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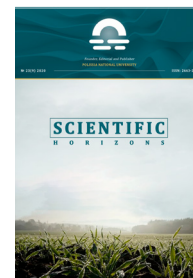
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## Seasonal and Age Dynamics of Passalurosis Invasion of Rabbits and Pathological and Histological Changes in this Nematodosis

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**Abstract.** The most common nematode in rabbits is passalurosis, and climate change in Ukraine in recent decades has altered the epizootic manifestation of this parasitosis, which predetermines the relevance of this study. Therefore, the purpose of this paper was to establish the epizootic situation regarding the passalurosis invasion and to investigate the pathological and histological changes in both the large and small intestines of rabbits with a high degree of damage by *Passalurus ambiguus*. The study used the McMaster method, the improved method of incomplete helminthological dissection according to K.I. Scriabin Private households in the Polissia zone of Ukraine were found to be the most vulnerable in terms of passalurosis infestation, where 37.70-41.67% of sick rabbits were registered. In the seasonal aspect, the highest infection of animals was found in winter (invasion extensiveness (IE) was 35.27%), with a peak in January (IE = 35.29%), the lowest – in summer (IE = 25.79%). Rabbits aged 1 to 2 years had the highest incidence of the pathogen *Passalurus ambiguus* (82.76%). It was found that with a high degree of damage (invasion intensiveness (II) –  $2446.67 \pm 422.11$  eggs in 1 g of faeces) rabbits with passalurosis had catarrhal and catarrhal-haemorrhagic inflammation, swelling of the mucous membrane of the intestines with dotted or striped haemorrhages. The destruction of the villi of the mucous membrane of the small, caecum, and colon, crypt cells and infiltration by lymphocytes and monocytes, severe swelling of the submucosal base and muscle membrane, and a state of granular dystrophy of all smooth muscle cells of the muscle membrane were established. The detection of seasonal, age-related, and pathological-histological changes in rabbits with passalurosis will allow controlling the degree of infection of animals and promptly taking effective countermeasures, which predetermines the practical value of this study

**Keywords:** nematodosis of rabbits, *Passalurus ambiguus*, passalurosis, invasion extensiveness and intensiveness, histological changes



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## INTRODUCTION

Rabbit breeding in Ukraine is a major area in the development of meat breeding. Parasitosis is a restraining factor in the development of the industry. In the body of an animal of this species, several types of parasites can be localized, which form a parasitocenosis, and determine the relevance of this study.

One of the most common nematodes in the parasitocenosis of rabbits is passalurosis, which is caused by the pathogen *Passalurus ambiguus Rudolphi, 1819* of the *Oxyuridae* family (Sheng *et al.*, 2015; Sultan *et al.*, 2015). Helminths parasitize in the caecum and large intestine of animals and lead to various digestive disorders, weight loss, and with a high degree of exposure even to death (Pilarczyk *et al.*, 2008; Abdel-Gaber *et al.*, 2019).

According to N.M. Hussein *et al.* (2022), infection of animals with the *Passalurus ambiguus* pathogen in rabbit farms in Egypt reaches 45%, and in Italy – up to 82.3% with the invasion intensiveness from a few eggs of the pathogen to about 200 specimens in 1 g of faeces (Nosal *et al.*, 2006). According to scientists, passalurosis of rabbits in Serbia was registered in 17.09% of animals (Ilić *et al.*, 2018), in Italy – 12.9% (Sergi *et al.*, 2018), in Poland – 21.9% (Kornaś *et al.*, 2015), in Iraq – 10% (Athraa, 2014), 32.37% (Marhoon, 2018), 52.1% (Al-Moula, 2005). That is, despite a sufficient number of reports, this disease stays relevant in rabbit farms around the world today.

When studying the age dynamics of invasion in rabbits, it was established that animals of different age groups were invaded differently. In rabbits aged under one year, the invasion intensiveness of *Passalurus ambiguus* was 5.8%, while in adults it increased to 23.3% (Ilić *et al.*, 2018). Older animals were more often infected with nematodes. As a result of reinfestation in 180-day-old rabbits, the intensiveness of *P. ambiguus* lesions increased 4.0 times (Prus *et al.*, 2021). With the age of the animals, the invasion extensiveness increases and ranges within 26.67-77.33% already in 12 months. The average invasion intensiveness in 4-month-old animals is 1,271 specimens of the pathogen, and in 5-7-month-old animals, it is 2,418 specimens. At the age of one year and older, with an increase in the invasion extensiveness, a simultaneous decrease in its intensiveness was detected (from 875 to 343 specimen in one animal). According to G. Sioutas *et al.* (2021), rabbits at 3-6 months are most prone to passalurous invasion, the invasion extensiveness in which was 60%, and the invasion intensiveness was higher in animals aged 6-12 months. However, according to M. Varga (2014), passalurosis is more dangerous for young than for adult rabbits.

In the seasonal aspect, according to N.M. Soroka and I.A. Berehovets (2011), in Ukraine passalurosis is registered almost throughout the year, but the peak of invasion is observed in the winter-spring period. In addition, I. Elshahawy and A. El-Goniemy (2018) noted that the most animals with passalurosis are registered in

summer (38.3%) and winter (33.3%). Its distribution, according to Z.K. Terentyeva *et al.* (2021) is contributed by the unsanitary conditions of keeping rabbits on unchanged bedding or floors. The optimal air temperature for egg development, according to V. Yevstafieva *et al.* (2021, 2022) is +35°C. This coincides with the reports of B. Boag (2001), who emphasizes that the maximum percentage of infected animals was detected in January, and the minimum infection of rabbits was registered already in April.

Pathogenic effect of passalurosis, according to H. Legendre *et al.* (2021), depends on the invasion intensiveness. Upon crawling out of the anus of the female, the pinworms cause severe itching in rabbits and thus disturb the animals. In rabbits, intensive reinvasion by the causative agent of passalurosis continually occurs, due to which mature parasites are simultaneously found in the intestines of animals next to young pinworms. The pinworms are tightly pressed to the intestinal mucosa, located between the intestinal villi and oppose peristalsis; penetrating in numerous instances into the Lieberkühn glands, pinworms cause strong mechanical irritation of the epithelial cells. Research by J. Vadlejch *et al.* (2010) found that the pathogens of *Passalurus ambiguus* are coprophages, although R. Frank *et al.* (2013) sometimes observed haematophagy in pinworms.

According to pathomorphological studies (Voitovska, 2019; Karamushka *et al.*, 2022), toxicosis phenomena are observed in the caecum, colon, mesenteric lymph nodes, liver, kidneys, heart muscle, in the lungs – signs of enterotoxemia, and in places of localization (caecum and colon intestines) areas of necrosis are formed. Exhaustion is noted in rabbits that died from passalurosis. Mesenteric lymph nodes are juicy, swollen, enlarged 2-3 times. Liquid contents in the large intestine. The mucous membrane of the appendix of the caecum is swollen, often with speckled or striped haemorrhages, easily peels off and separates from the muscle layer of the intestinal wall, traumatic injuries of varying intensity on the vulva and anus. In the chronic course of passalurosis, S.M. Mykhailiutenko *et al.* (2019) established pathomorphological changes in parenchymal organs (lymphohistiocytic interstitial hepatitis and fatty and granular dystrophy of hepatocytes were noted in the liver; necrotic, lipoid, and protein nephrosis, serous extracapillary glomerulonephritis, diffuse lymphohistiocytic interstitial nephritis in the kidneys; lymph node hyperplasia in the spleen). At a high invasion intensiveness by pinworms, the death of rabbits due to peritonitis and haemorrhagic colitis is noted.

The climate in Ukraine has changed a lot in recent decades, which has led to a change in the epizootic manifestation of parasitosis. With the change of climate in the country, the epizootic situation regarding this nematode of rabbits was not studied at all in the entire territory of Ukraine, with only individual regions described. Therefore, *the purpose of this study* was to

establish the epizootic situation regarding passalurosis in Ukraine and to investigate the pathological and histological changes in the intestines of rabbits with a high level of invasion intensiveness.

## MATERIALS AND METHODS

The spread of passalurosis infestation was investigated through clinical observation and coproscopic studies of 1,209 domestic rabbits (*Oryctolagus cuniculus*) aged from birth to 4 years in large and small private households from different regions of Ukraine: Volyn, Dnipropetrovsk, Zhytomyr, Zaporizhzhia, Ivano-Frankivsk, Kirovohrad, Lviv, Odesa, Poltava, Kharkiv, Kherson, Khmelnytskyi, Cherkasy. The age and seasonal dynamics of passalurosis were studied in rabbits in the territories of Dnipropetrovsk and Zaporizhzhia regions during 2014-2020. The study was conducted in the scientific laboratories of the Department of Parasitology and Veterinary Expertise of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University. *Passalurus ambiguus* eggs were identified based on their size and morphological characteristics using the atlas of differential diagnosis of helminthiasis by I.S. Dakhno et al. (2001). To determine the level of infection of rabbits with pathogens of *Passalurus ambiguus*, faecal samples were examined using McMaster slide (Cringoli et al., 2004). Therewith, 2 g of faeces were placed in a glass container, mixed with 28 ml of flotation solution (preferably ammonium nitrate), thoroughly mixed and filtered through a strainer. The filtered mixture was resuspended with a pipette, introduced into the counting chamber and left for flotation for 2-3 minutes. Under a microscope, all helminth eggs in the specified fields of two chambers were counted, multiplied by 50 and the number of eggs in 1 g of faeces was obtained.

Autopsies and examination of carcasses of slaughtered rabbits were performed using the improved method of incomplete helminthological dissection according to K.I. Skryabin in the generally accepted sequence (Skryabin, 1928). For histological examination, small pieces, no more

than 2 cm thick, of different sections of the intestine were taken. The sampled biological material was fixed in 10% neutral formalin solution. The obtained material was dehydrated in ethanol of increasing strength, embedded in paraffin according to generally accepted methods. Sections were made on a slide microtome (up to 5 µm thick) and stained with Karatsi's haematoxylin and eosin. Microscopy of the obtained histopreparations was performed under an MC 100 LED microscope, and photomicrography was performed through the NDPL-2 photo nozzle (2x) with a Canon DS12671 camera. Histological preparations were examined under a microscope at magnifications: ×100, 200, 400.

Statistical processing of experimental results to determine biometric indicators (arithmetic mean values (M) and their errors (m)) was calculated using the Microsoft Excel-16 program.

When working with animals, the provisions of Article 26 of the Law of Ukraine No. 3447-VI dated 10/16/2012 "On the Protection of Animals from Cruelty" (Law of Ukraine No. 3447-VI), "General Ethical Principles of Animal Experiments", approved on The First National Congress on Bioethics (Reznikov, 2003), the requirements of the European Convention "On the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" (European convention, 1986), the Declaration "On the Humane Treatment of Animals" (Universal Declaration, 2007).

## RESULTS AND DISCUSSION

The conditions of natural and climatic zones are of leading importance in the spread of parasitosis among rabbits. The territory of the flat part of Ukraine comprises three geographical zones: Polyssia, Forest-Steppe, and Steppe, each of which has climatic differences. To establish the epizootic situation regarding rabbit passalurosis, 1,209 animals from 13 regions of Ukraine, located in different natural and climatic zones, were investigated. Infestation of rabbits with passalurosis in different climatic zones is presented in Table 1.

**Table 1.** Invasion of rabbits with *Passalurus ambiguus* in households of Ukraine, depending on the natural and climatic zone (own data;  $M \pm m$ ,  $n = 1209$ )

| Natural and climatic zones | Infection rates | 2019                         | 2020                         |
|----------------------------|-----------------|------------------------------|------------------------------|
| Polissia(p)                | IE, %           | 41.67                        | 37.70                        |
|                            | II, eggs/g      | 161.67 ± 27.42               | 162.30 ± 28.84               |
| Forest-Steppe(l)           | IE, %           | 26.92                        | 26.06                        |
|                            | II, eggs/g      | 116.03 ± 19.20               | 105.45 ± 17.58               |
| Steppe(s)                  | IE, %           | 39.49                        | 37.19                        |
|                            | II, eggs/g      | 184.37 ± 17.41 <sup>ll</sup> | 178.24 ± 15.16 <sup>ll</sup> |
| On average                 | IE, %           | 36.27                        | 34.12                        |
|                            | II, eggs/g      | 163.20 ± 12.40               | 156.20 ± 11.04               |

**Note:** ll – significant ( $p < 0.01$ ) difference with the specified group of this year

**Source:** compiled by the authors

During 2019-2020, the highest number of passalurosis patients (41.67% and 37.70%) were registered in rabbits of personal subsidiary farms in the Polissia zone of Ukraine, simultaneously with an elevated level of invasion intensity ( $161.67 \pm 27.42$  and  $162.30 \pm 28.84$  eggs per 1 g of faeces, respectively). This zone includes the surveyed Volyn, Zhytomyr, Ivano-Frankivsk, and Lviv regions. This zone occupies the northern part of Ukraine. The climate of Polissia is continental, with mild winters and humid and warm summers. The average air temperature in July is  $+17$  to  $+19^\circ\text{C}$ , the average air temperature in January drops to  $-4.5$  –  $-7.8^\circ\text{C}$ . The average annual precipitation is 550-650 mm (Vrublevska *et al.*, 2012). According to the authors, the climate of Polissia

is the most favourable for the development of *Passalurus ambiguus* from the three climatic zones of Ukraine.

It has been established that passalurosis is a widespread nematodosis of rabbits, which was registered in households in all regions of Ukraine during 2019-2020. IE was, respectively, 36.27% and 34.12% (on average 35.20%). The number of parasites in the external environment, as well as changes in the physiological state of the body and the functioning of the immune system, depend on the season (Duda *et al.*, 2019; Duda *et al.*, 2020). During 2014-2020, in the conditions of farms of the Zaporizhzhia and Dnipropetrovsk regions, during coproscopic studies, seasonal patterns of infection of rabbits with *Passalurus ambiguus* were revealed (Table 2).

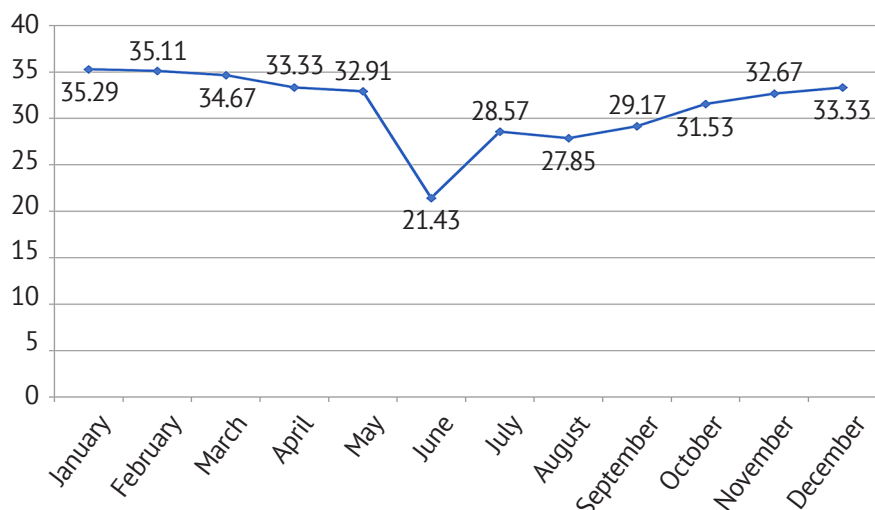
**Table 2.** Seasonal dynamics of passalurosis rabbit infestation indicators ( $M \pm m$ ,  $n = 928$ )

| Infection rates | Seasons            |                    |                  |                    |
|-----------------|--------------------|--------------------|------------------|--------------------|
|                 | Winter             | Spring             | Summer           | Autumn             |
| IE, %           | 35.27              | 34.50              | 25.79            | 31.27              |
| II, eggs/g      | $713.69 \pm 85.00$ | $207.86 \pm 39.64$ | $78.73 \pm 8.49$ | $200.32 \pm 42.14$ |

**Source:** compiled by the authors

According to the results presented in Table 2, it was found that in the summer period of the year, the lowest IE was found in rabbits with passalurosis (25.79%), the highest – in the winter period (35.27%). Therewith, the minimum value of the invasion intensiveness was also established in summer ( $78.73 \pm 8.49$  eggs in 1 g of faeces), the maximum – in winter ( $713.69 \pm 85.00$  eggs/g). Indicators of infestation by the causative agent of passalurosis of rabbits in spring and autumn were recorded at the same level, namely IE – 34.50% and 31.27%,

II –  $207.86 \pm 39.64$  eggs/g and  $200.32 \pm 42.14$  eggs/g, respectively. The dynamics of the monthly infection of rabbits with the causative agent of passalurosis was established (Fig. 1). The highest indicator of invasion extensiveness was found in January and February (35.29% and 35.11%, respectively). With the onset of spring (in May), the IE decreased to 32.91%, and in the summer, the IE was the lowest (June) and amounted to 21.43%. In autumn, the extent of passalurosis infestation in rabbits increased, reaching 32.67% in November.

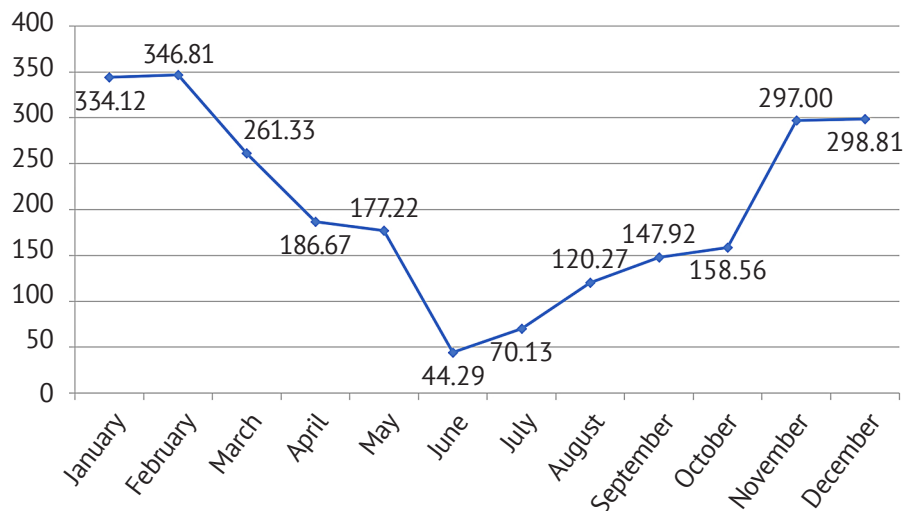


**Figure 1.** Dynamics of the invasion extensiveness of passalurosis in rabbits by month

**Source:** compiled by the authors

Similarly, depending on the season, the indicator of the invasion intensiveness (II) of passalurosis changed (Fig. 2). The highest II was observed in

January and February ( $344.12 \pm 69.82$  and  $346.81 \pm 71.15$  eggs/g, respectively), and the lowest – in June ( $44.29 \pm 11.45$  eggs/g).



**Figure 2.** Dynamics of the intensity of passalurosis infestation in rabbits by month

**Source:** compiled by the authors

According to the data obtained in large and small rabbit farms of Ukraine, the highest rate (35.29%) of infection of rabbits with passalurosis was noted in January. In summer (June) this rate is the lowest and was 21.43%, i.e.,

the peak of passalurosis infestation in rabbits observed in winter. When investigating the age dynamics of passalurosis infestation in rabbits, it was established that animals of different age groups were invaded differently (Table 3).

**Table 3.** Age dynamics of passalurosis rabbit infestation indicators ( $M \pm m$ ,  $n = 928$ )

| Infection rates | Age groups |                      |                       |                       |                       |                        |                       |                       |
|-----------------|------------|----------------------|-----------------------|-----------------------|-----------------------|------------------------|-----------------------|-----------------------|
|                 | 1-2 months | 2-3 months           | 3-4 months            | 4-5 months            | 5-6 months            | 6-9 months             | 9-12 months           | 1-2 years             |
| IE, %           | 0          | 23.53                | 44.44                 | 50.00                 | 56.67                 | 77.78                  | 81.48                 | 82.76                 |
| II, eggs/g      | 0          | 41.17<br>$\pm 17.27$ | 133.33<br>$\pm 60.09$ | 144.44<br>$\pm 42.95$ | 376.67<br>$\pm 96.45$ | 716.67<br>$\pm 110.33$ | 533.33<br>$\pm 89.47$ | 479.31<br>$\pm 91.96$ |

**Source:** compiled by the authors

The data presented in Table 3 indicates that no passalurosis infestation was detected among the rabbits at the age of 1-2 months. From the age of 2-3 months, the invasion extensiveness of pasalurosis increased and in animals aged 1-2 years it was registered in 82.76% of rabbits. The invasion intensiveness also increased in animals aged 6-9 months amounted to 716.67  $\pm$  110.33 eggs/g. In rabbits older than 9 months, the invasion intensiveness of passalurosis decreased and in animals aged

1-2 years it was 479.31  $\pm$  91.96 eggs in 1 g of faeces. The pathogenic effect of *Passalurus ambiguus* depends on the invasion intensiveness (Duda et al., 2019; Shevchik et al., 2021). As a result of helminthocoproscopic studies, it was established that rabbits suffering from passalurosis had different levels of invasion intensiveness (II): low level (II = 276.47  $\pm$  43.33 eggs in 1 g of faeces), medium level (II = 1293.75  $\pm$  275, 80 eggs in 1 g of faeces) and high level (II = 2446.67  $\pm$  422.11 eggs in 1 g of faeces) (Fig. 3).



**Figure 3.** Faecal samples of rabbits with different levels of invasion intensiveness

**Source:** photographed by the authors

It was found that with a high degree of damage to rabbits by passalurosis, catarrhal and catarrhal-haemorrhagic inflammation was observed in the

mucous membrane of the small intestine, the mucous membrane of the caecum was swollen, with dotted or striped haemorrhages (Fig. 4).

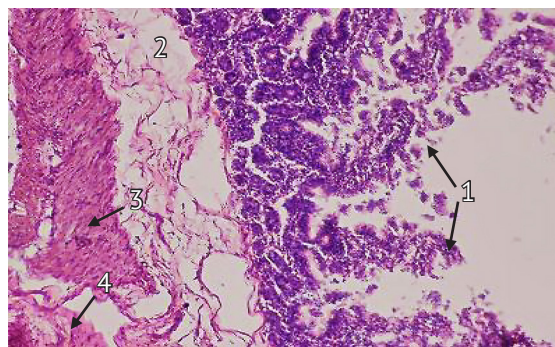


**Figure 4.** Small and large intestine of a rabbit affected by *Passalurus ambiguus*

**Source:** photographed by the authors

Upon conducting histological studies in the small intestine, distinct microscopic changes were established. Strong destruction of villi, disruption of the integrity of crypt cells and infiltration by lymphocytes and monocytes were found in the mucous

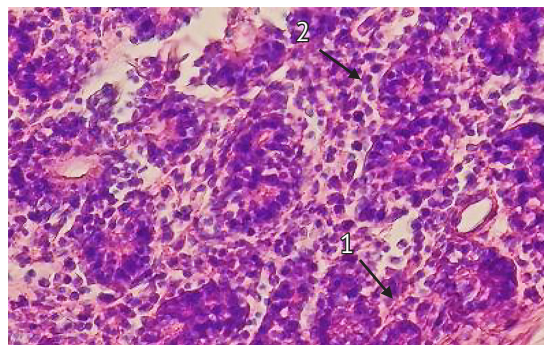
membrane. The submucosa base and muscle membrane are very swollen. All cells of the muscle sheath were in a state of granular dystrophy (Figs. 5; 6). No microscopic changes were detected in the serous membrane.



**Figure 5.** An empty stomach of a rabbit with passalurosis: 1 – destruction of villi; 2 – swelling of the submucosal base; 3 – granular dystrophy of muscle sheath cells; 4 – swelling of the muscle layer

**Note:** Karatsi's haematoxylin and eosin, x100

**Source:** photographed by the authors



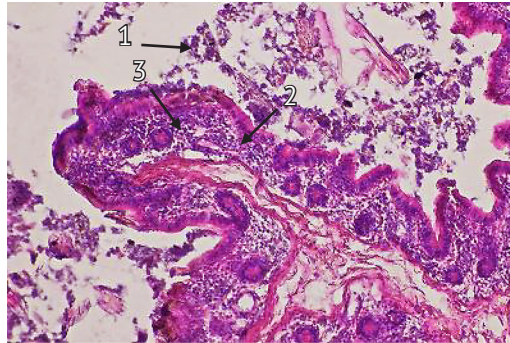
**Figure 6.** Fasting intestine of a rabbit with passalurosis: 1 – destruction of crypt cells; 2 – infiltration of the mucous membrane by lymphocytes and monocytes

**Note:** Karatsi's haematoxylin and eosin, x400

**Source:** photographed by the authors

A large amount of cellular detritus was found in the lumen of the caecum and colon of rabbits with passalurosis. Some of the crypts are destroyed. Relatively large granuloma-like clusters of lymphocytes and monocytes were found on the tops of individual folds of the mucous membrane. The mucous membrane is

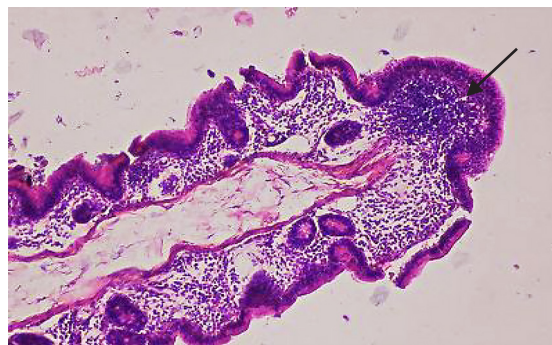
infiltrated by a considerable number of lymphocytes and monocytes. Oedema was found in the submucosa base (Figs. 7; 8), and in the muscle membrane – oedema, granular and hydropic dystrophy of smooth muscle cells. Some of the dystrophically changed cells were destroyed.



**Figure 7.** Caecum of rabbit with passalurosis: 1 – cellular detritus in the lumen; 2 – destruction of the crypt; 3 – infiltration of the mucous membrane by lymphocytes and monocytes; 4 – swelling of the submucosal base

**Note:** Karatsi's haematoxylin and eosin, x100

**Source:** photographed by the authors



**Figure 8.** Caecum of a rabbit with passalurosis: a granuloma-like accumulation of lymphocytes and monocytes at the top of the fold of the mucous membrane (pointed by an arrow)

**Note:** Karatsi's haematoxylin and eosin, x200

**Source:** photographed by the authors

Therefore, pathogens of *Passalurus ambiguus*, with a high degree of damage to the intestines of rabbits, led to the emergence of a large amount of cellular detritus in the lumen of the caecum and colon of rabbits, destruction of crypt cells, infiltration of the mucous membrane by lymphocytes and monocytes, and oedema in the submucosa and muscle sheath.

*Passalurus ambiguus* infestation is one of the most common nematode diseases in large and small rabbit farms of Ukraine, and according to the data obtained, the infestation of rabbits by the causative agent *Passalurus ambiguus* was 35.20%. Comparable results were obtained by G. Sioutas *et al.* (2021), who established that the level of infestation of rabbits with the pathogen *Passalurus ambiguus* in rabbit farms in Greece reaches 31% and I.A. Marhoon *et al.* (2018) found that rabbits

were affected by this pathogen in Al-Diwaniya province, Iraq – 32.37%. According to V. Yevstafieva *et al.* (2021), in the conditions of one-person farms of the Poltava region, passalurosis was found in an average of 82.09%, and according to the results of O.S. Klymenko (2015) – in 21.26% of rabbits, while the authors also claim that this is the most common helminthosis of rabbits.

According to the obtained results, it was established that the highest rate of passalurosis in rabbits (35.29%) is in January. In the spring, the invasion extensiveness decreases to 32.91%, and in the summer (June), this rate is the lowest and is 21.43%. That is, the peak of passalurosis invasion in rabbits is observed in the winter period. This coincides with the reports of B. Boag (2001), who indicates that the maximum percentage of infected animals was recorded in January,

and the minimum infection of rabbits was detected already in April. The peak of nematode infestation, according to N.M. Soroka *et al.* (2011), was observed in the winter-spring period.

Upon the investigation of the age dynamics of this invasion in rabbits, it was established that animals of different age groups were invaded differently. Passalurosis infestation was not detected among the rabbits at the age of 1-2 months. From the age of 2-3 months, its invasion extensiveness increased and in animals aged 1-2 years it was registered at 82.76%. The study results coincide with those obtained by G. Sioutas *et al.* (2021), who indicated that rabbits aged 3-6 months are most prone to passalurosis infestation, and the invasion intensiveness was higher in animals aged 6-12 months. At the same time, the invasion intensiveness of passalurosis, according to the present study, was increasing already in animals aged 6-9 months, its decrease was observed later against the background of a gradual increase in the infection rate of *Passalurus ambiguus* rabbits.

The studies investigating the effect of *P. ambiguus* on the body of rabbits are outdated and few. S.M. Mykhailoutenko *et al.* (2019) described pathomorphological changes in rabbits during the chronic course of passalurosis, i.e., with low II; Z.K. Terentyeva *et al.* (2021) described these changes for medium and high II, where the main structural changes were revealed only in the large intestine of rabbits.

According to the obtained data, catarrhal and catarrhal-haemorrhagic inflammation and swelling of the mucous membrane of the intestines with dotted or striped haemorrhages were observed in rabbits with a high degree of damage by passalurosis. J.R. Jenkins (2004) also noted that in the presence of up to 500 pinworms in the intestine there are no changes in the mucous membrane, in the presence of up to 1000 parasites – catarrhal inflammation develops, over 1000 passalurosis specimens – haemorrhagic colitis occurs. These data were confirmed by B. Lord (2012), who noted the death of rabbits from peritonitis and haemorrhagic colitis only at a high invasion intensiveness with pinworms. Similar pathomorphological changes in the intestines are described by Z.K. Terentyeva *et al.* (2021), who indicated serous-catarrhal and catarrhal-necrotic inflammations of the large intestine. Mesenteric lymph nodes are enlarged, hyperaemic, swollen. The mucous membranes of the caecum, rectum, and anus are hyperaemic, swollen, with point haemorrhages.

Therewith, according to the results of histological studies, it was established that the destruction of villi, crypt cells and infiltration by lymphocytes and monocytes, swelling of the submucosal base and muscular membrane, granular or hydropic dystrophy of the cells of the muscular capsule were found in the mucous membrane of the small intestine, caecum, and colon. The obtained data coincide with the scientific research

of M.N. Nermean *et al.* (2021), who identified similar pathomorphological changes, namely: *Passalurus ambiguus*, penetrating deep into the follicles, caused hyperplasia in the lymphoid tissue and cells of the follicular epithelium; granulomatous reaction was induced due to cellular damage and accumulation of chronic inflammatory oedema. Comparable results were also obtained by Szkucik *et al.* (2014), for chronic passalurosis, namely destruction of the epithelium of the large intestine, lymphohistiocytic infiltration and oedema of the submucosal base and muscle membrane.

According to M. Varga (2014), the changes in passalurosis of rabbits are associated with the tight pressing of nematodes to the mucous membrane between the intestinal villi, penetration into the Lieberkühn glands and strong mechanical irritation of the epithelial cells, as well as due to the effect on the mucous membrane caused by substances produced *P. ambiguus* (Vadlejch *et al.*, 2010; Duda *et al.*, 2019).

Thus, mechanical and toxic damage to the intestine by passalurosis with a high degree of damage led to catarrhal and catarrhal-haemorrhagic inflammation, swelling of the mucous membrane of the intestines with dotted or striped haemorrhages. In the mucous membrane of the small intestine, caecum, and colon, the destruction of villi, crypt cells and infiltration by lymphocytes and monocytes was detected, the submucosa base and the muscle membrane are strongly swollen, all smooth muscle cells of the muscle membrane are in a state of granular dystrophy.

## CONCLUSIONS

*Passalurosis ambiguus* infestation is one of the most common nematode diseases in rabbit farms of Ukraine, the infestation of rabbits by *Passalurus ambiguus* was 35.20%. Rabbits of private households in the Polissia zone of Ukraine were the most vulnerable in terms of passalurosis infestation, where 37.70-41.67% of sick animals were registered.

In terms of seasons, the highest infection rate for passalurosis of rabbits was detected in the winter (IE = 35.27%), the lowest – in the summer (25.79%). The peak of passalurosis infestation in rabbits was observed in January (35.29%), while in June, the invasion intensiveness is the lowest (21.43%). Passalurosis infestation was not detected among the rabbits at the age of 1-2 months. From the age of 2-3 months, its extensiveness increased. Rabbits aged 1 to 2 years had the highest incidence of pathogens *Passalurus ambiguus* (82.76%). The invasion intensity of passalurosis also increased and in animals aged 6-9 months was  $716.67 \pm 110.33$  eggs/g. In rabbits older than 9 months, II decreased and in animals aged 1-2 years it was  $479.31 \pm 91.96$  eggs in 1 g of faeces.

Mechanical and toxic damage to the intestine by passalurosis with a high degree of damage (II =  $2446.67 \pm 422.11$  eggs in 1 g of faeces) led to

a complex of pathomorphological changes: catarrhal and catarrhal-haemorrhagic inflammation, swelling of the mucous membrane of the intestines with dotted or streaked haemorrhages. Histological examination revealed the destruction of the villi of the mucous membranes of the small, caecum, and colon and cells of the crypts. In the lumen of the caecum and colon, a large amount of cellular detritus and relatively large granulo-loma-like accumulations of lymphocytes and monocytes

on the tops of single folds of the mucous membrane were found. In the submucous base, a strong oedema was established, and in the muscular shell – oedema, granular and hydropic dystrophy of smooth muscle cells. Some of the dystrophically changed cells were destroyed. In the future, it is planned to investigate the microscopic changes in other organs of rabbits with passalurosis with an elevated level of invasion intensiveness.

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### Сезонна та вікова динаміка пасалурозної інвазії кролів і патолого-гістологічні зміни за даного нематодозу

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**Анотація.** Найбільш поширеним нематодозом у кролів є пасалуроз, а зміна клімату в Україні за останні десятиліття призвела до зміни епізоотичного прояву даного паразитозу, що зумовлює актуальність вивчення цієї проблеми. Тому метою роботи було встановити епізоотичну ситуацію щодо пасалурозної інвазії та вивчити патолого-гістологічні зміни як у товстій, так і в тонкій кишках кролів за високого ступеню ураження *Passalurus ambiguus*. В роботі було використано метод МакМастера, удосконалений метод неповного гельмінтологічного розтину за К. І. Скрябіним. Найбільш неблагополучними щодо пасалурозної інвазії були виявлені приватні домогосподарства зони Полісся України, де реєстрували від 37,70 до 41,67 % хворих кролів. У сезонному аспекті найвищу зараженість тварин було встановлено взимку (екстенсивність інвазії (EI) становила 35,27 %), з піком у січні (EI = 35,29 %), найнижчу – влітку (EI = 25,79 %). Кролі віком від 1 до 2 років мали найвищу ураженість збудником *Passalurus ambiguus* (82,76 %). Було з'ясовано, що за високого ступеню ураження (інтенсивність інвазії (II) – 2446,67 ± 422,11 яєць в 1 г фекалій) кролів пасалурісами відмічено катаральне та катарально-гемарагічне запалення, набряклість слизової оболонки кишок з крапковими чи смугастими крововиливами та встановлено руйнування ворсинок слизової оболонки тонкої, сліпої й ободової кишок, клітин крипт і інфільтрацію лімфоцитами й моноцитами, сильний набряк підслизової основи та м'язової оболонки, стан зернистої дистрофії усіх гладких м'язових клітин м'язової оболонки. Виявлення сезонних, вікових та патолого-гістологічних змін у кролів за пасалурозу дозволить контролювати ступінь зараженості тварин та своєчасно вживати ефективні заходи боротьби, що зумовлює практичну цінність цієї роботи

**Ключові слова:** нематодоз кролів, *Passalurus ambiguus*, пасалуроз, екстенсивність та інтенсивність інвазії, гістологічні зміни

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## Influence of *Sitophilus Oryzae* on Biological Properties of *Mycobacterium Bovis*

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**Abstract.** The active spread of tuberculosis around the world has been an urgent issue for many decades, but even now, there is no data on all available methods of introducing the pathogen into a previously healthy territory and the mechanisms of mycobacteria activity, and this makes it impossible to eradicate the disease completely. The purpose of this study was to determine the effect of the rice weevil (lat. *Sitophilus oryzae*) on the dissociative forms of mycobacterium tuberculosis, their viability, biochemical properties and virulence through passage of the pathogen through the insect's body. To fulfil this purpose, the following methods were used: biological (experimental infection of laboratory animals), anatomical pathology and determining the viability of mycobacteria by counting colony-forming units. The effect on pathogenic properties of dissociative forms of *Mycobacterium bovis* (passage 118) after passage through the body of the rice weevil in an experiment on guinea pigs was determined and summarized. The change in the viability of mycobacteria isolated in the dynamics of the experiment in comparison with the original culture was described. The plasticity of the biochemical activity of the pathogen after passing through the body of the beetle was proven, and the influence of the macroorganism on the enzymatic properties of mycobacterium tuberculosis was analysed. The phenomena of the activation of enzymatic systems, which are associated with the adaptation and survival of mycobacteria in the conditions of a changed habitat, were substantiated. The practical value of this study lies in the improved understanding of the processes of the spread of mycobacterium tuberculosis and the established role of insects in the transmission of infection, with the prospect of developing measures to prevent and combat the disease

**Keywords:** mycobacteria, rice weevil, dissociative strain, biochemical properties, colony-forming units, variability



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## INTRODUCTION

The issue of the rapid spread of tuberculosis among animals and humans has been relevant for many years throughout the world, as well as in Ukraine. This disease poses a great epizootological threat and is accompanied by considerable economic and social losses (Pérez-Morote *et al.*, 2020). Two important problems are still the most understudied. The first is the development of anti-tuberculosis preparations to fight infection (Bihdan *et al.*, 2018; Gotsulya *et al.*, 2020; Hotsulia *et al.*, 2021). Considerable attention is paid to elucidating the antimicrobial effect of plant extracts and derivatives of 1,2,4-triazole-3-thiols (Palchykov *et al.*, 2019; Zazharskyi *et al.*, 2019; Zazharskyi *et al.*, 2020). The second is the question of determining all possible ways of the tuberculosis pathogen entering a previously safe territory, to interrupt the epizootological chain and prevent new foci of infection. The spread of mycobacterium tuberculosis is ensured by the exceptional resistance of mycobacteria to the action of physical and chemical factors due to the presence of lipids in the composition of the cell wall, their ability to change to adapt the microorganism in an unfavourable environment and to resist the protective forces of the macroorganism (Queiroz *et al.*, 2017). Changes in the morphology, biological and biochemical properties of *M. bovis* are not accidental, they are related to the maintenance of the viability of the microbe in the surrounding environment. It is proved that mycobacteria can change both genotypic and phenotypic traits. To survive, mycobacteria can change their morphological features, tincture, cultural properties, as well as virulence and enzymatic activity. A typical example of the variability of the causative agent of tuberculosis is the dissociation of mycobacteria. Dissociative forms of *M. bovis* are characterized by a change in the metabolic processes of the bacterial cell, which contributes to a wide range of survival of the microbe in the external environment (Tkachenko *et al.*, 2020).

As a result of adaptation to the conditions of existence, the pathogen has an expansive range of biological and mechanical vectors. *M. bovis* actively circulates in nature and maintains its viability due to the large number of potential reservoirs in nature. Crispell *et al.* (2019) described the possibility of both intraspecific and interspecific transmission of the pathogen. Some authors demonstrate that insects of various species can be a source of tuberculosis infection. Thus, Cano *et al.* (2018) indicate that Hemiptera insects are potential carriers of *Mycobacterium ulcerans*. Silva *et al.* (2022) found that Triatomine bugs are carriers of mycobacteria, namely species of *Mycobacterium tuberculosis complex*.

During the experimental infection of beetles of the rice weevil species (Latin *Sitophilus oryzae*) with *M. bovis* by the method of contamination of wheat with the pathogen's suspension, it was determined that the bacteria stay viable in the body of weevils until the 50<sup>th</sup> day, and are released by the beetles into the

environment until the 30<sup>th</sup> day after infection (Tkachenko *et al.*, 2021). However, the issue of changes in the biological properties of mycobacteria after passage through the body of beetles is understudied and requires further research. The expansion of knowledge in this area, namely on the change in the properties of the pathogen due to the replacement of the habitat, is important and explains the relevance of the subject under study.

*The purpose of this study* was to figure out the influence of the rice weevil *Sitophilus oryzae* on the biological and biochemical properties of dissociative forms of *M. bovis*, to investigate the mechanism of adaptation of mycobacteria in an unfamiliar environment. The tasks of the study: to establish changes in virulence, viability (by the method of counting the number of colony-forming units) and biochemical activity of mycobacterium after passage through the body of beetles in the dynamics of the experiment.

## MATERIALS AND METHODS

The research was conducted in the conditions of the educational laboratory of the Department of Infectious Diseases of Animals of the Dnipro State Agrarian and Economic University during 2020-2021. The subject of the research is guinea pigs and cultures of the dissociative form of *Mycobacterium bovis*: the initial culture of 118 passages (240<sup>th</sup> generation), which was stored at  $3.0 \pm 0.5^{\circ}\text{C}$  in the museum of the department, and cultures obtained after passage through the organism of the rice weevil (with beetle homogenate 30 days after infection; from the grain on which the insects were kept during the experiment after the second to fifth transplantation (on Days 8, 12, 20, and 30 of the experiment) to pre-sterilized grain). This paper is the second stage of the study. At the first stage, it was established that beetles can keep mycobacteria in their bodies for 50 days and release them, contaminating objects of the external environment for 30 days (the methodology and detailed results of the experiment can be found in the previous paper). To fulfil the purpose of this study, it was conducted in three stages:

**Stage I (biological research).** To determine the influence of beetles on the change in virulence of the dissociative form of *M. bovis*, the original culture of passage 118, cultures isolated from grain suspensions on Days 8, 12, 20, and 30, on which infected beetles were kept, were used, as well as the culture obtained from the homogenate of beetles on Day 30 of the experiment.

Guinea pigs were divided into 7 experimental groups and one control group (3 animals in each group). Animals of the Group I were infected with the original strain of mycobacteria, which was not exposed to the body of beetles; Group II – culture obtained from grain on Day 4 of the experiment, Group III – on Day 8, Group IV – on Day 12, Group V – on Day 20, Group VI – on Day 30, Group VII – culture obtained from the beetle

homogenate on Day 30 of the experiment. The control group stayed intact throughout the entire study period. Before infection, the animals were quarantined for 14 days, during which time they were subjected to an allergy test (tuberculinization) to rule out natural tuberculosis. Tuberculinization was carried out by injecting a process solution (25 IU in a volume of 0.1 cm<sup>3</sup>) of PPD-tuberculin for mammals intradermally, the reaction was recorded after 24 and 48 hours.

Guinea pigs were infected subcutaneously in the inner part of the thigh with a suspension of experimental mycobacteria in the amount of 1.0 mg of microbial mass per 1.0 cm<sup>3</sup> of isotonic 0.9% sodium chloride solution. During the experiment (90 days), the animals were clinically examined and weighed weekly, and on Days 30, 60, and 90, the sensitizing properties of dissociative forms of mycobacteria were determined before and after passage through beetles by tuberculinization. The result of the biological sample was evaluated by conducting an anatomical pathology, cultural, and microscopic examination. After the autopsy, pathological changes in the lungs, liver, spleen, and lymph nodes were determined. Virulence was assessed by the degree of damage to internal organs, which was expressed in points (M.V. Trius scheme). According to this scheme, specific lesions were expressed by pluses (“+” – single; “++” – several; “+++” – numerous nodules) followed by conversion into digital indicators (each “+” in lymph nodes equals 1; in the spleen – 2; in the liver – 3; in the lungs – 4), respectively, the maximum index of damage to the body is 30 points. In addition, a suspension was prepared from internal organs according to the method of A.P. Alikaeva. The resulting suspension was sown on Mordovsky (“Nove”) nutrient medium with a pH of 6.5 (an Adwa AD1030 pH meter was used to determine the pH value).

**Stage II (viability of mycobacteria).** At the second stage of the study, the viability of the microbial cells of the original culture of mycobacteria and cultures obtained after passage through the body of beetles was determined by evaluating colony-forming units (CFU) by conducting serial dilutions. To conduct the research, *Eppendorf*-type microtubes were used, in which sterile isotonic 0.9% sodium chloride solution was introduced in the amount of 0.5 cm<sup>3</sup> in the first microtube and 0.4 cm<sup>3</sup> in all subsequent microtubes. Test cultures were collected from the test tubes with a bacteriological loop, squeezed between two sheets of filter paper and weighed 50.0 mg (0.05 g) on a torsion balance. The weighted mass of mycobacteria was introduced into microtube No. 1 and suspended by thorough mixing with a bacterial loop. With an insulin syringe, 0.1 cm<sup>3</sup> of the obtained suspension was taken, after which it was introduced into microtube No. 2, mixed thoroughly, 0.1 cm<sup>3</sup> was taken again and transferred to the next test tube up to and including the tenth. Subsequently,

0.1 cm<sup>3</sup> of suspension was taken from each microtube with separate insulin syringes, which was placed in two tubes with Mordovsky nutrient medium and distributed on the surface. In the future, the test tubes were placed in a thermostat at +37 ± 0.5°C.

When determining the viability of microbial cells of the initial culture of dissociants and the culture obtained from grain on Day 8 of the experiment, the calculation was made in the eighth dilution, upon determining the number of CFU of the culture that grew from the sowing of homogenized beetles on Day 30 of the experiment and the culture obtained from grain on Day 12 of the experiment, counting was carried out in the seventh dilution. When determining the viability of cultures obtained from grain on Days 20 and 30 of the experiment, the calculation was made in the sixth dilution. These dilutions were chosen because at lower dilutions continuous growth was observed, and at higher dilutions colony growth was absent. After the occurrence of colony growth, the number of viable microorganisms in 1 gram of culture was calculated mathematically.

**Stage III (biochemical activity).** The initial culture of dissociants and cultures isolated after passage through beetles were examined for changes in biochemical activity. Catalase, peroxidase, dehydrogenase activity, the presence of nitrate reduction and the ability to hydrolyse Tween 80 were determined.

Catalase and peroxidase activity were determined simultaneously according to the modified method of Bogen (1981). A 2.0% solution of hydrogen peroxide and a 0.5% solution of pyrogallol A were added to the experimental cultures. Catalase activity was measured 15 and 30 minutes after the reaction was started. Reactions were assessed by visual inspection based on the turbulent flow and release of the number of oxygen bubbles: (+++) – considerable release of bubbles; (++) – moderate; (+) – single; (-) – no bubbles are released. Peroxidase activity of dissociants was determined after 1.5-2 hours by evaluating the above reaction. The calculation was carried out visually, determining the formation of brown pigment in the experimental colonies as a result of the transformation of pyrogallol due to the action of the peroxidase enzyme into purpurogallin in the presence of hydrogen peroxide: (+++) – dark brown colouration of the colonies; (++) – brown; (+) – pale brown; (-) – the colour does not change.

Dehydrogenase activity was established in agglutinating tubes. For this, 4.0 cm<sup>3</sup> of a suspension of microbial cells with a concentration of 10.0 mg/cm<sup>3</sup> in a phosphate buffer of pH 7.4-7.6 was mixed with 1.0 cm<sup>3</sup> of a 1.0% glucose solution and 0.1 cm<sup>3</sup> of 0.02% solution of methylene blue. Sterile vaseline oil was layered on the contents obtained in the test tube. The test tubes were incubated in a thermostat at +38 ± 0.5°C and the time of dye discolouration was monitored. The

result of the reaction was evaluated after 15-30 minutes and 24 hours. Test tubes with mycobacterial suspension and methylene blue without glucose served as controls.

At the next stage, the nitrate reductase activity of mycobacteria was evaluated. The presence of nitrate reduction was determined as follows: 10.0 mg of the wet biological mass of the experimental culture was weighed on a torsion balance and placed in a test tube containing 1.0 cm<sup>3</sup> of 0.067 M phosphate buffer in (pH .1) with 0.1% – with sodium nitrate solution. After suspension, the cultures were seasoned for 20-22 hours at +37.0 ± 0.5°C. Nitrate formation was checked by adding two drops of a 2.0% solution of paradimethylaminobenzaldehyde (P-dimethylaminobenzaldehyde) to a 1.0% solution of hydrochloric acid in a test tube.

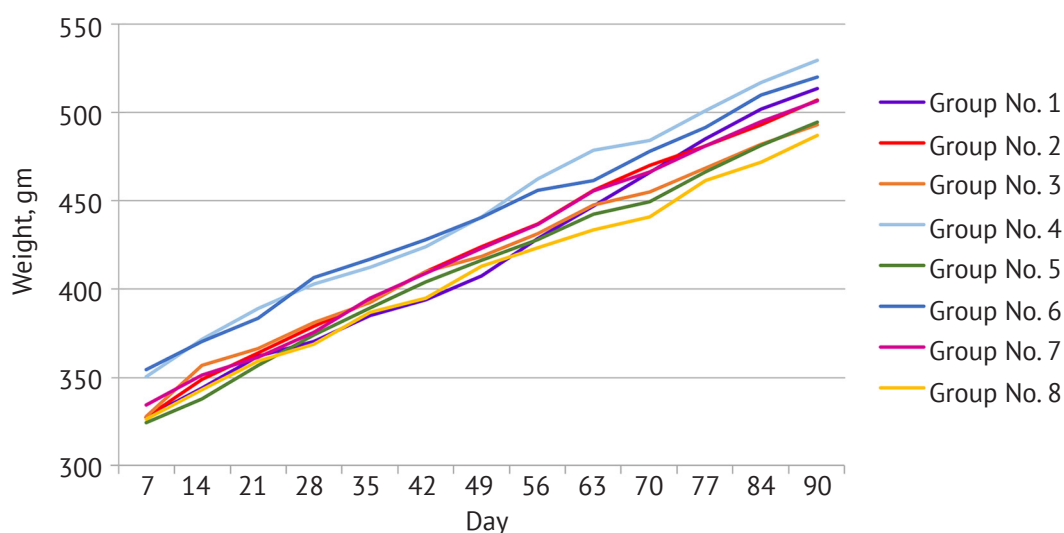
The hydrolysis reaction of Tween 80 was determined by the modified method of Wein. The following reagents were used for the reaction: 1/15 M phosphate buffer (pH 7.0) – 100.0 cm<sup>3</sup>, Tween 80 – 0.5 cm<sup>3</sup>, basic red 0.1% – 2.0 cm<sup>3</sup>. All reagents were mixed and poured into 4.0 cm<sup>3</sup> tubes and autoclaved for 15 minutes at +120.0±0.5°C. Subsequently, the test tubes were placed in a thermostat for 24 hours to check for sterility. Three bacteriological loops of each experimental culture were emulsified in test tubes with a substrate and incubated in a thermostat for 10 days. The reaction was recorded after 4 hours, on Days 5 and 10. The result

was considered positive if a pink or red colour appeared within 10 days, with a negative result, the colour did not appear. Data in the tables are presented as  $x \pm SD$  (standard deviation).

When working with animals, the provisions of Article 26 of the Law of Ukraine No. 5456-VI “On the Protection of Animals from Cruelty” dated 10/16/2012 (Law of Ukraine No. 3447-IV), “General Ethical Principles of Animal Experiments”, approved on The First National Congress on Bioethics (Reznikov, 2003), the requirements of the European Convention “On the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes” (European convention, 1986), the Declaration “On the Humane Treatment of Animals” (Universal Declaration, 2007).

## RESULTS AND DISCUSSION

The introduction of a suspension of experimental mycobacteria to guinea pigs did not cause clinical and pathological signs inherent in the course of tuberculosis in any of the experimental groups, an ulcer was not formed at the place of introduction of the suspension. During the experiment, the animals were active and gained weight (Fig. 1). When studying the sensitization of experimental cultures, it was established that guinea pigs do not respond to the administration of PPD-tuberculin in any of the experimental groups.



**Figure 1.** Dynamics of changes in body weight of experimental guinea pigs during the experiment

**Source:** compiled by the authors

3 months after infection, the animals were euthanized, and an anatomical pathology study was performed. In the internal organs (lungs, liver, spleen) and lymph nodes, no changes inherent in tuberculosis were detected. The index of damage to the body of experimental guinea pigs of all groups is 0 points. When inoculating the suspension from organs and lymph nodes on Mordovsky (“Nove”) nutrient medium, the growth of smooth, rounded, even-edged orange colonies was observed, which were

morphologically identical to the dissociative forms of *M. bovis* (passage 118) for 8-10 days from the organs of guinea pigs of I-IV and VII experimental groups. On Day 12, no growth was detected from the organs of guinea pigs of groups V-VI in test tubes from the control group of animals.

At the next stage of the study, the viability of microbial cells was established by calculating CFU. It was found that the number of colonies that grew from the original culture during the eighth dilution was

338.0 ± 29.7, and from the culture obtained on Day 4 of the experiment from the grain suspension – 321.0 ± 9.9; on Day 8 – 293.0 ± 15.6 colonies in 6.4×10<sup>-5</sup> mg of microbial mass, i.e., in 1 gram of culture 528125000.0≈5.3×10<sup>8</sup>, 501562500.0≈5.0×10<sup>8</sup> and 457812500.0≈4.6×10<sup>8</sup> viable microorganisms, respectively.

Viable mycobacteria in the culture obtained from the grain suspension on Day 12 of the experiment and the culture from the homogenate of beetles on Day 30 was calculated during the seventh dilution. It was found

that the number of colonies was equal to 350.0 ± 43.8 and 442.0 ± 32.5 in 3.2×10<sup>-4</sup> mg of bacterial mass, respectively. That is, 109375000.0≈1.1×10<sup>8</sup> and 138125000.0≈1.4×10<sup>8</sup> bacteria in 1 g of culture.

The CFU of the cultures obtained from the grain suspension Days 20 and 30 was calculated in the sixth dilution. It was found that 1.6×10<sup>-3</sup> mg of microbial mass contains 237.0 ± 39.6 and 209.0 ± 24.0 microbial units, i.e., 14812500.0≈1.5×10<sup>7</sup> and 13062500.0≈1.3×10<sup>7</sup> microbial units in 1 g of culture (Table 1).

**Table 1.** Evaluation of colony-forming units of the dissociative form of *Mycobacterium bovis* (118 passages) in the dynamics of the experiment

|   | Number of grown colonies on the nutrient medium |      |      |      |      |      |      |      |      |      |      | M ± m | The amount of CFU in 1 g of bacterial mass |                     |
|---|---|------|------|------|------|------|------|------|------|------|------|-------|--|---------------------|
|   | Dilution multiplicity                           |      |      |      |      |      |      |      |      |      |      |       |  |                     |
|   | I   | II   | III  | IV   | V    | VI   | VII  | VIII | IX   | X    |      |       |  |                     |
| Source culture (118 passage)  | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 317  | n.g. | n.g.  | 338.0 ± 29.7                               | 5.3*10 <sup>8</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 359  | n.g. | n.g.  |  |                     |
| Culture obtained from grain on Day 4 of the experiment                | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 314  | n.g. | n.g.  | 321.0 ± 9.9                                | 5.0*10 <sup>8</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 328  | n.g. | n.g.  |  |                     |
| Culture obtained from grain on Day 8 of the experiment                | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 282  | n.g. | n.g.  | 293.0 ± 15.6                               | 4.6*10 <sup>8</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 304  | n.g. | n.g.  |  |                     |
| Culture obtained from grain on Day 12 of the experiment               | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 319  | n.g. | n.g. | n.g.  | 350.0 ± 43.8                               | 1.1*10 <sup>8</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 381  | n.g. | n.g. | n.g.  |  |                     |
| Culture obtained from grain on Day 20 of the experiment               | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 209  | n.g. | n.g. | n.g. | n.g.  | 237.0 ± 39.6                               | 1.5*10 <sup>7</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 265  | n.g. | n.g. | n.g. | n.g.  |  |                     |
| Culture obtained from grain on Day 30 of the experiment               | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 192  | n.g. | n.g. | n.g. | n.g.  | 209.0 ± 24.0                               | 1.3*10 <sup>7</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 226  | n.g. | n.g. | n.g. | n.g.  |  |                     |
| Culture obtained from homogenized beetles on Day 30 of the experiment | Sample No. 1                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 419  | n.g. | n.g. | n.g.  | 442.0 ± 32.5                               | 1.4*10 <sup>8</sup> |
|   | Sample No. 2                                    | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | c.g. | 465  | n.g. | n.g. | n.g.  |  |                     |

**Source:** c.g. – continuous growth of colonies on nutrient medium; n.g. – no growth

It was found that beetles can reserve and secrete viable mycobacteria and contaminate objects in the environment. At the same time, the authors observed a tendency to decrease the viability of the pathogen isolated by weevils over time. The number of colony-forming units in cultures isolated from grain on Day 4 of the experiment decreased by 5.03% compared to the initial strain, on Day 8 – by 13.32%, on Day 12 – by 79.29%, on

Day 20 – by 97.20%, on Day 30 – by 97.53%, respectively. The viability of *M. bovis* in the body of beetles for 30 days (in the homogenate) decreased by 73.85% from the initial culture. This may indicate that the body of the beetle is a favourable environment for the reservation of mycobacteria, and therefore rice weevils may contribute to the activation of the epizootic process of tuberculosis and mycobacterial infections of animals. As a result

of conducting a biochemical study, it was established that indicators of the enzymatic activity of mycobacteria are variable. Passage of dissociative forms of *M. bovis* through

the body of the rice weevil led to an increase in the activity of the following enzymes: dehydrogenase, nitrate reductase, and the ability to hydrolyse Tween 80 (Table 2).

**Table 2.** Biochemical activity of the dissociative form of *M. bovis* (118 passages) in the dynamics of the experiment ( $n = 3$ )

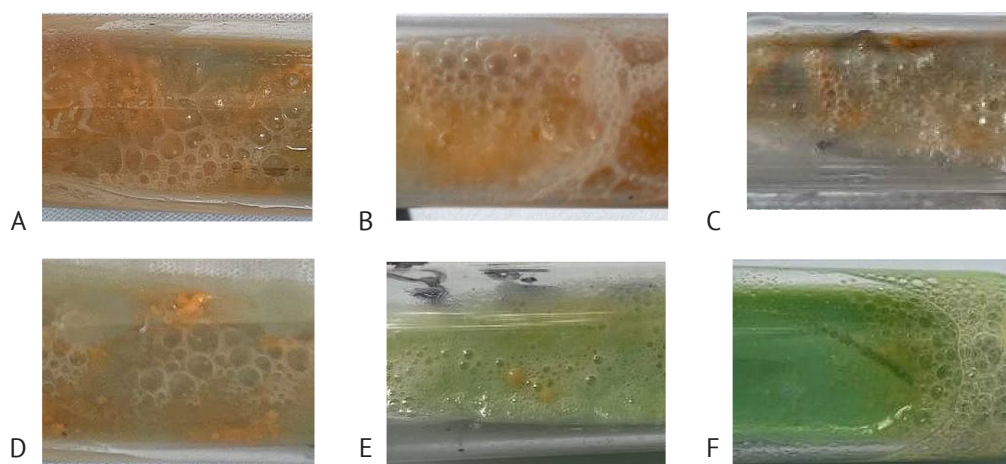
|   | Catalase activity |        | Peroxidase activity 2 h | Dehydrogenase activity |      | Nitrate reductase activity | Hydrolysis of Tween 80 |        |         |
|---|-------------------|--------|-------------------------|------------------------|------|----------------------------|------------------------|--------|---------|
|   | 15 min            | 30 min |                         | 15-30 min              | 24 h |                            | 4 h                    | 5 days | 10 days |
| Source culture (118 passage)  | ++                | ++     | -                       | -                      | -    | ±                          | -                      | -      | +       |
| Culture obtained from grain on Day 4 of the experiment                | ++                | ++     | -                       | -                      | -    | ±                          | -                      | -      | +       |
| Culture obtained from grain on Day 8 of the experiment                | ++                | ++     | -                       | -                      | ±    | +                          | -                      | -      | +       |
| Culture obtained from grain on Day 12 of the experiment               | ++                | ++     | -                       | -                      | ±    | +                          | -                      | -      | +       |
| Culture obtained from grain on Day 20 of the experiment               | ++                | ++     | -                       | -                      | ±    | +                          | -                      | -      | +       |
| Culture obtained from grain on Day 30 of the experiment               | ++                | ++     | -                       | -                      | ±    | +                          | -                      | -      | +       |
| Culture obtained from homogenized beetles on Day 30 of the experiment | ++                | ++     | -                       | -                      | ±    | +                          | -                      | -      | +       |

**Note:** + – positive reaction; - – negative reaction; ± – doubtful reaction

**Source:** compiled by the authors

The principle of the catalase reaction lies in the splitting of hydrogen peroxide under the action of the catalase enzyme into water and atomic oxygen, which is accompanied by the release of oxygen bubbles and the

transition of pyrogallol to purpurogallin in the presence of hydrogen peroxide under peroxidase. All cultures under study had moderate catalase activity (Fig. 2). Peroxidase activity was negative in all experimental samples.

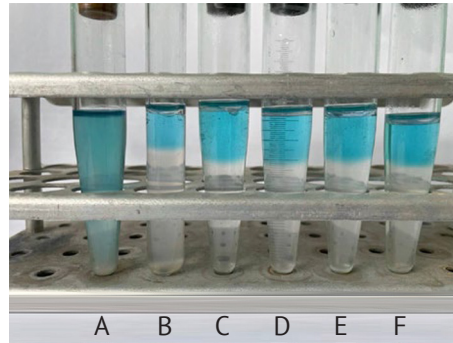


**Figure 2.** Calculation of catalase activity of experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, E – on Day 30, F – from homogenate of beetles on Day 30

**Source:** photographed by the authors

When studying the activity of dehydrogenase, no discoloration of the methylene blue solution was found in any of the cultures after 15-30 minutes. The dehydrogenase activity of the original culture and the culture obtained from grain on Day 4 after 24 hours

was also negative. However, cultures obtained from the homogenate of weevils on Day 30 of the experiment and from the beetle-contaminated grain on Days 8, 12, 20, and 30 of the experiment partially decolorized the solution in 24 hours (Fig. 3).

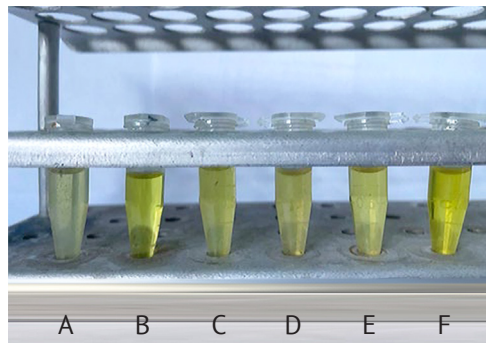


**Figure 3.** Calculation of dehydrogenase activity of experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, G – on Day 30, D – from homogenate of beetles on Day 30

**Source:** photographed by the authors

Nitrate reductase activity of mycobacteria lies in accounting for the reduction of nitrite from nitrate by visual observation of the colour reaction with P-dimethylaminobenzaldehyde, with a positive reaction, a yellow colour is formed, with a negative reaction, the colour of

the solution does not change. Investigating the activity of nitrate reduction, it was established that the result was questionable in the original culture and the culture obtained from grain on Day 4, while all other experimental cultures acquired the ability to reduce nitrates (Fig. 4).



**Figure 4.** Calculation of nitrate reductase activity of experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, G – on Day 30, D – from homogenate of beetles on Day 30

**Source:** photographed by the authors

The result of Tween 80 hydrolysis in all experimental cultures was negative after 4 hours and after 5 days. However, on Day 10 of observation, a positive

result (the formation of a pink-red colour) was found in all the samples under study (Fig. 5).



**Figure 5.** Calculation of hydrolysis reaction of Tween 80 in experimental cultures: A – culture obtained from grain on Day 4, B – on Day 8, C – on Day 12, D – on Day 20, E – on Day 30, F – from homogenate of beetles on Day 30

**Source:** photographed by the authors

Thus, the research results demonstrate that the passage of mycobacteria through the body of the rice weevil leads to certain changes in the biochemical properties of the bacterial cell. However, passage through insects does not lead to restoration of virulent properties of dissociative forms of the pathogen. Therewith, the number of viable cells in the mycobacteria isolated from the homogenate of beetles and grain contaminated by them decreases but stays sufficient to infect laboratory animals.

This study determined the effect of the rice weevil on the viability, pathogenicity, and biochemical properties of a dissociative strain of *M. bovis*. The obtained results demonstrate that the dissociative forms of *M. bovis* are non-pathogenic for guinea pigs. The animals did not develop an ulcer at the place of introduction of the suspension of the original strain of the pathogen and the cultures obtained as a result of passage through the body of the rice weevil. Allergic reaction to the introduction of PPD-tuberculin for mammals and anatomical pathology changes were not detected. The results of the experiment show that dissociants do not restore virulent properties after passing through a living organism, which indicates a persistent loss of pathogenicity of the original strain. However, despite the absence of visible clinical and anatomical pathology changes in guinea pigs, it was possible to isolate mycobacteria from their internal organs. The obtained data do not contradict the study of Tkachenko *et al.* (2020), which describes the absence of an infectious process in laboratory animals infected with dissociative forms of *M. bovis* after long-term storage. As is known, mycobacteria that have lost their virulent properties can survive and replicate in the macroorganism without causing immunopathological processes.

Scientific works of researchers have proven that mycobacteria can change to adapt to their surrounding environment and, accordingly, have a large number of reservoirs and factors of disease transmission (Bañuls *et al.*, 2015). Mycobacteria have high adaptive properties and can survive in adverse conditions (for instance, in conditions of starvation) without showing growth for up to two years with the ability to fully recover. When studying the viability of microbial cells after passing through the weevil, it was determined that mycobacteria stay viable in the beetle's body for a long time, and therefore it is a favourable environment for the pathogen to be reserved and can potentially contribute to the activation of the epizootic process of tuberculosis and mycobacterial infections of animals. Mycobacteria, due to the structure of the cell wall, are resistant to the action of gastric enzymes of insects and can be released by them with saliva and excrement. In addition, existing data suggest that infected insects can be captured by favourable animals. In their gastrointestinal tract, due to the action of gastric juice, insects are digested, but the acid-fast pathogen stays viable and can persist in

the macroorganism or be released into the environment and contaminate it. Fischer *et al.* (2003) conducted an experimental oral infection of *Blatta orientalis* cockroach nymphs with mycobacterial suspension and found that the pathogen accumulated in the insect's body and was released into the environment. The researchers isolated viable mycobacteria in cockroach excrement 3 days after infection, and in cockroach homogenate after 10 days, which also indicates the ability to reserve the pathogen for a long time.

Indicators of the enzymatic activity of mycobacteria are extremely important. Enzymes take part in ensuring the energy metabolism of the microbial cell and provide the protective function of the microbe and contribute to its adaptation to environmental factors (Arora *et al.*, 2018). As a result of conducting a biochemical study, it was established that indicators of the enzymatic activity of mycobacteria are variable. Li *et al.* (1998) and Manca *et al.* (1998) claim that catalase activity contributes to the resistance and survival of microorganisms and suggest that this enzyme is a factor in the virulence of mycobacteria. However, this study did not confirm this assumption.

Dehydrogenase activity is based on the detection of the redox enzyme dehydrogenase and metabolic products. This enzyme is responsible for the protection of the bacterial cell and adaptation in the conditions of the macroorganism, takes part in ensuring the antioxidant function of the microbe. The results confirm the existing data, the activity of dehydrogenase increased after the contact of mycobacteria with beetles. Comparable studies are demonstrated by other scientific papers indicating an increase in the activity of this enzyme after passing through a living organism (Tkachenko *et al.*, 2021).

Fritz (2002) states on the example of *M. bovis* BCG that nitrates are important for the metabolism of the microbial cell and take part in providing it with energy; moreover, they can maintain metabolism even without oxygen. The results of research by a number of scientists claim that the activity of nitrate reductase is partially responsible for the survival of mycobacteria in host cells (Hayashi *et al.*, 2010). The obtained results, according to which non-pathogenic dissociative forms of mycobacteria can stably reduce nitrates, do not contradict the studies of Sohaskey & Modesti. (2009), who claim that only the virulent strain of *M. bovis* has weak nitrate reductase activity.

Considering the opposite of the existing data, it can be concluded that relying solely on the ability of mycobacteria to reduce nitrates, it is not possible to unambiguously characterize the pathogenicity of the strain. It can be assumed that the activity of this enzyme is not directly related to the virulence of the pathogen. The ability of mycobacteria to hydrolyse Tween 80 is provided by mycobacterial phospholipase A, which catalyses lipid hydrolysis. The essence of the reaction is that Tween 80 binds neutral red and before the reaction the experimental solution has a straw-yellow colour, but

due to the release of neutral red, the colour is restored from pink to red (with a positive reaction). According to the assumption of Deb *et al.* (2009), this activity can contribute to the survival of the bacterial cell in conditions of starvation and be used by the pathogen as a carbon source.

The obtained data suggest that changes in the biochemical parameters of mycobacteria occur as a result of increased survival and adaptation of the microbe to new environmental conditions. Such assumptions do not contradict other studies on the high degree of variability of biological properties of *M. bovis*, including biochemical activity. It is believed that mycobacteria in unfavourable conditions of existence can maintain their viability for more than 650 days. At the same time, a decrease in the viability of the pathogen (decrease in CFU) is observed. In this study, against the background of a decrease in the number of CFUs in the microbial cell, the mechanisms of adaptation to the change in habitat are activated, the activity of redox enzymes increases, and the microbial cell is supplied with energy by increasing the reduction of nitrates.

Therefore, analysing the results of this study and the research of other scientists, it can be stated that the change in the properties of mycobacteria is related to the adaptation and survival of the microbial cell in a new habitat.

## CONCLUSIONS

Summarizing the obtained results, it becomes clear that mycobacteria can change the properties of the bacterial cell to adapt to existence in changed conditions. The biochemical activity of the pathogen undergoes primary changes, and this, in turn, may indicate that synthetase

systems, namely enzyme systems, are mainly responsible for the survival of the microbe. Enzymatic activity of dissociative forms of mycobacteria is variable. Cultures obtained after passage through the body of beetles acquire the ability to decolorize methylene blue solution in 24 hours (dehydrogenase activity) and have higher nitrate reductase activity compared to the original strain. Changes in the enzymatic activity of cultures after passing through the body of the rice weevil are associated with ensuring the adaptation of the microbial cell in a new habitat.

Analyzing the pathogenic properties of the pathogen, it can be stated that mycobacteria have a stable genome. Dissociative forms of *M. bovis* (118 passage, 240 generation) do not restore virulent properties after passage through the body of beetles, which indicates persistent changes in mycobacteria towards reduced pathogenicity. Rice weevils are incapable of increasing virulence. At the same time, it can be noted that dissociative forms of *M. bovis* have a stable genotype with loss of pathogenicity.

Estimating the number of CFU dissociants, we observe a tendentious loss of viability in the dynamics of the experiment. As a result of passage through the organism of the rice weevil, the number of viable mycobacteria decreased by 5.03-97.53% in grain contaminated with beetles during Days 8-30 after infection compared to the original strain, and by 73.85% in the homogenate from beetles after 30 days from the moment of infection. Prospects for further research are to improve disinsection measures and develop ways to interrupt the epizootic chain and prevent infected insects from entering the safe territory.

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## Вплив *Sitophilus oryzae* на біологічні властивості *Mycobacterium bovis*

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**Анотація.** Активне поширення туберкульозу по всьому світу є актуальною проблемою протягом багатьох десятиліть, але й дотепер немає даних щодо всіх наявних методів занесення збудника на раніше благополучну територію та механізмів діяльності мікобактерій, а це, в свою чергу, унеможливорює повне викорінення хвороби. Метою даної роботи було визначення впливу рисового довгоносика (лат. *Sitophilus oryzae*) на дисоціативні форми мікобактерій туберкульозу, їх життєздатність, біохімічні властивості та вірулентність шляхом пасажування збудника через організм комах. Для досягнення мети були застосовані наступні методи дослідження: біологічні (експериментальне інфікування лабораторних тварин), патологоанатомічні та визначення життєздатності мікобактерій методом підрахунку колонієутворюючих одиниць. Було визначено та узагальнено вплив на патогенні властивості дисоціативних форм *Mycobacterium bovis* (118-й пасаж) після пасажування через організм рисового довгоносика в експерименті на морських свинках. Описано тенденційну зміну життєздатності мікобактерій виділених у динаміці дослідів порівнянні з вихідною культурою. Доведено пластичність біохімічної активності збудника після проходження через організм жука та проаналізовано вплив макроорганізму на ферментативні властивості мікобактерій туберкульозу. Обґрунтовано явища активізації ферментативних систем, які пов'язані з адаптацією та виживанням мікобактерій в умовах зміненого середовища існування. Практична цінність роботи полягає в удосконаленні розуміння процесів поширення мікобактерій туберкульозу та встановленні ролі комах у передачі інфекції з перспективою розробки заходів профілактики та боротьби з захворюванням

**Ключові слова:** мікобактерії, рисовий довгоносик, дисоціативний штам, біохімічні властивості, колонієутворюючі одиниці, мінливість



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## Identification of the Bioaerosol Microbiota in Veterinary Clinics as the Key to Preventing Nosocomial Infection

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**Abstract.** Nosocomial infections are important in veterinary medicine clinics, as they can contaminate surfaces and infect animals through bioaerosol and are the cause of ineffective antibiotic therapy. This paper presents a study of the effect of disinfection on the microbiota of bioaerozoic veterinary clinics. The purpose of this study was to establish the composition of the bioaerosol microbiota in veterinary clinic premises before and after disinfection with ultraviolet bactericidal lamps. Bioaerosol samples were taken in the premises of veterinary clinics by sedimentation method. Identification of the isolated microbiota was performed using classical methods involving commercial test systems for identifying microorganisms. It was established that the permanent microbiota of the bioaerosol of veterinary clinics includes the following representatives of gram-positive genera: *Staphylococcus* (coagulase-negative species), *Streptococcus spp.*, *Micrococcus spp.*, and *Corynebacterium spp.* These bacterial genera were present in the bioaerosol of all rooms in 100% of cases. Gram-negative species of bacteria were found in small quantities in the bioaerosol of such rooms as for the primary examination and the manipulation area with boxes for keeping sick animals. Representatives of gram-negative species were detected in a considerably larger number from the bioaerosol of the dental operating room during the day of the clinic. After disinfection with bactericidal lamps, pathogens of nosocomial infections (*S. aureus*, *S. pseudintermedius*, *Acinetobacter baumani*, *P. aeruginosa*) were released in the bioaerosol of such rooms as the primary examination, the manipulation area with boxes for keeping sick animals, and the dental operating room. This indicates that bioaerosol can serve as a medium for the spread of nosocomial infections among animals in veterinary clinics. Thus, conducting microbiological monitoring of bioaerosol in veterinary clinics will allow identifying pathogens of nosocomial infections and introducing preventive measures for the spread of pathogens

**Keywords:** air microflora, disinfection with ultraviolet lamps, nosocomial pathogens, pathogenic bacteria



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## INTRODUCTION

The control of nosocomial infection in medical and veterinary institutions is crucial in the system of preventing the spread of antibiotic-resistant strains among patients and in the environment. Closed premises of veterinary clinics often create conditions for the circulation and transmission of nosocomial infection via airborne bioaerosol (Jeong *et al.*, 2022; Mocherniuk *et al.*, 2022; Zheng *et al.*, 2023). Nosocomial infection is considered a common cause of complications of the underlying disease among companion animals in veterinary clinics and quite often leads to mortality (Elnageh *et al.*, 2020). In addition, the infection of animals with nosocomial pathogens leads to a long stay in clinics, the use of a considerable range of antimicrobial preparations and the formation of pathogens with multi-resistance to antibiotics (Morgado-Gamero *et al.*, 2021). Therefore, it is important that the microflora circulating in the premises of veterinary clinics does not negatively impact the health of patients, especially those who have undergone surgery and are weakened (Sellera *et al.*, 2021). Therefore, microbiological monitoring of bioaerosol in veterinary clinics can provide information about the spread of nosocomial pathogens, transmission routes, and the state of anti-epidemic measures.

Microorganisms can spread indoors by air-droplet, contact, and transfer by organisms (fleas, ticks, other small animals) (Loncaric *et al.*, 2019). Transmission of an infection requires three elements: a source of pathogens, a susceptible organism, and a route of transmission (Berhilevych *et al.*, 2021; Kisera *et al.*, 2021). Such conditions are created in veterinary clinics, since bioaerosols are air pollutants. Bioaerosol is a collection of dead and living pathogenic and non-pathogenic microorganisms, epithelial cells, wool, hair, particles of plant origin, etc., which are suspended in the air (Tsay *et al.*, 2020). Exposure to bioaerosol from the premises of veterinary clinics poses a significant threat to the health of veterinary staff and patient animals, as it can transmit pathogens of airborne infections, acute toxic reactions, and allergies, according to (Chen *et al.*, 2022). Therefore, nosocomial pathogens can contaminate instruments, implants, equipment, environmental objects, and veterinary personnel by airborne droplets. In this regard, the airborne route of transmission of infections is an important one that needs to be addressed (Fahlgren *et al.*, 2010; Chai *et al.*, 2021).

Researchers (Horiuk *et al.*, 2020; Chueahiran *et al.*, 2021; Tamakan & Gocmen, 2022) state that bacteria inhabiting the mucous membranes of the nasal and oral cavity and the skin can be easily transmitted by airborne droplets, especially such dangerous bacteria as methicillin-resistant *Staphylococcus aureus* (*MRSA*). *MRSA* is one of the main organisms causing infections in hospitalized animal patients in veterinary clinics or animal holding boxes (Elnageh *et al.*, 2020). *MRSA* isolated from companion animals (dogs, cats) cannot be distinguished from *MRSA* isolated from different biotopes of

sick people. In addition, the authors (Chai *et al.*, 2021; Lee & Yoo, 2022) indicate that, apart from airborne transmission, veterinary staff in hospitals are also at risk for *MRSA* colonization and spreading this infection to other people. Researchers (Song *et al.*, 2021; Hamido *et al.*, 2022; Naziri *et al.*, 2022) indicate that microorganisms such as *S. aureus*, *Escherichia* spp., *Klebsiella* spp., *Salmonella* spp., *Serratia marcescens*, *Clostridium difficile* and *Acinetobacter baumannii*, were identified as pathogens of nosocomial infections in hospitalized dogs and cats. Specifically, they caused skin diseases, inflammation of the ears, diseases of the urinary system, and complicated the healing of wounds of various aetiologies.

Thus, the main pathogens of nosocomial infections of small animals are known and there are certain general recommendations for the prevention and control of nosocomial pathogens. However, the study of bioaerosol microflora of various objects in veterinary clinics will provide a better insight into the sources of infections, ways of transmission, and improve preventive measures for the spread of nosocomial pathogens, both among animals and veterinary personnel.

*The purpose of this study* was to determine the species composition of bioaerosol microbiota in different premises of veterinary clinics before and after disinfection using ultraviolet bactericidal lamps and to figure out the possible role of bioaerosol in the transmission of nosocomial pathogens.

## MATERIALS AND METHODS

The study was conducted during 2021-2022 in veterinary medicine clinics (n = 3) in Chernivtsi and Kolomyia (Ukraine). 504 samples of bioaerosol from the premises of veterinary clinics were investigated to determine the effect of disinfection with bactericidal lamps on the amount of residual microflora. Sanitation in these clinics included daily evening wet cleaning of rooms with bactericidal lamps and once every three days disinfection with a disinfectant with active substances: N-(3-aminopropyl)-N-dodecylpropane-1,3-diamine and N,N-didecyl-N,N-dimethylammonium chloride. Bactericidal lamps (15 W) were used for bioaerosol disinfection of premises, the duration of irradiation was 1 hour.

Bioaerosol (air) samples were taken in the premises of veterinary clinics by sedimentation method. For this, open Petri dishes with blood agar and selective media were placed in envelopes (four samples at the corners and the fifth in the centre) at 0.5 m from the wall and at a height of 1.6 m by 30 minutes, while the windows and doors in the rooms were closed. After 30 minutes of exposure, the dishes were closed, placed in a cooler bag, and delivered to the laboratory within 2 hours.

To isolate microorganisms, inoculation was carried out on the following media. Specifically, staphylococci and micrococci were isolated on blood agar containing 5% sodium chloride, enterococci – on *Bile*

*Esculin Azide Agar*, streptococci and corynebacteria – on *Streptococcus Selective Agar* and blood agar. Mushrooms were isolated on the Saburo medium. Enterobacteria (*Escherichia*, *Enterobacter*, *Citrobacter*, *Klebsiella*, etc.) were grown on Endo, Ploskirev, and Levin media. *Pseudomonas* were isolated on a medium containing acetamide, other non-fermenting bacteria (*Acinetobacter* spp. and *Alcaligenes* spp.) – on MPA, with incubation at 20°C for 3 days. Cultures were incubated in a thermostat at 37 ± 1°C for 24-48 hours to isolate mesophilic microorganisms, and fungi at +28 ± 1°C for 5 days. Isolated cultures were identified according to morphological, tinctorial, cultural, biochemical properties and signs of pathogenicity, which are described in Bergey's Manual of Systematic Bacteriology (Vos et al., 2011).

Statistical processing was performed by analysis of variance using Fischer criteria (ANOVA). Data are presented as x±SD (mean±standard deviation). The significance of the obtained data was assessed by the F-criterion with a confidence level of P<0.05, P<0.01, P<0.001 (considering the Bonferroni correction).

## RESULTS AND DISCUSSION

Earlier studies (Mocherniuk et al., 2022) found that the quantitative content of mesophilic bacteria in the bioaerosol of the premises of veterinary clinics increases during the working day. Furthermore, there is a significant difference in the number of bacteria in different rooms of the clinic, which depends on the intensity of operation of the rooms and the therapeutic and preventive manipulations that are carried out in them. It was also found that microbial contamination of bioaerosol increases considerably in winter, which can cause the spread of nosocomial pathogens among patient animals that are overexposed around the clock. Therefore, to establish the most common types of bacteria in the bioaerosol of the premises of veterinary clinics, the isolated microbiota were identified. Therewith, bioaerosol samples were taken in the middle of the working day and after sanitary measures were carried out in the evening, as described above. The results of identification of isolated microorganisms before disinfection are presented in Table 1.

**Table 1.** Identification of bioaerosol microbiota of the premises of veterinary clinics (n = 3) selected during the working day, %

| Microorganisms              | Frequency of m/o release from indoor bioaerosol: |                         |                    |   |                               |  |                                    |
|-----------------------------|--|-------------------------|--------------------|---|-------------------------------|--|------------------------------------|
|                             | room for initial inspection of animals, n = 36   | Ultrasound room, n = 36 | X-ray room, n = 36 | manipulation zone with boxes for keeping sick animals, n = 36 | dental operating room, n = 36 | operating room for soft tissue surgery, n = 36 | orthopaedic operating room, n = 36 |
| <b>Gram-positive:</b>       |  |                         |                    |   |                               |  |                                    |
| CoNS                        | 100  | 100                     | 100                | 100   | 100                           | 100  | 100                                |
| CoPS                        | 16.3***  | 5.4***                  | 2.7***             | 19.4***   | 27.7***                       | 0***   | 0***                               |
| <i>Streptococcus</i> spp.   | 100  | 100                     | 100                | 100   | 100                           | 100  | 100                                |
| <i>Micrococcus</i> spp.     | 100  | 100                     | 100                | 100   | 100                           | 100  | 100                                |
| <i>Corynebacterium</i> spp. | 100  | 100                     | 100                | 100   | 100                           | 100  | 100                                |
| <i>Enterococcus</i> spp.    | 11.1   | 2.7                     | 0                  | 13.8  | 16.6*                         | 0  | 0                                  |
| <i>Bacillus</i> spp.        | 27.7   | 5.4                     | 5.4                | 30.5  | 38.9*                         | 8.3  | 5.4                                |
| <b>Gram-negative:</b>       |  |                         |                    |   |                               |  |                                    |
| <i>Escherichia</i> spp.     | 2.7  | 0                       | 0                  | 0   | 2.7                           | 0  | 0                                  |
| <i>Enterobacter</i> spp.    | 2.7  | 0                       | 0                  | 0   | 8.3*                          | 0  | 0                                  |
| <i>Acinetobacter</i> spp.   | 11.1   | 0                       | 0                  | 8.3   | 19.4*                         | 0  | 0                                  |
| <i>Pseudomonas</i> spp.     | 11.1   | 0                       | 0                  | 11.1  | 16.6*                         | 0  | 0                                  |
| Fungi                       | 8.3  | 2.7                     | 2.7                | 16.6  | 30.5*                         | 0  | 0                                  |

**Note:** \* – P<0.05, P<0.01 relative to the frequency of isolation of microorganisms from other rooms; \*\*\* – P<0.001 relative to the frequency of CoNS selection according to the F-criterion (considering the Bonferroni correction); CoNS – coagulase-negative staphylococci; CoPS – coagulase-positive staphylococci

**Source:** compiled by the authors

The results of Table 1 show that the autochthonous microbiota of the bioaerosol of veterinary clinics includes the following genera of gram-positive

bacteria: *Staphylococcus* (coagulase-negative species), *Streptococcus* spp., *Micrococcus* spp. and *Corynebacterium* spp. These genera of bacteria were present in the

bioaerosol of all rooms in 100% of cases. Although the above types of bacteria are mostly non-pathogenic, researchers (Naziri *et al.*, 2022) indicate that they can cause infections of the skin and genitourinary system in companion animals. The high frequency of detection of these microorganisms in the bioaerosol of all premises during the working day (Smith *et al.*, 2019) is associated with the fact that they are common inhabitants of the skin of dogs, cats, and people, and are easily released into the environment on exfoliated epithelial cells, wool.

*Bacillus spp.* bacteria, which were isolated from almost 30% of the examined samples of the rooms for the primary examination and the manipulation zone with boxes for keeping sick animals, were the second most important in the composition of the gram-positive microbiota of the bioaerosol. Most often, species of *Bacillus spp.* were emitted from the air during the working day, especially from the dental operating room in 38.9% of cases. Coagulase-positive species of staphylococci were considerably less frequently isolated in the bioaerosol of veterinary clinics ( $P < 0.001$ ) than coagulase-negative species. They were not isolated from the air of two operating rooms, while from the bioaerosol of the premises of the ultrasound room and the X-ray room, the frequency of their release was 5.4% and 2.7%, respectively. The most frequently isolated types of coagulase-positive staphylococci were present in the bioaerosol of the dental operating room – in 27.7% of cases. The frequency of detection of these species from the premises for primary examination and manipulation zone with boxes for keeping sick animals was 16.3% and 19.4%, respectively. Considering this fact, the presence of coagulase-positive species of staphylococci in the bioaerosol of veterinary clinics can be a serious source of spread of infection by airborne droplets. Specifically, the transmission of pathogens most often occurs in examination rooms, diagnostic rooms, intensive care, holding boxes for sick animals, as well as after contact with veterinary personnel (Feßler *et al.*, 2018; Krapf *et al.*, 2019; Elnageh *et al.*, 2020).

From the identified gram-positive coccal microflora, *Enterococcus spp.* were least often released from the bioaerosol of veterinary clinics during the working day. Thus, from the air of the X-ray room and two operating rooms, they were not released at all, and from the bioaerosol of the ultrasound room – in no more than 2.7% of samples. Within 11.1-13.8% of the examined samples, these types of bacteria were present in the bioaerosol of the rooms for primary examination and the manipulation area with boxes for keeping sick animals. The most frequently isolated types were present in the bioaerosol of the dental operating room – in 16.6% of samples.

The identification of gram-negative bioaerosol microbiota of all premises of veterinary clinics indicates that these microorganisms belong to the allochthonous microflora of this research object. Since there are no representatives of gram-negative bacteria that would exceed 20% in terms of detection frequency. That is, the frequency of detection of representatives of gram-negative bacteria is significantly ( $P < 0.01$ ) lower than that of gram-positive species. The most frequently identified gram-negative bacteria were *Acinetobacter spp.* species, which were present in approximately 10% of the bioaerosol samples of the premises for primary examination and the manipulation area with boxes for keeping sick animals. However, most often these species were identified from the bioaerosol of the dental operating room – in 19.4% of samples. Other representatives of gram-negative microbiota, namely *Escherichia spp.* and *Enterobacter spp.*, were detected only from the bioaerosol of the premises for the primary examination and the dental operating room in 2.7% and 8.3% of the samples, respectively. Types of *Pseudomonas spp.* were also found in a few examined samples – 11.1% of them were found in the primary examination room and manipulation area with boxes for keeping sick animals. However, most frequently – from the bioaerosol of the dental operating room (16.6% of samples).

The minimum amount of fungal microflora from the examined samples (2.7%) was detected from the bioaerosol of the ultrasound room and the X-ray room. An increase in the frequency of detection of fungi up to 8.3% was observed in the air of the room for primary examination and up to 16.6% in the manipulation area with boxes for keeping sick animals. Most often, these microorganisms were present in the bioaerosol of the dental operating room in 30.5% of the samples under study. However, fungal microflora was not detected in the bioaerosol of two operating rooms – orthopaedic and soft tissues.

Therefore, the data of the study indicate that the microbiota of the bioaerosol of veterinary clinics is mainly represented by species of gram-positive coccal microflora, which are isolated from almost all premises. However, gram-negative bacterial species are found in small amounts in the bioaerosol of such rooms as the room for primary examination and the manipulation area with boxes for holding sick animals. Representatives of gram-negative species are detected in a considerably larger number from the bioaerosol of the dental operating room during the day of the clinic. Table 2 shows the results of a study on the detection and identification of microbiota of bioaerosol of veterinary clinics after disinfection using bactericidal lamps.

**Table 2.** Identification of bioaerosol microbiota of the premises of veterinary clinics (n = 3), after disinfection with ultraviolet lamps, n = 36

| Microorganisms              | Frequency of m/o release from indoor bioaerosol: |                         |                    |   |                               |  |                                    |
|-----------------------------|--|-------------------------|--------------------|---|-------------------------------|--|------------------------------------|
|                             | room for initial inspection of animals, n = 36   | Ultrasound room, n = 36 | X-ray room, n = 36 | manipulation zone with boxes for keeping sick animals, n = 36 | dental operating room, n = 36 | operating room for soft tissue surgery, n = 36 | orthopaedic operating room, n = 36 |
| <b>Gram-positive:</b>       |  |                         |                    |   |                               |  |                                    |
| CoNS                        | 100  | 83.3                    | 83.3               | 100   | 100                           | 86.1   | 83.3                               |
| CoPS                        | 2.8  | 0                       | 0                  | 8.4   | 8.4*                          | 0  | 0                                  |
| <i>Streptococcus</i> spp.   | 0  | 0                       | 0                  | 5.4   | 5.4*                          | 0  | 0                                  |
| <i>Micrococcus</i> spp.     | 100  | 100                     | 100                | 100   | 100                           | 100  | 100                                |
| <i>Corynebacterium</i> spp. | 100  | 0                       | 0                  | 100   | 100                           | 0  | 0                                  |
| <i>Enterococcus</i> spp.    | 0  | 0                       | 0                  | 2.8   | 5.4*                          | 0  | 0                                  |
| <i>Bacillus</i> spp.        | 8.3  | 2.7                     | 0                  | 5.5   | 8.3*                          | 0  | 0                                  |
| <b>Gram-negative:</b>       |  |                         |                    |   |                               |  |                                    |
| <i>Escherichia</i> spp.     | 0  | 0                       | 0                  | 0   | 0                             | 0  | 0                                  |
| <i>Enterobacter</i> spp.    | 0  | 0                       | 0                  | 0   | 0                             | 0  | 0                                  |
| <i>Acinetobacter</i> spp.   | 2.8  | 0                       | 0                  | 0   | 5.5*                          | 0  | 0                                  |
| <i>Pseudomonas</i> spp.     | 0  | 0                       | 0                  | 0   | 2.7*                          | 0  | 0                                  |
| Mushrooms                   | 0  | 0                       | 0                  | 8.3   | 11.1*                         | 0  | 0                                  |

**Note:** \* –  $P < 0.05$  relative to the frequency of isolation of microorganisms from other premises according to the F-criterion (considering the Bonferroni correction); CoNS – coagulase-negative staphylococci; CoPS – coagulase-positive staphylococci

**Source:** compiled by the authors

It was established (Table 2) that after disinfection of premises with ultraviolet lamps, the frequency of detection of microorganisms from bioaerosol significantly decreased, or bacteria were not isolated at all. Furthermore, gram-positive microorganisms of bioaerosol are more resistant to the influence of ultraviolet rays of lamps, compared to gram-negative bacteria. Therewith, among gram-positive bacteria, *Micrococcus* spp. bacteria were in the first place in terms of frequency of isolation, which were detected in 100% of cases from the bioaerosol of all premises after disinfection. Coagulase-negative staphylococci, which were isolated in 83.3-100% of samples, ranked second in frequency of detection from bioaerosol after exposure to ultraviolet rays. At the same time, coagulase-positive Staphylococcus species were detected much less frequently in air samples. Specifically, these species were found in 2.7% of samples in the bioaerosol of rooms for primary examination and 8.3% of samples in the air of the manipulation area with boxes for keeping sick animals and the dental operating room. Bacteria of the species *Corynebacterium* spp. were isolated in 100% of the samples under study from the bioaerosol of the rooms for the primary examination, the manipulation area with boxes for sick animals and the dental operating room. They were not

emitted from the air of other premises of veterinary clinics. The proposed disinfection with ultraviolet rays had a rather harmful effect on bacteria of *Streptococcus* spp. and *Enterococcus* spp., which were isolated only from the bioaerosol of two rooms of the manipulation area with boxes for keeping sick animals and the dental operating room in 2.8-5.4% of samples. Spore-forming bacteria species *Bacillus* spp. were released from the bioaerosol of four premises with a frequency of no more than 10%. Thus, bacteria were released from the air of the room for the primary examination of animals and the dental operating room in 8.3% of samples, and from the bioaerosol of the ultrasound cabinet and manipulation area with boxes for keeping sick animals – in 2.7% and 5.5% of samples, respectively.

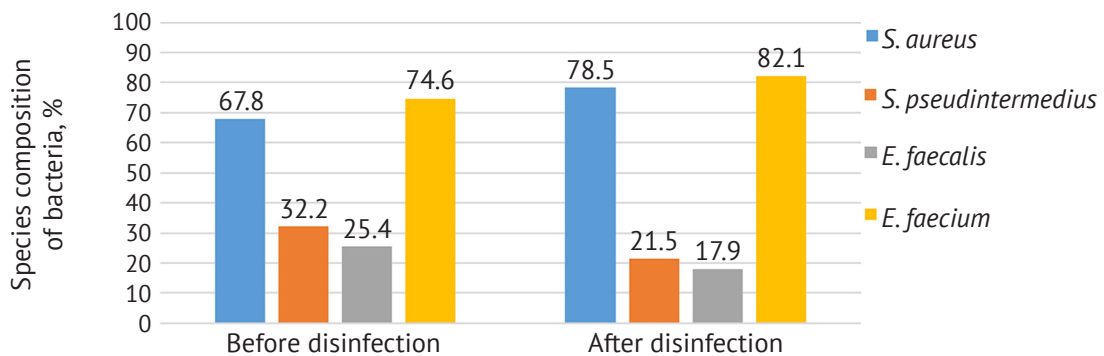
Among gram-negative bacteria from the bioaerosol of veterinary clinics after disinfection with bactericidal lamps, only *Acinetobacter* spp. from two rooms: for primary examination and dental operating room, with a frequency of 2.8% and 5.5% of samples, respectively. *Pseudomonas* spp. species were also isolated from the bioaerosol of the dental operating room in the minimum amount from the tested samples – 2.7%. Fungal microflora was present after treatment with ultraviolet lamps only in the bioaerosol of the manipulation area

with boxes for keeping sick animals and in the dental operating room in 8.3 and 11.1% of samples, respectively. No fungi were found in the air of other rooms.

Therefore, the identification of bioaerosol microbiota of the premises of veterinary clinics after disinfection with ultraviolet lamps established a significant decrease in the frequency of detection of all microorganisms. At the same time, in the bioaerosol of such premises as the primary examination, the manipulation area with boxes for keeping sick animals and the dental operating room, types of gram-positive bacteria were distinguished, especially coagulase-positive staphylococci and fungi. This suggests that under strong microbial contamination, bacteria can survive in the suspended phase of bioaerosol

and continue to be a source of infection. Therefore, the authors of this paper believe that added disinfection measures should be taken to reduce the risk of the spread of nosocomial pathogens in premises with an intensive movement of animals, where frequent diagnostic manipulations or dental treatments are carried out.

In general, gram-positive and gram-negative genera of opportunistic pathogens, which can be a source of nosocomial infection, were isolated from the bioaerosol of the premises of veterinary clinics. Figure 1 shows the results of a study of the identification of gram-positive opportunistic microbiota of the bioaerosol of the premises of veterinary clinics before and after disinfection using bactericidal ultraviolet lamps.



**Figure 1.** Identification of gram-positive opportunistic microorganisms from the bioaerosol of the premises of veterinary clinics ( $n = 3$ )

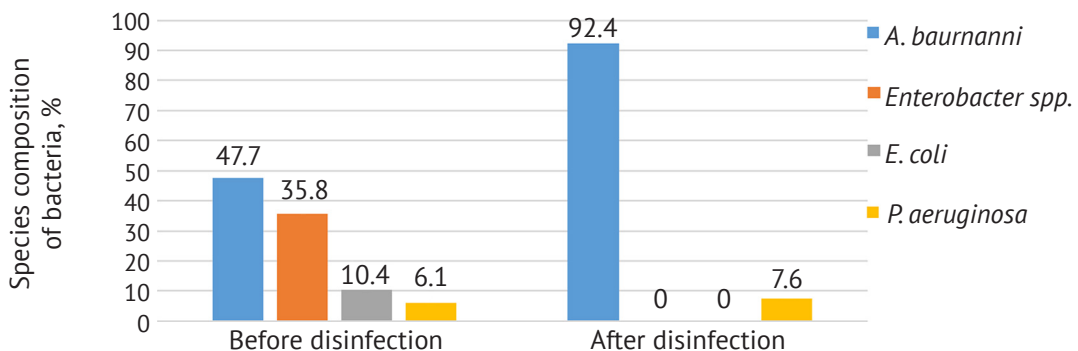
**Source:** compiled by the authors

As Figure 1 shows, among the isolated coccid bacteria from the bioaerosol before disinfection, coagulase-positive staphylococci were represented by two species: *S. aureus* and *S. pseudintermedius*. Therewith, *S. aureus* accounted for  $67.8 \pm 2.1\%$ , and *S. pseudintermedius* –  $32.2 \pm 0.8\%$ . According to (Murray *et al.*, 2018), *S. pseudintermedius* is a species that is mainly isolated from dogs and cats and is the cause of diseases such as pyoderma, otitis externa, etc. At the same time, *S. aureus* is a species that colonizes biotopes, both human and animal ones (Berhilevych *et al.*, 2021). Enterococci before disinfection in the bioaerosol were mainly represented by the *E. faecium* species, which accounted for

$74.6 \pm 2.4\%$  of the isolated cultures, and  $25.4 \pm 0.7\%$  belonged to *E. faecalis*.

Disinfection did not significantly change the ratio between bacterial species, although greater resistance of *S. aureus* and *E. faecium* to the action of ultraviolet radiation was noted. The share of these species in disinfected air increased by 10.7% and 7.5%, respectively. The detection of coagulase-positive staphylococci and enterococci in the bioaerosol of veterinary clinics after the action of bactericidal lamps indicates the imperfection of the introduced system of combating infectious agents in these clinics.

The identification of gram-negative opportunistic bacteria isolated from the bioaerosol is presented in Fig. 2.



**Figure 2.** Identification of gram-negative opportunistic microorganisms from the bioaerosol of the premises of veterinary clinics ( $n = 3$ )

**Source:** compiled by the authors

It was established (Fig. 2) that among the bacteria isolated from the bioaerosol before disinfection, the largest share was *Acinetobacter baumani* –  $47.7 \pm 1.3\%$ . *Enterobacter spp.* accounted for  $35.8 \pm 1.1\%$  of identified cultures, while the least were found for *E. coli* and *P. aeruginosa* –  $10.4 \pm 0.4\%$  and  $6.1 \pm 0.2\%$ , respectively.

After a one-hour exposure to ultraviolet lamps, *E. coli* and *Enterobacter spp.* cultures were not released from the indoor bioaerosol, which indicates the bactericidal effect of this disinfection regimen on these types of bacteria. At the same time, the cultures of *Acinetobacter baumani* stayed resistant to the action of ultraviolet rays, the share of which was  $92.4 \pm 3.8\%$  of the isolated gram-negative bacteria. In addition, *P. aeruginosa* was isolated from the bioaerosol of some rooms after disinfection, which accounted for  $7.6 \pm 0.3\%$  of cultures.

Therefore, the obtained data clearly shows that representatives of gram-negative microflora are more often released from the bioaerosol of the premises of veterinary clinics with an intensive movement of animals (examination room), where manipulations are carried out with the possible splashing of tissue particles, tooth enamel (dental operating room), in boxes for keeping sick animals, i.e., all this is connected with biological secretions from animals. Such results are consistent with research by (Sitkowska et al., 2015), that the microbiota of the bioaerosol of veterinary clinics is based on microorganisms that are representatives of the normal microbiota of closed premises of the housing stock, offices, i.e., gram-positive genera. Gram-negative microflora is present in the air in most cases due to violations of hygiene requirements, and in veterinary clinics in the premises of long-term or permanent stay of animals, for instance, in boxes after surgical intervention (Pertegal et al., 2022).

Considering the data obtained, the authors of this paper believe that the more frequent release of gram-positive microflora from indoor bioaerosol, compared to gram-negative, is explained by their greater resistance to the action of ultraviolet rays. This fact is confirmed by (Fahlgren et al., 2010), who found increased resistance of gram-positive microflora to strong sunlight during long-term exposure, compared to gram-negative. However, the survival of coagulase-positive staphylococci, enterococci, species of *Acinetobacter spp.*, *Pseudomonas spp.* after air treatment with ultraviolet lamps should be noted for such premises as the primary examination room, the manipulation area with boxes for keeping sick animals, and the dental operating room. That is, bacteria that, according to (Hritcu et al., 2020; Elnageh et al., 2020), are the cause of outbreaks of nosocomial infections among animal patients in veterinary clinics.

Outbreaks of nosocomial infection in hospitalized animals caused by the multiresistant species *Acinetobacter baumani* are reported by researchers (Kempf & Rolain, 2012). Therefore, it is necessary to pay attention to this type of bacteria in the practical activities of

veterinary clinics. Researchers (Giannouli et al., 2013) point to increased resistance to drying and the action of ultraviolet radiation in *Acinetobacter baumani*, as they can form dense biofilms on various surfaces and thereby survive in the hospital environment. The obtained studies indicate that some strains of opportunistic bacteria survive in the bioaerosol of the premises of veterinary clinics after exposure to ultraviolet rays of bactericidal lamps. This indicates that bioaerosol can serve as a medium for the spread of nosocomial infections among animals in veterinary clinics.

Therefore, today there is a considerable number of recommendations for the prevention and control of nosocomial infections in humane medicine, which are based on practical experience (Zheng et al., 2023). At the same time, there are no generally accepted recommendations or instructions for the supervision and control of nosocomial pathogens in veterinary clinics in Ukraine. This makes the system of preventive measures not too strong, since there are no stable, defined indicators that need to be focused on, and accordingly prevent the spread of nosocomial infection. Therefore, the authors of the present study believe that the development and implementation of a system for monitoring nosocomial pathogens in veterinary medicine clinics in Ukraine should complement the national strategy for combating antibiotic resistance of microorganisms. This will, firstly, reduce infection with resistant strains of animals, secondly, reduce the use of broad-spectrum antibiotics, and thirdly, prevent the spread of antibiotic-resistant bacteria in the environment of veterinary clinics between animals, between animals and veterinary and service personnel, and between personnel and other people and animals.

## CONCLUSIONS

It was established that bacteria of the *Micrococcus spp.*, *Staphylococcus* (coagulase-negative species), *Streptococcus spp.* and *Corynebacterium spp.* species were released from the bioaerosol of veterinary clinics during the working day in 100% of cases. Coagulase-positive species of staphylococci were present in indoor bioaerosols in 5.4-27.7% of samples. Gram-negative species of bacteria: *Escherichia spp.*, *Enterobacter spp.*, *Acinetobacter spp.* and *Pseudomonas spp.* were present in the bioaerosol of the dental operating room in 2.7-9.4% of the samples, and these types of bacteria were practically not isolated from the bioaerosol of other rooms. Thus, gram-positive bacterial species make up the permanent microflora of bioaerosol in veterinary clinics.

Disinfection with ultraviolet lamps ensured the destruction of gram-negative bacteria, which were practically not released from the bioaerosol of the premises, except for the dental operating room, where up to 5.5% of *Acinetobacter spp.* and *Pseudomonas spp.* bacteria were released from the air. Gram-positive microbiota was more resistant to the effects of ultraviolet

rays, as bacteria of the *Micrococcus spp.*, *Staphylococcus* (coagulase-negative species) and *Corynebacterium spp.* species were isolated from the bioaerosol in almost 100% of the samples.

The causative agents of nosocomial infections were identified from the indoor bioaerosol: *S. aureus* and *S. pseudintermedius*, *Acinetobacter baumani* and *P. aeruginosa*. This indicates that bioaerosol can serve as

a medium for the spread of pathogenic bacteria among animals in veterinary clinics. In addition, this provides a basis for the development of added sanitary measures to combat the spread of nosocomial pathogens. Prospects for further research lie in determining the sensitivity of the isolated microflora to antibacterial preparations and establishing the presence of antibiotic resistance plasmids.

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## Ідентифікація мікробіоти біоаерозолі ветеринарних клінік – запорука профілактики нозокоміальної інфекції

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**Анотація.** Внутрішньо лікарняні інфекції мають суттєве значення у клініках ветеринарної медицини, оскільки можуть контамінувати поверхні і заражати тварин через біоаерозоль та є причиною неефективної антибіотикотерапії. У роботі наведено дослідження впливу дезінфекції на мікробіоту біоаерозолі ветеринарних клінік. Мета роботи полягала у встановленні складу мікробіоти біоаерозолі приміщень ветеринарних клінік до та після знезараження ультрафіолетовими бактерицидними лампами. Проби біоаерозолі відбирали в приміщеннях ветеринарних клінік седиментаційним методом. Ідентифікацію виділеної мікробіоти проводили класичними методами із застосуванням комерційних тест-систем для ідентифікації мікроорганізмів. Встановлено, що до постійної мікробіоти біоаерозолі ветеринарних клінік можна віднести наступні представники грам позитивних родів: *Staphylococcus* (коагулазонегативні види), *Streptococcus spp.*, *Micrococcus spp.* та *Corynebacterium spp.* Дані роди бактерій були наявні в біоаерозолі всіх приміщень у 100 % випадків. Грамнегативні види бактерій зустрічалися в незначній кількості в біоаерозолі таких приміщень, як для первинного огляду та маніпуляційної зони з боксами для перетримування хворих тварин. У значно більшій кількості представники грамнегативних видів виявлялися з біоаерозолі стоматологічної операційної протягом дня роботи клініки. Після дезінфекції бактерицидними лампами у біоаерозолі таких приміщень, як первинного огляду, маніпуляційної зони із боксами для перетримування хворих тварин та стоматологічної операційної виділялися збудники нозокоміальних інфекцій (*S. aureus*, *S. pseudintermedius*, *Acinetobacter baumani*, *P. aeruginosa*). Це вказує на те, що біоаерозоль може слугувати середовищем для розповсюдження збудників нозокоміальних інфекцій серед тварин ветеринарних клінік. Отже, проведення мікробіологічного моніторингу біоаерозолі у ветеринарних клініках дозволить виявити збудників нозокоміальних інфекцій та запровадити профілактичні заходи щодо розповсюдження патогенів

**Ключові слова:** мікрофлора повітря, дезінфекція ультрафіолетовими лампами, внутрішньолікарняні збудники, патогенні бактерії



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## The Role of Winter Wheat Plant Height in the Formation of Grain Yield Depending on Varietal Characteristics and Weather Conditions

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**Abstract.** Winter wheat is one of the main food crops that ensures the national food security of Ukraine with general production and high-quality grain. The area of winter wheat sowing ranks first in Ukraine, and the production of high-quality grain is of particular relevance. In the technology of its cultivation, the selection of varieties is a decisive factor in increasing yield and improving the quality of grain. The purpose of this study was to determine the influence of weather conditions and variety characteristics during the year on plant height, lodging resistance, and yield of winter wheat. This paper presents data on the results of the research of 20 varieties of soft winter wheat in the conditions of the Research-to-Practice Centre of the Mykolaiv National Agrarian University from 2017 to 2022. During the study, generally accepted methods were used: system approach, system analysis, analysis and synthesis, field and statistical method. The study also analysed the influence of variety characteristics and weather conditions in the years under study on the yield of soft winter wheat. It was established that the optimal plant height of winter wheat varieties ranged within 94.9-100.7 cm, at which the highest grain yield is formed, from 7.09 t/ha in the Staleva variety to 7.73 t/ha in the Duma Odeska variety. An increase in the height of the winter wheat varieties under study by 4.0-6.4% led to a decrease in yield from 4.5 to 20.9%. It was established that the awned varieties of soft winter wheat formed a higher grain yield, compared to the thornless forms, which had a higher plant height and less lodging resistance. The conducted studies confirmed that, on average, the resistance to lodging was higher in the thornless forms of winter wheat plants, which is 0.4 points higher than in the awned ones. The higher average grain yield of soft winter wheat was formed by varieties of spiny forms, which is 5.8% more than that of awned varieties. The obtained results will contribute to the wide application of the varieties of soft winter wheat under study for this soil and climate zone, which will further contribute to the increase in yield and gross grain harvests

**Keywords:** soft winter wheat, lodging resistance, awned and awnless forms, air temperature, precipitation amount



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## INTRODUCTION

Food production is one of the strategic industries of any country in the world. In modern economic conditions, the importance of the agricultural sector in the economy is growing, since the sale of agricultural products benefits the competitiveness of Ukraine, i.e., its gross domestic product. An essential part of agricultural production is the production of food crops. For a long time, Ukraine has been among the world's top ten in terms of grain production per capita.

The yield of winter wheat varieties is affected by weather conditions during the growing years of the crop and agricultural techniques. Thus, Žofajová *et al.* (2017) and Berdnikova & Kucherak (2021) found that genotype, year, and location substantially affect yield and yield components. Nazarenko *et al.* (2020) and Demydov *et al.* (2021) compared winter wheat varieties of Ukrainian and French selection, according to the results of which 6 highly productive varieties were bred (Dyvo, Metelytsia (first group), Vekha (second), Fihura, Manella, Matrix (third)). Some varieties were bred in a separate year without a predominance of the standard on average: varieties Laval, Dijon, Polisianka, Zorepad Bilotserkivskiyi, Bodychek in the conditions of the 2016-2017 growing year; Nasnahy in the conditions of 2018-2019. The Pamiati Hirka variety showed a higher yield under conditions of two years of vegetation, without an advantage per the standard regarding three-year results.

Research by Bazalii *et al.* (2019) and Gamayunova *et al.* (2022) proved that in the conditions of southern Ukraine, it is necessary to grow plastic varieties of wheat with increased yield stability (Khersonska awnless, Khersonska 99, Znakhidka Odeska, Askaniiska, Klarysa), if strict stress limits are expected in the environmental conditions. Based on the results of research by L. Wu & H. Zhatova (2022), wheat varieties with high-performance parameters were selected. These are such varieties as Okhtyrchanka Yuvileina, Svitanok Myronivskiyi, Melodiia Odeska, Kubok, Zorepad, Ovidii, Shchedra Nyva, Oktava Odeska, and Slaven. In the studies of Markovska *et al.* (2018), Gavrilyuk (2016) the Konka winter wheat variety produced a seed yield of 3.59 t/ha, which is 8.2% more than Khersonska 99.

Djaman *et al.* (2018) and Harkness *et al.* (2020) established that grain yield depended on the variety of winter wheat, decreased over the years and ranged within 1.84-7.09 t/ha. TAM107 received the largest grain yield. M.M. Korkhova (2019) conducted a comparative analysis of varieties of soft winter wheat and spelt, which indicated that, on average, in 2016-2018, a higher height of plants (130.7 cm) was formed in the awnless variety Zoria Ukrainy compared to the awned ones under study, which is 6.7% more than the Yevropa variety, 25.9% more than the Vidrada variety, 32.9% more than the Shestopalivka variety, and 33.7% more than the Zysk variety. However, the higher yield (5.53-6.27) was formed by the awned varieties of wheat – Zysk,

Shestopalivka, Vidrada, and Yevropa, which is 29.8-38.1% more than the awnless variety Zoria Ukrainy.

Bondarenko & Nazarenko (2022), Panfilova (2021) claim that high-yielding genotypes form yield due to added spikes, have higher lodging resistance due to lower plant height and grain mass in the spike, respectively, and spend fewer nutrients on stem formation. Zhupina *et al.* (2022) found that the growth of plant height of hybrid populations of winter wheat leads to a decrease in grain yield. Thus, there is no consensus among scientists regarding the effect of plant height on lodging resistance and the formation of grain yield of soft winter wheat varieties of distinct morphological characteristics, since the research was conducted in years with different weather conditions. Furthermore, with the advent of new varieties in production, this issue requires more research.

*The purpose of this paper* was to establish varietal characteristics and the influence of weather conditions during the year on resistance to lodging and yield of winter wheat, as well as on plant height.

## MATERIALS AND METHODS

Experimental research was conducted for five years (2017-2022) in the conditions of the Educational Research-to-Practice Centre of the Mykolaiv National Agrarian University (ERPC MNAU), which belongs to the Southern Steppe zone of Ukraine. The experiment included 20 variants (winter wheat varieties), which were placed in the experimental field of the ERPC MNAU according to the method of complete randomization in threefold repetition. The area of the sowing plot was 70 m<sup>2</sup>, and the accounting plot was 35 m<sup>2</sup>. The agricultural technique of conducting the experiments was generally accepted for the existing zonal recommendations for the conditions of the Southern Steppe zone of Ukraine. Winter wheat was sown in the first decade of October, with a seeding rate of 4.5 million pcs./ha.

The soil of the experimental field is a typical southern chernozem, residual slightly saline heavy loam in the loess, humus content (0-30 cm) – 3.1-3.3%, soil solution is neutral (pH 6.8-7.2). On average, the arable layer contains 15-25 mobile forms of nitrates, 41-46 mobile phosphorus and 389-425 mg/kg exchangeable potassium.

The material for research was 20 varieties of soft winter wheat, the owners of which are leading scientific institutions of Ukraine and the near abroad: Ozerna, Staleva (Private Agricultural Breeding and Research Enterprise "Bor"); Kvitka Poliv, Lehenda Bilotserkivska (Bila Tserkva Research and Breeding Station of the Institute of Bioenergy Crops and Sugar Beet of the National Academy of Agrarian Sciences of Ukraine); Mudrist Odeska, Duma Odeska (Breeding and Genetics Institute – National Centre for Seed Science and Varietal Research); Koshova, Maria (Institute of Irrigated

Agriculture, NAASU); Zdobna, Dyvo (V. Ya. Yuriev Institute of Plant Breeding of the NAASU); MIP Assol, MIP Valencia (V. M. Remeslo Myroniv Institute of Wheat of the NAASU); Pamiati Hirka, Kraievyd (Institute of Agriculture, NAASU); Katarina, Centurion (Viterro SEED – Germany); Felix, PONTUKUS, Faustus, Glaucus (“Shtrube

Ukraine GmbH” LLC), which are registered in the State Register of Plant Varieties Suitable for Distribution in Ukraine in 2013-2019 (State Register of Plant Varieties..., 2022). Varieties of soft winter wheat were characterized by morphological features, namely by height, presence of awns or teeth, and maturity group (Table 1).

**Table 1.** Morpho-biological characteristics of soft winter wheat varieties

| No. seq. | Varieties              | Plant: by height (stem and spike) | Spike: awns or teeth of the lower flower scales | Ripeness group |
|----------|------------------------|-----------------------------------|---|----------------|
| 1        | Ozerna                 | short-stemmed                     | awns present                                    | mid-early      |
| 2        | Staleva                | short-stemmed                     | awns present                                    | mid-early      |
| 3        | Kvitka Poliv           | medium-grown                      | teeth present                                   | mid-early      |
| 4        | Lehenda Bilotserkivska | medium-grown                      | teeth present                                   | mid-season     |
| 5        | Mudrist Odeska         | short-stemmed                     | awns present                                    | mid-early      |
| 6        | Duma Odeska            | short-stemmed                     | awns present                                    | short-season   |
| 7        | Koshova                | short-stemmed                     | awns present                                    | short-season   |
| 8        | Maria                  | short-stemmed                     | awns present                                    | short-season   |
| 9        | Zdobna                 | short-stemmed                     | awns present                                    | mid-early      |
| 10       | Dyvo                   | short-stemmed                     | awns present                                    | mid-early      |
| 11       | MIP Assol              | medium-grown                      | teeth present                                   | mid-season     |
| 12       | MIP Valencia           | semi-dwarfs                       | awns present                                    | mid-season     |
| 13       | Pamiati Hirka          | short-stemmed                     | awns present                                    | mid-season     |
| 14       | Kraievyd               | short-stemmed                     | awns present                                    | mid-season     |
| 15       | Katarina               | semi-dwarfs                       | teeth present                                   | short-season   |
| 16       | Centurion              | semi-dwarfs                       | awns present                                    | mid-early      |
| 17       | Felix                  | semi-dwarfs                       | teeth present                                   | short-season   |
| 18       | PONTICUS               | semi-dwarfs                       | teeth present                                   | short-season   |
| 19       | Faustus                | semi-dwarfs                       | teeth present                                   | short-season   |
| 20       | Glaucus                | semi-dwarfs                       | teeth present                                   | mid-season     |

**Source:** compiled by the authors

The analysis of agroclimatic conditions uses data from the Pessl Instruments weather station (iMETOS), manufactured in Austria, which not only provides highly accurate local meteorological data, but also provides 6-day weather forecasts. Weather stations are equipped with sensors that determine the following indicators: amount of precipitation, air and soil temperature, air, soil, and leaf humidity level, wind speed, etc. The height of winter wheat plants was determined before harvesting on 100 plants permanently set aside for this purpose, using a measuring ruler evenly placed on the accounting area of each plot. The stems were measured from the surface of the soil to the top of the plant. The last indicator is the average height of plants on the plot.

Lodging resistance of soft winter wheat plants was evaluated from the beginning of this phenomenon to harvesting every 5-10 days (these observations could reveal the properties of individual crops returning to their previous vertical position) on a ten-point scale. Yield

was accounted continuously from the entire accounting area. Soft winter wheat was harvested by a SAMPO-500 combine harvester. After threshing, the thresher of the grain harvester was turned off at each plot, and the harvested grain was weighed and adjusted to the standard humidity (14%) and purity (100%). The research results, obtained in the form of analytical digital materials, are processed statistically and mathematically according using the methods of dispersion and variation analyses with Microsoft Excel and Agrostat computer software.

The years of research differed substantially in weather conditions. Thus, 2017/2018 and 2021/2022 agricultural years were moderately wet, during the growing season 338.5 and 331.6 mm of precipitation fell, respectively, while in 2019/2020 – 161.2 mm; 2018/2019 – 547.6 mm, and 2020/2021 – 603.7 mm. 2018/2019 and 2020/2021 were the most favourable in terms of weather conditions, and 2019/2020 was the least favourable. Such contrasting conditions allowed

the authors to investigate the influence of weather conditions on plant height, lodging resistance, and grain yield of soft winter wheat.

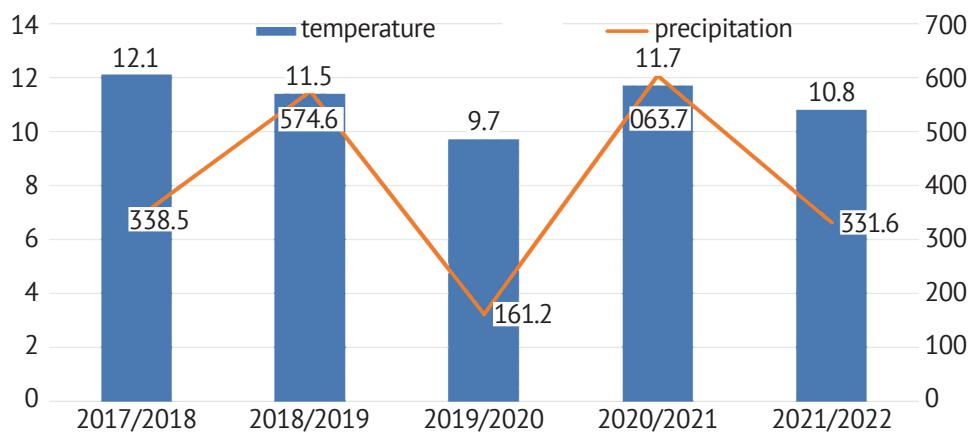
## RESULTS AND DISCUSSION

Plant height is a genetically inherited trait that characterizes the ecological plasticity of genotypes in various soil and climatic conditions and depends on weather conditions. Plant resistance to lodging and grain yield depends on the height and anatomical properties of the wheat stem.

M.M. Gavrilyuk (2019) conventionally divides winter wheat varieties by height into five types: dwarfs – under 60 cm; semi-dwarfs – 60-85 cm, which include MIP Valencia, Katarina, Centurion, Felix, PONTICUS, Faustus, and Glaucus; short-stemmed – 85-105 cm (Ozerna, Staleva, Mudrist Odeska, Duma Odeska, Koshova, Maria,

Zdobna, Dyvo, Pamiati Hirka, Kraievdyd); medium-sized – 105-120 cm (Kvitka Poliv, Lehenda Bilotserkivska, MIP Assol), and tall – over 120 cm. Most of the varieties included in the State Register (2022) belong to the short-stemmed type, but the share of semi-dwarf varieties has decreased. Most of the varieties of soft winter wheat under study are classified as short-stemmed and semi-dwarf, and only three are classified as medium-sized.

The formation of plant height was substantially influenced by weather conditions during the years of research. It was found that the greater precipitation, the taller the winter wheat plants. Thus, in years with a large amount of precipitation of 574.6 mm (2018/2019) and 603.7 mm (2020/2021), the plant height of the varieties of soft winter wheat under study was formed at 70.6-118.2 and 73.5-125.5 cm, respectively (Fig. 1).



**Figure 1.** Average air temperature and precipitation (2017/2018–2021/2022)

Source: compiled by the authors

On average, by varieties, the lower height of winter wheat plants (82.9 and 82.5 cm) was formed in 2017/2018 and 2021/2022 with precipitation of 338.5 mm and 331.6 mm, respectively. It was found that winter wheat plants of the varieties under study were formed lower in height (55.4-94.3 cm) in 2019/2020, when only 161.2 mm of precipitation fell, and the average annual

air temperature was 9.7°C. Over five years of research (2018-2022), the taller height of winter wheat plants was formed by the varieties Kvitka Poliv (107.4 cm) and Lehenda Bilotserkivska (106.5 cm), which is 6.2–40.2% taller the other varieties under study. The lowest plant height on average for 2018-2022 was formed by the varieties Katarina (64.2 cm) and Felix (69.2 cm) (Table 2).

**Table 2.** Height of soft winter wheat plants depending on varietal characteristics and weather conditions, cm

| No. seq. | Varieties              | Years |       |      |       |       | Average for 2018-2022 |
|----------|------------------------|-------|-------|------|-------|-------|-----------------------|
|          |                        | 2018  | 2019  | 2020 | 2021  | 2022  |                       |
| 1        | Ozerna                 | 83.3  | 99.0  | 79.0 | 105.3 | 86.3  | 90.6                  |
| 2        | Staleva                | 81.8  | 94.9  | 73.7 | 101.0 | 80.6  | 86.4                  |
| 3        | Kvitka Poliv           | 100.4 | 118.2 | 91.0 | 125.5 | 101.7 | 107.4                 |
| 4        | Lehenda Bilotserkivska | 101.7 | 115.2 | 94.3 | 122.5 | 98.7  | 106.5                 |
| 5        | Mudrist Odeska         | 91.2  | 108.2 | 88.8 | 114.0 | 91.2  | 98.7                  |
| 6        | Duma Odeska            | 85.9  | 100.7 | 75.3 | 106.0 | 86.6  | 90.9                  |

Table 2, Continued

| No. seq.                                      | Varieties     | Years |       |      |       |      | Average for 2018-2022 |
|---|---------------|-------|-------|------|-------|------|-----------------------|
|   |               | 2018  | 2019  | 2020 | 2021  | 2022 |                       |
| 7   | Koshova       | 84.7  | 100.5 | 80.0 | 106.0 | 85.3 | 91.3                  |
| 8   | Maria         | 84.5  | 102.2 | 83.0 | 106.7 | 85.4 | 88.8                  |
| 9   | Zdobna        | 86.2  | 101.2 | 76.7 | 106.5 | 84.1 | 90.9                  |
| 10  | Dyvo          | 81.8  | 94.7  | 72.8 | 99.7  | 78.3 | 85.5                  |
| 11  | MIP Assol     | 96.4  | 113.3 | 77.9 | 120.5 | 95.2 | 100.7                 |
| 12  | MIP Valencia  | 77.8  | 94.6  | 73.9 | 98.5  | 77.8 | 84.5                  |
| 13  | Pamiati Hirka | 89.9  | 105.9 | 83.1 | 113.8 | 88.8 | 96.3                  |
| 14  | Kraievyd      | 87.4  | 103.8 | 80.9 | 109.3 | 86.5 | 93.6                  |
| 15  | Katarina      | 61.0  | 70.6  | 55.4 | 73.5  | 60.3 | 64.2                  |
| 16  | Centurion     | 76.1  | 89.8  | 68.6 | 94.0  | 75.0 | 80.7                  |
| 17  | Felix         | 66.5  | 76.0  | 59.5 | 79.2  | 64.7 | 69.2                  |
| 18  | PONTICUS      | 77.2  | 90.7  | 68.5 | 96.5  | 77.4 | 82.1                  |
| 19  | Faustus       | 73.2  | 85.0  | 68.0 | 91.5  | 72.3 | 78.0                  |
| 20  | Glaucus       | 71.6  | 83.7  | 66.3 | 87.3  | 72.8 | 76.3                  |
| Average by variety                            |               | 82.9  | 97.4  | 75.8 | 102.  | 82.5 | 84.6                  |
| LSD <sub>0.05</sub> (cm), partial differences |               | 1.38  | 2.87  | 2.88 | 2.91  | 3.51 | 0.74                  |

Source: compiled by the authors

Thus, the highest plant height on average for soft winter wheat varieties (102.7 cm) was formed in 2021, which is 19.3% more than in 2018, 5.2% more than in 2019, 26.2% more than in 2020, and 19.7% more than in 2022. Based on the results of variance analysis, it was found that the smallest significant difference in factor A (Varieties) was 1.38 cm (2018), 2.87 cm (2019), 2.88 cm (2020), 2.91 cm (2021) and 3.51 cm (2022). Over the years of research, it was

established that winter wheat varieties Ozerna, Staleva, Katarina, Centurion, Felix, Ponticus, Faustus, Glaucus had a very high resistance to lodging – 9 points, varieties Maria, Dyvo, Kvitka Poliv, MIP Assol, MIP Valencia, Pamiati Hirka, Kraievyd, Centurion – high resistance (8.0-8.6 points), Mudrist Odeska variety – medium resistance (7.8 points), and the varieties Lehenda Bilotserkivska, Duma Odeska, Koshova, Zdobna – below average (6.2-6.6 points) (Fig. 2).

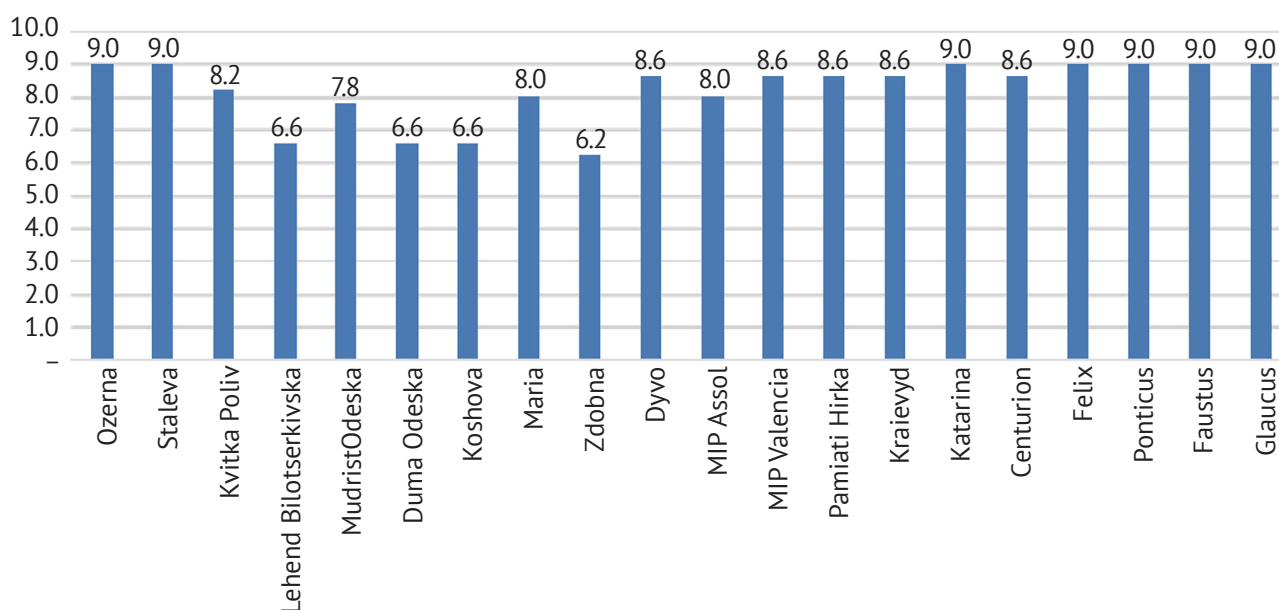


Figure 2. Resistance to lodging of soft winter wheat plants depending on the variety (average for 2018-2022), points  
Source: compiled by the authors

Previous studies by M. Korkhova & V. Mykolaichuk (2022) established that the yield of winter wheat varieties is substantially influenced by weather conditions in the years of research, namely the amount of precipitation and air temperature. According to the

research results, the highest grain yield (6.88 t/ha) on average for varieties was formed by winter wheat plants in 2019, which is 20.1% more than in 2018, 31.0% more than in 2020, 8.7% more than in 2021, and 21.8% more than in 2022 (Table 3).

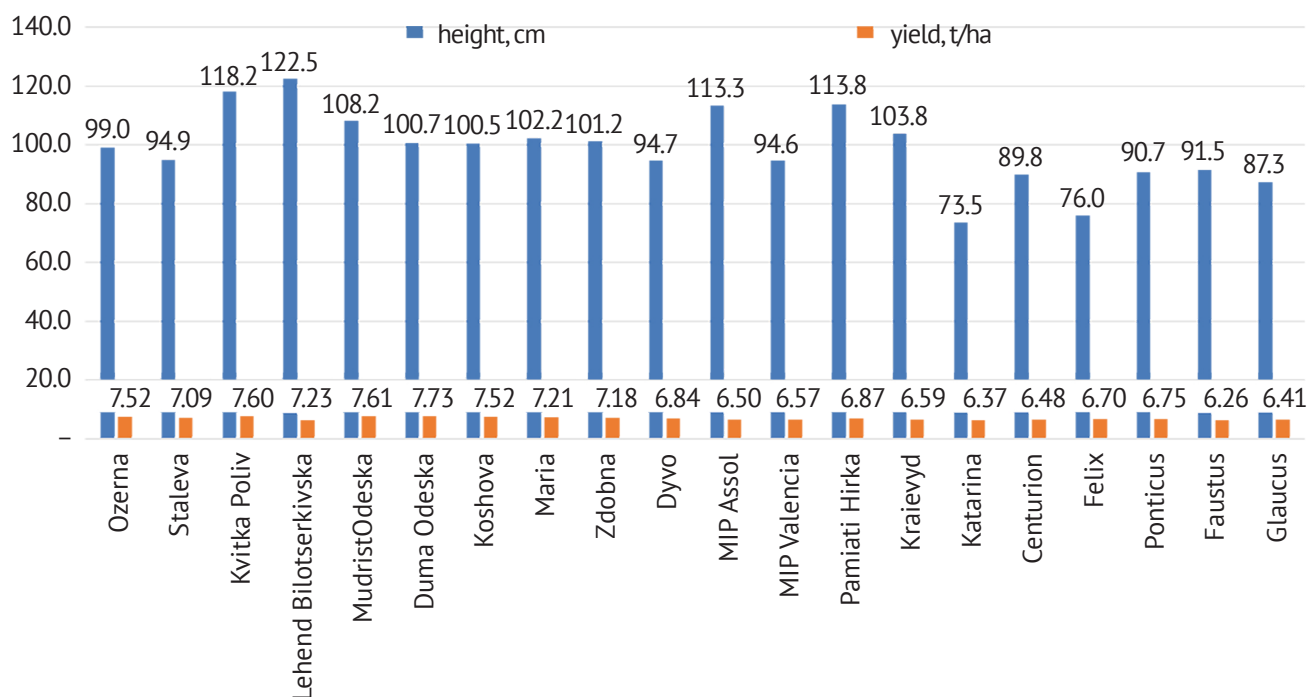
**Table 3.** Yield of soft winter wheat grain depending on varietal characteristics and the year of research, t/ha

| No. seq.  | Varieties              | Years |      |      |      |      | Average for 2018-2022 |
|---|------------------------|-------|------|------|------|------|-----------------------|
|   |                        | 2018  | 2019 | 2020 | 2021 | 2022 |                       |
| 1   | Ozerna                 | 5.34  | 7.52 | 5.57 | 6.53 | 5.87 | 6.17                  |
| 2   | Staleva                | 5.07  | 7.09 | 5.34 | 6.43 | 5.07 | 5.80                  |
| 3   | Kvitka Poliv           | 5.22  | 7.60 | 5.09 | 6.01 | 5.77 | 5.94                  |
| 4   | Lehenda Bilotserkivska | 4.72  | 6.15 | 4.56 | 6.23 | 5.73 | 5.48                  |
| 5   | Mudrist Odeska         | 6.07  | 7.61 | 4.97 | 6.21 | 5.12 | 6.00                  |
| 6   | Duma Odeska            | 5.42  | 7.73 | 5.18 | 6.41 | 6.47 | 6.24                  |
| 7   | Koshova                | 6.11  | 7.52 | 5.08 | 6.34 | 4.95 | 6.00                  |
| 8   | Maria                  | 6.25  | 7.21 | 4.91 | 6.02 | 5.02 | 5.88                  |
| 9   | Zdobna                 | 5.81  | 7.18 | 4.76 | 6.19 | 5.97 | 5.98                  |
| 10  | Dyvo                   | 5.50  | 6.84 | 4.71 | 6.08 | 5.11 | 5.65                  |
| 11  | MIP Assol              | 5.41  | 6.50 | 4.08 | 6.21 | 4.53 | 5.35                  |
| 12  | MIP Valencia           | 5.56  | 6.57 | 4.50 | 6.02 | 4.55 | 5.44                  |
| 13  | Pamiati Hirka          | 5.61  | 6.69 | 5.20 | 6.87 | 5.44 | 5.96                  |
| 14  | Kraievyd               | 5.50  | 6.59 | 5.08 | 6.48 | 6.00 | 5.93                  |
| 15  | Katarina               | 5.14  | 6.34 | 3.12 | 6.37 | 5.73 | 5.34                  |
| 16  | Centurion              | 5.37  | 6.48 | 4.87 | 6.02 | 5.39 | 5.63                  |
| 17  | Felix                  | 5.67  | 6.70 | 4.01 | 6.12 | 4.59 | 5.42                  |
| 18  | PONTICUS               | 5.77  | 6.75 | 4.13 | 6.42 | 4.63 | 5.54                  |
| 19  | Faustus                | 5.12  | 6.24 | 4.29 | 6.26 | 5.75 | 5.53                  |
| 20  | Glaucus                | 5.34  | 6.37 | 4.92 | 6.41 | 5.97 | 5.80                  |
| Average yield by variety                        |                        | 5.50  | 6.88 | 4.75 | 6.28 | 5.38 | 5.76                  |
| LSD <sub>0.05</sub> (t/ha), partial differences |                        | 0.22  | 0.25 | 0.20 | 0.24 | 0.26 | 0.07                  |

**Source:** compiled by the authors

Thus, in 2020, the lowest grain yield was obtained – 4.75 t/ha on average for varieties. On average, over five years of research, a higher yield (6.24 t/ha) was obtained in the Duma Odeska variety, which is 1.1-14.4% more than in other varieties under study. The lowest average grain yield was formed by the Katarina variety – 5.34 t/ha. In 2018, the best result in terms of yield was shown by the Maria variety (6.25 t/ha), in 2019 – by the Duma Odeska variety (7.73 t/ha), in 2020 – by the Ozerna variety (5.57 t/ha), in 2021 – Pamiati Hirka (6.87 t/ha), and in 2022 – the Duma Odeska variety (6.47 t/ha). The lowest grain yield in the same years was formed by the following varieties: Lehenda Bilotserkivska (4.72 t/ha and 6.15 t/ha) in 2018 and 2019, Katarina (3.12 t/ha) in 2020, Kvitka Poliv (6.01 t/ha) in 2021 and MIP Assol (4.53 t/ha) in 2022.

However, for each variety, there is an optimal plant height, at which it forms a higher grain yield. The present study confirms this. Thus, the highest grain yield (7.52 t/ha) was obtained in the Ozerna variety when the plant height was 99.0 cm, while the lowest yield was 5.34 t/ha (2018) with a plant height of 83.3 cm. For the Staleva variety, the optimal height of the plants, at which the crop is formed at the level of 7.09 t/ha, is 94.9 cm. A further increase in plant height to 101 cm (2021) led to a 9.3% decrease in yield. The varieties of winter wheat Kvitka Poliv, Lehenda Bilotserkivska and MIP Assol, which according to the height group are medium-sized, formed a higher grain yield (7.60, 6.23, and 6.50 t/ha, respectively), for plant heights of 113.3-122.5 cm (Fig. 3).



**Figure 3.** Optimum height and yield parameters of soft winter wheat varieties (average for 2018–2021), cm

**Source:** compiled by the authors

Optimum plant height parameters of wheat varieties of the short-stemmed winter group (Ozerna, Staleva, Mudrist Odeska, Duma Odeska, Koshova, Maria, Zdobna, Dyvo, Pamiati Hirka, Kraievdy), for which the grain yield was formed at 6.59–7.73 t/ha (2019 and 2021) were from 94.9 to 103.8 cm. Semi-dwarf varieties – Katarina, Felix, Ponticus, Faustus, MIP Valencia, Centurion, Glaucus – in 2021 produced a higher grain yield (6.37–6.75 t/ha) at plant heights of 73.5–94.6 cm.

An increase in the height of plants of the winter wheat varieties Mudrist Odeska, Duma Odeska, Koshova,

Maria, Zdobna, Dyvo, MIP Assol, MIP Valencia, Kraievdy, Centurion, Felix, Ponticus by 4.0–6.4% led to a decrease in the yield level from 4.5 (MIP Assol) to 20.9% (Kvitka Poliv). Many years of research by W.P. Bruening (2019) found that awned varieties of soft winter wheat formed a higher grain yield, compared to awnless forms, which had greater plant height and lesser lodging resistance. The present study partially confirms. Thus, the average plant height of the winter wheat varieties of awned forms was 89.9 cm, while in awnless ones – 85.6 cm, which is 4.4 cm less (Table 4).

**Table 4.** Winter wheat grain yield depending on the morphological characteristics of the variety (average for 2018–2022), t/ha

| Indicators                        | Varieties |         |
|-----------------------------------|-----------|---------|
|                                   | Awned     | Awnless |
| Average plant height, cm          | 89.9      | 85.6    |
| Average lodging resistance, score | 8.0       | 8.4     |
| Yield, t/ha                       | 5.89      | 5.55    |

**Source:** compiled by the authors

The average lodging resistance by varieties was higher in awnless forms and amounted to 8.4 points, which is 0.4 points higher than in spinous forms of winter wheat plants. It was found that among the winter wheat varieties under study, awned forms formed a higher grain yield (5.89 t/ha), which exceeded the yield of awnless forms by 5.8%. Among the varieties of winter

wheat under study, which were conditionally divided into three groups according to the group of maturity, the higher grain yield (5.88 t/ha) was formed by mid-early varieties (Ozerna, Staleva, Kvitka Poliv, Mudrist Odeska, Zdobna, Dyvo, Centurion), and the smallest – 5.66 t/ha – by short-season varieties (Duma Odeska, Koshova, Maria, Katarina, Pontikus, Faustus, Felix (Table 5).

**Table 5.** Grain yield of winter wheat depending on the maturity group of the variety (average for 2018-2022), t/ha

| Indicators                        | Varieties    |           |            |
|-----------------------------------|--------------|-----------|------------|
|                                   | Short-season | Mid-early | Mid-season |
| Average plant height, cm          | 80.7         | 91.5      | 93.0       |
| Average lodging resistance, score | 8.2          | 8.2       | 8.2        |
| Yield, t/ha                       | 5.71         | 5.88      | 5.66       |

**Source:** compiled by the authors

Thus, it can be assumed that awned short-season forms have greater resistance to drought, which is an essential indicator for the Southern Steppe zone of Ukraine, where studies were conducted. Li *et al.* (2022), Lollato *et al.* (2019) state that lodging resistance is highly dependent on the plant height of each variety. Therefore, the authors believe that this should be considered when choosing a variety for different soil and climatic conditions, predecessors, soil fertility. The present study does not support this statement. Thus, the medium-growing varieties Kvitka Poliv and MIP Assol, on average, over the years of research, had a high lodging resistance (8.0-8.2 points), while the short-stemmed ones (Zdobna, Koshova, and Duma Odeska) had an average resistance (6.2 points) and below average (6.0 points).

Research by Vlasenko *et al.* (2018) and Lavrinenko *et al.* (2020) conducted with 50 varieties of winter wheat found that the shorter the growing season of the genotype, the shorter the plants, which is also confirmed by the present study. It was found that among the 20 varieties under study, the highest plant height (76.3-106.5 cm) was formed by medium-ripening varieties Lehenda Bilotserkivska, MIP Assol, MIP Valencia, Pamiati Hirka, Kraievdyd, Glaucus, and the smallest (64.2-91.3 cm) – Duma Odeska, Koshova, Maria, Katarina, Ponticus, Faustus, and Felix.

Wang *et al.* (2022), Visioli *et al.* (2018) claim that as the height of winter wheat plants decreases, their productivity increases. Yes. Zaika (2021), F. Spolidorio (2019) prove that short-stemmed winter wheat has various sources of mechanisms affecting the redistribution of plastic substances from the vegetative to the generative part, so even tall varieties with high stem strength can exceed short-stemmed ones in terms of yield. This is confirmed by the present research.

Thus, on average, over the years of research (2018-2022), the yield of the medium-grown winter wheat variety Kvitka Poliv was 5.94 t/ha, which is 0.31-0.60 t/ha more than that of the semi-dwarf varieties Katarina, Centurion, Felix, Faustus, and Ponticus.

## CONCLUSIONS

According to the results of five-year research (2018-2022) in the conditions of the Southern Steppe of Ukraine with varieties of soft winter wheat with distinct morphological

characteristics, it was found that in the wet 2021, the height of plants of the short-stemmed varieties Mudrist Odeska (114.0 cm); Duma Odeska (106.0 cm); Maria (106.7 cm); Zdobna (106.5 cm); Pamiati Hirka (113.8 cm) and Kraievdyd (109.3 cm) were formed as medium-growing, while the varieties Kvitka Poliv and Lehenda Bilotserkivska, which belong to the group of medium-growing ones, formed a height of 125.5 and 122.5 cm, i.e., as in tall-growing. In the dry year of 2020, the semi-dwarf varieties Katarina (55.4 cm) and Felix (59.5 cm) formed the height of plants as dwarfs, and the short-stemmed ones – Ozerna (79.0 cm); Staleva (73.7 cm); Duma Odeska (75.3 cm); Koshova (80.0 cm); Maria (83.0 cm); Zdobna (77.6 cm); Dyvo (72.8 cm) – as semi-dwarves. The soft winter wheat variety MIP Assol, which in terms of height belongs to the medium-growing group, in 2020 formed the height of plants at the level of semi-dwarf plants – 77.9 cm.

The short-stemmed and semi-dwarf varieties Ozerna, Staleva, Katarina, Felix, Ponticus, Faustus, and Glaucus were very resistant to lodging (9 points), and the least resistant (6.2-6.6 points) were the medium-growing (Lehenda Bilotserkivska) and short-stemmed varieties (Duma Odeska, Koshova, Zdobna). The optimal height of winter wheat plants (94.9-100.7 cm) was established, at which a higher grain yield is formed at 7.09 t/ha in the Staleva variety to 7.73 t/ha in the Duma Odeska variety, which belong to groups of short-stemmed plants.

Thus, it was found that, on average, over five years of research (2018-2022), among the 20 varieties of winter wheat under study, spiny forms (Ozerna, Staleva, Mudrist Odeska, Duma Odeska, Koshova, Maria, Zdobna, Dyvo, MIP Valencia, Pamiati Hirka, Kraievdyd, Centurion) formed a 4.8% higher plant height and a 5.8% higher grain yield than awnless – Kvitka Poliova, Lehenda Bilotserkivska, MIP Assol, Katarina, Felix, Ponticus, Faustus, and Glaucus. Mid-season varieties (Ozerna, Staleva, Kvitka Poliv, Mudrist Odeska, Zdobna, Dyvo, and Centurion) produced 2.9% more grain yield than short-season ones (Duma Odesa, Koshova, Maria, Katarina, Ponticus, Faustus, and Felix) and 3.7% more than mid-season ones (Lehenda Bilotserkivska, MIP Assol, MIP Valencia, Pamiati Hirka, Kraievdyd, and Glaucus). In the future, it is planned to analyse the influence of weather conditions on the duration of the main interphase

periods, the coefficient of productive tillering, the number of internodes, their length, and the weight of grain from 1 ear, which will give a more complete characterization of the winter wheat varieties under study.

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### Роль висоти рослин пшениці озимої у формуванні врожайності зерна залежно від сортових особливостей та погодних умов

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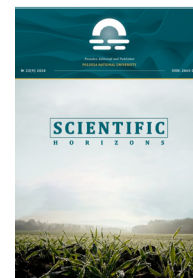
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**Анотація.** Пшениця озима є однією з основних продовольчих культур, що забезпечує національну продовольчу безпеку України загальним виробництвом та високоякісним зерном. Площа посіву пшениці озимої займає перше місце в Україні, а виробництво зерна високої якості має актуальне значення. У технології його вирощування вирішальним фактором підвищення врожайності та покращення якості зерна є добір сортів. Метою роботи було визначити вплив погодних умов та особливостей сорту протягом року на висоту рослин, стійкість до вилягання та врожайність пшениці озимої. У статті наведено дані про результати дослідження 20 сортів пшениці м'якої озимої в умовах Навчально-практичного центру Миколаївського НАУ з 2017 по 2022 роки. У ході дослідження використовувалися загальноприйняті методи: системного підходу, системного аналізу, аналіз та синтез, польовий та статистичний метод. Проаналізовано вплив особливостей сорту та погодних умов у досліджувані роки на врожайність пшениці м'якої озимої. Встановлено, що оптимальна висота рослин сортів пшениці озимої коливалась від 94,9 до 100,7 см, за якої формується найвищий врожай зерна на рівні від 7,09 т/га у сорту Сталева до 7,73 т/га у сорту Дума одеська. Збільшення висоти рослин досліджуваних сортів пшениці озимої на 4,0-6,4% призводило до зниження рівня врожайності від 4,5 до 20,9%. Встановлено, що остисті сорти пшениці м'якої озимої формували більшу врожайність зерна, порівняно з безостими формами, які мали більшу висоту рослин та меншу стійкість до вилягання. Проведеними дослідженнями підтверджено, що у середньому по сортах стійкість до вилягання була вища саме у безостих форм рослин пшениці озимої, що на 0,4 бали вище, ніж у остистих. Більшу середню урожайність зерна пшениці м'якої озимої сформовано сортами остистих форм, що на 5,8% більше, ніж у остистих. Отримані наукові результати досліджень сприятимуть широкому застосуванню досліджуваних сортів пшениці м'якої озимої для даної ґрунтово-кліматичної зони, що в подальшому сприятиме підвищенню врожайності та валових зборів зерна

**Ключові слова:** пшениця м'яка озима, стійкість до вилягання, остисті та безості форми, температура повітря, сума опадів

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## Efficiency of the Sowing Date of Spinach in the Southern Steppe Conditions of Ukraine

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**Abstract.** The population of Ukraine should receive fresh greens from early spring to autumn from open field production and the period of growing spinach will ensure the supply of fresh greens. The purpose of the research was to study the possibility of extending the period of fresh greens supplying from the open field due to the extension of the seed sowing period in the open field. The research was conducted in 2019-2021 in the conditions of the Southern Steppe of Ukraine. For the research, field, statistical, calculation-analytical and laboratory methods were used. The varieties Fantaziia and Malakhit were studied. The behaviour of plants after six sowing dates was examined. The control variant was the variety Fantaziia for sowing in the 1<sup>st</sup> decade of April. It was found that plants of both varieties of spinach had a larger leaf surface (114.2-127.7 cm<sup>2</sup>) in the early sowing period. Plants which were sown in August had a smaller leaf surface – 86.0-106.2 cm<sup>2</sup>. The highest yield of marketable green mass was obtained for sowing in the 3<sup>rd</sup> decade of April and 2<sup>nd</sup> decade of May. The variety Fantasia provided 22.9-23.0 t/ha, and the variety Malakhit provided 23.3-23.9 t/ha. The sowing date had a significant influence on the main chemical parameters of spinach of the Fantaziia and Malakhit varieties. The higher indicators were the sowing dates in the third decade of April and the second decade of May, when the brix reached the level of 6.4-6.8%, the sugars content – 2.3-2.4% and the content of vitamin C – 58-70 mg/100 g. It was established that the soil and climatic conditions of the Southern Steppe of Ukraine were suitable for sowing spinach in six dates and would ensure an uninterrupted supply of products from the 3<sup>rd</sup> decade of April to the end of September inclusive, and in years with favourable weather conditions in the 2<sup>nd</sup> to 3<sup>rd</sup> decades September to mid-October. Such approach will solve the problem of seasonality in the consumption of fresh vegetables. The sowing dates are recommended to agricultural producers of spinach in order to extend the period of receiving fresh greens from the 3<sup>rd</sup> decade of April to the end of September and in some years to the middle of October

**Keywords:** total leaves surface, leaves number, plant height, weight of the plant, beginning of rosette growth, technical maturity



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## INTRODUCTION

Establishment of optimal sowing dates for uninterrupted supply of marketable greens and consumption during the spring-summer-autumn period in fresh form, correlation between plant growth and productivity, determines the relevance of this study. It is known from research of Hospodarenko *et al.* (2008) that from early to late sowing, the accumulation of dry matters and brix consistently decreases. The intensity of the processes of plant and stem death during the spring-summer vegetation period decreases. According to the research of Ulianych *et al.* (2018), at the optimal sowing date plants are programmed for high yield, but their productivity decreases both at early and at late sowing date. In the first case, the plant forms a larger vegetative mass and, because of overgrowth, intensively uses reserve substances and becomes less resistant to adverse conditions. In addition, the plant of the early sowing is more damaged by pests, diseases, and is often weedier. Because weeds, by their biological characteristics, are more competitive with plants, they outgrow and shade them, take more nutrients and moisture. All this leads to slowdown in the growth of cultivated plants, thinning of crops and a decrease in yield founded by Korniyenko *et al.* (2015).

Plants of the late sowing grow and develop more slowly, do not have time to form a full-fledged rosette and sufficient above-ground mass and secondary root system shown in research of Ulianych *et al.* (2015).

The study of the dependence of the sowing date on soil fertility of Galla *et al.* (2013) and Barcelos *et al.* (2017) showed that it was necessary to sow earlier on poor soils, and later fertile ones, so that the plants did not overgrow. The optimal sowing date on fertilized fields is shifted 10-15 days later, compared to sowing on a less fertilized field mentioned in research of Gutierrez-Rodriguez *et al.* (2013). Positive impact on yield has organic fertilizers and sea weed extractions mentioned in researches of Kim *et al.* (2018), Xu & Leskovar (2015) and Fan *et al.* (2013). Using of bio stimulants, besides regular fertilizing has positive impact on yield and leaves quality was found in research of Fiorentino *et al.* (2018) and Kulkarni *et al.* (2019).

Sowing date is important as environmental temperature influencing on plants. In autumn, and spring the effect of the maximum, minimum and average temperature was dominant, but in summer, only the average and maximum temperature seemed significant concluded in research of Zuska *et al.* (2019). The sowing date varies depending on the biological characteristics of the variety, and for classic varieties, the interval of the optimal sowing period is longer. Studies of Khareba *et al.* (2012) are recommending calendar dates for sowing 6-12 days later, compared to previously grown varieties, which is due to the biological features of the modern variety. And this is a shortened epicotyl, faster passage of the interphase period, more intense formation of plastic substances and formation of above-ground mass.

The results of observations of the growth and development of plants in the autumn period showed by research of Golubkina *et al.* (2017). It showed that the degree of plant development, especially the accumulation of dry matter, was not directly related to the level of adaptive characteristics. They are formed higher during optimal and late sowing. Plants of different ages do not equally consume water from the soil. Van Treuren *et al.* (2012) shows then crops of early sowing, as they are more physiologically old, use less water than plants of optimal and late sowing.

For vegetables, in particular of spinach, the sowing date is important, because the optimal sowing date will help to increase the yield due to the full growth and development of the plant mentioned by M. Pollock (2012). Therefore, in order to obtain a high yield of green mass several times during the growing season in the Southern Steppe of Ukraine, research was conducted to study the effectiveness of the sowing date for growing spinach. As a result of the conducted experimental study, new elements of the technology of growing spinach were investigated. For the first time, in the study of by Panda *et al.* (2017), the biological ability of new varieties of spinach to form a high yield of marketable green mass and their actual productivity has been theoretically substantiated and experimentally proven.

*The aim of the current research* was to find the optimal timing of sowing spinach seeds and obtaining high yields.

## MATERIALS AND METHODS

The research on determining the optimal sowing date of spinach in the Southern Steppe of Ukraine was conducted in 2019-2021 in open field conditions on a field with drip irrigation. The influence of the sowing date on the growth, development and yield of spinach varieties Fantaziia and Malakhit was determined. The methodical recommendations of Bondarenko and Yakovenko (2001) were used.

In order to ensure a constant supply of fresh products during the period from spring to autumn, the following six sowing dates were studied: the 1<sup>st</sup> and 3<sup>rd</sup> decades of April, the 2<sup>nd</sup> decade of May, the 2<sup>nd</sup> decade of June, and the 1<sup>st</sup> and 3<sup>rd</sup> decades of August. The control variant was drilled in first decade of April. The research was conducted on light sandy chernozems. The humus horizon in the 0-20 cm soil layer contained 1% of humus, and 0,6% of humus in the 20-40 cm soil layer. Chemical characteristics of the soil: organic carbon – 2.1%, pH 6.9-7.4, P – 92 mg/kg-1, K – 101 mg/kg-1, N-NO<sub>3</sub> – 17 mg/kg-1. The electrical conductivity (EC) of the soil was measured by using a conductometer. P, K were determined by the AB-DTPA method according to Ryan *et al.* (2001). The sowing conducted by the density of 150 000 seeds per hectare. The total acreage for the experiment was 360 m<sup>2</sup>, for the single plot it was 60 m<sup>2</sup>.

The experiment was designed as a Full Randomized with four replications.

**Biometric research.** The length and width of the leaf, the surface of the leaf plate and the total leaf surface of the plant were determined. The height of the plant and the number of leaves on the plant were count. The sowing dates of spinach, plant productivity, vitamin C were studied. All analyses were performed in four repetitions.

**Plant material.** Spinach (*Spinacea oleraceae* L.) varieties Fantaziia and Malakhit.

The analysis of vitamin C was held as follows: Lyophilized samples of leaves (0.2 g) were crushed and adding to 30 ml of a 3% metaphosphoric acid solution with followed homogenized at 11,000 rpm for 2 min by using a homogenizer (T25 basic ULTRA-TURRAX – IKAWerke GmbH. & Co. KG, Staufen, Germany). The volume was adjusted to 50 ml with a 3% solution of metaphosphoric acid. The 2 ml of extract was centrifuged at 12000 rpm for 3 min. The supernatant was filtered through polyvinylidene difluoride membrane filter of 0.45  $\mu\text{m}$  (Whatman International Ltd., Maidstone, UK). The fresh samples were analysed by using an HPLC system equipped with a PU (pump 2089), MD (2010 UV), AS (autoinjector 2057) variable wavelength detector (JASCO Corp., Tokyo, Japan). The separation made on a Crest Pak C18S column (15094.6 mm, inner diameter, 5  $\mu\text{m}$ , JASCO Corp.). The peak was reading at 254 nm using a UV detector. The quantification was determining by external calibration with the ascorbic acid.

**Weather conditions during the research years.** According to the data of the Nova Kakhovka hydrometeorological station (n.d.), the hydrometeorological conditions of 2019 were characterized as a slightly lower amount of precipitation compared to the average long-term indicators. The amount of precipitation for this period in 2020 was significantly higher than in 2019. 2021 was the rainiest year. The most precipitation fell in June, which allowed for better forming of leaf mass the plants. The air temperature in 2019-2021 at the sowing time was slightly higher than the perennial one, which had a positive effect on the development of spinach plants.

**Statistical analysis.** The nutritional and chemical compositions were analysed in four replications. Results were expressed as averages. Chemical composition was analysed using analysis of variance with  $P \leq 0.05$  for yield, plant weight and productive moisture storage by using the program of statistical analysis (SAS) v. 9.1.3.

## RESULTS AND DISCUSSION

The sowing of the spinach at different dates shows a positive effect on the results of farming and allowed obtaining reliable data on the plasticity of the crop in terms of growing conditions. The assessment of the influence of the date of the seeds sowing on the growth

and development of the spinach under the conditions of growing in open field was conducted based on the fixation of the duration of the phenological phases of plant growth, their development and other indicators. The analysis of the data obtained as a result of research on the growth of the spinach plants and their development in the Southern Steppe of Ukraine showed that the duration of the periods from the germination to the onset of the main phenological phases was the shortest for sowing seeds in the I and II decades of August, regardless of the studied variety. The mass germination appeared on the 8<sup>th</sup> day in the control variety and on the 7<sup>th</sup> day in the Malakhit variety due to sowing seeds in the I decade of April. The mass germination of both researched varieties was noted for 6 days due to sowing seeds in the III decade of April and II decade of May, as well as in the Fantasia variety due to sowing in the II decade of June. During the August sowing dates, mass germination was recorded at 5<sup>th</sup> day, regardless of the studied variety, which can be associated with the influence of high environmental temperatures.

The phase of the appearance of the first true leaf in spinach was recorded after 10-17 days, depending on the variant of the experiment, and it was noted after 12-17 days for the Fantasia variety, and 10-15 days for the Malakhit variety. The plants reached the technical phase of maturity on the 40<sup>th</sup> day after the appearance of germination in both studied varieties due to sowing seeds in the III decade of April and on the 41<sup>st</sup> day due to sowing seeds in the II decade of May. The summer sowing date showed that the onset of the technical maturity phase did not depend on the variety and occurred on the 37<sup>th</sup> and 38<sup>th</sup> day.

The study of the effect of the variety and date of sowing seeds on the duration of the growth phases proves that for all sowing dates the advantage is observed in plants of the Malakhit variety - the phase of technical maturity came 1-4 days earlier than in the Fantaziia variety. In its turn, studies showed that the difference in the speed of passage of phenological phases by spinach plants was more pronounced depending on the date of sowing than on the variety.

The influence of the sowing date of spinach and the variety is characterized by differences in biometric indicators. The evaluation of the plants of the Fantaziia and Malakhit varieties in the phase of the beginning of rosette growth shows that plants had height of 6.3-7.3 cm due to the sowing in the III decade of April and in the II decade of May and were taller compared to the plants of later sowing. This fact can be explained by the fact that at lower temperatures the growing season is longer. An exception to this pattern can be considered the sowing period in the I decades of April, when at the beginning of the growing season, low temperatures, on the contrary, slowed down the growth and development of spinach plants (Table 1).

**Table 1.** The height of the spinach plant depends on the variety, cm

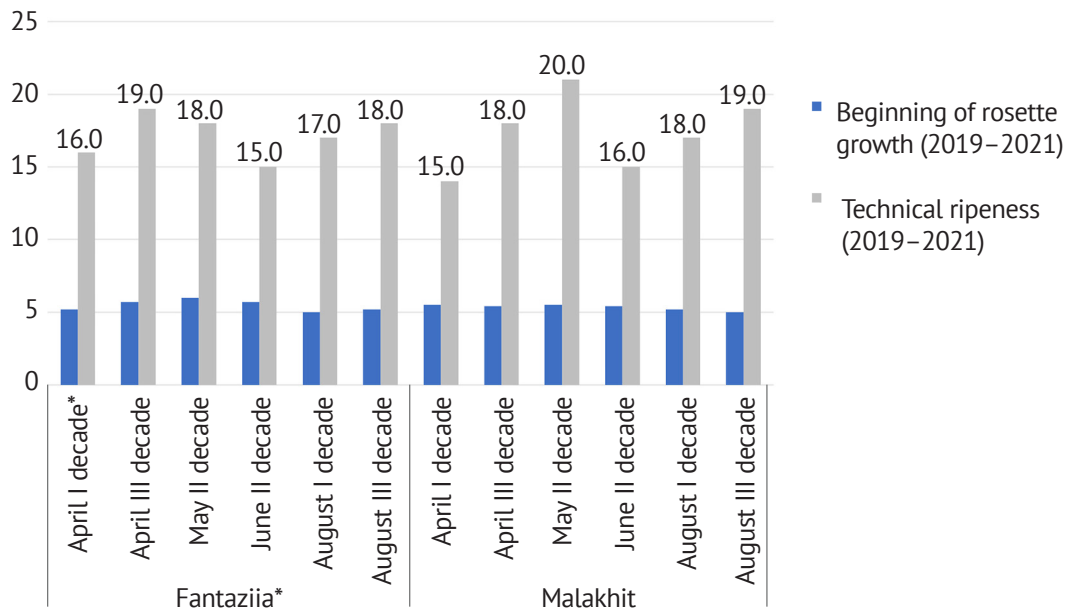
| Variety            | Sowing date       | Beginning of rosette growth |      |      |           | Technical maturity |      |      |           |
|--------------------|-------------------|-----------------------------|------|------|-----------|--------------------|------|------|-----------|
|                    |                   | 2019                        | 2020 | 2021 | 2019-2021 | 2019               | 2020 | 2021 | 2019-2021 |
| Fantaziia          | April I decade*   | 4.8                         | 5.4  | 5.1  | 5.1       | 24.4               | 23.8 | 26.2 | 24.8      |
|                    | April III decade  | 6.8                         | 7.4  | 7.1  | 7.1       | 30.5               | 25.4 | 30.8 | 28.9      |
|                    | May II decade     | 6.8                         | 7.3  | 6.7  | 6.9       | 27.5               | 26.3 | 26.1 | 26.6      |
|                    | June II decade    | 6.7                         | 6.3  | 6.5  | 6.5       | 19.3               | 19.7 | 19.2 | 19.4      |
|                    | August I decade   | 5.3                         | 5.4  | 4.9  | 5.2       | 16.2               | 15.9 | 15.7 | 15.9      |
|                    | August III decade | 5.4                         | 5.6  | 7.1  | 6.0       | 17.4               | 16.7 | 16.9 | 17.0      |
| Malakhit           | April I decade    | 5.8                         | 5.5  | 5.1  | 5.5       | 29.4               | 24.8 | 30.4 | 28.2      |
|                    | April III decade  | 7.4                         | 7.2  | 7.2  | 7.3       | 25.5               | 26.4 | 25.2 | 25.7      |
|                    | May II decade     | 5.7                         | 7.1  | 6.2  | 6.3       | 24.8               | 28.5 | 26.3 | 26.5      |
|                    | June II decade    | 4.8                         | 5.4  | 5.1  | 5.1       | 21.1               | 22.8 | 18.6 | 20.8      |
|                    | August I decade   | 5.3                         | 5.7  | 5.4  | 5.5       | 17.8               | 16.9 | 18.1 | 17.6      |
|                    | August III decade | 6.2                         | 5.8  | 5.1  | 5.7       | 16.9               | 17.8 | 17.5 | 17.4      |
| LSD <sub>0.5</sub> |                   | 0.4                         | 0.3  | 0.5  |           | 1.4                | 1.2  | 0.8  |           |

**Note:** \* – control variant

**Source:** compiled by the authors

It was noted that the height of spinach plants depended to a considerable extent on the sowing date and not on the variety. On average, over the years of research, the height of city spinach plants in the phase of green technical maturity, was 24.8-28.2 cm, depending on the variety due to sowing in the I decade of April. The research indexes differed and in the Fantaziia variety they increased to 26.6-28.9 cm, while in the Malakhit variety,

on the contrary, they decreased to 25.7-26.5 cm due to the sowing of spinach in the III decade of April and in the II decade of May. The indexes of both studied varieties exceeded the control by 1.8-4.1 cm and 0.9-1.7 cm, respectively. It has been established that in spinach the number of leaves per plant determines the potential value of the yield, therefore, in the conducted observations, attached great importance to this index (Fig. 1).



**Figure 1.** The dynamics of the leaves number increase of the spinach in different periods of growth and development depending on the variety and sowing date (2019-2021)

**Source:** compiled by the authors

In the phase of the beginning of plant rosette growth the number of leaves was, depending on the

investigated variety, from 5.0 to 6.0 pcs/plant. Observations of 2019-2021 showed us that early sowing

dates, especially the III decade of April, the II decade of May and the II decade of June, were more conducive for the Fantaziia variety, as one plant formed a larger number of leaves from 5.7 to 6.0 cs/plant. At the time when a significantly lower number of leaves from 5.0 to 5.2 pcs/plant was observed due to late sowing and sowing in the I decade of April. In its turn, in the Malakhit variety, the number of leaves varied slightly regardless of different sowing dates and ranged from 5.0 to 5.4 leaves per plant.

It was noted that the studied varieties had different numbers of leaves at the phase of the beginning of the rosette growth. High variability of the index was noted

in the Fantaziia variety with the highest index due to the sowing in the II decade of May. During the studies of the influence of the variety and sowing date on the number of leaves and their growth throughout the growing season, it should be noted that at the harvesting date the number of leaves increased by 2.5-3.5 times, from 5.0-6.0 to 14-21 pcs/plant. One of the important indexes of spinach plant growth, which determines their value as a green plant, is the leaf surface and the total leaves surface. The determination of these indexes depending on the date of sowing was conducted at the beginning of the rosette growth and in the phase of technical maturity of the greenery before the bolting (Table 2).

**Table 2.** The spinach leaf surface in different mode of growth and development depending on the variety and sowing date, cm<sup>2</sup>

| Variety            | Sowing date       | Beginning of rosette growth |      |      |           | Technical maturity |       |       |           |
|--------------------|-------------------|-----------------------------|------|------|-----------|--------------------|-------|-------|-----------|
|                    |                   | 2019                        | 2020 | 2021 | 2019-2021 | 2019               | 2020  | 2021  | 2019-2021 |
| Fantaziia          | April I decade*   | 18.8                        | 17.7 | 18.5 | 18.3      | 110.3              | 108.3 | 107.2 | 108.6     |
|                    | April III decade  | 20.4                        | 21.7 | 23.8 | 22.0      | 122.4              | 120.6 | 122.0 | 121.7     |
|                    | May II decade     | 21.5                        | 20.8 | 19.9 | 20.7      | 128.5              | 128.7 | 125.9 | 127.7     |
|                    | June II decade    | 21.8                        | 20.9 | 20.5 | 21.1      | 105.8              | 107.8 | 111.4 | 108.3     |
|                    | August I decade   | 17.4                        | 16.7 | 17.1 | 17.1      | 96.4               | 86.3  | 76.2  | 86.3      |
|                    | August III decade | 18.4                        | 18.1 | 18.5 | 18.3      | 84.6               | 88.4  | 85.1  | 86.0      |
| Malakhit           | April I decade    | 24.1                        | 21.3 | 21.7 | 22.4      | 120.1              | 110.6 | 115.2 | 115.3     |
|                    | April III decade  | 25.7                        | 22.1 | 22.9 | 23.6      | 130.1              | 121.4 | 124.7 | 125.4     |
|                    | May II decade     | 22.3                        | 21.5 | 22.2 | 22.0      | 115.7              | 110.5 | 116.5 | 114.2     |
|                    | June II decade    | 21.7                        | 20.7 | 22.5 | 21.6      | 114.4              | 117.7 | 118.0 | 116.7     |
|                    | August I decade   | 19.4                        | 18.3 | 18.8 | 18.8      | 111.5              | 101.5 | 105.6 | 106.2     |
|                    | August III decade | 19.1                        | 18.1 | 18.3 | 18.5      | 109.9              | 102.2 | 104.1 | 105.4     |
| LSD <sub>0.5</sub> |                   | 0.5                         | 0.2  | 0.3  |           | 4.3                | 3.9   | 2.8   |           |

**Note:** \* – control variant

**Source:** compiled by the authors

It was established that plants of the Fantaziia variety had a larger leaf surface due to sowing in the I decade of April – 22.0 cm<sup>2</sup>. The smallest leaf surface in the Fantaziia variety was found due to sowing seeds in the I decade of August – 17.1 cm<sup>2</sup>. At the same time, in the Malakhit variety, the smallest leaf surface was observed due to sowing in the last term – 18.5 cm<sup>2</sup>, and the largest due to the III decade of April – 23.6 cm<sup>2</sup>. The study of the influence of sowing dates on the leaf surface index proves that it is larger at the beginning of growth due to sowing in the III decade of April and the II decade of May, regardless of the variety.

At the phase of technical maturity, before harvesting the spinach plant in both researched varieties, a smaller leaf surface was observed in the August sowing dates and was from 86.3 to 86.0 cm<sup>2</sup> due to the Fantaziia variety and from 106.2 to 105.4 cm<sup>2</sup> due to the Malakhit variety. At the technical maturity mode, plants of the Fantaziia variety of spinach had a larger leaf surface due to sowing in the III decade of April and II decade of

May – 121.7 and 127.7 cm<sup>2</sup>, respectively, and in the Malakhit variety due to sowing in the III decade of April – 125.4 cm<sup>2</sup>. The study of the influence of the sowing date on this index proves that the surface of the leaf plate in spinach plants had bigger values at the beginning of growth in the early dates, apart from the first one.

During 2019-2021, the dynamics of the growth of the leaf surface depending on the date of sowing were studied in detail, and data were obtained and factors that could influence the increase in the leaves surface during the growing season and on the eve of harvesting, depending on the variety and the date of sowing in open field were analysed. The data received shows that the leaves surface of the spinach plants in 2019-2021 at the beginning of growth was found to have a larger the leaves surface of Fantaziia spinach plants that were sown in the II decade of May – 1.72 thousand m<sup>2</sup>/ha, which exceeded the control by 0.40 thousand m<sup>2</sup>/ha. In the Malakhit variety, during the three early sowings, the index almost did not vary and was in the range from

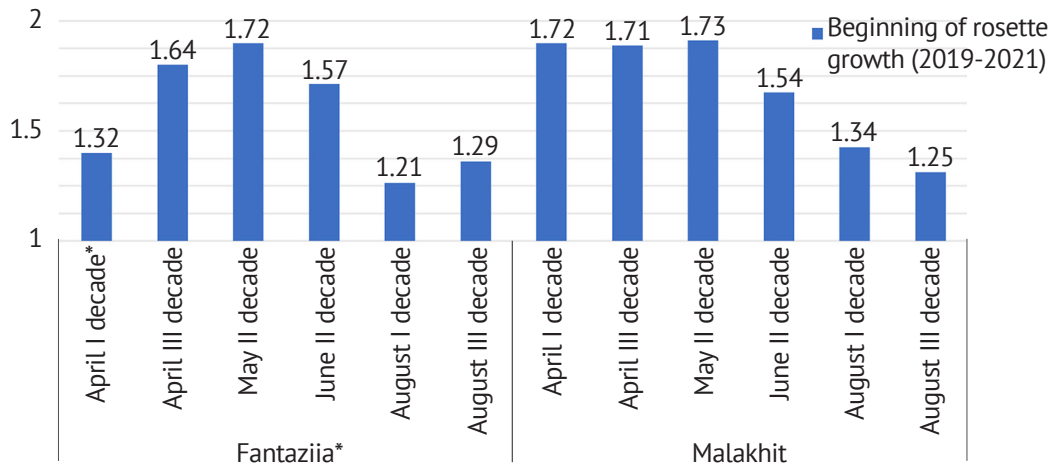
1.71 to 1.73 thousand  $m^2/ha$ , which exceeded the control by 0.39-0.41 thousand  $m^2/ha$ . A smaller leaves surface was obtained in the summer sowing dates in both studied varieties of spinach.

It was established that the August sowing dates did not contribute to obtaining a large leaves surface, and for sowing in the I and III decades of August it amounted to 1.21-1.29 thousand  $m^2/ha$  in the Fantaziia variety, according to the sowing date, which was lower than control by 0.11-0.03 thousand  $m^2/ha$ . In plants of the Malakhit variety, this index in August was 1.34 and 1.25 thousand  $m^2/ha$ , respectively. At the technical maturity mode, the leaf surface reached the value of 25.6 thousand  $m^2/ha$  of Fantaziia variety due to sowing in the I decade of April. In the Malakhit variety during the early sowing date the leaf surface was 23.9 thousand  $m^2/ha$ , which is lower than the control by 1.7 thousand  $m^2/ha$ .

The larger leaves surface was formed by spinach varieties Fantaziia and Malakhit due to the seeds sowing in the III decade of April – 30.8-33.4 thousand  $m^2/ha$ ,

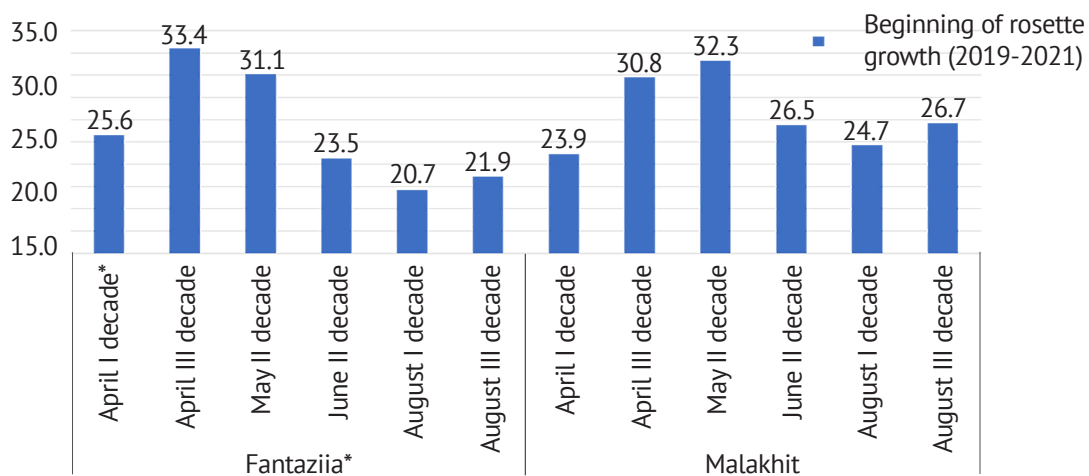
according to the variety, which exceeded the control by 5.2-7.8 thousand  $m^2/ha$ . In the second decade of May plants had a leaf surface in this phase of 31.1-32.3 thousand  $m^2/ha$ , according to the variety, which exceeded the control by 5.5-6.7 thousand  $m^2/ha$ . The leaves surface largely depended on the weather conditions of the year. Thus, plants grown in 2020 had the smallest leaf surface, which is explained by the unfavourable weather conditions of the year, especially the lack of moisture during the period of intensive plant growth and development.

Observation of the growth and development of plants showed that in the first period of growth, spinach grows slowly and forms an insignificant leaf mass, but during the period of technical maturity the plant significantly increases in size. In the phase of intensive growth, plants sown in the open field in the III decade of April had a larger leaves surface, regardless to the variety. At the time of the plant density forming the leaf surface was from 28.9 to 32.9 thousand  $m^2/ha$  (Figs. 2; 3).



**Figure 2.** The dynamics of the leaves surface increase on the beginning of rosette growth of spinach varieties Fantaziia and Malakhit depending on the sowing date (2019-2021), thousand  $m^2/ha$

Source: compiled by the authors



**Figure 3.** The dynamics of the leaves surface increase on the technical maturity phase of spinach varieties Fantaziia and Malakhit depending on the sowing date (2019-2021), thousand  $m^2/ha$

Source: compiled by the authors

The tendency of the leaves surface decreasing with each next sowing in the variety Fantaziia was observed. Thus, the leaves surface reached 33.4 thousand m<sup>2</sup>/ha due to second sowing date in the III decade of April. It decreased to 31.1 thousand m<sup>2</sup>/ha due to II decade of May and to 23.5 thousand m<sup>2</sup>/ha due to II decade of June. In the late summer sowing dates, the leaf surface had a minimum value and amounted to 20.7 thousand m<sup>2</sup>/ha due to sowing in the I decade of August, and 21.9 thousand m<sup>2</sup>/ha due to III decade, which was caused by high temperature conditions of growth.

In spinach varieties Fantaziia and Malakhit before harvesting the green mass, the plants had the largest leaves surface after sowing in the III decade of April – 33.4-30.8 thousand m<sup>2</sup>/ha and 31.1-32.3 thousand m<sup>2</sup>/ha in the II decade of May. Plants of the Fantaziia variety sown in the II decade of May had a smaller leaves surface on the plant – 31.1 thousand m<sup>2</sup>/ha. In the late summer sowing dates, the leaves surface of Fantasia variety was minimal and was 20.7 thousand m<sup>2</sup>/ha during sowing in the II decade of August, and 21.9 thousand m<sup>2</sup>/ha in the III decade, which was caused by high temperature conditions.

An index of the efficiency of varieties and growing periods of spinach in the open field is the output from one plant and from a unit of acreage. The results of the conducted research show that the variety and sowing date have a significant effect on the weight of the plant since the connection between the yield of commercial greens and the weight of one plant is quite high. It has been proven that the largest weight of a spinach plant

was obtained by using early sowing dates and when sowing spinach in the I decade of April. At the beginning of the rosette growth the mass of the Fantaziia variety over the years of research reached 2.0 g, in the III decade of April – in the II decade of May – 2.5-2.8 g.

In the Malakhit variety, in the I decade of April at the beginning of rosette growth, the weight of the plant reached 2.5 g. In the III decade of April and the II decade of May it reached from 2.7 to 2.8 g, respectively. Next years the weight of the plant was on the level from 2.3 to 2.8 g in accordance to sowing date. With the use of early sowing dates for sowing of spinach in the I decade of April in the phase of technical maturity, the mass of the Fantaziia plant over the years of research reached 57.5 g, in the III decade of April – II decade of May – from 66.4 to 70.0 g. At the next years the plant weight index was smaller and amounted to 60.5-65.0 g, depending on the sowing date. In the Malakhit variety, in the I decade of April in the phase of technical maturity, the weight of the plant reached 57.0 g, in the III decade of April and the II decade of May was from 62.4 to 67.3 g, respectively.

Therefore, later sowing dates in the Southern Steppe of Ukraine coincide with high temperatures during the growth of plants. It has a detrimental effect on plants, and therefore, in later sowing dates, especially in late summer, they were smaller in weight. The main assessment of the level of influence of the variety and sowing date on the growth and development of spinach plants of the Fantaziia and Malakhit varieties is conducted based on the results of the analysis of the productivity of marketable green mass (Table 3).

**Table 3.** The yield of spinach depending on the variety and sowing date, t/ha

| Variety           | Sowing date       | 2019 | 2020 | 2021 | 2019-2021 | ± to control |
|-------------------|-------------------|------|------|------|-----------|--------------|
| Fantaziia         | April I decade*   | 19.6 | 20.5 | 18.9 | 19.7      | 0            |
|                   | April III decade  | 20.9 | 24.7 | 23.3 | 23.0      | 3.3          |
|                   | May II decade     | 21.7 | 23.8 | 23.2 | 22.9      | 3.2          |
|                   | June II decade    | 21.3 | 22.2 | 21.9 | 21.8      | 2.1          |
|                   | August I decade   | 19.9 | 21.8 | 21.5 | 21.1      | 1.4          |
|                   | August III decade | 20.6 | 22.7 | 21.9 | 21.7      | 2.1          |
| Malakhit          | April I decade    | 19.8 | 22.8 | 19.6 | 20.7      | 1.1          |
|                   | April III decade  | 22.5 | 25.5 | 23.6 | 23.9      | 4.2          |
|                   | May II decade     | 20.4 | 24.7 | 24.9 | 23.3      | 3.7          |
|                   | June II decade    | 20.8 | 22.8 | 22.7 | 22.1      | 2.4          |
|                   | August I decade   | 19.9 | 20.2 | 21.6 | 20.6      | 0.9          |
|                   | August III decade | 20.6 | 21.6 | 22.8 | 21.7      | 2.0          |
| LSD <sub>05</sub> | Factor A          | 0.3  | 0.4  | 0.2  |           |              |
|                   | Factor B          | 0.7  | 0.6  | 0.5  |           |              |
|                   | Interaction AB    | 1.4  | 1.3  | 1.1  |           |              |

**Note:** \* - control variant

**Source:** compiled by the authors

According to the table data, it is possible to trace the change in yield index of spinach depending on the

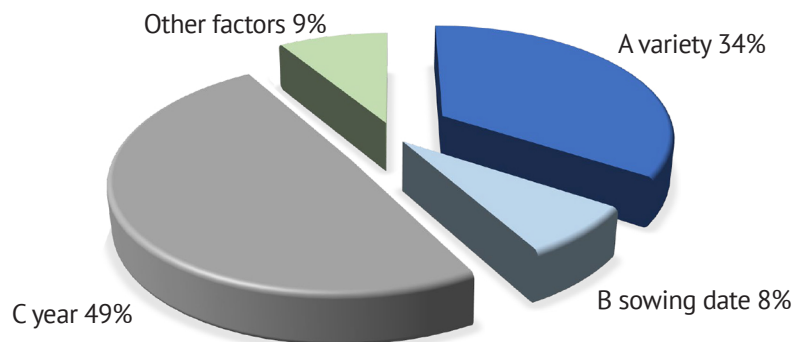
variety and date of sowing in open field and the year of research, when the weather conditions were not the same

and mostly dry in 2019. Accordingly, the yield analysis shows that it was lower in 2019 by 19.6-22.5 t/ha, which is explained by unfavourable weather conditions during the growing season. The years 2020 and 2021 were characterized by more favourable conditions, in which the yield of marketable green mass was higher and during the early sowing dates it reached the level of 23.2-24.7 t/ha in spinach of the Fantaziia variety and 23.6-25.5 t/ha in the Malakhit variety. It was received significant yield growth at level from 3.3 to 6,0 t/ha. At the next sowing dates in the II decade of June and I and II decades of August, the productivity of the Fantaziia variety was 21.5-22.7 t/ha and Malakhit 20.2-22.8 t/ha respectively.

On average, over the years of research, the highest yield, depending on the variety, was obtained for sowing in the III decade of April and the II decade of May in the Fantaziia variety – from 22.9 to 23.0 t/ha, in the Malakhit variety – from 23,3 to 23.9 t/ha, which is

higher than the control by 3.3-4.2 t/ha. A low yield was obtained from plants sown in the I decade of April and in summertime. So, when sowing spinach varieties Fantaziia and Malakhit in the I decade of April, the yield was only 18.9 and 19.6 t/ha, respectively, according to the studied variety. In the II decade of June, the yield of both varieties decreased to the level of 21.8-22.1 t/ha, which was higher than the control by 2.1-2.4 t/ha. During the late summer sowing dates in August, the yield of commercial greens was 20.6-21.7 t/ha, which is higher than the control by 0.9-2.1 t/ha.

The  $LSD_{0,5}$  in quantitative terms, according to factors A and B for spinach yield was 0.2-0.7 t/ha, which indicates reliable values between their replications and variants. Three factors together (year – 49%, genetical potential of variety – 34%, sowing date – 8%) form 91% of the spinach yield in the conditions of the Southern Steppe of Ukraine (Fig. 4).



**Figure 3.** The dynamics of the leaves surface increase on the technical maturity phase of spinach varieties Fantaziia and Malakhit depending on the sowing date (2019-2021), thousand m<sup>2</sup>/ha

**Source:** compiled by the authors

As a result of the conducted research, there was a need to establish important characteristics for spinach,

which was conducted on the basis of calculations of correlations between biometric and productive indexes (Table 4).

**Table 4.** Matrix of correlation analysis of biometric and productive indexes of the spinach (2019-2021)

| Index                        | Plant height, cm | The leaves number, pcs/plant | Plant weight, g |
|------------------------------|------------------|------------------------------|-----------------|
| Plant height, cm             | –                | –                            | –               |
| The leaves number, pcs/plant | 0.23             | –                            | –               |
| Plant weight, g              | 0.33             | 0.98                         | –               |
| Yield, t/ha                  | 0.57             | 0.75                         | 0.91            |

**Source:** compiled by the authors

It was found that there is a strong positive correlation between plant weight and the number of leaves ( $r=0.98$ ), the yield of spinach and the plant weight ( $r=0.91$ ). In parallel with the study of productivity, an assessment of the quality of products of spinach varieties was conducted according to chemical parameters in commercial products before harvesting, depending on the variety and date of sowing in open field (Table 5). The analysis of the

obtained data showed that the variety and sowing date influenced the main indexes of the chemical composition of the green mass of the spinach varieties Fantaziia and Malakhit. The higher content of chlorophyll (a+b) – 0.445 ml/l was observed in plants that were sown in the III decade of April. The content of nitrates in spinach plants was at a low level in the range from 48 to 55 mg/kg and did not exceed the MAC for green leaves of spinach.

**Table 5.** Indexes of the chemical composition of spinach in different periods of growth and development depending on the variety and date of sowing (2019-2021)

| Variety   | Sowing date       | Brix, % | Chlorophyll (a+b), ml/l | Nitrates, mg/kg | Sugar, % | Vitamin C, mg/100 g |
|-----------|-------------------|---------|-------------------------|-----------------|----------|---------------------|
| Fantaziia | April I decade*   | 6.3     | 0.449                   | 51              | 2.2      | 55                  |
|           | April III decade  | 6.7     | 0.449                   | 52              | 2.3      | 61                  |
|           | May II decade     | 6.4     | 0.442                   | 49              | 2.3      | 59                  |
|           | June II decade    | 6.4     | 0.429                   | 53              | 2.2      | 55                  |
|           | August I decade   | 5.4     | 0.429                   | 52              | 2.1      | 54                  |
|           | August III decade | 5.8     | 0.408                   | 54              | 2.1      | 54                  |
| Malakhit  | April I decade    | 6.1     | 0.440                   | 47              | 2.1      | 53                  |
|           | April III decade  | 6.2     | 0.442                   | 51              | 2.2      | 57                  |
|           | May II decade     | 5.2     | 0.421                   | 52              | 2.3      | 59                  |
|           | June II decade    | 5.3     | 0.422                   | 52              | 2.2      | 55                  |
|           | August I decade   | 5.3     | 0.420                   | 52              | 2.1      | 54                  |
|           | August III decade | 5.5     | 0.421                   | 53              | 2.1      | 55                  |

**Note:** \* – control variant

**Source:** compiled by the authors

The brix in spinach leaves of varieties Fantaziia and Malakhit was at the level of 5.1-6.8% and was higher than sowing in the III decade of April and II decade of May – 6.4-6.8%, which exceeded the control by 0.3-0.7%. The mass share of sugars, depending on the variety and date of sowing in open field, fluctuated between 2.1-2.4% and was almost at the same level. Plants grown during the early sowing dates were characterized by a higher sugar content. The content of vitamin C was in the range of 52-70 mg/100 g, depending on the date of sowing. Moreover, the vitamin C content was dominated by the sowing dates in the I and III decade of April – 50-60 mg/100 g and II decade of May – 58-70 mg/100 g. So, the date of sowing had a significant influence on the main chemical parameters of the spinach varieties Fantaziia and Malakhit, and in the leaves, the higher parameters were noted for the dates of sowing in the III decade of April and the II decade of May, where the brix reached the level of 6.4-6.8%, sugars content – 2.3-2.4%, vitamin C content – 58-70 mg/100 g.

The sowing date has quite significant influence on the plant's phenotype. It was proved by conducted research. The leaf surface and total leaves surface were under attention in research as direct influencer on productivity and result production in general. The leaf surface depended on growing conditions and variety. The general tendency was observed then due to mild temperature conditions plants have more time for forming big leaves and higher number of them. On the other hand, increasing of temperatures of environment make growing period shorter and leaves size smaller. The research of Mudau *et al.* (2018) confirms that term of growing is directly influencing on leaf size and as result on biomass production itself. Longer growing time is showing significant difference in leaf size and biomass production. It needs to indicate than by research of Conte

*et al.* (2008) was proved that climatic conditions slightly influenced visual quality of spinach leaves.

A. Bottino *et al.* (2009) and Gutiérrez-Rodríguez *et al.* (2013) conclude that dry matter is a direct indicator of leaves quality and their storage ability. The dry matters were in scope of the conducted research. The study showed that the degree of plant development, especially the accumulation of dry matter, was not directly related to the level of adaptive characteristics. The influence of the sowing date on the dry matter content was observed. It was found decreasing of their content at latest sowing dates. The highest content was found at Spring sowing and partly during Summer growing at hot climatic conditions. It a bit contradictory with research of V. Chernyshenko *et al.* (2017). The plants are forming higher content of dry matter during optimal sowing. Dry matter was depended on research variety as well in research of Alessa *et al.* (2017). It was shown in current research that varieties differently react on growing conditions and general tendency was not observed.

The sowing date still under attention of Ukrainian scientists and foreigners as well. The sowing date is quite important because it can determine growing result can has significant impact on spinach plants' productivity. The tendency of increasing of plant habitus and prolongation of the growing terms due to mild growing conditions observed. Similar tendency was shown by research of Polish scientist by E. Kunicki *et al.* (2010) which was conducted in two sowing dates only. However, instead of sowing at Spring and Autumn were investigated late Spring and Summer sowing dates for prolong possibility to obtain fresh greens during whole open field season. It was risky to grow spinach at hot conditions because yield can be decreased by 30% and more what was found by research of Tai *et al.* (2020). Conducted research partly confirmed such tendency.

The decreasing of yield was observed with growth of the day temperatures during the growing period for summer sowing dates. But decreasing was on the level not more than 10-14%. The higher impact on yield had cold weather and wet soil at the earliest sowing date. It was observed decreasing of yield more than 15% compared to the best variant of research. Flood and water stress was studied by C. Pereira *et al.* (2019) and M. Seymen (2021) and reported that overwatered soil has negative effect on the spinach plants' characteristic and yield itself.

Studying the timing of sowing garden spinach, we believe that in the conditions of the Steppe of Ukraine, these issues are quite relevant because spring and autumn allow to extend the timing of production and obtain high yields.

### CONCLUSIONS

The study of the influence of the sowing date on the leaves number revealed that when sowing of spinach varieties Fantaziia and Malakhit in the 3<sup>rd</sup> decade of April and the 2<sup>nd</sup> decade of May formed a higher number of leaves. The Fantaziia variety had 18-19 pcs/plant and Malakhit – 18-20 pcs/plant. More favourable conditions in 2020 made it possible to obtain an additional 2-4 leaves per plant. Spinach plants of both varieties had a larger leaf surface at the beginning of rosette growth during the early sowing dates - 114.2-127.7 cm<sup>2</sup>. Plants sown in August had a smaller leaf surface – 86.0-106.2 cm<sup>2</sup>.

Depending on the variety, the highest yield of marketable green mass was obtained for sowing in the 3<sup>rd</sup> decade of April and the 2<sup>nd</sup> decade of May in the Fantaziia variety – 22.9-23.0 t/ha, in the Malakhit variety – 23.3-23.9 t/ha, which was higher compared to control by 3.2-4.2 t/ha. The LSD<sub>0.5</sub> in quantitative expression according to factors A and B for spinach yield was 0.2-0.7, which indicates reliable values between their reps and variants. Three factors together (year, variety and sowing date) form 91.3% of the spinach yield.

It was established that the climatic and soil conditions of the Southern Steppe of Ukraine were suitable for sowing spinach in six sowing dates. The conveyor growing will ensure an uninterrupted supply of green products from the third decade of April to the end of September. In some years until mid-October. It will partially solve the problem of overcoming seasonality in the consumption of fresh green vegetables. The date of sowing had a significant influence on the main chemical parameters of spinach varieties. The higher indexes were noted for the dates of sowing in the 3<sup>rd</sup> decade of April and the 2<sup>nd</sup> decade of May, where the brix reached the level of 6.4-6.8%, sugars content – 2.3-2.4%, vitamin C content – 58-70 mg/100 g.

Taking into consideration the importance of fresh greens consumption during full year and positive impact on health, prospect research may be based on extension of terms of receipt of the fresh greens on account of using temporary tunnels in early spring and fixed tunnels and greenhouse in autumn-winter growing seasons.

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## Ефективність строків сівби шпинату в умовах південного Степу України

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**Анотація.** Населення України повинно отримувати свіжу зелень від ранньої весни до осені з відкритого ґрунту і строки вирощування шпинату забезпечать постачання свіжої зелені. Метою досліджень було вивчення можливості подовження періоду надходження свіжої зелені шпинату з відкритого ґрунту за рахунок розширення строків висівання насіння у відкритому ґрунті. Дослідження проводились у 2019–2021 рр. в умовах Південного Степу України. У дослідженні було використано польові, статистичні, розрахунково-аналітичні та лабораторні методи. Досліджувалися сорти Фантазія та Малахіт. Вивчали поведінку рослин залежно від шести строків сівби. Контрольним варіантом був сорт Фантазія за строком сівби I декада квітня. Виявлено, що рослини обох сортів шпинату мали більшу листову поверхню за ранніх строків сівби 114,2–127,7 см<sup>2</sup>. Рослини, які висівали в серпні, мали меншу площу листової поверхні – 86,0–106,2 см<sup>2</sup>. Найбільший урожай товарної зеленої маси отримано за сівби у III декаді квітня та II декаді травня і сорт Фантазія забезпечив 22,9–23,0 т/га, а сорт Малахіт – 23,3–23,9 т/га. Строк сівби мав значний вплив на основні хімічні показники шпинату городнього сортів Фантазія і Малахіт і вищими показниками відзначалися строки сівби у III-й декаді квітня та II-й декаді травня, де вміст сухої розчинної речовини досягав рівня 6,4–6,8 %, масова частка цукрів – 2,3–2,4 %, вміст вітаміну С – 58–70 мг/100 г. Встановлено, що ґрунтово-кліматичні умови Південного Степу України придатні для сівби шпинату городнього у шість строків і забезпечать безперебійне надходження продукції з III-ї декади квітня до кінця вересня включно, а у роки з сприятливими погодними умовами у II-й–III-й декадах вересня до середини жовтня, що вирішить проблему сезонності у споживанні свіжих овочів. Рекомендуємо сільгоспвиробникам досліджені строки сівби шпинату, щоб продовжити термін отримання свіжої зелені з третьої декади квітня до кінця вересня і в окремі роки до середини жовтня

**Ключові слова:** загальна площа листків, кількість листків, висота рослини, маса рослини, початок росту розетки, технічна стиглість



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## Technological and Nutritional Benefits of Amaranth Groats in Breadmaking

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**Abstract.** To increase the nutritional value of wheat bread as a staple food using non-conventional wholesome floury ingredients should meet high expectations of consumers. The study was aimed to investigate the effect of amaranth groats application into breadmaking focused on the technological qualities and biological value of wheat bread enriched with onion powder and safflower oil. The influence of scalded amaranth groats on the bread quality was measured by technological, physical, chemical, instrumental, and computational methods analysing raw materials and developed products. The use of the amaranth grain-derived ingredient at 4-8% as a valuable plant source in the wheat bread formulations enhanced the product consumer characteristics. The proofing of dough of the developed formulations was intensified followed by 6-20% increase in the bread specific volume and better organoleptic properties of bread. Amaranth groats showed high amino acid score for lysine (156%), phenylalanine and tyrosine (125%), and scores for threonine, valine, and cysteine were 2-fold to wheat flour scarce in bioactive compounds. The developed wheat-amaranth bread had an improved amino acid composition due to an increase in the number of essential amino acids, a 2.6 times higher protein utility, contributing to its digestibility. The main factor in changing the fatty acid composition of the developed products was the introduction of safflower oil with a high content of linoleic acid into the product. Introduction of scalded amaranth groats into bread formulations with vegetable-based additives is promising way to attribute the product with therapeutic, and health-improving properties

**Keywords:** amaranth groats, scalding, safflower oil, onion powder, wheat bread, amino acid composition, fatty acid composition



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## INTRODUCTION

Pseudo cereals, such as amaranth, have an exceptional nutritional as well as phytochemical profile with proteins of good balanced amino acid content. Due to the absence of gluten, these cereals can be included in the diet of people with gluten intolerance. Dietary fibre, vitamins, minerals, phenols, and other biologically active compounds found in pseudocereals have the potential to prevent chronic diseases such as cancer, diabetes, hypertension, and cardiovascular disease. Wheat bread is a staple food based on patent wheat flour, which is a reliable source of energy but not of high nutritional value. Thus, to explore the way of promoting health attributes of such staple food by using amaranth grain-derived products still underestimated in XXI century could be very beneficial for food value chain reevaluation.

Baraniak & Kania-Dobrowolska (2022) highlight the presence of bioactive compounds in pseudocereals, and their great potential for diverse application in processed food, such as e.g. bakery products. Guardianelli *et al.* (2022) demonstrated that the nutritional properties of wheat flour can be improved through mixing with other functional ingredients, and amaranth grain-derived products added to wheat flour brings considerable health benefits to the nutritional value due to high protein content, rich in lysine, precious dietary fibre, and lipids composition.

Shevkani *et al.* (2022) state that amaranth flour adds a light-yellow grain-like colour to the composite flour, which is described as nutty, earthy, and grassy. Amaranth flour protein receives high quality marks due to its rich content of lysine and methionine. It is also recognized as one of the best proteins of plant origin which is close to the ideal protein. Studies conducted by Castro-Martínez *et al.* (2012) testify that amaranth is one of the leaders among plants in terms of the content of lysine, threonine, arginine, phenylalanine. In a two-year study, Hlinková *et al.* (2013) pointed out found that amaranth seeds were also rich in 77% polyunsaturated fatty acids content, considerably presented by linoleic acid as a source of arachidonic acid, which is used to the basis for the synthesis of prostaglandins in the body.

Amaranth proteins digestibility is close to the digestibility of proteins of animal origin. Amaranth exceeds conventional crops by content lysine and methionine. Alicia Martinez-Lopez *et al.* (2020) consider this plant is a source of fibre, polyunsaturated acids, phytosterols, phospholipids, squalene, vitamins C, E, PP, B2, B1, A, folate, macro- and microelements (sodium, magnesium, potassium, phosphorus, calcium, iron, copper), which are crucial for the optimal functioning of the digestive, immune, and endocrine systems, etc. The authors indicate that 700-80% of amaranth grain tocopherols are represented by  $\beta$ - and  $\gamma$ -tocopherols, 20.0-25.0% by  $\delta$ -tocopherols, and 5.0-10.0% by  $\alpha$ -tocopherols.

Nauman Khalid *et al.* (2017) investigated safflower oil as a source of magnesium, vitamins (B1, B2, PP, E,  $\beta$ -tocopherol), which contains carotenoids. The oil extracted from the kernels of safflower seeds has a bitter taste and a floral smell. In terms of the balanced composition of unsaturated fats, this oil is superior to sunflower oil and, moreover, suitable to be hydrogenated to margarine of the best quality (Xin *et al.*, 2022).

Prokopov *et al.* (2018) claim that onion powders in confectionery, dairy, bakery, and pasta formulations can enrich their techno-functional properties. Bae *et al.* (2003) presented data on the quality characteristics of wheat bread with the addition of 2-8% onion powder. The colour index of bread with onion powder had lower lightness and higher redness and yellowness than that of the control bread. The specific volume of the onion powder-added bread increased, and the texture measurement showed that hardness, springiness, stickiness, and chewiness decreased as the amount of onion powder added was enlarged. Masood *et al.* (2020) was aimed to improve the nutritional quality of bread by adding onion peel extract (OPE) and onion powder (OP) at 1-7% replacement of wheat flour. The obtained properties illustrate that onion powder can serve as a good flavour ingredient for baking wheat bread.

*The purpose of this study* is to evaluate the effect of scalded amaranth grains on techno-functionality and biological value of wheat bread enriched with onion powder and safflower oil. The purpose was fulfilled by solving the following tasks: to investigate the chemical composition, technological properties of amaranth groats; to assess the effect of scalded amaranth groats on the breadmaking; to evaluate organoleptic, physico-chemical indicators of the quality of bread enriched with amaranth groats, onion powder and safflower oil; to estimate the biological value of the developed bread with scalded amaranth groats.

## MATERIALS AND METHODS

The following types of raw materials were used for the study: wheat flour; amaranth flour; amaranth groats; onion powder; pressed bakery yeast and salt (Table 1). Water was added to the formulations to dough moisture content of 43.5%. The moisture content of raw materials was determined by the thermogravimetric method according to DSTU ISO 712:2015 (2015) using a SESH-3M drying oven, Ukraine. Whiteness was determined as the light reflection of flour with the use of RZ-BPL-CM, Ukraine, according to DSTU 26361:2019 (2019). The flour water absorption capacity was determined for 100 g of flour during dough kneading by ISO 17718:2013 method (2013). Falling number was analysed according to Perten method of ISO 3093:2009 (2009) with the use of PChP-99-2, Ukraine. Autolytic activity was determined as the ability of flour to form sugars during heating according to the DSTU 3016-95 (1995) method.

**Table 1.** Bread with scalded amaranth groats and vegetable additives

| Sample | Quantity of raw materials in the recipe, g |                         |               |              |       |       |      |       |
|--------|--|-------------------------|---------------|--------------|-------|-------|------|-------|
|        | Wheat flour                                | Scalded amaranth groats | Safflower oil | Onion powder | Sugar | Yeast | Salt | Water |
| 1      | 600  | 0                       | 18            | 24           | 24    | 18    | 9.6  | 365   |
| 2      | 600  | 72                      | 18            | 24           | 24    | 18    | 9.6  | 312   |
| 3      | 600  | 144                     | 18            | 24           | 24    | 18    | 9.6  | 259   |
| 4      | 600  | 216                     | 18            | 24           | 24    | 18    | 9.6  | 206   |
| 5      | 600  | 288                     | 18            | 24           | 24    | 18    | 9.6  | 153   |
| 6      | 600  | 360                     | 18            | 24           | 24    | 18    | 9.6  | 100   |

**Source:** compiled by the authors

Baking loss of the bread was determined as the ratio of the weight of the bread removed from the oven, to the weight of the bread after 4 hours of storage at room temperature, expressed in %. Drying loss was measured as the ratio of the weight of the bread after 4 hours of storage at room temperature, to the weight of the bread after 24 hours of storage at room temperature, expressed in %. Bread shape stability was determined as the ratio of height to width of the central part of the loaf. Bread specific volume was analysed by millet seed replacement method according to AACC 10-05.01 (2000). Bread crumb porosity was determined using Zhuravlov's device (Hlinková *et al.*, 2013). Staleness of the bread was assessed during three days of storage of the loaves at room temperature. Moisture content of the crumb was analysed by the thermogravimetric method as the weight loss of 5 g of crushed bread crumb from the central part of the loaf according to AACC 44-15.02 (1999). Water absorption capacity of the crumb was determined as the amount of water absorbed by the crushed crumb, g per g of the dry crumb. Organoleptic properties of bread samples were analysed by a panel of experts by the following indicators: crust colour and smoothness; crumb colour, porosity structure, and rheological properties; aroma, taste, and chewiness of crumb, expressed in a five-point scale. The acidity of the bread crumb was determined by the titrimetric method (DSTU 7045:2009, 2009).

Crude protein content was analysed by the Kjeldahl method (DSTU 7169:2010, 2011). Crude fat content was determined by the Soxhlet method (ISO 6492:1999,

1999). Crude fibre content was analysed by sequential solvent extraction (ISO 6865:2000, 2000). Crude ash content was determined by burning the sample in a muffle furnace according to ISO 5984:2002 (2002). Carbohydrate content was calculated according to FAO recommendations. The amino acid composition was determined by ion-exchange liquid column chromatography on amino acid analyser T 339 (Mikrotechna, Czech Republic). Total protein utility, amino acid composition difference coefficient (AACDC) and protein utility (PU) were calculated by the method presented by Martinez-Lopez *et al.*, 2020.

The fatty acid composition of flour and bread samples was determined by gas chromatography on Agilent 6890B (Agilent Technologies, USA), equipped with the flame ionization detector (FID) and HP-Innowax capillary column. Experiments were performed in 3-5-fold repetition.

## RESULTS AND DISCUSSION

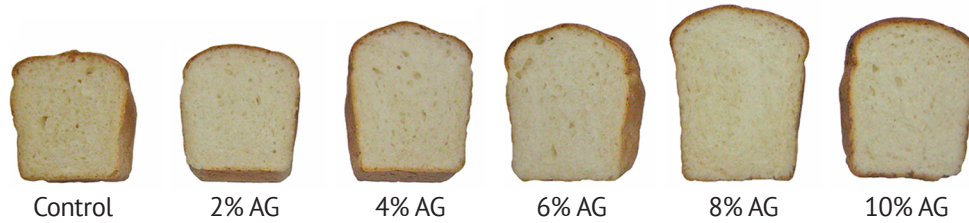
Table 2 demonstrates that the amaranth flour presented the lowest moisture content of 10%, while the onion powder had the lowest whiteness. The highest falling number was represented by wheat flour (610 s), which needed to be increased for optimising the dough rheological characteristics, through mixing with such ingredients as the amaranth grain-derived products. Water absorption capacity of the studied samples was related to the particle size distribution and chemical composition, varying between 144% (amaranth flour) to 500% (onion powder).

**Table 2.** Technological properties of composite flour ingredients

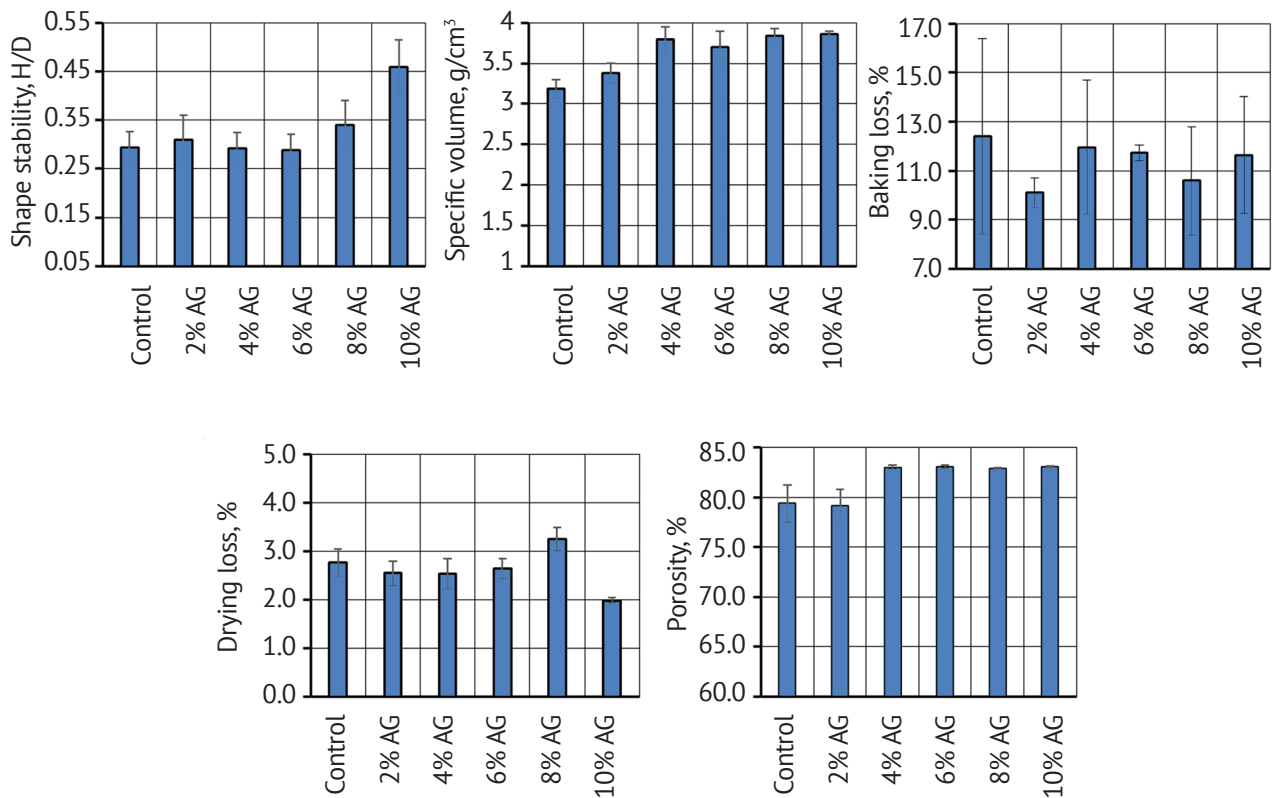
| Ingredient      | Moisture content, % | Whiteness, c.u. | Water absorption capacity, % | Falling number, s | Autolytic activity, % |
|-----------------|---------------------|-----------------|------------------------------|-------------------|-----------------------|
| Wheat flour     | 14.5 ± 0.2          | 61.6 ± 0.1      | 184.0 ± 11.3                 | 610.1 ± 9.9       | 31.6 ± 0.2            |
| Amaranth flour  | 10 ± 0.1            | 19.3 ± 0.1      | 224.1 ± 12.1                 | 70.5 ± 2.1        | 30.6 ± 0.2            |
| Amaranth groats | 11.8 ± 0.3          | 22.9 ± 0.1      | 144.0 ± 9.1                  | 61.1 ± 0.9        | 17.1 ± 0.2            |
| Onion powder    | 12.5 ± 0.2          | 11.8 ± 0.1      | 500.2 ± 15.3                 | 426.5 ± 10.6      | 34.3 ± 0.2            |

**Source:** compiled by the authors

Figure 1 presents the developed samples of wheat bread with scalded amaranth groats enriched with onion powder and safflower oil, when the technological characteristics of the samples are demonstrated by Figure 2.



**Figure 1.** Wheat bread with scalded amaranth groats (%), enriched with onion powder and safflower oil  
**Source:** photographed by the authors

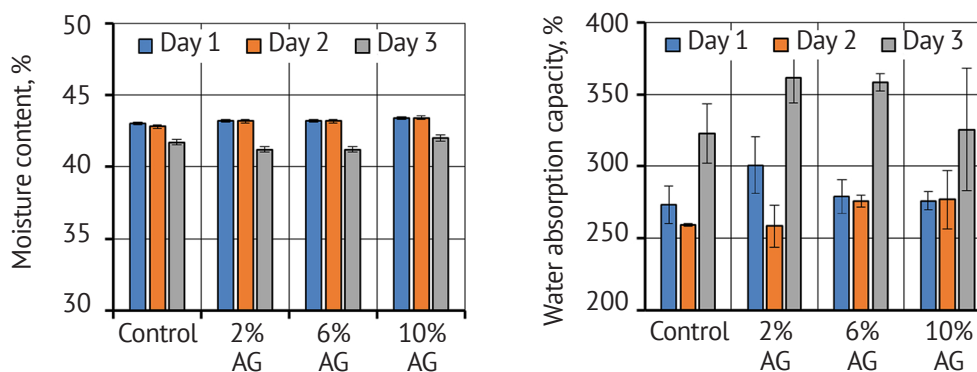


**Figure 2.** Technological characteristics of wheat bread with scalded amaranth groats (%)  
**Source:** compiled by the authors

Application of scalded amaranth groats into the wheat bread formulations led to a specific volume increase of 1.06-1.2 times with improved crumb colour, taste and aroma of bread. Also, the use of scalded amaranth groats as an ingredient in the bread formulations promoted 5% higher porosity of the products. Taking into account the change in the dough rheological characteristics, shape stability of the loaves increased drastically when 10% of scalded amaranth groats were introduced to the product. Crumb acidity of the samples with 8-10% of the amaranth grain-derived ingredient was slightly lowered in comparison to the control sample due to speedy proofing of the dough. Thus, scalded amaranth

groats application intensified the wheat dough fermentation which is in agreement with higher falling number of amaranth groats (Table 2). Considering the obtained effects, scalded amaranth groats could be a promising ingredient of the wheat bread at 4-8% amaranth groats content to the wheat flour in the formulation.

Moisture content and water absorption capacity of the bread crumb tend to decrease with the losing of bread freshness indicating its staling. The scalded amaranth groats application contributed to retaining more water by the bread crumb (Fig. 3) with an effect predominately on water absorption capacity of the crumb at 2-6% scalded amaranth groats after 3 days of storage.



**Figure 3.** Effect of scalded amaranth groats (%) of freshness of wheat bread

**Source:** compiled by the authors

Table 3 shows the chemical composition of wheat patent flour, amaranth groats, and the bread made with their use following the developed formulations including onion powder and safflower oil (Table 1). Such a combination of the ingredients has the potential to enrich the product with biologically active compounds

providing the quality of the product, which could meet the high expectations of consumers. The last, but not least benefit of using amaranth grain-derived products is related to its sustainability as a drought and pest-resistant crop capable to withstand climate change and global warming issues.

**Table 3.** Nutritional profile of amaranth groats and wheat bread enriched with scalded amaranth groats, onion powder and safflower oil

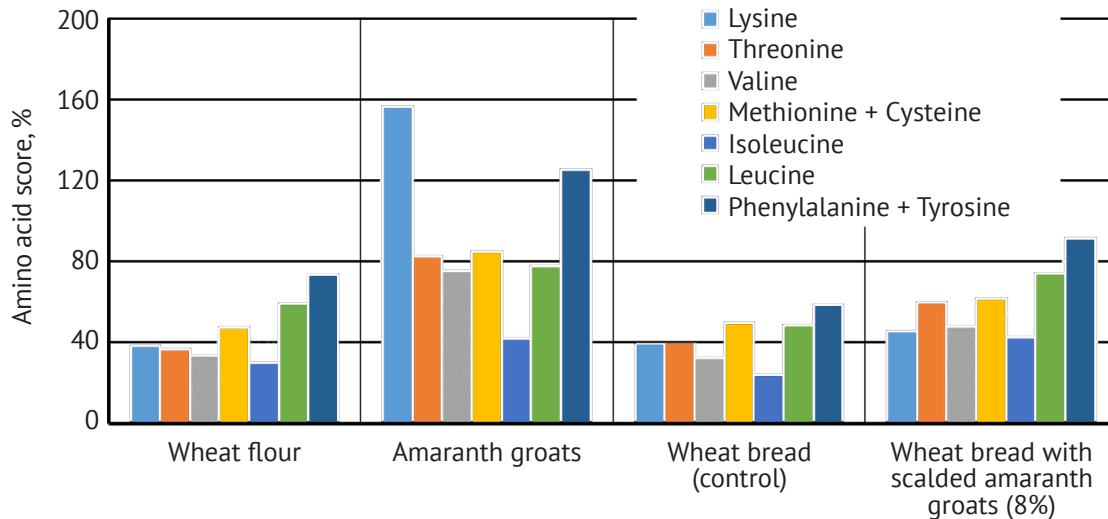
| Indicator               | Wheat flour  | Amaranth groats | Wheat bread with onion powder and safflower oil | Wheat bread with scalded amaranth groats, onion powder and safflower oil |
|-------------------------|--------------|-----------------|---|--|
| Crude protein, %        | 12.01 ± 0.22 | 3.68 ± 0.22     | 12.01 ± 0.16                                    | 11.46 ± 0.12   |
| Crude fat, %            | 1.82 ± 0.07  | 0.94 ± 0.06     | 1.55 ± 0.20                                     | 1.22 ± 0.17  |
| Crude fibre, %          | 0.18 ± 0.02  | 0.06 ± 0.02     | 0.24 ± 0.01                                     | 0.23 ± 0.01  |
| Crude ash, %            | 0.64 ± 0.02  | 0.89 ± 0.18     | 2.37 ± 0.13                                     | 2.15 ± 0.08  |
| Carbohydrate content, % | 85.35 ± 0.43 | 94.42 ± 0.40    | 83.83 ± 0.58                                    | 84.94 ± 0.04   |

**Source:** compiled by the authors

Compared to wheat patent flour which is scarce in bioactive compounds, amaranth groats as an ingredient for breadmaking showed 4-fold less crude protein content, 2-3-fold less crude fat and fibre, but higher by 10-50% total carbohydrates and ash content. This is related to the distribution of nutrients in the amaranth grain and thus, amaranth groats with removed fatty and proteinous other part of the seed during processing demonstrated mostly starchy perisperm with well-distributed essential mineral elements. To be emphasized, amaranth groats introduction to the wheat bread formulations could bring benefits to the consumer characteristics of the wheat bread, and make the application of other non-traditional

ingredients, which are prone to deteriorate the quality of the product, an appropriate option. Therefore, to determine the nutritional profile of the bread, the developed formulations with onion powder and safflower oil were used combined with 8% of scalded amaranth groats.

Only the difference in the chemical composition of the experimental and control sample of bread is statistically significant for the content of crude protein, which decreases by 5% for the experimental sample, and available carbohydrates, which increases by 1%. As Figure 4 shows, wheat flour has an inferior amino acid composition, while the limiting essential amino acids are isoleucine and valine.

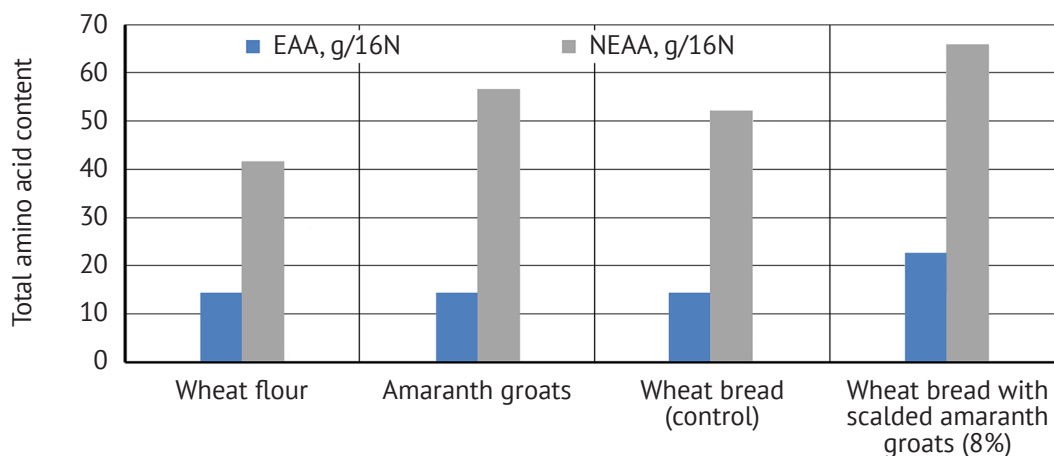


**Figure 4.** Amino acid composition of the used raw materials and developed wheat bread with different dosages of amaranth groats and onion powder (control and experiment)

Source: compiled by the authors

The amino acid composition of raw materials and bread made according to the developed recipe and technology was studied (Fig. 5). Amaranth groats differ substantially in their very high content of lysine (156%), phenylalanine and tyrosine (125%) relative to ideal protein and to wheat flour. Therewith, the contents of threonine, valine, methionine, and cysteine in amaranth groats are twice as high as for wheat flour, and are 75-85%.

Limiting are isoleucine and valine, as for wheat flour, but their crusts are much higher. Even though the rates of all essential amino acids are higher for amaranth groats in raw form, the difference coefficient of the amino acid rate is higher, the biological value is lower, as well as the utility of the protein, so the use of the groats requires its hydrothermal treatment to balance the amino acid score (Fig. 5, Table 4).



**Figure 5.** Amino acid composition of raw materials and bread made according to the developed recipe and technology

Source: compiled by the authors

**Table 4.** Amino acid composition of raw materials and developed functional bread

| Amino acid | Control (wheat flour) |          | Amaranth groats    |          | Wheat bread (control) |          | Wheat bread with scalded amaranthgroats (8%) |          |
|------------|-----------------------|----------|--------------------|----------|-----------------------|----------|--|----------|
|            | AG g/100 g protein    | Score, % | AG g/100 g protein | Score, % | AG g/100 g protein    | Score, % | AG g/100 g protein                           | Score, % |
| Lysine     | 2.12                  | 39       | 8.59               | 156      | 2.17                  | 39       | 2.51   | 46       |
| Histidine  | 1.07                  | -        | 2.49               | -        | 1.16                  | -        | 1.46   | -        |

Table 4, Continued

| Amino acid                  | Control (wheat flour) |          | Amaranth groats    |          | Wheat bread (control) |          | Wheat bread with scalded amaranthgroats (8%) |          |
|-----------------------------|-----------------------|----------|--------------------|----------|-----------------------|----------|--|----------|
|                             | AG g/100 g protein    | Score, % | AG g/100 g protein | Score, % | AG g/100 g protein    | Score, % | AG g/100 g protein                           | Score, % |
| Arginine                    | 2.54                  | –        | 14.46              | –        | 1.66                  | –        | 2.78   | –        |
| Aspartic acid               | 2.25                  | –        | 8.97               | –        | 2.79                  | –        | 3.26   | –        |
| Threonine                   | 1.47                  | 37       | 3.30               | 82       | 1.60                  | 40       | 2.40   | 60       |
| Serine                      | 3.02                  | –        | 4.36               | –        | 3.25                  | –        | 4.17   | –        |
| Glutamic acid               | 23.05                 | –        | 10.24              | –        | 24.29                 | –        | 30.35  | –        |
| Proline                     | 7.64                  | –        | 6.82               | –        | 8.71                  | –        | 9.93   | –        |
| Glycine                     | 2.54                  | –        | 5.96               | –        | 2.53                  | –        | 3.31   | –        |
| Alanine                     | 2.11                  | –        | 5.87               | –        | 1.80                  | –        | 2.75   | –        |
| Valine                      | 1.68                  | 34       | 3.76               | 75       | 1.64                  | 33       | 2.41   | 48       |
| Meteonin + cystine          | 1.66                  | 47       | 2.98               | 85       | 1.73                  | 50       | 2.15   | 62       |
| Isoleucine                  | 1.20                  | 30       | 1.67               | 42       | 0.96                  | 24       | 1.71   | 43       |
| Leucine                     | 4.14                  | 59       | 5.44               | 78       | 3.41                  | 49       | 5.21   | 74       |
| Phenylalanine + Tyrosine    | 4.42                  | 74       | 7.52               | 125      | 3.53                  | 59       | 5.50   | 92       |
| Total EAA                   | 16.69                 |          | 33.27              |          | 15.03                 |          | 21.89  |          |
| Biological value of protein |                       |          |                    |          |                       |          |  |          |
| Total protein utility       | 0.63                  |          | 0.44               |          | 0.56                  |          | 0.68   |          |
| AACDC, %                    | 15.47                 |          | 50.14              |          | 17.90                 |          | 17.77  |          |
| BV, %                       | 84.53                 |          | 49.86              |          | 82.10                 |          | 82.23  |          |

**Source:** compiled by the authors

As Figure 5 shows, according to all indicators, the control and experimental samples of the developed wheat bread with different dosages of amaranth groats and onion powder differ in the biological value of protein in favour of the latter. The values of essential amino acids increase by 1.1-1.45 times compared to the control without amaranth cereals and the protein utility in the product increases

by 2.6 times, which indicates an increase in its digestibility by the human body when using developed bread.

Lipids as fatty acids and their derivatives, as well as substances biosynthetically or functionally related to these compounds in the raw material and developed wheat bread with different dosages of amaranth groats and onion powder are presented in Table 5.

Table 5. Fatty acid composition of flour raw materials and bread

| Fatty acid | Wheat flour | Sample      |                 |                                |  |
|------------|-------------|-------------|-----------------|--------------------------------|--|
|            |             | Wheat flour | Amaranth groats | Onion bread with safflower oil | Onion bread with safflower oil and amaranth groats |
| C8:0       | Caprylic    | 0.01        | 0.02            | 0.01                           | traces   |
| C9:0       | Pelargonic  | –           | 0.01            | traces                         | –  |
| C10:0      | Capric      | –           | 0.03            | 0.01                           | traces   |
| C11:0      | Undecylic   | –           | 0.03            | 0.01                           | 0.01   |
| C12:0      | Isolauric   | –           | 0.01            | traces                         | –  |
| C12:0      | Lauric      | 0.03        | 0.37            | 0.04                           | 0.02   |
| C12:1      | Lauroleic   | –           | 0.05            | 0.01                           | –  |
| C13:0      | Tridecylic  | –           | 0.13            | 0.01                           | –  |
| C14:0      | Myristic    | 0.13        | 0.88            | 0.29                           | 0.21   |

Table 5, Continued

| Fatty acid<br>Wheat flour |                  | Sample      |                 |                                |  |
|---------------------------|------------------|-------------|-----------------|--------------------------------|--|
|                           |                  | Wheat flour | Amaranth groats | Onion bread with safflower oil | Onion bread with safflower oil and amaranth groats |
| C14:1                     | Myristoleic      | 0.02        | 0.38            | 0.02                           | 0.04   |
| C14:2                     | Tetradecadienoic | –           | 0.05            | –                              | –  |
| C15:0                     | Pentadecylic     | 0.07        | 0.19            | 0.11                           | –  |
| C16:0                     | Isopalmitic      | –           | 0.15            | 0.06                           | –  |
| C16:0                     | Palmitic         | 23.82       | 22.48           | 18.84                          | 16.61  |
| C16:1                     | Palmitoleic      | 0.29        | 0.89            | 0.64                           | 0.45   |
| C17:0                     | Margaric         | 0.13        | 0.70            | 0.27                           | 0.08   |
| C17:1                     | Heptadecenic     | 0.05        | 0.21            | 0.09                           | 0.03   |
| C18:0                     | Isostearic       | –           | 0.40            | –                              | –  |
| C18:0                     | Stearic          | 1.87        | 3.88            | 5.99                           | 4.59   |
| C18:1                     | Oleic            | 18.15       | 31.35           | 25.58                          | 24.63  |
| C18:2                     | Linoleic         | 49.85       | 30.87           | 41.61                          | 49.30  |
| C18:3                     | Linolenic        | 2.58        | 0.44            | 1.58                           | 0.96   |
| C20:0                     | Arachidic        | 0.30        | 0.50            | 0.93                           | 0.62   |
| C20:1                     | Gondoic          | 0.61        | 0.52            | 0.72                           | 0.45   |
| C21:0                     | Heneicosylic     | 0.08        | 0.12            | 0.46                           | 0.05   |
| C20:2                     | Eicosadienoic    | 0.79        | –               | 0.48                           | 0.55   |
| C20:4                     | Arachidonic      | 0.64        | 2.01            | 1.14                           | 0.67   |
| C22:0                     | Docosanoic       | 0.14        | 1.05            | 0.28                           | 0.23   |
| C22:3                     | Docosatrienic    | 0.06        | 0.30            | 0.04                           | 0.02   |
| C22:5                     | Docosapentaenoic | 0.36        | –               | 0.40                           | 0.25   |
| C22:6                     | Docosahexaenoic  | 0.03        | –               | 0.38                           | 0.19   |
| C22:6                     | Docosahexaenoic  | –           | 1.40            | –                              | –  |

**Source:** compiled by the authors

Free fatty acids (Table 6), and especially linoleic and linolenic acids, take part in the absorption of oxygen. It is assumed that the mechanism of these processes is the oxidation of free linoleic and linolenic acids of flour with the combined oxidation of sulfhydryl groups of gluten. Aerobic mixing of flour and water results in

the loss of free fatty acids within 10 min, but not the loss of other nonphosphorus lipids. Losses of free fatty acids can occur through lipoxidase oxidation of essential fatty acids and concomitant enzymatic oxidation of free fatty acids (Mercier & Gélinas, 2001).

**Table 6.** Features of the fatty acid composition of raw materials and bread with amaranth groats (8%) and vegetable additives

| Fatty acid spectrum indicator | Wheat flour | Amaranth groats | Bread enriched with onion powder and safflower oil | Bread enriched with amaranth groats, onion powder and safflower oil | Safflower oil |
|-------------------------------|-------------|-----------------|--|---|---------------|
| Saturated                     | 26.57       | 30.95           | 27.32  | 22.44   | 8.10          |
| Monounsaturated               | 19.11       | 33.40           | 27.06  | 25.58   | 11.20         |
| Polyunsaturated               | 54.32       | 35.27           | 45.62  | 51.95   | 80.70         |
| Unsaturated: saturated        | 2.76        | 2.22            | 2.66   | 3.45  | 11.35         |
| Essential                     | 1.15        |                 | 0.88   | 0.80  | –             |
| Omega-3                       | 4.40        | 3.85            | 3.97   | 2.63  | 0.30          |
| Omega-6                       | 49.91       | 31.42           | 41.65  | 49.32   | 80.40         |
| Omega 6:3                     | 11.33       | 8.17            | 10.49  | 18.77   | 268.00        |
| Omega-9                       | 19.11       | 33.40           | 27.06  | 25.61   | 11.20         |

**Source:** compiled by the authors

The obtained results from Table 6 are consistent with the data presented by Aliieva *et al.* (2022) and Ruyvaran *et al.* (2022), where the authors indicate that the use of amaranth grain processing products, namely amaranth groats, can have technological advantages along with increasing the biological value of bread with their use.

Thakur *et al.* (2022) conducted a study of grinding gluten-free amaranth grain into flour for nutrient composition, antinutrients, total and bioavailable minerals, fatty acids and amino acids, acid profile and functional properties of the flour. It was found that the fine fraction, which is 44% of the total amount of amaranth flour, contains more protein (19.7%), fat (8.54%), minerals (3.46%) and dietary fibre content (20.09%), as well as a higher total amino acid content with lysine as the main essential amino acid. The content of linoleic acid in fine flour was (44.8%). Coarsely ground flour was dominated by linoleic acid (37.2-44.8%), which is a polyunsaturated fatty acid, oleic acid (27.9-29.4%), and palmitic acid by (26.5-29.6%). Differentially sifted flour of amaranth grain fractions showed a wide distribution of nutrients and of the finely dispersed fraction. In this way, to compensate for the loss of free fatty acids in wheat flour during the production of bread, Guardianelli *et al.* (2022) suggested the use of amaranth flour with the indicated content of nutrients, including free fatty acids.

Ballester-Sánchez *et al.* (2019) proved that wheat flour has a very low falling number, indicating low alpha-amylase activity. In turn, Saubhik (2016) claimed that amaranth flour and amaranth groats have highly active alpha-amylase, which together with wheat flour will activate the dough ripening processes due to the breakdown of starch and the initiation of the release of sufficient maltose to feed the yeast. The lowest autolytic activity is inherent in amaranth groats.

Wolosik & Markowska (2019) analysed onion powder and proved that it has anti-inflammatory and decontaminating properties; even after baking, onion powder has a considerable part of useful substances. The paper (Castro-Martínez, 2012) examines the nature and biological activity of onion thiosulphinates, as well as their potential value as food preservatives for preserving food and extending its shelf life, and their effectiveness as food preservatives and chemical substitutes.

Comparing the research presented in this paper with the results known from individual literature sources, one can draw several important conclusions that reflect the further research prospects started by the authors to create fundamentally new varieties of wheat bread enriched with useful components of amaranth groats and plant additives. Firstly, the favourable increase in the total number of drops of the composition of wheat and amaranth flour leads to the achievement of the creation of high-quality consumer bread with high physical and chemical component indicators, which ultimately cannot but attract a wide range of Ukrainian consumers of the product with preventive and

curative properties. Secondly, the saturation of developed varieties of wheat bread, enriched by adding amaranth groats and plant additives with a sufficient amount of useful minerals, vitamins, flavour enhancers due to high-quality lipid components can create the conditions for placing such bread products in the range of the greatest consumer demand, provided effective management and quality control production. Thirdly, an attractive, readily available raw material environment will have a favourable effect on the improved production technology of a qualitatively new mass consumption product without any conversion of technological lines to produce new types of bread.

## CONCLUSIONS

It was established that the use of amaranth groats as a component of wheat bread, provided that it is hydrothermally processed and introduced into the dough recipe in the form of porridge, leads to the improvement of the technological properties of semifinished products during the ripening of the products and gives the products improved technological qualities. The use of amaranth groats as an ingredient in the recipe of bread enriched with onion powder and safflower oil substantially affects the growth of the dimensional stability of bread from 0.25 to 0.45, the increase in the specific volume of products by 6-20% and its porosity by 5%, subject to improvement the colour of the pulp, a more pronounced taste and aroma of bread with a slight decrease in the acidity of the pulp. The optimal introduction of amaranth groats in the form of porridge to the recipe of bread with onion powder and safflower oil is 4-8% by weight of wheat flour.

Amaranth groats differ substantially in their high content of lysine (156%), phenylalanine, and tyrosine (125%) relative to the amino acid scores of wheat flour. Therewith, the contents of threonine, valine, methionine, and cysteine in amaranth groats are twice as high as for wheat flour, and amount to 75-85%. Bread made according to the developed recipes with amaranth groats has a rate of essential amino acids 1.1-1.45 times higher than for the developed bread without amaranth groats, and 2.6 times higher protein utility in the product, which indicates an increase in its digestibility by the human body when using the developed bread.

Considering the fatty acid composition of the product, the proven improvement of the amino acid composition of the products as a result of enrichment with all essential amino acids and the growth of protein utility, the developed recipe and technologies are promising for introduction at enterprises of various power levels, which allows them to produce more competitive health products.

To increase the functional status of the developed bread with amaranth groats and plant additives in towards their enrichment with organic acids and increase the assimilation of essential metals that are part of amaranth grain processing products, further research

aimed at the development of bread based on sour-milk sourdough of spontaneous fermentation is relevant.

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## Технологічні та харчові переваги крупи амаранту в хлібопеченні

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**Анотація.** Підвищення біологічної цінності пшеничного хліба за рахунок використання нетрадиційної борошняної сировини, багатой на біологічно цінні компоненти, як продукту масового споживання має відповідати вимогам високої якості його споживчих властивостей. Мета роботи – дослідження впливу амарантової крупи на технологічні якості і біологічну цінність пшеничного хліба, збагаченого такими рослинними добавками, як цибулевий порошок і сафлорова олія. Вплив амарантової крупи на формування якості хліба визначали шляхом використання технологічних, фізичних, хімічних, інструментальних і розрахункових методів аналізу сировини і розроблених виробів. Застосування продуктів переробки зерна амаранту і сафлору як цінної продовольчої рослинної сировини у рецептурі пшеничного хліба призводило до поліпшення споживчих якостей хліба за умови 4–8 % введення амарантової крупи. Забезпечення інтенсифікації дозрівання тістових напівфабрикатів супроводжувалося поліпшенням органолептичних властивостей виробів, 6–20 % зростанням питомого об'єму. Встановлено, що амарантова крупа мала високий амінокислотний скор за лізином (156 %), феніланіном і тирозином (125 %), а скори за треоніном, валіном і цистеїном були удвічі вищими порівняно з пшеничним борошном, збідненим на біологічно цінні речовини. Розроблений пшенично-амарантовий хліб мав поліпшений амінокислотний склад за рахунок зростання скорів незамінних амінокислот, у 2,6 разів вищої утилітарності білку, що може сприяти його засвоюваності. Основним чинником зміни жирнокислотного складу виробів виступало введення до складу продукту сафлорової олії з високим вмістом лінолевої кислоти. Практична цінність роботи визначається тим, що такий хліб має всі ознаки профілактично-лікувального і оздоровчого продукту

**Ключові слова:** амарантова крупа, заварювання, сафлорова олія, цибулевий порошок, пшеничний хліб, амінокислотний склад, жирнокислотний склад

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## Indicative Features of Macrophyte Communities in the Assessment of Anthropogenic Load on Aquatic Ecosystems

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**Abstract.** Studying structural and functional biodiversity in relation to various environmental factors is currently extremely relevant because aquatic ecosystems are a significant source of biological diversity and make up a significant part of the biological productivity of the Earth, they perform many functions, and they are valuable and important for the stability of biotic communities. With this in mind, the task to determine the floristic composition of the Teteriv ecological corridor as a prototypical river landscape in the northern part of Ukraine, to analyse the structural and functional features of the species diversity of macrophytes therein, and to dissect this diversity into its component parts according to its place of origin, its life form, and its relationship to environmental factors was set. The number of species and their predicted coverage in areas with different anthropogenic pressures within the Teteriv ecological corridor were analysed within ecological zones based on the study's findings. It was demonstrated that the integrated ecological indicator of water quality was crucial to the growth of phytocenoses in high-anthropogenic-load regions. The communities may survive in environments where dissolved oxygen is low, muddy sediments are abundant, and anaerobic processes predominate in the transformation of substances. Additionally, they can propagate in floodplains, wet swampy ecotopes, and other environments where water is present for extended periods of time. Most of these communities are not picky about their habitat, as they may thrive in a variety of situations, including slightly acidic or neutral substrates, varying amounts of nitrogen and minerals in the soil, and mild salinization of the plant life. An increase in the number of representatives of individual ecogroups can attest to changes in the ecological state of aquatic ecosystems and have practical significance in detecting increased anthropogenic pressure on aquatic ecosystems

**Keywords:** indicators, phytocenoses, biodiversity, species, ecogroups



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## INTRODUCTION

Ecological monitoring of surface waters based on macrophyte reactions, as well as the study of the impact of various environmental factors on their diversitological and physiological indicators, is a common and proven area of hydrobiological research. The study of the reaction of macrophytes to the influence of anthropogenic factors can be attributed to the same direction. For example, using a diversitological approach to look at how stable the growth of higher aquatic plants is when water bodies get saltier may show early signs of deterioration.

Aquatic flora is a sensitive component of ecosystems that respond not only to environmental degradation but also to improvement (Elo *et al.*, 2018). This is because, as the effects of humans on river ecosystems are lessened, the original species mix of macrophyte groups is brought back, and at the same time, some species whose growth was once caused by the displacement of more sensitive species from ecological niches are brought back.

Determining the diversity of organisms and any shifts in their physiological and morphological characteristics is an important part of evaluating the health of aquatic ecosystems. Phytoindicators are useful because they can, according to some sources, (a) react to relatively weak loads because of the effect of dose accumulation; (b) account for the total effect of various anthropogenic factors; (c) record an abundance of chemical and physical parameters that characterize the state of the environment; (d) establish the rate at which environmental changes are occurring; (e) track the evolution of biogeocenoses; and (e) pinpoint the entry points of toxicants (Isaienko *et al.*, 2019; Hájek *et al.*, 2020; Fedonyuk *et al.*, 2020; Hu *et al.*, 2021).

Due to varying methodological techniques and distinct natural environments in which this research was conducted, several writers ambiguously interpret the suggestive features of higher aquatic plants and their groups. Submerged macrophytes, or plants most closely associated with the aquatic environment, respond to changes in the composition of the water very quickly (O'Hare *et al.*, 2018; Gurnell *et al.*, 2020; Dong *et al.*, 2022).

Species diversity is the main criterion for the state of aquatic ecosystems (Tanwir *et al.*, 2020; Fedoniuk *et al.*, 2020). Similar work was carried out in other parts of the world. Thus, similar studies noted the important role of imaging methods in the analysis of environmental quality (Fedoniuk *et al.*, 2019; Xu *et al.*, 2020).

The presence of *Isoëtes lacustris* L., *I. echinospora* Durieu, *Lobelia dortmanna* L. and *Miriophyllum alterniflorum* DC indicate the purity and oligotrophy of natural waters. *Potamogeton pussilus*, *P. trichoides* Cham. et Schldl., *P. gramineus* L. grow in the same water (Lindholm *et al.*, 2021).

Areas of water bodies that do not currently undergo anthropogenic eutrophication are overgrown with *Catabrosa aquatica* (L.) Beauv., *Elatine alsinastrum* L.,

*Ceratophyllum submersum* L., and reservoirs characterized by relatively clean water – *Glyceria plicata* Fries. Aschers, *Potamogeton alpinus* Balb., and *P. trichoides* Cham. Et Schlecht. The same group of plants includes *Potamogeton lucens* L. (Sand-Jensen *et al.*, 2017). According to some sources, *Miriophyllum spicatum* L. very sensitive to industrial and municipal effluents (Rameshkumar *et al.*, 2019; Ceschin *et al.*, 2021). That is why other authors defined it as an indicator of reservoirs with high mineral content that were subject to strong anthropogenic eutrophication (Vardanyan & De, 2021; Lin *et al.*, 2021).

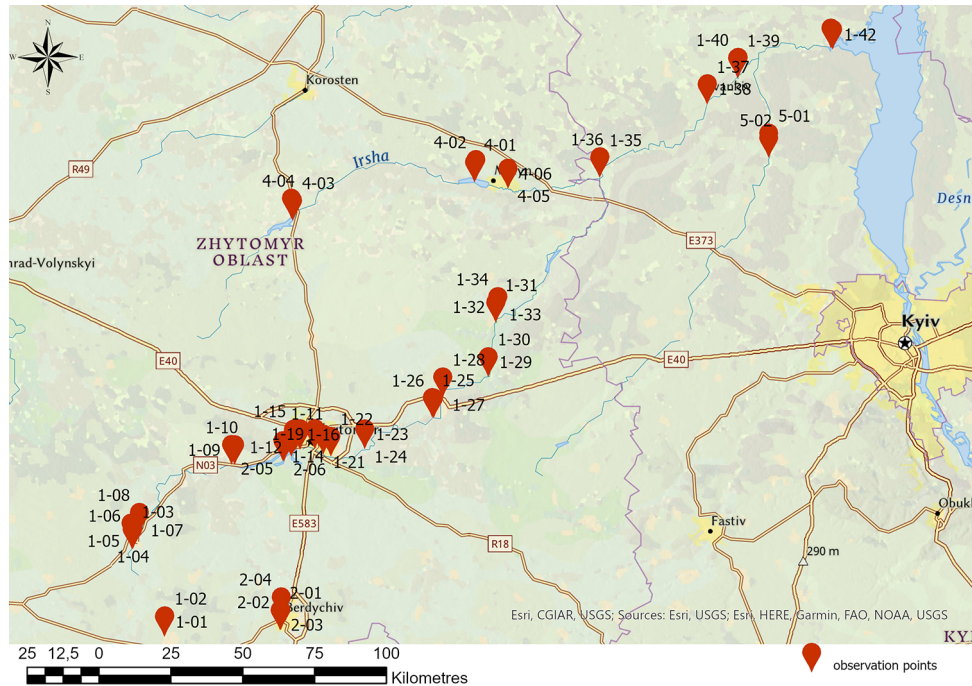
The group of indicator plants that indicate the mesotrophic and eutrophic nature of water bodies is quite significant (Gil *et al.*, 2020). Other groups of mesotrophic species include *Sparganium emersum* Rehm., eutrophic species – *Equisetum fluviatile* L., *Typha angustifolia* L., *T. latifolia* L., *Potamogeton natans* L., *Sagittaria sagittifolia* L., *Utricularia australis* etc. (Roth *et al.*, 2020; Orlov *et al.*, 2021; Szpakowska *et al.*, 2021). The rapid growth of duckweed could be a sign that pollution from agriculture and industry is hurting aquatic ecosystems (Fedoniuk *et al.*, 2022).

The indicator potential of aquatic macrophytes is highly ambiguous (Fares *et al.*, 2020). However, the prevalence of macrophytes – plant species with wide geographical ranges that may require various environmental conditions even within their range – cannot be relied upon as a reliable predictor of a certain degree of the environmental component. In phytocenoses, far fewer macrophytes than eurytopic ones often serve as accurate markers of any given natural situation. The development of an understanding of the level of water body pollution by the structure of macrophyte groups, the set of species, and their productivity appears to be the most promising route in light of this (Joniak *et al.*, 2017; Reid *et al.*, 2019; Kataki *et al.*, 2021). Biomonitoring the dynamics of littoral overgrowth later can say a lot about how water quality changes both in the past and in the present.

The goal of this work was to figure out the structure and function of species diversity in the Teteriv ecological corridor in areas with different levels of development of the floodplain of the river landscape, and to find the most important ecogroups and biomorphs in relation to different environmental factors.

## MATERIALS AND METHODS

The study was carried out from 2011 to 2020 in 78 sites within the wooded part of the ecological corridors of the Teteriv-River cascade. From among different types of water basins, the following rivers were chosen: the Teteriv River and its tributaries – the Gnylopiat, the Irsha, the Guiva, the Zdvyzh, as well as rather big water reservoirs – Chudniv, Vidsichne, Zhytomyr, Irshansk, Malyn (Fig. 1).



**Figure 1.** The distribution of higher aquatic vegetation metering points on the territory of Ukraine

**Source:** Google Earth 6.2.2.6613 (n.d.), aerial photo with author's tags

Water samples were taken at the 78 points described above. The choice of research points was based on the following criteria: places of wastewater discharge and intensive surface runoff, etc.). The structural characteristics of the typical river ecosystems of the ecological corridors of the Teteriv-River cascade were analysed in the first stage of research. Retrospective data from the Ukrainian Hydrometeorological Center (n.d.) and the State Agency of Water Resources of Ukraine (n.d.) in the Zhytomyr region (from 1975 to 2010), as well as hydro-chemical and hydro-physical studies in the period 2011-2020, were used to conduct this research, obtained at monitoring sites No 1-01...1-10, 1-21...1-28, 1-31...1-34, 1-37...1-42, 2-01...2-04, 4-01...4-06 together with the State Agency of Water Resources of Ukraine (n.d.), survey data in points 1-11...1-13, 1-04, 1-15...1-20, 1-29, 1-30, 1-35, 1-36, 2-05, 2-06, 3-01 obtained individually.

At the same points as water sampling, descriptions of the species composition of plant organisms were carried out. Woodwiss's method (Woodwiss, 1964) was used for research. When analysing a sample of higher aquatic plants from the accounting points, the data was compared with the list of indicator species, in which certain species have a certain class of water purity.

Life forms of macrophytes-indicators were analysed according to the classical classification of Hejny (1960). The distribution of aquatic macrophyte species by habitat type was carried out according to Meusel *et al.* (1965). Classification of aquatic macrophytes in relation to environmental factors was carried out based

on indicator scales of Ellenberg *et al.* (1967) and unified scales of Didukh & Plyuta (1994), which are adapted to the geographical and territorial conditions of Ukraine, according to which the grouping of species was based on the value of ecological indices of plants in relation to light, thermal regime, continentality, humidity, acid, nitrogen and salt regimes.

## RESULTS AND DISCUSSION

The structure and function of aquatic phytocenoses are an important way to figure out how stable they are. From 2011-2020, a study of some small rivers in the Zhytomyr region was conducted. As already mentioned, within the observation points, 43 species were described, which in total, from the point of view of taxonomy, belonged to 3 divisions, the most numerous of which was Magnoliophyta, which included 41 species. One species was observed within the Equisetophyta and Filicophyta divisions. 95.3% of the species in the Teteriv Ecological Corridor (TEC) belong to the Magnoliophyta division. When it comes to higher aquatic plants, this region has only one species of fern and one species of horsetail in its phytocenoses. This is in line with general trends in the development of higher aquatic plants in temperate-cool climates.

At the same time, the number of species in each department is about the same. There are 15 species in the class Liliopsida and 14 species in the class Magnoliopsida. The Equisetophyta and Filicophyta divisions remain with one species in each of these divisions (Table 1).

Table 1. Systematics of aquatic flora within the TEC

| Division                       | Class                           | Order                    | Family                       | Genus              | Number of species |
|--------------------------------|---------------------------------|--------------------------|------------------------------|--------------------|-------------------|
| Magnoliophyta -<br>Angiosperms | Liliopsida -<br>Monocotyledons  | <i>Alismatales</i>       | Alismataceae Vent.           | Alisma L.          | 1                 |
|                                |                                 |                          |                              | Sagittaria L.      | 1                 |
|                                |                                 |                          | Araceae Juss.                | Acorus L.          | 1                 |
|                                |                                 |                          | Butomaceae Rich.             | Butomus L.         | 1                 |
|                                |                                 |                          | Hydrocharitaceae Juss.       | Elodea Michx. "    | 1                 |
|                                |                                 |                          |                              | Hydrocharis L.     | 1                 |
|                                |                                 |                          | Lemnaceae SF Gray            | Lemna L.           | 2                 |
|                                |                                 |                          |                              | Spirodela Schleid. | 1                 |
|                                |                                 |                          | Potamogetonaceae Dumort.     | Potamogeton        | 6                 |
|                                |                                 |                          | Poales -                     | Cyperaceae Juss.   | Carex L.          |
|                                |                                 | Scirpus L.               |                              |                    | 2                 |
|                                |                                 | Poaceae                  |                              | Glyceria R. Br.    | 1                 |
|                                |                                 | Poaceae Barnhart         |                              | Phragmites Adans.  | 1                 |
|                                |                                 | Typhaceae Juss.          |                              | Sparganium L.      | 1                 |
|                                | Typha L.                        |                          | 2                            |                    |                   |
|                                | Asparagus                       | Iridaceae Juss.          | Iris L.                      | 1                  |                   |
|                                | Magnoliopsida -<br>Dicotyledons | Asterales                | Asteraceae Dumort.           | Bidens L.          | 1                 |
|                                |                                 | Brassicales              | Brassicaceae Burnett         | Rorippa Scop.      | 1                 |
|                                |                                 | Saxifragales             | Haloragaceae R. Br.          | Myriophyllum L.    | 2                 |
|                                |                                 | Lamiales                 | Lamiaceae Lindl.             | Lycopus L.         | 1                 |
|                                |                                 |                          |                              | Lythrum L.         | 1                 |
|                                |                                 |                          |                              | Trapa L.           | 1                 |
|                                |                                 | Caryophyllales           | Polygonaceae Juss.           | Polygonum L.       | 1                 |
| Ranunculales                   |                                 | Ranunculaceae Juss.      | Ranunculus L.                | 2                  |                   |
| Malpighiales                   |                                 | Salicaceae Mirb.         | Salix L.                     | 1                  |                   |
| Ceratophyllales                |                                 | Ceratophyllaceae SF Gray | Ceratophyllum L.             | 1                  |                   |
| Nymphaeales                    | Melanthiaceae Batsch.           | Nuphar Smith.            | 1                            |                    |                   |
|                                |                                 | Nymphaea L.              | 1                            |                    |                   |
| Equisetophyta -<br>Horsetail   | Equisetopsida -<br>Horsetail    | Equisetales              | Equisetaceae Rich.<br>ex DC. | Equisetum L. -     | 1                 |
| Filicophyta -<br>Ferns         | Polypodiopsida -<br>Ferns       | Salviniales              | Salviniaceae Dumort.         | Salvinia Seguiet   | 1                 |

Source: compiled by the authors

The most numerous, in terms of the number of species within the TEC, was the order *Alismatales*, within which 6 families and 9 genera were noted, which are represented by a total of 15 species. The order *Poales*, with 4 families and 6 genera, within which 11 species were recorded, was also numerous. It should be noted that both orders belong to the class Liliopsida. Within the class *Magnoliopsida*, orders *Saxifragales*, *Ranunculales*, *Nymphaeales*, and *Myrtales* are presented in the number of 2 species each. All other orders were counted within the studied phytocenoses of one species.

Thus, the distribution of hydrophytes by taxonomic parameters to identify general trends observed within the plant groups of temperate latitudes on the Eurasian continent was allowed (Dubina & Shelyag-Sosonko, 1984).

The distribution of species represented by the minimum number within families and genera indicates the absence of polymorphism in the studied biocenoses, and biomorphs of the described species were practically not observed. In general, according to the species composition of vascular plants, most are angiosperms.

The species diversity of the TEC is spread out based on how closely they are related to growing conditions. The group of surface-water-air species is the most common, making up about 53.8% of all species. It should be noted that 100% of surface-water-air species were rooted plants. The group of flood-water-air plants was less numerous, occupying a total of 23.1%. Unlike the last group, this one had both rooted and floating species (10.3% and 12.8% of the total number of species, respectively). Submerged

plants within the TEC were also represented only by rooted species (20.5%). And the least numerous was the group of submerged plants – 2.6%.

The distribution of species diversity of the TEC according to the spectrum of zonal chorological groups proposed by Meusel & Jäger (1989) showed that most phytocenoses belong to the boreo-meridional chorological group – 34.5%, plurizonal species – 25.6%, boreal-meridional – 18%, and submeridional type was about 10%. Three types of latitudinal areas: temperature-meridional, temperature-boreal, and boreal-tropical, are occupied by 7.7% and temperature-tropical type – about 5%. According to regional chorological groups by longitudinal habitats, the group of species with a circumpolar type of habitat was the most numerous – 53.8, the chorological Euro-Asian group – 25.6%, and the European group – 10.3%.

Important, in terms of understanding the patterns of spatial distribution of aquatic macrophytes, is their classification by life forms. However, one of the most comprehensive and accurate, in authors' opinion, is the classification of Dubina *et al.* (1993), which optimally reflects the features of natural-territorial complexes within which the studied phytocenoses are formed, namely considering fluctuations in river ecosystems and floodplain drainage, several ecological and biological features of the ontogenesis of macrophytic species, etc. This classification assumes the division of macrophytes into deep-water, littoral shallow-water, limous marsh, and terrastic terrestrial ecotopes.

According to this classification, 5 groups and 9 types of biomorphs were noted within the TEC. The

group of hydromorphic species turned out to be the most numerous, covering about 53.85% of all biomorphs belonging to it. The most numerous types (25.6%) of biomorphs within this class are aérohydatophytes (*Iris pseudacorus*, *Lycopus europaeus* L., *Myriophyllum spicatum* L., *M. verticillatum* etc.), which take place in the limnophase and coastal ecophase. The other two types of biomorphs of this class are quite numerous: euhydatophytes occupy 12.8% and pleistophytes – 15.38%.

The gelomorph group of biomorphs occupies a smaller share of the total amount of species diversity – 38.5%. However, it should be noted that this group contains the most numerous types of biomorph-ochtohydrophytes – in which most of their existence is associated with coastal, swamp, and terrestrial ecophases and only for a short time – with the limnophase. This type covers about 31% of the entire species diversity of the TEC. The other two types of biomorphs of the gelomorph group are present in small quantities: hydrochtohydrophytes – 2 species and efochtohydrophytes – 1.

The most common for the analysis of macrophytes are the Ellenberg indicator scales (1967) and the Didukh scales (2011), both adapted to the natural and climatic conditions of Ukraine. According to the distribution on the thermoclimatic scale of Ellenberg, almost three times fewer shares were occupied by species with ecological index 5 (*Alisma plantago-aquatica* L., *Lemna minor* L., *Lythrum salicaria* L., etc.), – 23% of the species diversity of phytocenoses. A significantly smaller share was occupied by species with ecological indices 7 and 8-15% (*Ceratophyllum demersum* L., *Schoenoplectus mucronatus* (L.) Palla, *Typha angustifolia* L., etc) (Fig. 2).

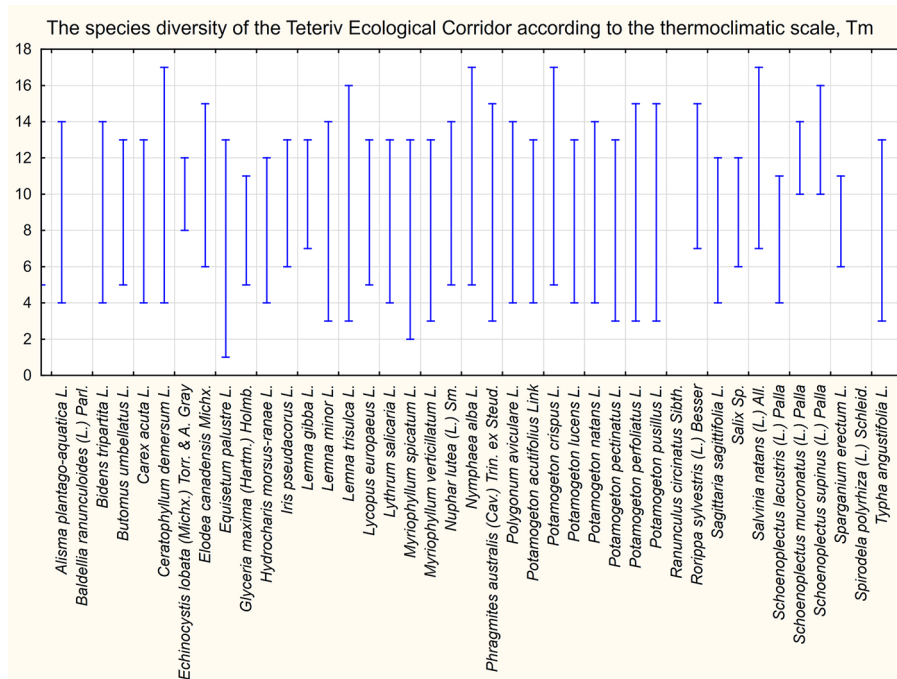


Figure 2. Distribution of species diversity of the TEC according to the thermoclimatic scale, Tm

Source: compiled by the authors

According to the Didukh scale, the largest share was occupied by species with tolerance ranges of 3-13 (9.76%), 4-13 (9.76%), and 4-14 (9.76%) (*Myriophyllum verticillatum* L., *Potamogeton pectinatus* L., *Typha angustifolia* L.). The distribution of the obtained data by ecogroups shows a significant predominance of submesothermic species, which occupied a total of 55%, with a larger share in this group occupied by hemieurytopic species (32.5%) than by eurytopic and hemistenotopic (10 and 12.5%, respectively). The group of submicrothermal hemieurytopic organisms was also significant (17.5%). Within the study area there are ecogroups and

biomorphs of species characteristic of temperate latitudes with a wide range of tolerance to thermoclimatic regimes (stenotic species were not observed at all). Almost 77% of the total number of species are hemieurytopic and eurytopic species.

Thus, the development of submicrothermal species (*Equisetum palustre* L., *Myriophyllum spicatum* L., *Myriophyllum verticillatum* L., *Potamogeton pectinatus* L., etc.) is suppressed by the deterioration of the overall integrated water quality index, with a clear tendency to reduce the coverage area of the observed species ( $r = -0.294$ ) and their number ( $r = -0.506$ ), (Table 2).

**Table 2.** Analysis of the relationship between the temperature patterns of macrophyte ecogroups and an integrated ecological index of water quality (N=5)

| Ecogroup       | Correlation coefficient | t <sub>Student's</sub> * | Correlation conclusion | by projective coating   |                          |                        |
|----------------|-------------------------|--------------------------|------------------------|-------------------------|--------------------------|------------------------|
|                |                         |                          |                        | Correlation coefficient | t <sub>Student's</sub> * | Correlation conclusion |
| Submicrotherms | -0.294                  | -2,264                   | reverse                | -0.506                  | -4,312                   | reverse                |
| Submesotherms  | -0.249                  | -1,886                   | missing                | -0.611                  | -5,677                   | reverse                |
| Mesotherms     | 0.097                   | 0.717                    | missing                | 0.033                   | 0.243                    | missing                |

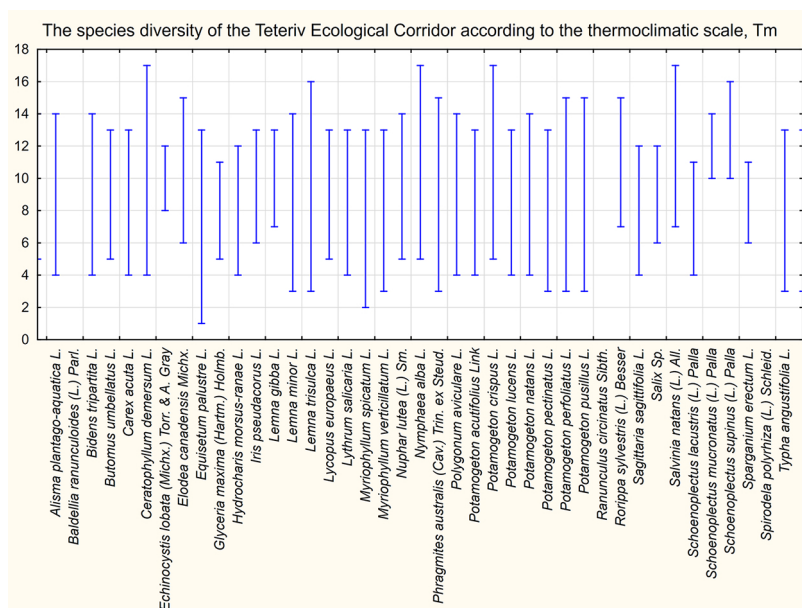
**Note:** \*in this table and all the following ones the weight of the relationship on the effectiveness of the feature is equated to the critical tabular value  $t_{Student's} = 2.00$

**Source:** compiled by the authors

A decrease in the number of species under the influence of anthropogenic pressure occurs in the ecogroup of submesothermic species. However, unlike the previous ecogroup of submicrothermal species, in this one, projective coverage does not vary significantly due to the presence of more tolerant species. However, it should be noted that anthropogenic pressure has a clearly defined negative impact on the number of species in this ecogroup, which is confirmed by a close inverse correlation ( $r = -0.611$ ). Mesothermal species had the most stable growth in the presence of

pollution caused by humans, and there was no significant dependence on environmental degradation reactions.

The ombroregime is one of the most important environmental factors, which integrates the impact of precipitation, evaporation from the soil surface, and thermal resources of the territory. Most species of the TEC have a wide range of tolerance to the ombroregime. Thus, more than half of the species have such values for the upper and lower limits, the difference between which exceeds 10 units (*Elodea canadensis* Michx., *Lemna trisulca* L., *Ceratophyllum demersum* L., etc.), (Fig. 3).



**Figure 3.** Distribution of species diversity of the TEC according to the thermoclimatic scale, Tm

**Source:** compiled by the authors

Based on the ombre regime scale, all of the species in the TEC were put into 5 ecogroups. Of these, mesoaridophytes (20%), subaridophytes (37.5%), and subumbrophytes (27.5%) were the most common. In most ecogroups, the aridity-humidity of the climate did not cause

significant changes, except for the mesoaridophyte ecogroup, where the overall decrease in projective coverage ( $r = -0.310$ ) was accompanied by the loss of phytocenoses of some species (*Typha anphastifolia* L., *Typha angustifolia* L., *Potamogeton pectinatus* L., etc.) ( $r = -0.210$ ) (Table 3).

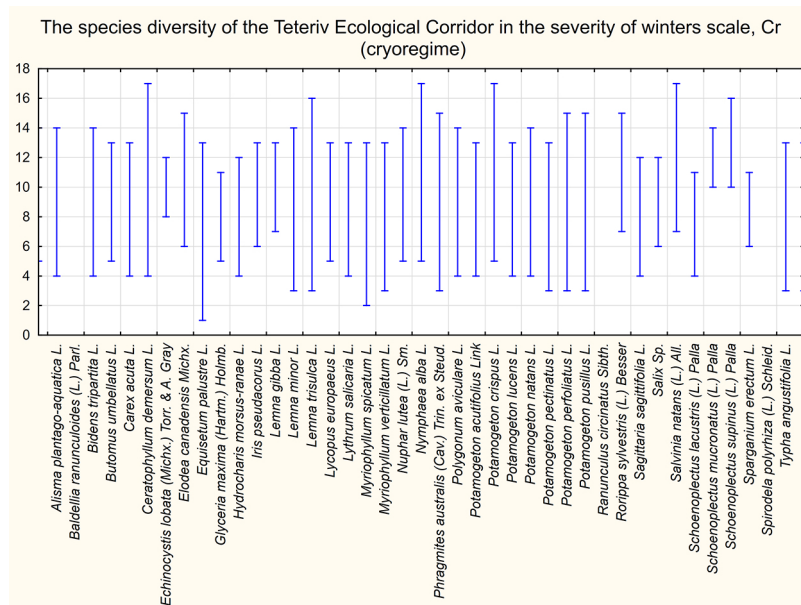
**Table 3.** Analysis of the relationship between the aridity-humidity patterns of macrophyte ecogroups and an integrated ecological index of water quality (N=5)

| Ecogroup        | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion | Correlation coefficient  | t <sub>Student's</sub> | Correlation conclusion |
|-----------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
|                 | by projective coating   |                        |                        | by the number of species |                        |                        |
| Semiaridophytes | -0.258                  | -1,961                 | missing                | <b>-0.490</b>            | -4,126                 | missing                |
| Mesoaridophytes | <b>-0.310</b>           | -2,397                 | reverse                | <b>-0.280</b>            | -2,140                 | reverse                |
| Subaridophytes  | -0.254                  | -1,931                 | missing                | <b>-0.501</b>            | -4,249                 | reverse                |
| Subombrophytes  | -0.101                  | -0.745                 | missing                | <b>-0.316</b>            | -2,445                 | missing                |
| Semiombrophytes | 0.009                   | 0.063                  | missing                | 0.046                    | 0.339                  | missing                |

**Source:** compiled by the authors

In the subaridophyte ecogroup, the decrease in the number of species ( $r = -0.501$ ) occurred against the background of the stability of projective coatings due to the presence of species sufficiently resistant to anthropogenic pollution species, which quickly filled the ecological niches freed from more sensitive species (*Potamogeton natans* L., *Potamogeton perfoliatus* L., *Schoenoplectus lacustris* (L.) Palla, *Carex acuta* L. etc). For

other ecogroups, no significant dependencies on water quality were found. In terms of natural and climatic conditions, the Polissya region is a zone of significant temperature differences. This is especially true in the winter when sub-zero temperatures cause the formation of ice cover and cannot but affect the conservation of aquatic macrophytes. That is why their attitude to the cryoregime of the study area is also very important (Fig. 4).



**Figure 4.** The species diversity of the TEC in the severity of winters scale, Cr (cryoregime)

**Source:** compiled by the authors

Thus, in accordance with the Didukh scale, most species were characterized by a significant range of tolerance to cryotherapy: 1-15 (*Ceratophyllum demersum* L., *Elodea canadensis* Michx., *Lemna trisulca* L., *Phragmites australis* (Cav.) Trin. Ex Steud., *Polygonum hydropiper* L., etc) – 23.08%, 1-13 (*Alisma plantago-aquatica* L., *Lycopus europaeus* L., *Sparganium erectum* L., *Myriophyllum spicatum* L.) – 10.26%, 5-12 (*Potamogeton acutifolius*, *Potamogeton lucens* L. etc) – 10.26%.

The distribution of species diversity by ecogroups in relation to the cryotherapy showed that most species have a high amplitude, so the largest share in species diversity was occupied by subcryophytes – 53.84%, of which eurytopic species – 33.33 % and hemieurytopic – 17.95%. Slightly fewer hemieurytopic hemicryophytes – 12.82% and hemieurytopic cryophytes and acriophytes – 5.13% in each ecogroup, which are indicators of moderate and mild winter periods. In general, 97% of the species belong to

the eco-groups of species, which can store seeds or other parts at very low temperatures and have a wide range of tolerance to the cryo-regime. According to research, the

projective areas of the main ecogroups of macrophytes in relation to cryoregime did not vary under the influence of anthropogenic pressure on aquatic ecosystems (Table 4).

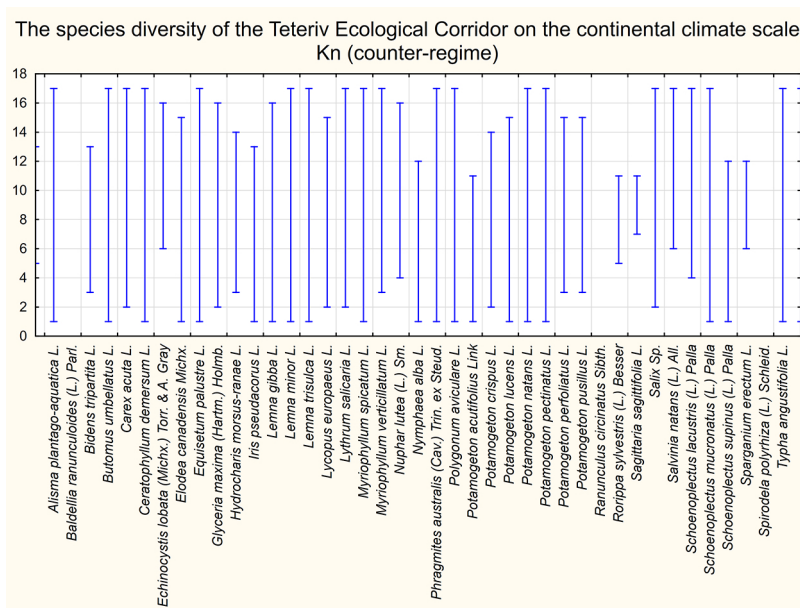
**Table 4.** Analysis of the relationship between the cryotherapy patterns of macrophyte ecogroups and an integrated ecological index of water quality (N = 55)

| Ecogroup       | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion | Correlation coefficient  | t <sub>Student's</sub> | Correlation conclusion |
|----------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
|                | by projective coating   |                        |                        | by the number of species |                        |                        |
| Cryophytes     | -0.132                  | -0.976                 | missing                | -0.117                   | -0.866                 | missing                |
| Subcryophytes  | -0.197                  | -1,479                 | missing                | <b>-0.457</b>            | -3,777                 | reverse                |
| Hemicryophytes | -0.165                  | -1,231                 | missing                | <b>-0.298</b>            | -2,297                 | reverse                |

Source: compiled by the authors

However, there is a pronounced negative impact of anthropogenic pressure and deterioration of water quality on ecological groups of subcryophytes and hemicryophytes, which is confirmed by the presence of close inverse correlations ( $r = -0.457$  and  $r = -0.298$ , respectively). The decrease in the total number of species and the stability of coverage areas show that space is being taken up by species that are less affected by human activities. Many hemieurytopic (41.03%) and eurytopic (33.33%)

species play a big part in these changes. According to Ellenberg's ecological scale, the range of scores ranged from 1 to 4. More than half of the species had a score of 4-51.5%, i.e., they were characterized by a wide range of tolerance to the continental climate. According to the Didukh scale, a similar trend was noted. The largest share of species was characterized by a wide range of tolerance: 1-17 (*Alisma plantago-aquatica* L., *Butomus umbellatus* L., *Ceratophyllum demersum* L., etc) – 35% and others (Fig. 5).



**Figure 5.** The species diversity of the TEC on the continental climate scale, Kn (counter-regime)

Source: compiled by the authors

In general, according to the continental scale, all species diversity in the TEC covered 4 ecogroups. The largest share of species belonged to the eco-group of hemicontinentals – 70.0%, less-homo-oceanic – 20.0%, and sub-oceanic and subcontinental together accounted for about 10%. In terms of ecological amplitude, the absence of stenotic species across all ecogroups should be noted. The largest share in both ecogroups was occupied by eurytopic species – 60% within the hemicontinental ecogroup and 10% by hemioceanists.

Hemieurytopic species accounted for 7.5%, 5%, and 5% of hemicontinentals, hemioceanists, and subcontinentals, respectively.

The influence of anthropogenic factors on projective coverings in terms of ecogroups on a continental scale did not show a consistent pattern. However, there was still a negative impact on the number of species (Table 5). Thus, it was most pronounced in the ecogroups of hemioceans and hemicontinentals ( $r = -0.461$  and  $r = -0.581$ , respectively). The presence

of a significant proportion of eurytopic species, which allows them to occupy free ecological niches in the fall of more sensitive to anthropogenic factors species,

plays a significant role in maintaining the total number of individuals in terms of ecogroups, as it did in the previously described groups.

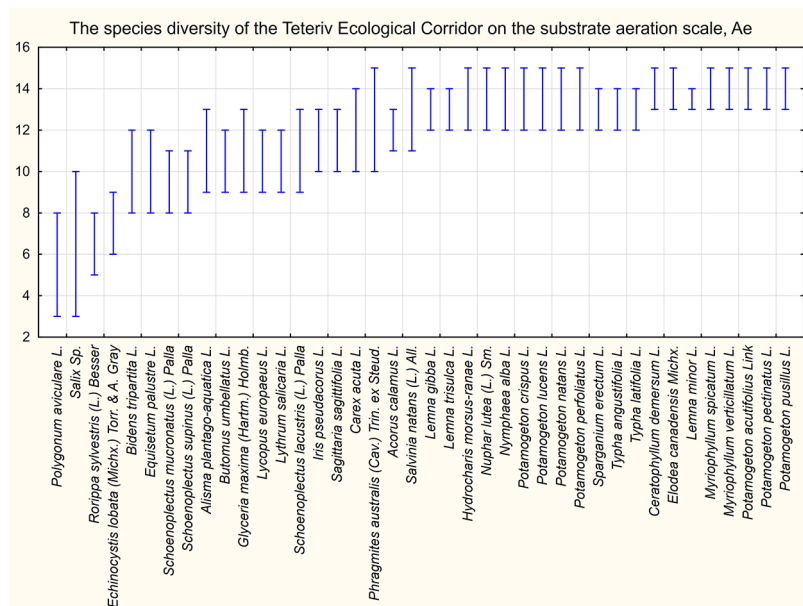
**Table 5.** Analysis of the relationship between the continentality patterns of macrophyte ecogroups and an integrated ecological index of water quality (N = 55)

| Ecogroup         | Correlation coefficient | $t_{\text{Student's}}$ | Correlation conclusion | Correlation coefficient  | $t_{\text{Student's}}$ | Correlation conclusion |
|------------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
|                  | by projective coating   |                        |                        | by the number of species |                        |                        |
| Hemioceanists    | -0.137                  | -1,016                 | missing                | <b>-0.461</b>            | -3,817                 | reverse                |
| Hemicontinentals | -0.249                  | -1,893                 | missing                | <b>-0.581</b>            | -5,244                 | reverse                |
| Subcontinental   | -0.201                  | -1,508                 | missing                | -0.155                   | -1,156                 | missing                |

**Source:** compiled by the authors

Aeration is an important indicator that determines the nature of physicochemical processes in substrates. For the aquatic environment, this indicator is usually important because it determines the oxidation

of organic and mineral substances, and thus determines the intensity of the self-cleaning processes. The most numerous groups of species with a tolerance range were: 12-15-17.5% (Fig. 6).



**Figure 6.** The species diversity of the TEC on the scale of substrate aeration, Ae

**Source:** compiled by the authors

All species of macrophyte diversity within the TEC in terms of substrate aeration were divided into 6 ecogroups. The largest share was occupied by stenotic hyperaerophobes – 48.8%, which indicates a low level of dissolved oxygen in water, the presence of silty deposits and the predominance of anaerobic processes of transformation of substances.

This is supported by data from hydrochemical analyses of water, where Biological Oxygen demand (BOD) and Chemical Oxygen Demand (COD) values exceeded the corresponding maximum permissible concentration (MPC) by 1.5-2.3 times in some cases. The share of subaerophobes, aerophobes, and megaaerophobes was significant in the studied phytoceoses – 9.8%, 19.5%,

and 14.7%, respectively. The presence of these species is due to the processes of glaciation of coastal soils and constant excessive moisture of the territory with an unsatisfactory aeration regime, which inevitably leads to waterlogging of the floodplain. The total share of species adapted to exist in sufficient aeration regimes – subaerophiles and hemiaerophiles, accounted for a small share – just over 7%. All species diversity had a rather narrow range of ecological amplitude, as evidenced by a significant percentage of stenotic (53.7%) and hemitenotopic (46.3%). In response to anthropogenic pollution of aquatic ecosystems, the parameters of different ecogroups in relation to the aeration of the substrate varied differently (Table 6).

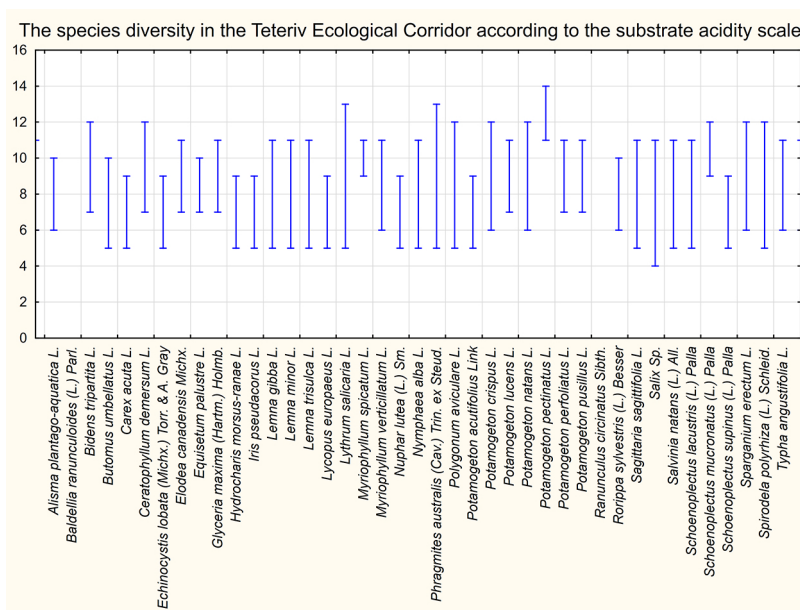
**Table 6.** Analysis of the relationship between the substrate aeration (Ae) patterns of macrophyte ecogroups and an integrated ecological index of water quality (N = 55)

| Ecogroup        | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion | Correlation coefficient  | t <sub>Student's</sub> | Correlation conclusion |
|-----------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
|                 | by projective coating   |                        |                        | by the number of species |                        |                        |
| Subaerophiles   | <b>-0.443</b>           | -3,631                 | reverse                | <b>-0.499</b>            | -4,236                 | reverse                |
| Hemiaerophobes  | <b>-0.501</b>           | -4,257                 | reverse                | <b>-0.424</b>            | -3,444                 | reverse                |
| Subaerophobic   | -0.033                  | -0.242                 | missing                | -0.142                   | -1,054                 | missing                |
| Aerophobic      | <b>-0.382</b>           | -3,042                 | reverse                | <b>-0.313</b>            | -2,423                 | reverse                |
| Megaaerophobes  | -0.148                  | -1,102                 | missing                | -0.251                   | -1,905                 | missing                |
| Hyperaerophobic | -0.056                  | -0.411                 | missing                | -0.379                   | -3,009                 | reverse                |

Source: compiled by the authors

Close correlation with inverse links and negative effects have been established between the deterioration of water quality and the number of subaerophiles, hemiaerophobes, and aerophobes, which manifested itself in both a general reduction in the number of species and a general suppression of populations as well as a reduction in the overall projective coverage of relevant ecogroups. Under conditions of anthropogenic pressure, the physicochemical composition of the habitat of species is of

great importance. Thus, especially in places of wastewater inflow, there are significant variations in the pH of the aquatic environment. The largest share of species of aquatic phytocenoses had the Ellenberg scale values indices 6, 7, and 8, which indicate the confinement of such species to neutral, slightly alkaline, and alkaline substrates. Their share was almost 90% of all species' diversity. The values of ecological indices according to Didyk acidity scale showed a wide range of changes (Fig. 7).



**Figure 7.** The species diversity in the TEC according to the substrate acidity scale

Source: compiled by the authors

Most species had a wide range of tolerance to environmental acidity – from 5 to 9 points, or 17 %. Although a retrospective analysis of water quality data for the period since 1947 showed an average pH value of 7.49 and the presence of a significant proportion of hemistenotopic subacidophiles (26.3%), the negative impact of contaminated effluents associated with significant pH variations within the study area. It should be noted that most of the species in these ecogroups are hemistenotopic and hemievryptopic biomorphs. The

presence of neutrophilic and basophilic species is more like how the conditions and chemical properties of water have changed over time. The inflow of acidified wastewater in recent decades has significantly adjusted the diversity of this area, and thus the sensitivity of individual ecogroups to the acidity of the substrate is obvious. As the correlation analysis of the relationships between total projective coverings and the number of species of individual ecogroups showed, they all changed due to the transformation of the aquatic environment under

the influence of anthropogenic factors. In particular, sub-acidophiles and basophiles saw a decrease in the total projective cover (Table 7), as well as a decrease in the number of species ( $r = -0.519$  and  $r = -0.284$ , respectively).

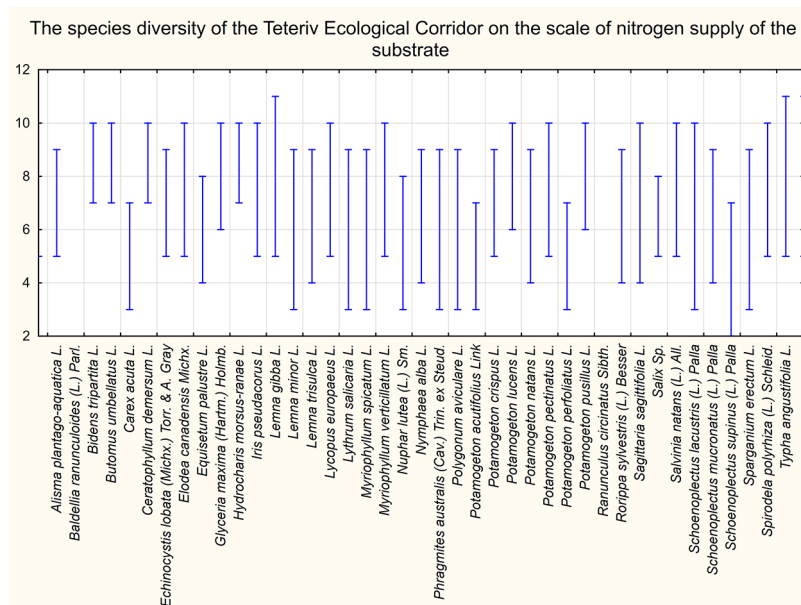
**Table 7.** Analysis of the relationship between the substrate acidity ( $R_c$ ) patterns of macrophyte ecogroups and an integrated ecological index of water quality ( $N = 55$ )

| Ecogroup       | Correlation coefficient | $t_{\text{Student's}}$ | Correlation conclusion | Correlation coefficient  | $t_{\text{Student's}}$ | Correlation conclusion |
|----------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
|                | by projective coating   |                        |                        | by the number of species |                        |                        |
| subacidophiles | <b>-0.371</b>           | -2,936                 | reverse                | <b>-0.519</b>            | -4,458                 | reverse                |
| neutrophils    | -0.122                  | -0.904                 | missing                | <b>-0.516</b>            | -4,430                 | reverse                |
| basophils      | <b>-0.271</b>           | -2,071                 | reverse                | <b>-0.284</b>            | -2,179                 | reverse                |

**Source:** compiled by the authors

In the neutrophil ecogroup, where the projective cover was stable, there was a decrease in the number of species and an increase in species that were less affected by pollution from humans, such as *Potamogeton lucens* L., *Potamogeton perfoliatus* L., *Elodea canadensis* Michx., etc. Aquatic plant species with a significant range of ecological indices in relation to the nitrogen supply of the environment have been recorded. According to the Ellenberg scale, the most numerous were groups of

plants with indices 6, 7, and 8 (27.0%, 29.7%, and 27.0%). An oligotrophic type of nutrition is observed for almost 8% of species (2 and 3 ecological indices). According to the Didukh tolerance scale JP (2011), most species had narrow ranges: 3-7 – 14.6%, 5-10 – 19.5% (Fig. 8). The distribution of species by ecogroups in relation to nitrogen supply showed that the largest shares are nitrophils (61%) and heminitrophils (37.8%), which tend to place with high concentrations of nitrogen compounds in water.



**Figure 8.** The species diversity of the TEC on the scale of nitrogen supply of the substrate

**Source:** compiled by the authors

Hemievritopic species occupy the largest share within both ecogroups, at 63.41%. The presence of species belonging to such a variety of ecogroups in terms of nitrogen supply indicates a significant variation in plant living conditions on this indicator of significant anthropogenic pressure within the TEC, namely intensive agricultural production, and wastewater from urban areas. The ecogroups of geminitrophils and nitrophils were the most sensitive to the

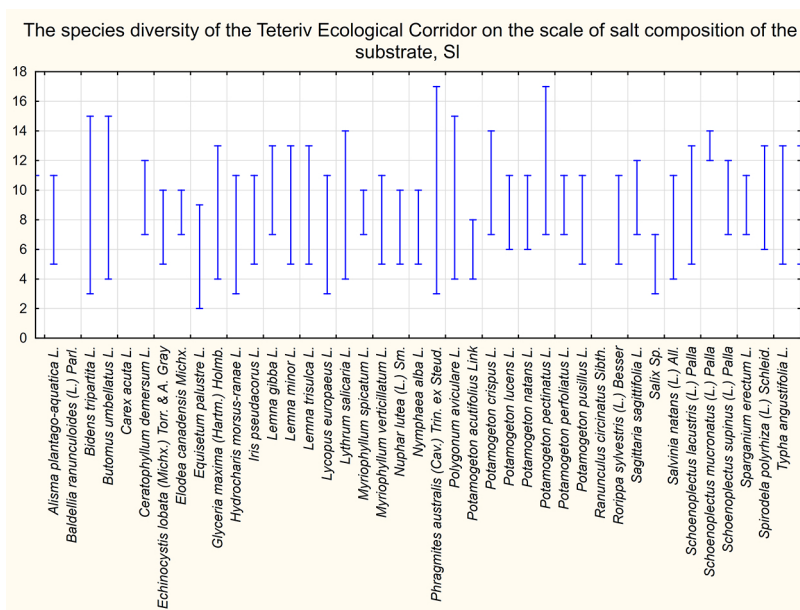
influence of anthropogenic factors, but the nature of reactions within these ecogroups differed. In heminitrophils with deteriorating water quality, there is a decrease in the number of species ( $r = -0.352$ ) against the background of stability of the total projective cover of the ecogroup. In nitrophils, the general decrease of species ( $r = -0.555$ ) with deterioration of water quality was accompanied by a decrease in projective coverings ( $r = -0.391$ ) (Table 8).

**Table 8.** Analysis of the relationship between the ratio of digestible forms of nitrogen in the substrate of macrophyte ecogroups and an integrated ecological index of water quality (N=55)

| Ecogroup       | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion | Correlation coefficient  | t <sub>Student's</sub> | Correlation conclusion |
|----------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
|                | by projective coating   |                        |                        | by the number of species |                        |                        |
| Heminitrophils | -0.069                  | -0.507                 | missing                | <b>-0.352</b>            | -2,765                 | reverse                |
| Nitrophiles    | <b>-0.391</b>           | -3,117                 | reverse                | <b>-0.555</b>            | -4,902                 | reverse                |
| Eunitrophiles  | -0.056                  | -0.413                 | missing                | -0.183                   | -1,366                 | missing                |

Source: compiled by the authors

Analysis of species diversity on a scale relative to the salt regime showed that a significant proportion of species have a wide range of tolerances: 5-11 – 8.2% and 5-13 – 8.2% (Fig. 9).



**Figure 9.** The species diversity of the TEC on the scale of salt composition of the substrate, SI

Source: compiled by the authors

Based on how the plant ecogroups were spread out in relation to the salt content in the TEC, semieutrophs (22.0%) and eutrophs (58.6%) were the most common, which tend to have high salt concentrations in their substrates. In both groups, hemistenotopic species occupy a larger share (56.1%) than less-hemievrytopic (31.7%). Eurytopic species are represented only within the eutrophic ecogroup (4.9%), stenotic-only within the eutrophic (4.9%), and glycotrophic (2.4%).

A small share of the total species diversity was occupied by species that tend to salt-poor substrates or mesotrophic substrates –12.2%. In general, the distribution of macrophyte species by industry shows that species adapted to existence in mineral-rich substrates predominate,

and the presence of subglycotrophic and glycotrophic species indicates the presence of excess HCO<sub>3</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, and Cl-salts and traces of salt salinity in the substrate.

Different ecogroups of macrophytes were affected by the deterioration of water quality in different ways, according to the industry. Projective coverage of ecogroups varied slightly, except for subglycotrophs and glycotrophs. In particular, the first group showed a decrease in the total projective coverage of the ecogroup (r = -0.271) against the background of a stable number of species, and for the second, the deterioration of water quality led to an increase in the number of species (r = 0.567) and the expansion of their projective coverage (r = 0.607) (Table 9).

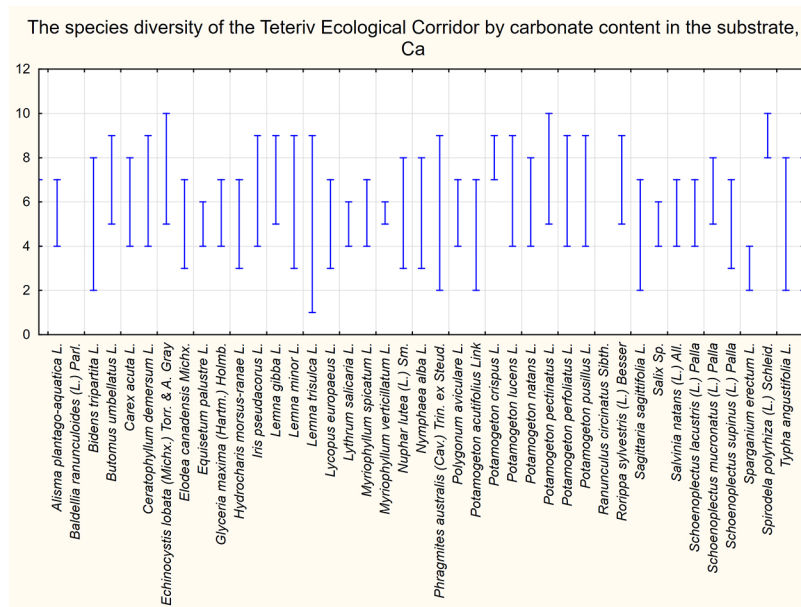
**Table 9.** Analysis of the relationship between the salt regime of the substrate (SI) of macrophyte ecogroups and an integrated ecological index of water quality (N=55)

| Ecogroup       | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion |
|----------------|-------------------------|------------------------|------------------------|-------------------------|------------------------|------------------------|
| Mesotrophs     | -0.179                  | -1,338                 | missing                | -0.219                  | -1,645                 | missing                |
| Semievotrophs  | -0.169                  | -1,263                 | missing                | <b>-0.323</b>           | -2,509                 | reverse                |
| Eutrophies     | -0.242                  | -1,833                 | missing                | <b>-0.550</b>           | -4,842                 | reverse                |
| Subglycotrophs | <b>-0.271</b>           | -2,071                 | reverse                | -0.250                  | -1,897                 | missing                |
| Glycotrophs    | <b>0.607</b>            | 5,618                  | direct                 | <b>0.567</b>            | 5,056                  | direct                 |

Source: compiled by the authors

For semieutrophic and eutrophic ecogroups, the deterioration of water quality led to a decrease in the number of species against the background of insignificant variations in the total groups of projective coatings. Mesotrophs were found to be the least sensitive to anthropogenic pressure, with no significant dependencies on the number of species or their projective

coatings within the study area. The information on the distribution of macrophyte species diversity in relation to soil carbonate content is intriguing. Most species have narrow ranges of ecological valence to this factor. And the most represented ranges, 4-7 and 4-9, cover almost 30% of species (Fig. 10). Carbonatophobes (2.44%) and hemicarbonatophobes (68.29%) are prevalent.

**Figure 10.** The species diversity of the TEC by carbonate content in the substrate

Source: compiled by the authors

Stenotic and hemistenotopic biotopes are more common in ecogroups, which also shows that carbonate salinization isn't allowed in the study area.

Different ecogroups were affected by deteriorating water quality in relation to the presence of carbonates. (Table 10).

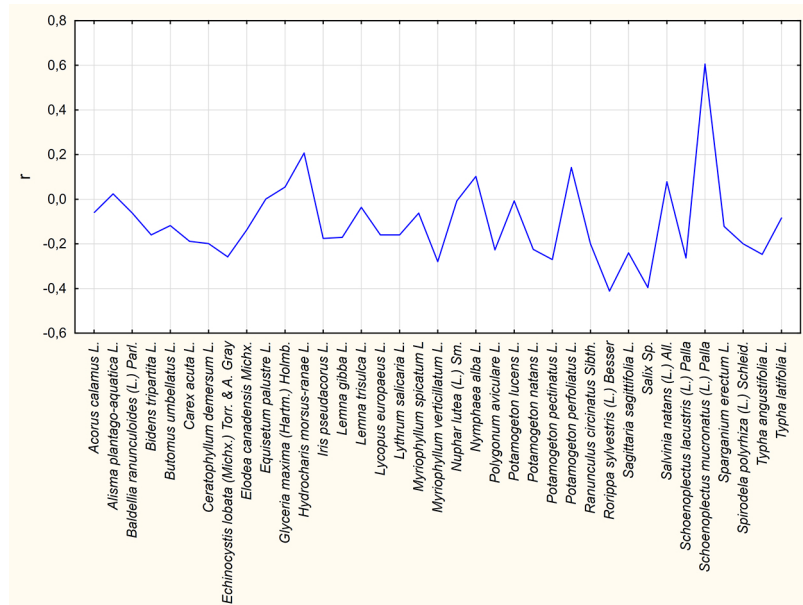
**Table 10.** Analysis of the relationship between the carbonate content in the substrate (Ca) of macrophyte ecogroups and an integrated ecological index of water quality (N = 55)

| Ecogroup            | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion | Correlation coefficient | t <sub>Student's</sub> | Correlation conclusion |
|---------------------|-------------------------|------------------------|------------------------|-------------------------|------------------------|------------------------|
| Carbonatophobes     | -0.118                  | -0.875                 | missing                | -0.118                  | -0.875                 | missing                |
| Hemicarbonatophobes | <b>-0.331</b>           | -2,581                 | reverse                | <b>-0.578</b>           | -5,211                 | reverse                |
| Acarbonate          | -0.128                  | -0.950                 | missing                | <b>-0.323</b>           | -2,510                 | reverse                |
| Hemicarbonate       | -0.196                  | -1,469                 | missing                | -0.116                  | -0.863                 | missing                |

Source: compiled by the authors

Thus, the most sensitive group was the hemicarbonat ecogroup, where, along with a decrease in the number of species, there was a decrease in their projective coverage. On the other hand, in the acarbonate group, the loss of water-sensitive *Rorippa sylvestris* (L.)

Besser was accompanied by overgrowth of *Potamogeton lucens* L. and *Schoenoplectus mucronatus* (L.) Palla. So, research has shown that there are a few ecogroups and species that have changed the most in response to changes in water quality (Fig. 11).



**Figure 11.** Correlation relationships ( $r$ ) of macrophyte distribution in terms of projected coverage based on the integrated environmental water quality index (IE)

Source: compiled by the authors

Thus, the negative impact and close feedback between these indicators for the species *Trapa natans* L. (Michx.) Torr. & A. Gray, *Myriophyllum verticillatum* L., *Rorippa sylvestris* (L.) Besser, *Salix* Sp. etc. In addition, the positive effect of deteriorating water quality on the increase of projective coatings of some species of macrophytes, in particular, *Hydrocharis morsus-gapae* L., *Potamogeton perfoliatus* L., *Salvinia natans* L., *Schoenoplectus lacustris* L. etc.

Wetlands are vital, self-sufficient ecosystems that aid in the dispersal of a wide variety of plant life. Related research supports this claim (Finlay et al., 2020; Cruz et al., 2020). According to Vestergaard et al. (2000), these aquatic communities play a crucial role in conservation efforts over the long run. Wetlands operate as natural pollution filters and store water after heavy rains, so it's no surprise that Khan et al. (2022) found that they also help reduce pollution.

Wetland vascular plant diversity has been categorized in numerous ways based on the findings of different studies. For instance, Khan et al. (2022) categorized wetland plants using Cook's (1996a) taxonomy. Although Ellenberg's systems are widely recognized for the European region, they are missing several of the species that was discovered in the research. As a result, Didukh's system was heavily utilized, albeit modified to better suit Polissya Ukraine.

Members of the Potamogetonaceae and Cyperaceae families, which thrive in open places because to favourable factors like strong light, are disproportionately common, according to certain authors (De Oliveira et al., 2020; Haq et al., 2021). This research uncovered similar patterns.

Through this investigation, it was possible to deduce the relationships between species' ecological characteristics and their growing environment. There is a lack of research on this problem in wetlands, even though it is generally accepted that ecological development conditions affect species richness through decreased nutrient availability and slower rates of nitrogen mineralization and nitrification (Rajilesh et al., 2016; Cruz et al., 2020). The relevance of water composition and lighting conditions for their maintenance was highlighted by the current study, which showed a correlation between practically all ecogroups of macrophytes and the ecological parameters of locales. Vestergaard and Sand-Jensen (2000), two other authors, validate these findings as well.

Analysis of species distribution patterns revealed that aquatic habitats, home to species with a wide variety of thermoregime, ombroregime, continentality, and cryoregime tolerances, was predominant in the Teteriv ecological corridor. They are adaptable to a wide variety of environments, including those with a constant over

wetting of the floodplain, wet swamp ecotopes, and places with temporary over wetting, low levels of dissolved oxygen in the water, the presence of muddy sediments, the predominance of anaerobic processes of transformation of neutral or, in some cases, slightly acidified substrates, a diverse supply of nitrogen and mineral composition, and the presence of traces of phosphorus. The following ecogroups are particularly vulnerable because of the degree to which their evolution is altered by the effect of anthropogenic factors.

### CONCLUSIONS

The conducted studies showed the presence of species belonging to 3 divisions, and Magnoliophyta was the most numerous (41 species). The number of species is distributed almost evenly among the divisions – 15 and 14 species within the classes Liliopsida and Magnoliopsida. The group of surface-aquatic-aerial species was the most numerous, accounting for about 53.8% of the total number of species. Most phytocenoses belong to the boreo-meridional chorological group with a circumpolar range type. Thus, because of phytoindication analysis of macrophytes, a significant diversity of ecomorphs and groups of species in response to major abiotic environmental factors, formed mainly on nutrient-rich, acid-neutral, and nitrogen-rich substrates. The analysis of the distribution of species diversity showed that the Teteriv ecological corridor is dominated by aquatic ecosystems inhabited by species with wide ranges of tolerance to thermoregime, ombroregime, continentality, and cryoregime, which are able to exist in ecotopes of constant over wetting of the floodplain,

wet swamp ecotopes, and places with temporary over wetting, a low level of dissolved oxygen in the water, the presence of muddy sediments and the predominance of anaerobic processes of transformation of substances neutral in acidity, and in some cases slightly acidified substrates, with a significant range of nitrogen supply and mineral composition, with the presence of traces of salt salinization of the substrate, but unable to exist in the conditions carbonate salinity.

The development of various ecological groups is significantly adjusted by the influence of anthropogenic factors, so the ecogroups are defined as sensitive: in relation to the thermal regime – submicrothermal and submesothermal; by aridity-humidity – mesoaridophytes and subaridophytes; in relation to the cryoregime – subcryophytes and hemicryophytes; according to the scale of continentality – hemioceanists and hemicontinentals; according to soil moisture – hygromesophytes, hydrophytes, hygrophytes and subhydrophytes; in relation to aeration of the substrate – subaerophiles, hemiaerophobes and aerophobes, hyperaerophobes; according to the reaction of the environment – neutrophils, heminitrophils and nitrophils; according to substrate branching – subglycotrophs, glycotrophs, semieutrophs and eutrophs; according to the content of carbonates in the substrate – hemicarbonatophobes and acarbonatophiles.

The prospects of further research are related to the determination of morphological features of species belonging to different ecogroups, which can be used for bioindication of anthropogenic pressure on aquatic ecosystems.

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## Індикаційні особливості макрофітних угруповань в оцінці антропогенного навантаження на водні екосистеми

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**Анотація.** Вивчення структурно-функціонального біорізноманіття у взаємозв'язку з різними екологічними чинниками наразі є надзвичайно актуальним, оскільки водні екосистеми є вагомим джерелом біологічного різноманіття і складають значну частину біологічної продуктивності Землі, виконують багато функцій, є цінними і важливими для стабільності біотичних угруповань. З огляду на це, було поставлено завдання визначити флористичний склад Тетерівського екологічного коридору як прототипового річкового ландшафту північної частини України, проаналізувати структурно-функціональні особливості видового різноманіття макрофітів у ньому та розчленувати це різноманіття на складові частини за місцем походження, життєвою формою та відношенням до факторів середовища. За результатами дослідження проаналізовано кількість видів та їх прогнозоване покриття на ділянках з різним антропогенним навантаженням в межах Тетерівського екологічного коридору в розрізі екологічних зон. Показано, що інтегральний екологічний показник якості води є визначальним для зростання фітоценозів у регіонах з високим антропогенним навантаженням. Угруповання можуть виживати в умовах низького вмісту розчиненого кисню, великої кількості мулистих відкладів та переважання анаеробних процесів у перетворенні речовин. Крім того, вони можуть поширюватися в заплавах річок, вологих болотистих екотопах та інших середовищах, де вода присутня протягом тривалого часу. Більшість цих угруповань не вибагливі до середовища існування, оскільки можуть процвітати в різних ситуаціях, включаючи слабкокислі або нейтральні субстрати, різний вміст азоту та мінеральних речовин у ґрунті, а також помірне засолення рослинного покриву. Збільшення чисельності представників окремих екоугруповань може свідчити про зміни екологічного стану водних екосистем та мати практичне значення при виявленні посилення антропогенного тиску на водні екосистеми

**Ключові слова:** індикатори, фітоценози, біорізноманіття, види, екоугруповання

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## Impact of Munitions Corrosion Processes on Groundwater Contamination and Techniques for Their Safe and Cost-Effective Disposal: A Case Study of a Village

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**Abstract.** The relevance of the study lies in the fact that the ammunition that has entered the groundwater (deeper than 250 m), as a result of its disposal, poses serious threats to the entire Donetsk region water area. The purpose of the study was to investigate the impact of munitions corrosion on the aquatic environment and to consider the most effective and environmentally friendly methods of disposal. To conduct the research following scientific methods were used: methods of analysis, deduction, synthesis, and formalization method. It was found that each method of ammunition disposal led to a negative impact on the environment both in the short and long term. The literature of recent developments in munitions corrosion and munitions recycling was analyzed. It was summarized that there was an urgent need to introduce a system for cleaning up contaminated territories, to take control over the implementation of legislative environmental standards, to use new biological methods for elimination of the consequences of the impact of ammunition on the ecosystem of the region, and to switch to more environmentally friendly types of weapons. The practical value of the article is in search of ways to prevent an environmental and humanitarian catastrophe in the war zone in the East of Ukraine

**Keywords:** open burning, uranium, disposal, toxic substances, water and soil contamination



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## INTRODUCTION

Military equipment in today's world is an important issue for all countries. However, when it is placed into the water, it leads to dangerous ecological impacts on humans and the environment. Also, most munitions are sources of mercury, filling the marine environment with its concentrations. Mostly after military equipment has been used, surplus ammunition is being dumped into the sea, rivers and also into groundwater. The risk of explosion with this type of disposal cannot be excluded. In recent years, the impact of chemical munitions aggregate emissions, their corrosion, and their influence on public health and ecosystem has become an ever-increasing issue. Munitions contain large amounts of substances (mainly heavy metals, also trinitrotoluene (TNT) or hexanitrodiphenylamine) which can be dangerous for the environment. Corrosion processes are also the cause for the rate of ejection of hazardous materials (explosives) that may threaten the aquatic ecosystem, and after all may enter the food chain, and human body during water consumption. Moreover, considering the fact that the only source of drinking water in Donetsk region is the Siversky Donets-Donbas Channel.

Also, according to the source (Using groundwater in..., 2020), there is a high content of chemical and toxic elements in groundwater in Donetsk region. Various factors influence the corrosion process. For example, the speed and rate of the process may differ depending on the time under water, and the type of munition material. The thickness and quality of coating materials are also important. When assessing the corrosion of the material, attention should also be given to the level of penetration into the sediment (Silva & Chock, 2016). Toxic substances potentially released from dumped munitions are added to the total mass of pollutants present in the aquatic ecosystem. There is uncertainty about what kind of large-scale threat humanity might face and what would be the most cost-effective methods of remediation or beneficial impacts in different disposal areas (Betdowski *et al.*, 2020). The possibility of people coming into contact with hazardous waste of military origin is now extremely high. However, there are also suggestions to find replacements for toxic substances used in munitions production to reduce the impact on the environment.

For example, in (Ferreira *et al.*, 2016), the authors evaluated and equate the environmental and toxicological influences related to the life cycle of four 9 mm ammunition: a combination of two types of shells with two types of primer (lead primer; non-lead primer). Moreover, opportunities have been identified to enhance the environmental indicators of these munitions. The results showed that projectile and primer alternatives reduced exposure, mainly toxicity to humans due to the replacement of lead. However, alternative ammunition (with composite projectile) demonstrates a higher influence on ecotoxicity because of copper emissions,

perhaps associated with the erosion of the projectile in the barrel of the weapon. Improper disposal of ammunition can affect a number of areas, affecting the economy of Ukraine, in particular: sea communication lines (trade); marine infrastructure, transmission through the gas lines, and oil pipelines; fishing; bases of the North Atlantic Treaty Organisation (NATO), etc. The dumping of munitions poses a direct hazard to all living things, and the degree of the threat depends on the condition the hazard sources themselves have been in for as long as they have spent in a corrosive water environment (Miętkiewicz, 2020).

Alboloushi *et al.* (2020) studied the environmental pollution situation in the Gulf of Kuwait as a result of military operations in the Persian Gulf region in 1991. Scientists determined that only natural uranium remained in the seawater, while depleted uranium has not been detected. It is thus clear that even in the case of the decomposition of uranium-containing munitions, it is possible for the ecosystem to fully recover from radioactive substances, although this requires some time. The study by Beck *et al.* (2022) examines the damage from munitions that have been present in the coastal waters of the southwestern Baltic Sea since the two world wars. Scientists claim that from explosives contained in munitions, marine biota accumulates toxic elements. Biota is food for larger marine life, such as commercial fish species (flounder). Even outside the munitions dump, TNT was found in the fish. At this stage, the toxic substances in marine inhabitants consumed by humans do not pose a threat to health and life, but researchers focus on the continued corrosion of the walls of munitions, which will significantly increase the number of harmful substances in coastal waters and organisms that live there.

Scharsack *et al.* (2021) in addition to studying the effects of munitions in marine waters considered climate change as one of the reasons for the increased harmfulness of toxic substances to marine organisms. Wars are not only a danger to the environment, but also a danger to the habitat. Global warming entails a chain of other climate changes that can lead to environmental disasters. In the face of massive storms, typhoons, and volcanic eruptions, the corrosion of munitions in the water will accelerate exponentially, as will the overall damage from the toxins entering the water. However, there is a positive trend in this process: due to an increase in the temperature of the water, the process of processing biota chemicals munitions will be significantly accelerated. Zhang *et al.* (2021) team also investigated the environmental impact of depleted uranium munitions, but the burials were not in water but in soil. The focus of the study was to establish a link between the toxicity of munitions and soil moisture levels. The corrosion started in corner cracks, edges, and small pits of ammunition, and accelerated with increasing soil

moisture. The results of this study show the influence of soil moisture on the corrosion rate and the migration rate of DU metal.

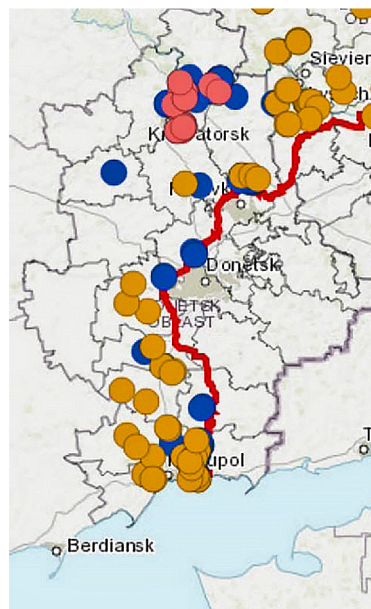
A. Souchen (2022) conducted a study on the process of disposing of the Canadian state of surplus ammunition and military equipment used in World War II. The need for disposal triggered three main reasons, among which are the post-war recycling crisis, the desire to prevent deflation of the economy, and the unprofitability of leftovers. Military materials were processed and used in the production of products for a peaceful life. The positive side of the policy was also negative: often the prices of reproducible products were overvalued and sales were artificially reduced. Ferreira *et al.* (2019) in their research proposed an alternative model of ammunition processing, which has a much smaller impact on the environment. Scientists speak about the use of energy material in ammunition in the production of explosive materials for civilian use. This approach to recycling is seen as a replacement for the usual combustion processes in complex waste incineration plants, as it has limitations on the capacity of the incinerator, electricity consumption, and high cost.

Thus, *the purpose of this research* is to address the environmental issue associated with ammunition dumping in groundwater. The study also considers the impact of progressive corrosion of notorious munitions and analyses safe and cost-effective methods of disposal.

## MATERIALS AND METHODS

Within the framework of this study, the features of the Donetsk region were studied as an example of a region of active hostilities. The data to create the work was collected between 2019 and 2021. As research methods, articles in scientific journals from such databases as ScienceDirect, Science, Nature Publishing Group, Taylor and Francis, Science, and SpringerLink were analysed. Ukrainian news portals were also used, providing open sources and data on the state of groundwater, issues of drinking water for the Donetsk region population, and questions about the imminent disposal of unsuitable ammunition for the storage of new batches. Through the media, it was possible to identify the main health concern of the Donetsk region population – the lack of clean drinking water and the impact of military action on the population. The Ukrainian Ministry of Defence, together with the British charity and American non-profit organisation HALO Trust, demonstrated a map (Figure 1) of Donetsk region with information on areas contaminated by mines and explosive remnants of war (The Ministry of Defense presented..., 2018). The marked bright circles indicate how the terrain is filled with munitions, minefields, etc. The “cleanest” spaces are mostly in the eastern and Western parts of the Donetsk and Luhansk regions, making the results in ammunition disposal methods considered to be of concern

to this area on account of their safety. Furthermore, the disposal requires underground and buried storage and landfill sites away from the public and potentially unsafe areas. In Ukraine, an ammunition recycling programme was already introduced in 2008-2017, where 474 thousand tons of ammunition had to be processed. To conduct the research following scientific methods were used: methods of analysis to identify the main toxic materials from the munitions in the region; deduction to assess the level of the environmental danger of such toxic materials for the villages; synthesis to select the most effective ways to minimize a negative influence to the Donetsk and Luhansk regions' water ecology; and formalization method to illustrate the corrosion and dissolution of depleted uranium processes as a chemical expressions.



**Figure 1.** Donetsk and Luhansk regions territory information map

**Source:** The Ministry of Defense presented..., 2018

The following section is divided into two main stages where important aspects of the study are addressed. Firstly, the process of munitions corrosion and its impact on water, the environment and the population were considered. Studies listing the elements that can be formed by ammunition corrosion have been cited. Such elements include: uranium (including its oxides), mercury, lead, copper, tungsten, arsenic, and zinc. All of these elements can have negative consequences and affect human health and vital activity and environmental safety. This paper also reviewed studies in analysing the behaviour of munitions corrosion in water in general, including oceans, sea, rivers, groundwater, etc., as the species of bacteria found in oceans, freshwater, etc., are very similar.

The second stage dealt with the area of ammunition disposal methods. Classifications were given and the latest innovative technologies proposed by various authors, such as thermal salt recovery, oxidation, solvent extraction, biodegradation, alkaline hydrolysis and green technology, were listed. When searching for keywords related to this work, the results show a number of concerns expressed by the population of Donetsk region regarding a large amount of ammunition disposal, military activities and flooding of mines, which have a detrimental impact on livelihoods. The cited case studies can be used as a basis for further investigations. All of the analysed studies involving ammunition disposal can be implemented at any location.

## RESULTS AND DISCUSSION

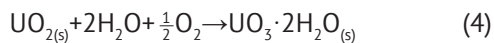
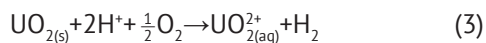
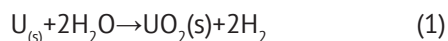
**Corrosion of ammunition.** In the traditional classification, two types of underwater ammunition are distinguished: those that contain dangerous elements and those that do not. Hazardous elements are various types of detonators, explosives, chemical warfare agents, and fuses. When items are thrown with a specific purpose during an event, they are defined as detonators and are classified as Unexpected Ordnance Disposal (UOD). Corrosion of ammunition in water occurs due to the flow of environmental, electrochemical, and microbiological processes. First, there is an ejection of soluble metal cations (electrolyte) and the passage of electrons through the metal, to the neighbouring cathode sections of the metal surface. Here the consumption process happens, due to which the amount of diluted oxygen and water molecules at the cathode is reduced in order to form hydroxide ions. The electrochemical cell obtained as a result of the described process leads to corrosion of the solid metal at the anode sites. This is because of the ejection of metal ions into the mud, due to which localized holes are formed on the surface, and the reaction products of soluble metal cations and hydroxide anions are also precipitated. The described process has the form of amorphous metal hydroxides and oxyhydroxides in neighbouring large cathode surfaces. The described process is called general or "homogeneous" corrosion. The overall corrosion rate is determined by the presence of dissolved oxygen and the level of electron consumption that occurs during the reaction to ensure charge balance (Silva & Chock, 2016). Sulphide-reducing bacteria (SRB) found in waters should be investigated very carefully, just like bottom sediments. Due to them, biofilms form on metal surfaces, which can make the corrosion process more difficult. This process is called microbiologically influenced corrosion (MIC). The intensification and potential of corrosion processes are determined by the presence of such bacteria and their populations (Cybulska et al., 2020).

The improvement of military hardware and the increased usage of projectiles with urbanisation disrupt infrastructure development. However, the removal

of ammunition can be fraught with technical risks that may cause even greater harm and threat to the environment, and in some cases, for example, for large unexploded ordnance, it is considered an impracticable option using currently available disposal methods. Donetsk region has a single source of water supply – the Siverskyi Donets River. In the research (Nikolopoulou et al., 2022), the authors assessed the pollution of this artery. Patterns of new pollutants along with nutrients and metals have been explored in surface water and groundwater samples. The results thus show that warfare and mine flooding have had harmful impact on the population. This requires groundwater pumping to avoid the ingress of toxic substances into the groundwater, at the very least, and also to avoid groundwater seepages. Previously, groundwater has already been investigated by means of an ecological-geological survey in the Donetsk region at a distance of 30 km from Donetsk (Water of questionable quality..., 2019). The results showed that all elements of the periodic table were present in the soil. This is already a sign that corrosion together with improper disposal has significantly affected the environment. For example, in the village of Yaremivka, the results of water analyses show high levels of nitrates along with sulphate shale (Special project, 2021). Negative results are also shown near Toretsk and in the village of Novhorodske – the samples contain high salinity, which already implies that it is no longer suitable for drinking (JCCC: Toretsk threatens..., 2017). It is also worth noting that in the village of Solnetsvka, as in many other villages in the region, there is no centralised water supply, residents complain about water quality, which is confirmed by the results of analyses (Humenyuk & Nazarov, 2018).

For villages in Donetsk region, there is no other water source than the Siversky Donets-Donbas (Dangerous water and soil..., 2019), and given that all groundwater is saturated with toxic elements, it cannot be used. Ignoring this issue will lead to an environmental catastrophe, the consequences of which cannot be reversed. In work Bełdowski et al. (2019) the results show that flooded munitions in the water are sources of mercury deposited as bottom sediments. Bełdowski et al. (2016a) investigated the disposal site of chemical munitions within the Gotland Basin. The findings suggest that the analysed area may be characterised by huge number of objects and that these sediments might contribute to the spreading of pollutants in the bottom sediments. Military training activities, especially those involving munitions, can have a negative impact on the surface soil as they deposit metals such as Pb, Sb, Cu, As, Zn and W that pose risks to human health and the safety of the environment. Military projectiles mainly consist of a steel penetrating element, followed by a Pb/Sb bullet coated with a Cu, Zn and Pb alloy shell (Barker et al., 2021). However, it is important to consider that the toxic, weakly radioactive element uranium U is widely

used in munitions during wartime conflicts. Flooded uranium is a co-product of the Uranium-235 fortification process, which is used in nuclear reactors and weapons. The production of 1 kg of fuel from enriched uranium containing 235U yields approximately 5 kg of depleted uranium waste. Depleted uranium is used in many areas, such as in counterweights for aircraft, in the capacity of catalysts in the oil and gas sectors and for radiation protection. Depleted U is thermodynamically volatile and thus prone to corrosion in the natural environment. The corrosion and dissolution of depleted uranium occur as follows:



The level of depleted uranium corrosion is much higher in water than in the air. However, the content of water in the soil influence on the erosion mechanism of depleted uranium, but it is important to consider activity parameters such as soil pH. Thus, it was shown by Lotufo *et al.* (2021) that the corrosion level of depleted uranium was influenced by the soil's water content. From the perspective of influence on soil micro- and macrofauna, depleted uranium corrosion causes a marked decrease in the variety of the microbial community. In the material written by Papageorgiou *et al.* (2022) there are samples from groundwater have been examined and the results show that approximately 10% of the groundwater has a uranium concentration exceeding the World Health Organization provisional norm of 30 µg/l for drinking water. Explosions of depleted uranium release particles of uranium oxide, such as  $UO_2$  and  $UO_3$ , which spread into the air for about 10 km, thus increasing the contamination radius with long-lasting effects on animals, insects, water, food and air. The study conducted by M.R. Joyce and K.H. Holton (2020) outlines four possible ways of ingestion: inhalation of depleted uranium powder, swallowing through infected food products and liquids, shrapnel injuries or dermal suction of dust. The same data are reported by the author of the research work "Metals/Metalloids: Environmental Exposure and Health Effects" (Danesi, 2019) – depleted uranium weapons leave fragmented or undamaged penetrating particles along with dust, which, if inhaled, are highly likely to be acquired, especially by persons spending some time in vehicles affected by the ammunition. All the studies carried out so far showed that exposure doses in the open air were so low that they did not pose a significant risk. For those

spending at least 10 hours a year in vehicles affected by depleted uranium munitions, the risk of fatal cancer is slightly higher (~0.2%).

Plaza *et al.* (2018) report that ammunition is an underestimated source of lead. That is, pollution by lead through bioaccumulation and lack of biodegradation should be expected to become a worldwide issue that affects the health of all living beings. Lead is extremely toxic for humans, and the presence in food of the rest emissions could be very dangerous for health. T. Schupp (2021) investigated areas where relatively high Pb inputs to soil had previously been observed. The results show that such areas require long-lasting groundwater monitoring, even if Pb accumulation is considered insignificant. In the work created by Straumer *et al.* (2020) the relationship between impact of fish to dumped munitions and histopathological changes in the liver is investigated. Fish from the "reference (clean) site" showed less pathology compared to those from the area where munitions were disposed of. The study of corrosion products from the decomposition of ammunition, which have been under water for more than 60 years, was carried out by Li *et al.* (2016). To conduct the study, scientists used various technologies, including EDXA (energy dispersive X-ray spectroscopy) combined with XRD (X-ray structural analysis) and Raman spectroscopy.  $\beta$ -Fe $2(OH)3Cl$  together with Fe $3O_4$  and  $\beta$ -FeOOH – this is how the primary phase was determined after treatment of the inner layer. The researchers found a thick layer of nodule that was outside the iron hydroxychloride layer. The nodule layer consisted of an outer greyish-white layer of CaCO $3$  and an internal black layer of FeCo $3$ . The authors of the study attribute the formation of FeCo $3$  to the exchange process of Fe $2+$  ions with Ca $2+$  ions in the CaCO $3$  layer. The exchange process occurred during the movement of Fe $2+$  ions to the external coral sheet from the corroding steel.

Findings of Beldowski *et al.* (2016b) show that dumped munitions can degrade to inorganic arsenic and that the disposal areas studied contain high levels of arsenic compared to the reference areas. Thus, the materials that make up ammunition are generally highly susceptible to oxidation and corrosion (e.g., uranium is highly sensitive to hydrogen, oxygen, carbon and nitrogen), which requires preventive treatment. The dissolution of harmful substances in water is considered an important, first stage in their decomposition. Corrosion of munitions refers to a phenomenon in which the object itself and the environment are subjected to chemical, electrochemical or physical attack, resulting primarily in deterioration of the environment and the release of toxic substances. Preventing and controlling such a situation means that methods and technical measures for prevention and long-term protection must be applied, based on a study of corrosion principles and the reaction mechanism of munitions in the environment. In worst-case situations of chemical weapons corrosion,

specific instructions on how to avoid contact or how to handle military items need to be developed. The presence of munitions on sediments also adversely affects cables, main gas and oil pipelines, etc.

Groundwater is an important component of the natural water cycle. It connects the Earth's surface and surface receiving waters. The Earth's population, fauna and flora, i.e., its entire environment, attract negative consequences due to the pollution of groundwater and soil through the corrosion of munitions. The groundwater of Donetsk region has poor water quality. It can no longer be used as a drinking source. It is directly involved in the process of leaching ammunition parts, which forms a plume of contamination. The speed of the corrosion process and extrication all of the chemical and toxic materials later are depend on the conditions in which the munition lies. Usually, the oxygen and entraining current encourage the destruction of the weapons. That is, the corrosion of the munitions and the associated possible leakage into oxygen-containing layers of water and sediments leads to a diffuse release of the explosive substances and chemical warfare agents they contain. Conventional munitions, especially those containing trinitrotoluene, are poisonous to microorganisms and aquatic vegetation.

That is, different toxic substances are released during the corrosion process, depending on the ammunition material. When water contacts with the toxic substances, it could be a dissolvent or suspending substance. Therefore, chemicals seep into the ecology system, initially spreading pointwise, probably entering into an equilibrium process of sorption/desorption in sediments and eventually spreading more widely because of water processes and humans' activities. Ingestion and inhalation of corrosion products are potential routes of radiation exposure. Thus, drinking water and consuming food contaminated with the substances listed above is harmful to human health. It is therefore worth focusing on the purity of groundwater used for public consumption. Also, as effective measures to reduce the entry of such toxic substances into the soil, measures should be taken to reduce their Pb content, and the use of depleted uranium in ammunition construction should be banned. Munitions require corrosion protection methods and techniques at all stages: design, construction, operation and disposal.

**Disposal of ammunition.** The most common and long-standing methods of ammunition disposal are:

1. Open burning is one of the most common disposal methods, carried out in especially remote locations (e.g., military landfills). The process is carried out as follows: the ammunition is burned on the ground using fuel. The combustion temperature does not exceed 500°C, there may be residues of waste products.

2. Open detonation is also a frequently used method of disposal, but there are some limits to its applicability. The process is carried out by detonating the munition

with an explosive charge, resulting in the complete chemical decomposition of the object, but particles of heavy materials may remain, which are detrimental to the quality of the air and soil.

3. Closed detonation, similar to the open detonation method, but with the advantage of controlling emissions to the environment (Zhu *et al.*, 2021).

Ammunition storage, and its safe disposal at Ukrainian military bases, is a pressing issue. Pavlograd Chemical Plant is one of the leading enterprises in Ukraine engaged in ammunition disposal. The company contributed to the development and implementation of integrated processes for the disposal of ammunition, which have a favourable impact on the country's economy, and the development of recycling technologies for material resources such as metals, plastics, etc. Zhu *et al.* (2021) presented a detailed study on the dangers of six different methods of ammunition disposal. The results showed that the closed detonation method brought about the highest hazard and the highest risk, followed by open burning and open detonation. There are also disposal methods that have been used for quite a long time. The basic principles of such techniques are briefly outlined below. The mechanical disassembly method (designed for large calibre ammunition) – the idea is to separate the shell from the casings using pulling machines or by breaking them out. Disposal method using an electric arc, i.e., the direct impact of an electric arc on a cartridge case, with further destruction of the ammunition cartridges. The firing method has advantages such as security, cost-effectiveness, etc. An electric incinerator is the most economical yet effective method of disposing of munitions. Its high efficiency in handling ammunition is due to the fact that the disposal process is fully automated for small arms and has a reserve of recyclable material from individual items (casings, shell casings, etc.). Moreover, the operation of this furnace is not dangerous for the personnel (Yang *et al.*, 2019).

At each stage of the munitions manufacturing process – production, transport, storage, distribution and disposal – environmental degradation occurs through the introduction of explosive contaminants in the form of pure liquid or solid particles due to leaching, trace particles, contaminant spills, fully or partially detonated and unexploded munitions. Wastewater from ammunition is generated during the production and disposal of ammunition containing residual energetic substances. Despite the necessity of removing energy pollutants from munitions wastewater, practical treatment faces three obstacles. The first obstacle is that energy substances are biochemically unstable. The second is that the physiological destruction of these nitro elements releases nitrates (NO<sub>3</sub><sup>-</sup>) and/or nitrites (NO<sub>2</sub><sup>-</sup>), which causes secondary contamination. The third obstacle is that such substances usually coexist in the wastewater of ammunition and must be removed. The study by Zheng *et al.* (2022) has succeeded in developing a

technology that is two to three orders of magnitude superior to other methods. The authors of another study (Pirzadah *et al.*, 2022) propose a method of phytoremediation, which involves the inherent ability of plants to bind these explosive compounds in their original forms or to modify and decompose them into inert forms. In response to the need for safe disposal, plasma arc technology with the ability to safely dispose of munitions in an environmentally acceptable manner is given in work created by Gonçalves *et al.* (2022). This technology has been installed and operated in the Western District, North Carolina since 2001.

Thermal saline disposal, solvent extraction, oxidation, alkaline hydrolysis, and biodegradation are becoming more and more common as new methods of ammunition disposal. The alkaline hydrolysis method can rightly be considered the most popular among all the above mentioned approaches. This method is the removal of nitro groups from nitrocellulose, followed by the formation of nitrites and nitrates. The formation of the latter becomes possible due to the course of chemical reactions such as substitution and hydrolysis (Li *et al.*, 2019). S.A. de Oliveira *et al.* (2018) compared the influence of three ways of disposing of ammunition on the environment. Among the three methods analysed were open blasting; a combination of incineration including the air pollution control, open burning, recuperation of some energy materials, and recycling of the metal; burning in a stationary incinerator with control of the air pollution level combined with the recycling of metal. The results indicate that the biggest impact is the recycling of munition and minimizing the extending of air pollution after incineration. The lowest degree of satisfaction with disposal tasks was traced as a result of the open detonation.

Corrosion processes are now also taking place for metal shells of ammunition once thrown into the water. It is impossible to determine the exact duration of the complete completion of the corrosion process. However, complete corrosion has a number of disadvantages. For example, it is very difficult to determine the fact of lost ammunition due to the absence of metal cases as such. Or high levels of chemical release from decomposition into the environment. Also, one should not ignore the change in climatic conditions, which reduces the possibility of predicting the future fate of the ammunition being dropped to zero. The solubility of the object, water temperature, oxidation, and hydrolysis are of paramount importance in determining the bioavailability and continued residence of chemicals in the water, which as a result can affect the ecosystem as a whole. The location of the munitions dump is also a fact that deserves attention, as it has certain environmental parameters, similar to the types and quantities of chemical and conventional munitions dumped. The creation of a monitoring system by drilling deep wells at the disposal site can solve the problem of monitoring the state of groundwater pollution.

It is also worth considering the possibility that explosive residues could be washed away by rain, which would affect groundwater. This issue can also be solved by the introduction of treatment plant systems. However, the popular physical and chemical approaches to cleaning up polluted areas are costly. There is a growing trend of munitions waste management towards the use of 'green' technologies that will benefit water, soil, etc. One such alternative is the plant and microbe clean-up method, which has the benefits of being low-cost, highly efficient, environmentally friendly and also cost-effective. Microbes are unique, characterised by versatile and prolific decomposition functioning systems that promote favourable degradation and recycling of toxic substances as energy resources, adapting well to changes in the environment. Plants absorb harmful substances from groundwater and are subsequently converted.

There are two categories of green technologies with which it is possible to dispose of toxic substances that are contained in the soil. The first one is ex situ technologies (with soil removal). The second is known as in situ technologies (without soil removal). The bioremediation processes of the first technology require the extraction of soil that has been contaminated or the extraction of groundwater that has been contaminated. The in-situ technology makes it possible to process contaminated objects directly at their location. S. Anand & S.M. Celin (2017) demonstrated the positive results of an attempt to use microbes in the purification process, including them in ammunition. The safety of the process of neutralizing toxic materials using this technology was ensured. The gradual reduction process of TNT, which resulted in the formation of hydroxy-amino-dinitrotoluene (C<sub>7</sub>H<sub>7</sub>N<sub>3</sub>O<sub>5</sub>), 4-amino-2, 6-dinitrotoluene, 2-amino-4, 6-dinitrotoluenes, various azox compounds, 2, 6-aminonitrotoluenes and 2, 4-diaminonitrotoluenes, was preceded by the inclusion of the bacterial organisms *Pseudomonas putida* GG04 and *Bacillus* SF in the munitions. The latter process was carried out in order to improve the bioremediation of 2, 4, 6-trinitrotoluene remains. A description of the experience of using plants during processing is also present by Zheng *et al.* (2022).

Thus, the use of "green" technologies is popular, effective, and most importantly environmental in the disposal of ammunition for cleaning from toxic substances. Microbes are widely used in the restoration of heavy metals on contaminated sites. This technology is expected to gain traction and displace chemical processes, but a further extensive investigation is needed to achieve this. Such techniques need to be introduced into Donetsk and Luhansk regions, as much of the area is already occupied by minefields and munitions, making disposal difficult. While all disposal options have advantages and disadvantages, they must be preceded by a thorough risk assessment, both of the potential proliferation of the munitions and of the consequences of the various disposal options.

## CONCLUSIONS

The widespread and significant effects of soil contamination due to munitions waste from military operations in Donetsk and Luhansk regions contribute significantly to the global loss of productive soils and landscapes. The danger posed by the dumping of munitions in groundwater from various quarters should be considered. The accumulation of harmful substances in different parts of the food chain, as well as their direct toxicity, should be considered in the risk assessment to the same extent as the adverse effects of direct exposure to substances. The study found that industrial processes, military activities, disposal processes using old methods such as open burning, open detonation, and the release of munitions in inappropriately designated areas generate and release large quantities of explosive and toxic substances. The discharge and detonation of these explosive chemicals pose a danger to natural resources and living beings, polluting the soil and groundwater. The study also has pointed to the underestimation of the disposal of munitions, leading to the conclusion that the issue until now has been poorly studied and remains overlooked. The lack of studies on conventional munitions can be a

serious obstacle to further risk assessments for humanity. Underestimating the issue can have unpredictable environmental and economic consequences.

Donetsk and Luhansk regions need to introduce a system of monitoring and cleaning the condition of the soil, and groundwater. State programmes must be implemented, considering the selection of areas outside of military operations, and wellfields. Each method of ammunition disposal leads to a negative impact on the environment both in the short and long term. Therefore, it is important to pay attention to the method of treating the effects of munitions disposal using plants and indigenous microbes. Successful use of "green" technological concepts will reduce the risks to the environment and the population of Donetsk region. The studies on the replacement of ammunition calibres with more environmentally friendly calibres are also worth paying attention to. This requires the following measures to be taken: reduction of energy required for production; replacing traditional cartridges (which use brass) with plastic, cardboard or, eventually, using ammunition without cartridge cases; replacing copper and nylon as materials for the composite projectile.

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## **Вплив процесів корозії боєприпасів на забруднення ґрунтових вод і методики їхньої безпечної та економічно вигідної утилізації: аналіз прикладів села**

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**Анотація.** Актуальність дослідження полягає в тому, що боєприпаси, які потрапили в підземні води (глибше 250 м), в результаті їх утилізації, створюють серйозні загрози для всієї акваторії Донецької області. Метою роботи було дослідити вплив корозії боєприпасів на водне середовище та розглянути найбільш ефективні та екологічно безпечні методи їх утилізації. Для проведення досліджень були використані наступні наукові методи: методи аналізу, дедукції, синтезу та метод формалізації. Встановлено, що кожен метод утилізації боєприпасів призводить до негативного впливу на навколишнє середовище як у короткостроковій, так і в довгостроковій перспективі. Проаналізовано літературні джерела останніх розробок з питань корозії боєприпасів та утилізації боєприпасів. Узагальнено, що існує нагальна потреба у впровадженні системи очищення забруднених територій, запровадженні контролю за виконанням законодавчих екологічних норм, використанні нових біологічних методів для ліквідації наслідків впливу боєприпасів на екосистему регіону та переходу на більш екологічно безпечні види озброєнь. Практичне значення статті полягає у пошуку шляхів запобігання екологічній та гуманітарній катастрофі в зоні бойових дій на Сході України

**Ключові слова:** відкрите спалювання, уран, захоронення, токсичні речовини, забруднення води та ґрунту

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## Problems and Prospects of State Financial Control and Analysis of Local Budget Execution

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**Abstract.** In the conditions of the constant increase of global challenges and socio-economic instability, the problem of the effectiveness of state financial control is one of the central ones for modern Ukraine. The purpose of the research was to identify key problems of government financial control and to assess the implementation of local budgets. The main methods for the study of the subject matter were theoretical approaches (analysis of Ukrainian and international theoretical positions), analysis and synthesis, statistical analysis, systematic analysis, terminological analysis, method of graphical representation of statistical data, and analysis of legislative and programme documents. As a result, it was confirmed that necessity to develop the state financial control system and to create a basis for improving the effectiveness of local budget execution had increased. The statistical data for the last three years (in some cases for 10 years) were analysed, which made it possible to describe the current situation in the field of public financial control and execution of local budgets in Ukraine. Shortcomings, problems, obstacles, limitations, and perspectives for public financial control development and the execution of local budgets are identified. The results obtained have theoretical and practical significance. The application of theoretical approaches in the study complements the theoretical provisions financial control of government. The results are of practical importance, especially in the implementation of national policy in the field of state financial control in the context of the crisis caused by the pandemic and its socio-economic consequences

**Keywords:** mechanisms of state financial control, national financial system, local budget, financial security of the state, analysis of performance indicators



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## INTRODUCTION

Timely identification and prevention of problems in the public financial control system are necessary to overcome the current crisis situation and develop the overall Ukrainian financial system efficiency (Cherbeyako & Ryabokon, 2016). The execution of local budgets in Ukraine has some problematic issues. The identification of such features, as well as tools and ways to eliminate them, is an urgent research vector under the current conditions of socio-economic uncertainty. In this regard, it is advisable to make a scientific assessment of the Ukrainian and international experience in the execution of local budgets, effective mechanisms, and practices used in budget regulation. In recent years, the attention of many Ukrainian and international researchers has been directed towards the investigation of control systems, public financial management, and municipal financial management. The main promising vector of development identified in the course of research is the creation of an integrated financial system. A system that would ensure the minimisation of the cost and maximisation of revenues to the municipal budget.

Modern theoretical approaches to the development of financial control of the state were studied by such researchers in the field of financial management as J. Keynes (2017), J. Koltaia *et al.* (2021), Gibbons *et al.* (2021), I. Schumpeter (2007). The problem of the improvement of financial control at the macro and micro levels, and the execution of local budgets, was investigated by I. Alekseev and N. Yaroshevich (2007), A. Goray (2018), E. Gutzeit (2008), V. Malyshko *et al.* (2017). The analysis of the papers (Roberts & Kwon, 2022; Kozarenko, 2015) showed that they were based on a fairly extensive existing theoretical and methodological base, but the need for systematic development of the issues under study in the context of new challenges and socio-economic instability was traced.

The novelty of the research is conditioned by the analysis of the object of the study in the context of modern global challenges and socio-economic instability in the world (Lavezzolo *et al.*, 2018). Difficult socio-economic conditions in 2020 were created by the significant impact of the COVID-19 pandemic. During this period, systemic changes took place in the entire global economy, and there was a need for mechanisms to develop the effectiveness of government financial control and the execution of local budgets (Seregin, 2016; Starostenko & Bulgakov, 2006). Therefore, new research, scientific refinement of mechanisms and tools to develop the efficiency of government financial control and the execution of local budgets are needed.

*The aim of the research* is to evaluate the effectiveness of local budget execution and calculate the main problems of financial control of the state. Disclosure of the goal implies the solution of several tasks:

- to investigate the national and successful international experience in the execution of local budgets and state financial control;
- to analyse scientific methods and approaches to the study of financial control of the government and budget execution;
- to examine statistical indicators that reflect the dynamics of development public state financial control system and execution of local budgets;
- to disclose issues and perspectives for improving the system of public financial control and execution of local budgets;
- To identify opportunities and areas to improve an effectiveness of the system of public financial control and execution of local budgets in terms of socio-economic uncertainty.

## MATERIALS AND METHODS

The following methods were used to achieve the goal: dialectical, systematisation and generalisation, concretisation, and terminological, analysis, synthesis, statistical analysis, statistical comparisons, comparative law method (used in the adaptation of effective world practices to Ukrainian conditions). Empirical methods used in the study included observation and system monitoring. The study pays considerable attention to identifying gaps in theory and practice, due to the increase in acute crisis socio-economic conditions in the world. Using the methods of concretisation and generalisation, the main problems and gaps in research and theory of government financial control and execution of local budgets were identified.

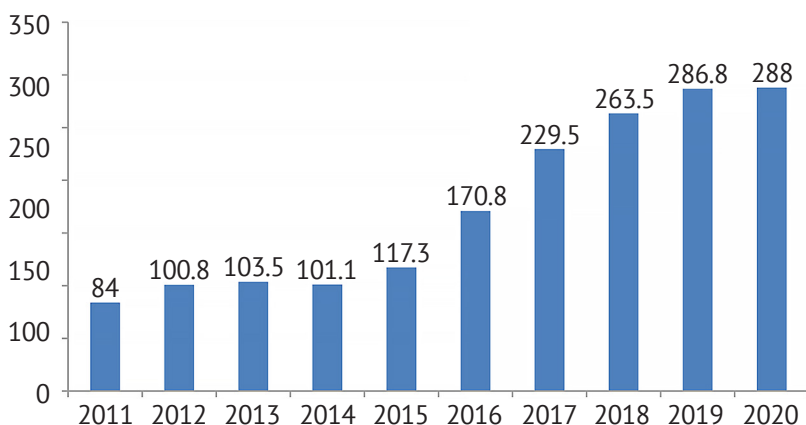
The dynamics of the national experience in the execution of local budgets and state financial control has been studied over the past three years (over 10 years according to some indicators). The analysis of the revenue structure of local budgets of European countries and Ukraine (in comparison) is carried out.

During the research, the following data were studied: statistical data of the State Treasury Service of Ukraine (2022), the State Statistics Service of Ukraine (2022), the Ministry of Finance of Ukraine (2022), and the European Commission (2022). The research was done in several stages. At the first stage, the analysis of methodological approaches to the problem of state financial control and execution of local budgets was held. The purpose, tasks, and methods were defined, and the main hypothesis was put forward. The expediency of using a synergetic approach in improving financial control has been investigated. At the second stage, sources of information were selected and data reflecting the provision of state financial control and local budget implementation were analysed: the dynamics of incomes and costs of local budgets of Ukraine, the proportion of own incomes of regional budgets in incomes of Ukrainian consolidated budget,

the amount of budgets of regions of Ukraine, the volume of controlled fiscal and material resources, the structure of violations, the structure of incomes to local budgets of Ukraine and European countries. At the third stage the analysis was carried out and theoretical and applied conclusions were formed. The experience of Ukrainian financial control in Ukraine, features and efficiency of execution of local budgets in Ukraine and some European countries was systematized and generalized. The study results can be used by employees of state and financial management bodies, and specialists of local municipal self-government.

## RESULTS AND DISCUSSION

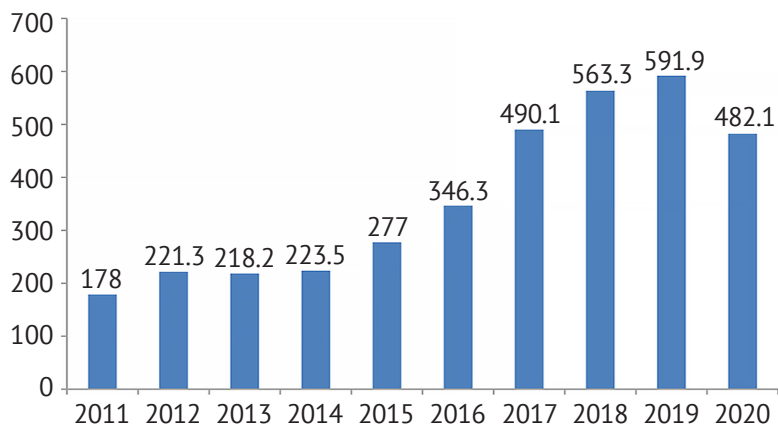
Most of the government's fiscal resources are concentrated in the national and local budgets. System of Ukrainian budget is structured as follows: two blocks – the state budget and the local budget. Local budgets include regional budgets, district budgets, and district budgets of local self-government. Accordingly, local government budgets include: territorial community budgets, village budgets, city budgets, budgets of associations (villages and towns), and budgets of districts in cities. Ukrainian local budget structure: "General Fund", "Special Fund" and additional "Reserve Fund". To assess the dynamics of incomes of Ukrainian local budgets, we chose indices for a significant time series: from 2011 to 2020 (Fig. 1).



**Figure 1.** Dynamics of incomes of local budgets of Ukraine, billion UAH for 2011-2020 (excluding inter-budget transfers)  
**Source:** compiled by the authors

An investigation of the dynamics of local budget incomes for the period from 2011 to 2020 showed a trend of slight growth from 2011 to 2020 and a trend of active growth since 2016 (Fig. 1). From 2011 to 2015, the amount of local budget revenues increased by UAH 33.3 billion, and for the period from 2016 to 2020 – by UAH 117.2 billion. In 2021, the trend of active growth of local budget revenues has continued. Moreover, for 9 months of 2021 (compared to the same period in

2020), local budget revenues increased by 20.1%. This amounts to 248 billion UAH. In 2021, the increase in the amount of personal tax income was 19.5%, the land fee was 14.9%, the real estate tax was 38.3%, and the single tax was 21.1%. Inter-budget transfers to local budgets for 9 months of 2021 – UAH 120 billion 872.8 million, which is 95.3% of the planned. Analysis of cost dynamics of local budgets of Ukraine for the period from 2011 to 2020 is presented in Figure 2.



**Figure 2.** Dynamics of expenditures of local budgets of Ukraine for 2011-2020, UAH billion (excluding inter-budget transfers)  
**Source:** compiled by the authors

In the dynamics of local budget costs there is a correlation with the dynamics of local budget incomes for the same period. A slight increase from 2011 to 2015 - from 178.3 billion UAH to 277 billion UAH. From 2016 to 2019 – from 346.3 billion UAH to 591.9 billion

UAH. But in 2020 recorded a decrease in local budget expenditures, which amounted to 482.1 billion UAH. The dynamics of the proportion of own incomes of local budgets in consolidated budget revenues in Ukraine for the period from 2010 to 2020 is presented in Table 1.

**Table 1.** Proportion of own incomes of local budgets in revenues of the consolidated budget in Ukraine, %

| Year | Fraction, % |
|------|-------------|
| 2010 | 27.3        |
| 2011 | 21.1        |
| 2012 | 22.6        |
| 2013 | 23.3        |
| 2014 | 22.2        |
| 2015 | 18          |
| 2016 | 21.8        |
| 2017 | 22.6        |
| 2018 | 22.2        |
| 2019 | 22.3        |
| 2020 | 23.4        |

**Source:** compiled by the authors

As shown in Table 1, the highest rate was in 2010 – 27.3%, and the lowest in 2015 – 18%. In other years the share of own incomes of local budgets fluctuated between 21-23%. It is this indicator that reflects the self-sufficiency and the possibility of socio-economic growths of the territories of the country. Implementation of local budgets in the legal aspect is the responsibility of the state local administration, the executive bodies of the relevant local councils or village heads. General organization and management of local budget execution, coordination of activities of participants of the budget

process on issues of budget execution are carried out by local financial bodies. A local financial authority is an institution that, in accordance with Ukrainian legislation, performs the functions of drafting and implementation of local budgets, control over the expenditure of funds by administrators of budgetary funds and other functions associated with the administration of local budget funds. In the execution of local budgets, the treasury service of budgetary funds is applied. The body empowered to keep records and prepare reports on the execution of local budgets in Ukraine is the treasury body (Table 2).

**Table 2.** The size of the budgets of the regions of Ukraine in 2020

| Area                   | Income (million UAH) | Expenses (mln UAH) |
|------------------------|----------------------|--------------------|
| Dnipropetrovsk Oblast  | 9266.7               | 9266.6             |
| Kharkiv Oblast         | 5881.9               | 5881.9             |
| Lviv Oblast            | 5134.6               | 5134.6             |
| Kyiv Oblast            | 4992.3               | 4995.3             |
| Odesa Oblast           | 4824.6               | 4827.2             |
| Donetsk Oblast         | 4566.2               | 4566.2             |
| Zaporizhia Oblast      | 4439.1               | 4439.1             |
| Vinnitsia Oblast       | 4309.6               | 4302.6             |
| Poltava Oblast         | 4238.0               | 4297.2             |
| Zhytomyr Oblast        | 3290.0               | 3287.2             |
| Khmelnytskyi Oblast    | 2843.2               | 2842.9             |
| Sumy Oblast            | 2821.6               | 2821.6             |
| Cherkasy Oblast        | 2677.0               | 2673.6             |
| Mykolaiv Oblast        | 2619.2               | 2611.2             |
| Ivano-Frankivsk Oblast | 2523.5               | 2515.4             |

Table 2, Continued

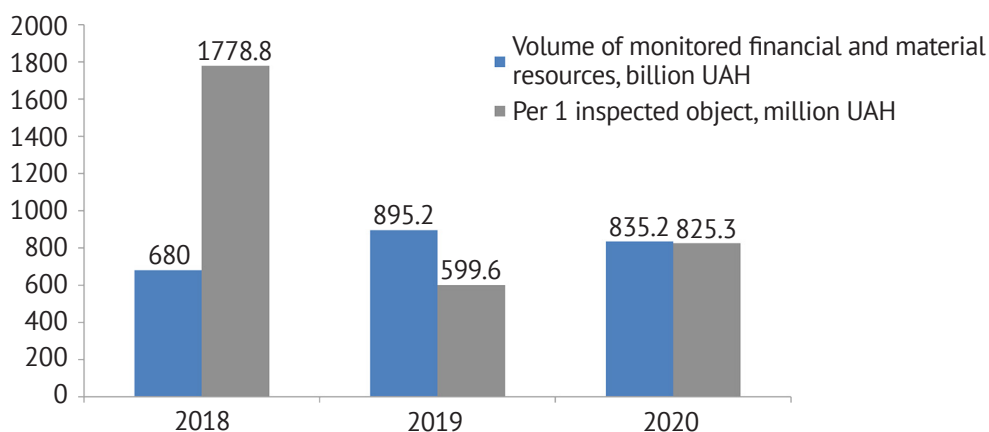
| Area                       | Income (million UAH) | Expenses (mln UAH) |
|----------------------------|----------------------|--------------------|
| Kirovohrad Oblast          | 2523.2               | 2695.1             |
| Chernihiv Oblast (project) | 2520.4               | 2517.4             |
| Volyn Oblast (project)     | 2410.9               | 2410.9             |
| Rivne Oblast               | 2400.0               | 2400.0             |
| Kherson Oblast             | 2193.4               | 2187.3             |
| Zakarpattia Oblast         | 2084.0               | 2069.4             |
| Ternopil Oblast            | 2086.2               | 2086.0             |
| Luhansk Oblast             | 1925.0               | 1925.0             |
| Chernivtsi Oblast          | 1682.3               | 1679.8             |

**Source:** prepared by the authors on the basis of the source Ministry of Finance of Ukraine (2022)

Analysis of the size of regional budgets of Ukraine in 2021 showed that the Dnipropetrovsk Oblast has the largest budget – revenues of UAH 9 billion 266.7 million, and expenditures of UAH 9 billion 266.6 million (Table 2). The Dnipropetrovsk Oblast has allocated UAH 2.4 billion from the state budget, which will be spent on road repairs (UAH 847 million) and education (UAH 767 million). In the region, –UAH 130 million has been allocated for measures to combat COVID-19. For various development projects – more than UAH 1.5 billion. The second largest budget region in Ukraine is the Kharkiv Oblast. Revenues and expenses at the beginning of 2021 were planned in the amount of UAH 6 billion 881.9 million. About UAH 2 billion has been allocated for education, almost UAH 485 million for healthcare, and UAH 548 million for social protection and welfare. In the Lviv Oblast, in the budget for 2021, revenues and expenses are planned in the range of UAH 5 billion 134.6 million. Of these, UAH 277 million has been allocated for state investment

projects, and 1.4 billion UAH for the implementation of regional programmes. Regions with budget deficits: Poltava Oblast (59.2 million), Odesa Oblast (2.6 million), Kirovohrad Oblast (171.9 million), and Kyiv Oblast (3.0 million). The largest budget deficit, with a significant gap from other deficit regions, in the Kirovohrad Oblast is 171.9 million UAH (Ministry of Finance of Ukraine, 2022).

In 2021, the State Audit Service completed 10.9 thousand inspections. Including: 1.4 thousand – audits and inspections, procurement monitoring – 9.3 thousand events, and state financial audit – 151. Organisations, state-owned and municipal enterprises have estimated the loss of income owed to them to be almost 5.5 billion UAH. Of these, budgets – about 3.8 billion UAH, which is 40.1% of the total amount of violations related to the use of state and municipal resources (Ministry of Finance of Ukraine, 2022). The volume of financial and material resources covered by the control in January – December 2019-2020 is shown in Figure 3.



**Figure 3.** The volume of financial and material resources covered by the control in January-December 2019-2020

**Source:** compiled by the authors based on the source The state of financial and budgetary discipline in the use of budget funds, state and municipal property for 2020, 2019 and 2018 (2022)

The total volume of financial and material resources covered by the control in 2019, compared to 2018, increased from UAH 680 billion to UAH 895.2 billion. But in 2020, there was a slight decrease in

this indicator: from UAH 895.2 billion to UAH 835.2 billion. And based on 1 verified object, the volume of resources for the same years decreased significantly (from UAH 1778.8 million to UAH 599.6 million), and

then increased slightly to UAH 825.3 in 2020 (Ministry of Finance of Ukraine, 2022). In general, the results of audits and inspections revealed losses of financial and

material resources for more than UAH 154.4 billion. The structure of violations that led to resource losses for 2018, 2019, and 2020 is shown in Table 3.

**Table 3.** The structure of violations that caused the loss of financial and material resources identified by the State Audit Service of Ukraine in January-December 2018-2020, in %

|                                     | 2018 | 2019 | 2020 |
|-------------------------------------|------|------|------|
| Lost financial resources            | 42.5 | 23.8 | 98.2 |
| Inappropriate spending of resources | 2.9  | 5.8  | 0.1  |
| Illegal spending of resources       | 52.5 | 64.2 | 0.2  |
| Lack of resources                   | 2.1  | 6.2  | 1.5  |

**Source:** prepared by the authors on the basis of the source State of financial and budgetary discipline in the use of budgetary funds, state and municipal property for 2020, 2019 and 2018 (2022)

The highest % of violations occurred in the group of illegal spending of resources: in 2018 – 52.5%, and in 2019 – 64.2%. But in 2020, the percentage ratio is changing dramatically – non-targeted expenses began to account for 0.1% of violations, and the lost financial

resources increased to 98.2%. In 2018 and 2019, the share of lost financial resources was high, but not as much as in 2020. In 2018 – 42.5%, and in 2019 – 23.8%. Structure of incomes to Ukrainian local budgets for 2018, 2019, and 2020 is presented in Table 4.

**Table 4.** The structure of revenues to local budgets of Ukraine for 2018, 2019, 2020, million UAH

|                  | 2018   | 2019   | 2020   |
|------------------|--------|--------|--------|
| Tax revenue      | 85385  | 94811  | 95970  |
| Non-tax revenues | 29129  | 26105  | 21752  |
| Transfers        | 304672 | 260302 | 163846 |
| Other            | 3308   | 3575   | 5136   |

**Source:** made by the authors on the basis of the source State of financial and budgetary discipline in the use of budgetary funds, state and municipal property for 2020, 2019 and 2018 (2022)

Table 4 shows that the main source of revenues to local budgets are tax revenues, which have increased by approximately 10 million UAH over the analysed years. Non-tax revenues and transfers have been decreasing for three

years. Moreover, the volume of transfers has almost halved by 2020. In this regard, a comparative analysis with a similar structure of incomes to the local budget of Ukraine and some European countries will be informative (Table 5).

**Table 5.** The structure of revenues to local budgets of Ukraine and European countries, in %

| Country        | Tax revenues | Non-tax revenues | Transfers | Other |
|----------------|--------------|------------------|-----------|-------|
| Ukraine        | 34           | 7                | 57        | 1     |
| Sweden         | 74           | 20               | 3         | 1     |
| France         | 45           | 33               | 18        | 4     |
| United Kingdom | 15           | 13               | 70        | 2     |
| Switzerland    | 48           | 26               | 15        | 11    |
| Norway         | 44           | 38               | 18        | 0     |
| Netherlands    | 13           | 67               | 20        | 0     |
| Italy          | 38           | 49               | 12        | 1     |
| Germany        | 60           | 26               | 14        | 0     |
| Denmark        | 53           | 38               | 7         | 2     |

**Source:** compiled by the authors based on data of European Commission (2022)

As can be seen from Table 5, the structure of the sources of income of local states in Europe is dominated by tax and non-tax revenues, which approximately account for 81% to 95% of all sources of income. The United Kingdom is an exception in this situation – the share of transfers is 70% and these transfers are usually targeted grants for the performance of specific tasks by municipal authorities. The main sources of tax revenues of most countries are personal income tax and income tax. Another significant source of income are property taxes (Roberts & Kwon, 2022). Among all the analysed countries, with the exception of the UK, Ukraine leads in terms of attracted transfers – 57% (2020) and has the lowest non-tax revenue – 7% (2020).

The role of state financial control has significantly increased in the context of new global challenges and socio-economic uncertainty. Control in the sphere of public administration is of particular importance (Alekseev & Yaroshevich, 2007). This is conditioned by the fact that financial security and national economic development overall depend on the effectiveness of its functioning. Theoretically, the definition of control has many manifestations. But in economic theory, control is increasingly being studied from a systemic perspective, since this concept is a complex and multilevel system that develops according to the basic laws of the functioning of economic systems. The systematic approach allows considering the financial control system as an organic unity of its types: state, public, audit, and internal. The new theoretical substantiation of financial control focuses on the development of the concept of its systemic organisation (entrepreneurs) (Vygovskaya, 2013).

When solving the key issues in the financial control system development, some researchers suggest using synergetic mechanisms, the possession of which, in their opinion, helps to choose the optimal path of development (Vygovskaya, 2013). Synergetics is the doctrine of interaction, applicable in almost all spheres (Bogter & van Helden, 2021). In the context of control, this works as follows: for a more effective functioning of systemic financial control, it is advisable to develop a functional non-interference of state, public, audit, and internal control, which together form the national system of financial control. The synergistic effect can be not only positive, but also negative (in the case when the factors that combine contradict each other). Therefore, there is a difficulty in planning the effects and their implementation. In the financial control system, to achieve synergetic effects, it is advisable to have an agreed system of goals of its participants for the harmonisation of the control process (Financial System Soundness, 2021). Therefore, the problems of contradictions between state and internal financial control can be solved using approaches that are not mutually exclusive, that is, they can be applied in a complex (synergistically).

Approach 1. Development of the legislative financial control framework and development of a concept for the development of a balanced Ukrainian of financial control system in, standardisation of all types of control, considering the emergence of new conditions of socio-economic instability. Moreover, the concept of state financial control is the main one, that is, it is advisable to develop partial concepts in relation to it: public control, internal control, and audit.

Approach 2. Development of the public financial control system and improvement of interaction and coordination of its individual units. In modern Ukraine, there remains a situation of incompleteness in the formation of the regulatory framework of state control, which leads to the concentration of the efforts of the control bodies exclusively on their own departmental interests, and this, in turn, entails duplication of functions by the subjects of control. This disadvantage can be offset by ensuring systematic control. And to achieve a synergistic impact and improve the coordination of control bodies, it is advisable to finalise the concept of Ukrainian of financial control system and a law (Law of Ukraine..., 1993) with provisions (special section) on the procedure for the interaction of subjects of state financial control with other controlling organisations.

Approach 3. Improvement and modernisation of internal financial control systems of enterprises. That is, the development of internal control systems not only to undergo a further external audit, but also to improve the management of internal business processes. In economically developed countries, in state financial control systems, control units that have a higher status focus precisely on the results of audits and evaluation of the activities of lower controlling structures than directly on their own control.

Approach 4. The use of audit and public financial control systems in order to minimise and eliminate disagreements. Under the circumstances of modern global challenges and socio-economic instability, these types of control are of particular importance.

The priority condition for improvement of economic systems sustainability, is the harmonisation of the objectives of the entire system and its individual elements (Widanaputra & Mimba, 2014). The financial control system allows receiving information about the presence of contradictions, which would allow purposefully eliminating them. This is conditioned by the increased mutual responsibility of various control subjects to ensure the objectivity and quality of control reports, a large number of control objects, limited resources for control, and the common problems of different types of financial control (Gutzeit, 2008). Various types of control (state, public, external audit, internal) have a lot in common. Therefore, it is possible to identify common problems that require solutions within the improvement of the general theory of control: firstly, the

problem of evaluating the effectiveness of control systems; secondly, the problem of standardisation; thirdly, the problem of increasing the level of objectivity and independence of control.

### CONCLUSIONS

The hypothesis suggested in the present research was proved about the significantly increasing need to develop public financial control system and create prerequisites for efficiency improvements of local budget execution. In the system of public financial control in conditions of socio-economic instability, it is advisable to ameliorate the regulatory and legislative framework and develop a balanced system of control. The recommendation is to create a system of standardization of all types of state financial control. This is particularly required under the conditions of increasing global socio-economic challenges and instability. The most important direction to improve efficiency of public financial control and execution of local budgets is to minimize the public debt, which significantly reduces the efficiency of budgets at various levels and is a significant constraint on their

development. The presence of debt forms a policy of reducing expenditure items for budgets of all levels, therefore, an effective measure to prevent an imbalance is a policy of actively increasing budget revenues-tax and non-tax (which in Ukraine, as the analysis showed, are among the lowest among European countries).

It is advisable to enhance the effectiveness of local budget execution in Ukraine and the management of municipal finances through the following measures. Firstly, increase the share of tax and non-tax incomes to local budgets (focusing on the average indicators of developed European countries). Secondly, assist public administration bodies in the improvement of the quality of management of regional and local finances (budgets). Thirdly, ensure the development of programme-targeted methods of budget management at the regional level. Another priority is the improvement of the controlling part of the state financial policy. Namely: bringing control to a level that allows reducing high rates of violations that cause losses of financial and material resources – illegal spending of resources and shortfall of financial resources.

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## **Проблеми та перспективи державного фінансового контролю та аналізу виконання місцевих бюджетів**

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**Анотація.** В умовах постійного наростання глобальних викликів та соціально-економічної нестабільності проблема ефективності державного фінансового контролю є однією з центральних для сучасної України. Мета статті полягала в тому, щоб виявити основні проблеми державного фінансового контролю та провести оцінку виконання місцевих бюджетів. Основні методи до дослідження заявленої у статті проблеми: теоретичні підходи (аналіз українських та міжнародних теоретичних положень), аналіз і синтез, статистичний аналіз, систематичний аналіз, термінологічний аналіз, метод графічного представлення статистичних даних, аналіз законодавчих і програмних документів. У результаті проведеного дослідження підтвердилася висунута автором гіпотеза про значно зростаючу необхідність удосконалення, посилення системи державного фінансового контролю та створення передумов для підвищення ефективності виконання місцевих бюджетів. Виявлено недоліки, проблеми, перешкоди, обмеження та перспективи розвитку державного фінансового контролю та виконання місцевих бюджетів. Отримані результати мають теоретичну та практичну значущість. Застосування теоретичних підходів при дослідженні доповнює теоретичні положення державного фінансового контролю. Практичну значущість мають результати особливо при проведенні національної політики у сфері державного фінансового контролю в умовах кризи, спричиненої пандемією та її соціально-економічних наслідків. За результатами дослідження сформульовано пропозиції щодо вдосконалення системи державного фінансового контролю та виконання місцевих бюджетів

**Ключові слова:** механізми державного фінансового контролю, національна фінансова система, місцевий бюджет, фінансова безпека держави, фінансова безпека держави

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## Sources of Financing for Increasing the Competitiveness of the Food Industry in Azerbaijan

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**Abstract.** The state policy of the government of Azerbaijan is aimed at the sectoral diversification of the economy of the country, in which the food industry occupies a significant place. Since for a long time there has been a raw materials-based model of the economy in Azerbaijan, investment funds have not been not directed to increase the competitiveness of the food industry in order to operate successfully in the global market. The main aim of the research is to investigate the opportunity of using various sources of financing, which are required to improve the Azerbaijani food industry competitiveness. The methods of summary and grouping, analysis, synthesis, induction, deduction, were used in order to reach the goal. It was shown that the food industry in the state could be improved by increasing the climate investment and the share of outside and joint financing sources. During the research conducted it was determined that over the past 15 years, the government of Azerbaijan has implemented more than 70 national and industry specific programs. The work separately points out the State Programs I and II on the economic and social development of the regions, which were introduced in the period from 2004 to 2013. For increasing the food sector competitiveness in Azerbaijan, additional financial resources, which reached \$65 billion in 2012, were attracted in the country; loans to strengthen the competitiveness of the food industry increased by 9.7 times in the period between 2006 and 2021, and the investment volume will exceed \$3 billion in the result. The positive impact of the measures, which were taken, was reflected in the creation of additional 1.2 million jobs in the food industry in the Republic of Azerbaijan. The study results showed that internal sources were the largest share in the financing of the food industry in the country, while the due attention was not paid to the development of international cooperation aimed at increasing external and joint sources of financing. The practical significance of the results of the study presented in this article lies in its providing of the basis for subsequent areas of study of the topic under consideration, as well as for the development of state programs on the improving of the food sector in Azerbaijan

**Keywords:** consumer market, customer orientation, investments, international cooperation, non-resource economy



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## INTRODUCTION

The food industry of Azerbaijan has a great potential in the national economy. After the collapse of the Union of Soviet Socialist Republics, Azerbaijan received raw material resources, which have already been depleted by now, and the country needs to use the existing potential to develop the non-resource of economy branch. There are internal as well as external factors that influence the urgency of the food sector development. Azerbaijan is an industrial-agrarian state, and the food industry occupies a leading position among the republic's processing industries. The insufficient development level of the food industry, due to low competitiveness, is evidenced by the ranking of the states of the world community when it comes gross domestic product, in which Azerbaijan ranks 76<sup>th</sup> (Food Safety Agency..., 2022). This situation is explained by the low level of competitiveness of food industry enterprises, which have practically not been modernized over the years of Azerbaijan's independence, suspended their activities on a large scale, and reduced production volumes.

It should be pointed out that the development of the food industry is one of the main directions for the strategic development of the economy in the Republic of Azerbaijan in "State Program for the Social and Economic Development of the Regions of the Republic of Azerbaijan in 2019-2023" (Decree of the..., 2019), which is currently being implemented. At the same time, state resources for financing the modernization of equipment of food industry enterprises, the development and implementation of innovative technologies for processing food products, and training the personnel of food industry enterprises in Azerbaijan were virtually not allocated. Since the enterprises of the food industry in Azerbaijan are privately owned, the search for funds to increase competitive advantages falls on the owners.

The need to develop the competitiveness of the food sector in the country is due to the current world economy situation, which has faced the problem of hunger in developing countries. This problem has become especially acute against the backdrop of the war waged by Russia against Ukraine, as a result of which food products produced in Ukraine are not supplied to the world market. To solve the problem of the food crisis, Azerbaijan needs to attract investments to increase the competitive advantages of produced food in the country in a short time, since in order to import food products produced in Azerbaijan, these products must meet international quality standards. According to the head of the Small and Medium Business Development Agency (SMBDA) of the Republic of Azerbaijan (2022) O. Mammadov, the export potential of the food industry is huge; it is justified by the fact that approximately 10 thousand business entities are registered in Azerbaijan every year; and their activities are carried out in the food production market. Therefore, the study of the possibility of using various sources of financing for the food industry

in Azerbaijan, aimed at increasing the level of competitiveness of the industry's enterprises, is relevant.

The importance of increasing the competitiveness of Azerbaijan's food industry enterprises has been widely studied by various authors. The high level of significance of involvement of the country in the food industry development in the state has been determined. But, at the same time, the main bias is made on the need and importance of the formation of new enterprises for the processing of agricultural products, without at all considering the possibility of attracting various investment funds for the development of the industry (Babayeva, 2018). There is evidence of the possibility of reducing the level of resource costs in the agricultural sector, but the financial support of the innovative technologies is still not provided (Hajiyeva, 2021). In addition, the issues of financing the agrarian economy branch in the state are not considered in article of A.D. Iskenderova *et al.* (2021). At the same time, this study proved the feasibility of increasing the level of competitiveness of food industry enterprises by optimizing supply chains. The article of N.R. Aliyeva (2021) proved the necessity and effectiveness of developing the food sector in the Republic of Azerbaijan through the introduction of green technologies. The disadvantage of the article is that it does not present calculations for the financing of the proposed innovations. The article of M. Suleymanov (2022) presents evidence of the importance and necessity of introducing the ideas of the scientific and technological innovations – innovative developments in order to achieve an enhance in the share, in the overall volume of the regional gross product of the Nakhichevan Autonomous Republic, enterprises and organizations involved in the production of food products; issues of financing the proposed directions for increasing competitive advantage are not considered.

Based on the above mentioned facts, it can be concluded that by now, a broad ground has been developed for conducting research on the expediency of using various sources of financing to increase the food field competitiveness in the Republic of Azerbaijan. *The purpose of the article* to study the possibility of using various sources of financing, and which are required to increase the competitiveness of the food industry in Azerbaijan, is finally justified.

## MATERIALS AND METHODS

To carry out this research the process was divided into separate stages. Collection of the statistical reporting data was done at the first stage. This data was presented on the official websites State Statistics Committee of the Republic of Azerbaijan (2022), Small and Medium Business Development Agency (SMBDA) of the Republic of Azerbaijan (2022), Food Safety Agency of the Republic of Azerbaijan (2022) over the last 15 years. Since the study involves the collection of a large amount of data,

the method of summary and grouping was used, which is the most appropriate at this stage.

An analysis of the collected material was carried out at the next stage; an insufficient level of use of the competitive potential of food industry enterprises was proved via using data analysis methods (Kelley, 2022). The method of analysis was used at this stage of the study, since there is a need for a detailed consideration of the object of the study, practical decomposition of the collected data into components to track existing trends. It was possible to prove the necessity and expediency of considering various sources of financing for the food branch development in state on the basis of the above-mentioned method.

The third stage of the study is to consider the possibility of using internal sources of financing, consisting of the financial resources of the budgetary and non-budgetary funds of Azerbaijan, the funds of the Azerbaijani population, the own funds of Azerbaijani enterprises and organizations, which will be used later to increase the competitiveness of Azerbaijan's food industry enterprises. It should be pointed out that the presented article considers several sources of funding, in addition to internal sources, external and joint sources are also considered. External sources of financing include funds received from foreign partners. Joint sources of financing for the sector development in the state include funds from international associations and corporations operating in various countries, including Azerbaijan.

It is explained by the functioning of the market model of the Azerbaijani economy, which involves the selection of the optimal ratio of funds raised and the result obtained. The induction methods were used at this stage (Yang *et al.*, 2022). The validity of using this research method is explained by the fact that, given the results of the analysis of the indicators of competitiveness of Azerbaijan's food sector business, it is required to determine the possibility of using internal, external and joint investments.

The fourth stage of the research involves the use of method of analysis to calculate the volume and share ratio of the use of various investment sources. Since internal funding may not be enough for the introduction of innovative production technologies, staff training, and the establishment of new companies, then by using the deduction method, it will be possible to select other sources of financing, which include exclusively external and joint sources (Bradford & Weisberger, 2021). Furthermore, a review of the ongoing situation of the global financial marketplace was carried out at this stage of study, which makes it possible to consider and assess the opportunity of attracting investment funds to the food industry in Azerbaijan from various sources, taking into consideration financial market analysis data.

The deduction method was used at the fifth stage of the study, which helped in drawing conclusions based

on logical conclusions that rely on the results of the data obtained in the previous stages of the study (Javed, 2022). The need to use this particular research method is substantiated by the need to obtain one's own conclusions, which were drawn after carrying out analytical and generalizing procedures, indicating the possibility and necessity of using internal, external, joint sources of financing for food industry enterprises, directions for increasing the level of competitiveness of these enterprises.

## RESULTS

Among the sectors of the Azerbaijani economy, the non-primary sector is developed more actively by the government nowadays, in view of the depletion of natural resources. To this end, Development Concept "Azerbaijan 2020: A look into the future" (Decree of the..., 2012) has already been implemented, and "State program for the Social and Economic Development of the Regions of the Republic of Azerbaijan in 2019-2023" (Decree of the..., 2019) is currently being implemented, one of its priority tasks is to enhance the competitiveness level of food industry business. According to Small and Medium Business Development Agency (SMBDA) of the Republic of Azerbaijan (2022) 75.6% of the overall number of food industry businesses of the republic are micro and small enterprises, which influence the activity of these enterprises in the implementation of measures to increase their competitiveness in a positive way, including innovative activity. The need to stimulate an increase in the level of competitiveness of enterprises of food industry in Azerbaijan is evidenced by statistical data, according to which, 180 enterprises of food industry were liquidated in 2016-2021 due to the influence of subjective and objective reasons, while 20% of the overall number of companies working in the market, small, micro and medium-sized enterprises operate in the red – the number of total losses in 2021 was 1063 million manats. According to Food Safety Agency of the Republic of Azerbaijan (2022), the first place among the countries pursuing an active investment policy is actually occupied by the United Kingdom, which invested \$1234759000 in the economy of Azerbaijan in 2021. It is followed by Turkey – \$530051000, United States of America – \$410960000, Malaysia – \$324857000, Cyprus – \$231309000, Japan – \$215820000, Iran – \$209390000, Norway – \$121722000, Georgia – \$74264000, United Arab Emirates – \$48222000.

The low rate of implementation of measures to increase competitiveness by food industry enterprises indicates the need to search for various sources of investment, which are required for the improving and realization of measures dedicated at increasing the competitiveness of the food industry in Azerbaijan. These directions should not be limited solely to the upgrading production and the implementation of innovative developments in production activities that require constant

updating due to rapid wear and tear. The measures to improve the quality of work of labor resources, such as timely training of personnel, full-fledged motivation of personnel, and the prevention of internal labor conflicts, which also have a negative impact on the competitiveness of enterprises, should also be included. Thus, according to the Food Safety Agency of the Republic of Azerbaijan (2022), during 2006-2021, the quality of products decreased by 40% at 25% of food industry enterprises in Azerbaijan due to a low level of employee motivation, and the level of competitiveness of products decreased by 28% at 45% of food industry enterprises

in Azerbaijan as a result of internal labor conflicts. The mentioned facts influence the economic sustainability of Azerbaijan's food industry enterprises in a negative way and are a significant obstacle to the realization of Azerbaijan's export potential in international markets, where the level of competition is quite high.

As a result of the data analysis on investing in measures to increase the competitiveness of food industry enterprises in 2021 relative to the data of 2006, financing of the non-state sector at the expense of internal funds increased by 44.43 times, external financing increased by 21.36 times, joint financing – by 30.38 times (Table 1).

**Table 1.** Volumes of investment in increasing the competitiveness of food industry enterprises

| Years | Internal funds                        |  |  | External sources                      |  |                                       | Joint investment                      |   |                                      |
|-------|---------------------------------------|--|--|---------------------------------------|--|---------------------------------------|---------------------------------------|---|--------------------------------------|
|       | Volume of investments, million manats | Growth rate over the previous year (%) | Share in total domestic investment (%) | Volume of investments, million manats | Growth rate over the previous year (%) | Share in total foreign investment (%) | Volume of investments, million manats | Growth rate compared to the previous year (%) | Share in total joint investments (%) |
| 2006  | 57.11                                 | 110.10                                 | 44.13                                  | 82.02                                 | 88.3                                   | 35.99                                 | 13.91                                 | 101.30  | 38.88                                |
| 2007  | 72.51                                 | 126.96                                 | 56.42                                  | 105.69                                | 128.86                                 | 46.38                                 | 17.82                                 | 128.08  | 49.80                                |
| 2008  | 86.50                                 | 119.30                                 | 66.56                                  | 157.87                                | 149.38                                 | 69.27                                 | 24.44                                 | 137.14  | 68.29                                |
| 2009  | 134.29                                | 155.24                                 | 33.30                                  | 247.90                                | 157.02                                 | 10.88                                 | 38.22                                 | 156.39  | 106.80                               |
| 2010  | 155.32                                | 115.66                                 | 29.13                                  | 341.26                                | 137.66                                 | 14.97                                 | 49.66                                 | 129.93  | 138.77                               |
| 2011  | 198.97                                | 128.10                                 | 53.10                                  | 562.67                                | 164.88                                 | 24.69                                 | 76.16                                 | 153.38  | 21.28                                |
| 2012  | 284.33                                | 142.90                                 | 28.78                                  | 1223.90                               | 217.52                                 | 53.71                                 | 150.82                                | 198.02  | 42.15                                |
| 2013  | 384.74                                | 135.31                                 | 29.04                                  | 2454.41                               | 200.54                                 | 10.77                                 | 283.91                                | 188.24  | 79.34                                |
| 2014  | 815.58                                | 2.1 times                              | 27.56                                  | 3115.38                               | 126.93                                 | 13.67                                 | 393.10                                | 138.46  | 10.98                                |
| 2015  | 1152.45                               | 141.30                                 | 67.60                                  | 3101.30                               | 99.55                                  | 13.61                                 | 425.37                                | 108.21  | 11.89                                |
| 2016  | 1105.98                               | 95.97                                  | 51.01                                  | 2672.91                               | 86.19                                  | 11.73                                 | 377.89                                | 88.84   | 10.56                                |
| 2017  | 937.11                                | 84.73                                  | 72.07                                  | 2303.22                               | 86.17                                  | 10.11                                 | 324.03                                | 85.75   | 90.55                                |
| 2018  | 1743.90                               | 186.09                                 | 41.89                                  | 1767.48                               | 76.74                                  | 77.56                                 | 351.14                                | 108.36  | 98.12                                |
| 2019  | 1636.71                               | 93.85                                  | 59.39                                  | 1158.34                               | 65.54                                  | 50.83                                 | 279.51                                | 79.60   | 78.11                                |
| 2020  | 1693.21                               | 103.45                                 | 62.86                                  | 1743.02                               | 150.47                                 | 76.48                                 | 343.62                                | 122.94  | 96.02                                |
| 2021  | 2537.39                               | 149.86                                 | 52.43                                  | 1751.90                               | 100.51                                 | 76.87                                 | 428.93                                | 124.83  | 11.99                                |

**Source:** compiled by the author in accordance with data provided by Food Safety Agency of the Republic of Azerbaijan (2022)

In 2022 \$19.7 billion was attracted from all sources of financing as a result of internal labor conflicts, to increase the competitiveness of Azerbaijan's food industry enterprises, which is 18% more than in 2021. 42% micro, small and medium enterprises are among these food industry enterprises. The sources

of financing for ensuring the competitiveness of the food sector are the funds of enterprises, the budget and extra-budgetary funds of the country, credit funds of banking institutions, personal savings of Azerbaijani citizens, more detailed figures are shown below (Table 2).

**Table 2.** Funding for enhancing the competitiveness of the food industry by investment source

| Years | Funds of enterprises and organizations | Own funds of the population | Bank loans | Budget resources | Extrabudgetary funds | Joint investment |
|-------|--|-----------------------------|------------|------------------|----------------------|------------------|
| 2006  | 71.2                                   | 4.7                         | 13.6       | 2.9              | 0.9                  | 6.7              |
| 2014  | 82.6                                   | 5.7                         | 5.4        | 3.4              | 1.3                  | 1.6              |
| 2015  | 70.7                                   | 3.8                         | 5.8        | 14.2             | 4.1                  | 1.4              |
| 2016  | 59.8                                   | 3.4                         | 5.3        | 24.8             | 5.4                  | 1.3              |
| 2017  | 47.6                                   | 3                           | 6          | 28.9             | 7.6                  | 6.9              |
| 2018  | 49.4                                   | 5                           | 7.4        | 23.3             | 7.2                  | 7.7              |

Table 2, Continued

| Years | Funds of enterprises and organizations | Own funds of the population | Bank loans | Budget resources | Extrabudgetary funds | Joint investment |
|-------|--|-----------------------------|------------|------------------|----------------------|------------------|
| 2019  | 50.9                                   | 3.8                         | 6.5        | 32.9             | 5.7                  | 0.2              |
| 2020  | 45                                     | 2.7                         | 6.3        | 42.7             | 3.1                  | 0.2              |
| 2021  | 44.6                                   | 2.6                         | 4.9        | 43.8             | 3.7                  | 0.4              |

**Source:** compiled by the author given the data provided by Food Safety Agency of the Republic of Azerbaijan (2022)

Due to the data presented in Table 2, 71.2% were attracted from the own funds of enterprises, 13.6% were investors from banking institutions, 4.7% of measures to enhance the level of food industry companies were financed at the expense of the population, 2.9% – extrabudgetary funds, 6.7% – joint investments in 2006. By 2014, the share of own funds of enterprises accounted for 82.6%, the share of own funds of the population – 5.7%, the share of bank loans – 5.4%, the share of extra-budgetary funds – 1.3%, and joint investments 1.6%. In 2021, the share of investments by enterprises and organizations in improving the competitiveness of the food industry decreased to 44.6%, the share of own funds of the population decreased to 2.6%, the bank loans share reduced to 4.9%, the share of budget funds in financing the increase in the competitiveness of the food economy sector increased to 43.8%, the share of extra-budgetary funds was 3.7%, the share of joint investments was 0.4%. The data allow noting the upward trend in the budgetary funds of the Republic of Azerbaijan as a source of financing, while the share ratio of other sources of financing showed a downward trend. At the same time, the total indicator of sources of financing showed growth, with the exception of bank loans.

This trend is explained by the fact that over the past 15 years, the Government of Azerbaijan has developed and adopted more than 70 programs of a national and narrow sectoral format for implementation. The I and II State Programs for the social and economic regions development in Azerbaijan, which were implemented in 2004-2013, should be highlighted separately. More financial resources were attracted from domestic sources, the amount of which was equal to \$65 billion by 2012 in order to realize their implementation. Over the period from 2006 to 2021, funding for developing the food industry increased by 9.7 times, to a level exceeding \$3 billion. The effectiveness of the implemented measures can be judged by the creation of 1.2 million additional jobs in the food industry in Azerbaijan (Ibrahimli, 2022). According to Small and Medium Business Development Agency (SMBDA) of the Republic of Azerbaijan (2022), a significant role in the process of formation of internal sources of financing for increasing the competitiveness of food industry enterprises belonged to small and medium-sized companies. Meanwhile, only 60% of small and medium-sized companies in the food industry in the state were profitable, the volume of investments is dedicated to enhance the competitiveness of the food industry has grown every year (Table 3).

**Table 3.** Financial resources allocated by small, medium and large food industry enterprises to increase the competitiveness of their enterprises (million manats)

| Years       | Small business enterprises | Medium and large enterprises |
|-------------|----------------------------|------------------------------|
| 2006        | 23.3                       | 92.5                         |
| 2016        | 23.0                       | 89.3                         |
| 2017        | 53.7                       | 246.2                        |
| 2018        | 114.1                      | 281.1                        |
| 2019        | 44.5                       | 309.7                        |
| 2020        | 86.4                       | 423.7                        |
| 2021        | 261.4                      | 586.5                        |
| Growth rate | 2021 to 2006               | at 11.2 times                |
|             | 2021 to 2020               | 2 times                      |
|             |                            | at 6.3 times                 |
|             |                            | by 138.4%                    |

**Source:** compiled by the author given the data provided by Food Safety Agency of the Republic of Azerbaijan (2022)

The data presented in Table 3 indicates that small businesses operating in the food economy sector in 2021 compared to the data of 2006 increased the amount of funding allocated to increase their competitiveness by 11.2 times, while the growth of investment funds allocated for the specified goals, by enterprises

of large and medium-sized businesses amounted to 6.3 times. The increase in funding for building competitive advantages by large and medium-sized enterprises amounted to 138.4% relative to the data of 2020, and small enterprises allocated 3 times more financial resources. Considering the total number of food industry

enterprises in Azerbaijan from 2006 to 2021, it should be emphasized that the overall number of operating enterprises decreased by 235, medium and large enterprises decreased by 99 units. The food industry of Azerbaijan in 2021 structurally consisted of 79.3% of small enterprises. According to the data of 2022, funds were allocated to the improvement of the food sector in the amount of \$7.3 billion during the period from January to May. Thus, the growth of financial resources amounted to 27.4%, comparatively with the same period in 2021. Domestic sources of funding accounted for 73.4%. In 2006-2021, the food industry market of Azerbaijan was actively developing, as a result, factories for the production of ice cream in the Absheron region, the cultivation and processing of Aspi winery grapes were built and put into operation. The Azersun Holding and Gilan Holding enterprises continued their work, producing more than 120 types of food products exported to 40 countries of the world (Ibrahimli, 2022).

According to the assessment of the effectiveness of the financial resources usage allocated to increase the competitiveness of the food industry in the country in 2021, the enterprises restructured 2.1 times more than the sold products, the value growth of fixed production assets was 160.6%, business profitability increased 17.9 times, net profit increased 18.4 times compared to 2006. During the specified period, the indicator of the average level of renewal of fixed production assets was fixed at the level of 5.96% (Ranking of countries ..., 2021). Meanwhile, the high depreciation level of fixed production assets of food industry enterprises in Azerbaijan should be noted, which in 2006-2020 fluctuated at the level of 51.4-56.9%, the level of capital productivity – 1.04-1.46 manats, demonstrating an upward trend in 2020-2021. Given these factors, it can be affirmed that the financial resources are not used effectively enough, despite some improvements. There is still a need for financial sponsorship and other types of financial support which will enhance the competitiveness of the food economy sector in Azerbaijan with an enhancement in the share of investment from foreign funds (ElShazly, 2020; Lingnau *et al.*, 2022).

## DISCUSSION

To solve the low competitiveness of the food sector problem in Azerbaijan, it is necessary to introduce innovative technologies for processing agricultural products of Azerbaijan without taking into account financial needs, which must be attracted to build up competitive advantages that are not limited solely to the modernization of agricultural products in Azerbaijan. It is also required to use the results of earlier marketing research and monitor the state and needs of the market at the present stage of development by conducting new marketing research that will reveal the actual needs of the market that are not yet satisfied or the level of competitiveness of the food sector in Azerbaijan is insufficient, and will

help to determine the directions in which it is required to invest attracted financial resources (Babayeva, 2018). Given the fact that until now the leadership of the food industry in Azerbaijan has not been able to independently cope with the problem of increasing their competitiveness, it is required to think about attracting external consultants. Since more than 70% of food industry enterprises in Azerbaijan are small and medium-sized ones, the use of external consulting can be considered appropriate (Azerbaijan – Competitiveness List, 2022).

A.D. Iskenderova *et al.* (2021) believe that it is required to enhance the competitive advantages of the food economy sector in Azerbaijan through the introduction of resource and energy-efficient technologies in the agricultural sector of Azerbaijan, which is a significant structural component of the food industry in Azerbaijan. Since scientists A.D. Iskenderova *et al.* (2021) do not consider at all the sources of financing of the directions, which they proposed to solve issues of the food industry in Azerbaijan, it can be assumed that they also share the opinion that financing should come from domestic sources, which contradicts the results of the study presented in this article. It is also impossible to agree that it is required to introduce only resource- and energy-saving technologies developed in Azerbaijan. Since the world community is an interconnected mechanism and Azerbaijan occupies a significant place in the global food market, in order to successfully compete with international companies, enterprises and organizations of the food industry in Azerbaijan need to introduce innovative technologies developed in other countries, the effectiveness of which has already been proved by the example of enterprises and food industry organizations of other countries. It is necessary to ensure the joint development of the food industry and the oil sector of Azerbaijan. The Azerbaijan economy is resource-oriented, and internal funding for the food industry in Azerbaijan is carried out at the expense of funds received by the country after the sale of oil, gas, refined products (Djella *et al.*, 2019).

N.R. Aliyeva (2021) disagrees with the statement drawn from the results of this study presented in this article that for increasing the competitiveness of the food economy sector in Azerbaijan, it is required to take measures to increase the share of external sources of financing. N.R. Aliyeva (2021) believes that in order to increase the competitive advantages of enterprises and organizations of the food industry in Azerbaijan, it is necessary to attract more domestic resources to the tasks that are addressed in the state programs implemented by the Azerbaijani government. The results of the study presented in this article show that the domestic financial resources are not enough, it is required to develop such areas as co-financing and develop relationships with international partners to attract external sources of financing. At the same time, these steps should be implemented at the level of individual companies operating

in the food industry of Azerbaijan. Although the importance of the adopted state programs of Azerbaijan cannot be underestimated, their implementation contributes to the improvement of the investment climate of the entire state, because precisely the favorable investment climate of the state influence positively on the international cooperation development, and also helps to attract foreign financial resources. The implementation of government state programs is required to be coordinated with the actions of the heads of enterprises and organizations of the food sector in Azerbaijan. All actions together should be effective in order to increase the share of external and joint sources of financing.

A.D. Iskenderova *et al.* (2021) do not see the need to improve the investment climate in Azerbaijan, use various sources of financing aimed at increasing the competitive advantages of Azerbaijan's food industry enterprises, but focus on the development of supply chains in the agrarian sector of Azerbaijan and, due to this, increase the profitability of the agrarian sector enterprises as a structural element of the food industry in Azerbaijan. Despite the fact that the optimization of the logistics component has a beneficial effect on the level of competitiveness of enterprises and organizations in the Azerbaijani agricultural sector and the entire food industry in Azerbaijan, this is not enough. An obligatory condition for the growth of the competitiveness of enterprises and organizations of the food industry in Azerbaijan is the use of various sources of financing for measures to increase the competitiveness of the food industry in Azerbaijan.

M. Suleymanov (2022) supports A.D. Iskenderova *et al.* (2021), does not support the results of the study presented in this article, and believes that funds should be invested in innovative developments. The effectiveness of their research M. Suleymanov (2022) proves on the example of the Nakhichevan Autonomous Republic. He does not see the need to develop commercial relations in the field of finding various sources of financing for other areas of developing the food industry in Azerbaijan. Given this, it can be affirmed that M. Suleymanov (2022) does not agree with the need to conduct research in this direction and considers it inappropriate to continue developments aimed at finding means of attracting investments aimed at improving the investment attractiveness of the food industry companies and organizations in Azerbaijan.

R.N. Sultanova (2020) can be considered a supporter of the results of the study, since the scientist conducts research in the field of ensuring the food security in Azerbaijan through the formation of a competitive consumer market in Azerbaijan. But this support is partial, since the food industry is only a part of the consumer market, and it is impossible to ensure comprehensive food security for the entire country only in this way. And since in order to ensure food security it is required to build effective international relations, as a result of which

it will be possible to attract financial resources aimed at increasing the competitiveness of consumer market enterprises in general and the food industry in particular, it can be affirmed that R.N. Sultanova (2020) agrees with the gained results of this research. It can be concluded that the food sector development will allow the economy of Azerbaijan to reorient itself to a non-raw material model of the economy, and to this end it is necessary to increase competitiveness (Overview foreign economic ..., 2022).

## CONCLUSIONS

According to the results of the presented research, it can be summarized that as a result of the state programs being implemented by the government of Azerbaijan in the field of developing areas of non-resource-based economy, it was possible to track that attracting exclusively domestic financial resources to develop the enterprises and organizations of the food industry in Azerbaijan is not enough. In 2006, 71.2% was financed from own funds of enterprises, 13.6% from bank loans, 4.7% from state funds, 2.9% from extra-budgetary funds and 6.7% from co-investment. By 2014, 82.6% was financed from the own funds of enterprises, 5.7% from state self-financing, 5.4% from bank loans, 1.3% from extra-budgetary funds and 6.7% from co-investment. Investments – 1.6%; in 2021, the share of investments of enterprises and organizations in improving the competitiveness of the food industry will decrease to 44.6%, the share of own funds of the population – to 2.6%, the share of bank loans – to 4.9%, the share of financial resources in improving the competitiveness of the food industry will increase to 43.8%, extra-budgetary funds – 3.7%, co-investment – 0.4%.

Investments of foreign partners and joint investments should be more widely used as sources of financing in Azerbaijan. Another source that will expand the opportunities for increasing the competitiveness of the food economy branch in the state is the issuance of corporate securities by enterprises and organizations operating in the food market of Azerbaijan with their subsequent sale in the securities market. To attract financial resources, it is necessary to expand the network of joint ventures and foreign organizations, as well as create new holding companies, financial and industrial groups, special economic zones, technology parks, industrial centers and innovation infrastructures in the main cities of the Republic of Azerbaijan. The above mentioned actions will contribute to the innovative activity of food industry enterprises and organizations and increase their attractiveness as objects for financial investment. The study of the rating attractiveness of Azerbaijan's foreign partners is promising research for the feasibility of developing partnerships in the field of attracting investments in the food industry of Azerbaijan, it is a continuation of the study.

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## Джерела фінансування для підвищення конкурентоспроможності харчової промисловості в Азербайджані

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**Анотація.** Державна політика уряду Азербайджану спрямована на галузеву диверсифікацію економіки країни, значне місце в якій займає харчова промисловість. Оскільки тривалий час в Азербайджані існувала сировинна модель економіки, інвестиційні кошти не спрямовувалися на підвищення конкурентоспроможності харчової промисловості для успішної роботи на світовому ринку. Основною метою дослідження є вивчення можливості використання різних джерел фінансування, необхідних для підвищення конкурентоспроможності харчової промисловості Азербайджану. Для досягнення поставленої мети використовувалися методи зведення та групування, аналізу, синтезу, індукції, дедукції. Було показано, що харчову промисловість у державі можна покращити шляхом збільшення кліматичних інвестицій та частки зовнішніх та спільних джерел фінансування. У ході проведеного дослідження було встановлено, що за останні 15 років уряд Азербайджану реалізував понад 70 національних і галузевих програм. У роботі окремо відзначаються Державні програми I і II з економічного і соціального розвитку регіонів, які були введені в період з 2004 по 2013 рік. Для підвищення конкурентоспроможності харчового сектора в Азербайджані були виділені додаткові фінансові ресурси, які досягли 65 мільярдів доларів США. 2012, були залучені в країні; кредити на посилення конкурентоспроможності харчової промисловості зросли в 9,7 рази в період з 2006 по 2021 роки, а в результаті обсяг інвестицій перевищить \$3 млрд. Позитивний вплив вжитих заходів відобразився у створенні додаткових 1,2 млн робочих місць у харчовій промисловості Азербайджанської Республіки. Результати дослідження показали, що у фінансуванні харчової промисловості країни найбільшу частку займають внутрішні джерела, а розвитку міжнародної співпраці, спрямованої на збільшення зовнішніх та спільних джерел фінансування, не приділяється належної уваги. Практичне значення наведених у статті результатів дослідження полягає в тому, що вони стануть основою для наступних напрямків дослідження теми, що розглядається, а також стануть основою для розробки державних програм удосконалення харчової промисловості Азербайджану

**Ключові слова:** споживчий ринок, клієнтоорієнтованість, інвестиції, міжнародна кооперація, нересурсна економіка

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## Prospective Areas for Managing the Financial Potential of Sustainable Development of Territorial Communities

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**Abstract.** Low income and considerable amounts of local budget expenditures, dependence on interbudgetary transfers, and the lack of an effective financial resource management system do not allow local authorities to fully implement the functions assigned to them and fulfil the community's financial potential. In this context, the search for promising areas for managing the financial potential of sustainable development of territorial communities is considered particularly relevant. The purpose of this paper is the theoretical and methodological substantiation of promising areas for managing the financial potential of sustainable development of territorial communities. It has been established that in the conditions of martial law, the tasks of local authorities have significantly expanded. Specifically, the issues of social and household services for internally displaced people, the formation of food reserves, and the application of security measures to protect the community, the implementation of which require adequate funding, have become relevant. It has been proven that local budget revenues are a key indicator of the financial potential of territorial communities. Using the method of economic and mathematical modelling, it is argued that the progress of the financial potential of territorial communities both during the martial law and in the post-war period will depend on capital investments in the development of the economy, the growth of the employment of the working population, as well as the increase in its income as the main source of the formation of financial bases of local budgets. It has been empirically confirmed that the state of war and the need for post-war reconstruction of territorial communities requires their classification depending on the degree of human, infrastructural, and economic losses. It has been proven that such a gradation allows developing an algorithm for managing the financial potential of sustainable development of territorial communities depending on their immediate needs and requests, and for state authorities and management bodies to find out the necessary amounts of financial and other support as soon as possible. The need to develop conceptual approaches to the development of a sophisticated management mechanism for the restoration of the financial potential of communities synchronized with the EU membership plan is argued. Such approaches can be used by local government and management bodies when developing strategic plans for the sustainable development of territorial communities, developing financial potential capable of ensuring the implementation of measures to manage their economic, social, and environmental advance

**Keywords:** decentralization, financial resources, sustainable development, martial law, local budget revenues



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## INTRODUCTION

The decentralization reform gave impetus to the transformation of the system of territorial organization of power by uniting territorial communities and focused attention on local development as the basis of the socio-economic progress of the state. Communities received a wider range of powers in managerial decision-making, as well as other opportunities for the development and use of financial resources. Despite certain positive changes in the system of financial support of territorial communities, the problems of low income and considerable amounts of local budget expenditures, the absence of an effective system for managing financial resources, which does not allow local authorities to fully implement the functions assigned to them and reveal the financial potential of the community, are still relevant.

The military aggression that began on February 24, 2022 on the part of the Russian Federation forced to change the vector of development of territorial communities and management of their financial potential. The problem worsened because with the beginning of hostilities, numerous territorial communities found themselves under temporary occupation, with some of them suffering varying degrees of destruction. The introduction of martial law by the country's central government forced a change in the system of relations between state and local authorities to ensure the coordination of the actions of all power structures for the fastest liberation and restoration of the country. In the conditions of martial law, the tasks of local authorities have expanded significantly. Apart from the direct performance of its key functions aimed at meeting the needs of the population of territorial communities, the issues of social and household services for internally displaced people, the formation of food reserves, the application of measures to protect the community, etc., were brought up to date. Territorial communities are becoming financially more dependent on the state budget. At the same time, despite the continuation of martial law, it is necessary to have a clear plan for the recovery and growth of the financial potential of territorial communities to promptly rebuild the destroyed territories and to facilitate the return of citizens to their permanent places of residence. In such conditions, the issue of managing the financial potential of territorial communities, as the basis of their sustainable development, becomes particularly relevant.

*The purpose of this study* is to develop theoretical and methodological approaches to the justification of promising areas for managing the financial potential of sustainable development of territorial communities. The tasks of this study are as follows: to establish factors influencing the financial potential of territorial communities based on the analysis of the publications of Ukrainian and foreign scientist; to assess the impact of key factors on the financial potential of territorial communities using methods of economic and

mathematical modelling; to propose a recovery algorithm and point to promising areas for managing the financial potential of territorial communities.

## LITERATURE REVIEW

Even in the pre-war period, the territorial communities of Ukraine differed in the level of economic development, financial and human potential. Even though the decentralization reform contributed to the filling of local budgets with added amounts of financial resources, the regions of Ukraine continued to show a different level of progress. As a result, numerous territories managed to fulfil their financial potential and achieved a considerable degree of financial capacity (the ability to independently finance the needs of the community); others, on the contrary, could not get rid of financial dependence. In this context of the issue under study, the papers of leading scientists, which cover the theoretical foundations of managing the financial potential of territorial communities and the factors that have the principal influence on their development, deserve special attention.

Methodological approaches to the assessment of influencing factors on the financial and economic potential of regions are laid down by classical and neoclassical economic theories. The main sources of growth of the region's economy, as a key component of its financial potential. According to these theories, there is an increase in labour and capital, an increase in labour productivity and technological advance. Corresponding models of economic growth were developed in the studies of T. Malthus (1895) and J. Hicks (1939). The progress of these fundamental theories took place in the papers of the American economist E. Denison (1984), who substantiated that financial and economic growth is determined not so much by the number of spent factors of production, but by the growth of their quality and, above all, by the improvement of the quality of the workforce. J. Barney (1991) and K. Wilkinson (1991) covered the issue of managing the financial potential of territories. L. Van de Pole (2015) substantiated the importance of modelling for stimulating the economic growth of rural areas. Ukrainian researcher M. Skrypnychenko (2007) developed an original model construction of economic development based on endogenous factors as indices of integral indicators. The studies of S. Karpets (2012) illustrate the models of socio-economic development of Ukraine. Theoretical and applied aspects of the influence of various factors on the development of territorial communities for modelling their socio-economic development are outlined by R. Bihun, V. Lytvyn, and N. Oleksiv (2021). The analysis of the mentioned sources proved that such factors influencing the development of the financial potential of territorial communities as the income of local budgets, investments in economic development, employment of the population of the respective territory, etc., stay

outside the focus of leading researchers and confirmed the importance of further investigation in this area.

K. Andersson *et al.* (2015) investigated other aspects of the development of territorial communities, namely the issue of territorial decentralization. The influence of financial decentralization on the development of rural areas was studied by O. Dyakonenko (2018). Features of the development of financial resources of the regions became the subject of research by M. Brady and K. Walker (2018). V. Semianovskiy (2018) theoretically justified the development of the intellectual potential of territorial communities. A. Dvigun (2020) investigated new opportunities for communities that opened up during the decentralization in Ukraine. The problems of territorial communities' access to financing are covered in the papers by E. Girolami *et al.* (2022). O. Levchenko, N. Velychko, and L. Kovshun (2018) investigated and described the administrative mechanisms of community development as the basis for the growth of their financial and economic potential. M. Olchak (2020) proposed the application of an integrated approach to managing the sustainable development of territorial communities, considering the key aspects of their functioning: economic, social, and environmental. V. Dovzhenko, A. Voytenko, and M. Plotnikova proposed the use of European management experience for planning the development of territorial communities (2020). T. Zinchuk and M. Patynska-Popeta (2020) developed the innovative model of united territorial communities, which promotes business development in rural areas. The study by M. Datsyshyn (2022) covers the issues of restoring local self-government after the war. The analysis of the above-mentioned studies suggests that financial potential is a complex concept, under which one should understand the ability, opportunities, and prospects of attracting and using the available and potential resources of the territorial community to ensure its sustainable development. At the same time, the conducted study proved that the promising areas of managing its development are still understudied and poorly described in literary sources.

O. Borodina and I. Prokopa (2019) considerably contributed to shaping the national paradigm for the development of rural territorial communities. D. Dema, I. Abramova, and L. Nedilska (2019) investigated the financial and economic conditions of the development of rural areas. National regional financial policy and its influence on rural development were investigated by L. Dorokhova and N. Kurovska (2019). S. Obushnyi (2019) studied the issues concerning the improvement of financing of social services in rural territorial communities. Improvement of access to public services in the countryside, as well as institutional aspects of the involvement of rural communities in the inclusive development of territorial communities, found further development in the works of I. Prokopa (2022), O. Shkuratov,

O. Khohulyak, and T. Kushniruk (2022) investigated the financial provision of rural areas. Despite a considerable number of studies justifying various aspects of the development of territorial communities, including rural ones, the unsatisfactory state of their financial potential and the lack of effective mechanisms for its management indicate the need for separate research in this area.

## MATERIALS AND METHODS

To fulfil the purpose of this study and to solve its tasks, both general scientific and special methods of cognition were applied, namely: scientific synthesis, comparison, and systematization – to substantiate the economic content of the concepts and categories; abstract-logical – to summarize the theoretical and methodological principles of managing the financial potential of territorial communities based on sustainable development; statistical and economic – to find the specific features of fulfilling the financial potential of territorial communities; correlation-regression analysis – to determine the influence of various factors on the financial potential of territorial communities; comparative analysis and synthesis – to develop promising areas for managing the financial potential of territorial communities based on sustainability; logical generalization – to substantiate conclusions and formulate proposals; tabular and graphic – for visualization of the results obtained. The information basis of this study included the papers of Ukrainian and foreign authors; regulatory and reference materials; official materials of the State Statistics Service of Ukraine (2020), the Ministry of Development of Communities and Territories of Ukraine (2021; 2022), electronic publications and Internet resources on the topic under study, etc.

## RESULTS AND DISCUSSION

### ***Assessment of the influence of key factors on the financial potential of territorial communities***

Based on a critical analysis of scientific literature and the author's personal generalizations, it was established that the income of local budgets per 1 person of the population of the respective territorial unit should be considered the indicator of the state of development of the financial potential of territorial communities. It is assumed that this indicator should be assessed considering numerous factors, among which the principal ones were singled out as follows: the volume of capital investments (except for investments from the state budget), the unemployment rate of the working population, the disposable income of the population, labour productivity, gross regional product, the number population of working age, etc. This hypothesis was confirmed using methods of economic and mathematical modelling. During the study, such factors as gross regional product, the number of the working-age population, labour productivity, the number of small and medium-sized

business entities, etc., showed a fairly close correlation with the result indicator (correlation coefficient  $R^2 > 0.9$ ) and were not included in the model. At the same time, such indicators as the index of industrial production, the volume of export of goods, and some others are low (correlation coefficient  $R^2 < 0.2$ ), which became the reason for their deviation.

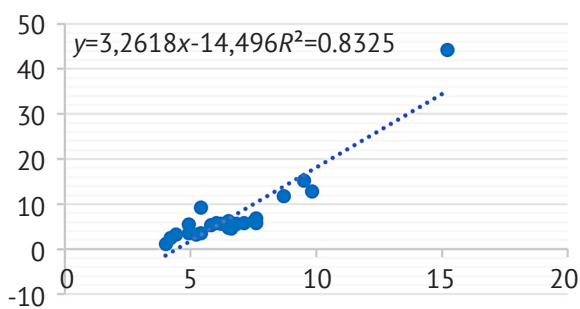
Proceeding from this, the following independent variables served as the basis for the construction of the correlation-regression model:

$X_1$  – volumes of capital investments (except for investments from the state budget) calculated per 1 person of the population in thous. UAH;

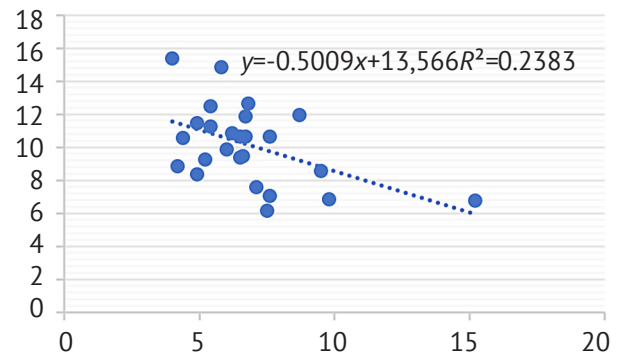
$X_2$  – the unemployment rate of the population aged 15-70 in % to the economically active population of the corresponding age;

$X_3$  – disposable income of the population per person.

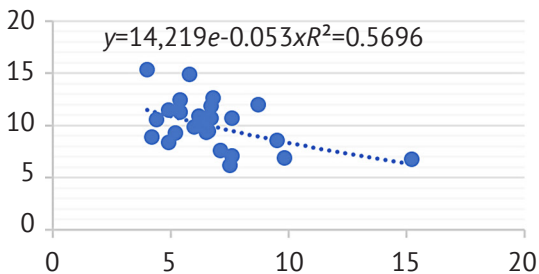
The model was constructed based on data from the monitoring of socio-economic development of regions for 2020, which was performed by the State Statistics Committee of Ukraine and the Ministry of Development of Communities and Territories of Ukraine. Calculations were performed using the Microsoft Excel software package. Using graphical visualization, the paired interdependence of local budget revenues (excluding transfers) with the selected indicators was proved (Fig. 1).



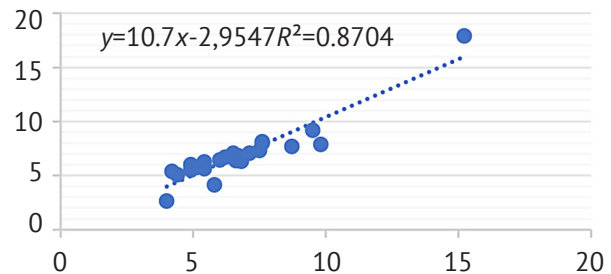
Linear dependence of local incomes on the volume of capital investments (excluding investments from the state budget)



Linear dependence of local incomes on the unemployment rate of the population aged 15-70



Exponential dependence of local incomes on the unemployment rate of the population aged 15-70



Linear dependence of local incomes on available incomes of the population

**Figure 1.** Pairwise interdependence of local budget revenues (excluding transfers) with selected indicators  
**Source:** developed by the authors based on source data

It was established that the revenues of local budgets (dependent variable  $Y$ ) have a high degree of correlation with factor characteristic  $X_1$ , the correlation coefficient  $R^2 = 0.8325$ , a linear correlation between the variables is observed. When graphically visualizing the linear dependence of the  $Y$  variable on the  $X_2$  factor, the correlation coefficient  $R^2 = 0.2383$  showed a weak relationship between the features, while the construction of the exponential trend line proved a sufficient

degree of dependence between them ( $R^2 = 0.5696$ ). The dependent variable  $Y$  revealed a high degree of linear correlation on factor  $X_3$  ( $R^2 = 0.8704$ ).

Graphical calculations regarding the presence of correlation between variables will be confirmed by constructing a correlation matrix, where the number of observations is  $n = 25$ , and the number of independent variables is 3. Considering the variable  $Y$ , the dimension of the matrix equals 5 (Table 1).

**Table 1.** Correlation matrix showing the degree of dependence between variables

| Variables      | Y       | X <sub>1</sub> | X <sub>2</sub> | X <sub>3</sub> |
|----------------|---------|----------------|----------------|----------------|
| Y              | 1       | 0.8124         | -0.4882        | 0.903          |
| X <sub>1</sub> | 0.8124  | 1              | -0.3674        | 0.8302         |
| X <sub>2</sub> | -0.4882 | -0.3674        | 1              | -0.5707        |
| X <sub>3</sub> | 0.903   | 0.8302         | -0.5707        | 1              |

**Source:** developed by the authors based on source data

The data presented in the table indicate a strong positive linear correlation, when the increase of one value affects the growth of another, between the variables: X<sub>1</sub> and Y, X<sub>2</sub> and Y, X<sub>3</sub> and Y, X<sub>2</sub> and X<sub>1</sub>, X<sub>3</sub> and X<sub>1</sub>, X<sub>3</sub> and X<sub>2</sub>; the data also indicates the average negative closeness of connection, when an increase in one value leads to a decrease in another, between variables: X<sub>2</sub> and Y, X<sub>2</sub> and X<sub>1</sub>, X<sub>3</sub> and X<sub>2</sub>.

The regression equation in the model under study took the following form:

$$Y = 2.6452 + 0.09963X_1 - 0.02015X_2 + 0.05143X_3 \quad (1)$$

The economic content of the obtained correlation-regression equation is as follows:

– the volumes of local budget revenues (without considering transfers from the state budget) per 1 person of the population of the respective administrative territorial unit (resultative indicator Y) in the absence of X<sub>i</sub> would amount to 2.6452 thous. UAH;

– coefficient b<sub>1</sub> shows that with an increase in the volume of capital investments (excluding investments from the state budget) calculated per 1 person of the population (X<sub>1</sub>) by 1 thous. UAH, the resulting indicator Y increases by 0.09963 thous. UAH;

– coefficient b<sub>2</sub> indicates that with an increase in the unemployment rate of the working-age population aged 15-70 in % to the economically active population of the corresponding age (X<sub>2</sub>) by 1%, the resulting indicator decreases by 0.02015%;

– coefficient b<sub>3</sub> proves that with an increase in the available income of the population per 1 person (X<sub>3</sub>) by 1 thous. UAH, Y increases by 0.05143 thous. UAH.

Based on the maximum coefficient β<sub>3</sub>=0.099, it can be concluded that factor X<sub>1</sub> has the greatest influence on the result Y. That is, the obtained correlation-regression equation proves that capital investment in the development of the economy of territorial regions has a considerable impact on one of the effective indicators of their financial potential, namely the income of local budgets.

The next stage of the study was the analysis of the reliability and adequacy of the constructed model. For this purpose, the multicollinearity of the factors embedded in the model was verified. The indicator was calculated using Microsoft Excel software. The calculation

results showed that r(X<sub>1</sub>X<sub>2</sub>) have |r|>0.7, which indicates the multicollinearity of factors X<sub>1</sub> and X<sub>3</sub>. For the rest of the factors |r|<0.7, which indicates the absence of multicollinearity between them. However, the obtained data are insufficient to formulate final conclusions.

Further statistical analysis of the obtained regression equation, which involved verifying the significance of the equation and its coefficients, showed as follows: the average error of approximation is 10.22%, which is within the permissible values and indicates the good quality of the model; the variance estimate is 14.738, which indicates a normal distribution of the model data; the unbiased variance estimate is 0.7018; the standard error of Y estimate is 0.838, indicating a low degree of standard error.

A critical component of a qualitative regression analysis is the determination of autocorrelation, which guarantees the absence of correlation between any residuals (deviations) of the model. To determine the degree of autocorrelation of the model under study, the autocorrelation coefficient was calculated, and its significance was verified using the standard error criterion.

Using the calculation table, we get as follows:

$$r_1 \approx \frac{\sum \epsilon_t \cdot \epsilon_{t-1}}{\sum \epsilon_t^2} = \frac{-3.583}{14.738} = -0.243 \quad (2)$$

Since -0.483 < r<sub>1</sub> = -0.243 < 0.483, the property of independence of residuals is fulfilled. There is no autocorrelation in the model under study.

The Durbin-Watson test is best known for confirming the absence or presence of autocorrelation between model data. For the model under study, the Durbin-Watson exponent will be as follows:

$$DW = \frac{35.75}{14.74} = 2.43 \quad (3)$$

Autocorrelation of residuals is absent if the condition: 1.5 < DW < 2.5 is fulfilled. Since for the constructed model 1.5 < 2.43 < 2.5, it can be concluded that there is no autocorrelation of the residuals.

The closeness of the joint influence of the factors on the resulting feature was estimated using the multiple correlation coefficient. For the model under study, calculations were made using known linear pair correlation values and β-coefficients.

$$R = \sqrt{0.912 \cdot 0.356 + (-0.488) \cdot (-0.0207) + 0.933 \cdot 0.59} = \sqrt{0.885} = 0.941 \quad (4)$$

The obtained values of the multiple correlation coefficient  $R=0.941$ , as well as the adjusted correlation coefficient:

$$R^2 = 1 - (1 - 0.8854) \cdot \frac{25 - 1}{25 - 3 - 1} = 0.869 \quad (5)$$

testify to the high quality of the model.

The determination coefficient  $R^2=0.8854$  was found, which proved the high quality of the model.

$$R^2 = 1 - \frac{s_{\epsilon}^2}{\Sigma(y_i - \bar{y})^2} = 1 - \frac{14.738}{128.59} = 0.8854 \quad (6)$$

The hypothesis about the overall significance of the model was tested using the *F-criterion* ( $F_{kp}$ ):

$$F = \frac{R^2}{1 - R^2} \cdot \frac{n - m - 1}{m} = \frac{0.8854}{1 - 0.8854} \cdot \frac{25 - 3 - 1}{3} = 54.079 \quad (7)$$

It was established that since the real value of  $F$  is greater than the *tabular* one, the determination coefficient is statistically significant, and the regression equation is statistically reliable in the model under study (i.e., the  $b_i$  coefficients are jointly significant).

Thus, the conducted study allowed building a multiple regression equation  $Y=2.6452+0.09963X_1-0.02015X_2+0.05143X_3$ , the economic content of which is as follows: an increase in the volume of capital investments by 1 thous. UAH per 1 person of the population leads to the income growth of the budgets of territorial communities by 0.09963 thous. UAH; with an increase in the unemployment rate of the working-age population by 1%, the corresponding revenues of local budgets decrease by 0.02015%; with an increase in the available income of the population by UAH 1 thous., the effective indicator increases by 0.05143 thous. UAH. The calculations proved that the greatest influence on the incomes of local budgets has such a factor as the available incomes of the population in the calculation per 1 person. The statistical significance of the equation was confirmed using the determination coefficient and the *F-criterion*. It is proved that in the constructed model, 88.54% of the total variation of the outcome characteristic  $Y$  is explained by the change in factors  $X_j$ .

Considering the conclusions obtained as a result of correlation-regression modelling, the advance of the financial potential of territorial communities both during the martial law and in the post-war period

will depend on capital investments in the economy development, the growth of the employment of the able-bodied population, as well as increase in its income as the main source for forming the financial base of local budgets. Therewith, the differentiation between the degrees of development of territorial communities intensified with the beginning of military operations on the territory of Ukraine. Local authorities of the temporarily occupied territorial communities or those in the zone of active military operations have completely or partially lost the opportunity to develop and use financial resources to meet even the primary needs of the population living there. Some, already liberated territories, have a greater or lesser degree of destruction of infrastructure, production facilities, are more manageable. Here, local self-government is slowly starting to resume work, using all available resources to meet the needs of communities and ensure their financial capacity. Some territorial communities, mainly in the western regions of Ukraine, suffered much less, but their financial and economic potential also changed. So far, they have sheltered millions of internally displaced people and assumed responsibility for their socio-economic protection.

#### **Algorithm for restoring the financial potential of territorial communities**

Prior to the outbreak of the war, I. Prokopa (2022) formulated the conceptual principles of identifying territories not by administrative, but by functional characteristics, and methodologically approached the classification of territorial communities of Ukraine according to functional types with a predominantly urban, urban-rural, and predominantly rural population. The key need for such identification was caused by the fact that communities are entrusted with the responsibility for the development of their territories, and this, according to the scientist, corresponds to one of the goals of the national regional policy. The state of war and the need for post-war reconstruction of territorial communities actualizes the issue of their division depending on the degree of human, infrastructural, and economic losses. Such gradation allows developing an algorithm for managing the financial potential of sustainable development of territorial communities depending on their immediate needs and requests (the tentative structure of which is illustrated in Figure 2), and for state authorities and management bodies to promptly find the necessary amounts of financial and other support.



**Figure 2.** Algorithm for restoring the financial potential of territorial communities

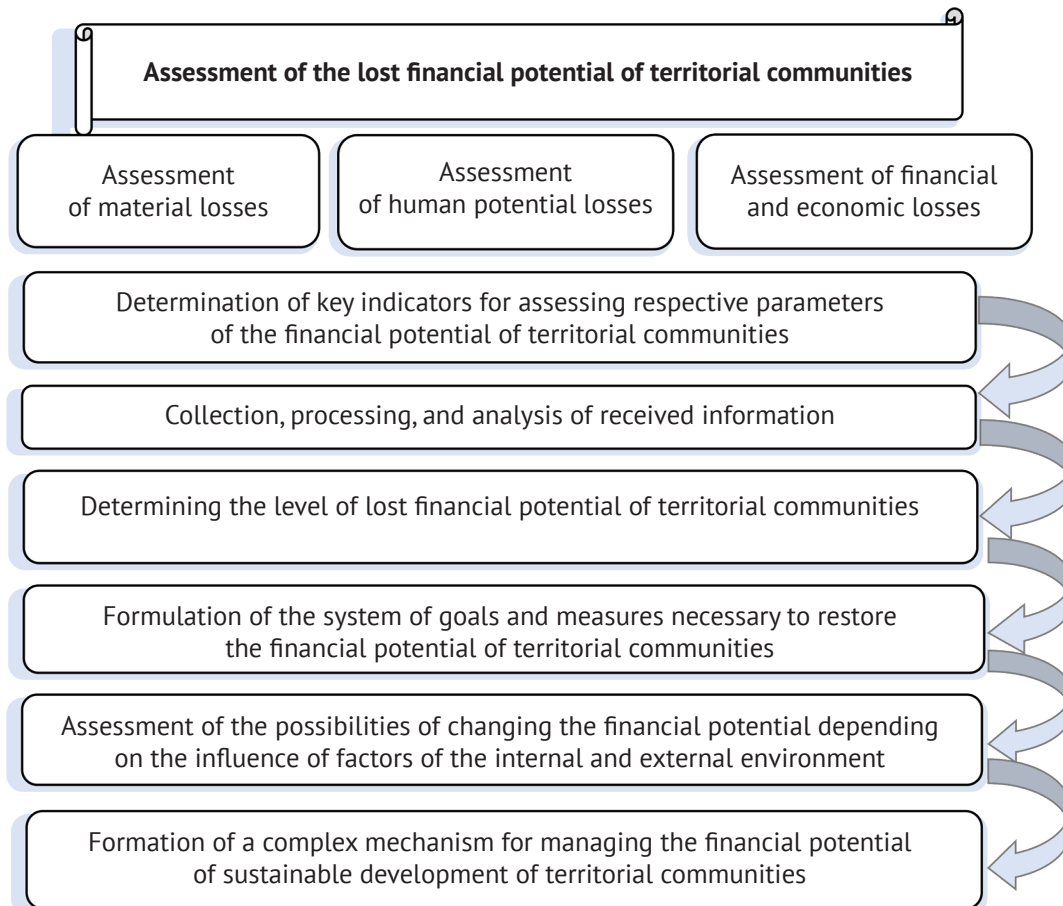
**Source:** developed by the authors

Specialists of state authorities and local self-governments are developing methodical approaches to the assessment of damage caused to territorial communities as a result of the military aggression of the Russian Federation. In these processes, the reference point is mainly made on the inventory and assessment of the value of the lost property. To form a holistic mechanism for managing financial potential and development of territorial communities, the authors of this study suggest focusing attention on the loss of human potential, which underlies or the economic activity of any enterprise and is the main payer of personal income tax – a key source of income for local budgets. This refers to the fact that territorial communities have suffered various human losses from death, departure abroad or to other, safer, regions of Ukraine. Therefore, it is worth developing tools and creating conditions for the fastest

possible return of refugees and internally displaced people to their permanent places of residence.

To restore the financial potential of territorial communities, it is important to focus on the direct assessment of economic losses, not so much on property losses as on the losses of a certain set of benefits that enterprises could have, but did not, as a result of military actions. Here, it is important to focus on identifying lost and forming new logistics relationships with suppliers and contractors, buyers and customers, etc. Undoubtedly, the search vector should be shifted towards the member states of the European Union, completely moving away from economic dependence on the Russian Federation, Belarus, etc.

The proposed approaches should become the basis for assessing the financial potential of territorial communities depending on the degree of damage, losses of economic and human potential, etc. (Fig. 3).



**Figure 3.** System for assessing the financial potential of territorial communities

*Source:* compiled by the authors

### **Promising areas of financial potential management of territorial communities**

Determination of key indicators for assessment, collection, analysis, and processing of received information, determination of the level of lost financial potential of territorial communities should become the basis for formulating a system of goals and measures necessary for its restoration, as well as the formation of a complex management mechanism. A key component of such a mechanism can be participative strategic planning, which opens the possibility of involving a wide range of stakeholders, conducting analysis and assessment of the state of business development and the financial potential of the community. While planning, it is worth clarifying the interdependence of such factors as the reorientation of business structures to European sales markets and the growth of the gross regional product – the basis of the revenue base of local budgets. Attention should also be paid to the possibility of community participation in programs of grant support by EU countries, and their impact on the future infrastructural development of the regions should be assessed.

It is necessary to strategically calculate how the introduction of EU standards will affect the profitability of business, since a significant increase in the cost

of products is expected, which in the conditions of low solvent demand of the population and economically unstable business can become a financial burden both for the population of communities and for entrepreneurs. Labour migration to EU countries may cause labour shortages and raising social standards will require added costs from local budgets for their implementation. Improving social standards – the quality of education, medicine, and other social services – will require added expenditures from local budgets. Considering the outlined proposals and provisions of the Association Agreement with the EU, each community can conduct its own strategic analysis, factoring in individual socio-economic features of development, resource and labour potential, and other factors.

An important European practice is participatory budgeting, during which the formation and implementation of local budgets takes place with the involvement of citizens. The population takes part in the development of recommendations for the distribution of the community budget, monitors and controls the use of funds. Participatory budgeting is a manifestation of democracy. At the same time, it strengthens the responsibility of local bodies and citizens to each other. The population of the community, involved in managing

budget funds, has an interest in more efficient use of available resources, and local self-government bodies have added motivation to use them more transparently.

In Ukraine, in parallel with the decentralization reform, an initiative to create participatory budgets was launched. The content of the project lies in providing an opportunity for any resident to take part in the allocation of local budget funds through the creation of projects for the improvement of the city or voting for them. Residents of the community can submit a project related to improving life in the city, take part in the competition and monitor its implementation. Together with foreign partners, an online platform "Public Project" was created in Ukraine, where project initiatives are displayed. Currently, most large cities of Ukraine have their own public budget. Predictive indicators of public budgets can become a component of the strategy for developing the financial potential of communities.

An important aspect of managing the financial potential of territorial communities is the restoration of vertical and horizontal communication between state and local self-government bodies, the population, business structures, and other institutions in the liberated and temporarily occupied territories. Presently, the primary task is the return of control of the Ukrainian authorities over the entire territory of Ukraine and the establishment of cooperation with the legitimate representatives of the territorial authorities. The restoration or formation of horizontal cooperation between local self-government bodies should be based on the principles of comprehensive mutual assistance and support, including financial one. Territorial communities less affected as a result of the war, in conditions where the state budget for 2023 prioritizes the financing of security and defence complexes of Ukraine, should maximally aid the recovery of more affected regions. Financial support for displaced people from affected communities, creation of conditions for business relocation, search for specialists capable of filling a niche in the workforce, investment in the reconstruction and development of the economy of these territories – these

are the tools that will ensure the integrity, sovereignty, and financial stability of Ukraine in the future.

## CONCLUSIONS

The analysis of the studies of leading researchers allows concluding that financial potential of a territorial community is a complex concept, under which one should understand the ability, opportunities, and prospects of attracting and using the available and potential resources of the territorial community to ensure its sustainable development. It is proven that the definition of the system of goals and objectives for the restoration of the financial potential of territorial communities should be correlated with the conceptual approaches to the country's restoration strategy laid down by the government. It is argued that in this process it is important to predict and establish the necessary complementary changes in the economic environment, which will allow increasing the financial potential of territorial communities through the ability to effectively form and distribute not only their resources, but also the financial support of European partners.

The creation of a solid basis for the sustainable development of territorial communities in the post-war period through the formation of an open, competitive, socially responsible economy integrated into the European community is considered relevant. Territorial communities – safe and comfortable for people to live in. The outlined goals and tasks should underlie the development of conceptual approaches to the formation of a complex management mechanism for the restoration of the financial potential of communities synchronized with the EU accession plan. The implementation of the proposed measures will create the basis for an effective mechanism for managing the financial potential for the sustainable development of territorial communities. As a result, growth of the local economy and financial self-sufficiency of territorial communities, improvement of the level and quality of life of the population of communities, maintenance of ecological balance, preservation, and improvement of local territories are expected.

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## Перспективні напрями управління фінансовим потенціалом сталого розвитку територіальних громад

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**Анотація.** Низький рівень доходів й значні обсяги витрат місцевих бюджетів, залежність від міжбюджетних трансфертів, відсутність ефективної системи управління фінансовими ресурсами не дозволяє органам місцевої влади повною мірою реалізувати покладені на них функції й розкрити фінансовий потенціал громади. В такому контексті пошук перспективних напрямів управління фінансовим потенціалом сталого розвитку територіальних громад вбачається особливо актуальним. Метою статті є теоретико-методологічне обґрунтування перспективних напрямів управління фінансовим потенціалом сталого розвитку територіальних громад. Встановлено, що в умовах воєнного стану завдання місцевих органів влади значно розширилися, а саме актуалізувалися питання соціально-побутового обслуговування внутрішньо-переміщених осіб, формування продовольчих резервів, застосування безпекових заходів для захисту громади, виконання яких потребують належного фінансування. Доведено, що доходи місцевих бюджетів є ключовим індикатором фінансового потенціалу територіальних громад. З використанням методу економіко-математичного моделювання аргументовано, що поступ фінансового потенціалу територіальних громад як під час воєнного стану, так і в повоєнний період залежатиме від капітальних інвестицій у розвиток економіки, зростання зайнятості працездатного населення, а також збільшення його доходів, як основного джерела формування фінансової бази місцевих бюджетів. Емпірично підтверджено, що військовий стан та необхідність повоєнної відбудови територіальних громад потребує їх класифікації залежно від ступеня людських, інфраструктурних та економічних втрат. Доведено, що така градація дозволить розробити алгоритм управління фінансовим потенціалом сталого розвитку територіальних громад залежно від їх безпосередніх потреб та запитів, а органам державної влади та управління – якомога швидше з'ясувати необхідні обсяги фінансової та іншої підтримки. Аргументовано необхідність розробки концептуальних підходів до формування комплексного механізму управління відновленням фінансового потенціалу громад синхронізованого з планом набуття членства у ЄС. Такі підходи можуть використовувати органи місцевої влади та управління при розробці стратегічних планів сталого розвитку територіальних громад, формуванні фінансового потенціалу спроможного забезпечити реалізацію заходів з управління їх економічним, соціальним й екологічним поступом

**Ключові слова:** децентралізація, фінансові ресурси, сталий розвиток, воєнний стан, доходи місцевих бюджетів

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## Modeling the Influence of Startup Ecosystem Components: Entrepreneurial Aspect

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**Abstract.** Defining the basis for the development of a country's start-up ecosystems as the basis for activating entrepreneurship is an urgent task of restoring the country's economy. The purpose of this study is to identify ways to improve the country's start-up ecosystem based on the construction of economic and mathematical models for activating business activities. The research methodology is based on statistical research methods, namely dynamics analysis; to assess the strength of the influence of each component of the start-up ecosystem on the change in position in the rating, regression correlation analysis was chosen, which allows identifying the strength of the influence of factors on the final indicator. The components of the start-up ecosystem were investigated, which means an interactive and interdependent set of institutions whose activities create an environment for the qualitative and quantitative growth of start-ups as subjects of innovative entrepreneurship development. The use of statistical analysis methods for the data of the countries, which were grouped into 5 clusters, allowed determining the absolute changes in the values of the Global Start-up Ecosystem Index rating indicators from Start-up Blink: rating change, quantitative component, qualitative component, business environment, general summary. Based on regression-correlation analysis, economic-mathematical models were built, which describe the influence of the components of the country's ecosystem on the change in the rating positions in the section of four clusters, demonstrating: a strong connection between the factors and the result (Clusters 2, 4). Weak connection for the countries of Clusters 3, 5, which indicates the dependence of the development of start-up ecosystems on other factors that are not considered in their description and which affect the development of start-ups and entrepreneurship in the countries of Clusters 3, 5. The practical value of this paper is that the results of the study can serve as the basis for the local and state authorities to form strategies to develop start-up ecosystems at the national and regional level

**Keywords:** start-up, country ecosystem, economic and mathematical model, entrepreneurship, rating



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## INTRODUCTION

The development of entrepreneurship is the key to the economic growth of a country with a market economy, which determines the relevance and necessity of the formation of a comprehensive ecosystem of entrepreneurship development. Its important condition is the creation of not just new firms and start-ups, but rather high-growth firms (HGFs), which cannot be ensured without using an ecosystem approach to entrepreneurship.

Turning to the sources of the mentioned approach, the very term “ecosystem” came into economic circulation from biology. It was first mentioned in the economic context in the work of James Moore (1993), who coined the term “entrepreneurial ecosystem” and believed that the ecosystem includes both the internal and external environment. Thus, apart from owners and employees, adding government agencies, competitors, suppliers, etc. In their study, E. Stam and B. Spiegel (2016) consider the entrepreneurial ecosystem as “a set of interdependent actors and factors that are coordinated in such a way as to ensure productive entrepreneurship in a certain territory”, while not identifying with them clusters and innovative systems. What is important is the emphasis that these scientists put – the spread to a certain territory and the shift of attention to entrepreneurship as a whole, rather than the concentration on an individual subject.

The entrepreneurship ecosystem includes such “elements as individuals, organizations, and institutions outside of entrepreneurship that encourage or hinder a person’s decision to be an entrepreneur or influence their success in starting an entrepreneurial activity” (Aliabadi *et al.*, 2022). This definition focuses on certain components of the ecosystem that should interact with each other. Therewith, the emphasis is on starting one’s own business, and not on its growth in the form of the existence of fast-growing companies that cause development.

An interesting idea (Porev, 2018) is that the presence of entrepreneurial structures in the economic system ensures the presence of entrepreneurial ecosystems as an environment for the growth of firms. R. Brown and K. Mason (2017) do not limit the role of entrepreneurial ecosystems to the development of start-ups and believe that the role of large firms is to attract skilled labour, build human potential capable of start-up activities.

In addition, the development of entrepreneurial ecosystems was considered in the studies of O’Connor *et al.* (2018), as well as Haustov *et al.* (2022). Thus, an entrepreneurial ecosystem can be considered as a certain interacting set of institutions and organizations that enter into an interdependent relationship, creating an environment for the successful start, operation, and rapid growth of enterprises.

Further research of the scientific heritage indicates the relevance of investigating start-up ecosystems and their development vectors. Thus, according to Creating a Future-Ready Start-up Ecosystem (2022), the

authors identified the obstacles that prevent the start-up ecosystem from reaching its full potential, identified new opportunities, and developed actionable strategies that can help the ecosystem reach new heights. (Agnihotri, 2018) described the modern start-up ecosystem and proved that start-up creation makes “business” a career worthy of attention outside the conventional trade community, a political path for start-up creation and success in scale improvement, combining simplicity and efficient execution with results based on continuous improvement. The authors (Cukier & Kon, 2018) proposed a maturity model for start-up ecosystems that helps understand their evolution and dynamics. Moreover, such a system can serve as a basis for stakeholders in less mature ecosystems to analyse their environment, identify weaknesses, and suggest policies and practical actions to improve their ecosystems after some time.

According to (Segers, 2019), the entrepreneurial start-up ecosystem as a concept appeared quite recently and serves as a framework that allows understanding the environment and its favourable characteristics for entrepreneurial prosperity. An essential element of the new regional entrepreneurship ecosystem is Student Start UP, a joint venture between the University of Applied Sciences and Hasselt University that focuses on student entrepreneurship. Authors such as (Jacobides *et al.*, 2018) note two approaches to understanding the start-up ecosystem. In the first approach, the ecosystem is perceived as a group of organizations that are largely interdependent in relation to factors of production and output; in the second – as a system of interdependent technologies. These approaches define two areas of research related to ecosystem analysis: 1) in the field of strategic management; 2) in the field of technology management.

*The purpose of this study:* to establish the vectors of development of the modern start-up ecosystem of countries based on economic and mathematical modelling to ensure the development of entrepreneurship. The task of the research: to cover the essence and components of the start-up ecosystem; to determine dynamic changes in the ranking of countries in terms of the components of the Global Start-up Ecosystem Index from Start-up Blink, which reflects the country’s start-up ecosystem (quantitative component, qualitative component, business environment); to build mathematical models that describe the influence of each component of the start-up ecosystem on the change in the ranking position.

## MATERIALS AND METHODS

The data of the Global Start-up Ecosystem Index 2021 according to Start-up Blink (2021), which is the largest comprehensive rating of start-up ecosystems of 100 countries and 1,000 cities of the world since 2013, formed the information base of this study. It presents the ranking of countries, where the Total Score (corresponds

to the position in the ranking) and Rank Change (shows the change in the ranking in 2021 relative to 2020) are noted. The choice of this particular rating is conditioned upon the presence of a structured start-up ecosystem, which is described by components containing appropriate indicators and descriptions. The methodology for calculating the start-up ecosystem index Start-up Blink contains 3 components: quantitative, qualitative indicators, and assessment of the business environment.

The quantitative component shows the level of activity of the ecosystem through its stakeholders and other indicators, such as the number of start-ups, the number of coworking spaces, the number of accelerators, the number of meetings related to start-ups, which allows establishing the level of activity of the start-up ecosystem. The qualitative component of the rating examines the parameters that indicate the qualitative results achieved by the ecosystem. These parameters include an analysis of the popularity of the best start-ups in the ecosystem: traffic, domain rating, customer base; availability of branches and research centres; international technology corporations; branches of multinational companies; investment volume; number of start-up employees; availability of unicorn companies, exit companies, and pantheons. The business environment assessment component combines business and economic indicators at the national level, which focus on overall indicators related to infrastructure, the business environment, and the ability of start-up founders to work freely in each country. The main components of the business environment component are: ease of doing business and registering companies; availability of the internet and its speed; investment in R&D; availability of various technological services (payment portals, travel exchange programs, cryptocurrencies); number of patents per capita; level of English language proficiency, etc.

The methodology of this study is based on statistical methods, namely dynamic analysis, which is used

to investigate the absolute change in the ranks of countries in the Global Start-up Ecosystem Index ranking from Start-up Blink, which allows determining dynamic changes by year (2021 to 2020), as well as structural shifts that led to such changes, in terms of components of the index (quantitative component, qualitative component, and business environment).

The dynamics were investigated in terms of groups of countries, which were unified using cluster analysis, which allowed forming 5 clusters, presented in detail in the studies (Dymchenko *et al.*, 2022; Kyzym *et al.*, 2022). To assess the impact of changes in each component of the start-up ecosystem, a regression-correlation analysis was chosen, which allows finding the impact of factors on the final indicator. For the best understanding of how the selected indicators affect the resulting indicator ( $y$ ), a single multivariate regression equation is constructed, which has the following form (1):

$$y=f(\beta, x)+\varepsilon \quad (1)$$

where  $x=x(x_1, x_2, \dots, x_n)$  is the vector of independent (explanatory) variables;  $\beta$  is the vector of parameters that fall under the definition;  $\varepsilon$  is the random error (deviation);  $y$  is the dependent variable (the one that is explained).

The calculated coefficients  $\beta$  near the arguments show that when the variable  $x$  increases by one, the average value of  $y$  will increase by the corresponding value of the coefficient  $\beta$ . The “+” sign next to  $\beta$  shows a direct relationship between the corresponding arguments and the occupied value, the “-” sign is the opposite.

The constructed model is verified for authenticity. The most general assessment in this case was provided by correlation and determination coefficients. The relationship between the factors ( $x$ ) and the result ( $y$ ) is evaluated using the multiple correlation coefficient ( $R$ ) and the coefficient of determination ( $R^2$ ) according to the Chaddock scale (Table 1).

**Table 1.** Chaddock scale

| The magnitude of the absolute value of the correlation coefficient | Characteristics of the linear relationship between random variables |
|--|---|
| up to 0.3  | Almost absent   |
| 0.31-0.5   | Weak  |
| 0.51-0.7   | Noticeable  |
| 0.71-0.9   | Strong  |
| 0.91-0.99  | Very strong   |

**Source:** (Kushnir & Zavalniuk, 2018)

Within the framework of this study, the influencing factors are as follows: quantitative component ( $x_1$ ), qualitative component ( $x_2$ ); business environment ( $x_3$ ), and the

resulting indicator ( $y$ ) is Change ranking. According to the given methodology, a regression-correlation analysis is performed for each cluster, which allows determining

the influence of factors for different types of start-up ecosystems. In addition, within each cluster of start-up ecosystems of the countries included in the rating, a model is built, and its reliability is verified. To generalize the results, a tabular method is also used, which allows visually presenting the changes in the constituent factors and the performance indicator; generalize the model and indicators of its significance and reliability.

## RESULTS AND DISCUSSION

To find the influence of each component (quantitative, qualitative, and business environment) on the overall result and the rating and in the cluster, the dynamics of their change were analysed. The results for clusters are presented in Tables 2-6. Thus, Cluster 1 will be analysed in more detail, which includes only one country that is the absolute rating leader – the USA (Table 2).

**Table 2.** Dynamics of changes in start-up ecosystems in Cluster 1 countries

| Country       | Rating change | Quantitative component | Qualitative component | Business environment | Overall summary |
|---------------|---------------|------------------------|-----------------------|----------------------|-----------------|
| United States | 0             | -0.33                  | 1.29                  | 0.3                  | 1,253           |

**Source:** compiled by the authors

Analysis of Cluster 1 proved that the United States still dominates the global start-up ecosystem between 2020 and 2021. In 2021, the USA maintained a significant gap between itself and the rest of the world. Therewith, the gap in the overall score slightly decreased between the United States and lower-ranked countries. If one analyses each component in more detail, it is clearly visible in Table 2 that the quantitative component characterizes a downward trend by -0.33, while other components show growth. Thus, the absolute growth rate in 2021 was 1.253.

It is also important to note that according to the Global Start-up Ecosystem Index 2021 (Start-up Blink, 2021), 12 US cities are in the top 30 cities of this rating, and 267 US cities are in the top 1000 (in 2021, 28 new cities appeared in the global ranking). The world leader in innovation is still San Francisco (first

place in the rating, 328,996 points), which is home to the world's largest innovation centre, Silicon Valley. US dominance is observed in several industries: e-commerce and retail technology, marketing and sales technology, healthcare, and social and leisure. The global open Internet, the dominance of the English language in the world, opportunities for financing and support from both private and public entities, and immediate access to global markets allow the United States to stay a leading country, according to the Enterprise Development Fund (2022).

Therefore, the analysis of Cluster 1 suggests that the USA is still the land of opportunity, which most vividly represents the world's innovative and breakthrough technological opportunities. Next, the study analysed the ecosystems of the countries included in Cluster 2, which is presented in Table 3.

**Table 3.** Dynamics of changes in start-up ecosystems in Cluster 2 countries

| Country         | Rating change | Quantitative component | Qualitative component | Business environment | Overall summary |
|-----------------|---------------|------------------------|-----------------------|----------------------|-----------------|
| United Kingdom  | 0             | 0.99                   | 2.95                  | 0.37                 | 4.313           |
| Israel          | 0             | 1.56                   | 6.81                  | 0                    | 8.333           |
| Canada          | 0             | 0.25                   | 1.8                   | 0.11                 | 2.156           |
| Germany         | 0             | 0.62                   | 2.57                  | 0.09                 | 3.283           |
| Australia       | -2            | -0.16                  | 2                     | 0.01                 | 1.855           |
| Sweden          | 4             | -0.41                  | 4.41                  | 0.1                  | 4.1             |
| China           | 7             | 0.8                    | 5.13                  | 0.23                 | 6.156           |
| Switzerland     | 0             | 1.01                   | 2.75                  | -0.14                | 3.62            |
| Singapore       | 6             | 1.5                    | 3.97                  | -0.28                | 5.176           |
| The Netherlands | -5            | 0.69                   | -0.04                 | -0.01                | 0.648           |
| France          | 0             | 0.83                   | 2.62                  | 0.22                 | 3.67            |
| Estonia         | -2            | 0.39                   | 1.46                  | 0.48                 | 2.325           |
| Finland         | -1            | 0.43                   | 1.6                   | 0.24                 | 2.278           |
| Spain           | -6            | 0.54                   | -0.49                 | 0.27                 | 0.325           |
| Lithuania       | -1            | 0.49                   | 0.38                  | 0.52                 | 1.376           |
| Russia          | 0             | 0.23                   | 0.8                   | 0.27                 | 1.288           |
| Ireland         | 0             | 0.32                   | 2.35                  | 0.33                 | -2.997          |

**Source:** compiled by the authors

Cluster 2 includes 17 leading countries that have moderate both positive and negative changes in rank, including no change in rank. Cluster 2 analysis showed that in 2021, it was Spain that underwent substantial negative changes, which, after leaving the top 10, took the 15<sup>th</sup> place in the world, i.e., it has a drop in rank by 6 positions. Such negative changes occurred due to a drop in the qualitative component (-0.49), while other components have a slight increase. The Netherlands also has a negative trend, with its rating falling by 5 positions, which was negatively affected by the quality component (-0.04) and the business environment (-0.01). Therewith, the quantitative component and overall result have improved.

A positive trend of +7 positions is occupied by the start-up ecosystem of China, where all components have considerable improvements. Thus, the overall result increased by as much as 6.156. Such a sharp jump speaks of China's transition from a low-tech developing country to an advanced technological powerhouse that should inspire any other country with similar aspirations.

Singapore's ecosystem is also showing a positive trend. This country has risen 6 steps from 16<sup>th</sup> place in the world in 2020 to 10<sup>th</sup> in 2021. Such a considerable increase in rank demonstrates how fast Singapore's start-up ecosystem is developing. Its start-up ecosystem has a high quantitative and qualitative component, but the business environment has a rather low value and is characterized by a drop (-0.28) compared to most other countries in the top ten (excluding China).

Most of the start-up ecosystems of the world's countries (Great Britain, Israel, Canada, Germany, Switzerland, France, Ireland) stayed in the same place as they were in 2020, but their components have some negative and positive trends. Thus, for instance, Ireland has consistently ranked 18<sup>th</sup>, the components of its start-up ecosystem are improving, but, with such positive changes, its overall result has a drop of (-2.997). The results of the dynamic analysis of indicators that characterize the country's place in the ranking and the components of its start-up ecosystem according to cluster 3 are presented in Table 4.

**Table 4.** Dynamics of changes in start-up ecosystems in Cluster 3 countries

| Country      | Rating change | Quantitative component | Qualitative component | Business environment | Overall summary |
|--------------|---------------|------------------------|-----------------------|----------------------|-----------------|
| South Korea  | 0             | 0.33                   | 1.87                  | 0.12                 | 2.325           |
| India        | 3             | 0.25                   | 2.24                  | 0.66                 | 3.135           |
| Japan        | 0             | 0.66                   | 2.04                  | -0.19                | 2.508           |
| Denmark      | 0             | 0.69                   | 1.76                  | -0.14                | 2.299           |
| Belgium      | 1             | 0.82                   | 0.95                  | -0.04                | 1.727           |
| Brazil       | -4            | -0.03                  | 0.97                  | 0.04                 | 0.968           |
| Taiwan       | 4             | 0.59                   | 0.58                  | 0.73                 | 1.902           |
| Portugal     | 4             | -0.07                  | 1.93                  | 0.04                 | 1.898           |
| Austria      | 0             | 0.47                   | 1.08                  | 0.31                 | 1.856           |
| Italy        | -4            | 0.17                   | 0.67                  | 0.21                 | 1.045           |
| Poland       | -3            | 0.21                   | 0.81                  | 0.34                 | 1.346           |
| Norway       | 2             | 0.27                   | 1.35                  | 0.09                 | 1.719           |
| Bulgaria     | -3            | -0.11                  | 0.05                  | 0.37                 | 0.31            |
| Chile        | -2            | -0.27                  | 0.48                  | 0.3                  | 0.503           |
| Croatia      | 2             | -0.16                  | 0.86                  | 0.29                 | 0.984           |
| Mexico       | 3             | 0.1                    | 0.58                  | 0.34                 | 1.018           |
| Argentina    | -1            | 0.14                   | 0.23                  | 0.19                 | 0.558           |
| Romania      | 4             | -0.16                  | 0.7                   | 0.18                 | 0.723           |
| Luxembourg   | -3            | 0.27                   | 0.23                  | -0.41                | 0.09            |
| Turkey       | 5             | -0.04                  | 0.5                   | 0.1                  | 0.557           |
| Colombia     | -1            | -0.03                  | 0.39                  | -0.07                | 0.284           |
| South Africa | 4             | 0.16                   | 0.52                  | -0.04                | 0.622           |
| Thailand     | 0             | 0                      | 0.15                  | -0.01                | 0.133           |
| Philippines  | 1             | 0.1                    | 0.09                  | -0.12                | 0.063           |
| Iceland      | 3             | 0.2                    | 0.27                  | -0.01                | 0.47            |
| Cyprus       | -2            | -0.03                  | 0.13                  | -0.63                | -0.543          |

Table 4, Continued

| Country         | Rating change | Quantitative component | Qualitative component | Business environment | Overall summary |
|-----------------|---------------|------------------------|-----------------------|----------------------|-----------------|
| North Macedonia | 2             | -0.08                  | 0.52                  | 0.11                 | 0.553           |
| Vietnam         | 0             | 0.09                   | 0.15                  | -0.06                | 0.177           |
| Malta           | 1             | 0.28                   | 0.09                  | 0.05                 | 0.411           |
| Kenya           | 1             | -0.02                  | 0.33                  | 0.08                 | 0.384           |
| Nigeria         | 5             | 0.03                   | 0.48                  | 0.17                 | 0.674           |
| Jordan          | 3             | 0.02                   | 0.21                  | 0.02                 | 0.258           |
| Liechtenstein   | 1             | 0.13                   | 0.06                  | -0.02                | 0.165           |
| Lebanon         | 0             | 0.09                   | 0.02                  | -0.11                | 0.006           |
| Jamaica         | -3            | 0                      | 0.06                  | -0.09                | -0.029          |
| Georgia         | -1            | 0.01                   | 0.05                  | -0.06                | 0.001           |
| Ghana           | 4             | 0.02                   | 0.09                  | 0.05                 | 0.166           |
| Panama          | 4             | 0.04                   | 0.05                  | 0.05                 | 0.138           |
| Qatar           | 0             | 0.04                   | 0.05                  | 0.03                 | 0.119           |
| Cape Verde      | 4             | 0.1                    | 0.03                  | 0                    | 0.136           |
| Mongolia        | 5             | 0.06                   | 0.03                  | 0.04                 | 0.143           |
| Kuwait          | 2             | 0                      | 0.06                  | 0.02                 | 0.08            |
| Bangladesh      | 5             | 0.04                   | 0.06                  | 0.04                 | 0.132           |
| Somalia         | 1             | 0.05                   | 0.03                  | -0.03                | 0.059           |
| Nepal           | 2             | 0.03                   | 0.02                  | 0.02                 | 0.071           |

Source: compiled by the authors

The ecosystems of Cluster 3 countries are almost entirely characterized by an increase in absolute value, except for Cyprus (-0.543) and Jamaica (-0.029); as well as positive shifts in the quality component. Downward changes occur in the quantitative component and in the

business environment. The countries that had an increase in all components and, accordingly, an increase in the total, also had a negative change in the rating (Argentina, Italy, Poland). An analysis of the dynamics of changes in start-up ecosystems in Cluster 4 countries is presented in Table 5.

Table 5. Dynamics of changes in start-up ecosystems in Cluster 4 countries

| Country              | Rating change | Quantitative component | Qualitative component | Business environment | Overall summary |
|----------------------|---------------|------------------------|-----------------------|----------------------|-----------------|
| United Arab Emirates | -18           | 0.38                   | 2.24                  | 0.72                 | 3.338           |
| New Zealand          | -14           | 0.46                   | 0.96                  | 1.21                 | 2.622           |
| Malaysia             | -8            | 0.31                   | 0.59                  | 0.31                 | 1.216           |
| Indonesia            | -9            | 0.1                    | 0.67                  | 0.4                  | 1.172           |
| Uruguay              | -15           | 0.07                   | 1.08                  | 0.99                 | 2.141           |
| Bahrain              | -9            | 0.14                   | 0.09                  | 0.22                 | 0.458           |
| Egypt                | -11           | 0.1                    | 0.22                  | 0.21                 | 0.535           |
| Saudi Arabia         | -17           | 0.18                   | 0.15                  | 0.33                 | 0.655           |
| Pakistan             | -7            | 0.01                   | 0.07                  | 0.05                 | 0.136           |
| Kazakhstan           | -10           | 0.08                   | 0.06                  | 0.1                  | 0.235           |
| Sri Lanka            | -7            | 0.07                   | 0.02                  | 0.06                 | 0.141           |

Source: compiled by the authors

The growth of the start-up ecosystems of the countries of the Cluster 4 in 2021 occurred as a result of

the improvement of quantitative, qualitative indicators, and business environment indicators. Considering each

of the countries where rapid growth took place separately, today the UAE ranks 2<sup>nd</sup> in the level of innovation development in the Middle East, and Dubai entered the world's top 50 in terms of innovation software and data and a high concentration of technological innovation. There was also a significant increase in Abu Dhabi, which rose 146 positions in the ranking of 1000 cities of the World Ecosystem Index and ranked 169<sup>th</sup>. Among the

UAE's successful start-ups is the Middle East's first unicorn, Careem, which was sold to Uber for nearly 3 billion USD. Another positive element is the constant reform of business by the government, and the adoption of laws that favour entrepreneurs and the banking system, which ensures the development of innovations (Dymchenko et al., 2022). An analysis of the dynamics of changes in start-up ecosystems in Cluster 5 countries is presented in Table 6.

**Table 6.** Dynamics of changes in start-up ecosystems in Cluster 5 countries

| Country                | Rating change | Quantitative component | Qualitative component | Business environment | Overall summary |
|------------------------|---------------|------------------------|-----------------------|----------------------|-----------------|
| Czech Republic         | -6            | 0.08                   | 0.54                  | 0.14                 | 0.806           |
| Ukraine.               | -5            | <b>0.17</b>            | <b>-0.09</b>          | <b>0.46</b>          | <b>0.648</b>    |
| Latvia                 | -6            | 0                      | 0.17                  | -0.23                | -0.063          |
| Slovenia               | -11           | -0.12                  | 0.09                  | -0.76                | -0.797          |
| Hungary                | -12           | 0.06                   | 0.03                  | -0.76                | -0.659          |
| Serbia                 | -11           | -0.2                   | 0.1                   | -0.83                | -0.934          |
| Greece                 | -10           | -0.18                  | 0.09                  | -0.94                | -1.023          |
| Slovakia               | -5            | -0.05                  | 0.08                  | -0.9                 | -0.876          |
| Peru                   | -6            | 0.09                   | -0.04                 | -0.47                | -0.409          |
| Armenia                | -8            | -0.15                  | 0.07                  | -0.67                | -0.751          |
| Belarus                | -4            | -0.02                  | 0.03                  | -0.11                | -0.102          |
| Rwanda                 | -4            | 0.01                   | 0.1                   | -0.08                | 0.046           |
| Moldova                | -13           | -0.09                  | 0.04                  | -0.4                 | -0.444          |
| Albania                | -6            | -0.01                  | 0.05                  | -0.1                 | -0.059          |
| Tunisia                | -5            | 0.02                   | 0.04                  | -0.1                 | -0.043          |
| Bosnia and Herzegovina | -12           | -0.04                  | 0.04                  | -0.15                | -0.149          |
| Ecuador                | -15           | -0.03                  | 0.01                  | -0.16                | -0.183          |
| Azerbaijan             | <b>-19</b>    | <b>-0.05</b>           | <b>0.02</b>           | <b>-0.2</b>          | <b>-0.23</b>    |
| Paraguay               | -11           | -0.03                  | 0.04                  | -0.1                 | -0.107          |
| Morocco                | -12           | 0                      | 0.03                  | -0.05                | -0.036          |
| Dominican Republic     | -18           | -0.09                  | 0.02                  | -0.15                | -0.211          |
| Uganda                 | -8            | 0                      | 0.02                  | -0.05                | -0.027          |

**Source:** compiled by the authors

The analysis of Cluster 5 countries shows some ambiguity, despite the only general trend – a considerable drop in the overall ranking of countries (from -5 to -19). Foremost, the Czech Republic should be singled out, which, having a drop of 6 positions in the overall rating, shows an increase in the absolute indicator by 0.806, which is due to the growth of all three components. A similar situation is observed in the start-up ecosystems of two countries of the cluster – Ukraine and Rwanda, which have an absolute growth rate that is due to the growth of two of the three components. Ukraine has an absolute growth of 0.648 due to the growth of the qualitative component (+0.17) and the improvement of the business environment (+0.46) and a slight reduction in the qualitative component (-0.09). All countries of the

cluster, except the Czech Republic and Ukraine, show a deterioration in the business environment. Only 5 start-up ecosystems of the cluster countries have an increase in the quantitative component (Czech Republic, Ukraine, Peru, Rwanda, Tunisia) and 3 countries do not have changes in this component (Latvia, Morocco, Uganda). Only two start-up ecosystems of this cluster's countries have a decrease in the quality component – Ukraine and Peru. All other countries show growth according to this indicator.

To assess the impact of changes in each component of the country's start-up ecosystem ( $x_1$  – quantitative component;  $x_2$  – qualitative component;  $x_3$  – business environment) on the change in rating for each cluster, a regression analysis was conducted, the results of which are summarized in Table 7.

**Table 7.** The results of the regression analysis for the start-up ecosystems of the countries of Clusters 2-5

| Cluster | Model  | R        | R <sup>2</sup> | F        |
|---------|--|----------|----------------|----------|
| 2       | $y = 1.73 - 1.41x_1 - 0.34x_2 + 4.67x_3$     | 0.76275  | 0.58179        | 20.86752 |
| 3       | $y = 40.58 - 9.83x_1 - 14.83x_2 + 10.16x_3$  | 0.36834  | 0.13567        | 6.74993  |
| 4       | $y = - 8.41 - 0.14x_1 - 2.31x_2 - 3.89x_3$   | 0.717234 | 0.51442        | 2.47192  |
| 5       | $y = - 10.04 + 28.43x_1 + 7.89x_2 - 3.04x_3$ | 0.48585  | 0.23605        | 1.85394  |

**Source:** compiled by the authors

Table 7 shows that start-up ecosystems of cluster countries have different values of  $R$  and  $R^2$ , which demonstrate a rather low correlation ( $R^2 < 0.4$  is characterized as low). The coefficients near  $x$  show different strength and direction of influence on the change rating.

Cluster 2 is the most closely correlated ( $R^2 = 0.58179$ ), while  $x_1$  and  $x_2$  are inversely correlated and have a weak effect on  $y$ . In Cluster 4,  $R^2 = 0.5144$ , and all coefficients have an inverse effect on the change in rating ( $y$ ). Thus, it is recommended for the countries of Cluster 2 to develop the quantitative and qualitative components that, albeit insignificantly (indicators near  $x$  have low values), but adversely affect changes in the rating, and to maintain the development of a business environment that has the maximum impact on the change in positions in the rating. For the development of the start-up ecosystems of Cluster 4 countries, it is necessary to develop all components more intensively to ensure the growth of positions in the rating.

Thus, the mathematical models built based on regression-correlation analysis, which describe the influence of the country's start-up ecosystem components on the change in the ranking positions in the section of four clusters demonstrate a strong connection between the factors and the result according to the Chadcock scale (Cluster 2 and 4 with values  $R = 0.76275$  and  $R = 0.717234$ , respectively); weak connection for start-up ecosystems of Cluster 3 and 5 countries. The latter indicates the dependence of the development of start-up ecosystems on other factors that are not considered in the description of the start-up ecosystem and which affect the development of start-ups and entrepreneurship in the countries of Clusters 3 and 5.

First of all, the proposed clustering of start-up ecosystems of countries has nothing to do with the formation of clusters as a combination of several homogeneous elements that can be considered as a separate unit with certain properties. Groups of countries formed based on clustering have similar characteristics of start-up ecosystems, which can be considered for adopting best practices, forming strategic development documents by the governments of countries to improve individual start-up ecosystems.

Proceeding from the research results of P.T. Roundy and L. Burke-Smalley (2021), the development of entrepreneurial ecosystems should be based on a holistic approach from political institutions (governments of countries) and include: subjects of entrepreneurial

activity in the ecosystem; resource providers in the ecosystem; business connections in the ecosystem and the business environment of the ecosystem; development of indicators to find the strengths and weaknesses of individual ecosystems and approaches to measurement.

R. Brown and K. Mason (2017), emphasizing the uniqueness of ecosystems, generally speak about the lack of a standard development strategy. At the same time, the study indicates that there are signs that start-up ecosystems in different countries can be grouped together and have common development vectors. Thus, it can be concluded that the start-up ecosystem of each country is unique, but has common trends that hinder or, on the contrary, help it grow in the current rankings, which allows developing recommendations for improving the ecosystem for a cluster of countries.

The results of this study can be considered to a certain extent as a continuation and substantiation of the prerequisites for the development of entrepreneurial ecosystems (Roundy P.T. and Burke-Smalley, L., 2021) in terms of the intervention of the governments of countries in the formation of a favourable environment, the creation of prerequisites for rapid growth and the provision of general vectors of development individual firms and start-ups. The relevance of this issue is reflected in the set of programs and legal acts aimed at promoting the development of entrepreneurship at various levels – from international to regional. At the international level, according to the European Commission (2022), numerous projects are presented that help start a business in the EU: Start-up Europe (2022), Start-up Europe Partnership (2022), InvestEU Portal (2022). This also includes information on obtaining financing, establishing a European company, taxes on companies in the EU, digitalization of business and sales in the EU.

The work of the Entrepreneurship Development Fund (2022), which helps businesses in cooperation with banks in obtaining financing, was intensified, and the Ukrainian Start-up Fund (2022) was launched. The latest initiative is preconditioned by the fact that start-ups, as a special form of starting one's individual business based on innovative solutions, directly play a substantial role in the development of entrepreneurship. This allows discussing the start-up ecosystem. It is the aforementioned that predetermines the need to investigate the issue of modelling the start-up ecosystem as a prerequisite for increasing entrepreneurial activity.

## CONCLUSIONS

The start-up ecosystem is understood as an interacting and interdependent set of institutions whose activities create an environment for qualitative and quantitative growth of start-ups as subjects of innovative entrepreneurship development. The components of the start-up ecosystem include the environment, qualitative and quantitative characteristics that correlate with the vision of the components in the Global Start-up Ecosystem Index from Start-up Blink.

The use of statistical analysis methods for the data of the countries, which were grouped into 5 clusters, allowed determining the absolute changes in the values of the Global Start-up Ecosystem Index rating indicators from Start-up Blink: rating change, quantitative component, qualitative component, business environment, general summary. In Cluster 1, the United States is a single country, and the position of its ecosystem is characterized by leadership and a considerable gap from others. The start-up ecosystem of almost all countries in Cluster 2 demonstrate positive dynamic shifts in all components, which lead to the growth of the overall result. Ecosystems of Cluster 3 countries are almost completely described by the growth of the overall total,

which is entirely conditioned upon the growth of the qualitative component with various dynamic changes in other components of the country's ecosystem, which leads to positive shifts in the rating. The ecosystems of Cluster 4 countries are described by the growth of all components of the ecosystem, but insufficient to change their positions in the rating. Ecosystems of Cluster 5 countries have a drop in the rating with an increase in the qualitative component and a reduction in other components.

Mathematical models built based on regression-correlation analysis, which describe the influence of the components of the country's ecosystem on the change in the ranking positions in four clusters demonstrate: a strong connection between factors and the result (Clusters 2, 4); weak connection for countries in Cluster 3, 5. The latter testifies to the dependence of the development of start-up ecosystems on other factors that are not considered in their description and which affect the development of start-ups and entrepreneurship in countries of Cluster 3, 5. These results are the basis for developing recommendations, start-ups and plans for the development of start-up ecosystems at the national and regional levels as prerequisites for the activation of entrepreneurial activity.

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## Моделювання впливу складових стартап екосистем: підприємницький аспект

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**Анотація.** Визначення засад розвитку стартап екосистем країни як основи активізації підприємництва є нагальним завданням відбудови економіки країни. Мета роботи полягає у визначенні шляхів покращення стартап екосистем країни на основі побудови економіко-математичних моделей для активізації підприємницької діяльності. В основу методології дослідження покладено статистичні методи дослідження, а саме: аналіз динаміки; для оцінки сили впливу кожної компоненти стартап екосистем на зміну позиції в рейтингу було обрано регресійно-кореляційний аналіз, який дозволяє виявити силу впливу факторів на кінцевий показник. Досліджено складові екосистем стартапів, під якою розуміється взаємодіюча та взаємозалежна сукупність інституцій, діяльність яких створює середовище для якісного та кількісного зростання стартапів як суб'єктів інноваційного розвитку підприємництва; використання методів статистичного аналізу до даних країн, які було згруповано в 5 кластерів, дозволило визначити абсолютні зміни величин показників рейтингу Global Startup Ecosystem Index від StartupBlink: зміна рейтингу, кількісна складова, якісна складова, бізнес середовище, загальний підсумок; побудовано на основі регресійно-кореляційного аналізу економіко-математичні моделі, які описують вплив складових екосистем країни на зміну позицій рейтингу в розрізі чотирьох кластерів демонструють: сильний зв'язок між факторами та результатом (кластери 2, 4); слабкий зв'язок для країн кластеру 3,5, останнє свідчить про залежність розвитку стартап екосистем від інших факторів, які не враховані в їх описі та які впливають на розвиток стартапів та підприємництва в країнах кластерів 3, 5. Практична цінність роботи полягає в тому, що результати роботи є базисом для формування стратегій розвитку стартап екосистем на національному та регіональному рівні органами місцевої та державної влади

**Ключові слова:** стартап, екосистема країни, економіко-математична модель, підприємництво, рейтинг

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