrocondensate using highly permeable selective polymer

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membranes. It was established that the average composition of pyrocondensate contains up to 58.4% of hydrocarbons suitable for obtaining gasoline, kerosene and diesel fractions, as well as 20% of unsaturated hydrocarbons and 20% of pyrocarbon with heavy pyrolysis resin. The effectiveness of membrane technology for separating pyrocondensate with the production of gasoline (8.2%), kerosene and diesel (23.9%) fractions with low sulphur and unsaturated hydrocarbon content has been proven. The characteristics of the fractions obtained met the requirements of standards for their use as finished commercial products. The optimal separation temperatures for each fraction were determined: gasoline – 50°C, kerosene – 70°C, diesel – 85°C. The dependence of membrane permeability on temperature and selectivity for unsaturated hydrocarbons and sulphur was established. The separation process ensured the simultaneous removal of pyrocarbon and heavy pyrolysis resin, which simplifies the technology and increases its efficiency. The proposed catalytic methods for processing pyrocarbon sediment and unsaturated hydrocarbon residues allow for the complete processing of pyrocondensate from 96% to 98%. The possibility of using membrane technology for the efficient processing of pyrocondensate with minimal impact on the environment has been confirmed. Analytical and experimental principles for calculating membrane separation devices and their productivity have been developed. The proposed solutions can be applied to the production of high-quality fuel fractions from waste materials

Keywords: fractional composition; pyrolysis; petroleum polymer resins; catalytic treatment; diesel fraction

Introduction

In the modern world, considerable attention is paid to technologies for processing industrial waste and obtaining marketable products while improving environmental performance. In this context, the processing of polymer waste, in particular worn car tyres, is of great interest. This type of waste ranks first in terms of tonnage compared to other polymer waste. In Ukraine, the annual increase in worn tyres is 250-300 thousand tonnes, and the level of their utilisation does not exceed 10%. The accumulation of such waste creates a significant anthropogenic load on the environment and requires the introduction of effective technologies for comprehensive recycling. One of the most promising areas in this context is thermochemical processing, in particular pyrolysis, which allows valuable fuel and chemical products to be obtained (Boichenko *et al.*, 2024).

Scientist I.Ya. Zakhara (2025) analysed modern approaches to the disposal and processing of worn car tyres, focusing on the environmental and technological aspects of existing methods. The author showed that traditional methods, in particular incineration and mechanical processing, were accompanied by a significant environmental burden, while thermochemical methods, primarily pyrolysis, were considered more promising in terms of obtaining valuable products and reducing harmful emissions. K.A. Ibragimov *et al.* (2020) investigated the composition, properties and uses of liquid pyrolysis products, paying particular attention to catalytic methods of their processing. The authors established the patterns of selective hydrogenation and oligomerisation of unsaturated hydrocarbons and showed the prospects for obtaining petroleum polymer resins and fuel fractions, while noting the technological limitations associated with coking and the loss of part of the hydrocarbon raw materials.

The article by I. Shlonchak & O. Batrachenko (2024) examined modern approaches to the disposal of car tyres in Ukraine, taking into account global experience. The authors analysed the main processing technologies and identified their advantages and disadvantages. They also emphasised the need to develop infrastructure and improve the rubber waste management system in the Ukrainian context. The work by

K. Hrynyshyn *et al*. (2022) showed that low-temperature pyrolysis allows obtaining pyrocondensate with a clearly defined fractional composition, including gasoline and diesel fractions, as well as heavy residue. The authors emphasised the possibility of comprehensive use of tyre processing products and their return to the production cycle.

Experimental studies by A. Khurmamatov & K. Akhmedova (2025) also confirmed the effectiveness of pyrolysis as a tyre processing technology. This article examines in detail the physicochemical properties of pyrolysis oil, gas and solid phase. The authors found that the synthetic fuel obtained could be a promising energy resource, provided that the process is further refined and optimised. The importance of pyrolysis as an environmentally sound way to reduce rubber waste and reuse it was also emphasised. The work of A. Ranskyi & B. Korinenko (2023) considered the prospects of obtaining synthetic oil as an alternative energy resource through the pyrolytic processing of polypropylene waste. The authors showed that thermochemical decomposition of polymers allowed plastic waste to be converted into liquid hydrocarbon products suitable for further use in the fuel and chemical industries. The study emphasised the importance of developing pyrolysis technologies as an effective way to dispose of polymer waste while obtaining valuable products. The article by V. Chernega (2024) analysed the problem of worn car tyre accumulation from an economic and environmental point of view. The author emphasised that used tyres are one of the most common and dangerous types of polymer waste, and their reuse and recycling are essential for reducing the anthropogenic impact on the environment. The work justified the need to introduce modern tyre disposal technologies that would ensure not only an environmental effect but also economic feasibility through the recovery of secondary resources.

The work of W. Jerzak *et al*. (2024) expanded scientific understanding of the pyrolysis process through the use of modern analytical methods and different types of reactors. The authors focused on the influence of heating rate and catalyst introduction method on the formation of aromatic hydrocarbons and raw soot yield. The study also demonstrated the complexity of the thermal decomposition mechanisms of tyre raw materials and the relationship between process conditions and the chemical composition of products. The work of N.H. Zerin *et al*. (2023) stated that pyrolysis is a more environmentally friendly method of processing waste tyres compared to traditional methods, as it allows waste to be converted into oil, gas and solid phase (char) without causing harmful emissions during combustion. The study also summarises the influence of process parameters (temperature, residence time, particle size) on the yield and composition of pyrolysis products, highlighting the technological complexity of this method.

Thus, there is a pressing need to improve technologies for processing pyrocondensate obtained from the pyrolysis of car tyres to increase its efficiency and obtain commercial products with predictable physical and chemical properties. The aim of the work was to determine the optimal technological parameters of the process of separating pyrocondensate obtained by pyrolysis of worn car tyres and extracting pyrocarbon and heavy pyrolysis resin from it to obtain pure hydrocarbon fractions using membrane technology. To achieve this aim, the following objectives were set: to determine the composition of pyrocondensate from the pyrolysis of worn car tyres; to carry out membrane separation of pyrocondensate to obtain commercial products for use in the automotive industry; to determine the optimal conditions for pyrocondensate separation.

Materials and Methods

The object of the research was the process of obtaining pure hydrocarbon fractions from pyrocondensate. The subject of the research was a comprehensive approach to pyrocondensate processing by combining traditional methods and unique membrane pervaporation technologies. The study used pyrocondensate obtained at a low-temperature pyrolysis plant at the Rosava private joint-stock company (Bila Tserkva, Ukraine) as a result of the pyrolysis of worn car tyres. The initial pyrocondensate was characterised by a complex multicomponent structure containing up to 58.4% hydrocarbons (light, medium and heavy fractions), about 20% unsaturated hydrocarbons, and 20% pyrocarbon with heavy pyrolysis resin. The content of sulphur, unsaturated hydrocarbons, water and solid impurities was determined in accordance with DSTU 7687:2015 (2015).

The study used highly permeable selective polymer membranes specially designed for the separation of complex organic mixtures. The membranes were selected taking into account their resistance to organic solvents, process temperature and selectivity to pyrocondensate fractions. The membrane separation process was carried out in an experimental laboratory membrane unit with the ability to regulate temperature (from 30 to 90°C) and pressure (up to 10 bar). For each fraction (gasoline, kerosene, diesel), the optimal process temperatures were set: 50°C, 70°C and 85°C, respectively. The research was conducted in the educational laboratory of membrane technology at the Department of Biotechnics and Engineering, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute". All analyses of the pyrocondensate and the separated fractions were performed using certified equipment on a YL6100 chromatograph (YoungIn Chromass) in the laboratories of the research institute of the state fuel company MASMA. The following methods and tests were used to assess the quality and composition of the products.

The fractional composition of the gasoline fraction was determined in accordance with DSTU EN 228:2022 (2022), which provided for the determination of fractional distribution temperatures by distillation; the fractional composition of the kerosene and diesel fractions was determined according to the DSTU EN 590:2022 (2022), which includes measuring distillation points to assess the composition of fractions; the sulphur content in the fractions was determined in accordance with ISO 8754 (2003) using energy-dispersive X-ray fluorescence analysis, which allows the concentration of sulphur in petroleum products to be accurately determined; the iodine number (a characteristic of the degree of unsaturation of organic compounds) was determined according to EN 16300:2012 (2012) using the calculation method for fatty acid methyl esters (FAME) based on gas chromatography data. Gas chromatography-mass spectrometry (GC-MS) was used for detailed determination of the component composition of the pyrocondensate and its fractions, as well as for identifying impurities and target hydrocarbons. Fourier-transform infrared spectroscopy (FTIR) was applied to analyse functional groups in the fractions, enabling assessment of the degree of saturation and the presence of impurities. Water content was determined by coulometric titration using the Karl Fischer method. Viscosity measurements were performed with a rotational viscometer to evaluate the suitability of the fractions for use as fuels. Storage stability was assessed by an accelerated ageing method (24 hours at 80°C) followed by analysis of changes in viscosity and composition.

The choice of these methods was dictated by the need to obtain complete and reliable information on the composition, purity, physicochemical properties and stability of pyrocondensate fractions. The application of international standards ensured the comparability of the results with modern requirements for the quality of fuel products and allowed an objective assessment of the effectiveness of membrane technology for industrial use. The component composition of pyrocondensate, both raw and subjected to catalytic treatment in the presence of KTK-1, was studied by gas-liquid chromatography. Figure 1 presents the schematic technological diagram for the production of petroleum polymer resins in the presence of the KTK-1 catalytic complex.

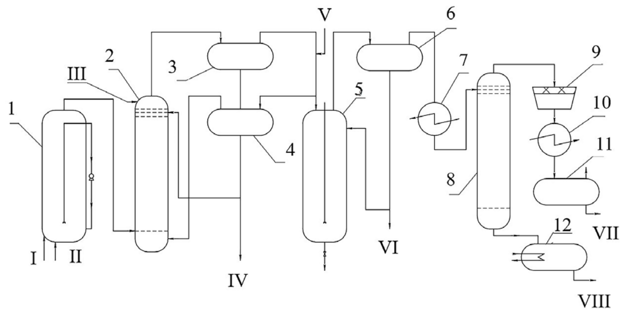

Figure 1. *Schematic process flow diagram for the production of petroleum polymer resins in the presence of the KTK-1 catalytic complex*

Note: 1 – reactor; 2, 5 – reactor-washer; 3, 4 – settler; 6 – separator; 7 – heater; 8 – distillation column; 9 – air cooler; 10 – condenser; 11, 12 – storage tanks. I – pyrocondensate; II – catalytic complex; III – alkali solution; IV – deactivated catalyst; V, VI – water; VII, VIII – finished product

Source: V.T. Dumskyi *et al.* (2014)

Polymerisation was carried out by a continuous circulation method in reactor 1, into which pyrocondensate I and catalytic complex II were fed from below. Reactor 1 was equipped with a special device designed to ensure effective circulation of the reaction mixture and maximum conversion of unsaturated hydrocarbons. In this way, the possibility of accidental breakthrough, monitored by chromatographic analysis, was virtually eliminated. The resulting polymerisate from reactor 1 was fed to reactor-washer 2, where the catalytic complex was decomposed using alkali solution III, which was subsequently discharged from the top of the reactor and, through separators-settlers 3 and 4, directed for water washing V in the reactor-washer. In the separators, the polymerisate was separated from the circulating alkali solution (in separators-settlers 3 and 4), part of which was discharged to a wastewater treatment unit. The deactivated catalyst IV was removed from the bottom of separator-settler 4.

The neutralised polymerisate, via separator 6, where water VI was removed and discharged from the unit, was fed to heater 7 and then to the top of distillation column 8. Unpolymerised hydrocarbons from the top of column 8 were directed to air cooler 9 and then to condenser 10, from which they were transferred to storage tank 11 and subsequently to storage. The resin melt VIII from the bottom of column 8 was collected in tank 12, from which it was pumped out of the unit for dissolution or granulation. The proposed process scheme for pyrocondensate processing made it possible to remove a gasoline fraction purified from unsaturated hydrocarbons, which could be used either for alkylation with ethylene or for the production of high-purity aromatic hydrocarbons, while reducing the number of stages in the financially costly hydrogenation process.

According to the proposed fundamentally new scheme for pyrocondensate processing using KTK-1, targeted synthesis of modified petroleum polymer resins was achieved through the rational utilisation of unsaturated hydrocarbons. These resins possess a set of properties required for their use as components of polymer-bitumen compositions, binding agents in paint and varnish materials, effective plasticisers for rubber compounds, and vulcanising agents for electrical insulation coatings. One of the most reliable and economical methods for improving the quality of road bitumen is protection against ageing and drying. The possibility of using petroleum polymer resins as effective additives to road bitumen is confirmed by research results (Ibragimov *et al.*, 2020).

Problem statement. The high content of pyrocarbon in pyrocondensate obtained from the pyrolysis of worn car tyres significantly complicated the application of known technologies for its processing. The methods of processing pyrocondensate from polymer waste described in the literature were technologically complex even under conditions of significantly lower pyrocarbon content, which in such systems was on average four times lower than in pyrocondensate from worn car tyres. With an increased pyrocarbon content, the implementation of these technologies was impossible due to the intensive deposition of pyrocarbon and heavy pyrolysis resin on the surface of catalysts in the processes of hydrogenation and catalytic treatment, as well as on the contact elements of equipment during fractionation in rectification columns. Pyrocondensate processing was carried out in stages, involving the preliminary separation of pyrocarbon and heavy pyrolysis resin, followed by the processing of purified pyrocondensate using traditional technologies. Membrane technology using the pervaporation method was used to remove pyrocarbon and heavy pyrolysis resin. The use of pervaporation membranes is due to the fact that they are semi-permeable, have no pores and exhibit anti-adhesion properties with respect to pyrocarbon and resins, so that such contaminants cannot be deposited on their surface. Two processes took place simultaneously in the apparatus with such membranes: the passage of hydrocarbons contained in the pyrocondensate through the membrane wall due to molecular diffusion and the precipitation of pyrocarbon and heavy pyrolysis resin at the bottom of the apparatus during the separation process. The process of separating pyrocondensate into fractions (gasoline, kerosene, diesel) and extracting pyrocarbon and heavy pyrolysis resin from it was carried out on an experimental setup, the diagram of which is shown in Figure 2.

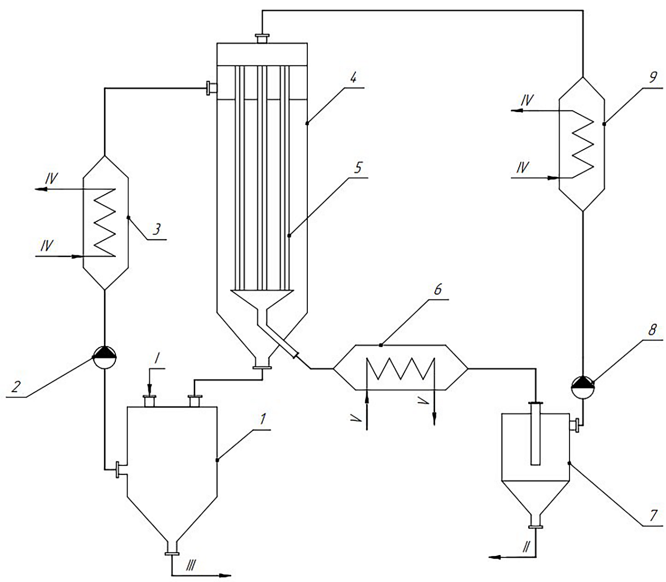

Figure 2. *Schematic diagram of the experimental unit for the separation of pyrocondensate*

Note: 1 – receiving tank, 2 – gear pump, 3 – heat exchanger, 4 – membrane apparatus, 5 – membrane element, 6 – condenser refrigerator, 7 – fraction collector, 8 – steam-gas compressor, 9 – calorifier. I – pyrocondensate, II – fractions, III – pyrocarbon and heavy pyrolysis resin, IV – hot coolant, V – cold coolant

Source: authors' own work

Before starting work, the devices that were part of the installation were were purged with nitrogen. The output pyrocondensate I was loaded into receiving tank 1, from which it was fed by gear pump 2 through heat exchanger 3, where it was heated to a set temperature, into membrane element 4 on the distribution device. The distribution device ensured uniform irrigation of the outer surface of the tubular membrane elements 5. In the membrane apparatus, the heated pyrocondensate flowed in the form of a thin film over the outer surface of the tubular membrane elements. A heated nitrogen-vapor mixture (a mixture of nitrogen and hydrocarbon vapors) was pumped along the inner surface of the membrane elements using a steam-gas compressor 8 under a slight excess pressure (0.01-0.02 MPa). In this case, certain fractions of hydrocarbons passed through the walls of the membrane elements due to molecular diffusion and mixed in the form of vapour (pervaporation process) with the nitrogen-vapour mixture that was pumped along the inner surface of the membranes. The nitrogen-vapor mixture enriched with the hydrocarbon fraction in a saturated state from the membrane apparatus entered the condenser refrigerator 6, where condensation took place. Condensate II entered the fraction collector 7, from which it was periodically drained, and the nitrogen-steam mixture from the fraction collector was sucked out by a steam-gas compressor 8, heated in a calorifier 9 and fed into the membrane apparatus.

The spent pyrocondensate from the membrane apparatus was drained into a membrane vessel, where pyrocarbon and heavy pyrolysis resin were precipitated from it. The precipitation process is related to the fact that heavy pyrolysis resin exhibits high adhesion to pyrocarbon and dissolves well in hydrocarbon fractions, which are removed from the pyrocondensate by membrane elements during the pervaporation process. Thus, as the content of hydrocarbon fractions in the pyrocondensate decreased, pyrocarbon and heavy pyrolysis resin III were deposited, which were periodically removed from the bottom of the receiving tank. The pyrocondensate from the receiving tank was fed back into the plant's apparatus by a gear pump. Thus, the technological process of pyrocondensate separation at the experimental plant was carried out in a closed cycle.

Results and Discussion

Worn car tyres are a complex multi-component raw material containing a rubber matrix, carbon black, metal cord, textile impurities and a number of organic and inorganic additives. Their accumulation creates a significant anthropogenic load on the environment, and traditional methods of disposal do not ensure the effective extraction and use of the material and energy potential of this type of waste. In this context, chemical processing methods, in particular pyrolysis, are considered a technologically feasible way of comprehensive tyre disposal. Pyrolysis of car tyres allows the organic component of the raw material to be converted into pyrolysis oil and gas, and the inorganic component into a solid carbon phase (carbon black) and metal cord. However, scientific research shows that the yield, fractional composition and physicochemical properties of pyrolysis products depend significantly on the temperature regime, heating rate, process duration, reactor type, and the presence or absence of catalysts. The lack of standardised approaches to assessing process parameters complicates the scaling of technologies and their implementation in industrial practice. In addition, an important engineering and technological task is to evaluate the quality of the pyrolysis products obtained in terms of their further use – pyrolysis oil as a raw material for fuels or chemical synthesis, gas as an energy resource, and solid carbon phase as a filler or functional material. Particular attention should be paid to the analysis of the content of sulphur, nitrogen, oxygen-containing compounds and ash components, which determine the environmental and operational characteristics of the products (Hrynyshyn *et al.,* 2021).

Chromatographic studies show that unsaturated hydrocarbons are almost completely converted during the catalytic treatment of pyrocondensate (98.7-99.8% conversion) and no unsaturated hydrocarbons remain in the pyrocondensate. The optimal conditions obtained formed the basis for the synthesis of petroleum polymer resins for catalytic processing of pyrocondensate. Gas chromatography makes it possible to separate the complex mixtures of hydrocarbons contained in the pyrocondensate fractions into individual components according to their retention times (Table 1). Each fraction is characterised by its own set of peaks corresponding to different organic compounds. The gasoline fraction is characterised by the presence of light hydrocarbons, therefore the main peaks are observed at the beginning of the chromatogram (Fig. 3).

Table 1. *Chromatographic analysis of the obtained liquid fractions of pyrocondensate*

Retention time (min)	Gasoline fraction	Kerosene fraction	Diesel fraction
2-5	High peaks (C5-C7)	Low peaks	Absent
5-10	Medium peaks (C7-C9)	Start of peaks	Absent
10-15	Low peaks (C9-C10)	High peaks (C10-C12)	Start of peaks
15-25	Absent	Medium peaks (C12-C15)	High peaks (C15-C18)
25-40	Absent	Absent	Medium/high peaks (C18-C22)

Source: developed by the authors

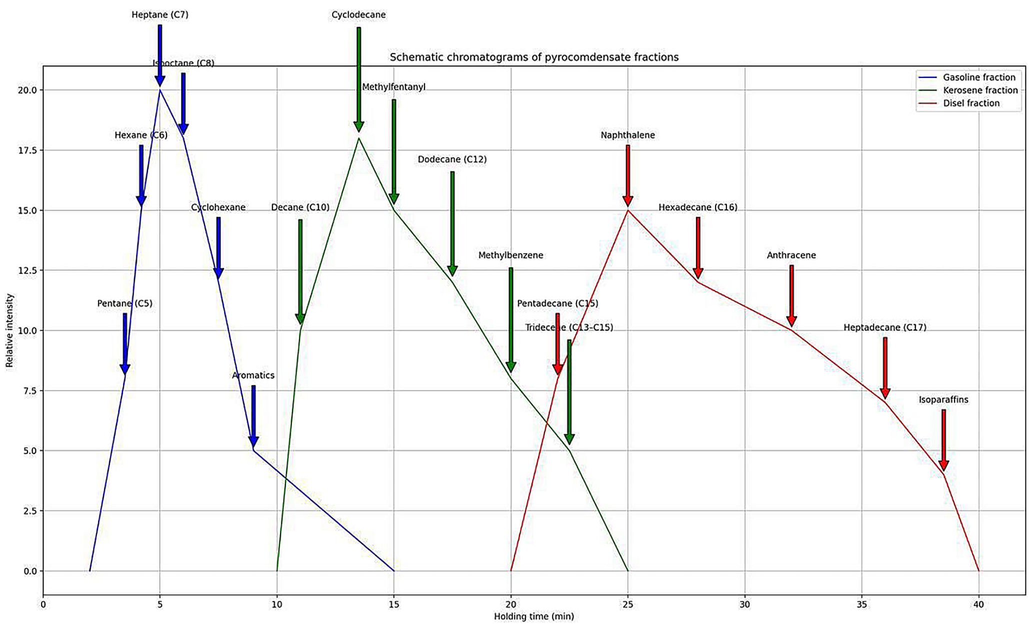

Figure 3. *Chromatogram of gasoline, kerosene and diesel fractions*

Source: developed by the authors

The kerosene fraction contains medium-boiling components, with peaks located in the middle of the chromatogram. The diesel fraction has the longest retention time, corresponding to heavy hydrocarbons. The peak height reflects the relative concentration of the component. The peaks of the gasoline fraction are located closer to the beginning of the chromatogram (short retention time). The peaks of the kerosene and diesel fractions are shifted to the right, corresponding to an increase in molecular weight and retention time. The designation of the peaks (Fig. 3) and their identification (Table 2-4) are presented on the chromatogram.

Table 2. *Gasoline fraction (retention time 2-15 min)*

Peak	Retention time (min)	Identified component	Explanation
1	~3.5	Pentane (C5)	Light alkane
2	~4.2	Hexane (C6)	Main component of the gasoline fraction
3	~5.0	Heptane (C7)	Important for octane rating
4	~6.0	Isooctane (C8, isomer of octane)	Responsible for anti-knock properties
5	~7.5	Cyclohexane	Cycloalkane
6	~9.0	Aromatic hydrocarbons (benzene, toluene)	Small amount of aromatics

Source: developed by the authors

Table 3. *Kerosene fraction (retention time 10-25 min)*

Peak	Retention time (min)	Identified component	Explanation
1	~11.0	Decane (C10)	Medium boiling alkane
2	~13.5	Cyclodecane	Cycloalkane
3	~15.0	Methylnaphthalene	Aromatic hydrocarbon
4	~17.5	Dodecane (C12)	Medium chain alkane
5	~20.0	Methylbenzene	Aromatic component
6	~22.5	Tridecene (C13-C15)	Heavier alkanes

Source: developed by the authors

Table 4. *Diesel fraction (retention time 20-40 min)*

Peak	Retention time (min)	Identified component	Explanation
1	~22.0	Pentadecane (C15)	Heavy alkane
2	~25.0	Polycyclic aromatic hydrocarbon (naphthalene)	Aromatic component
3	~28.0	Hexadecane (C16)	Alkane
4	~32.0	Polycyclic aromatic hydrocarbon (anthracene)	Heavy aromatic
5	~36.0	Heptadecane (C17)	Heavy alkane
6	~38.5	Isoparaffins	Branched hydrocarbons

Source: developed by the authors

Identification of the components was carried out using gas chromatography-mass spectrometry (GC-MS), which provides retention times and mass spectra for accurate substance determination. Comparison with spectral libraries (for example, NIST) made it possible to confirm the identity of the peaks. Standard samples were used to calibrate the holding time. Pyrocondensate obtained as a result of pyrolysis of worn car tyres is an opaque black liquid with a characteristic odour. Its composition is shown in Table 5.

Table 5. *Composition of pyrocondensate from the pyrolysis of worn car tyres*

Parameter	Value
Density	0.921 g/cm^3
Sulphur content	1.6% by mass
Unsaturated hydrocarbon content	20% by mass

Table 5. *Continued*

Parameter	Value
Pyrocarbon content	9% by mass
Heavy pyrolysis resin content	11% by mass

Source: developed by the authors

As can be seen from Table 5, the pyrocondensate contains a large amount of pyrocarbon and heavy pyrolysis resin, a significant quantity of unsaturated hydrocarbons and sulphur, and has a density close to that of heavy crude oil. It was established that the pyrocondensate comprises paraffinic-naphthenic, cyclic aromatic, polycyclic and olefinic hydrocarbons, as well as oxygen-containing compounds. The gasoline fraction isolated from the pyrocondensate using the experimental unit is a clear, colourless liquid with a characteristic gasoline odour. Its fractional composition and properties are presented in Table 6.

Table 6. *Composition and characteristics of the gasoline fraction of pyrocondensate obtained from the pyrolysis of worn car tyres*

Parameter	Value
Pyrocondensate output	26.3% by mass
Density	0.786 g/cm^3
Fractional composition:	
Initial boiling point	50℃
10% boiling point	90℃
50% boiling point	151℃
90% boiling point	190℃
Final boiling point	210℃
Sulphur content	0.05% by mass
Unsaturated hydrocarbon content	0.6% by mass

Source: developed by the authors

According to Table 6, it has been established that the fractional composition and density of the gasoline fraction are very close to the standard indicators for gasoline. It contains insignificant amounts of sulfur and unsaturated hydrocarbons, but according to the distillation curve, it has a boiling point that is 20-25℃ higher than that of automotive gasoline. The kerosene fraction obtained from pyrocondensate in the experimental setup is a transparent, colourless liquid with a characteristic kerosene odour. Its fractional composition and indicators are given in Table 7.

Table 7. *Composition and characteristics of the kerosene fraction of pyrocondensate from the pyrolysis of worn car tires*

Parameter	Value
Pyrocondensate output	8.2% by mass
Density	0.852 g/cm^3
Fractional composition:	
Initial boiling point	180℃
10% boiling point	198℃
50% boiling point	221℃
90% boiling point	232℃

Table 7. *Continued*

Parameter	Value
Final boiling point	240℃
Sulphur content	0.08% by mass
Unsaturated hydrocarbon content	0.8% by mass

Source: developed by the authors

According to the main indicators (density, acceleration curve, sulphur content), the kerosene fraction obtained meets the requirements of DSTU 7687:2015 (2015). The kerosene fraction of pyrocondensate obtained from the pyrolysis of worn car tyres demonstrates a set of characteristics that make it possible to assess it as potentially suitable for use as a fuel component or as technical kerosene, subject to appropriate finishing treatment. The yield of the kerosene fraction is 8.2% by mass of the pyrocondensate. This is a relatively small but typical proportion for pyrolysis products of tyres, where a significant part is accounted for by the gasoline fraction, heavy distillates and resins. This yield indicates that the kerosene fraction is not the main target product of the process, but can be considered a valuable by-product that increases the overall energy and economic efficiency of pyrocondensate processing. The density of 0.852 g/cm^3 is within the range typical for commercial kerosene (approximately 0.78-0.86 g/cm^3 depending on the application – aviation, technical, lighting, etc.). This indicates: a sufficiently balanced hydrocarbon composition (no excessive amount of heavy components); potential compatibility with existing fuel systems in terms of density. This density level indirectly indicates a predominance of C10_{10}10–C14_{14}14 hydrocarbons characteristic of kerosene in the composition of the fraction. Distillation behaviour is key to assessing the suitability of a fraction for use as fuel. Boiling point: 180°C: this corresponds to the lower limit of the kerosene fraction and indicates the absence of excess volatile gasoline-type components that could lower the flash point and impair safety characteristics. 10% boiling off at 198℃ and 50% at 221℃: these values correlate well with the typical distillation characteristics of kerosene, reflecting a fairly narrow and technologically convenient boiling range. 90% evaporation at 232°C and end of boiling at 240°C: the small gap between 90% and the end of boiling indicates: relative homogeneity of the fraction, a small amount of heavy "tail" components, which has a positive effect on the completeness of combustion and reduces the formation of carbon deposits. Overall, the distillation curve shows that the distillation characteristics of this fraction are close to those of commercial kerosene, which is regulated by the relevant DSTU EN and ISO standards.

The sulphur content of 0.08% by mass is relatively low for pyrolysis products of tyres, which are traditionally characterised by an increased content of sulphur-containing compounds due to the composition of rubber compounds. This indicator is acceptable for most technical applications of kerosene; it may be close to the requirements of certain fuel standards or require minimal additional desulphurisation depending on the intended use (especially for environmentally sensitive aviation or energy fuels). Low sulphur content reduces the corrosive activity of the product and SOx_xx emissions during combustion. The unsaturated hydrocarbon content of 0.8% by mass is low, which has several important consequences: storage stability is improved (unsaturated compounds are prone to oxidation and the formation of resinous products); the risk of varnish and carbon deposits in fuel systems is reduced; this characteristic brings the obtained fraction closer to hydrotreated petroleum kerosene. The fact that the pyrolysis fraction has such a low unsaturated hydrocarbon content indicates the effectiveness of the membrane separation technology and the previous purification stages. Thus, in terms of key indicators (density, distillation curve, sulphur content), the kerosene fraction obtained meets the requirements (implicitly – the current standards for kerosene fuel). The analysed parameters confirm this: the density corresponds to the typical range for kerosene; the distillation characteristics indicate the formation of a "correct" boiling range without an excess of light or heavy impurities; the sulphur and unsaturated hydrocarbon content is acceptably low for technical and energy fuels.

Thus, based on the characteristics given, the kerosene fraction of pyrocondensate from the pyrolysis of worn car tyres can be considered potentially suitable for use: as a component of blended kerosene fuels after minimal refinement (if necessary, additional purification or mixing with petroleum fractions); as technical kerosene for power plants, boilers, combustion processes, etc. In addition, the results obtained demonstrate the promise of membrane technology for pyrolysis condensate processing as a tool for the maximum possible utilisation of tyre pyrolysis products in the fuel and energy cycle, which has both economic and environmental benefits (reduction of tyre waste and replacement of part of traditional petroleum resources). The diesel fraction is a transparent light-yellow liquid with a characteristic odour. Its fractional composition and quality indicators are presented in Table 8.

Table 8. *Fractional composition and indicators of the diesel fraction*

Parameter	Value
Pyrocondensate output	23.9% by mass
Density	0.889 g/cm³
Fractional composition:	
Initial boiling point	240℃
10% boiling point	264℃
50% boiling point	294℃
90% boiling point	327℃
Final boiling point	350℃
Sulphur content	0.12% by mass
Unsaturated hydrocarbon content	1.05% by mass

Source: developed by the authors

According to the main indicators (density, acceleration curve, sulphur content), the obtained diesel fraction meets the requirements of the standards. The sediment deposited in the receiving tank during the pyrocondensate separation process had a density of 1.242 g/cm^3 and a sulphur content of 4.47% by mass. It consisted of 45% pyrocarbon by mass (with a pyrocondensate yield of 9% by mass) and 55% heavy pyrolysis resin by mass (with a pyrocondensate yield of 11% by mass). The residue formed after the pyrocondensate separation process consisted of unsaturated hydrocarbons (with a pyrocondensate yield of 19.51% by mass), had a density of 0.85 g/cm^3 and contained 3.4% by mass of sulphur. As can be seen from Tables 6-8, the gasoline, kerosene and diesel fractions obtained as a result of membrane separation of pyrocondensate have a low content of sulfur and unsaturated hydrocarbons, which negatively affect fuel quality. To reduce the content of these components in fuel, technologically complex processes are used, such as hydrotreating and hydrogenation on highly active catalysts. The use of membrane pervaporation technology allows fractions with a low content of these ingredients to be obtained. These fractions can be used to produce high-quality automotive fuels. Most importantly, used car tyres are used in its production. In addition, thanks to complex mass transfer and diffusion processes that occur at the molecular level during pervaporation, pyrocarbon and heavy pyrolysis resin are removed from the pyrocondensate.

In membrane separation, an important characteristic of the membrane is its permeability. Knowing the permeability of the membrane, it is possible to calculate the productivity of the membrane apparatus. The permeability of the membrane during pervaporation depends on the temperature of the separation process; it increases with increasing temperature. At a certain temperature, the selectivity of the membrane φ (separation quality) begins to decrease. Thus, the calculated permeability of the membrane is taken to be the permeability it has at the highest temperature, provided that φ = 1. Figure 3 shows the dependence of membrane permeability on the temperature of the separation process of different fractions at φ = 1. As can be seen from the graphs in Figure 4, the optimal separation temperature is: 50°C for the gasoline fraction, 70°C for the kerosene fraction, and 85°C for the diesel fraction. The permeability at these temperatures is: for gasoline m = 4.9 kg/(m^2·h), kerosene fraction m = 3.8 kg/(m^2·h), diesel fraction m = 2.5 kg/(m^2·h).

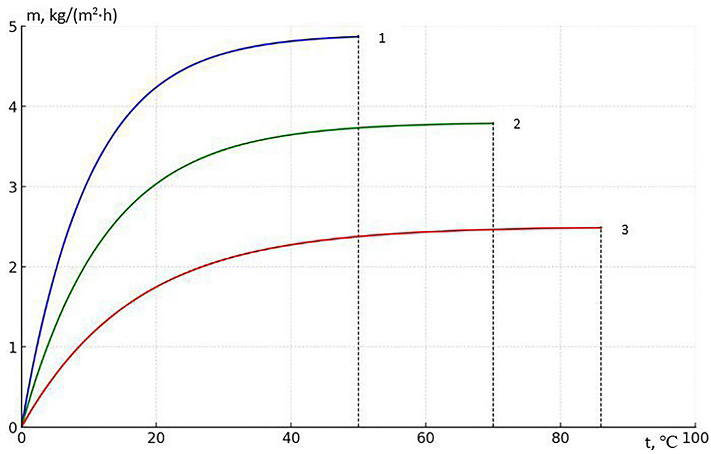

Figure 4. *Dependence of membrane permeability (m) on temperature for different fractions*

Note: 1 – gasoline fraction, 2 – kerosene fraction, 3 – diesel fraction

Source: developed by the authors

The sulphur content in fractions obtained during pyrocondensate separation is crucial when using them as commercial products. The main factor determining the sulphur content in fractions during the separation of pyrocondensate on pervaporation membranes is the temperature at which the process is carried out. Thus, studies were conducted on an experimental setup to investigate the effect of temperature on the sulphur content in fractions. Figure 5 shows the dependence of the sulphur content in the fractions on the temperature of the separation process, assuming that the selectivity is maintained at φ = 0.9988 – 0.9995 (relative to sulphur) and the maximum permeability of the membranes. As can be seen from the graphs, the sulphur content in the fractions obtained at a selectivity of 0.9988 to 0.9995 at maximum membrane permeability is: for the gasoline fraction 0.05% by mass; for the kerosene fraction 0.08% by mass; for the diesel fraction 0.12% by mass. When the optimum temperature of the separation process is reduced, the sulphur content in the fractions decreases slightly, but the permeability of the membranes decreases significantly. The content of unsaturated hydrocarbons in pyrocondensate reaches 20% by mass, which leads to the formation of resins. Thus, one of the main tasks in the membrane separation of pyrocondensate is to obtain fractions with a low content of unsaturated hydrocarbons. As studies show, the selectivity of membranes (in relation to unsaturated hydrocarbons) at maximum permeability is: for the gasoline fraction φ = 0.994; for the kerosene fraction φ = 0.9992; for the diesel fraction φ = 0.9897. Figure 6 shows the dependence of membrane permeability on temperature for unsaturated hydrocarbons.

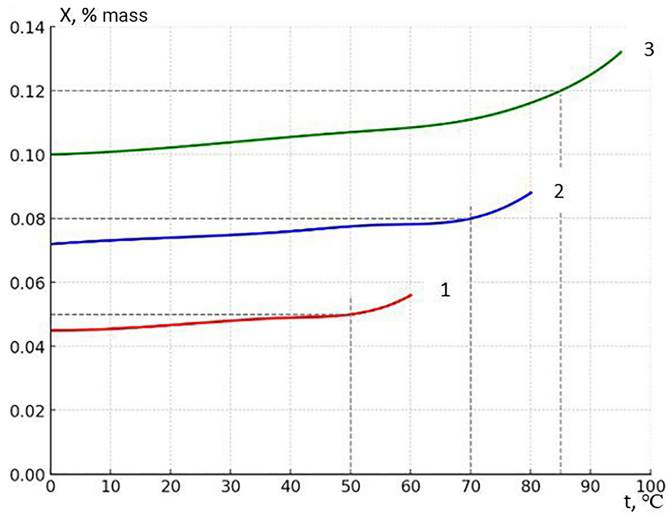

Figure 5. *Dependence of sulphur content in fractions on the temperature of the pyrocondensate separation process*

Note: 1 – gasoline fraction; 2 – kerosene fraction; 3 – diesel fraction

Source: developed by the authors

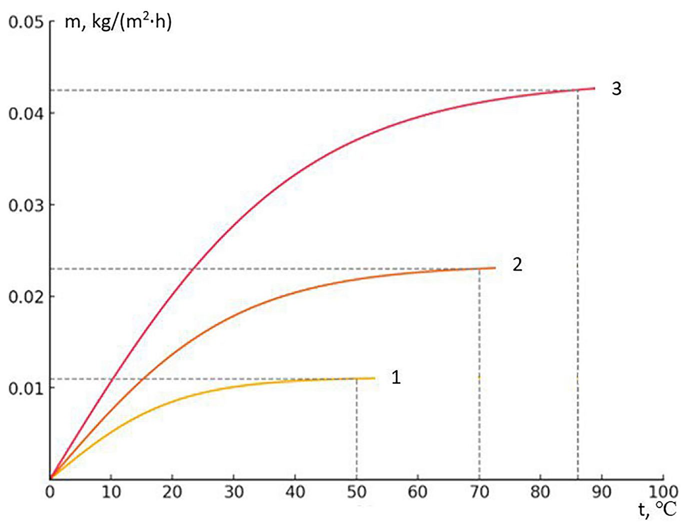

Figure 6. *Dependence of membrane permeability on temperature*

Note: 1 – membranes for gasoline fraction; 2 – membranes for kerosene fraction; 3 – membranes for diesel fraction

Source: developed by the authors

As can be seen from the graphs, the maximum permeability of membranes at optimal temperatures for unsaturated hydrocarbons is: for gasoline fraction membranes m = 0.0114 kg/(m^2·h); for kerosene fraction membranes m = 0.0224 kg/(m^2·h); for diesel fraction membranes m = 0.043 kg/(m^2·h). If the membrane permeability for the fractions is compared with the permeability for unsaturated hydrocarbons, it is 430 times higher for the gasoline fraction and 60 times higher for the diesel fraction than the permeability for unsaturated hydrocarbons. Such a large difference in permeability is due to the structure of the pervaporation membranes, on which their selectivity depends. This allows fractions with a very low content of unsaturated hydrocarbons to be obtained. The sulphur content in gasoline, kerosene and diesel fractions is influenced by the sulphur concentration in the pyrocondensate fed to the membrane apparatus for separation. The more sulphur in the pyrocondensate, the more sulphur in the fractions obtained by membrane separation. In this case, a "plasticising effect" occurs, which is associated with the fact that during the diffusion of saturated hydrocarbon molecules through the membrane, a very small amount of molecules of substances containing sulphur and sulphur compounds dissolved in these hydrocarbons can penetrate. Figure 7 shows the dependence of the sulphur content in the gasoline, kerosene and diesel fractions obtained by membrane separation of pyrocondensate on the sulphur content in the pyrocondensate fed to the membrane apparatus for separation.

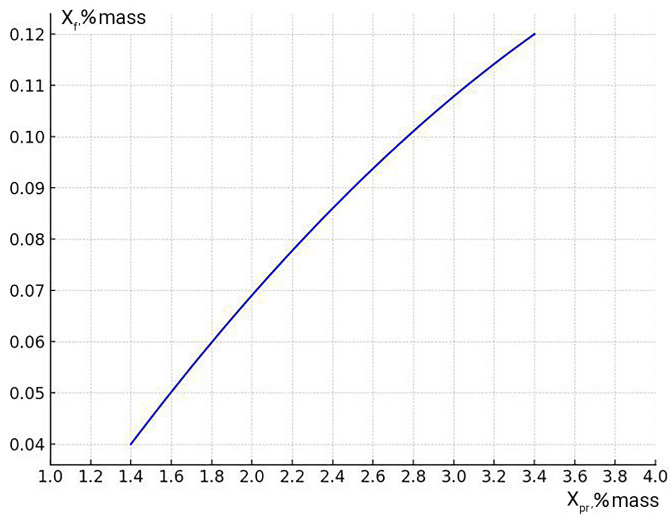

Figure 7. *Dependence of the sulphur content in the fractions X_f on the sulphur content in the pyrocondensate X_{pr}, fed to membrane separation*

Source: developed by the authors

During the separation of pyrocondensate in an experimental setup, in addition to gasoline, kerosene and diesel fractions, pyrocarbon sediment with heavy pyrolysis resin is also formed, as well as a residue containing unsaturated hydrocarbons. The sediment can be briquetted and used as fuel. The residue is a mixture of unsaturated hydrocarbons with sulphur-containing compounds, which can be hydrogenated on highly active catalysts to obtain saturated and aromatic hydrocarbons.

The results obtained in this work regarding the composition, properties and processing directions of pyrocondensate obtained in the process of pyrolysis of worn car tyres are generally consistent with the data presented in the works of other authors, but have a number of fundamental differences that determine the scientific novelty and practical value of the research. In the studies by K. Hrynyshyn *et al.* (2021), the complex multicomponent nature of pyrocondensate is noted, which contains aliphatic saturated and unsaturated hydrocarbons, aromatic and polycyclic aromatic compounds, sulphur-containing components, as well as pyrocarbon and resinous impurities. Similar conclusions are presented in the works of S. Magagula *et al.* (2023), which emphasise that it is the high proportion of unsaturated and aromatic hydrocarbons that causes the instability of pyrocondensate and limits its direct use as motor fuel. The data obtained in this work from the chromatographic analysis of gasoline, kerosene and diesel fractions confirm these patterns and indicate the presence of a wide range of C_5-C_{22} hydrocarbons with an increase in retention time as the molecular weight of the components increases.

The influence of temperature conditions and process parameters on the composition of pyrolysis products has been extensively studied in the works of L.M. Markina & M.S. Kryva (2018). In particular, it has been established that an increase in pyrolysis temperature contributes to the intensification of the destruction of the polymer matrix of tyres and an increase in the proportion of light aromatic hydrocarbons, but can lead to secondary condensation reactions and the formation of resins. Similar trends were found in this work: it was shown that with an increase in temperature and processing time, the content of unsaturated hydrocarbons decreases, with the most intense transformations occurring in the first two hours of the process, and a further increase in temperature does not significantly improve the characteristics of the final product. In a thorough review, S. You *et al.* (2018) analysed the current state and prospects of waste gasification from the perspective of obtaining synthesis gas and biochar, with a focus on high-temperature processes and their energy efficiency. The authors emphasised that the practical implementation of gasification is accompanied by the complexity of equipment design and limited possibilities for selective control of the composition of liquid by-products. In contrast to such approaches, the membrane separation of pyrocondensate proposed in this work is oriented towards mild process conditions and targeted fractionation of liquid products without the need for extreme temperatures.

At the same time, the majority of published studies are focused primarily on optimising pyrolysis conditions or on the subsequent catalytic treatment of pyrocondensate. In the works of S. Papari *et al.* (2021) and M. Mello *et al.* (2023), the main focus is on the processes of hydrotreating, desulphurisation and catalytic upgrading of liquid pyrolysis products. In a thorough review, S. Papari *et al.* (2021) analysed modern approaches to the pyrolytic processing of polymer waste to obtain fuels and chemical products, with a focus on the selection of temperature regimes, reactor types and catalysts. The authors showed that the improvement in the quality of liquid products is achieved mainly through catalytic effects and the intensification of thermochemical processes, while the issue of physical separation of pyrocondensate is practically not considered. In their work, M. Mello *et al.* (2023) conducted a detailed analysis of the pyrolysis processes of worn car tyres and the subsequent desulphurisation of pyrolysis oil. The authors focused on methods of hydrotreating, extraction and catalytic treatment aimed at reducing the content of sulfur-containing compounds, while noting the complexity of such technologies and their energy intensity.

D. Zheng *et al.* (2023) considered the targeted production of valuable aromatic compounds of the BTX fraction through control of pyrolysis conditions, whereas N. Lee *et al.* (2021) investigated the catalytic pyrolysis of polymer waste with emphasis on the influence of zeolite catalysts. The fundamental distinction of the present work lies in the application of membrane pervaporation technology for the separation of pyrocondensate into marketable fuel fractions with the simultaneous removal of pyrocarbon and heavy pyrolysis resin. In contrast to the conventional approaches described in the cited sources, membrane separation enables selective fractionation based on differences in molecular size and diffusion capacity of the components, thereby reducing the content of unsaturated and sulphur-containing compounds in the final fractions without the use of severe thermocatalytic conditions.

In addition, this work implements a comprehensive approach that combines membrane separation with subsequent catalytic oligomerisation of residual unsaturated hydrocarbons in the presence of the KTK-1 catalytic complex. Similar oligomerisation processes are described in the works of H. Ibragimov *et al*. (2013) and S. Boichenko *et al*. (2025), however, these studies do not consider preliminary membrane purification of pyrocondensate, which limits the selectivity of the process and control of the product composition. The results obtained in the current work indicate the high efficiency of combining catalytic treatment and membrane pervaporation technology for processing pyrocondensate from the pyrolysis of worn car tyres. The almost complete conversion of unsaturated hydrocarbons confirms the feasibility of using the catalytic stage as a preliminary purification stage, which minimises the formation of resinous products and increases the stability of the fuel fractions obtained.

Thus, the results obtained confirm the promise of membrane pervaporation technology as an effective tool for incorporating pyrolysis products from worn car tyres into the fuel and energy cycle, which has both economic and environmental advantages. Thus, the results of this work not only confirm the basic patterns established by other authors regarding the composition and properties of pyrocondensate, but also complement them by proposing an effective combined approach to its deep processing. The use of membrane technologies in combination with catalytic processes creates the conditions for obtaining stable fuel fractions with predictable physicochemical properties and increases the overall efficiency of waste tyre recycling.

Conclusions

The study proposes and experimentally confirms an effective method for processing pyrocondensate obtained from low-temperature pyrolysis of worn car tyres using highly permeable selective polymer membranes of the pervaporation type. It has been established that the average composition of pyrocondensate includes 58.4% by mass of hydrocarbons suitable for obtaining fuel fractions, about 20% of unsaturated hydrocarbons, approximately 20% of pyrocarbon together with heavy pyrolysis resin, and 1.6% by mass of sulphur. Membrane separation allows simultaneous separation of commercial fractions and precipitation of pyrocarbon with heavy resin directly in the apparatus, which greatly simplifies the technological scheme. The average yields of target products are 8.2% by mass of the gasoline fraction and 23.9% by mass of the total kerosene and diesel fractions, giving a total yield of fuel fractions of about 32.1% of the initial pyrocondensate.

The optimal process temperatures for achieving maximum membrane permeability with high selectivity are 50°C for the gasoline fraction with a permeability of 4.9 kg/(m^2·h), 70°C for the kerosene fraction with a permeability of 3.8 kg/(m^2·h) and 85°C for the diesel fraction with a permeability of 2.5 kg/(m^2·h). Under these conditions, the residual sulphur content in the fractions is 0.05% in the gasoline fraction, 0.08% in the kerosene fraction and 0.12% by mass in the diesel fraction, and the content of unsaturated hydrocarbons is significantly reduced due to the high selectivity of the membranes for these compounds. The fractions obtained in terms of fractional composition, density, distillation curve, sulphur content and stability meet the requirements of standards DSTU EN 228:2022 and DSTU EN 590:2022 and can be used as commercial fuel products or their components. The total degree of pyrocondensate processing, taking into account the catalytic refinement of unsaturated hydrocarbon residues and the use of sediment as fuel, reaches 96-98%. The process takes place under mild conditions, without aggressive reagents, with low energy consumption and minimal impact on the environment. Further research should focus on scaling up the technology, increasing the durability of the membranes and optimising their composition for industrial implementation.

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Conflict of Interest

None.

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Високопроникні селективні полімерні мембрани для розділення піроконденсату

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Анотація. Метою роботи було визначення оптимальних технологічних параметрів для отримання чистих фракцій вуглеводнів та вилучення пірокарбону та важкої піролізної смоли. В роботі використовувалася методика мембранного розділення піроконденсату за допомогою високопроникних селективних полімерних мембран. Встановлено, що середній склад піроконденсату, який містить до 58,4 % вуглеводнів, придатних для отримання бензинової, керосинової та дизельної фракцій, а також 20 % ненасичених вуглеводнів та 20 % пірокарбону з важкою піролізною смолою. Доведена ефективність мембранної технології для розділення піроконденсату з отриманням бензинової (8,2 %), керосинової та дизельної (23,9 %) фракцій з низьким вмістом сірки та ненасичених вуглеводнів. Характеристики одержаних фракцій відповідали вимогам стандартів для використання їх як готових товарних продуктів. Визначено оптимальні температури процесу розділення для кожної фракції: бензинової – 50 °C, керосинової – 70 °C, дизельної – 85 °C. Встановлено залежність проникності мембран від температури та селективності щодо ненасичених вуглеводнів та сірки. Процес розділення забезпечив одночасне висадження пірокарбону та важкої піролізної смоли, що спрощує технологію та підвищує її ефективність. Запропоновані каталітичні методи переробки осаду пірокарбону та залишку ненасичених вуглеводнів, що дозволяє досягти повної переробки піроконденсату від 96 % до 98 %. Підтверджено можливість використання мембранної технології для ефективної переробки піроконденсату з мінімальним впливом на навколишнє середовище. Розроблені аналітичні та експериментальні засади для розрахунку апаратів мембранного розділення та їхньої продуктивності. Запропоновані рішення можуть бути застосовані для виробництва високоякісних паливних фракцій з відпрацьованих матеріалів

Ключові слова: фракційний склад; піроліз; нафтополімерні смоли; каталітична обробка; дизельна фракція

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Diagnostic tests for fatty hepatosis in high-yielding cows

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Abstract. The research relevance is determined by problems in the dairy farming sector related to the development of diseases in high-yielding cows during the transition period. Early diagnosis of metabolic disorders during the transition period is necessary for the timely prevention of complications and the development of irreversible changes in the body of cows and the subsequent culling of animals from the herd. The study aimed to confirm the development of fatty hepatosis in dairy cows during the transition period, identify its possible causes, and determine effective measures for the prevention of this disease and its consequences. For this purpose, clinical diagnostic methods and biochemical studies of cow blood serum were used as early markers of metabolic changes in the animals' bodies. The study determined that clinical signs of pathology are manifested in only 12-20% of cows from each physiological group. Changes in the biochemical parameters of cows' blood serum depending on the physiological period had unique characteristics. During the study, changes were found in such blood serum parameters as total protein, albumin, globulin, protein coefficient, urea, urea nitrogen, aspartate aminotransferase activity, glucose, total calcium, inorganic phosphorus, calcium-phosphorus ratio, carotene, lipoproteins, thymol test, non-esterified fatty acids, and vitamin E. The study established that for timely detection of metabolic disorders in cows, it is necessary to conduct regular medical examinations of the herd covering all physiological groups of animals, including blood tests, and, based on the results obtained, to apply correction methods in a timely manner. Identifying the causes of metabolic disorders and pathological changes in the

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liver and calcium-phosphorus metabolism in cows during different physiological periods will make it possible to select appropriate prevention methods and prevent economic losses from milk yield reduction and culling of cows in the conditions of a particular farm

Keywords: hepatolipidosis; hyperglobulinaemia; non-esterified fatty acids; lipid metabolism; triacylglycerols; hypoglycaemia

Introduction

Cattle breeding is substantial in animal husbandry, especially in terms of milk and meat production. A highly productive cow can produce the maximum amount of milk by utilising its genetic potential only if it is in excellent health. However, health of each cow directly depends on many factors, including feeding, housing and operating conditions, proper management, lactation/gestation period, etc. As noted by S. Göncü & M.G. Sucak (2024), deterioration of health among cows decreases quantity and quality of milk produced, increases costs and reduces farm profitability. Therefore, monitoring and timely treatment of sick cows are vital for dairy farms. Diseases that occur in cows on farms can be caused by various factors and are substantial causes of economic losses. To prevent spread of these diseases in cows, livestock enterprises must apply methods of care, feeding and herd management, vaccination, hygiene, regular testing and biosecurity control. However, each farm must also conduct internal investigation into root causes, identifying common problems in unique circumstances. Based on above, instead of treating cows for common diseases on farms, permanent solution should be to identify preventive measures with an emphasis on the causes of these diseases.

In contrast, G. Buonaiuto *et al.* (2023) considered that high culling rates in dairy farming indicate poor animal welfare, suboptimal farming conditions and inefficient use of animal resources, which undermines the sustainability of dairy sector. High replacement rates lead to reduced herd productivity and increased replacement costs. In Holstein-Friesian cows, maximum milk production occurs only in third lactation, and a full return on initial investment in their rearing usually occurs at the end of the second lactation. Thus, culling cows during first two lactations negatively affects the profitability of the farm. M.E. Elmhadi *et al.* (2022) noted that it is common practice in the modern intensive management system to feed ruminants with large amounts of highly concentrated feed. As a result, these animals suffer from metabolic diseases. In dairy herds, one or more metabolic disorders can affect every second cow.

V. Tufarelli *et al.* (2024) argued that during perinatal period, dairy cows are usually in a state of negative energy balance (NEB), during which they mobilise fat reserves in the body to provide themselves with the energy they need at this stage. According to Y. Mekuriaw (2023), feeding and housing methods, as well as breed differences, can lead to different NEB statuses in dairy cows. Holstein breeds mobilise significantly more body energy at the beginning of lactation than Danish Red and Jersey breeds. First-calf heifers are more prone to metabolic stress during the transition period than cows with repeated lactations. Therefore, the duration and severity of NEB in dairy cows depend on how the cows are managed. Although NEB is common in high-producing dairy cows, with consequences ranging from mild to severe, its extent and severity can be minimised through proper feeding and the addition of feed supplements. Cows fed a diet with a higher energy value had a better energy balance. In general, NEB is associated with adverse effects on cow health, milk production and fertility, which in turn affects farm profitability.

P. Melendez & P. Pinedo (2024) argued that in this complex scenario, the liver is considered a key organ in regulating overall metabolism, especially in high-yielding dairy cows, which can exceed its normal function, leading to a series of local and systemic inflammatory and degenerative processes that jeopardise the overall health of the animal. M. Holzhauer & J.F. Valarcher (2024) believed that in the early stages of lactation, cows usually develop fatty hepatosis, which is associated with various health problems. Development of this disease is associated with a higher susceptibility to inflammation and infections such as mastitis and endometritis due to decreased immunity. In addition, cows with fatty hepatosis are more likely to develop ketosis, displaced abomasum, hoof health problems, and decreased productivity and reproductive capacity in first weeks/months after calving. Therefore, fatty hepatosis problems in herd must be diagnosed early in transition period to prevent such disorders. The study aimed to determine health status of herd of high-yielding cows at all stages of lactation period, to identify clinical and metabolic disorders in their bodies, and to take timely preventive measures to prevent development of pathologies and their complications.

Literature Review

The state of dairy farming worldwide is characterised by continued growth in cow productivity and a reduction in feed costs per unit of production. However, increased production inevitably leads to a deterioration in herd health. According to C. Zhang *et al.* (2023), during the first month of lactation, 5-10% of high-yielding cows suffer from severe fatty hepatosis, and 30-40% of cows develop mild fatty hepatosis, indicating that almost 50% of these cows are at risk of metabolic disorders. Cows with fatty hepatosis usually do not show clinical signs, but this condition is associated with other health and production problems and, as a result, with huge economic losses on dairy farms. Despite the economic impact of this disease, it is often misidentified or ignored due to diagnostic difficulties.

D. Kirovski & Z. Sladojevic (2017) concluded that the high energy requirements of high-yielding dairy cows immediately after calving lead to increased mobilisation of fat, usually subcutaneous fat. It is released into the blood and transported mainly to the liver, but also to the muscles and kidneys. The degree of fat loss depends on the cow's body condition and energy deficit. Subcutaneous fat and skeletal muscle decrease after calving, with well-fed cows losing 2.5 times more muscle mass than thin cows. The loss of physical condition is due to the general mobilisation of the body (fat and protein).

During lactation, energy expenditure in cows increases significantly, which, in the context of reduced dry matter intake, leads to the development of a negative energy balance. The mobilisation of fats from depots leads to increased concentrations of non-esterified fatty acids in the blood. Their excess can cause the development of metabolic disorders. In particular, the liver of cattle has a limited ability to convert non-esterified fatty acids. They can be oxidised or excreted from hepatocytes as very low-density lipoproteins. When the functional capacity of the liver is reached, triacylglycerols and acetyl-CoA begin to accumulate in it. The synthesis and accumulation of triacylglycerols in the liver determine the high risk of developing "fatty liver syndrome" in cows with lipolysis syndrome. This contributes to the accumulation of triacylglycerols and a deficiency of lipotropic substances in the diet, since, according to J.J. Gross (2022), microbial fermentation products from the rumen alone, especially at the beginning of lactation, are insufficient to meet the needs of high-yielding dairy cows. A deficiency of methionine, lysine, histidine and B vitamins synthesised by microorganisms may be the primary limiting factor not only in milk production but also a significant cause of fatty hepatosis in cows. In addition, an apparent deficiency of lipases for the hydrolysis of triacylglycerols from the cytosolic storage pool may limit their incorporation into very low-density lipoproteins (VLDL), thereby reducing the removal of triacylglycerols from hepatocytes.

P. Melendez & P. Pinedo (2024) believed that the transition period is usually characterised by a curvilinear decline and then an increase in dry matter intake, with the lowest point occurring around calving. In addition, the energy requirements for lactogenesis in cows increase sharply immediately after calving. As a result, cows develop a typical postpartum negative energy balance characterised by hypoglycaemia, as glucose is used for lactose synthesis during the milk production process. These metabolic reactions are the result of increased synthesis of IGF-I and growth hormone, decreased synthesis and release of insulin, and a surge of glucagon and catecholamines, which in turn activate hormone-sensitive lipase and other enzymes in adipose tissue, triggering the typical breakdown of triglyceride ester bonds, releasing glycerol and non-esterified fatty acids into the bloodstream. In contrast, V. Yefimov *et al.* (2023) noted that the development of fatty hepatosis reduces glucagon levels and the intensity of gluconeogenesis in the liver, which leads to a decrease in blood glucose levels and a reduction in insulin secretion. As a result, lipid mobilisation in tissues increases, fatty infiltration of the liver occurs, and ketogenesis intensifies. This leads to the development of a "circle" and the deepening of liver pathology and, as a result, the removal of the most productive cows from the herd.

C. Zhang *et al.* (2023) divided fatty hepatosis into mild, moderate, and severe fatty liver dystrophy. The latter is divided into non-encephalopathic severe fatty hepatosis and hepatic encephalopathy. The pathology of mild and moderate fatty liver disease is less studied than the pathology of severe fatty liver disease. In severe cases, the accumulation of triacylglycerols in the cytoplasm of hepatocytes is accompanied by a disruption in the structure and functioning of the liver, causing hypoglycaemia and ketonaemia (Radostits, 2000). D. Kirovski & Z. Sladojevic (2017) noted that serum glucose levels are a good indicator of liver function, and a decrease in its concentration may reflect fat infiltration in animals with high lipomobilisation. In the subclinical form of fatty hepatosis, low glucose levels and high levels of non-esterified fatty acids in the serum of animals are closely related to the level of triacylglycerol in the liver. Fatty hepatosis occurs when the concentration of lipids exceeds the liver's ability to oxidise and excrete them. Excess lipids are stored as triacylglycerols in the liver, which is associated with a decrease in metabolism.

Given the large number of biomarkers that may indicate functional changes in the liver, biochemical abnormalities are usually non-specific, as there is no single metabolite that can be considered reliable for the diagnosis of fatty liver disease in dairy cows. Only a comprehensive and combined analysis of all available serum metabolites, combined with clinical symptoms or their absence, can provide a more accurate diagnosis of fatty liver disease in dairy cows. The final confirmation of the diagnosis of fatty hepatosis can only be made by taking a biopsy to determine the lipid content in liver cells. However, this method is not suitable for monitoring the health of cows during routine herd screening on industrial farms.

Materials and Methods

The research was conducted in 2024 in industrial farming conditions in eastern Ukraine on Holstein cattle. The research was conducted during routine medical examinations of the farm, which is free from infectious and invasive diseases. All animals were routinely vaccinated against anthrax. Twice a year, routine deworming of all adult cattle and routine diagnostic tests for tuberculosis, leukaemia and brucellosis were conducted. Cows were kept in typical tethered housing. Lighting in the room where cows were kept was 200 lux. Floor for cows was covered with rubber mats. Feeding was conducted at a feeding table at the level of front legs. Animals were provided with water from the water supply through automatic drinkers.

Depending on the physiological period of lactation or dry period on the farm, cows were divided into 5 physiological groups: 3 lactation periods and 2 dry periods. For the experiment, 30 animals were selected for each group. Each physiological group received a unique diet. The diet of cows on the farm consisted of following components: hay, straw, corn silage, haylage, compound feed, which includes sunflower seed meal, wheat, chalk, salt, dry matter premix, milk premix (for cows in the relevant groups), buffer, absorbent, and bypass. Components of the diet were calculated and added to diet in amounts necessary for corresponding physiological period of cows and their needs during this period. Medical examinations were conducted to identify pathologies at an early stage and prevent further development of diseases and culling of cows. Clinical condition of the cows and results of their blood biochemical analysis were examined. For biochemical blood test, five cows were selected from each physiological group. For this purpose, 5 groups of animals were formed, corresponding to each physiological period, of which 2 groups of cows were in dry period, 60 and 20 days before calving, and 3 groups of cows were in lactation period, 5 days, 70-80 days and 175-185 days of lactation.

Clinical condition of cows was determined using standard methods – during examination, physique was assessed, and mucous membranes, condition of coat and skin, lymph nodes, organ systems and individual organs were examined. Blood was collected from cows in sterile disposable vacuum tubes with a clotting activator. During transport, blood samples were stored in a cooler bag with ice packs, separated by a layer of cotton wool to inhibit biochemical processes in whole blood cells and, as a result, changes in parameters. During medical examination at dairy farm, all bioethical requirements regarding animals were ensured, following the Law of Ukraine No. 249 (2012) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

During the study, biochemical parameters of blood were determined, including total protein, albumin, globulin, protein coefficient, urea, creatinine, aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, and alkaline phosphatase, glucose, total calcium, inorganic phosphorus, carotene, lipoproteins, thymol test, non-esterified fatty acids, vitamins A and E. Biochemical parameters were determined using an automatic biochemical analyser Biochem-200, HTI (USA), and vitamins on a high-performance liquid chromatograph LC 300, PerkinElmer, (USA) at Biosafety Centre for Biosafety and Environmental Control of Agricultural Resources of Dnipro State Agrarian and Economic University. Statistical processing of research results was performed using Microsoft Excel 2016 computer program.

Results and Discussion

During the diagnostic stage of medical examination, the study determined that clinical symptoms of the disease manifested themselves in only 12-20% of cows from each physiological group. The symptoms observed included slight depression, low mobility, pale pink conjunctiva and pulse rate at the upper limit of physiological fluctuations or slightly increased. Appetite in cows is reduced, taste distortion is noted, and rumen contraction is weak, in some cows below normal. Body temperature within physiological limits. The area of liver dullness in some cows is enlarged and extends beyond the last rib. Lymph nodes accessible for clinical examination are normal. To obtain the most complete picture of the level and state of metabolism in lactating cows, laboratory blood tests were performed. During the analysis of blood biochemical parameters, a range of changes in metabolism were found in cows of all physiological groups. An increase in the total protein content in the blood serum of cows at 70-80 days and 170-185 days of lactation (Table 1) compared to the maximum indicator of physiological fluctuations occurs due to an increase in the content of globulin fraction proteins. According to V.V. Sakhniuk *et al.* (2017), hyperproteinemia is a substantial indicator of hepatodystrophy, diagnosed in 60-80% of sick cows. An increase in the content of globulin fraction proteins above the upper normal range by 9.7%–40% was determined in the blood serum of cows of all physiological groups, with the exception of the late dry period group (20 days before calving). Hypergammaglobulinaemia occurs during all immunological reactions, accompanied by increased globulin synthesis and caused by an increase in the content of almost all classes of immunoglobulins and non-specific antibodies in many bacterial infections, chronic hepatitis, hepatodystrophy, cirrhosis of the liver, etc. In fatty hepatodystrophy, an increase in the globulin fraction in the blood serum of cows develops as a result of irritation of the immunocompetent cells of the mononuclear system. On the other hand, a decrease in the globulin content in the blood serum of cows during late dry period (Table 2) to normal levels is the result of the transfer of blood immunoglobulins to the mammary gland tissues during the preparation of the cow for calving and the formation of a pool of colostral antibodies in them for secretion in colostrum in the first hours after calving.

Table 1. *Biochemical indicators of cows' blood during lactation, M ± m, n = 5*

No.	Indicators	5th lactation day	70-80th lactation day	170-185th lactation day	Norm
1	Total protein, g/l	67.60 ± 3.40	80.00 ± 1.10	77.00 ± 1.38	55-75
2	Albumin, g/l	29.20 ± 1.02	31.00 ± 0.63	32.20 ± 0.20	30-35.5
3	Globulins, g/l	38.40 ± 2.86	49.00 ± 1.00	44.80 ± 1.28	30-35
4	Protein coefficient, units	0.76 ± 0.05	0.64 ± 0.02	0.74 ± 0.02	06-1.1
5	Thymol test, unit S-H	4.40 ± 0.55	5.38 ± 0.55	4.32 ± 0.50	0-4
6	Urea, mmol/L	3.32 ± 0.57	1.96 ± 0.25	2.32 ± 0.25	2.8-5.8
7	Urea nitrogen, mg%	6.36 ± 1.08	3.78 ± 0.49	4.46 ± 0.46	8-14
8	Creatinine, μmol/L	64.40 ± 3.33	62.40 ± 5.10	72.20 ± 1.93	45-140
9	AST, units per litre	112.60 ± 16.27	149.40 ± 20.69	96.60 ± 7.00	10-50
10	ALT, units per litre	19.40 ± 3.64	26.00 ± 1.52	27.00 ± 1.67	10-40
11	De Ritis index (AST/ALT), units	6.06 ± 0.64	5.88 ± 0.98	3.58 ± 0.30	1.0-3.4
12	Alkaline phosphatase, units per litre	93.00 ± 9.68	128.20 ± 15.31	99.40 ± 10.96	20-150
13	Glucose, mmol/L	2.26 ± 0.09	2.34 ± 0.08	2.58 ± 0.12	2.5-4.16
14	Vitamin E, mcg/ml	1.48 ± 0.15	1.79 ± 0.35	2.18 ± 0.41	3-14
15	Vitamin A, mcg/100 ml	21.04 ± 3.41	34.32 ± 6.36	43.49 ± 2.59	12.5-50.0
16	Carotene, mcg%	353.40 ± 38.68	367.80 ± 55.48	394.40 ± 64.75	275-965
17	Total lipoproteins, mg%	627.20 ± 35.35	925.60 ± 77.97	796.00 ± 73.19	400-800
18	Total calcium, mmol/l	2.16 ± 0.05	2.20 ± 0.03	2.36 ± 0.04	2.43-3.1
19	Ca/P, units	1.52 ± 0.04	1.38 ± 0.02	1.60 ± 0.07	1.2-1.6
20	Inorganic phosphorus, mmol/l	1.46 ± 0.07	1.60 ± 0.03	1.46 ± 0.04	1.45-1.94

Source: compiled by the authors

Table 2. *Biochemical blood parameters of cows 2 months and 20 days before calving, M ± m, n = 5*

No.	Indicators	60 days before calving	20 days before calving	Norm
1	Total protein, g/l	73.20 ± 2.44	63.60 ± 1.54	55-75
2	Albumin, g/l	33.80 ± 0.58	35.00 ± 0.32	30-35.5
3	Globulins, g/l	39.40 ± 2.91	28.60 ± 1.63	30-35
4	Protein coefficient, units	0.88 ± 0.09	1.22 ± 0.09	0.6-1.1
5	Thymol test, unit S-H	4.04 ± 0.63	3.58 ± 0.81	0-4
6	Urea, mmol/L	2.02 ± 0.18	3.74 ± 0.17	2.8-5.8

***Table 2.** Continued*

No.	Indicators	60 days before calving	20 days before calving	Norm
7	Urea nitrogen, mg%	3.86 ± 0.32	7.14 ± 0.30	8-14
8	Creatinine, μmol/L	51.80 ± 6.49	47.80 ± 0.58	45-140
9	AST, units per litre	84.60 ± 9.00	78.00 ± 6.27	10-50
10	ALT, units per litre	23.20 ± 1.71	20.60 ± 1.21	10-40
11	De Ritis index (AST/APT), units	3.70 ± 0.29	3.84 ± 0.37	1.0-3.4
12	Alkaline phosphatase, units per litre	91.60 ± 11.73	80.80 ± 8.49	20-150
13	Glucose, mmol/L	2.26 ± 0.11	2.24 ± 0.04	2.5-4.16
14	Non-esterified fatty acids, mmol/L	-	0.59 ± 0.17	up to 0.25
15	Vitamin E, mcg/ml	7.01 ± 0.72	4.22 ± 0.53	3-14
16	Vitamin A, mcg/100 ml	27.20 ± 3.64	29.68 ± 5.22	22.5-80.0
17	Carotene, mcg%	264.60 ± 30.30	318.80 ± 38.60	275-965
18	Total lipoproteins, mg%	720.20 ± 32.23	909.00 ± 48.12	400-800
19	Total calcium, mmol/l	2.12 ± 0.04	2.02 ± 0.06	2.43-3.10
20	Ca/P, units	1.06 ± 0.24	0.86 ± 0.11	1.2-1.6
21	Inorganic phosphorus, mmol/l	2.26 ± 0.30	2.44 ± 0.27	1.45-1.94

Source: compiled by the authors

Five days after calving, a decrease in albumin content below the lower limit of physiological fluctuations was found in the blood serum of cows. This is a consequence of liver damage caused by lipomobilisation syndrome. According to V.V. Sakhniuk *et al.* (2017), in the blood serum of 85-90% of cows with hepatodystrophy, the proportion of albumin (11-36%) decreases with a simultaneous increase in the relative and absolute content of globulins (beta and gamma fractions). Hypoalbuminaemia in cows can develop as a result of protein starvation and is a typical sign of liver disease, since albumin synthesis occurs only in hepatocytes. Hypoalbuminaemia also occurs in various diseases, including hepatitis, hepatosis, ketosis, liver abscesses, etc. In addition, it indicates chronic liver pathology, since only about one-third of the total amount of albumin is found in blood plasma, with the rest located in the extravascular space.

Elevated thymol test values in the blood serum of cows of all physiological groups by 1-34.5% compared to the upper limit of physiological fluctuations, with the exception of the late dry period group (20 days before calving), indicate dysproteinemia and confirm pathological changes in the liver of the studied animals. The thymol test has high sensitivity and the ability to detect even minor disturbances in liver function. It determines the level of protein fractions in blood serum, which change when liver function is impaired, and can be used to detect damage and inflammatory processes in liver tissue at an early stage, which is necessary for the timely treatment and prevention of diseases in productive animals.

The urea content in the blood serum of cows 70-80 days after the start of lactation, 170-185 days of lactation and 60 days before calving was lower by 30%, 17.1% and 27.9%, respectively, compared to the minimum physiological fluctuation. Impaired urea synthesis may also indicate liver damage in these animals. According to V.V. Vlizlo *et al.* (2021), indicators of liver function are the levels of total protein, albumin and urea in blood serum, and a decrease in their concentration may reflect fatty infiltration in animals with high lipomobilisation. In particular, the urea content in the blood serum of cows with fatty hepatosis is lower than in clinically healthy animals. It is noted that the urea-forming function of hepatocytes is quite stable; its violation indicates significant changes in the liver parenchyma and may be accompanied by syndromes of hepatic encephalopathy and hepatic coma. Urea is the product of protein metabolism. During urea synthesis, ammonia, which is quite toxic to animals, especially to the cells of the nervous system, is neutralised. A decrease in the urea content in the blood serum of animals is observed in cases of alimentary exhaustion and severe liver pathology. Even when 85% of the liver parenchyma is affected, urea synthesis is maintained. Since the cows studied were not clinically exhausted, significant liver damage was implied. R. Djoković *et al.* (2019) indicated that nitrogen metabolism indicators in their studies decreased in cows after calving compared to lactating cows, which is a consequence of the development of fatty hepatosis.

For cattle, the study of AST activity in blood serum is indicative, since AST has high activity in the cells of large animals, and ALT has high activity in small ones. Therefore, the de Ritis index increases by 5-78% in the blood serum of cows in all study groups compared to the upper limit of physiological fluctuations. According to E. Bombik *et al.* (2020), liver enzyme activity in the blood correlates with liver damage due to fatty infiltration. AST is the most sensitive indicator of liver condition during the transition period. Fatty infiltration of the liver causes damage to liver tissue and a general increase in enzyme activity, in particular AST and others, indicating damage to hepatocytes (Andjelić, 2022). With the development of fatty hepatosis in cows, an increase in AST activity above the upper limit of physiological fluctuations occurs already with ultramicroscopic changes in the organ, when the activity of other enzymes is still changing little. V.I. Levchenko *et al.* (2002) found that hyperfermentaemia depends on the amount of fat deposited in the liver parenchyma. During the medical examination of cows, an increase in AST activity was recorded in all physiological groups compared to the upper limit of physiological fluctuations, with the highest indicator in the group of cows on the 70^{th}-80^{th} day of lactation, almost three times higher, 2.25 times higher 5 days before calving, at 170-185 days of lactation by 93.2%, 60 days before calving by 69.2% and by 56% in the group of cows 20 days before calving. Data from V. Yefimov *et al.* (2023) confirm the assumptions of the current study and suggest that the development of cytolysis syndrome is determined by the anatomical features of the liver: pronounced hyperfermentaemia of cytosolic enzymes develops immediately after damage to the plasma membrane of hepatocytes and quickly reaches high levels. The highest AST activity increases during the acute process, while in the chronic process, this hyperfermentemia is less pronounced. Changes in serum enzyme activity may be the result of increased enzyme activity in cells (mainly hepatocytes), but may also be the result of cell damage. When fat infiltrates the liver, damage occurs in its tissues, and the levels of enzymes indicating liver damage (AST) usually increase. R. Djoković *et al.* (2019) found that AST activity in their studies was significantly ($P < 0.05$) higher in the blood serum of cows at the beginning of lactation than at the end of gestation, confirming damage to hepatocytes by steatosis and the release of this enzyme into the blood. AST is the most sensitive biomarker for diagnosing liver conditions during the transition period. This condition may be associated with fat deposits released at the beginning of lactation and may cause metabolic problems in the form of mild fatty liver dystrophy. This fact is confirmed by data from other scientists, C.K. Cebra *et al.* (1997), who claimed that in severe fatty hepatosis, AST activity was 83% sensitive and 62% specific, while only 8% of animals had abnormally elevated serum bilirubin levels. M. Krsmanović *et al.* (2016) stated that AST activity is closely related to the degree of fatty hepatosis, which can be used in the diagnosis of this disease in cows, even in subclinical cases.

Hypoglycaemia in cows at the beginning of lactation was observed throughout the lactation period, except for cows at 170-185 days of lactation. Hypoglycaemia may be caused by insufficient gluconeogenesis in the liver, as well as mammary gland activity and increased lactose synthesis in lactating cows. Hypoglycaemia is also often recorded in liver pathology, especially in cows with fatty hepatosis. Therefore, a decrease in this indicator below the reference values is another confirmation of the development of fatty hepatosis in the studied cows. L.S. Caixeta & B.O. Omontese (2021) believed that the body attempts to eliminate hypoglycaemia by activating the process of gluconeogenesis through an increase in glucocorticoid levels. Hypersecretion of glucocorticoids causes the mobilisation of fats from depots, which, when they enter the liver, cause fatty infiltration of hepatocytes. In addition, the development of fatty hepatosis reduces the intensity of gluconeogenesis in the liver, which leads to a decrease in serum glucose levels and a decrease in insulin secretion. A decrease in the level of these metabolites contributes to the increased mobilisation of lipids from adipose tissue, causing the development of fatty liver infiltration and increased ketogenesis.

A 14% higher level of non-esterified fatty acids compared to the upper limit of physiological fluctuations in the last dry period (20 days before calving) indicates the intensive development of lipomobilisation syndrome, which is characteristic of the transition period in cows. According to I. Lakić *et al.* (2018), the level of non-esterified fatty acids in the blood of cows is the most reliable indicator of negative energy balance and lipomobilisation during lactation. Elevated levels of circulating non-esterified fatty acids result from the remodelling of adipose tissue, which leads to their absorption by the liver in proportion to their levels in blood plasma. The simultaneous lack of oxaloacetate, which is largely used for gluconeogenesis, along with elevated levels of non-esterified fatty acids, leads to the accumulation of acetyl-CoA in the liver. Subsequently, instead of utilising acetyl-CoA through the complete Krebs cycle, incomplete oxidation (ketogenesis) and/or re-esterification occurs, followed by storage of triglycerides in the liver. If the concentration of non-esterified fatty acids exceeds the processing capacity of hepatocytes, such as during excessive lipid mobilisation, liver function is impaired due to the accumulation of triglycerides, causing fatty liver dystrophy and excessive production of ketone bodies such as β-hydroxybutyrate. A massive influx of unesterified fatty acids causes lipotoxicity in the liver of dairy cows. As a result, the balance between oxidation and antioxidant capacity in the liver is disrupted due to the formation of excess lipid peroxides and reactive oxygen species, leading to oxidative stress (Konvičná *et al.*, 2015). In addition to oxidative stress, apoptosis and inflammation can also be triggered by lipotoxicity induced by non-esterified fatty acids (Li *et al.*, 2020).

Thus, lipolysis becomes excessive, and glucose balance is seriously disrupted. Therefore, the studied changes in the content of non-esterified fatty acids and glucose in the blood serum of cows during the transition period (20 days before calving, 70-80 days of lactation) are key and trigger the mechanism of fatty hepatosis, the consequences of metabolic changes that are also observed in other physiological periods (5 days after calving, 70-80 and 175-180 days of lactation). In addition, increased concentrations of non-esterified fatty acids in the blood serum of cows affect inflammatory reactions and enhance the production of active oxygen species in cows during the transition period. According to L.M. Sordillo & W. Raphael (2013), oxidative stress can cause additional lipolysis, thus entering a vicious circle of lipolysis and the production of reactive oxygen species. High concentrations of non-esterified fatty acids and the production of reactive oxygen species are characteristics of metabolic stress and are recognised risk factors for the development of diseases in cows during the transition period, such as mastitis, retained placenta, ketosis and fatty hepatosis. Since vitamin E is considered an important antioxidant, sufficient levels of it in the blood serum should prevent the development of these diseases in cows, especially during the critical period of increased non-esterified fatty acids. According to fundamental research conducted by L. Pinotti *et al.* (2020) based on disease risk and immune function in dairy cows, the concentration of vitamin E in blood serum should be greater than approximately 3 μg/ml during the perinatal period. Below this threshold, vitamin deficiency occurs, specifically hypovitaminosis E.

A decrease in vitamin E content in cow blood serum 5 days after calving by more than 2 times compared to the lower limit of physiological fluctuations (1.48 ± 0.15 μg/ml) may be the result of its release in large quantities along with colostrum. A. Abuelo *et al.* (2015) noted that the cause of the decrease in vitamin E content after calving may also be inflammation in the liver of dairy cows during the transition period. This reduces the production of various vitamin-carrying proteins by the liver and, as a result, lowers the level of vitamin E in the blood plasma of animals. On the other hand, low vitamin E levels in the blood serum of cows during the 70-80 and 175-180 days of lactation (1.79 ± 0.35 and 2.18 ± 0.41 μg/ml, respectively) indicate a deficiency of this vitamin in the bodies of the studied cows throughout the entire lactation period. According to S. Haga *et al.* (2015), a study of vitamin E distribution in cattle tissues showed that the liver is central in regulating its distribution, as evidenced by the high hepatic expression of six genes associated with this vitamin. Therefore, low vitamin E levels in the blood serum of the studied cows may indicate significant changes in liver function, particularly antioxidant function.

An increase in the content of total lipoproteins in the blood serum of cows of two physiological groups by 13.6% and 15.7% (20 days before calving and 70-80 days of lactation, respectively) compared to the maximum physiological fluctuation may indicate the most critical periods for metabolic changes and the development of diseases in the animals' bodies. A study of the total calcium content in the blood serum of the cows studied showed a decrease in cows of all physiological groups by 2.9-16.9% compared to the lower limit of physiological fluctuations (Tables 1, 2). On the one hand, this may indicate an insufficient content of this macroelement in the feed, and on the other hand, the reason may be a violation of its absorption and metabolism in the animal's body. O.M. Chekan & V.V. Stryzhyus (2025) indicated that the reasons for the decrease in calcium content in the blood serum of cows in the postpartum period are disturbances in its absorption from the gastrointestinal tract, as well as its assimilation in the body of cows as a result of a lack of vitamin D and parathyroid hormone. P.R. Aziz *et al.* (2025) believed that a strong negative energy balance is a common cause affecting adaptation mechanisms, which can lead to increased cortisol levels in cows' blood and calcium homeostasis disorders. According to I. Horalska & A. Pavliuk (2021), calcium deficiency in the blood serum of cows is considered a risk factor for the development of fatty hepatodystrophy or its consequence. In addition, hypocalcaemia disrupts the functional state of the kidneys and heart, characterised by signs of acute inflammation in cardiomyocytes, typical of myocarditis. Therefore, low serum calcium levels in cows are a significant problem, especially during the transition period.

An increase in inorganic phosphorus content in the blood serum of cows 60 and 20 days before calving by 16.5% and 25.8%, respectively, is a negative factor, especially in the context of low total calcium content. This imbalance disrupts the Ca:P ratio in the blood serum of cows in the last trimester of pregnancy, which can negatively affect foetal growth and skeletal formation during this period. The assessment of biological markers and their correlation with each other can provide additional insight into the mechanisms underlying the development of fatty hepatosis. Changes in the liver parenchyma can be both functional and reversible. Therefore, it is necessary to address preventive measures to avoid the progression of pathological processes in the liver of lactating cows and the development of secondary diseases, including dietary adjustments, the use of hepatoprotectors, minerals, vitamins, lipotropic and other biologically active substances. These actions will primarily be aimed at maintaining homeostasis, stimulating the development of regenerative processes in the liver, positively affecting intracellular metabolism, and contributing to an increase in the quantity and improvement in the quality of products produced.

Conclusions

Examination of high-yielding cows during herd screening revealed significant changes in their health status. The cows examined showed signs of chronic and/or subacute liver disease and calcium-phosphorus metabolism disorders, characterised by mild clinical symptoms that were only evident in some animals (12-20%), in particular: slight depression, low mobility, decreased appetite, distorted taste, weak rumen contraction, enlarged liver dullness extending beyond the last rib. Depending on the period of lactation or dryness, changes in the biochemical parameters of cow blood serum have unique characteristics. Cows' blood parameters 5 days after calving are characterised by increased aspartate aminotransferase activity, positive thymol test, hyperglobulinaemia, decreased albumin, urea nitrogen, glucose, vitamin E and hypocalcaemia. Cows' blood parameters at 70-80 days of lactation are characterised by an increase in total protein, total lipoproteins, aspartate aminotransferase activity, a positive thymol test, hyperglobulinaemia, and a decrease in urea, urea nitrogen, glucose, vitamin E, and hypocalcaemia. Blood parameters in cows at 170-185 days of lactation are characterised by an increase in total protein, aspartate aminotransferase activity, a positive thymol test, hyperglobulinaemia and a decrease in urea, urea nitrogen, vitamin E and hypocalcaemia. Blood parameters in cows 60 days before calving are characterised by increased aspartate aminotransferase activity, positive thymol test, hyperglobulinaemia, hyperphosphataemia, decreased urea, urea nitrogen, glucose, carotene, hypocalcaemia and decreased Ca: R ratio. Blood parameters in cows 20 days before calving are characterised by an increase in total protein, total lipoproteins, non-esterified fatty acids, aspartate aminotransferase activity, hyperphosphataemia and a decrease in urea nitrogen, glucose, hypocalcaemia and the Ca: P ratio.

For timely detection and prevention of clinically pronounced symptoms in cows, it is necessary to conduct regular medical examinations of the herd, including blood tests, and, based on the results obtained, apply corrective measures in a timely manner. A promising area for future research is the correction of diets for cows of all physiological groups and the development of effective preventive measures that include macro- and microelements, vitamins, hepatoprotectors, and other substances that affect the correction of metabolic processes in the body of animals.

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Conflict of Interest

None.

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Діагностичні дослідження за жирового гепатозу у високопродуктивних корів

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Анотація. Актуальність дослідження зумовлена проблемами в секторі молочного тваринництва пов'язаними з розвитком захворювань високоудійних корів у транзитний період. Рання діагностика метаболічних розладів в транзитний період необхідна для своєчасного попередження ускладнення і розвитку незворотних змін в організмі корів та послідуючому вибракуванні тварин із стада. Метою цього дослідження було підтвердити розвиток у молочних корів транзитного періоду жирового гепатозу, визначити можливі його причини і визначити дієві кроки для профілактики цього захворювання та його наслідків. Для цього були використані клінічні методи діагностики та біохімічні дослідження сироватки крові корів, як ранні маркери зміни метаболізму в організмі тварин. Встановлено, що клінічно ознаки патології проявляються лише у 12-20 % корів із кожної фізіологічної групи. Зміни біохімічних показників сироватки крові корів залежно від фізіологічного періоду мали свої особливості. Під час дослідження були виявлені зміни в таких показниках сироватки крові, як загальний білок, альбуміни, глобуліни, білковий коефіцієнт, сечовина, азот сечовини, активність аспартатамінотрансферази, глюкоза, кальцій загальний, фосфор неорганічний, кальцій-фосфорне співвідношення, каротин, ліпопротеїди, тимолова проба, неестерифіковані жирні кислоти, вітамін Е. Встановлено, що для своєчасного виявлення метаболічних порушень у корів необхідно проводити регулярну диспансеризацію стада охоплюючи всі фізіологічні групи тварин, включаючи аналіз крові та, на основі отриманих результатів вчасно застосовувати методи корекції. Визначення причин порушення обміну речовин та патологічних змін в печінці і кальцій-фосфорному обміні в організмі корів у різні фізіологічні періоди дасть можливість підібрати належні методи профілактики та запобігти зменшенню економічних втрат від недоотримання молока і вибракування корів в умовах конкретного господарства

Ключові слова: гепатоліпідоз; гіперглобулінемія; неестерифіковані жирні кислоти; обмін ліпідів; триацилгліцероли; гіпоглікемія

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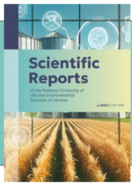

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The effect of monochromatic light on the blood picture and functional state of the hematopoietic system in laying hens

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Abstract. This study aimed to investigate the influence of monochromatic lighting on the haematological indices of Hy-Line W-36 laying hens. In the course of the experiment, four groups of birds were exposed to monochromatic lighting of different wavelengths: blue, green, yellow and red. The haematological study included analysis of leukocyte, erythrocyte and thrombocyte count, haemoglobin concentration, haematocrit and erythrocyte sedimentation rate (ESR). It was found that the colour composition of light affects the hematopoietic system of hens. The activity of white blood cells gradually decreased in the series of transition from short- to long-wavelength light, which indicates the weakening of immune stimulation under the influence of warm light. The content of haemoglobin and haematocrit increased under the yellow and red light, which indicates the activation of erythropoiesis and an increase in the volume of formed blood elements. The number of erythrocytes increased in the yellow and red spectrum, while thrombocytes are most sensitive to long wave radiation, which indicates the activation

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of the megakaryocytic lineage. The ESR decreased with an increase in the wavelength, which indicates the stabilisation of blood viscosity and a decrease in the aggregation of erythrocytes. One-way analysis of variance showed a significant effect of the colour composition of light on all studied indices. Thus, the analysis of experimental data indicated a clear dependence of the haematological status of laying hens on the spectral composition of lighting. In particular, replacing the blue light with red light resulted in a decrease in the number of leukocytes by 45.6% ($p < 0.001$), while the content of haemoglobin increased by 51.8% under the red spectrum compared to the blue one ($p < 0.001$). Additionally, the red light exposure led to an increase in the number of erythrocytes by 52.2% ($p < 0.001$) and a decrease in their sedimentation rate by 22.6% ($p < 0.001$). The results of the study are the scientific basis for the development of recommendations on the use of monochromatic and mixed lighting modes for optimising the productivity and increasing the stress resistance of laying hens

Keywords: leukocytes; erythrocytes; thrombocytes; haemoglobin; haematocrit; spectral composition; immune activity

Introduction

Lighting is one of the key factors that affect the physiological state and productivity of laying hens in the conditions of modern poultry farming. A number of studies indicate the importance of the spectral composition of light, including monochromatic light, in the control of biological processes. The effect of different wavelengths of light on the haemopoietic system of birds is poorly understood and requires further experimental studies to determine the mechanisms of the effects. In the study of M. Shahraki *et al.* (2025), an experimental and modelling approach was used to evaluate the impact of different light intensities (10, 40, 60 and 100 lx) on the morphofunctional state and productivity of young quails. Along with traditional physiological techniques, mathematical modelling methods were used to determine the quantitative dependencies of biological reactions on the parameters of the light environment. The bird productivity was estimated based on live weight gain, feed conversion ratio, meat quality, the rate of lipid peroxidation, the activity of antioxidant enzymes, and the immune status. An integrated analysis demonstrated that a moderate light intensity of 50 to 55 lux is physiologically optimal, ensuring the maximum realisation of growth potential, improvement of feed efficiency, decrease in the level of oxidative stress and increase in immune responsiveness. The data obtained indicate a direct dependence of metabolic processes on the photic effects.

The study by K. Mustafa & I. Al-Kirkuki (2025) investigated the effect of lighting regimens, including a five-hour dark period during the day, applied at different periods of the postnatal ontogenesis (after the 1st, 2nd or 3rd weeks of life), on the productivity of Ross broiler chickens during a 42-day growing period. Compared to continuous lighting, the application of the lighting regimen with a dark period starting from the 1st week of life was accompanied by an increase in the daily average weight gain and improvement of feed conversion ratio, with no negative effect on feed consumption and survival rate of the flock. The results indicate that the optimisation of the light regimen plays an important role in the harmonisation of growth processes and energy metabolism. A systematic review published by Y. Huang *et al.* (2026) summarises the available data on the role of light exposure in the physiological functions of poultry, with a particular emphasis on the fact that light not only performs a visual function but also plays an essential role in regulating the circadian rhythms of physiological functions. The experiment showed that lighting parameters – spectral composition, intensity, duration and type of light source – influence neuroendocrine axes, the secretion levels of melatonin, gonadotrophic hormones and growth hormone, and also influence metabolic processes, immune competence and behavioural responses in poultry. According to the authors, this necessitates an integrated approach to the development of lighting regimens aimed at increasing productivity and ensuring the welfare of poultry.

In the experiment conducted by L. Galan *et al.* (2025), it was found that monochromatic light of different parts of the spectrum has different effects on the functional activity of the endocrine system of broilers by affecting complex neuroendocrine mechanisms that regulate growth, metabolic processes and stress responsiveness. Stimulation of somatotropin secretion and activation of early myogenesis was observed under the influence of green light, while blue light contributed to preserving the muscle mass at later ontogenetic stages and stabilising the pro-oxidant-antioxidant balance. The obtained results indicate the possibility of purposeful spectral light correction of the hormonal status and optimisation of productive qualities. Meanwhile, the analysis of the literature shows that the data on the effect of monochromatic lighting of different spectra on the productive and reproductive qualities of laying hens are fragmented and sometimes contradictory, especially in relation to the combined effect of wavelength, illuminance and photoperiod on the productive and reproductive qualities of laying hens. In the absence of unified, scientifically based recommendations for industrial keeping conditions, further studies are needed to delve deeper into the mechanisms of photoneuroendocrine regulation. The study of K. Takeshima *et al.* (2026) investigated the effects of natural daylight spectrum and artificial light spectrum (in the feeding area) on the growth performance, the rate of sexual maturation and reproductive traits of female Ross 708 broiler breeders reared under a precision feeding system. It was found that continuous red light supplementation delayed the onset of sexual maturation, delayed the onset of egg laying (by about four weeks) and decreased egg production. Conversely, the green spectrum of light did not have any negative effect on reproductive performance and helped to maintain circadian rhythmicity in physiological functions.

The review of G. Gržinić *et al.* (2023) considered the environmental and biomedical impact of intensive poultry farming in relation to the environmental and human health risks posed by the gaseous emissions (ammonia, nitrogen oxides, methane), bioaerosols, pathogenic micro-organisms and residues of antimicrobials. It was concluded that intensive poultry production contributes significantly to anthropogenic pressure, the dissemination of antibiotic resistance and poses a risk to the health of farm workers and the population in the surrounding areas. This implies the need for environmentally focused measures to be taken in the course of the development of this industry. It was established in the Ukrainian research of Yu.V. Osadcha (2021) that the wavelength of light can modulate the non-specific adaptive responses in hens. Thus, the spectral composition of light affects the phase structure of the adaptation process, the activity of the antioxidant system and metabolic indices. In the joint study of Yu.V. Osadcha & H.I. Sakhatsky (2021), the correlation dependence between the spectral characteristics of lighting and indices of viability and reproductive capacity of hens was found, which indicates the expediency of spectral optimisation of the lighting regime.

Although there is already a rather large number of scientific studies, the complex mechanisms of the action of monochromatic light of different spectral composition on the neuroendocrine regulation of the reproductive function of laying hens have not been sufficiently studied. In particular, there are not enough data on the function of the hypothalamic-pituitary-gonadal axis, as well as on the secretion of melatonin and gonadotropins under the conditions of commercial production. In this regard, this study aimed to conduct a comprehensive analysis of the effect of various monochromatic light spectra on the haematological indices of laying hens and to reveal possible neuroendocrine mechanisms of physiological reactions observed in the study.

Materials and Methods

The experimental study used egg-laying hens of the Hy-Line W-36 cross from a commercial flock. The experiments were conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (European Union, 2010). The study was carried out in a modern facility for the production of table eggs from 2022 to 2024. Four groups of laying hens were formed for the experiment and housed in poultry buildings equipped with 12-tier cage battery systems of the Big Dutchman type (Germany). The lighting regime during the rearing of pullets and after their transfer to the production flock complied with the recommendations of the cross developer (Hy-Line W-36 Final Hybrid, Management Guide, 2020).

From 18 weeks of age, light intensity in the poultry houses was maintained at 30 lx with a 12hour photoperiod. Thereafter, until the hens reached 30 weeks of age, day length was gradually increased to 16 hours. Subsequently, the lighting regime with an intensity of 30 lx and a 16-hour photoperiod was maintained until the end of the laying period. The only difference between the poultry houses concerned the type of LED luminaires used to create distinct spectral lighting regimes (Table 1). The peak emission wavelength of each monochromatic LED luminaire was determined using an MK 350 UPRtek spectrometer (UPRtek, Taiwan).

Table 1. *Experimental design for investigating the effect of light wavelength on the viability and productivity of laying hens*

Characteristics	Hen group			
	1	**2**	**3**	**4**
Light wavelength, nm	~490	~540	~580	~660
Spectral colour	blue	green	yellow	red
Number of cages	4,704			
Number of birds per cage	101			
Number of birds per group	475,104			
Stocking density, birds/m^2	24.9			
Space allowance, cm^2/bird	401.4			
Cage dimensions, cm: – length – depth	 362 112			
Cage area, cm^2	40,544			
Number of drinker nipples per cage	12			
Feeding front, cm	7.2			
Poultry house dimensions, m: – length – width – height	 110 26.5 15.0			
Poultry house volume, m^3	43,725			
Poultry house area, m^2	2,915			

Source: developed by the authors

The experimental groups of laying hens were kept under different monochromatic lighting conditions that varied in spectral characteristics. The experimental groups were exposed to LED light sources with peak wavelengths of approximately 490 nm (blue spectrum), 540 nm (green spectrum), 580 nm (yellow spectrum) and 660 nm (red spectrum). Each group consisted of 4,704 cages, with 101 birds in each cage, resulting in a total of 475,104 hens per group. The stocking density was 24.9 birds per m^2 and the floor space per bird 401.4 cm^2. The size of the cages was 362 × 112 cm with a total area of 40,544 cm^2. Each cage was equipped with 12 nipple drinkers; the feeding space was 7.2 cm per bird. The hens were kept in poultry houses 110 × 26.5 × 15.0 m in size with a total area of 2,915 m^2 and a volume of 43,725 m^3. During the entire experiment, the birds were kept under microclimatic conditions that corresponded to the current standards and recommendations of the HyLine W-36 crossbreed developer (Hy-Line W-36 final hybrid. Content guide, 2020). Feeding was carried out with complete compound feeds formulated according to DSTU 4120-2002 (2003); the quality of drinking water met the requirements of DSTU 7525:2014 (2015). The automated cup feeders and nipple drinkers provided the birds with constant access to feed and water.

In each experimental group, 30 blood samples were collected from laying hens at 18 weeks of age (at the start of the study) and again at 52 weeks of age. Blood sampling was carried out from the axillary vein with a volume of 1.0 to 1.5 ml and placed into tubes with K_2 EDTA as an anticoagulant. Sampling of the birds and the selection of animals were carried out in accordance with generally accepted methodological approaches approved by the ASVCP (2012). The haematological studies were carried out on a Micros 60 haematology analyser (Horiba Ltd., Japan) at the Bald laboratory (certificate No. LB/02/2016). Commercial reagents supplied by Horiba Ltd. (USA) were used, including the diluent ABX Minidil LMG, lysing reagent ABX Minilyse LMG, cleaning solution ABX Cleaner, deproteinising agent ABX Miniclair, reagent container ABX Minipack LMG, and the control set 2N, 1H, 1L Para 12 Extend.

The following haematological parameters were determined: erythrocyte, leukocyte and thrombocyte counts by the electrical impedance method (conductometric method); haemoglobin concentration by spectrophotometry; and haematocrit by whole-blood integration. Mean erythrocyte volume, mean haemoglobin content per erythrocyte, mean corpuscular haemoglobin concentration, erythrocyte distribution width, and mean thrombocyte volume were calculated from the recorded primary measurement data. Using the conductometric method, the differential count of leucocytes (monocytes, lymphocytes, eosinophils, basophils and heterophils) was also determined. The reference values of haematological parameters were used according to the data of N.C. Jain (1993).

For comparison between groups, Student's t-test, one-way analysis of variance (ANOVA) and Tukey-Kramer post hoc test were used. Normality of data distribution was tested by the Kolmogorov–Smirnov test. In the case of non-normal distribution, the non-parametric Mann-Whitney U test was used. Significance was set at $p < 0.05$.

Results and Discussion

The results show that the spectral characteristics of light influenced the leukocyte count in hens (Table 2, Figs. 1, 2). The maximum values were found under the blue light. Transition to the green light led to a 19.6% decrease in the leukocyte count compared to the initial level ($p < 0.001$). The yellow spectrum provoked a 24.7% decrease in the leukocyte count in comparison with the blue light group ($p < 0.001$). The most pronounced changes were observed under the red light, where a 45.6% decrease in the leukocyte count was found compared with the blue light ($p < 0.001$). The results of pairwise comparison between the groups are as follows: between the green and yellow, 6.4% ($p < 0.001$); between the yellow and red, 20.9% ($p < 0.001$). Thus, an increase in the wavelength of the lighting led to a consistent and significant decrease in the leukocyte count in the hens, which indicates a decrease in immune activation. Changes in the leukocyte component of the blood of hens under the influence of different light spectra are associated with the complex interaction between the neuroendocrine and immune systems.

Table 2. *Haematological parameters of hens depending on the intensity of the light stimulus, M ± m, n = 30*

Parameter	Group/light wavelength, nm				Reference values
	1	2	3	4	
	~490	~540	~580	~660	
	blue	green	yellow	red	
Leukocytes, thousand/μl	52.6 ± 0.45	$42.3 \pm 0.42^{***}$	$39.6 \pm 0.45^{***\circ\circ\circ}$	$28.6 \pm 0.25^{***\circ\circ\circ\prime\prime}$	20-40
Haemoglobin, g/dl	8.3 ± 0.19	$9.6 \pm 0.13^{***}$	$11.9 \pm 0.15^{***\circ\circ\circ}$	$12.6 \pm 0.18^{***\circ\circ\circ\prime\prime}$	7-13
Haematocrit, %	32.0 ± 0.25	31.9 ± 0.28	$34.8 \pm 0.32^{***\circ\circ\circ}$	$34.7 \pm 0.41^{***\circ\circ\circ}$	22-35
Erythrocytes, million/mm³	2.3 ± 0.06	2.3 ± 0.04	$2.9 \pm 0.03^{***\circ\circ\circ}$	$3.5 \pm 0.08^{***\circ\circ\circ\prime\prime}$	2.5-3.5
Thrombocytes, thousand/mm³	22.6 ± 0.53	$29.1 \pm 0.63^{*}$	$56.8 \pm 0.96^{***\circ\circ\circ}$	$62.3 \pm 1.12^{***\circ\circ\circ\prime\prime}$	32-100
ESR, mm/h	6.2 ± 0.07	$5.4 \pm 0.04^{***}$	$5.0 \pm 0.09^{***\circ\circ\circ}$	$4.8 \pm 0.21^{***\circ\circ\circ}$	4.0-6.5

Note: *$p < 0.001$ compared with the first group; °$p < 0.05$, °°$p < 0.001$ compared with the second group; ‘$p < 0.05$, “$p < 0.001$ compared with the third group

Source: developed by the authors

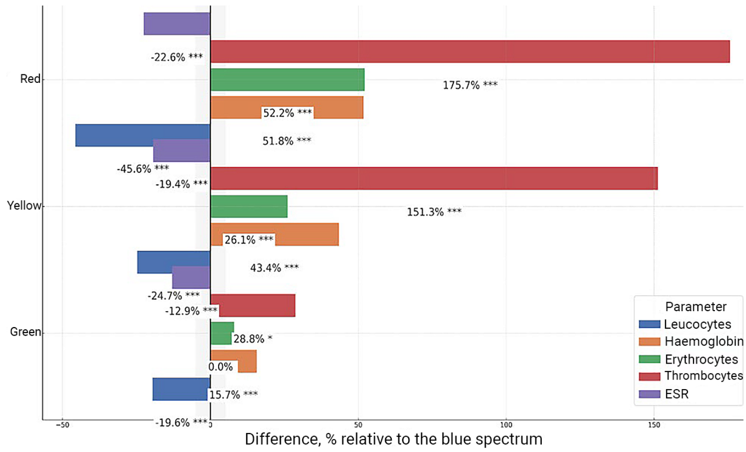

Figure 1. *Percentage differences in haematological parameters of hens under different light wavelengths*

Source: developed by the authors

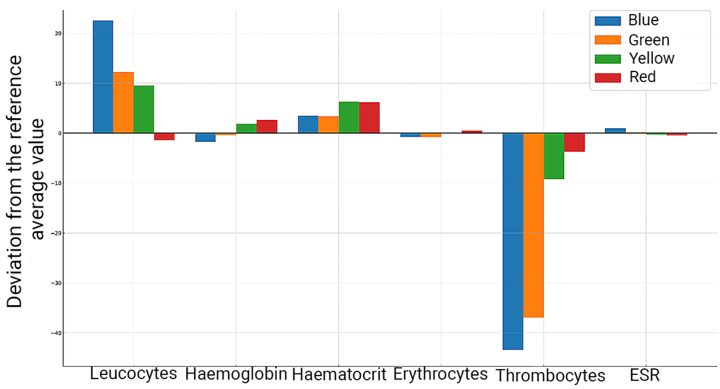

Figure 2. *Deviations of haematological parameters of hens from reference values*

Source: developed by the authors

Leukocyte count in the peripheral blood of hens decreased with an increase in the wavelength of the light from blue to red, with differences between groups of 45-46%. This is an adaptive restructuring of neuroendocrine and immune regulatory mechanisms in response to the change in the spectral composition of the lighting. Light is perceived not only by retinal photoreceptors but also through the skull by hypothalamic photoreceptors and brain structures that regulate the secretion of gonadotropin-releasing hormone (GnRH) and gonadotropin-inhibitory hormone (GnIH). In another experiment, M. Baxter *et al*. (2014) proved the importance of the red light for the stimulation of the reproductive axis in hens in the absence of retinal photoperception, thus validating the crucial role of transcranial photoreceptors in the regulation of the hypothalamic-pituitary system. Further, M. Baxter & G.Y. Bédécarrats (2019) showed that the spectrum of light affects reproductive traits in laying hens, thus, once again, underscoring the systemic nature of photoneuroendocrine response. Therefore, a decrease in the number of leukocytes in the current experiment with the increase of wavelength can be interpreted as an indirect effect of the activation of the reproductive axis and redistribution of metabolic resources in favour of the reproductive function. Blue light with a shorter wavelength is known to enhance the production of melatonin, which, being a central hormone of the circadian system and immune response, can, according to C.H. Teo *et al*. (2021), influence GnIH expression, thus ensuring elevated immune activity in the form of higher levels of leukocyte count in the peripheral blood.

With the increase of wavelength, transcranial photostimulation of the hypothalamic structures is enhanced, which leads to the activation of the hypothalamic-pituitary-gonadal axis and enhanced secretion of oestradiol and progesterone (Lewis *et al*., 2000). Summarising the data presented by P.D. Lewis & R. Gous (2009), it can be concluded that the spectral sensitivity of poultry covers both the visible and ultraviolet parts of the spectrum, and that different wavelengths have different effects on neuroendocrine regulatory mechanisms. It is also noted that the short-wave and ultraviolet parts of the spectrum are mainly involved in the behavioural reaction, while long-wave radiation is more effective for photostimulation of deep-seated photosensitive brain structures, which agrees with the hypothesis of the enhanced transcranial activation of the hypothalamic centres by red light. An increase in the concentration of these steroid hormones has a pronounced immunomodulatory effect, which consists of reducing the proliferation and release of leukocytes into the peripheral blood. E.M. Hassanein *et al*. (2024) in their analysis of the physiological effects of GnRH and related hormonal changes underscore a close relationship between the activation of the reproductive axis and systemic metabolic and immune reactions, which corroborates the treatment of the reduced number of leukocyte count as an element of endocrinologically mediated adaptation reaction. Therefore, the decline in the number of leukocytes along the blue–red axis represents a physiological trade-off between the immune system and reproduction.

The concentration of haemoglobin increased with the increasing length of the light wavelength. Compared to the blue light, the concentration of haemoglobin under the green, yellow, and red light increased by 15.7% ($p < 0.001$), 43.4% ($p < 0.001$), and 51.8% ($p < 0.001$), respectively. The difference in haemoglobin concentration between the green and yellow light was 23.9% ($p < 0.001$) and between yellow and red light, 6.1% ($p < 0.05$). Similarly, the haematocrit under the yellow and red lights was greater than that under the blue light by 9.1% and 8.8%, respectively ($p < 0.001$). These results are in line with those of I.C. Dunn *et al*. (2009), who found that oestradiol promotes the production of red blood cells by increasing the responsiveness of progenitor cells to erythropoietin and enhancing the proliferation of erythroid progenitors. However, it should be noted that these authors conducted their studies on mammals, and therefore, one should be cautious when extrapolating these mechanisms to birds due to the known species-specific features of erythropoiesis in hens. The data of this study can be considered indirect evidence in support of this hypothesis; however, to confirm it, it is necessary to measure the level of erythropoietin and oestradiol. In addition, J. Li *et al.* (2014) studied how oestrogen regulates the expression in the liver of hens of two key gene-molecular markers of yolk protein, *vitellogenin II* (VTG II) and *very low-density apolipoprotein II* (ApoVLDL II), and the type of receptor mediating this effect (ER-α, ER-β, or GPR30). The authors showed that oestrogen (17β-estradiol) significantly increased the expression of VTG II and ApoVLDL II mRNAs in hepatocytes of laying hens, and that this stimulation occurred mainly through ER-α, but not through ER-β or GPR30. These mechanisms contribute to an increase in the oxygen-carrying capacity of the blood, which is necessary to provide energy for reproductive functions. The rise in the number of red blood cells in the blood of hens is a manifestation of the stimulation of the erythropoietic component of haematopoiesis due to the neuroendocrine effect and an increase in the secretion of steroid hormones. This is in line with F. Zhang *et al.* (2024), who wrote about the effect of low-intensity red and near-infrared light (600-1,100 nm) on tissues through photobiomodulation, including stimulation of cellular processes. Their article will be useful for those interested in this issue and the effects of individual wavelengths of light on biological objects.

The platelets showed the greatest sensitivity to the change in the light spectrum. The number of these cells under the green spectrum was 29.0% ($p < 0.05$) higher than under the blue spectrum; under the yellow spectrum, it was 151.0% ($p < 0.001$) higher and under the red spectrum it was 175.7% ($p < 0.001$) higher. When comparing green and yellow light, the number of platelets increased by another 95.6% ($p < 0.001$), and when comparing yellow and red light, it increased by 9.0% ($p < 0.05$). These data are in agreement with the information of K. Tsutsui *et al.* (2010), which summarises the role of gonadotropin-inhibitory hormone (GnIH) as a key factor regulating the hypothalamicpituitary-gonadal axis and controlling the reproductive functions of birds, including the mechanisms of gonadotropin secretion inhibition. It has been shown that GnIH suppresses the synthesis and release of luteinising hormone and follicle-stimulating hormone and, thereby, controls follicular growth and ovulation. Activation of GnIH secretion may weaken egg production, while the regulation of this system is necessary for the optimal realisation of the reproductive potential of laying hens. In addition, C. Hanlon *et al.* (2022) presented a detailed review of the non-gonadal roles (i.e. outside the reproductive glands) of 17β-estradiol (E2) in laying hens and in broiler breeder hens, as well as its influence on individual physiological processes associated with reproductive function. The authors showed that, apart from the ovaries, E2 is produced in the heart, liver, muscles, brain, fatty tissue, and other organs, and that, through its receptors (ERα, ERβ, GPR30), it controls the processes of yolk formation, metabolism, bone formation, and the oviposition cycle, and, as a consequence, affects egg production and quality, both in laying and in broiler breeder hens.

Erythrocyte sedimentation rate showed a consistent decrease with increasing light wavelength. The ESR under green light was 12.9% lower compared to blue light; under yellow light, 19.4% lower; and under red light, 22.6% lower ($p < 0.001$). The ESR decrease is related to a decrease in immune stress, a membrane-stabilising effect on erythrocytes and a decrease in their aggregation ability in the state of elevated secretion of steroid hormones. L. Liu *et al.* (2015) and S. Li *et al.* (2016) came to similar conclusions. They studied the impact of various monochromatic light colours (blue, green, red and white as control) on laying performance and the expression of oestrogen receptors (ERα, ERβ) and progesterone receptor (PR) in ovarian follicles. Their results showed that blue and green light increase serum oestradiol and progesterone concentrations, up-regulate the expression of ER and PR in granulosa cells of the follicles, and improve egg production and peak laying period, suggesting that the reproductive axis in hens is regulated by light colour.

Thus, the light spectrum is a physiological factor regulating neuroendocrine, immune, and haematopoietic adaptive responses in hens. Short-wavelength light enhances immune response, while long-wavelength light enhances reproductive response, erythropoiesis and thrombopoiesis, thereby promoting better functional adaptation of the haematological system in the context of modern poultry farming. These observations are supported by the findings of N. Tez & M. Akşit (2024), who evaluated the effects of red monochromatic LED light compared with white LED light on laying hen performance in terms of sexual maturity age, egg production, egg quality, feed intake and certain behavioural traits in enriched cages. The researchers reported that red LED light accelerated sexual maturity and peak laying and enhanced egg production and egg weight when compared to white light. However, no significant differences were noted among groups for feed intake, feed conversion, most egg quality traits or behavioural characteristics.

One-way analysis of variance showed that light wavelength had a significant impact on all the assayed haematological parameters (Table 3). In case of leukocytes, the highest proportion of variance was explained by the factor "light spectrum" $F = 684.2$; $p < 0.001$ ($\eta^2 = 0.89$), which corresponds to 45%-46% intergroups difference. There were also significant differences in haemoglobin – $F = 312.5$; p <м0.001 ($\eta^2 = 0.78$), haematocrit – $F = 97.4$; $p < 0.001$ ($\eta^2 = 0.62$), erythrocytes – $F = 421.7$; $p < 0.001$ ($\eta^2 = 0.84$), thrombocytes – $F = 859.3$; $p < 0.001$ ($\eta^2 = 0.91$), ESR – $F = 204.8$; $p < 0.001$ ($\eta^2 = 0.73$). The η^2 values show strong and stable effects of light spectrum on haematopoiesis, which are particularly sensitive in the white blood cell and megakaryocyte lineage.

Table 3. *Influence of lighting spectrum on the haematological characteristics of hens (one-way ANOVA)*

Haematological parameter	F-value	p-value	η^2 (proportion of variance)	Effect description
Leucocytes	684.2	<0.001	0.89	Very strong effect; intergroup differences up to 45%-46%
Haemoglobin	312.5	<0.001	0.78	Strong effect of the light spectrum
Haematocrit	97.4	<0.001	0.62	Moderately strong effect
Erythrocytes	421.7	<0.001	0.84	Very strong effect
Thrombocytes	859.3	<0.001	0.91	Maximally pronounced effect
ESR	204.8	<0.001	0.73	Strong effect

Source: developed by the authors

The photoregulatory mechanisms seem to be involved in the united effect of the light spectrum on the neuroendocrine and immune systems. The effect of the short-wavelength blue light is likely related to the high immune activity, which maintains the higher levels of leukocytes and ESR. The long-wavelength light, in turn, probably excites transcranial hypothalamic photoreceptors that stimulate the reproductive axis and promote an increase in oestradiol and progesterone levels. The latter induces erythropoiesis and thrombopoiesis and increases the levels of haemoglobin, haematocrit and erythrocytes. It partially suppresses immune function and decreases ESR. Thus, the spectral composition of light regulates the ratio of reproductive function and immune activity to improve functional adaptation of the haematopoietic system.

Conclusions

The spectral composition of light has a determining role in the control of the haematological condition of laying hens. The short-wavelength blue light is characterised by enhanced leukocytogenesis and higher erythrocyte sedimentation rate values, which can be attributed to the activation of immune function and the predominance of immunoregulatory mechanisms. As the wavelength of the light increases (green, yellow, red), we observed a stepwise increase in the concentration of haemoglobin, haematocrit, and the content of erythrocytes and thrombocytes, which is evidence of the activation of erythro- and megakaryocytopoiesis and enhanced oxygen-transport function of the blood. Leuko- and megakaryocytic lineages of haematopoiesis are the most sensitive to the spectral composition of light, which highlights their key role in the adaptation of the organism to the light environment. The decrease in erythrocyte sedimentation rate observed under the long-wave light is related to the stabilisation of the physicochemical properties of the blood and the development of adaptive mechanisms directed toward the maintenance of homeostasis in conditions of increased reproductive load. In general, the data indicate that the light spectrum is a valid means to control the functional activity of the haematopoietic system and can be used as a regulated parameter for improving the physiological status and productivity in laying hens.

The results of this experiment showed that lighting spectral effects on haematological traits of laying hens. In the case of switching blue to red light, the significant decrease of leukocyte by 45.6% ($p < 0.001$), increase of haemoglobin by 51.8% ($p < 0.001$) in red light compared with blue light, the significant increase of erythrocyte by 52.2% ($p < 0.001$) and significant decrease of erythrocyte sedimentation rate by 22.6% ($p < 0.001$) in red light were detected. Future research should focus on investigating the impact of certain combinations of the spectrum of lighting on physiological traits and adaptive reactions in laying hens in order to improve these traits. The study of the interactions among light spectrum, reproduction and production traits (laying, egg quality, morpho-functional state of the reproductive organs) and the use of the cellular and molecular methods for the understanding of the functions of neuropeptides and hormonal pathways in the realisation of lightdependent effects are of great interest.

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Conflict of Interest

None.

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Вплив монохромного світла на гематологічні показники та функцію системи кровотворення курей-несучок

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Анотація. Метою дослідження було вивчити вплив монохромного освітлення на гематологічні показники курей-несучок кросу Hy-Line W-36. Було проведено експеримент із чотирма групами птиці, де відмінністю слугував колір світла: блакитний, зелений, жовтий та червоний. Аналіз гематологічних параметрів включав визначення кількості лейкоцитів, еритроцитів, тромбоцитів, концентрації гемоглобіну, гематокриту та швидкості осідання еритроцитів (ШОЕ). Результати показали, що спектральний склад освітлення суттєво впливає на систему кровотворення у курей. Лейкоцитарна активність поступово зменшувалася при переході від коротких до довгих хвиль, що свідчить про зниження імунної активації під дією теплого світла. Концентрація гемоглобіну та гематокрит збільшувалися при використанні жовтого і червоного світла, що відображає стимуляцію еритропоезу та збільшення об'єму формених елементів крові. Кількість еритроцитів зростала у жовтому та червоному спектрах, тоді як тромбоцити проявляли найбільшу чутливість до довгих хвиль, що свідчить про активацію мегакаріоцитарного ростка. ШОЕ навпаки знижувалося при збільшенні довжини хвилі, демонструючи стабілізацію в'язкості крові та зменшення агрегації еритроцитів. Однофакторний дисперсійний аналіз підтвердив статистично значущий вплив спектрального складу світла на всі досліджені параметри. Проведений аналіз експериментальних даних засвідчив істотну залежність гематологічного статусу курей-несучок від спектральних характеристик світлового режиму. Зокрема, при зміні блакитного освітлення на червоне встановлено зниження кількості лейкоцитів на 45,6 % ($p < 0,001$), водночас рівень гемоглобіну зростав на 51,8 % у червоному спектрі порівняно з блакитним ($p < 0,001$). Крім того, використання червоного світла супроводжувалося достовірним збільшенням чисельності еритроцитів на 52,2 % ($p < 0,001$) та зменшенням швидкості їх осідання на 22,6 % ($p < 0,001$). Отримані результати формують науково обґрунтовану основу для розроблення рекомендацій щодо застосування монохроматичних і комбінованих світлових режимів з метою оптимізації продуктивності та підвищення стресостійкості курей-несучок

Ключові слова: лейкоцити; еритроцити; тромбоцити; гемоглобін; гематокрит; спектральний склад; імунна активність

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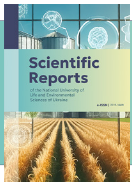

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Features of spheroidogenesis of L929 cell line during prolonged three-dimensional cultivation

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Abstract. Cellular 3D cultures, in particular spheroids, are widely used to study intercellular interactions and test biocompatibility and toxicity, as they more accurately reproduce physiological conditions compared to 2D cultures. The aim of the study was to investigate the features of spheroid formation in L929 cell line in static suspension culture during long-term cultivation. To obtain spheroids, a suspension with a concentration of 2×10^6 cells/ml was placed in a 24-well plate coated with a 2% agar solution. Cultivation was carried out for 34 days. Every three days, half of the medium volume was replaced. The average diameter and volume of the spheroids were determined. The presence of necrotic cells in the spheroids was assessed by double staining of the spheroids with fluorescein diacetate and ethidium bromide. It was found that maintaining L929 cell line in a static suspension culture allowed spheroids with an average diameter of 118 μm to be obtained without the presence of a necrotic nucleus. Spheroid formation was a multi-step process that included initial cell aggregation, followed by compaction of the spheroids, which were kept in a floating state for one month of cultivation. During the study, it was found that L929 cell line spheroids were formed in several stages, including aggregation, compaction, and stabilisation of cells. All spheroids retained their spherical shape and remained viable, showing no signs of necrotic changes even on the 34th day of cultivation. The results of the study can be used to improve methods for testing biocompatibility and cytotoxicity, particularly in the development of new drugs and biomaterials using 3D cultures of L929 cell line

Keywords: 3D cultivation; spheroids; fibroblast cell line; static suspension culture; morphological analysis

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Introduction

Cell 2D and 3D cultures are widely used in modern medical and biological research. When analysing the results obtained in 2D culture, it should be borne in mind that they only partially reflect the physiology of natural tissues. Cells that are forced to grow in a single plane as a monolayer can change their phenotypic and functional properties due to cytoskeletal rearrangement and disruption of cell-cell and cell-extracellular matrix contacts (Jubelin *et al.*, 2022). For this reason, studies of the toxicity of various substances and materials on 2D cultures do not provide adequate data for predicting cellular responses in the body. 3D models, in which cells are in three-dimensional cellular interactions, are closer to reality. As a result, 3D cultures (spheroids, organoids) are widely used to study intercellular interactions and cell differentiation, assess the toxicity of substances and the effectiveness of potential drugs, as components of bioengineered structures, and in regenerative medicine (Cardoso *et al.*, 2023).

Researchers C. Jubelin *et al.* (2022) summarised current approaches to the use of three-dimensional *in vitro* models in cancer research, in particular spheroids and organoids. The authors showed that 3D cultures better reproduce the architecture of tumour tissue, intercellular interactions, and oxygen and nutrient gradients compared to 2D cultures. Their advantage for evaluating the effectiveness of antitumour drugs and studying the mechanisms of drug resistance was emphasised. In 3D cell culture, a number of methods are used to induce spheroid formation by limiting cell adhesion and enhancing intercellular interactions. Thus, N.E. Ryu *et al.* (2019) noted that the use of non-adhesive surfaces, micro-well systems, and dynamic culture conditions is an effective approach to stimulate the self-organisation of cells into three-dimensional spheroid structures. In turn, A.P.P. Guimaraes *et al.* (2024) emphasised the importance of controlled cell aggregation methods, in particular the “hanging drop” technology, which allows the size and morphology of spheroids to be regulated and ensures reproducibility of results in 3D cultures.

It should be noted that the mechanisms of spheroid formation differ depending on the culture conditions. Under scaffold-based technology, spheroids are formed thanks to an artificial extracellular matrix that provides physical support for cells to attach and undergo three-dimensional reorganisation. At the same time, virtually all cells, including highly differentiated ones, participate in the formation of spheroids. K. Unnikrishnan *et al.* (2021) emphasised that artificial matrices are not limited to reproducing the architectural and biomechanical characteristics of the extracellular matrix, but also play an active role in regulating cell behaviour, in particular the processes of cell proliferation, migration and differentiation, as well as in the formation of spatial gradients of nutrients and oxygen within three-dimensional cell structures.

Under scaffold-free conditions, spheroid formation occurs through cell self-assembly. As noted by K.C. Liu *et al.* (2024), under such conditions, active synthesis and secretion of endogenous extracellular matrix components are observed, which contributes to the formation of 3D structures that are as close as possible to *in vivo* conditions. D. Stern-Tal *et al.* (2022) emphasised that scaffold-free approaches reduce the influence of foreign materials on the cellular response and allow the study of natural mechanisms of cell aggregation and spatial organisation. Unlike scaffold technology, this allows for the selection of stem/progenitor cells capable of forming cell colonies. The scaffold-free method of obtaining spheroids is the most promising for isolating and maintaining progenitor cells from different organs. One of the scaffold-free methods for obtaining spheroids is the use of a surface with low adhesive properties. Y. Xie *et al.* (2024) showed that in such a static suspension culture, the principle of reduced adhesion of the culture surface is used: the attachment of cells to the surface is suppressed, so they try to realise their ability to attach through interaction with other cells and spontaneously form spheroids. Agar, chitosan, hyaluronic acid, etc. are used to create a low-adhesion surface.

The process of spheroid formation involves changes in cell interaction and cytoskeletal organisation, as well as the synthesis of the extracellular matrix. It is important to note that the main stages of spheroid formation are standard but may have specific features depending on the source of the cells or the method used to obtain the spheroids. Epithelial cells often show delayed aggregation, reflecting their dependence on polarisation and the development of specialised intercellular contacts (tight and adhesive contacts, desmosomes) (Bharathan *et al*., 2024). In comparison, mesenchymal-derived cells, including the L929 line, typically aggregate more rapidly and form spheroids. A. Moisieiev *et al.* (2024) found that L929 cells have the ability to form spheroids in static suspension culture on low-adhesive surfaces. G.A. Bozhok *et al.* (2019) studied such spheroids only in the initial period (7-8 days), while the dynamics of their further existence under 3D culture conditions remain unknown.

Thus, the scientific literature notes the importance of using 2D and 3D cell cultures in biomedical research, in particular for studying intercellular interactions, assessing the toxicity and efficacy of drugs, and developing bioengineered structures and regenerative medicine. Despite the wide range of applications of 3D cultures, the details of the dynamics of spheroid formation of L929 cell line under conditions of prolonged 3D cultivation remain unexplored, which is relevant for assessing biocompatibility and cytotoxicity over a long period of time. In this regard, the aim of the study was to investigate the stages of spheroid formation in L929 cell line culture on a low-adhesive surface during prolonged 3D cultivation.

Materials and Methods

The study was conducted at the Department of General and Clinical Pathology of the Medical Faculty of V.N. Karazin Kharkiv National University in collaboration with the Department of Cryoendocrinology of the Institute of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine in 2021-2022. To study the stages of spheroid formation, the L929 cell line was selected, which is a transplantable adhesive cell line obtained from the subcutaneous connective tissue of C3H/An mice. Due to its high proliferative properties and genetic stability, the line is widely used in laboratory and screening analyses in accordance with the international standard ISO 10993-5:2022 (2022) for assessing the biocompatibility (Sharma *et al.*, 2016) and cytotoxicity of substances (Cannella *et al.*, 2019). In addition, as shown by researchers F. Ehrhart *et al.* (2009) and A. Moisieiev *et al.* (2024), it has the ability to grow in 3D culture with the formation of spheroids.

L929 cell line were cultured in DMEM/F12 medium (Biowest, France) with the addition of benzylpenicillin (200 IU/ml), streptomycin (200 μg/ml), amphotericin B (2.5 μg/ml) and 10% foetal calf serum (Biowest) in a CO_2 incubator at 37°C and 5% CO_2. Initially, the cells were cultured under standard conditions in polystyrene flasks (SPL Life Sciences, Korea). The seeding density was 4×10^4 cells per 1 cm^2 of surface area. After the formation of a confluent monolayer, the cells were detached from the surface using a 0.5% trypsin solution prepared in physiological saline (Novopharm-Biosynthesis, Ukraine) and Versen's solution (Biowest, France) in a 1:1 ratio. To obtain spheroids, a suspension with a concentration of 2×10^6 cells/ml was transferred to a 24-well plate coated with a 2% agar solution (Ferak, Germany). The agar solution was sterilised by autoclaving, then heated in a boiling water bath to a liquid state and coated the bottom of the wells (250 μl/well) under sterile conditions. After the agar layer had solidified, the cells were seeded. Cultivation was carried out for 34 days. Every 3 days, ½ of the medium volume was replaced.

Microphotography was performed using an AmScope XYL-403 inverted microscope (PRC) with a digital camera. The AxioVision Rel. 4.6 programme (Carl Zeiss, Germany) was used for morphometric analysis of the spheroids. The programme tools were used to determine the average diameter of the spheroids, from which their average volume (V) was calculated using the formula:

$$V = \pi/6d^3, \quad (1)$$

where d – spheroid diameter.

The presence of necrotic cells was assessed by double staining of spheroids with fluorescein diacetate (FDA) and ethidium bromide (EB) using a Zeiss LSM 510 META confocal microscope (Carl Zeiss, Germany). Confocal images were obtained at an excitation wavelength of 488 nm for FDA and 543 nm for EB. Statistica 10 software (StatSoft, USA) was used for statistical analysis of the data. Normality of distribution was determined using the Shapiro-Wilk W-test. The data are presented as the mean ± standard deviation. The sample size (n) was 25 spheroids for each cultivation term.

Results and Discussion

After 2 days of cultivation on a low-adhesive surface, single cells and small aggregates of 2-3 cells were observed (Fig. 1). During the first week of cultivation, the cells formed loose clusters, which gradually increased in size due to aggregation and, possibly, proliferation. The process of aggregation and proliferation continued for the next 3-4 days. On day 7, the shapeless cell aggregates became denser and acquired a more spherical shape. On day 9, it was noticeable that the spheroids were becoming denser. In addition, large clusters began to appear, which may be the product of spheroid fusion. Such conglomerates of spheroids were not used in further analysis.

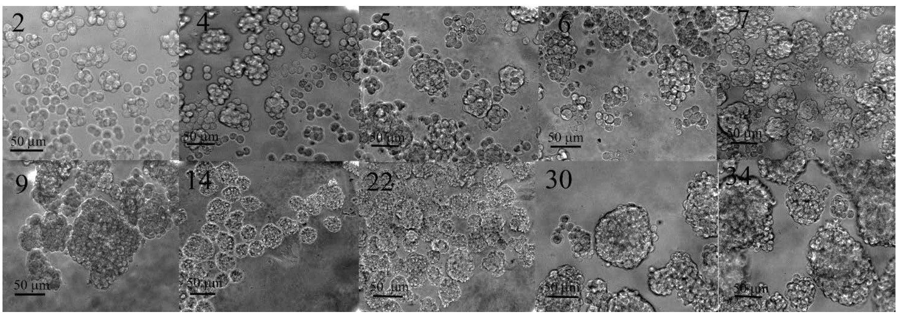

Figure 1. *Stages of spheroid formation from L929 cell line during cultivation in low-adhesion conditions for 34 days*

Note: the number indicates the number of days of cultivation

Source: developed by the authors

In the next period (14-34 days), along with an increase in size, the spheroids became more spherical in shape, and individual cells became less distinct. However, not all cells merged, so single cells and small aggregates were observed in the culture along with the spheroids. This may be due to the dynamic process of proliferation and exfoliation of cells in spheroids. After 30 days of cultivation, the spheroids remained floating in the culture medium, while the process of their fusion was permanently observed, indicating the preservation of intercellular adhesion and the dynamic restructuring of three-dimensional cell aggregates (Fig. 2).

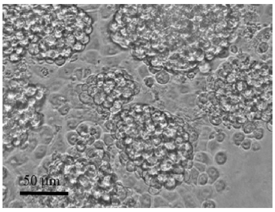

Figure 2. *Attachment of spheroids formed during cultivation of L929 cell line in low-adhesion conditions for more than 30 days*
Note: around the spheroids, there are noticeable clusters of cells that have migrated and formed a monolayer
Source: developed by the authors

The fusion of spheroids was accompanied by an increase in their size and a change in morphology, which may be associated with prolonged maintenance of scaffold-free culture conditions and the absence of stable adhesion to the surface. After more than 30 days, gradual biodegradation of the agar coating was observed, accompanied by a loss of low-adhesion conditions. This led to the spheroids attaching to the bottom of the culture dish, from which cells began to migrate, forming a monolayer. These morphological changes emphasise the critical role of stable low-adhesion conditions in maintaining spheroid culture during long-term cultivation. The quantitative dynamics of spheroid sizes from L929 cells during 35 days of cultivation are shown in Figure 3.

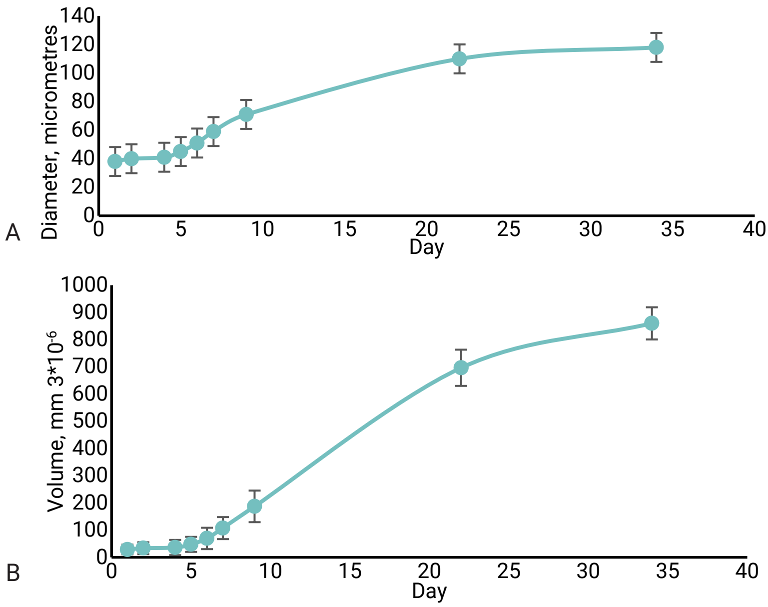

Figure 3. *Dynamics of spheroid sizes from L929 cell line*
Note: A – average diameter and B – average volume of spheroids obtained over 34 days
Source: developed by the authors

The initial diameter of the cellular aggregates averaged 38 ± 3 µm, while their volume was $28 \pm 18 \times 10^{-6}$ mm^3, reflecting the formation of small primary cellular conglomerates. During the process of primary aggregation up to day 5, the linear dimensions did not increase, indicating the stability of the initial cellular aggregates and a limited number of cell divisions at this stage. Morphological analysis showed that by day 7 the aggregates acquired a spherical shape, reflecting the process of cellular self-organisation and optimisation of intercellular contacts. After this point, a sharp increase in both the diameter and the volume of the spheroids was observed. It is likely that from this stage the change in spheroid size was associated not only with further aggregation but also with cellular proliferation within the three-dimensional structures. The increase in size was accompanied by a more compact and homogeneous spheroid morphology, indicating active intercellular adhesion and the organisation of extracellular matrix produced by the cells themselves. By day 34, the spheroids reached a mean diameter of 118 ± 36 µm and a mean volume of 859.85 ± 59.1 mm^3, demonstrating a substantial increase compared with the initial state and indicating the effectiveness of three-dimensional cultivation in maintaining the viability and functional activity of L929 cells over a prolonged period. Such data emphasise the phase-dependent nature of spheroid development: the initial formation and stabilisation of aggregates transitions into a phase of active growth, in which cellular proliferation and the strengthening of intercellular contacts predominate, ensuring the maintenance of structural integrity and functional organisation of the three-dimensional culture. At these average sizes, spheroids formed from the L929 cell line did not exhibit a necrotic core, as evidenced by the absence of red staining in the FDA and EB test (Fig. 4).

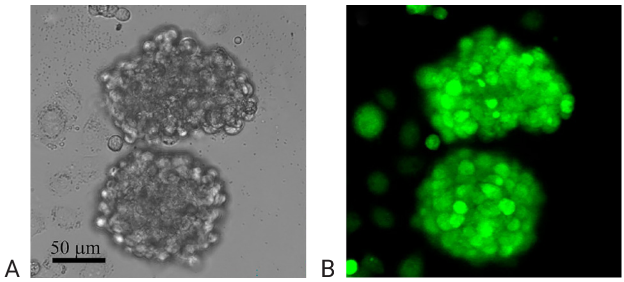

Figure 4. *Viability of cells in spheroids according to FDA/EB staining*
Note: A – light microscopy; B – fluorescence microscopy
Source: developed by the authors

To assess cell viability based on plasma membrane integrity and enzymatic activity, a test using FDA and EB was used. FDA passively diffuses through the plasma membrane of viable cells. Inside the cell, under the action of intracellular esterases, FDA is hydrolysed to fluorescein, which is retained in the cytoplasm and emits green fluorescence, reflecting esterase activity and membrane integrity. EB enters only cells with impaired membrane integrity, binds to nucleic acids and emits an intense red fluorescence, indicating cell death or severe damage. The analysis showed high cell viability and sufficient diffusion of nutrients and oxygen to the centre of the spheroids with an average diameter of 118 ± 36 µm, which allows maintaining the functional activity of cells even in a three-dimensional structure. The results obtained emphasised the phase nature of spheroid development: the initial stabilisation of aggregates transitions into active growth with cell proliferation, the formation of spherical morphology and the maintenance of metabolic stability within the three-dimensional culture, which makes such aggregates relevant models for in vitro studies. Thus, as a result of the experiments, it was established that when using a low-adhesive agar coating, L929 cell line quickly organise into aggregates, after which, from the 7th day, in the process of compaction, they form spheroids that remain in a floating state for up to 34 days.

Continuing the analysis of the data obtained, it can be noted that at different stages of cultivation, changes in the structure of spheroids are observed, which is the result of active proliferation and organisation of cells. This opens up opportunities for a more detailed discussion of the mechanisms that regulate these processes. The results of the presented study showed that L929 cell line actively form spheroids when cultured on low-adhesive surfaces, which is consistent with the literature data on the mechanisms of spheroid formation. As noted by K. Białkowska *et al.* (2020), 3D spheroids are extremely important for research in biology because they provide an opportunity to recreate the natural conditions of the cellular environment, in particular cell-cell and cell-ECM interactions, which are limited in 2D cultures. They also emphasised that spheroid formation in 3D cultures depends on a number of factors, including culture conditions and cell type, which are critical for analysing biocompatibility and cytotoxicity *in vitro*.

Research by N.E. Ryu *et al.* (2019) showed that the first stage of spheroid formation is characterised by the initial aggregation of individual cells into loose clusters, which was also observed in the current study. This stage is mainly regulated by passive physical processes and early intercellular recognition events, as emphasised by Y. Zhou *et al.* (2017). Intravital observations of cell culture on non-adhesive surfaces allowed L. Adenis *et al.* (2020) to identify four phases characteristic of several cell lines during spheroid formation. The first phase is the random movement of individual cells. The second phase is the formation of aggregates of 5-10 cells. The third phase is the restructuring of aggregates, during which the cells inside are constantly moving and reorganising. During this phase, the process of cell proliferation begins. A few hours after formation, the aggregates condense and reorganise into a three-dimensional shape, which is the final phase of spheroid formation. Proliferation continues, constantly increasing the size of the spheroids. The cells inside the spheroids actively remodel their microenvironment by synthesising and depositing extracellular matrix components such as collagen, fibronectin and laminin. This endogenous extracellular matrix provides additional mechanical support to the spheroids and modulates their sensitivity to xenobiotics.

The rate of progression of the phases, density, and size of aggregates/spheroids may vary depending on both the culture conditions (static culture, surface properties, medium composition) and the characteristics of epithelial, mesenchymal, stem, or tumour cells, as demonstrated by L. Adenis *et al.* (2020). Moreover, not all cell lines form compact spheroids when cultured in static suspension culture. The key difference is that L929 spheroids derived from fibroblasts are less prone to early necrosis. In contrast, according to Y. Tan *et al.* (2020), more metabolically active or sensitive epithelial cancer cell lines are prone to earlier hypoxia and central necrosis. In the current study, L929 cells underwent all phases of spheroid formation, including initial aggregation, maturation and restructuring of aggregates, compaction and stabilisation due to extracellular matrix deposition.

During spheroid formation, cells organise themselves into a 3D structure, leading to the formation of gradients of metabolites and gases, such as oxygen, within the spheroid. As established by M. Acland *et al.* (2018), oxygen diffusion into the spheroid is limited to a cell layer of 150-200 μm. As the size of the spheroid increases, oxygen diffusion becomes more difficult, which can lead to the formation of necrotic zones in the centre (Barisam *et al.*, 2018). Since the average size of the spheroids in the experiments did not exceed 120 μm, no necrotic zones were observed, confirming effective oxygen diffusion and maintenance of cell viability in all parts of the spheroid.

Thus, the experiments revealed that L929 cell line effectively form spheroids when cultured on low-adhesive surfaces, confirming their ability to self-organise and aggregate in three-dimensional conditions. The dynamics of spheroid formation includes several phases, starting from the initial aggregation of cells to their subsequent compaction and stabilisation due to the formation of an extracellular matrix. The observed changes in the size and morphology of spheroids are consistent with the literature data on the mechanisms of spheroid formation in other cell lines. Under cultivation conditions of up to 34 days, L929 spheroids do not show necrotic areas, confirming effective oxygen diffusion in three-dimensional aggregates, preserving cell viability. These results highlight the importance of three-dimensional cultures for maintaining cell functional activity in long-term experiments, as well as for further application in biomedical research, such as biocompatibility and cytotoxicity testing.

Conclusions

This article presents the results of a study of the stages of spheroid formation in L929 cells during prolonged three-dimensional cultivation on a low-adhesive surface. During the study, the morphological and quantitative dynamics of cell aggregate formation were monitored over 34 days, and their viability within three-dimensional structures was assessed. It was found that L929 cells in static suspension culture rapidly form primary aggregates, which from the 7th day become denser and acquire a spherical shape. Subsequently, the spheroids remain in a floating state, demonstrating active intercellular adhesion and the formation of an endogenous extracellular matrix, which maintains their structural integrity and functional activity throughout the entire cultivation period. With an average diameter of about 118 micrometres, no necrotic nuclei were found, indicating sufficient diffusion of nutrients and oxygen to the central zones of the spheroids. Thus, spheroid formation is a multistage process that includes initial cell aggregation, followed by compaction and stabilisation of three-dimensional structures over one month of cultivation.

Summarising the data obtained, it can be stated that the proposed approach using a low-adhesive agar coating allows obtaining stable three-dimensional models of L929 cell culture relevant for *in vitro* studies. Spheroids of this line are characterised by predictable growth dynamics, preservation of viability and absence of early necrosis, which makes them suitable for assessing biocompatibility, cytotoxicity and modelling cell responses in a three-dimensional environment. Thus, the results are of methodological and practical importance for the improvement of 3D models in biomedical research. Promising areas for further research include studying the effect of various characteristics of low-adhesive surfaces on the formation rate and stability of spheroids, detailed assessment of intracellular proliferation and apoptosis, analysis of the diffusion of nutrients and pharmacological agents into aggregates, as well as the application of the obtained three-dimensional models for testing cellular responses to external stimulants and therapeutic drugs.

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Conflict of Interest

None.

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Особливості сфероїдогенезу клітин лінії L929 при тривалому тривимірному культивуванні

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Анотація. Клітинні 3D-культури, зокрема сфероїди, широко використовуються для вивчення міжклітинних взаємодій та тестування біосумісності та токсичності, оскільки вони точніше відтворюють фізіологічні умови в порівнянні з 2D-культурами. Метою дослідження було вивчити особливості сфероїдоутворення клітин лінії L929 у статичній суспензійній культурі протягом тривалого терміну культивування. Для отримання сфероїдів суспензію з концентрацією 2×10^6 клітин/мл приміщували у 24-лунковий планшет з покриттям 2 % розчином агару. Культивування проводили впродовж 34 діб. Кожні три доби здійснювалась заміна половини об’єму середовища. Визначався середній діаметр та об’єм сфероїдів. Наявність некротичних клітин у складі сфероїдів оцінювалась за допомогою подвійного забарвлення сфероїдів флуоресцеїндіацетатом і етидієм бромідом. Було встановлено, що підтримка клітин лінії L929 у статичній суспензійній культурі дозволяла отримувати сфероїди з середнім діаметром 118 мкм без наявності некротичного ядра. Формування сфероїдів було багатоступеневим процесом, що включало початкову агрегацію клітин, після якої відбувалась компактизація сфероїдів, які утримувались у флотуючому стані протягом одного місяця культивування. Протягом дослідження було виявлено, що сфероїди клітин лінії L929 утворювались через кілька етапів, включаючи агрегацію, ущільнення та стабілізацію клітин. Всі сфероїди зберігали свою сферичну форму та залишалися життєздатними, не демонструючи ознак некротичних змін навіть на 34-й день культивування. Результати дослідження можна використати для вдосконалення методів тестування біосумісності та цитотоксичності, зокрема при розробці нових лікарських засобів та біоматеріалів за допомогою 3D-культур клітин лінії L929

Ключові слова: 3D-культивування; сфероїди; клітинна лінія фібробластів; статична суспензійна культура

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