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# НАУКОВІ ГОРИЗОНТИ

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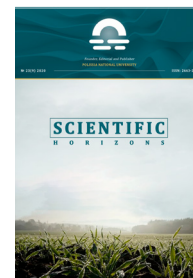
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## Effect of Fertiliser on Changes in Labile and Water-Soluble Forms of Humus in Short-Term Rotations

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**Abstract.** Mobile (labile and water-soluble) forms of humus are one of the basic components of effective soil fertility and a precondition for high productivity of crop rotations. As a result of fermentation, these forms of humus are mineralised and take part in plant nutrition, and some of them, being included in mobilisation processes, transition into stable humus substances. Therefore, it is important to investigate agrotechnological factors for managing their dynamics and redistribution in the soil environment during the growing season of agricultural crops. The purpose of the study: to investigate the effect of complex application of mineral and organic (conventional and alternative) fertilisers on the change of water-soluble and labile forms of humus during the growing season of agricultural crops grown in short-term rotations. The following research methods were used in this study: field, laboratory-analytical, computational-comparative, mathematical-statistical. Higher level of labile accumulation ( $359.59 \text{ mg kg}^{-1}$  of soil) and water-soluble ( $11.69 \text{ mg kg}^{-1}$  of soil) humus forms under winter wheat crops occur when the predecessor of the crop in the crop rotation is meadow clover. The application of  $N_{60}P_{90}K_{90}$  specifically for winter wheat and 40 t/ha of manure in the conventional fertilisation system of grain-grass crop rotation contributes to the formation of  $529.07$  and  $20.20 \text{ mg kg}^{-1}$  of soil of the organic substances under study. The application of  $N_{120}P_{100}K_{100}$  and 40 t/ha of manure for corn for grain yields  $567.42$  and  $22.55 \text{ mg kg}^{-1}$  of soil, and  $N_{90}P_{90}K_{90}$  and 40 t/ha of manure for potatoes yields  $543.66$  and  $21.75 \text{ mg kg}^{-1}$  of mobile compounds humus. The obtained research results can serve as a basis for the development of highly efficient environmentally friendly farming systems and can be used for further scientific research on the development of ways and directions for managing humus-forming processes in the soil environment

**Keywords:** humus substances, organic-mineral systems, secondary products, green manure, multicultural complexes



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## INTRODUCTION

Ensuring food security is a defining priority of the state, which is based on the conditions of stabilisation and rationalisation of the use of land potential, improving the efficiency of the agricultural sector of the economy (Kaminsky & Saiko, 2013; Tanchik, 2009; Oliver & Gregory, 2015).

Achieving an elevated level of agricultural production productivity by introducing agricultural technologies of diverse levels of intensity in modern conditions should be synchronised with strict compliance with the conditions of environment-stabilising functioning of soil systems and regimes, ensuring the possibility of preserving and reproducing soil fertility (Carberry *et al.*, 2013; Gura & Mnkeni, 2019; Neugschwandtner *et al.*, 2014).

A functional indicator for assessing the safety of agricultural systems is indicators of the humus state of the soil cover. The humus complex, being an integrated indicator of soil fertility, represents its energy potential, nutritional capabilities, directly affects agro-physical, agrochemical, and physico-chemical properties, microbiological activity, and erosion resistance (Wozniak, 2019; Raupp, 2001; Rumpel & Kögel-Knabner, 2011). The amount of humus accumulation in the soil depends on the ratio between the mineralisation and humification of organic substances. The primary products of humus-forming processes are mobile (unstable) organic compounds (water-soluble and labile humus), which are chemically unstable and under certain conditions, namely with the activation of enzyme systems and increased oxidative processes, can be mineralised to both intermediate and final decay products, replenish soil reserves with available forms of plant nutrition elements and use them for growth, development, and bio-production. Some mobile organic compounds are involved in further synthesis processes to form stable humus (Haynes, 2005). The amount of water-soluble and labile humus substances varies during the growing season of agricultural crops (Kopecký *et al.*, 2022; Bongiorno *et al.*, 2019).

Fertiliser and crop rotation are essential agrotechnological factors influencing the formation of mobile humus forms (Tian *et al.*, 2017; Kopecký *et al.*, 2021).

Scientifically based crop rotations, which are multicultural complexes of plants, are arranged in time and spatial dimensions in such a way that allows the most effective use of their biological potential of productivity and contribute to the optimisation of humus-forming processes, ensure a deficit-free balance of humus and nutrients and prevent the phenomena of soil fatigue and degradation (Kachmar *et al.*, 2019; Van Eerd *et al.*, 2014). Scientific studies have established that with root and post-harvest residues of agricultural crops grown in rational crop rotations, more organic matter enters the soil environment than with organic fertilisers (N'dayegamiye *et al.*, 2017). Partial accumulation of organic matter in the soil occurs even during the period of active plant development in spring and summer due to root system regeneration, root secretions, and microbiological activity (King & Bless, 2017; Campbell *et al.*, 2000; McDaniel *et al.*, 2014).

The integrated use of organic and mineral fertilisers constitutes a prerequisite for maintaining crop rotation productivity, preserving and reproducing soil fertility. Organic-mineral fertiliser systems ensure the supply of organic substances to the soil system from the outside and contribute to increasing the yield of cultivated crops, the secondary products of which can serve as an added source of organic replenishment. However, in modern conditions, the livestock industry does not ensure sufficient amounts of conventional organic fertilisers, and therefore it is crucial to use alternative sources to replenish organic substances in the soil – crop secondary products, green mass of green manure crops (oilseed radish, white mustard, Perko, yellow lupine, winter rapeseed) (Degodyuk *et al.*, 2012; Triberti *et al.*, 2016; Bronick & Lal, 2005).

Studies conducted at the Institute of Agriculture of the Carpathian Region of the National Academy of Agrarian Sciences of Ukraine have established that the complex use of green fertilisers and straw on a low background of mineral fertilisers is equivalent to manure both in terms of impact on crop rotation productivity and soil fertility (Kachma *et al.*, 2020). Thus, the scientific justification of the areas of activating humus-forming processes by rationalising organic-mineral fertiliser systems in scientifically based crop rotations is critical for environmentally safe farming and determines the agronomic strategy for increasing soil productivity and managing their fertility.

*The purpose of the study:* to investigate the dynamics of mobile forms of humus of grey forest soil under the conditions of experimental modelling of the flow directions of soil-forming processes under various levels of anthropogenic loads in crop rotations. To achieve this purpose, the following tasks were set: 1) to investigate the influence of precursors and fertiliser systems on changes in mobile humus compounds during the growing season of winter wheat in variety crop rotations for different predecessors; 2) to assess the possibility of accumulation of unstable forms of humus under corn in grain crop rotation for conventional and alternative fertiliser systems; 3) to investigate the redistribution of water-soluble and labile humus for the main phases of potato development in vegetable rotation at various levels of anthropogenic loads.

## MATERIALS AND METHODS

The study was conducted during 2018-2020 in the experimental training ground of the Institute of Agriculture of the Carpathian Region, which is located in the village of Stavchane of the Lviv District of the Lviv Oblast. The study was carried out under the conditions of a two-factor stationary experiment, which has the long-term status and is entered in the Register of Stationary Experiments of Ukraine (certificate number – 053). The experiment was laid in 2001 on grey forest surface-ogled soil. The number of factors studied is 2 (First-order plots – short-term rotation systems, second – fertiliser systems).

The experiment investigates 9 field variable crop rotations (3-4-5-field) with saturation of grain crops from 50% to 100%, on the use of conventional (combination of mineral fertilisers and manure) and alternative (layout of mineral fertilisers, straw, crop green manure) organic-mineral fertiliser systems and without fertiliser application (control). Experimental data covered in this paper are obtained from six crop rotations of the experiment: 1) peas – winter wheat – winter wheat – oats (four-field grain rotation); 2) peas – winter wheat – corn (grain) – oats (four-field grain rotation); 3) meadow clover – winter wheat – winter wheat – spring barley + meadow clover (four-field grain-grass rotation); 4) meadow clover – winter wheat – potatoes – spring barley + meadow clover (four-field vegetable rotation); 5) buckwheat – winter wheat – potatoes – spring barley (four-field grain-row crop rotation); 6) corn (green mass) – winter wheat – buckwheat – soy – winter wheat (five-field grain-row crop rotation). In the conventional fertiliser system, mineral fertilisers were applied against the background of manure (40 tonnes once per rotation for row crops, and in 1<sup>st</sup> and 3<sup>rd</sup> crop rotations – for winter wheat in repeated crops) in the following dosage: winter wheat –  $N_{60}R_{90}K_{90}$ , spring barley –  $N_{60}R_{60}K_{60}$ , oats –  $N_{40}R_{40}K_{40}$ , buckwheat –  $N_{60}R_{60}K_{60}$ , peas –  $N_{45}R_{45}K_{45}$ , soybeans –  $N_{45}R_{45}K_{45}$ , potatoes –  $N_{90}R_{90}K_{90}$ , corn –  $N_{120}R_{100}K_{100}$ . In an alternative system, at half doses of mineral fertilisers against the background of ploughing all secondary products of cultivated crops, oilseed radish was sown once per rotation on green manure in post-harvest crops (for the same crops where manure was applied in the conventional system, while full doses of mineral nutrition were applied). The repetition of the options is threefold, the arrangement is consistent. The total area of the plot by crop rotation factor was 864 m<sup>2</sup> (72 mx12 m), by fertiliser: total – 96 m<sup>2</sup> (12 mx8 m), accounting – 60 m<sup>2</sup> (10 mx6 m). The crops were entered into the rotation simultaneously in all fields.

The influence of fertiliser systems on the change of labile and water-soluble forms of humus were investigated under winter wheat of the Poliska 90 variety, corn on grain of the Zakarpatska Zubovydna variety, and potatoes of the Oksamyt variety.

The soil of the experimental plots is grey forest surface-gleyed coarsely dusty light loamy with the following agrochemical properties (before laying the experiment): humus content 1.67-1.71%, the sum of the absorbed bases 4.4-5.0 mg-eq kg<sup>-1</sup> soil, alkaline hydrolysis nitrogen 9.2-9.9, mobile phosphorus and exchangeable potassium 10.8-11.13 and 9.3-9.5 mg kg<sup>-1</sup> of soil, respectively. The reaction of the soil pH<sub>KCl</sub> solution is 4.70-4.84, hydrolytic acidity is 2.26 mg-eq kg<sup>-1</sup> of soil.

Soil samples of experimental variants and their preparation for laboratory and analytical work were taken according to DSTU 4287:2004 (2005) and DSTU ISO 11464-2001 (2002). Labile humus was determined in the obtained soil samples according to DSTU 4732:2007 (2007b), water-soluble humus – according to DSTU 4731:2007 (2007a).

The values of these indicators were determined from the arable layer (0-30 cm) for three years (2018-2020) in 3 repetitions and in 2 analytical parallels (in general,  $n=18$ ).

Statistical analysis of results, namely the analysis of variance ANOVA (under conditions of significance level  $\alpha=0.05$ ) was performed using Excel 11.0.6560.0.

The study employed general scientific and special research methods. Using a field experiment, data on the variability of labile and water-soluble forms of humus under the influence of agrotechnological factors were obtained; laboratory and analytical methods provided quantitative characteristics of the redistribution of mobile humus substances in the soil environment; computational and comparative methods justified the magnitude of changes in the indicators under study; mathematical and statistical reliability of experimental material and mathematical calculations was evaluated.

## RESULTS AND DISCUSSION

The study of the redistribution of mobile organic substances in the soil under winter wheat was carried out in five short-term rotations with different crop precursors and conventional and alternative fertiliser systems.

It was found that the accumulation of labile forms of humus was considerably influenced by precursors. Parallel studies of the authors conducted under a spring grain crop – barley, also confirmed differences in the accumulation of unstable humus compounds in the soil after winter wheat and potatoes (Kachmar *et al.*, 2020). The influence of precursors (corn, barley, oats) on potato yield has been proven by Canadian scientists (N'Dayegamiye *et al.*, 2017). Similar patterns have been observed by other authors (Gura & Mnkeni, 2019; Van Eerd *et al.*, 2014; McDaniel *et al.*, 2014).

Analysis of soil samples on variants without fertiliser (control) showed an elevated level of values of labile organic compounds after meadow clover in vegetable rotation and after peas in grain rotation. At the time of sprouting of winter wheat in terms of crop rotations, they were 359.59 mg kg<sup>-1</sup> and 341.88 mg kg<sup>-1</sup> of soil. Its predecessors – winter wheat (grain-grass crop rotation) and buckwheat (grain-row crop rotation) – provided the formation of 330.54 mg kg<sup>-1</sup> and 312.54 mg kg<sup>-1</sup> of soil of labile forms of humus. Their lowest values were formed after corn to grain and amounted to 290.40 mg kg<sup>-1</sup> of soil. This influence of precursors can be explained by their biological characteristics, namely the amount of organic residues left in the soil, the ability to accumulate symbiotic nitrogen in legumes. The duration of the period between harvesting the predecessor and sowing winter wheat is also important. Studies by Kachmar *et al.* (2020) found that the larger it is, the deeper is the magnitude of the mineralisation processes of organic substances that have entered the soil environment and the immobilisation of the formed compounds into labile forms of humus (Table 1).

**Table 1.** Influence of fertiliser systems and precursors in crop rotations on changes in labile humus content during the growing season of winter wheat ( $m \pm m$ ; 2018-2020)

No. and type of crop rotation	Fertilisation of winter wheat	From labile humus, mg kg <sup>-1</sup> of soil		
		Phases of plant development		
		Sprouts	Earing	Complete ripeness
<b>Predecessor – meadow clover</b>				
4-field vegetable	Control	359.59±1.62	318.17±1.65	326.37±2.07
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	478.64±3.18 <sup>a</sup>	435.59±1.74 <sup>a</sup>	449.76±2.15 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub>	439.92±1.38 <sup>a,b</sup>	405.82±2.41 <sup>a,b</sup>	416.04±2.76 <sup>a,b</sup>
<b>Predecessor – peas</b>				
1 grain	Control	341.88±1.70	295.20±3.51	307.00±2.02
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	459.17±1.79 <sup>a</sup>	418.94±1.87 <sup>a</sup>	432.55±2.55 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub> + s.p.	417.88±2.21 <sup>a,b</sup>	377.69±1.59 <sup>a,b</sup>	385.26±2.93 <sup>a,b</sup>
<b>Predecessor – buckwheat</b>				
5 row-crop grain	Control	312.26±1.84	269.96±2.44	276.44±2.99
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	438.32±1.74 <sup>a</sup>	394.66±2.40 <sup>a</sup>	405.96±2.92 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub> + s.p.	393.46±1.40 <sup>a,b</sup>	337.24±2.66 <sup>a,b</sup>	347.11±1.89 <sup>a,b</sup>
<b>Predecessor – winter wheat</b>				
3 grain-grass	Control	330.54±1.84	287.61±1.68	293.59±2.27
	Manure, 40 t/ha + N <sub>60</sub> R <sub>90</sub> K <sub>90</sub>	529.07±2.02 <sup>a</sup>	492.23±1.37 <sup>a</sup>	509.92±2.04 <sup>a</sup>
	Green manure + N <sub>60</sub> R <sub>90</sub> K <sub>90</sub> + s.p.	486.21±1.42 <sup>a,b</sup>	449.90±1.93 <sup>a,b</sup>	465.26±2.37 <sup>a,b</sup>
<b>Predecessor – corn</b>				
6 row-crop grain	Control	290.40±1.40	247.17±2.34	251.32±2.48
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	467.16±1.76 <sup>a</sup>	429.40±1.33 <sup>a</sup>	442.62±1.62 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub>	369.63±1.45 <sup>a,b</sup>	328.49±1.68 <sup>a,b</sup>	337.56±2.25 <sup>a,b</sup>

The level of significance of differences between the average values of indicators of the variants under study according to ANOVA data:  $P < 0.001$

**Note:** s.p. – secondary products; indices a, b – the level of significance of the difference for each control, and the subsequent fertiliser system

The use of organic-mineral fertiliser systems in crop rotations had a considerable impact on the formation of labile forms of humus.

Complex application directly for winter wheat N<sub>60</sub>R<sub>90</sub>K<sub>90</sub> and 40 t/ha of manure in the conventional fertiliser system of grain-grass crop rotation contributed to the formation of 529.07 mg kg<sup>-1</sup> of soil of the organic substances under study. For the introduction of the same level of mineral nutrition of plants under the culture, and the organic component – under the predecessors, higher values of labile humus compounds (478.64 mg kg<sup>-1</sup> of soil) were formed in a vegetable rotation after meadow clover. The data obtained are consistent with studies performed at the National Centre “Institute of Agriculture of the National Academy of Sciences”, according to which the introduction of an organic-mineral fertiliser system in a ten-field crop rotation provided the highest values of labile humus of grey forest soil in winter rye crops (Raupp, 2001). The positive effect of organic-mineral and organic fertilisers on the redistribution of labile humus forms in the soil was noted in previous studies by O. Kachmar *et al.* (2019), as well as a number of other scientists (Raupp, 2001; Bongiorno *et al.*, 2019; Bronick & Lal, 2005).

Joint combination of green mass of oilseed radish grown in post-harvest crops against the background of compressed straw of winter wheat with the introduction of N<sub>60</sub>R<sub>90</sub>K<sub>90</sub> in an alternative fertiliser system, grain-grass crop rotation contributed to the formation of 486.21 mg kg<sup>-1</sup> of soil of labile forms of humus. The combination of half doses of mineral fertilisers and post-harvest products (straw) of peas in the grain crop rotation and buckwheat in the grain-row crop rotation provided the values of the indicators under study at the level of 417.88 and 393.46 mg kg<sup>-1</sup> of soil. The lowest values of labile forms of humus were formed under winter wheat crops in grain-row crop rotation after corn to grain under an alternative fertiliser system upon applying half doses of mineral fertilisers and amounted to 369.63 mg kg<sup>-1</sup> of soil.

Analysis of the dynamics of labile forms of humus during the growing season of winter wheat showed a decrease in their values during crop earing in all crop rotations, both on non-fertilised variants and for both organic-mineral fertiliser systems under study. This is explained by the activation of mineralisation processes due to the intake of sufficient heat into the soil during this period of winter wheat development and the

increased consumption of released nutrients by plants for crop formation. The authors' research found that in fertilised areas the amount of labile humus compounds decreased by 9.0-7.8% in fruit crop rotation, by 8.8-9.6% in grain, by 9.9-14.3% in grain-row rotation with buckwheat crop as the predecessor, by 7.0-7.5% in grain-grass rotation, by 8.1-11.1% in grain-row rotation with corn as the predecessor.

By the end of the winter wheat growing season, an increase in the amount of labile humus was observed in all variants under study. Evidently, this is due to a decrease in the needs of the crop for nutrients, a shift in the chemical equilibria of "synthesis – decomposition" of mobile humus substances towards their immobilisation, and the involvement of organic matter that has entered the soil environment with organic litter in mineralisation processes. In the phase of full ripeness of the crop, the highest amount of labile humus substances was on fertilised versions of grain-grass crop rotation and amounted to 465.26-509.92 mg kg<sup>-1</sup> of soil. The lowest level of accumulation of labile humus on organic-mineral backgrounds was observed in grain-row crop rotations. In the conditions of the conventional system with direct application

of N<sub>60</sub>R<sub>90</sub>K<sub>90</sub> under the culture after buckwheat as the predecessor, their amount was 405.96 mg kg<sup>-1</sup> of soil, according to an alternative system using N<sub>30</sub>R<sub>45</sub>K<sub>45</sub> after corn – 337.56 mg kg<sup>-1</sup> of soil.

Studies of changes in water-soluble forms of humus under winter wheat showed that their number depended on fertiliser systems, the precursor of the crop in rotation and the phase of its vegetation (Table 2). It was found that in the control variants, higher values of this indicator were observed after meadow clover in a vegetable rotation. At the time of sprouting of the crop, they were at the level of 11.69, in the earing phase – at 10.24, at full ripeness – at 10.65 mg kg<sup>-1</sup> of soil. Analysis of the influence of fertiliser systems on humus-forming processes reveals the advantages of direct application of a complex of organic and mineral components for winter wheat. Thus, the combined use of 40 t/ha of manure and N<sub>60</sub>R<sub>90</sub>K<sub>90</sub> in the grain-grass crop rotation, the accumulation of 20.20 mg kg<sup>-1</sup> of soil of water-soluble humus compounds. Complex application of green mass of post-harvest oilseed radish, winter wheat straw and N<sub>60</sub>R<sub>90</sub>K<sub>90</sub> in the same crop rotation, contributed to the formation of their number at the level of 18.40 mg kg<sup>-1</sup> of soil.

**Table 2.** Dynamics of water-soluble humus during the growing season of winter wheat depending on fertiliser systems and precursors (m±m; 2018-2020)

No. and type of crop rotation	Fertilisation of winter wheat	From water-soluble humus, mg kg <sup>-1</sup> of soil		
		Phases of plant development		
		Sprouts	Earing	Complete ripeness
<b>Predecessor – meadow clover</b>				
4 vegetable	Control	11.69±0.20	10.24±0.28	10.65±0.25
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	18.97±0.23 <sup>a</sup>	16.74±0.26 <sup>a</sup>	17.00±0.23 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub>	14.93±0.24 <sup>a,b</sup>	12.90±0.24 <sup>a,b</sup>	13.24±0.23 <sup>a,b</sup>
<b>Predecessor – peas</b>				
1 grain	Control	11.53±0.20	10.23±0.26	10.36±0.28
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	18.47±0.35 <sup>a</sup>	16.33±0.39 <sup>a</sup>	16.63±0.34 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub> +s.p.	14.76±0.26 <sup>a,b</sup>	12.68±0.28 <sup>a,b</sup>	12.88±0.25 <sup>a,b</sup>
<b>Predecessor – buckwheat</b>				
5 row-crop grain	Control	11.20±0.26	9.99±0.22	10.14±0.23
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	18.06±0.28 <sup>a</sup>	15.36±0.37 <sup>a</sup>	15.58±0.39 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub> +s.p.	14.59±0.24 <sup>a,b</sup>	12.49±0.23 <sup>a,b</sup>	12.73±0.34 <sup>a,b</sup>
<b>Predecessor – winter wheat</b>				
3 grain-grass	Control	11.44±0.23	9.76±0.32	9.92±0.35
	Manure, 40 t/ha + N <sub>60</sub> R <sub>90</sub> K <sub>90</sub>	20.20±0.48 <sup>a</sup>	17.83±0.49 <sup>a</sup>	18.23±0.47 <sup>a</sup>
	Green manure + N <sub>60</sub> R <sub>90</sub> K <sub>90</sub> +s.p.	18.40±1.07 <sup>a,b</sup>	15.65±0.84 <sup>a,b</sup>	15.86±0.81 <sup>a,b</sup>
<b>Predecessor – corn on green mass</b>				
6 row-crop grain	Control	11.15±0.24	9.93±0.29	10.09±0.26
	N <sub>60</sub> P <sub>90</sub> K <sub>90</sub>	16.81±0.27 <sup>a</sup>	15.22±0.28 <sup>a</sup>	15.51±0.28 <sup>a</sup>
	N <sub>30</sub> P <sub>45</sub> K <sub>45</sub>	14.43±0.22 <sup>a,b</sup>	12.18±0.20 <sup>a,b</sup>	12.41±0.20 <sup>a,b</sup>

The level of significance of differences between the average values of indicators of the variants under study according to ANOVA data: P<0.001

**Note:** s.p. – secondary products; indices a, b – the level of significance of the difference for each control, and the subsequent fertiliser system

Observations of the dynamics of water-soluble humus substances during the growing season of the crop

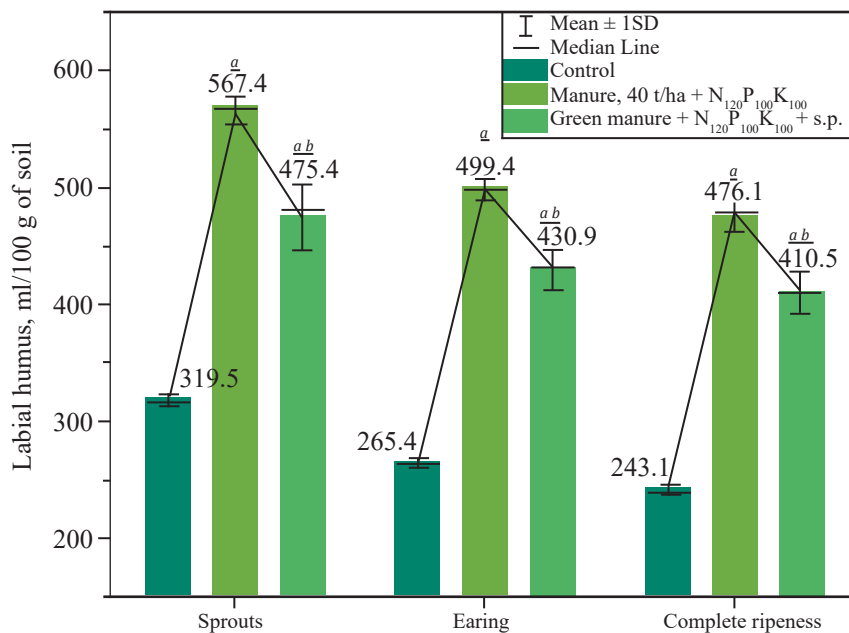
showed that in all crop rotations, their highest values were at the time of sprouting of winter wheat, decreased

to the earing phase due to the active consumption of decomposition products by plants and increased to full ripeness due to the predominance of immobilisation processes over mineralisation.

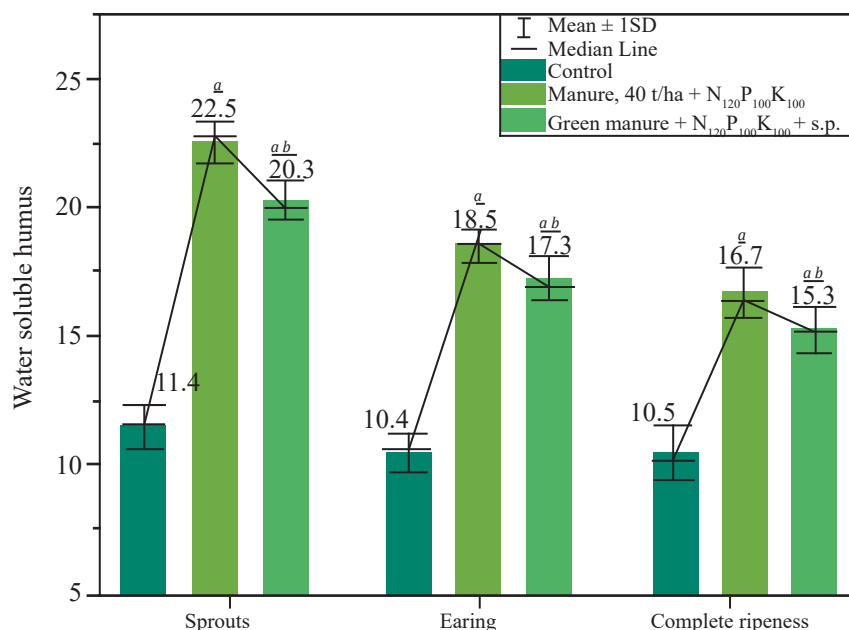
The study of changes in mobile forms of humus under corn (row-crop) was carried out in one (grain) crop rotation. Its predecessor was winter wheat. Variants without fertilisation and with a complex application of 40 t/ha of manure and  $N_{120}R_{100}K_{100}$  in the traditional fertiliser system were studied and the same level of mineral nutrition of plants against the background of secondary products (straw) of winter wheat and green mass of oilseed radish in an alternative system.

It was found that at the time of corn germination on the control variants, the content of labile humus was  $319.63 \text{ mg kg}^{-1}$ , water-soluble –  $11.42 \text{ mg kg}^{-1}$  of soil. Organic-mineral fertiliser systems provided

a considerable increase in these indicators. On alternative fertiliser options, labile humus values were  $475.44 \text{ mg kg}^{-1}$ , water-soluble –  $20.30 \text{ mg kg}^{-1}$  in the conventional case, their values were higher and amounted to 567.42 and  $22.55 \text{ mg kg}^{-1}$  of soil. In the subsequent phases of the growing season of the crop, the content of the organic compounds under study in the soil environment decreased and acquired the lowest values at full ripeness of corn. In comparison with the germination phase, this decrease in labile and water-soluble humus indicators was 24.0% and 8.5%, respectively, for the control variants, 16.1% and 26.0% for the introduction of 40.0 t/ha of manure and  $N_{120}R_{100}K_{100}$  mineral fertilisers, 13.6% and 24.8% for the composition of green mass of oilseed radish, secondary products of winter wheat and  $N_{120}R_{100}K_{100}$  mineral fertilisers (Fig. 1, 2).



**Figure 1.** Changes in the content of labile humus under various fertiliser systems under corn to grain ( $m \pm m$ ; 2018-2020)  
**Note:** s.p. – secondary products; indices a, b – the level of significance of the difference for each control, and the subsequent fertiliser system, respectively,  $P < 0.001$



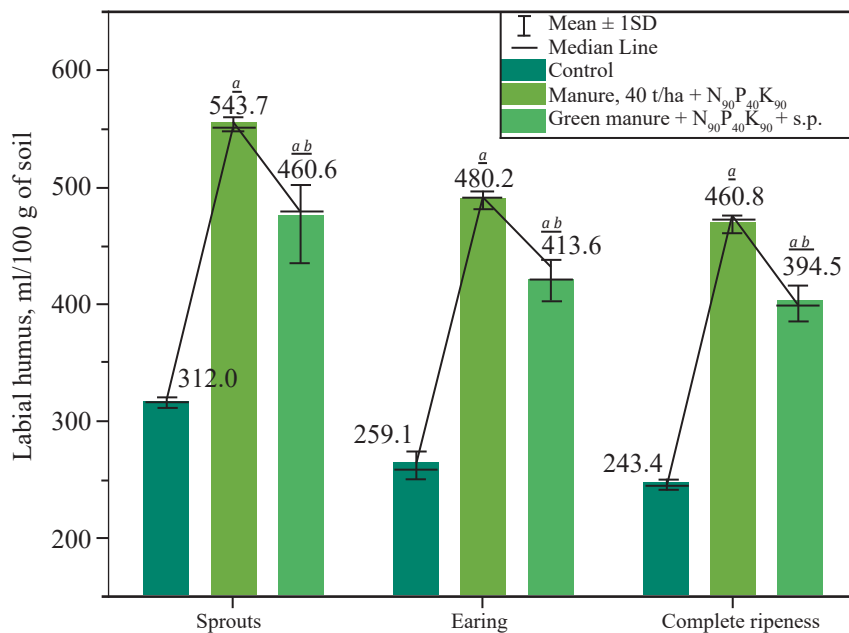
**Figure 2.** Changes in the content of water-soluble humus in various fertiliser systems under corn to grain ( $m \pm m$ ; 2018-2020)  
**Note:** s.p. – secondary products; indices a, b – the level of significance of the difference for each control, and the subsequent fertiliser system, respectively,  $P < 0.001$

A comparative analysis of the dynamics of changes in mobile forms of humus under winter wheat and corn showed differences in the redistribution of humus substances in the final phases of vegetation. This can be explained by the difference in the maturation period that occurs in grain crops at the end of July with high microbiological activity of the soil and in mid-September in row crops, when there is a decrease in these processes due to changes in the heat and water regimes of the soil environment.

In the vegetable rotation, changes in unstable forms of humus were observed under the row potato

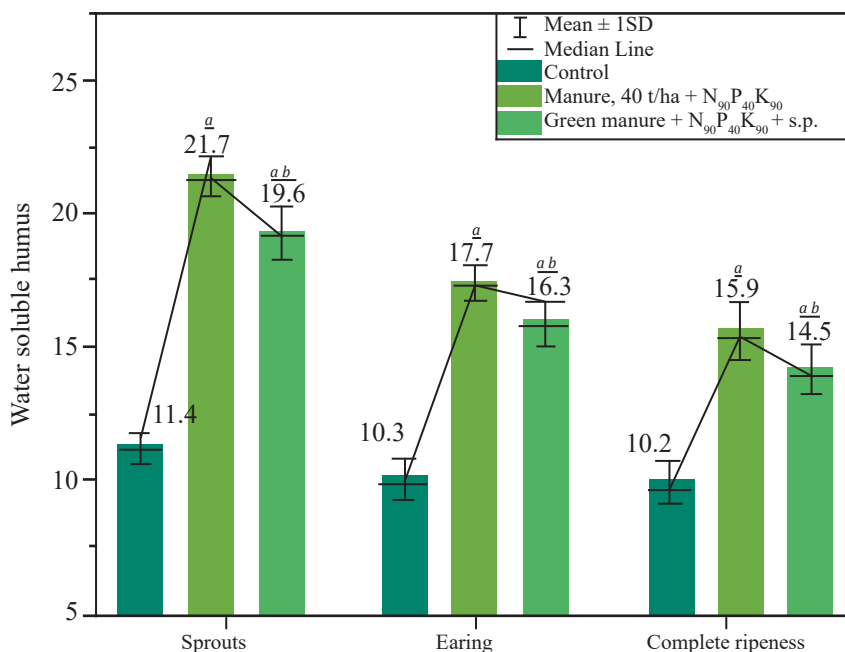
crop, the predecessor of which was also winter wheat. Variants without fertilisation and with complex application in the conventional fertiliser system of 40 t/ha of manure and  $N_{90}P_{40}K_{90}$  were studied and the same level of mineral nutrition of plants against the background of secondary products (straw) of winter wheat and green mass of oilseed radish in an alternative system.

It was found that the dynamics of the organic substances under study in terms of fertiliser variants and vegetation phases of the crop was similar to that observed under corn crops (Fig. 3, 4).



**Figure 3.** Changes in labile humus content under various potato fertiliser systems ( $m \pm m$ ; 2018-2020)

**Note:** s.p. – secondary products; indices a, b – the level of significance of the difference for each control, and the subsequent fertiliser system, respectively,  $P < 0.001$



**Figure 4.** Changes in the water-soluble humus content in various fertiliser systems under potatoes ( $m \pm m$ ; 2018-2020)

**Note:** s.p. – secondary products; indices a, b – the level of significance of the difference for each control, and the subsequent fertiliser system, respectively,  $P < 0.001$

Higher level of labile accumulation (543.66-460.60 mg kg<sup>-1</sup> of soil) and water-soluble (21.75-19.57 mg kg<sup>-1</sup> of soil) compounds are observed during the sprouting of the crop on organic-mineral backgrounds. By the end of the growing season, their number decreased and by the time of full ripeness was 460.81-394.48 mg kg<sup>-1</sup> of soil of labile and 15.88-14.45 mg kg<sup>-1</sup> of soil of water-soluble humus.

## CONCLUSIONS

The formation and redistribution of unstable organic compounds in short-term rotations during the growing season of agricultural crops are considerably influenced by precursors and fertiliser systems.

The highest values of indicators of labile and water-soluble humus of grey forest surface-gleyed soil under winter wheat crops in the germination phase in the control variants were after the predecessors of meadow clover (359.59 mg kg<sup>-1</sup> of soil of labile and 11.69 mg kg<sup>-1</sup> of soil of water-soluble humus) and peas (341.88 and 11.53 mg kg<sup>-1</sup> of soil, respectively), the lowest – after corn per green mass (290.40 and 11.15 mg kg<sup>-1</sup> of soil).

The use of organic-mineral fertiliser systems provided an increase in the content of mobile forms of humus substances. Direct application under winter wheat N<sub>60</sub>R<sub>90</sub>K<sub>90</sub> and 40 t/ha of manure in the conventional system contributed to the formation of 529.07 mg kg<sup>-1</sup> of labile compounds and 20.20 mg kg<sup>-1</sup> of soil of water-soluble humus. The combination of the same level of mineral nutrition, predecessor straw and post-harvest green mass of oilseed radish in an alternative system yielded 486.21 and 18.40 mg kg<sup>-1</sup> of soil of mobile humus substances. The introduction of the mineral component of fertiliser systems for winter wheat, and the organic component – remotely – for its predecessors, formed lower values of unstable humus compounds for all the crop rotations under study.

Organic-mineral fertiliser systems contributed to the activation of humus-forming processes in row crops. Application of N<sub>120</sub>R<sub>100</sub>K<sub>100</sub> and 40 t/ha of manure under corn for grain provided a 1.8-fold increase in the content of labile and 2.0-fold increase in water-soluble humus compared to the control. Complex application of N<sub>90</sub>R<sub>90</sub>K<sub>90</sub> and 40 t/ha of manure for potatoes contributed to the growth of these indicators by 1.7 and 1.9 times, respectively.

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## Вплив удобрення на зміну лабільних і водорозчинних форм гумусу в короткоротаційних сівозмінах

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**Анотація.** Однією з базових складових ефективної родючості ґрунтів та визначальною умовою забезпечення високої продуктивності сівозмін є рухомі (лабільні і водорозчинні) форми гумусу. В результаті ферментних процесів, вони мінералізуються і приймають участь у живленні рослин, а частина їх, включаючись в мобілізаційні процеси, переходять у стабільні гумусові речовини. Тому важливим є вивчення агротехнологічних факторів управління їх динамікою й перерозподілом в ґрунтового середовищі впродовж вегетації сільськогосподарських культур. Мета досліджень: вивчити вплив комплексного внесення мінеральних і органічних (традиційних та альтернативних) добрив на зміну водорозчинних і лабільних форм гумусу впродовж вегетації сільськогосподарських культур, вирощуваних у сівозмінах короткої ротації. В дослідженнях були застосовані наступні методи досліджень: польовий, лабораторно-аналітичний, розрахунково-порівняльний, математично-статистичний. Вищий рівень накопичення лабільних (359,59 мг кг<sup>-1</sup> ґрунту) і водорозчинних (11,69 мг кг<sup>-1</sup> ґрунту) форм гумусу під посівами пшениці озимої відбувається, коли попередником культури у сівозміні виступає конюшина лучна. Застосування безпосередньо під пшеницю озиму N<sub>60</sub>P<sub>90</sub>K<sub>90</sub> та 40 т/га гною у традиційній системі удобрення зерно-трав'яної сівозміни сприяє утворенню 529,07 і 20,20 мг кг<sup>-1</sup> ґрунту досліджуваних органічних речовин. Внесення N<sub>120</sub>P<sub>100</sub>K<sub>100</sub> і 40 т/га гною під кукурудзу на зерно забезпечує формування 567,42 і 22,55 мг кг<sup>-1</sup> ґрунту, а N<sub>90</sub>P<sub>90</sub>K<sub>90</sub> і 40 т/га гною під картоплю 543,66 і 21,75 мг кг<sup>-1</sup> ґрунту рухомих сполук гумусу. Отримані результати досліджень можуть бути основою для розробки високоефективних екологічно безпечних систем землеробства та використані для подальших наукових досліджень з розробки шляхів та напрямів управління гумусотвірними процесами в ґрунтового середовищі

**Ключові слова:** гумусові речовини, органо-мінеральні системи, побічна продукція, сидерат, полікультурні комплекси



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## Diversity of the Entomocomplex of the Grass Stand of a Hemp Field in The North-Eastern Forest-Steppe of Ukraine

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**Abstract.** The agrobiocenosis of the grass stand of hemp field is a specific plant biotope, which is a place of shelter, feeding, resettlement, and breeding for many insect populations, which in one way or another affecting the growth, development and yield of hemp plants. Taking this into consideration awareness of the species composition of phytophagous insects as well as the complex of their natural enemies and neutral species became especially relevant awareness due to the necessary to develop an effective system of protection of hemp in the context of current conditions. The aim of the research is to improve the ecologically oriented system of hemp protection by means of studying the taxonomic composition of entomofauna in the grass stand, as well as the trophic and ecological structure of insect groups associated with that habitat. The studies was conducted in 2019-2021 on the basis of the Northeast Agricultural Institute of the National Academy of Agrarian Sciences out during the vegetation of plants by means of mowing with a standard entomological net every ten days, from 10.00 till 15.00 o'clock when the insects were the most active. The current taxonomic composition of entomocomplex of the grass stand in hemp field is represented by 174 species of insects that belong to 76 families and 9 orders. The Coleoptera turned out to be the largest in terms of the species diversity and the number of individuals (56 species from 16 families and 74.6% of the number of captured insects). Were also detected insects from the orders Hymenoptera (31 species from 15 families), Hemiptera (30 species from 11 families), Diptera (20 species from 12 families), Homoptera (17 species from 8 families), Lepidoptera (12 species from 8 families), Orthoptera (4 species from 3 families), Neuroptera (3 species from 2 families), Thysanoptera (one species) were also detected. In the trophic structure of the entomofauna in grass stand of hemp field, 85.9% of the number and 59.8% of the species diversity account for phytophagous insects. Pests of hemp were 39 species of insects from 22 families, and 6 orders. Among them, 36 species, which accounted for 18.7% of the total number of specimens, were polyphagous and three, or 81.3%, were specialized species. The presence of insect pests in the grass stand of hemp field was characterized by oligodominance, as evidenced by quantitative and qualitative data, and indices of species diversity. Thus, the dominance structure is represented by one eudominant (*Psylliodes attenuata* – 81.1%), one subdominant (*Mordellistena parvula* – 4.72%), four recedents (*Lygus pratensis*, *L. rugulipennis*, *Lygocoris pabulinus*, *Stictocephala bisonia* – 8.6%) and, 33 subrecedents (5.58%). The obtained research results will be used in order to solve the problems related to the danger of basic phytophagous insects during the vegetation period of cannabis sativa plants and to develop a modern environmentally-oriented strategy to control their numbers and harmfulness

**Keywords:** agrobiocenosis, species composition, trophic structure, insect pests, dominance classes



## INTRODUCTION

Hemp seeds (*Cannabis sativa* L.) are a highly valuable fibre crop, the history of cultivation and comprehensive use of which began in ancient times (Clark & Merlin, 2016; Long *et al.*, 2017). The wide importance and benefits of hemp are determined by economically valuable characteristics, which allows the full use of all the components of the plant for the production of numerous environmentally friendly products with many applications, which every day occupy leading positions in the world and Ukrainian markets (Crini *et al.*, 2020; Bojko *et al.*, 2018). The issues of healing biocenoses and remediation of areas contaminated with radionuclides, heavy metals, and chemical compounds by cultivating hemp in such areas are becoming increasingly relevant (Placido & Lee, 2022; Wu *et al.*, 2021).

Growing crops is fraught with risks. Along with a natural disaster (drought, flood, hail, fires, etc.), cultivated plants are at risk from their natural consumers – pests. It is known that more than 10,000 species of insect pests can damage cultivated plants worldwide (Dhaliwal *et al.*, 2007). Phytophagous insects are thought to destroy about 18-20% of the global crop yield per year (Oerke, 2006; Sharma *et al.*, 2017). Hemp is no exception and has crop losses from harmful insect species.

Thousands of years of specialisation and intensification of crop production against the background of the influence of global climate change in particular environmental conditions contributed not only to the development of a certain species composition of insects, changes of the dominant phytophages, but also to the expansion of new areas of their existence. Every year, the entomocomplex of hemp is supplemented by introduced species that are more adapted to new trophic conditions, which previously did not have considerable economic importance (Küçüktopçu *et al.*, 2020; Ajayi & Samuel-Foo, 2021).

As the acreage under hemp continues to grow both in the world and in Ukraine (Zuk-Golaszewska & Golaszewski, 2018; Gruzinska *et al.*, 2020), considering the specific features of the hemp industry, the concentration of crops increases, and therefore a harmful entomocomplex accumulates. Given this, it is relevant to determine the species composition of insect pests, as well as the complex of their natural enemies and neutral species inhabiting plants. Knowledge of the species composition and harmful stages of phytophagous insects at various stages of hemp development is necessary for the development of efficient environmentally oriented control of their abundance and harmfulness.

*The purpose of this study* is to improve the environmentally oriented system of protection of hemp crops by investigating the taxonomic composition, the number of general and harmful entomofauna in the herbage, as well as the trophic and ecological structure of insect groups during the growing season of the crop in the north-eastern part of the Left-Bank Forest-Steppe of Ukraine.

## LITERATURE REVIEW

Due to the morphological and biological characteristics of plants, the herbage of hemp is particularly attractive for a diverse entomological fauna and is a plant biotope for the existence of numerous populations of arthropods. The diversity of ecological niches is primarily determined by the trophic relationships of insects in hemp agrocenosis (Cranshaw *et al.*, 2019).

It is known that the entomofauna of hemp seeds can include 180-300 species (Lago & Stanford, 1989; McPartland, 1996) and, depending on the geographical area, number about 20-150 specialised and polivorous phytophagous insects (McPartland *et al.*, 2000; Trotus & Naie, 2008; Fedorenko *et al.*, 2016), which can considerably harm the germinating seed and root system in the soil, and the aboveground vegetative and reproductive part of the plant in the herbage (Cranshaw *et al.*, 2019; Pivtoraiko *et al.*, 2020).

Climate change due to the global increase in air temperature and uneven precipitation in particular soil and climatic conditions of the region largely determine the distribution features and changes in the population density of serious insect pests (Skendžić *et al.*, 2021), including in agrocenoses of hemp seeds (Ajayi & Samuel-Foo, 2021). Taking this into account, in different geographical areas of hemp cultivation, there are differences in the species composition of entomofauna and the structure of dominance of phytophagous insects in the hemp field. Thus, on the American continent, the entomocomplex is represented by a richer species diversity, which is confirmed by studies in the United States in the southern state of Mississippi, where more than 300 species of insects have been identified. Among them, 69 species were identified that used hemp plants as a source of physiological nutrition. The majority (43 species) fed on sap, 15 species were leaf eaters, nine collected or fed on pollen, and the rest – on plant roots (Lago & Stanford, 1989). Similar studies in eastern Colorado identified harmful, beneficial, and neutral insects from 142 genera that belonged to 73 families and 15 orders. The most harmful insects include *Helicoverpa zea* Bodd., *Grapholitta delineaana* Walk., and *Phorodon cannabis* Pass (Schreiner & Cranshaw, 2021).

The entomocomplex of the hemp field in Europe is characterised by a slightly smaller variety of species. For example, in Germany, 129 species of insects were recorded, among which 51 species are potentially dangerous for hemp plants. Special attention should be paid to *Autographa gamma* L., *Agromyza strigata* Meig., *Eupteryx atropunctata* Goeze, *Lygus rugulipennis* Popp., *Tipula paludosa* Meig., *P. cannabis* Pass., and *Psylliodes attenuata* Koch. (Gottwald, 2002). In Poland, there are 27 species of phytophagous insects of hemp seeds (Barko *et al.*, 2018). The dominant and most dangerous species include *Phorodon humuli* Schr., *Ostrinia nubilalis* Hbn., *P. attenuata* Koch., and *A. gamma* L. In the central regions of the Irkutsk region, in Russia, the fauna of insect pests in the hemp stand includes about 18 species, and the main ones are bedbugs (Hemiptera) from the genus *Lygus* spp. (50% of the total population) and representatives of the

family Pentatomidae (15.7%). A high number of *Cardipennis rubripes* Hust., *Trichiocampus cannabis* Xiao & Huang and *P. attenuata* Koch is also noted. (Shylenkov & Tolstonogova, 2006). About seven main insect pest species of hemp were noted in Slovenia, of which *P. attenuata* Koch., *G. delineana* Walk., *O. nubilalis* Hbn., *P. cannabis* Pass and several species of leafhoppers caused the most economically significant losses of hemp production (Lepidoptera: Noctuidae) (Cizej & Policnik, 2018).

## MATERIALS AND METHODS

The research was conducted during the vegetating season of 2019-2021 in the conditions of the research and trial facility of the Institute of Agriculture of the North-East of the National Academy of Agrarian Sciences (IANE NAAS), Sumy Oblast, Sumy district, the village of Sad. The research site is geographically located in the north-eastern part of the Left-Bank Forest-Steppe of Ukraine at geographical coordinates 50.8846°N, 34.6961°E. The climate is temperate continental with warm long summers and moderately cold winters and frequent thaws, the average annual air temperature is +7.4°C. The average annual precipitation is about 593 mm. The average long-term relative humidity is within 77%. Monitoring of the entomocomplex was carried out in seed-growing hemp crops of Ukrainian selection – Glesia. Hemp was grown for bilateral use with 45 cm between rows. The seeding rate was 1.0 million pcs/ha. Its predecessor is winter wheat.

The total number of insect species in the entomofauna of the hemp grass stand was determined during the spring-summer vegetating season by mowing with a standard entomological net. Accounting began with the phase of two pairs of real leaves of the culture. For this, the authors of this study carried out decadal mowing from 10:00 to 15:00, when insects were most active. Each sample comprised 100 strokes (10 strokes in 10 places on two diagonals of the field). After each sample, all insects were selected from the net and soaked with acetic acid ether (ethyl acetate). The collected entomological material from the stain was disassembled separately for each sample on a sheet of white paper, then the insects were laid out on cotton mattresses measuring 12x20 cm and 3-5 mm thick. Each mattress was placed in a paper envelope with a label insert (Poljakov *et al.*, 1984; Omeliuta *et al.*, 1986). The reliability of determining the species affiliation of insects was confirmed by specialists of the I.I. Schmalhausen Institute of Zoology of the National Academy of Sciences of Ukraine.

To characterise the species structure of the entomocomplex of the hemp field, the total number of individuals and the degree of dominance were determined for each individual species (Fasulaty, 1971). Dominance classes for detected insect pests in the grass stand of a hemp field were set on a scale as follows: mass species, or eudominants (31.7-100%), common or dominants (10.1-31.6%), infrequent or subdominants (3.2-10.0%), rare, or recedents (1.1-3.1%), random, or subprecedents (<1.0%) (Stöcker & Bergmann, 1977). Generally accepted indices were used to characterise the species diversity of insects (Lebedeva *et al.*, 2004).

The Margalef's species richness index was calculated according to the Formula (1):

$$D_{Mg} = \frac{S-1}{\ln N} \quad (1)$$

where  $S$  is the number of types, pcs;  $N$  is the total number of individuals of all species, specimen.

The value of the Shannon's index was determined according to the Formula (2):

$$H' = -\sum P_i \ln P_i \quad (2)$$

where  $P_i$  is the proportion of individuals of each species.

The Simpson's index indicators were calculated according to the Formula (3):

$$D = 1 - \left( \frac{\sum n(n-1)}{N(N-1)} \right) \quad (3)$$

where  $n$  is the number of individuals for each species, specimen;  $N$  is the total number of individuals of all species, specimen.

The value of the Berger-Parker's index was determined according to the Formula (4):

$$d = 1 / \frac{N_{max}}{N} \quad (4)$$

where  $N_{max}$  is the number of individuals of the most numerous species, specimen;  $N$  is the total number of individuals of all species, specimen.

The Piel alignment index was calculated according to the formula (5):

$$E = \frac{H'}{\ln S} \quad (5)$$

where  $N'$  is the Shannon index;  $S$  is the number of types.

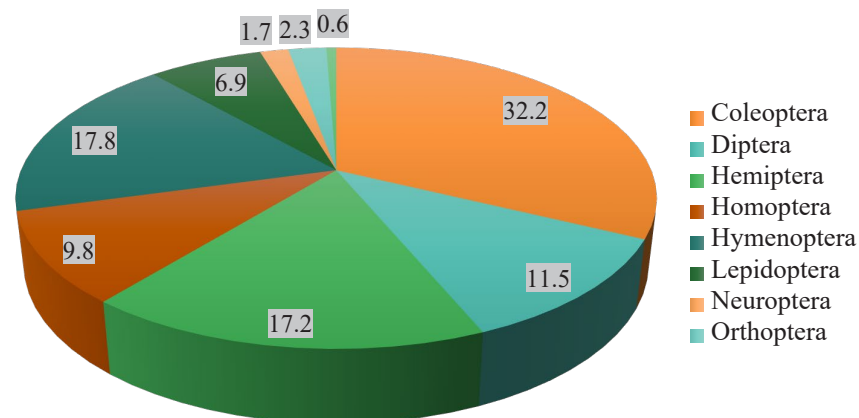
Mathematical calculations and visualisation of the obtained data were performed using the Microsoft Office Excel 2016 software package.

## RESULTS AND DISCUSSION

174 species of insects belonging to 76 families and 9 orders were caught in the grass stand of a hemp field. The greatest diversity of species was characterised by an order of Coleoptera (Coleoptera), which was represented by 56 species (32.2% of the total entomocomplex) from 16 families. The main number of representatives of this order was the leaf-eating family (Chrysomelidae) – 16 species (28.6% of the total number of the order). Curculionidae – seven species (12.5%), Coccinellidae – six species (10.7%), Mordellidae – four species (7.1%) were also noted for their considerable species diversity. The families Anthicidae, Elateridae, Malachiidae, Oedemeridae, Scarabaeidae had three species each (or 5.4% each), Staphylinidae – two species (3.6%), Bruchidae, Cantharidae, Carabidae, Cerambycidae, Lathridiidae, Nitidulidae – one species each, which was 1.8% each, respectively. The order of Hymenoptera (Hymenoptera) included 31 species (17.8% of the entomofauna biodiversity) from 15 families. The largest species diversity was observed in the following families: Ichneumonidae – six species (19.4%), Braconidae – five species (16.1%), and Chalcididae – four species (12.9% of all representatives of the order). Andrenidae, Formicidae,

Halictidae, Proctotrupidae included two species, or 6.5% each. Apidae, Aphelinidae, Chrysididae, Cynipidae, Megachilidae, Pompilidae, Sphecidae, Tenthredinidae

accounted for one species, or 3.2 % of all hymenopteran insects (Fig. 1).



**Figure 1.** Diversity of entomocomplex in the grass stand of a hemp field (mowing with an entomological net, total for 2019-2021), %

Hemiptera in the herbaceous entomocomplex of the hemp agrobiocenosis were represented by 30 species (17.2% of the total diversity) from 11 families. Among the insects of this order, the Miridae family was most fully represented – 12 species (or 40.0% of the total number). The Pentatomidae family was also distinguished by a considerable variety – five species (16.7%). Lygaeidae included three species (10.0%), Rhopalidae and Piesmatidae – two species each (6.7% each). Other families (Anthocoridae, Coreidae, Cydnidae, Nabidae, Pyrrhocoridae, Tingidae) were represented by one species, or 3.3% each. The order of Diptera was characterised by a considerable diversity – 20 species (11.5%) from 12 families. It was based on the families Anthomyiidae and Syrphidae – three species each (or 15.0% each). Other families (Agromyzidae, Asilidae, Tachinidae, Tephritidae) included two species of flies (or 10.0% each). Bibionidae, Calliphoridae, Chloropidae, Opomizidae, Sarcophagidae and Tipulidae were represented by one species (or 5.0% each).

The order of Homoptera in the grass stand of a hemp field numbered 17 species (9.8% of the total) from 8 families. The most diverse (6 species or 35.3%) was the family of Cicadelidae. Aphididae was represented by three species (17.6%). Psyllidae and Jassidae included two species each (11.8% each). Cercopidae, Delphacidae, Dictyopharidae, and Membracidae accounted for one species, or each for 5.9% of the insects of this order. The order of Lepidoptera was represented by 12 species (6.9%) from eight families in the structure of

the entomocomplex. Among the order, the families Noctuidae, Pyralidae, Tineidae, Tischeriidae were the most complete in terms of the number of species – two species each (or 16.7%). The families of Geometridae, Nymphalidae, Plutellidae, and Tortricidae were represented by one species, or 8.3% each. Substantially fewer species diversity were found in the following orders: Orthoptera – four species (2.3%) from three families and Neuroptera – three species (1.7%) from two families. The smallest share of species diversity (0.6%) in the agrobiocenosis of the hemp field was made up of the order of Thysanoptera, which had one species.

In terms of the number of insects in the grass of the hemp field, the order of Coleoptera prevailed – 74.6% of all insects. The highest number of individuals was represented by the Chrysomelidae family, which accounted for 88.8% of all Coleoptera insects and 66.3% of the total entomocomplex of the hemp agrobiocenosis. In this family there were a lot of earth fleas, mainly from the genera *Altica* sp., *Chaetochnema* sp., *Longitarsus* sp., *Phyllotreta* sp., *Psylliodes* sp. The Mordellidae family had a fairly considerable number of specimens, with a share of 4.3% in the total entomocomplex. A slightly smaller number of insects were represented by the families of Lathridiidae and Coccinellidae. Representatives of the following families were found singly: Carabidae, Cerambycidae, Malachiidae, Oedemeridae, Bruchidae, Staphylinidae, Cantharidae, Elateridae, Anthicidae, Nitidulidae, Scarabaeidae, and Curculionidae (Table 1).

**Table 1.** Composition and abundance of entomofauna in the grass stand of sown hemp (mowing with an entomological net, total for 2019-2021)

Order	Family	Number of instances	Share, %
Coleoptera	Anthicidae	10	0.04
	Bruchidae	6	0.02
	Cantharidae	7	0.03
	Carabidae	1	0.004
	Cerambycidae	2	0.01
	Chrysomelidae	17760	66.26
	Coccinellidae	395	1.47
	Curculionidae	63	0.24
	Elateridae	7	0.03
	Lathridiidae	545	2.03
	Malachiidae	4	0.01
	Mordellidae	1145	4.27
	Nitidulidae	14	0.05
	Oedemeridae	4	0.01
	Scarabaeidae	27	0.10
Staphylinidae	6	0.02	
Diptera	Agromyzidae	165	0.62
	Anthomyiidae	560	2.09
	Asilidae	32	0.12
	Bibionidae	2	0.007
	Calliphoridae	3	0.01
	Chloropidae	48	0.17
	Opomizidae	13	0.05
	Sarcophagidae	1	0.004
	Syrphidae	13	0.05
	Tachinidae	13	0.05
	Tephritidae	7	0.03
	Tipulidae	2	0.007
Hemiptera	Anthocoridae	1032	3.85
	Coreidae	24	0.09
	Cydnidae	1	0.004
	Lygaeidae	15	0.06
	Miridae	1884	7.02
	Nabidae	35	0.13
	Piesmatidae	10	0.04
	Pyrrhocoridae	6	0.02
Hemiptera	Pentatomidae	281	1.05
	Rhopalidae	74	0.28
	Tingidae	2	0.007
Homoptera	Aphididae	275	1.03
	Cercopidae	15	0.06
	Cicadelidae	106	0.40
	Delphacidae	3	0.01
	Dictyopharidae	1	0.004
	Jassidae	14	0.05
	Membracidae	368	1.37
	Psyllidae	87	0.32

Table 1, Continued

Order	Family	Number of instances	Share, %
Hymenoptera	Apidae	13	0.05
	Andrenidae	2	0.007
	Aphelinidae	1	0.004
	Braconidae	139	0.52
	Chalcididae	153	0.57
	Chrysididae	44	0.16
	Synipidae	46	0.17
	Formicidae	203	0.76
	Ichneumonidae	77	0.30
	Halictidae	9	0.03
	Megachilidae	1	0.004
	Pompilidae	1	0.004
	Proctotrupidae	7	0.026
	Sphecidae	7	0.026
	Tenthredinidae	4	0.01
Lepidoptera	Geometridae	1	0.004
	Noctuidae	162	0.60
	Nymphalidae	12	0.04
	Pyrilidae	29	0.11
	Plutellidae	49	0.20
	Tineidae	44	0.16
	Tischeriida	22	0.08
	Tortricidae	10	0.04
Neuroptera	Chrysopidae	187	0.70
	Hemerobiidae	4	0.01
Orthoptera	Acrididae	6	0.02
	Phaneropteridae	8	0.04
	Tettigoniidae	33	0.12
Thysanoptera	Aeolothripidae	442	1.65
Total		26804	100.0

The order of Hemiptera also had high rates of occurrence of individuals, the share of which was 12.6%. The basis was the families Miridae – 7.0%, and Anthocoridae – 3.9% of the complete collection of the herbaceous entomocomplex. Other representatives (Cydidae, Tingidae, Pyrrhocoridae, Piesmatidae, Lygaeidae, Coreidae, Nabidae, and Rhopalidae) had smaller numbers. Homoptera and Diptera insects were also quite noticeable in the herbage of agrocenosis, which comprised 3.2% each of the total entomofauna of hemp seeds. Among the order of Homoptera, the families of Membracidae, Aphididae, and Cicadellidae were the most numerous from the total entomofauna of the herbage with a share of 1.4%, 1.0%, and 0.4%, respectively. Among the Diptera insects, the Anthomyiidae species was the most numerous – 2.1%.

The number of insects was smaller in the orders

of Hymenoptera – 2.6% and Thysanoptera – 1.7% of the total collection. Most of them were represented by entomophages – parasites and predatory species. The population density of Lepidoptera insects was not high and accounted for 1.2% of the total collection. The highest number of individuals was represented by the Noctuidae family – 0.6%. The number of insects of other orders (Neuroptera and Orthoptera) was less than 1.0% in the total entomocomplex of the hemp field.

Notably, apart from the identified insects, spiders (Araneae: Thomisidae) also inhabited the grassland. Their share in the total collection was 1.8%. Over the years of research, the value of the Margalef's species richness index in the grass stand of hemp agrocenosis was 16.97; the Shannon's index indicator was 1.916; the Simpson's index value was 0.573; the Berger-Parker's index indicator was 0.649; the Piel's alignment index was 0.371 (Table 2).

**Table 2.** Indicators of biodiversity of the entomocomplex registered in the herbage of a hemp field (mowing with an entomological net, total for 2019-2021)

Biodiversity indices	Indicator
Total number of families	76
Total number of types	174
$D_{Mg}$	16.97
$H'$	1.916
$D$	0.573
$d$	0.649
$E$	0.371

**Note:**  $D_{Mg}$  is the Margalef's diversity index;  $H'$  is the Shannon's diversity index;  $D$  is the Simpson's dominance index;  $d$  is the Berger-Parker's dominance index;  $E$  is the Piel's alignment index

Considering the specific features of life and eating habits of individual insect species, the captured entomocomplex of hemp herbage was divided into ecological groups according to the type of food and trophic specialisation of insects. Thus, the highest diversity (104 species or 59.8%) and population size (85.9%) were noted among phytophagous insects, of which

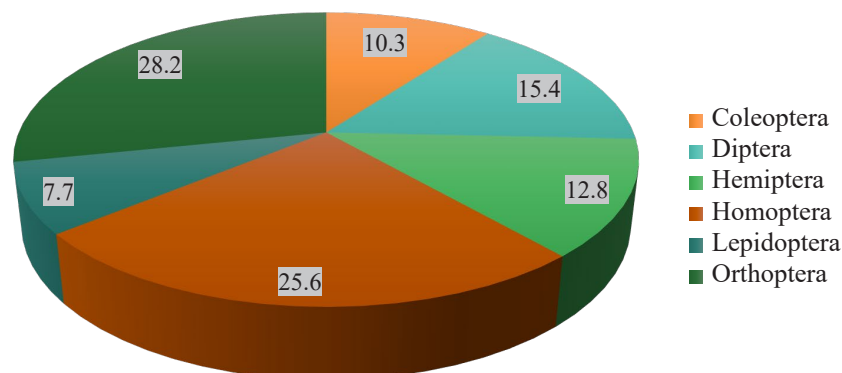
37.5% of species can harm hemp plants. The group of entomophages was represented by 50 species (28.7% of the diversity), and their share was 11.0%. Neutral species included 20 representatives (11.5%) with a share of 3.1%. The ratio of the number of entomophages to phytophages in the herbage of hemp seeds was 1:8 (Table 3).

**Table 3.** Trophic structure of the entomocomplex of the herbage of the hemp field (mowing with an entomological net, total for 2019-2021)

Trophic specialisation	Number of types	%	Number of instances	%
Phytophages	104	59.8	23022	85.9
Entomophages	50	28.7	2945	11.0
Neutral species	20	11.5	837	3.1
Total	174	100.0	26804	100.0

Among the harmful entomofauna caught in the grass stand of hemp agrobiocenosis, a considerable diversity of the species composition of hemp pests was found, including 39 species from 22 families and 6 orders. Among them, Coleoptera accounted for 28.2%, Diptera – 7.7%, Hemiptera – 25.6%, Homoptera – 12.8%, Lepidoptera – 15.4%, and Orthoptera – 10.3% (Fig. 2). Among

the identified species diversity, the majority of insects (36 species or 92.3%) are polyphages, whose nutrition and reproduction occurs on many species of cultivated and wild plants, and three species (7.7%) are specialised phytophagous insects of hemp plants. Therewith, the share of the former two accounts for 18.7% and 81.3% of the total number of insect pests in the hemp stand.

**Figure 2.** Taxonomic structure of the harmful entomocomplex of hemp grass stand (mowing with an entomological net, total for 2019-2021), %

It was established that among the complex of insect pests of hemp sown, the largest both in terms of the number and quantitative composition of species is

the order of Coleoptera. Thus, a total of 18,609 specimens (86.74% of the harmful entomocomplex), 11 species and 5 families were identified. The largest species

diversity (three species each) was observed in the family of Elateridae, Mordellidae, and Scarabaeidae. Curculionidae and Chrysomelidae included one species each (Table 4). Among the representatives of this order, the largest number of specialised phytophages was noted – the *Psylliodes attenuata*, being eudominant in the entomocomplex with a share of 81.1%. The subdominant

was *Mordellistena parvula* – 4.72%. Other beetles: Curculionidae – one species (*Tanymecus palliatus*), Elateridae – three species (*Agriotes sputator*, *Lacon murinus* and *Melanotus brunnipes*), Mordellidae – two species (*Mordellistena connata* and *M. variegata*), Scarabaeidae – three species (*Cetonia aurata*, *Oxythyrea funesta*, and *Maladera holosericea*) were sub-recedents.

**Table 4.** Species composition and dominance of insect pests in the hemp field stand (mowing with an entomological net, total for 2019-2021)

Order	Family	Species	Number of instances	%	Dominance class
Coleoptera	Chrysomelidae	<i>Psylliodes attenuata</i> (Koch, 1803)	17398	81.10	E
	Curculionidae	<i>Tanymecus palliatus</i> (Fabricius, 1787)	33	0.15	SR
	Elateridae	<i>Agriotes sputator</i> (Linnaeus, 1758)	3	0.01	SR
		<i>Agrypnus murinus</i> (Linnaeus, 1758)	3	0.01	SR
		<i>Melanotus brunnipes</i> (Germar, 1824)	1	0.005	SR
	Mordellidae	<i>Mordellistena connata</i> (Ermisch, 1969)	125	0.58	SR
		<i>M. parvula</i> (Gyllenhal, 1827)	1013	4.72	SD
<i>M. variegata</i> (Fabricius, 1798)		6	0.03	SR	
Coleoptera	Scarabaeidae	<i>Cetonia aurata</i> (Linnaeus, 1758)	1	0.005	SR
		<i>Oxythyrea funesta</i> (Poda, 1761)	25	0.12	SR
		<i>Maladera holosericea</i> (Scopoli, 1772)	1	0.005	SR
Diptera	Agromyzidae	<i>Liriomyza</i> sp. (1)	62	0.29	SR
		<i>Phytomyza atricornis</i> (Meigen, 1838)	103	0.48	SR
	Tipulidae	<i>Tipula paludosa</i> (Meigen, 1830)	2	0.01	SR
Hemiptera	Coreidae	<i>Coreus marginatus</i> (Linnaeus, 1758)	24	0.11	SR
	Lygaeidae	<i>Sphragisticus nebulosus</i> (Fallen, 1807)	5	0.02	SR
	Miridae	<i>Adelphocoris lineolatus</i> (Goeze, 1778)	49	0.23	SR
		<i>Lygus pratensis</i> (Linnaeus, 1758)	465	2.17	R.
		<i>L. rugulipennis</i> (Poppius 1911)	673	3.14	R.
		<i>Lygocoris pabulinus</i> (Linnaeus, 1761)	339	1.58	R.
		<i>Polymerus cognatus</i> (Fieber, 1858)	2	0.01	SR
		<i>P. vulneratus</i> (Panzer, 1806)	20	0.09	SR
	Pentatomidae	<i>Dolycoris baccarum</i> (Linnaeus, 1758)	148	0.69	SR
		<i>Palomena prasina</i> (Linnaeus, 1761)	54	0.25	SR

Table 4, Continued

Order	Family	Species	Number of instances	%	Dominance class
Homoptera	Aphididae	<i>Aphis fabae</i> (Scopoli, 1763)	168	0.78	SR
		<i>Phorodon cannabis</i> (Passerini, 1860)	41	0.19	SR
	Cercopidae	<i>Philaenus spumarius</i> (Linnaeus, 1758)	15	0.07	SR
	Cicadellidae	<i>Eupteryx atropunctata</i> (Goeze, 1778)	47	0.22	SR
	Membracidae	<i>Stictocephala bisonia</i> (Kopp & Yonke, 1977)	368	1.72	R.
Lepidoptera	Noctuidae	<i>Autographa gamma</i> (Linnaeus, 1758)	27	0.13	SR
		<i>Helicoverpa armigera</i> (Hübner, 1808)	136	0.63	SR
	Nymphalidae	<i>Vanessa cardui</i> (Linnaeus, 1758)	12	0.06	SR
Lepidoptera	Pyrilidae	<i>Ostrinia nubilalis</i> (Hübner, 1796)	17	0.08	SR
		<i>Loxostege sticticalis</i> (Linnaeus, 1761)	11	0.05	SR
	Tortricidae	<i>Grapholitta delineana</i> (Walker, 1863)	10	0.05	SR
Orthoptera	Acrididae	<i>Chortippus</i> sp. (2)	6	0.03	SR
	Tettigoniidae	<i>Tettigonia viridissima</i> (Linnaeus, 1758)	33	0.15	SR
	Phaneropteridae	<i>Phaneroptera falcata</i> (Poda, 1761)	8	0.04	SR
Total:			21454	100.0	-

**Note:** E – eudominant (31.7-100%); D – dominant (10.1-31.6%); SD – subdominant (3.2-10.0%); R – recedent (1.1-3.1%); SR – subrecedent (<1.0%)

The species composition of insect pests of the order of Hemiptera was represented by 10 species from four families and numbered 1,779 specimens (8.29%). Among them, most insects (six species) belonged to the family of Miridae. The diversity of shield bugs (Pentatomidae) included two species, edge bugs (Coreidae) and ground bugs (Lygaeidae) – one species each. Three species of Miridae were found to be the regulars – *Lygus rugulipennis*, *L. pratensis*, and *Lygocoris pabulinus*. The other seven species of bugs are classified as sub-recedents: Coreidae – one species (*Coreus marginatus*), Lygaeidae – one species (*Sphragisticus nebulosus*), Pentatomidae – two species (*Palomena prasina* and *Dolycoris baccarum*), Miridae – three species (*Adelphocoris lineolatus*, *Polymerus cognatus* and *P. vulneratus*).

The order of Homoptera numbered 639 specimens of phytophagous insects (2.98%) – five species from four families. The greatest diversity was found in the family of Aphididae – two species. The families of Cercopidae, Cicadellidae, and Membracidae had one species each. Among the Homoptera, one species was a recedent: Membracidae – *Stictocephala bisonia* (1.72%). The other four types were classified as sub-recedents: Aphididae – two species (*Aphis fabae* and *Phorodon cannabis*), Cicadellidae – one species (*Eupteryx atropunctata*), Cercopidae – one species (*Philaenus spumarius*).

Among the Lepidoptera, 213 specimens (0.99%) of phytophagous insects were caught – six species of insects from four families. The families of Noctuidae and Pyralidae comprised two species each, Nymphalidae and Tortricidae – one species each. All representatives of this order were few, that is, they belonged to sub-recedents.

Diptera phytophages numbered 167 specimens (0.78%) and were represented by three insect species from two families. Most of the representatives were a small family of Agromyzidae. All insects in this series were sub-recedents.

Among the Orthoptera hemp pests, 47 specimens (0.22%) of four insect species from three families were caught. All four representatives were sub-recedents: Tettigoniidae – one species (*Tettigonia viridissima*), Phaneropteridae – one species (*Phaneroptera falcata*), Acrididae – two species (*Chortippus* sp.)

Over the years of research, the main indices of the species diversity of the complex of insect pests of the herbage of seed hemp had low values, which indicates oligodominance in the station, that is, the predominance of several species. Thus, the Margalef's species richness index was 3.810, while the Shannon's index value was 0.960, the Simpson's index value was 0.337, the Berger-Parker's index value was 0.811, and the Piel's alignment index was 0.262 (Table 5).

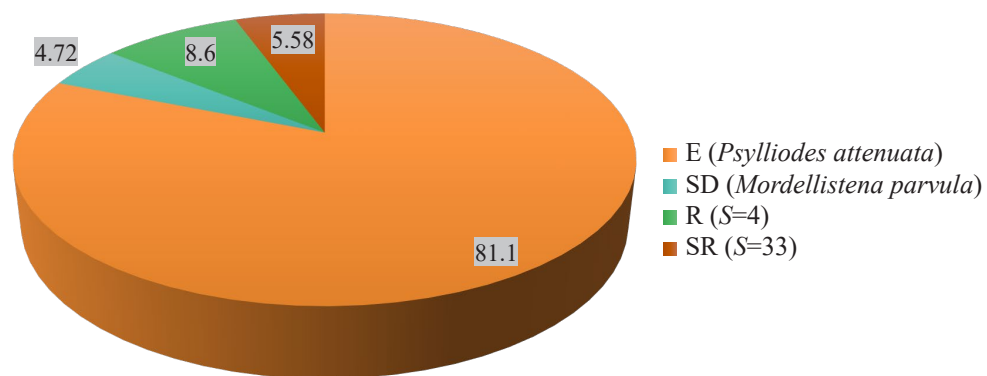
**Table 5.** Indicators of biodiversity of the insect pest complex in the herbage of a hemp field (mowing with an entomological net, total for 2019-2021)

Biodiversity indices	Indicator
Total number of families	22
Total number of types	39
$D_{Mg}$	3.810
$H'$	0.960
$D$	0.338
$d$	0.811
$E$	0.262

**Note:**  $D_{Mg}$  is the Margalef's diversity index;  $H'$  is the Shannon's diversity index;  $D$  is the Simpson's dominance index;  $d$  is the Berger-Parker's dominance index;  $E$  is the Piel's alignment index

Analysing the dominance distribution of insect pests of seed hemp, it was established that in the structure the eudominant (*Psylliodes attenuata*) made up

81.1%, the subdominant (*Mordellistena parvula*) – 4.72%, recedents – 8.6%, subrecedents – 5.58% (Fig. 3).



**Figure 3.** Structure of dominance of insect pests in the herbage of a hemp field (mowing with an entomological net, total for 2019-2021), %

Thus, the study indicates a high population adaptability and dominance of the main specialised insects in the grass stand of hemp agrogenosis.

Similar data were obtained upon investigating the entomofauna of hemp sown in Central Moldova, where 20 species of phytophagous insects feeding on these plants were found in the herbage. Among them, Coleoptera accounted for 35%, Lepidoptera – 30%, Diptera – 15%, Homoptera and Heteroptera – 10% each. Of these, there were 16 species (75%) of sub-recedents, two (10%) recedents, one (3%) subdominant, and two (10%) eudominants (Trotus et al., 2011). The study of the entomocoplax of the grass stand of a hemp field and the analysis of its trophic structure in the Eastern Polissia of Ukraine, where hemp farming is a traditional industry, revealed 117 species of insects from 57 families and eight orders, including 18 species – phytophages of hemp. Notably, polyivorous insect pests in the entomocoplax of hemp grass stand are represented by 15 species, specialised – by three. The dominant and particularly dangerous was the hemp flea (*P. attenuata*) (Fedorenko et al., 2016; Kabanets, 2013; Kabanets & Fedorenko, 2014).

Notably, there has been a tendency to increase the species diversity and abundance of harmful entomocoplax. The authors of this study believe that this may

be due to both climatic (an increase in the average annual air temperature) and agrotechnological factors (due to non-compliance with scientifically sound crop rotations, optimal land use structure, area expansion, and an increase in the concentration of thick-stemmed crops (corn, sunflower)), which have insect pests in common with hemp.

## CONCLUSIONS

In the conditions of the north-eastern part of the Left-Bank Forest-Steppe of Ukraine in 2019-2021, the structure of the entomological complex of the grass stand of a hemp field, the trophic specialisation of insects in it were studied, the most numerous species and the degree of their dominance were identified.

It was established that the modern entomocoplax in the north-eastern part of the Left-Bank Forest-Steppe of Ukraine is represented by 174 species of insects belonging to 76 families and 9 orders, of which the largest species diversity (32.2%) and the number of insects (74.6% in the structure of the entire entomocoplax) was characterised by the order of Coleoptera. In terms of trophic specialisation, most species (59.8% of the total diversity) and the highest number (85.9%) were phytophagous insects. The main pests in the grass stand

of a hemp field were 39 species from 22 families and 6 orders. Most of the species (36 or 92.3%) belonged to polyphages, and three species (7.7%) were specialised with their share of numbers in the harmful entomocomplex – 18.7% and 81.3%, respectively. Biodiversity indices of phytophagous insects indicate an oligodominant structure of the entomocomplex. According to the degree of dominance, the study distinguished one eudominant – *Psylliodes attenuata* (81.1%), one sub-recedent – *Mordellistena parvula* (4.72%), four recedents (*Lygus pratensis*, *L. rugulipennis*, *Lygocoris pabulinus*, *Stictocephala bisonia*), which comprised a total of 8.6% and 33 sub-recedents with a share of 5.58% in the general structure of insect pests of the herbaceous agrobiocenosis of seed hemp.

The obtained research results will be used upon solving problems related to the danger of the main phytophagous insects during the growing season of cannabis plants and developing a modern environmentally oriented strategy for controlling their abundance and harmfulness.

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### Різноманіття ентомокомплексу травостою конопляного поля у північно-східному лісостепу України

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**Анотація.** Агробіоценоз травостою конопляного поля являє собою специфічний рослинний біотоп, який є місцем укриття, живлення, розселення та розмноження численних популяцій комах, які тою чи іншою мірою впливають на ріст, розвиток і врожайність рослин конопель. З огляду на це особливої актуальності набуває знання видового складу комах-шкідників, комплексу їх природних ворогів та нейтральних видів, які заселяють травостій конопляного поля, що необхідно для розробки ефективної екологічно орієнтованої системи захисту конопель посівних у сучасних умовах. Мета дослідження – удосконалення екологічно-орієнтованої системи захисту конопель посівних за рахунок вивчення таксономічного складу, чисельності загальної та шкідливої ентомофауни у травостої, а також трофічної й екологічної структури угруповань комах, які пов'язані з цим місцем існування. Дослідження проводились упродовж вегетаційних періодів 2019–2021 рр. в умовах науково-експериментальної бази Інституту сільського господарства Північного Сходу. Обліки комах здійснювали методом косіння стандартним ентомологічним сачком один раз у декаду з 10.00 до 15.00 години дня, коли комахи були найбільш активні. Сучасний таксономічний склад ентомокомплексу травостою конопляного поля представлений 174 видами комах, які належать до 76 родин і 9 рядів. Найбільшим за різноманіттям видового складу та чисельністю особин був ряд Coleoptera (56 видів з 16 родин та 74,6% від чисельності відловлених комах). Також виявлено комах з рядів Hymenoptera (31 вид з 15 родин), Hemiptera (30 видів з 11 родин), Diptera (20 видів з 12 родин), Homoptera (17 видів з 8 родин), Lepidoptera (12 видами з 8 родин), Orthoptera (4 види з 3 родин), Neuroptera (3 види з 2 родин), Thysanoptera (один вид). У трофічній структурі ентомофауни травостою конопляного поля 85,9% чисельності та 59,8% видового різноманіття припадає на комах-фітофагів. Шкідниками конопель посівних були 39 видів комах з 22 родин та 6 рядів. Поміж них 36 видів, що склали 18,7% від загальної чисельності, є поліфагами та три або 81,3% – спеціалізованими видами. Присутність комах-шкідників у травостої конопель характеризувалась олігодомінантністю, про що свідчать якісно-кількісні показники та індекси видового різноманіття. Так, структура домінування представлена одним еудомінантом (*Psylliodes attenuata* – 81,1%), одним субдомінантом (*Mordellistena parvula* – 4,72%), чотирма рецедентами (*Lygus pratensis*, *L. rugulipennis*, *Lygocoris pabulinus*, *Stictocephala bisonia* – 8,6%) та 33 субрецидентами (5,58%). Отримані результати досліджень будуть використані при вирішенні проблем, пов'язаних з небезпечністю основних комах-фітофагів під час вегетації рослин конопель посівних, та розробці сучасної екологічно-орієнтованої стратегії контролю їх чисельності й шкідливості

**Ключові слова:** агробіоценоз, видовий склад, трофічна структура, комахи-фітофаги, класи домінування



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## Comparative Study of Sodium-Dependent Glucose Co-Transporters in Kidneys of Ostrich Chickens

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**Abstract.** In the changing internal and external conditions, maintenance of a constant internal environment – homeostasis – plays a significant role in the proper functioning of the organism. Kidneys play a key role in the homeostasis of glucose, in which the sodium-dependent glucose co-transporters contribute to renal glucose reabsorption. Although the localisation of Na<sup>+</sup>-glucose co-transporters has been extensively covered in animals' kidneys, the localisation of the transporters in birds' kidneys is still understudied. The purpose of this study was to immunolocalise the sodium-dependent co-transporters SGLT1 and SGLT2 in kidneys of ostrich chickens of different ages. In the study, kidney material derived from fifteen ostriches was divided equally into three age groups – 1-, 7-, and 14-days-old ostrich chickens. The polyclonal antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) served as primary antibodies and were used together with the IHC kit (Abcam, UK). With the AxioCam HRc camera (Germany) connected to the microscope Zeiss Axioplan-2 Imaging (Germany), the photos were taken and saved to the computer. As the result of the study on ostrich chickens of different ages, SGLT1 was noted to be localised in the renal straight proximal tubules and SGLT2 in the proximal convoluted tubules of nephron. The immunohistochemical locations of sodium-dependent glucose co-transporters revealed to be similar in ostriches' kidneys of all age groups. The staining for SGLT2 was noted to be more intensive compared to the staining for SGLT1. As avian kidneys have unique morphological and functional features compared to animals, it is recommended that further studies would be performed on the renal tissue of different avian species

**Keywords:** SGLT1, SGLT2, chicken, immunohistochemistry, renal proximal tubules



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## INTRODUCTION

The central source of energy in the body is glucose (Hruby, 1997). Kidneys contribute to glucose homeostasis by filtering and reabsorbing glucose by Na(+)-coupled glucose transporters. The sodium-dependent glucose co-transporter 1 (SGLT1) and sodium-dependent glucose co-transporter 2 (SGLT2) are the members of the solute carrier 5 (SLC 5A) gene family (Mota *et al.*, 2015; Wright, 2021). SGLT1, encoded by the SLC 5A1 gene (Liang *et al.*, 2021), has been reported as the high affinity Na<sup>+</sup>-glucose co-transporter, while SGLT2 encoded by the SLC 5A2 gene – as the low affinity Na<sup>+</sup>-glucose co-transporter (Kanai *et al.*, 1994). In humans, SLC 5A1 gene encodes the production of the SGLT1 protein to line the epithelial cells of the kidney tubules of the nephron for glucose uptake into cells (Lodish *et al.*, 2016). SGLT2, found solely in kidney tubules, resorbs in conjunction with SGLT1 glucose into the blood from the forming urine (Sen & Heerspink, 2021). SGLT proteins use energy from a gradient of sodium ions generated by an ATPase pump to transport glucose across the apical membranes. SGLT1 and SGLT2 are known as symporters, as sodium and glucose are moved in the same direction across the cell membrane.

Based on literature, the exact localisation of SGLT1 and SGLT2 in the kidneys has been investigated by immunohistochemistry using antibodies to the cloned transporters (Cramer *et al.*, 1992; Kim, 2019; Vrhovac *et al.*, 2015). The locations of both transporters are well-established in mammals – SGLT2, responsible for reabsorption of 80-90% of the glucose filtered by the glomerulus, has been described to be located in the beginning parts of the proximal tubule (Wright, 2021; Bonora *et al.*, 2020), while SGLT1, responsible for the remaining glucose absorption, has been localised in more distal sections of the proximal tubule of the kidney (Sędzikowska & Szablewski, 2021; Uehara-Watanabe *et al.*, 2022). In greater details, the proximal tubule can be divided into two sections known as *pars convoluta* and *pars recta* based on particular functional differences. The convoluted part can be divided into two segments designated as S1 and S2. In case of such marking, the *pars recta* of the proximal tubule are marked as S3. While SGLT1 is only found in the apical membrane of the S3 segment, SGLT2 in mammals has been noted to localise in the proximal tubules of the S1 and S2 segments (Ghezzi *et al.*, 2018). The membrane proteins of SGLT1 and SGLT2 have been detected in the apical membrane of the renal tubular cells where they are responsible for glucose reabsorption from the glomerular filtrate in the proximal tubule.

Despite punctual description of Na<sup>+</sup>-glucose cotransporters in mammals' kidneys, there are still large knowledge gaps regarding the localisation of the SGLTs in birds' kidneys. This is explained by the fact that, unlike mammals, the avian kidneys have several unique morphological and functional features (Yang & Nishimura, 2021; El-Bakary *et al.*, 2015). Due to the scarce information available on glucose transportation in avian kidneys on the molecular level, *the purpose of*

*this study* was the immunohistochemical localisation of SGLT1 and SGLT2 in the kidneys of ostrich chickens of various ages in their first post-hatching weeks.

## MATERIAL AND METHODS

Fifteen female African Black ostriches (*Struthio camelus var. Domesticus*) reared at the ostrich farm Ozolini AB, located in Latvia's Jēkabpils district took part in the current study. The ostriches were kept in boxes with a heated sand floor. Commercial ostrich chick feed Strus Premium-Strus 1 (Cargill, Poland) and water *ad libitum* was available during the entire experimental period. Feed ingredients (%): barley – 36.8; oats – 10; wheat – 18.2; wheat bran – 5; rapeseed oil – 3; chalk – 2; soy pellets – 22; Dolfos StrusMix PS-3; Calculated chemical analysis, (%): Protein – 17.6, Carbohydrates – 37.2; Fat – 9.7; Cellulose – 5.6; Calcium – 0.8. Ostriches were divided into three age groups: 1-, 7-, and 14-days-old. Every experimental group comprised five birds. On day 1, 7, and 14, according to the experimental groups, ostriches were anaesthetised by intramuscular injection of 1 ml solution holding equal volumes of 10% ketamine and 2% xylazine to reduce the pain before euthanasia. Thereafter, euthanasia by intracardial injection with 0.5 ml of 20% pentobarbital was conducted.

Tissue sections 0.5-1.0 cm in diameter were removed from renal cortex and medulla, fixed in 10% neutral buffered formalin at room temperature for 48 h, dehydrated in a tissue processor (TISSUE-TEK II, Japan) and embedded into paraffin according to a standardised histological procedure for tissue processing (Carson, 1997). Thereafter, slices 6 µm thick were cut using the microtome Microm HM360 (USA), floated on Poly-L-Lysine coated slides (O. Kindler GmbH, Freiburg, Germany), dried at 44°C for 12 h, followed by deparaffinisation with xylene. The rehydration of the slices was carried out in a graded series of ethanol and the immunohistochemical staining using the Immunohistochemistry kit (IHC kit, Abcam, UK) according to the manufacturer's guidelines. The slices were pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6) for 20 minutes and incubated with primary polyclonal antibodies Rabbit anti-SGLT1 and Rabbit anti-SGLT2 (Abcam, UK) in 1/1,000 dilution for 30 min at 37°C. Biotinylated secondary antibody and streptavidin-conjugated peroxidase were used for detection using DAB (3,3'-diaminobenzidine tetrahydrochloride) as chromogen. Negative controls contained antibody diluent (Dako, S3002, Denmark) instead of primary antibodies. Human kidney tissue sections for identifying SGLT-2 and SGLT-1 were used as positive controls available for comparison on Abcam antibody producer's homepage as examples for the antibodies' immunohistochemistry on paraffin-embedded tissues (IHC-P) (Official website of Abcam, 2022).

The Zeiss Axioplan-2 Imaging Microscope (Germany) was established for photography of the slices. The photos were saved to the computer and analysed visually using the camera AxioCam HRC (Germany) connected to the microscope.

The experiments were performed according to the guidelines laid down by the European Communities Council Directive of 22 September 2010 (2010/63/EU) and the Ethical Committee of Latvia University of Life Sciences and Technologies has approved the experiments (protocol number 2014/2).

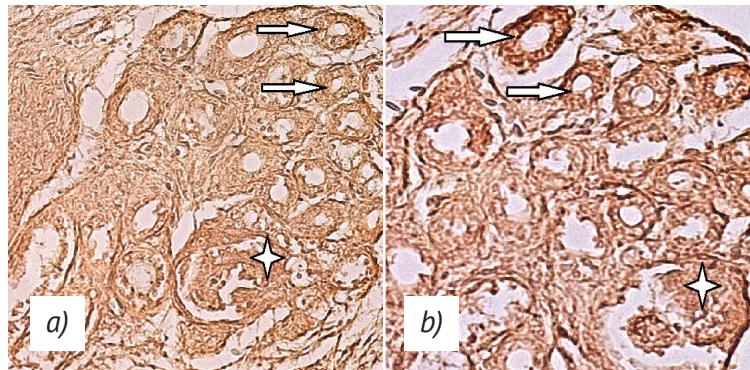
## RESULTS AND DISCUSSION

The immunohistochemical locations of SGLT1 and SGLT2 in kidneys of ostriches of different ages was performed. The immunohistochemical study revealed the

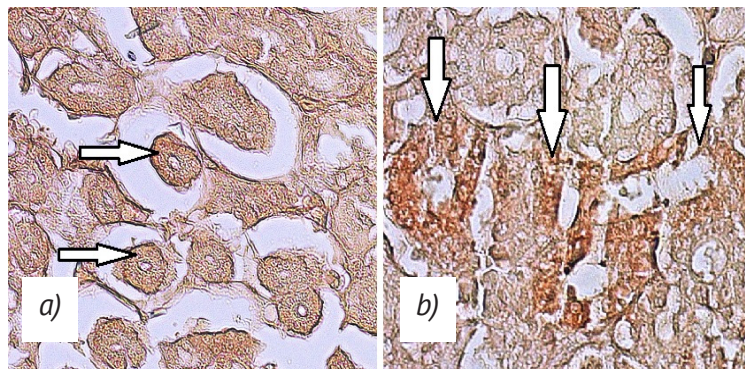
localisation of the both types of sodium-dependent glucose co-transporters in chicken of all age groups – 1-, 7-, and 14-days old ostriches. Compared visually, in different experimental age groups the staining intensities and localisation of SGLT1 and SGLT2 appeared to be similar in the kidneys of ostrich chickens.

### Immunolocalisation of SGLT 1

The staining for SGLT1 was noted to be poor in renal corpuscles and in most of the renal tubules, as demonstrated in Figures 1a, 2a.



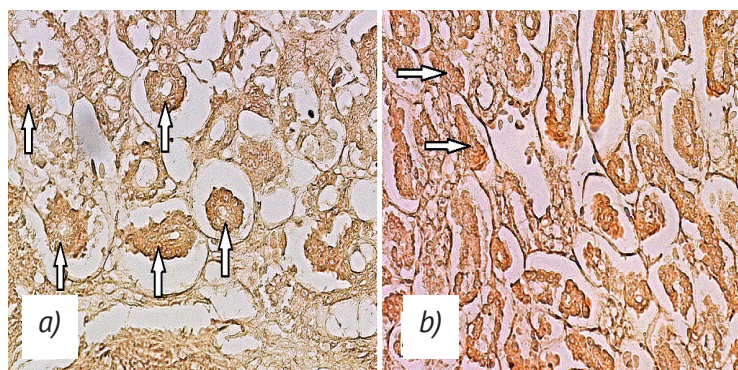
**Figure 1.** The immunolocalisation of SGLT1 and SGLT2 in 1-day-old ostrich chickens' kidneys: a) SGLT1 immunolocalised in renal proximal tubules (arrows). Note the poorly stained renal corpuscle (asterisk). Obj. 200x; b) Renal cortical proximal tubules strongly stained for SGLT2 (arrows) of the 1day old ostrich chickens' kidneys. Renal corpuscles poorly stained for SGLT2 (asterisk). Obj. 200x



**Figure 2.** The immunolocalisation of SGLT1 and SGLT2 in 7-days-old ostrich chickens' kidneys: a) SGLT1 immunolocalised in renal proximal tubules in medullary rays (arrows). Obj. 200x; b) SGLT2 (arrows) immunolocalised strongly in renal cortical proximal tubules of the 7-days-old ostrich chickens' kidneys. Obj. 200x.

Strong staining for SGLT1 was detected in the proximal tubules in medullary rays as well as in the

tubules of the outer stripe of renal medulla (Figures 2a, 3a).



**Figure 3.** The immunolocalisation of SGLT1 and SGLT2 in 14-days-old ostrich chickens' kidneys: a) SGLT1 immunolocalised in in the epithelial cells of the tubules of the outer stripe of renal medulla (arrows). Obj. 200x; b) Strong staining for SGLT2 (arrows) in renal cortical proximal tubules of the 14 days old ostrich chickens' kidneys. Obj. 200x

### Immunolocalisation of SGLT2

Strong staining for SGLT2 was detected to be in the renal cortical proximal convoluted tubules (Figs. 1b, 2b, 3b) in chicken of different ages. Compared the intensities of the staining for SGLT1 and SGLT2, the staining for SGLT2 was noted to be more intensive.

Based on the branching pattern of the ureter, the avian kidney can be divided into renal lobes and renal lobules (Liebich, 2019). The medullary region, which drains into the secondary branches of the ureter together with the region of the cortex drained by that medullary tissue, forms the renal lobes. Including both the medullary tissue and the cortical tissue that it drains, each of the renal lobes is composed of several renal lobules. The renal lobules drain into ureter's tertiary branches, which combine and form the secondary ureteral branches. Enclosing blood vessels and loops of Henle of juxtamedullary nephrons, the renal lobule's medullary parts comprise bundles of collecting tubules (*tubuli colligentes medullares*). As in histological sections of avian kidney's the renal lobes and lobules are seen at various levels, the cortical and medullary regions appear like intermingled. Like in mammals, nephrons are the smallest functional units of the kidney consisting of renal corpuscles and tubular apparatus. However, whilst the renal corpuscles are considerably smaller than in mammals, there is a higher amount of corpuscles per volume unit of kidney tissue (Koenig et al., 2016). The avian kidneys, divided into two zones, the cortex and medulla, have two types of nephrons unlike mammals, who have one type. These "reptilian-type" nephrons are located in the cortex without Henle's loop, and the "mammalian-type" nephrons are in the medulla and have a Henle's loop (Casotti & Braun, 2000; Cazimir et al., 2008). In birds that have a high ability for water conservation, the medullary regions (and thus the loops of Henle) are particularly well-developed, with each medullary region draining only a relatively small area of cortex. This arrangement presumably allows for production of more concentrated urine. The ability to produce hyperosmotic urine in avians is limited compared to that of mammals (Sjaastad et al., 2016). While loop nephrons produce concentrated urine, their contribution to ureteral urine is made by nephrons without loops (Ghezzi et al., 2018). Histologically, large cortical areas with small medullary islands can be identified. The number of medullary islands varies in different breeds of birds (Nickel et al., 2004).

In the organism, the kidneys are the main excretory and osmoregulatory homeostatic organs (Imenez Silva & Mohebbi, 2022). Kidneys contribute to the organism's homeostasis by removing the metabolic waste substances from blood and their excretion in the urine, which prevents toxic waste products from accumulating in the body, and by regulating the inorganic ion and water balance through filtering water and harmful substances from blood (Yang & Nishimura, 2021). In the healthy kidneys, the total amount of the filtered glucose in the glomerulus has to be reabsorbed along the nephron (Mota et al., 2015). Sudden decrease in renal excretion, e.g., during acute renal impairment, is accompanied by retention of uremic toxins and metabolic

residues in plasma and impaired regulation of fluid and electrolyte homeostasis, causing high morbidity and mortality (Nespoux et al., 2019). SGLT1 and SGLT2 take part in the renal glucose reabsorption from the glomerular filtrate.

The earlier research has shown that in healthy mammals, glucose is filtered by the renal glomeruli and the filtered glucose is reabsorbed in the tubular system mainly by the proximal tubules (tubuli proximales) (Nespoux et al., 2019). In the S1/S2 segments of the proximal tubules, apical low-affinity Na<sup>+</sup>-glucose co-transporter SGLT2 handles the major glucose uptake to the cytoplasm from the lumen (Vallon et al., 2011; Umino et al., 2018). The glucose that was not resorbed by SGLT2 reaching to the proximal tubule's S2/S3 segment will be resorbed by SGLT1, GLUT1, and GLUT2 (Rieg et al., 2014). As the SGLT-mediated glucose transport depends on Na<sup>+</sup> concentration gradient, SGLT1 serves as a minor active transporter. Together with SGLT1, SGLT2 resorbs glucose from blood-forming urine. It has been noted that of all tubular glucose reabsorption, SGLT1 accounts for about 10% (Koepsell & Vallon, 2020). In rats, SGLT1 has been identified on the apical side of the epithelial cells of the renal proximal tubules. In mammals' kidneys, SGLT1 and SGLT2 have been localised in the S1/S2 and S3 segments of the proximal tubules, noted in the brush border membranes of the tubular epithelial cells. Comparing the results previously described for immunolocalisation of the Na<sup>+</sup>-glucose co-transporters SGLT1 and SGLT2 in mammalian kidney tissue with current data in ostrich kidneys, immunolocalisation was found to be similar (Sen & Heerspink, 2021; Vallon et al., 2011; Sano et al., 2020).

When comparing the intensity of SGLT1 and SGLT2 staining, it was noted that SGLT2 staining was more intense compared to the intensity of SGLT1 staining, which is due to greater reabsorption of glucose through SGLT2 in the nephron (Vallon et al., 2011).

### CONCLUSIONS

In the current immunohistochemical study in ostrich chickens, localisation in the kidneys of SGLT1 and SGLT2 was revealed. The immunolocalisation of SGLT1 and SGLT2 in chickens of different ages was similar: SGLT1 was immunolocalised on the apical side of the epithelial cells of the direct proximal tubules in the brain rays (corresponding to the S3 segment), while SGLT2 was immunolocalised in the convoluted proximal tubules in the cortical region (corresponding to the S1/S2 segments). Due to the lack of knowledge about Na<sup>+</sup>-glucose co-transporters in avian kidneys, it would be highly informative to conduct additional comparative studies on the immunolocalisation of sodium-dependent glucose co-transporters in ostrich kidneys, as well as in the kidney tissue of other bird species, since avian kidneys have many unique morphological and functional features compared to animals.

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## Порівняльне дослідження натрій-залежних котранспортерів глюкози в нирках страусових куриць

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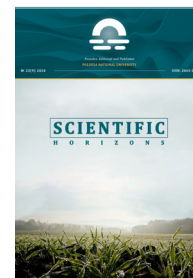
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**Анотація.** У змінних внутрішніх та зовнішніх умовах підтримання постійної внутрішньої середовища – гомеостазу – грає головну роль правильному функціонуванню організму. В організмі нирки відіграють важливу роль у гомеостазі глюкози, при цьому натрійзалежні котранспортери глюкози сприяють реабсорбції глюкози у нирках. Хоча локалізація котранспортерів Na<sup>+</sup>-глюкози у нирках тварин широко охарактеризована, досі недостатньо інформації про локалізації транспортерів у нирках птахів. Метою цього дослідження була імунолокалізація натрійзалежних котранспортерів SGLT1 і SGLT2 у нирках курчат страусів різного віку. Під час дослідження нирковий матеріал отримано від 15 страусів, розділених порівну на три вікові групи – 1-добові, 7-денні та 14-денні страусові курчата. Матеріал діаметром 0,5–1,0 см фіксували в 10 % забуференому нейтральному формаліні, зневоднювали, заливали в парафін; після цього вирізали і депарафінізували зрізи товщиною 6 мкм з подальшим імуногістохімічним забарвленням поліклональними первинними антитілами Rabbit anti-SGLT1 і Rabbit anti-SGLT2 (Abscam, Великобританія) відповідно до рекомендацій виробника (IHC kit, Abscam, UK). Фотографії препаратів були зроблені мікроскопом Zeiss AxioPlan-2 Imaging (Німеччина) та збережені на комп'ютер для аналізу під візуальним контролем за допомогою камери (AxioCam HRc, Німеччина), підключеної до мікроскопа. У нашому дослідженні виявлена імуногістохімічна локалізація SGLT1 у епітеліальних клітинах прямих проксимальних канальців мозкових променів та SGLT2 у проксимальних звивистих канальцях нефрону. Виявлено подібність імуногістохімічної локалізації натрійзалежних котранспортерів глюкози у нирках страусів усіх вікових груп. Відзначено, що фарбування SGLT2 було більш інтенсивним, ніж фарбування SGLT1. Оскільки нирки птахів мають унікальні морфологічні та функціональні особливості в порівнянні з тваринами, рекомендується проводити подальші дослідження ниркової тканини різних видів птахів

**Ключові слова:** SGLT1, SGLT2, курка, імуногістохімія, проксимальні канальці нирок



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## Anaerobic Fermentation of Chicken Manure and Methods for Intensifying Methane Output

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**Abstract.** A considerable share in the formation of food security of the population is occupied by the poultry industry, which is one of the most economically attractive and competitive, as evidenced by the annual steady growth dynamics of the production of valuable food products – poultry meat and eggs, characterised by high nutritional value, excellent dietary and taste qualities. The increase in poultry production leads, respectively, to the intensification of production on the one hand, and on the other hand – the accumulation of waste due to an increase in the number of poultry. One of the priority areas for solving the problem of environmental safety in the poultry industry is the processing of animal by-products through enzymatic fermentation, which yields an added energy product and organic-mineral fertiliser, thereby preventing methane emissions into the atmosphere, and therefore global warming. Therefore, the search for ways to intensify the methane output from chicken manure upon anaerobic fermentation, namely by adding various substances, was the purpose of the planned study. The experiment was conducted using laboratory, analytical, and mathematical-statistical methods. According to the results of experimental studies, a positive effect of FeO, Fe<sub>2</sub>O<sub>3</sub> was established, *Basidiomycota* fungal-based bio-compositions, biologics – Meganit Nirbator, Reduklin T, Reduklin Compost and a complex preparation for activating enzymatic processes in chicken manure on the processes of anaerobic bio-fermentation and growth of methane content (CH<sub>4</sub>) from chicken manure (*in vitro*) against the background of an increase in the pH value to 9.05-9.3 with a simultaneous lower level of carbon dioxide (CO<sub>2</sub>). Best results for increasing the volume of CH<sub>4</sub> output from the fermented substrate, by 15.7-18.8%, was observed in variants with a complex preparation for activating enzymatic processes in chicken manure. Application of *Basidiomycota* fungal-based bio-composition contributes to an increase in methane emissions from the test substrate by 5.4-9.6%, and biologics – Meganit Nirbator, Reduklin T and Reduklin Compost cause an increase in the volume of this gas output, respectively, by 5.6-9.4%, 9.5-14.2%, and 7.1-12%. Adding FeO and Fe<sub>2</sub>O<sub>3</sub> to chicken manure causes an increase in the level of CH<sub>4</sub> emissions by 4.1-7.4% and 5.8-11.2%, respectively. Thus, the results obtained indicate the expediency of using the studied substances in the processing of chicken manure in biogas plants to intensify the methane yield, which will minimise the adverse impact of intensive management of the poultry industry on the state of the environment

**Keywords:** poultry industry, animal by-products, methanogenesis, test substances, greenhouse gases



## INTRODUCTION

Agriculture constitutes a priority sector of the Ukrainian economy, which allows obtaining considerable amounts of food, both for saturating the Ukrainian market and for exporting abroad (Zakharchenko, 2107). The products of agricultural enterprises are the key to food security and independence of the country (Zakharchenko, 2107; Selivestrova, 2018). In the overall structure of agriculture, animal husbandry is a strategically important industry, as it accounts for more than 38% of gross output (Selivestrova, 2018; Khodorchuk *et al.*, 2014). The development of animal husbandry, on the one hand, provides the population with high-quality, high-calorie, and dietary food products, and the crop industry, namely agriculture – with organic fertilisers, and on the other hand – leads to an increase in the anthropogenic load on the environment, and therefore adversely affects the state of public health (Demchuk *et al.*, 2010; Palapa *et al.*, 2016).

One of the most promising branches of animal husbandry is poultry farming, the share of which in the industry structure is quite considerable and accounts for about 40-65% of the total production of livestock products and is also one of the main producers of animal protein necessary for the human body (Fudrycko *et al.*, 2019). Today, poultry farming is the only industry in Ukraine that has been increasing its capacity over the past decade (Selivestrova, 2018; Boroday *et al.*, 2017). However, the growth of the poultry population in poultry farms is accompanied not only by an increase in the volume of the main products – eggs and meat, but also by the development and accumulation of animal by-products in a much larger amount, including chicken manure (Fudrycko *et al.*, 2019; Boroday *et al.*, 2017; Zhukov, 2016). One bird releases 1.1-1.5 times more manure per day compared to the amount of feed it consumes during this period (Skliar *et al.*, 2018). It is known that during the year, 250-300 eggs are obtained from the laying hen, i.e., 15-18 kg of egg mass, which exceeds its weight by more than five times, while during this period the hen releases 40-65 kg of manure with a humidity of 65-75% (Zhukov, 2016). Thus, considering the above, the poultry industry is one of the environmental pollutants, since the use of manure without appropriate preparation as fertilisers adversely affects the ecological state of territories, which carries a direct biological threat due to the presence of pathogens of infectious and especially invasive diseases (Demchuk *et al.*, 2010; Shevtsova & Solohub, 2019; Bolan *et al.*, 2010). As a result, ecosystems in the zone of functioning of poultry farms gradually lose their biological balance and ability to self-regulate, and they experience a sharp deterioration in the growth and development of flora and fauna (Boroday *et al.*, 2017). Forests and fields adjacent to poultry enterprises are beginning to act as distribution centres for pathogenic microorganisms and heavy metals, etc., instead of a buffer function (Shevtsova & Solohub, 2019). Thus, unsatisfactory storage and irrational use of manure not only causes considerable damage to the environment, polluting land reservoirs, soils, and groundwater, but also causes the loss of an enormous amount

of high-quality organic fertiliser needed for agricultural land (Demchuk *et al.*, 2010; Shevtsova & Solohub, 2019; Bolan *et al.*, 2010). Long-term accumulation of waste causes intensive fermentation processes, as a result of which gaseous aeropolutants – methane, carbon dioxide, etc. – evaporate into the atmosphere and enter in large quantities (Khodorchuk *et al.*, 2014; Caro, 2019; Rubežius *et al.*, 2020).

The growing concentration of greenhouse gases in the atmosphere increases the greenhouse effect, adversely affects natural processes due to intensive retention of thermal radiation, which contributes to excessive warming of the planet, an increase in the number of natural disasters and cataclysms (desertification, landslides, hurricanes), etc. (Khodorchuk *et al.*, 2014; Vorobel *et al.*, 2021; Monteny *et al.*, 2001). Climate change due to the greenhouse effect is one of the most global environmental problems of modern times (Khodorchuk *et al.*, 2014; Demchuk *et al.*, 2010; Caro, 2019). As a result of the analysis of literature sources, it was found that the potential of methane influence on atmospheric heat retention is 21-34 times stronger than carbon dioxide, and therefore, in conditions of increasing the average annual temperature, CH<sub>4</sub> emission into the atmosphere worsens these conditions (Caro, 2019; Mitkov *et al.*, 2012; Asgedom & Kebreab, 2011). At the same time, methane can remain in the atmosphere for up to 12 years (Mitkov *et al.*, 2012). Hence, reducing the CH<sub>4</sub> output is more effective in preventing climate change than reducing CO<sub>2</sub> emissions. The concentration of methane in the atmosphere has more than doubled over the past two centuries, and carbon dioxide has increased by more than 25% (Binkovska & Shanina, 2016). Thus, in modern conditions, due to the active development of the poultry industry, and therefore a considerable amount of waste accumulation, which, albeit a valuable raw material, is not always used, the urgency of the problem of environmental protection increases. Considering the above, the issue of reducing greenhouse gas emissions from animal by-products is relevant, which requires thorough research and constitutes an essential aspect in the functioning of agro-industrial enterprises.

Anaerobic bio-fermentation of organic animal waste, namely poultry farming is an effective and rational way of their neutralisation, processing, and disposal in modern economic conditions, and therefore the vector of solving problems of eliminating the available and preventing further environmental pollution and ensures waste-free production, i.e., it allows increasing the quantity and improving the quality of organic fertiliser and obtaining an additional energy carrier – biogas, the main component of which is methane (Soluk *et al.*, 2015; Dere *et al.*, 2017; Wang *et al.*, 2019). The use of animal by-products due to the presence of a considerable raw material potential suitable for fermentation in the agricultural sector as alternative energy sources is one of the promising, environmentally friendly and energetically profitable areas of bioenergy today, reducing the amount

of pollutants released into the atmosphere (Panchuk & Shlapak, 2016; Abbasi *et al.*, 2012; Ulusoy *et al.*, 2021). Processing of organic waste from poultry farms by biological fermentation is of great environmental importance, since all chemicals contained in organic waste are completely disposed of (Mitkov *et al.*, 2012). Furthermore, the bio-fermentation process allows destroying the pathogenic microflora and weed seeds, i.e., improves the properties of manure as fertilisers, thereby minimising water, air, and soil pollution (Demchuk *et al.*, 2010; Mitkov *et al.*, 2016; Binkovska & Shanina, 2016).

Methane fermentation is a multi-stage process of microbiological transformation of an organic substrate carried out by a complex consortium of anaerobic bacteria in an oxygen-free environment to final products, mainly methane (55-70%) and carbon dioxide (30-45%) and less than 1% of other gases (hydrogen sulphide, ammonia, aromatic hydrocarbons, etc.) (Dere *et al.*, 2017; Adekunle & Okolie, 2015; Ziemiński & Frąć, 2012). First, during hydrolysis, under the action of microorganisms, high-molecular organic compounds of raw materials (proteins, lipids, polysaccharides) are destroyed to low-molecular fatty acids and alcohols, and then they are oxidised by acetogenic bacteria to form organic acids (lactic, propionic, acetic, etc.) (Demchuk *et al.*, 2010; Soluk *et al.*, 2015; Panchuk & Shlapak, 2016). Hydrolysis also produces hydrogen and carbon dioxide. The above-mentioned compounds, as well as the organic substrate, serve as further nutrient substrates for the development of methane-forming bacteria (methanogens), which carry out the final stage of fermentation-methane synthesis (Abbasi *et al.*, 2012; Ziemiński & Frąć, 2012; Shtatskyi *et al.*, 2013). It is at this stage that 90% of all methane is synthesised, and 70% of it is formed from acetic acid, and therefore the level of the latter constitutes a factor determining the rate of methane formation (Soluk *et al.*, 2015; Shtatskyi *et al.*, 2013). Notably, upon methane fermentation, up to 83% of the energy of fermented glucose is preserved, and therefore methanogenesis is the most energy-efficient way to transform the energy of organic substances (Shtatskyi *et al.*, 2013).

A promising substrate to produce biogas is chicken manure, due to the increased content of organic matter and the inherent greater ability to biological decay, compared to other animal waste (Dere *et al.*, 2017). Given this, the poultry industry is not only a producer of environmental pollution, but also a potential donor of alternative energy due to the use of organic waste biomass, which allows transforming the manure from harmful to the environment to profitable and useful related products, thereby ensuring high competitiveness and profitability of the industry, turning it into a highly efficient sector of the economy.

Thus, if the reduction and increase in the number of animals is suspended, biogas can become a relatively inexpensive alternative to natural gas, and compared with other renewable energy sources, it is very flexible in use, namely it is used in three important areas – the production of heat, electricity, and fuel. Thus, considering the relevance of ensuring timely and proper disposal

of animal by-products in the conditions of intensification of poultry farming, it becomes relevant to develop and search for effective means and methods to activate methanogenesis, which will speed up the fermentation processes of waste, thereby contributing to the growth of production of renewable energy resources, obtaining valuable humus, and ensuring the main component of value-preservation and protection of the environment, and therefore minimise the consequences of global warming.

*The purpose of this study* was to investigate the anaerobic fermentation of chicken manure (*in vitro*) and establish the effectiveness of the influence of the substances under study on the intensification of methane output, which directly determines the value of biogas.

## MATERIALS AND METHODS

Experimental studies on the effectiveness of the influence of the substances under study on the emission of greenhouse gases – methane and carbon dioxide from chicken manure, were carried out using laboratory methods – to determine the level of greenhouse gas release; analytical methods – to analyse and justify the results obtained; mathematical and statistical methods – to assess the reliability of research results. The study is based on the methodology of O.H. Skliar, R.V. Skliar, and S.M. Hryhorenko (2019). Sampling of the substrate under study, the chicken manure without bedding, was carried out in the farm “Zakhid-Ptytsia” of the Lviv Oblast. The methane fermentation process was carried out *in vitro* using sealed containers to maintain hermeticity and ensure anaerobic conditions. To maintain the stability of the bio-fermentation process and avoid its inhibition by ammonium nitrogen and sulphides, biomass (chicken manure) was diluted with water to a humidity of 92%, since it is known that for the effective anaerobic fermentation, and therefore an increase in methane yield, high humidity substrates are necessary. During the experiment, the fermented mass was mixed by periodically shaking the containers to destroy and prevent the formation of a crust, which leads to a delay in the release of gases. The bio-fermentation conditions were identical, both in the control version, where anaerobic digestion of the substrate was carried out at the expense of the natural microflora of the manure, and in experimental analogues with the introduction of the substances under study.

Experimental studies were carried out within 26 days, and after the stages of hydrolysis, oxidation, and acetogenesis at the beginning of methane formation (day 17), the substances under study were added to fermented chicken manure – methane generating raw materials in an effective pre-established and economically justified optimal dose of 3%: Variant I – control (without adding substances); II – FeO, 9 g; III – Fe<sub>2</sub>O<sub>3</sub>, 9 g; IV – *Basidiomycota* fungal-based bio-composition (Institute of Agroecology and Nature Management), 9 ml; V – biological product Meganit Nirbator (PE “Eksinvest”), 9 ml; VI – biological product Reduklin T (PE “Agro-Admiral”), 9 ml; VII – biological product

Reduklin Compost (PE "Agro-Admiral"), 9 ml; VIII – complex preparation for activating enzymatic processes in chicken manure (Institute of Agriculture of the Carpathian region), 9 ml. During the experiment in the *in vitro* conditions, on the 17<sup>th</sup> day and every three days, the level of greenhouse gas emissions from the substrate under study (CH<sub>4</sub>, CO<sub>2</sub>) was determined in control and in experimental analogues. The released amount of the gases under study upon anaerobic fermentation of chicken manure was measured using a portable gas analyser – Dozor S-M-5 (Certificate of verification of the device type UA.TR.001 212-18 and certificate of conformity UA.TR.002.CB.1234-19). In the experiment, the acidity of the test substrate was also determined (at the beginning of experimental studies and after completion) using the Tur n5170 pH meter device.

Statistical analysis of the obtained research results was carried out using a standard package of statistical software, *Microsoft EXCEL* and *AtteStat* using variational statistics methods. Arithmetic mean values (M) and their errors (m) were calculated. The difference between the arithmetic mean values was considered statistically significant at: \* $P < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

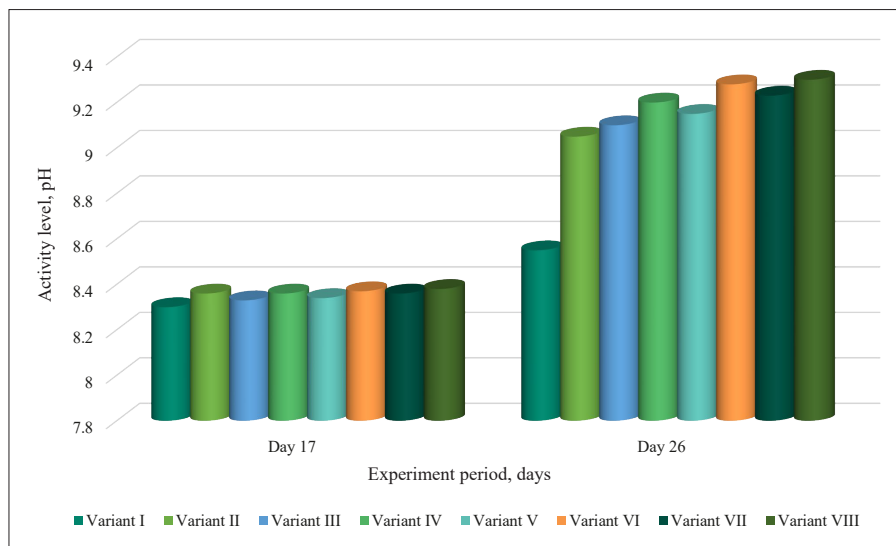
## RESULTS AND DISCUSSION

To ensure maximum efficiency of the anaerobic fermentation process, i.e., active reproduction and enzymatic activity of microorganisms, it is necessary to maintain optimal conditions, namely the temperature regime and acidity (pH) of the medium, etc. (Dere *et al.*, 2017; Adekunle & Okolie, 2015; Ziemiński & Frąc, 2012). According to several studies, it has been established that the amount of methane produced during anaerobic digestion is largely determined by temperature (Dere *et al.*, 2017; Ziemiński & Frąc, 2012; Polishchuk *et al.*, 2013). In particular, the minimum temperature at which methanogenesis occurs is 6°C, while its value below 0°C is maintained by microorganisms, but the metabolic process completely stops (Vorobel *et al.*, 2021; Soluk *et al.*, 2015; Ziemiński & Frąc, 2012). An increase in temperature contributes to a higher rate and degree of destruction of organic raw materials, but at a level of more than 70°C, methane-forming microorganisms die, and at a value above 45°C, the methane concentration in the total volume of gases decreases, despite the improvement in the conditions of their development (Skliar *et al.*, 2018; Soluk *et al.*, 2015; Polishchuk *et al.*, 2013). The above can be explained by the fact that at elevated temperatures, carbon dioxide dissolved in the substrate is more intensively converted to a gaseous state and released, and the more of it passes into a gaseous

form, the lower the proportion of methane will be (Polishchuk *et al.*, 2013). In addition, anaerobic fermentation at elevated temperature increases the sensitivity of microorganisms to its permissible fluctuations, which causes a decrease in their metabolic activity and ability to reproduce, so sharp changes in this indicator are not desirable (Ziemiński & Frąc, 2012; Polishchuk *et al.*, 2013). Considering the above, experimental studies were carried out at a temperature within 33°C, i.e., under the mesophilic regime, which is described by the highest stability of anaerobic digestion and minor temperature fluctuations are allowed without disrupting the process.

The duration of substrate bio-fermentation is also one of the key indicators affecting the properties of manure, their digestibility, reduction of toxic substances and harmful microorganisms, etc. (Skliar *et al.*, 2018; Soluk *et al.*, 2015). In particular, the fermentation exposure is set depending on the temperature regime within the following limits: at 10-25°C for 30 days, at 25-40°C – for 10-20 days, at 45-55°C – for 4-8 days (Adekunle & Okolie, 2015; Polishchuk *et al.*, 2013). In this experiment, the duration of the substrate bio-fermentation process was 17 days.

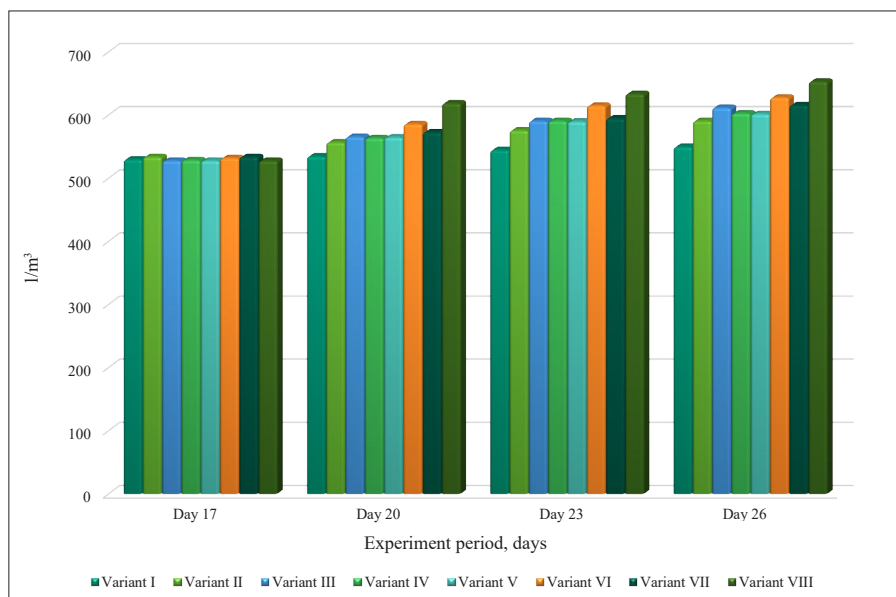
The stability of methanogenesis is considerably influenced by the hydrogen index, which determines the vector of anaerobic fermentation (Dere *et al.*, 2017; Ziemiński & Frąc, 2012; Polishchuk *et al.*, 2013). According to literature sources, it is known that a low pH level inhibits the growth of methanogenic bacteria and reduces the methane yield, specifically when this indicator decreases below 6.5, the gas yield worsens by 30-40%, and the development of methane microflora almost completely stops at pH 6.0 (Polishchuk *et al.*, 2013). In this experiment, upon determining the pH in the control variant (without adding substances) of chicken manure before and after the study (*in vitro*) was completed it was found that this indicator was within 8.35-8.55, i.e., it had an alkaline reaction of the medium. During experimental studies, there was a change in the acidity index. During the period of the bio-fermentation process in the experimental variants, after the introduction of the substances under study into the substrate, an increase in the enzymatic activity of the fermented mass was observed, and after the studies were completed, the pH level increased to 9.05 in variants using FeO, to 9.1 – using Fe<sub>2</sub>O<sub>3</sub>, to 9.2 – using *Basidiomycota* fungal-based bio-compositions, to 9.15 – using Meganit Nirbator biologics, to 9.28 – using Reduklin T biologics, to 9.23 – using Reduklin Compost biologics, to 9.3 – using a complex preparation for activating enzymatic processes in chicken manure (Fig. 1).



**Figure 1.** The level of acidity of chicken manure in variants using the substances under study

Therefore, maintaining optimal parameters of methane fermentation is a substantial factor determining the yield of biogas, the speed of the process and the methane content, and ultimately the efficiency of processing organic raw materials. At the same time, a promising area for intensifying the methane fermentation process is the use of stimulants that help improve the vital activity

of microorganisms that carry out transformation processes. The analysis of the obtained results shows that the substances under study, which contributed to the highest pH indicator in the fermented chicken manure, and therefore to the acceleration of bio-fermentation processes, simultaneously showed the most effective influence on the methane output increase (Fig. 2).



**Figure 2.** Volume of methane output from chicken manure in variants using the substances under study

The data obtained from experimental studies indicate that the intensity of the anaerobic fermentation process and the emission of greenhouse gases – methane and carbon dioxide from the substrate of chicken manure is determined by the duration of the experiment. With increasing fermentation time, the methane content increases, which indicates an improvement in the biogas quality.

According to the results of the conducted studies, it was established that upon anaerobic fermentation of chicken manure (in vitro) in variants using FeO,

the methane yield from the test substrate increases, depending on the day of the experiment, namely: on day 20-22 l/m<sup>3</sup> (P<0.01) or 4.1%; on day 23-31 l/m<sup>3</sup>-5.7%; on day 26-41 l/m<sup>3</sup>, i.e., 7.4%, relative to the control analogue. Introduction of Fe<sub>2</sub>O<sub>3</sub> to fermented chicken manure causes an increase in greenhouse gas emissions, such as methane, relative to the control variant, depending on the day of research, namely: by 31 l/m<sup>3</sup> (P<0.001), i.e., 5.8% – on day 20; by 46 l/m<sup>3</sup> (P<0.01) or 8.5% – on day 23; by 62 l/m<sup>3</sup> (P<0.01) or 11.2% – on day 26.

Analysis of the data obtained during this study shows that the addition of the *Basidiomycota* fungal-based bio-composition to the substrate under study upon anaerobic fermentation (*in vitro*) promotes an increase in the volume of CH<sub>4</sub> output for 20-26 days, respectively, by 29-53 l/m<sup>3</sup> (P<0.05), which is 5.4-9.6% as a percentage compared to the control variant.

According to the results of studies, it was found that upon using the biological product Meganit Nirbator, there is an increase in the level of methane emissions from fermented chicken manure, relative to the control analogue, depending on the day of research, respectively: on day 20-30 l/m<sup>3</sup> (P<0.05), i.e., 5.6%; on day 23-45 l/m<sup>3</sup> (P<0.05) or 8.3%; on day 26-52 l/m<sup>3</sup> (P<0.01), which is 9.4%.

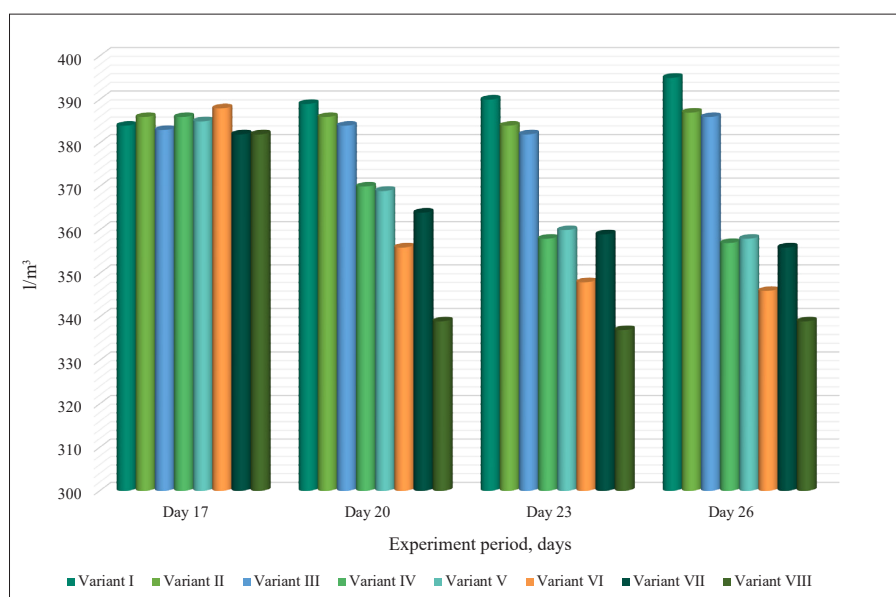
In variants with the introduction of Reduklin T into the test substrate, the volume of CH<sub>4</sub> output increases depending on the day of the experiment, namely: by 51 l/m<sup>3</sup> (P<0.05) or 9.5% – on day 20; by 70 l/m<sup>3</sup> (P<0.05) or 12.8% – on day 23; by 78 l/m<sup>3</sup> (P<0.05) or 14.2% – on day 26 of the experiment, compared to the control variant. Based on the conducted studies, it was found that adding the biological product Reduklin Compost to fermented chicken manure upon anaerobic fermentation (*in vitro*) causes an increase in methane emissions by 20-26 days, respectively, by 38-66 l/m<sup>3</sup> (P<0.05-0.01), i.e., 7.1-12%, relative to the control variant.

During the same period of experimental studies (20-26 days), the volume of CH<sub>4</sub> output from the test substrate upon using a complex preparation to activate enzymatic processes in chicken manure, it was higher than the control, respectively, by 84-103.5 l/m<sup>3</sup> (P<0.05-0.001), which is 15.7-18.8%.

Thus, considering the obtained research results, the highest level of methane released from fermented chicken manure in all experimental versions is observed on the 26<sup>th</sup> day of the experiment – by 7.4-18.8%. At the same time, the most pronounced effect (15.7-18.8%) on the growth of the CH<sub>4</sub> output from the test substrate, a complex preparation for activating enzymatic

processes in chicken manure was developed during the experiment. The intensification of the process of bio-fermentation of chicken manure and the highest level of methane in the experimental variants under study is probably conditioned upon the wide component composition of bio-stimulants that provide methanogenic microorganisms with a sufficient amount of required nutrients, including mineral elements (Demirel & Scherer, 2011). The above is confirmed by the studies of several researchers on the crucial role of mineral elements (Fe, Ni, Co, Mo, W, and Se), enzymes, microorganisms in the growth and metabolism of methanogens and for further acceleration of methane biosynthesis, as well as improving the stability of anaerobic fermentation (Zhang et al., 2013; Christy et al., 2014; Romero-Güiza et al., 2016). A mineral element such as Fe increases the production of acetate and at the same time can directly serve as an electron donor for the reduction of CO<sub>2</sub> to CH<sub>4</sub> (Romero-Güiza et al., 2016). The results obtained during the experiment on increasing the efficiency of methanogenesis, namely the formation of methane from the fermented substrate, are consistent with the studies of E. Abdelsalam et al. (2015), conducted in laboratory conditions using chlorine compounds CoCl<sub>2</sub>, NiCl<sub>2</sub> and FeCl<sub>3</sub>. Researchers J. Ahamed et al. (2016), R. Zeng et al. (2021) and J. Pan et al. (2019) obtained comparable results regarding the stabilisation of the anaerobic process, and consequently an increase in the yield of biogas with a high methane content from poultry industry waste using silica gel and biochar.

Analysis of experimental data shows that simultaneously with an increase in methane levels upon anaerobic fermentation of chicken manure (*in vitro*) in all experimental versions, carbon dioxide emissions are reduced (Fig. 3). When Fe compounds are added to the test substrate in the forms of FeO and Fe<sub>2</sub>O<sub>3</sub> for 20-26 days, there is a decrease in the volume of CO<sub>2</sub>, respectively, by 3-9 l/m<sup>3</sup>, i.e., 0.8-2.3%.



**Figure 3.** Volume of carbon dioxide output from chicken manure in variants using the substances under study

Introduction of *Basidiomycota* fungal-based bio-compositions to fermented chicken manure according to the mesophilic regime of anaerobic fermentation (*in vitro*) helps reduce the carbon dioxide release, depending on the day, respectively, by 19 l/m<sup>3</sup> (P<0.01) or 4.9% – on day 20; by 32 l/m<sup>3</sup> or 8.2% – on day 23; by 38 l/m<sup>3</sup> (P<0.01) or 9.6% – on day 26, relative to the control variant.

Based on the conducted studies, it was found that the use of Meganit Nirbator biologics causes a decrease in CO<sub>2</sub> yield from the test substrate, respectively, on day 20-20 l/m<sup>3</sup> (P<0.01) or 5.1%; on day 23-30 l/m<sup>3</sup> (P<0.01) or 7.7%; on day 26-37 l/m<sup>3</sup> (P<0.01) or 9.4%, relative to the control variant.

Analysis of the results obtained shows that upon adding the Reduklin T biological product, there is a decrease in carbon dioxide emissions from fermented chicken manure, depending on the day of the experiment, respectively, by 33 l/m<sup>3</sup> (P<0.05) or 8.5% – on day 20; by 42 l/m<sup>3</sup> (P<0.01) or 10.8% – on day 23; by 49 l/m<sup>3</sup> (P<0.01) or 12.4% – on day 26, compared to the control variant.

In variants with the introduction of the biological product Reduklin Compost for the study period (20-26 days), the yield of CO<sub>2</sub> decreases from the test substrate, respectively, by 25-39 l/m<sup>3</sup> (P<0.05-0.01) or 6.4-9.9%, relative to the control variant.

According to experimental data, it was found that upon using a complex preparation to activate enzymatic processes in chicken manure, the volume of carbon dioxide output from the fermented substrate, depending on the day of the experiment, is lower, respectively, on day 20-50 l/m<sup>3</sup> (P<0.01) or 12.9%; on day 23-53 l/m<sup>3</sup> (P<0.01) or 13.6%; on day 26-56 l/m<sup>3</sup> (P<0.01) or 14.2%. Thus, analysing the research results, on day 26 of the experiment, the lowest level of CO<sub>2</sub> emission was established in all experimental variants upon mesophilic bio-fermentation (by 2.1-14.2%) from chicken manure. In particular, the most effective reduction of carbon dioxide output from the fermented substrate was shown by a complex preparation for activating enzymatic processes in chicken manure, namely – by 12.9-14.2%, depending on the day of the experiment (20-26 days). Considering the above, it is experimentally confirmed

and economically justified that the test substances FeO, Fe<sub>2</sub>O<sub>3</sub>, *Basidiomycota* fungal-based bio-composition, biologics – Meganit Nirbator, Reduklin T, Reduklin Compost and a complex preparation for activating enzymatic processes in chicken manure effectively influence the growth of CH<sub>4</sub> yield volume from a fermented substrate in the mesophilic mode of anaerobic fermentation (*in vitro*) with a simultaneous decrease in CO<sub>2</sub> release.

Thus, of the substances under study, the most effective in increasing methane emissions at a lower level of carbon dioxide from the fermented substrate was found in variants using a complex preparation to activate enzymatic processes in chicken manure and, accordingly, their positive effect on the process of methanogenesis decreases in the following sequence: Reduklin T – Reduklin Compost – Fe<sub>2</sub>O<sub>3</sub> – *Basidiomycota* fungal-based bio-composition – Meganit Nirbator – FeO.

## CONCLUSIONS

The effectiveness of the substances under study, namely FeO, Fe<sub>2</sub>O<sub>3</sub>, *Basidiomycota* fungal-based bio-compositions, biologics – Meganit Nirbator, Reduklin T, Reduklin Compost and a complex preparation for activating enzymatic processes in chicken manure to intensify the bio-fermentation and methane release from chicken manure (*in vitro*) is theoretically justified, which is conditioned upon changes in the pH of the substrate to the alkaline side, while reducing the level of carbon dioxide emissions. It has been experimentally confirmed that the most influential on methanogenesis, and therefore the growth of CH<sub>4</sub> yield from chicken manure developed is a complex preparation for activating enzymatic processes in chicken manure (15.7-18.8%), biologics – Reduklin T (9.5-14.2%) and Reduklin Compost (7.1-12%). Thus, the increase in the level of methane upon the use of the substances under study indicates the prospects of their application in the processing of chicken manure in biogas plants for a comprehensive solution simultaneously with the energy and environmental problem, i.e., to reduce environmental pollution with intensive management of the poultry industry, which allows improving the profitability of both husbandry and crop production by obtaining high-quality humus suitable for organic production.

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## Анаеробне бродіння курячого посліду та способи інтенсифікації виходу метану

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**Анотація.** Вагому частку у формуванні продовольчої безпеки населення займає галузь птахівництва, яка є однією із найбільш економічно привабливих та конкурентоспроможних, про що свідчить щорічна стійка динаміка зростання виробництва цінних продуктів харчування – м'яса птиці і яєць, що характеризуються високою поживністю, відмінними дієтичними й смаковими якістьми. Нарощування продукції птахівництва зумовлює відповідно інтенсифікацію виробництва з одного боку, а з іншого – накопичення відходів внаслідок зростання чисельності птиці. Одним із пріоритетних напрямів вирішення проблеми екобезпеки в галузі птахівництва є переробка побічної продукції тваринного походження завдяки ферментативному зброджуванню, що дозволяє одержати додатковий енергетичний продукт та органо-мінеральне добриво, тим самим запобігаючи викидам метану в атмосферу, а відтак і глобальному потеплінню. Тому, пошук способів інтенсифікації виходу метану з курячого посліду при анаеробному бродінні, зокрема шляхом додавання різних речовин був метою запланованих досліджень. Експеримент проведено із використанням лабораторних, аналітичних і математико-статистичних методів. За результатами експериментальних досліджень встановлено позитивний вплив FeO, Fe<sub>2</sub>O<sub>3</sub>, біокомпозиції на основі грибів *Basidiomycota*, біопрепаратів – Меганіт Нірбатор, Редуклін Т, Редуклін Компост і комплексного препарату для активації ферментативних процесів у курячому посліді на процеси анаеробної біоферментації та зростання вмісту метану (CH<sub>4</sub>) з курячого посліду (*in vitro*) на фоні зростання показника рН до 9,05–9,3 за одночасного нижчого рівня вуглекислого газу (CO<sub>2</sub>). Найкращі результати щодо підвищення обсягу виходу CH<sub>4</sub> із зброженого субстрату – на 15,7–18,8 % спостерігали у варіантах із комплексним препаратом для активації ферментативних процесів у курячому посліді. Застосування біокомпозиції на основі грибів *Basidiomycota* сприяє зростанню рівня емісії метану з досліджуваного субстрату на 5,4–9,6 %, а біопрепарати – Меганіт Нірбатор, Редуклін Т та Редуклін Компост обумовлюють збільшення обсягу виходу цього газу, відповідно, на 5,6–9,4 %, 9,5–14,2 % і 7,1–12 %. Внесення у курячий послід FeO та Fe<sub>2</sub>O<sub>3</sub> викликає зростання рівня емісії CH<sub>4</sub> на 4,1–7,4 % й 5,8–11,2 %. Отже, одержані результати вказують на доцільність використання досліджуваних речовин при переробці курячого посліду в біогазових установках для інтенсифікації виходу метану, що дасть можливість мінімізувати негативний вплив інтенсивного ведення галузі птахівництва на стан навколишнього середовища

**Ключові слова:** галузь птахівництва, побічні продукти тваринного походження, метаногенез, досліджувані речовини, парникові гази



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## Linear Growth of Representatives of Wheat Seeds Mycobiota

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**Abstract.** Seed-born fungi of wheat interact with the plant at various stages of its development and with each other. With the highest growth rate, they should be isolated because of competition. The purpose of this study was to compare the growth of colonies on a nutrient medium for the gradation of fungal genera and species from wheat seeds according to aggressiveness. These data helped concluding on the effectiveness of mycoexpertise of winter wheat seeds. Potato-glucose agar (PGA) was used for the analysis of the fungal complex. Seven-day fungal cultures were sown in the centre of Petri dishes. Linear growth of fungal colonies on PGA with gentamicin was determined. The specific features of the development of 12 representatives of seeds mycobiota from the Northeast of Ukraine of the 2017-2019 harvest were investigated. The dominance of *Alternaria* sp. and a slight release of *Fusarium* sp. were established by analysis of the fungal complex. The first comparison of the linear growth of *Fusarium graminearum*, *F. poae*, and *Alternaria tenuissima* in 2017 showed that *Fusarium* colonies grow faster on nutrient medium. In 2018, the growth characteristics of *A. arborescens*, which quickly became dominant in wheat seeds mycoflora, and the little-common *Trichothecium roseum* were studied in detail. By comparing the growth of fast-growing *F. graminearum* with the common *Aureobasidium pullulans* and the aggressive *Nigrospora oryzae*, the fastest development of the third and the slowest of the second species was established. *F. poae* filled the Petri dish on day 6, *Penicillium* – on day 22. In 2019, in the first experiment comparing *F. poae*, *F. sporotrichioides*, and *A. avenicola*, the second species had the worst growth rates. It became the second fastest growing colony in the study of the growth of seven species in the second experiment. Isolates of *N. oryzae* in 2018 were more aggressive than in 2019. Specific features of colony growth on PGA did not affect the effectiveness of the analysis of mycobiota of winter wheat seeds. *N. oryzae* had the highest radial speed under the general dominance of *Alternaria* sp. *Fusarium* sp. (*F. poae*, *F. sporotrichioides*, *F. verticillioides*, and *F. graminearum*) and *B. sorokiniana* developed rapidly. *A. arborescens* and *A. avenicola* grew at the same level as *A. pullulans*. *Penicillium* and *T. roseum* lagged behind other fungi in speed and filled Petri dishes for the longest time. *A. tenuissima* had the lowest radial growth rate

**Keywords:** fungal seed complex, colony growth, potato-glucose agar, winter wheat



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## INTRODUCTION

The mycobiota of wheat seeds is a dynamic system comprising varied species of fungi. Its composition is determined by abiotic and biotic factors. The seeds contain fungi that do not have phytotoxic effects on the plant (endophytes) and phytopathogenic species. The negative effects of phytopathogens are associated with the production of secondary metabolites that are dangerous to plants, animals, and humans. The pathogenicity of fungi is because they produce phytotoxins, enzymes, effector proteins, etc. (Peng *et al.*, 2021). The composition of seed mycobiota includes fungi that contaminate agricultural products with mycotoxins. These are *Fusarium* sp., *Penicillium* sp., *Aspergillus* sp., *Alternaria* sp., *Cladosporium* sp., *Cochliobolus* sp. etc. Mycotoxins contaminate 20-25% of food crops in the world (Eskola *et al.*, 2020). Studies on the co-cultivation of fungi and bacteria revealed the synthesis of some of these metabolites, which allowed researchers to determine the cause of mycotoxins – competition between different representatives of the plant microbiome (Venkatesh & Keller, 2019). The endophytic microbiota is associated with the host plant throughout its ontogenesis. Endophytes have a positive effect on plants, increasing drought resistance and resistance to pathogens, stimulating plant growth and development (Hardoim *et al.*, 2015; Shahzad *et al.*, 2018; Kuźniar *et al.*, 2020).

The study of mycobiota in Ukraine, as in the world, is not permanent, is not included in state monitoring, but only depends on the scientific interest of researchers. For the last 20 years, Ukrainian scientists isolated and identified the following genera in the from wheat seeds: *Acremoniella* sp., *Alternaria* sp., *Aspergillus* sp., *Cladosporium* sp., *Cochliobolus* sp., *Curvularia* sp., *Epicoccum* sp., *Fusarium* sp., *Mucor* sp., *Nigrospora* sp., *Penicillium* sp., *Phoma* sp., *Stemphylium* sp., and *Sordaria* sp. A study of 70 samples of wheat grain harvested in 2016 and 2017, collected in collective farms, the private sector, elevators, breeding stations, and regional seed inspections of three zones of Ukraine showed that 1 g of wheat grain in Ukraine contained from  $1.12 \times 10^3$  to  $6.5 \times 10^4$  CFU, which averaged to  $3.3 \times 10^4 \pm 3.2 \times 10^4$ . Inside the seeds were 11 representatives of the fungal complex. The most common were *Alternaria* spp. (67% of samples), *Aspergillus* spp. (37%), *Phoma exiqua* (30%), less frequently identified *Fusarium* spp. and *Mucor* spp. (in 19% of samples) (Ostrovskiy *et al.*, 2018). The 2016-2017 analysis of fungi isolated from wheat seeds of two varieties (Levada and Podolyanka) in Poltava Oblast showed the presence of 8 species/genera: *Alternaria alternata* (Fr.) Keissl., *Tilletia caries* (DC.) Tul. & C. Tul., *Fusarium* spp., *Cladosporium herbarum* (Pers.) Link, *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Mucor* spp., *Penicillium* spp., and *Aspergillus* spp. Among the fungi of the mycoflora, only one species dominated – *A. alternata* (Pospelov *et al.*, 2020). The study of mycobiota of wheat seeds from the Right Bank Forest-Steppe of Ukraine from different varieties showed the presence of the following fungi: *Alternaria tenuis* Nees, *Fusarium graminearum* Schwabe, *Nigrospora oryzae* (Berkeley et Broome) Petch., *Aspergillus niger* Tieghem, *Penicillium* Link. The highest

intensity of spore formation was observed in *A. niger* and *Penicillium* – from 3.2 to 12 million units/ml. High intensity of spore formation was inherent in *A. tenuis* and *F. graminearum* species and ranged from 1.4 to 7.2 million units/ml. (Mostovyyak *et al.*, 2020). Analysis of wheat seeds with black point grown in Kyiv Oblast in 2018-2019 allowed identifying 13 species from 9 genera: *Alternaria*, *Fusarium*, *Curvularia*, *Bipolaris*, *Aspergillus*, *Acremoniella*, *Stemphylium*, *Sordaria*, and *Epicoccum*. *Alternaria* fungi dominated: *A. tenuissima* (Nees & T. Nees: Fr.) Wiltshire, Trans., and *A. infectoria* E.G. Simmons were most often isolated (Golosna, 2021).

The species composition of fungi mycobiota seeds determines the spectrum of secondary metabolites. Ukrainian scientists identify species of the most harmful and common representatives of the seed complex of fungi. The first representatives include belonging to *Fusarium* sp., the second – to *Alternaria* sp. According to the latest research, seven species of fungi of the genus *Fusarium* have been identified in 109 samples of winter wheat seed material from 78 districts of 21 regions of Ukraine: *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. graminearum*, *F. langsethiae* Torp & Nirenberg, *F. poae* (Peck.) Wollenw., in Lewis, *F. sporotrichioides* Sherb. and *F. tricinctum* (Corda) Sacc. *F. graminearum* was the most common species in the country (the share of detection was 71%) (Gritsev *et al.*, 2018). Analysis of isolates of the genus *Alternaria* from different regions of Ukraine during 2012-2013 showed the dominance of *A. tenuissima* (70%) and a considerable percentage of *A. infectoria* (25.6%) (Golosna, 2015).

Mycobiota fungi of wheat seeds interact not only with the plant, but also with each other. Admittedly, all the features of their interaction *in vivo* may be unknown, but *in vitro* studies provide insight into some of them. Therefore, *the purpose of this study* was to compare the growth of colonies on agar medium to understand the effect of fungal aggressiveness on the composition of mycobiota of winter wheat seeds.

## MATERIALS AND METHODS

The study was conducted during 2017-2019. Mycobiota fungi of winter wheat seeds were isolated from 43 samples obtained from agricultural enterprises of different districts and scientific institutions of the North-East of Ukraine. The authors of this study grew some wheat in the conditions of educational and scientific production complex of Sumy National Agrarian University. Before the analysis, the seeds (200-400 from the sample) were washed under running water for one hour, disinfected with 1% potassium permanganate solution for 1-2 minutes. The seeds were spread on a potato-glucose agar. 25 seeds were placed in one Petri dish. Petri dishes were incubated for seven days in a thermostat at a temperature of 20°C for germination of fungal colonies. Species were identified by various scientific studies: *Fusarium* sp. – by Leslie & Summerell (2006), Gagkaeva *et al.* (2011); *Alternaria* sp. – by Hannibal (2011), Woudenberg *et al.* (2013); *Aureobasidium pullulans* (de Bary)

G. Arnaud – by Zalar *et al.* (2008); *Nigrospora oryzae* (Berkeley et Broome) Petch. – by Wang *et al.* (2017); *Trichothecium roseum* (Pers.) Link – by Watanabe (2002); *B. sorokiniana* – by Manamgoda *et al.* (2014). The identified fungi were seeded in pure culture on PGA with gentamicin. Mycelial growth was determined by growing fungi in Petri dishes on PGA. For this, a seven-day growing was involved. The fungi were placed with a needle at the centre of dishes. Colonies grew in a thermostat at 20°C, 22°C, 23–24°C. The incubation period depended on the growth characteristics of the fungi (7–25 days). To identify the linear growth, the diameter of the colonies was measured in two perpendicular directions. The radial growth rate of colonies was determined according to the formula (1) (Poliksenova *et al.*, 2004):

$$Kr = (r - r_0)/t \quad (1)$$

where  $Kr$  is the radial growth rate of colonies, mm/day;  $r$  is the radius of the colonies at a given time, mm;  $t$  is the time from sowing to the moment when the colony will have a radius  $r$ , day.

Repetition depended on the experiment: three

to five times. Statistical analysis of the results was performed according to the method of one-way analysis of variance in Excel, calculating the  $LSD_{05}$  according to Dospekhov (1985).

## RESULTS AND DISCUSSION

According to the authors' observations, *Alternaria* fungi dominated the mycoflora of winter wheat seeds grown in the North-East of Ukraine (2021). *Fusarium* fungi were quite rare. Usually, one infected grain of this genus was found in a Petri dish. In 2017, the authors of this study concluded that *in vitro* conditions are better for the development of *Alternaria* sp. than *Fusarium* sp. The definition of *Alternaria* sp. recommends using Potato-Carrot Agar (PCA), Hay Infusion Agar (HAY), and V-8 (Vegetable Juice Agar) (Gannibal, 2011). *Fusarium* sp. is better determined on Carnation Leaf-piece Agar (CLA), Spezieller Nährstoffarmer Agar (SNA), and Potato Dextrose Agar (PDA) (Leslie & Summerell, 2006).

Therefore, the growth of fungi was investigated on the PGA medium (Table 1). The number of repetitions was 5 times.

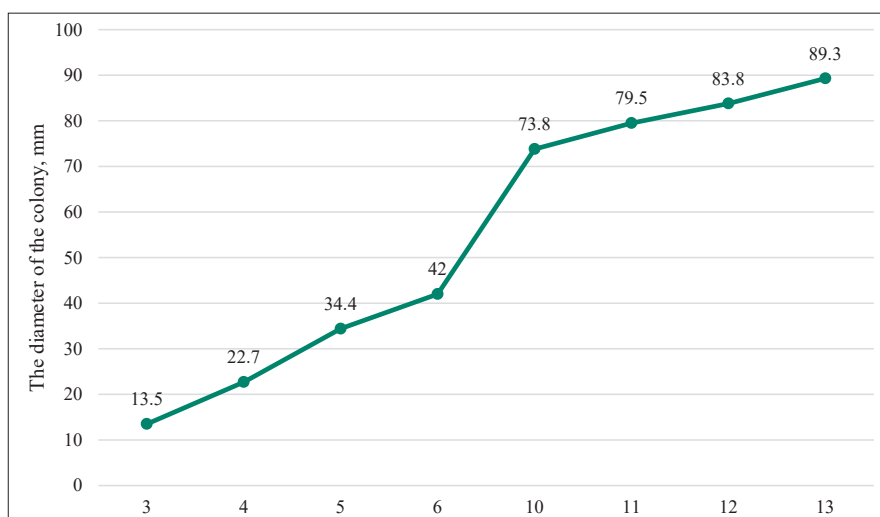
**Table 1.** Growth of mycelium on PGA medium (20°C, 2017)

Fungal species	The diameter of the colony, mm		
	Day 3	Day 4	Day 7
<i>F. graminearum</i>	17.4x15.8	24.8x25	51.7x55
<i>F. poae</i>	10x10	22.3x21.3	52.5x49.3
<i>A. tenuissima</i>	7x7.8	12.4x11.4	33.8x33.8
$LSD_{05}$	1.8	4.1	3.4

The assumption turned out to be wrong, which is confirmed by the data from Table 1. *Fusarium* fungi grew much faster on the medium than *A. tenuissima*. If *Fusarium* sp. indeed were present in a batch of grain in considerable quantities, they would quickly inhibit the development of *Alternaria* fungi, which was observed in 2016 on the Samuray variety. *F. graminearum* had higher colony growth rates on PGA than *F. poae*. When studying the growth rate of six species of *Fusarium* fungi on the KGA, the highest rate was found

in *F. graminearum* – 23.6 mm/day. *F. poae* has also been classified as a fast-growing species. Its growth rate was 21.7 mm/day (Shashko, 2020).

In 2018, the authors of this study decided to investigate the specific features of the growth of fungi on a nutrient medium in more detail. *A. arborescens* E.G. Simmons appeared unexpectedly and quickly dominated the mycoflora of wheat seeds. The specific features of the growth of this species on a nutrient medium were investigated (Fig. 1).



**Figure 1.** The diameter of *A. arborescens* (growing at 22°C) (2018)

This species developed quite rapidly compared to other *Alternaria* fungi. On days 13-14, the fungi colony completely filled the Petri dish. The average radial growth rate was 3.4 mm/day. By Day 3, the fungi developed slowly. From Day 4, the active development of the fungal colony began. Its maximum growth rate was observed on Day 5. Then the figure started to gradually decrease. The lowest growth rate of the colony was observed on Day 12 of fungal development.

The occurrence of *A. pullulans* in 2016 was

insignificant. Gradually, the amount of these fungi increased. Therefore, it was interesting to investigate their behaviour on medium without other fungi. During the isolation of fungi from the seeds, *N. oryzae* had increased aggression compared to other fungi. If they germinated from seed, no fungal colonies developed with them. Therefore, these fungi would develop separately on the medium. Thus, the authors started investigating the growth of fungi on the PGA (Table 2). Repeatability – three times.

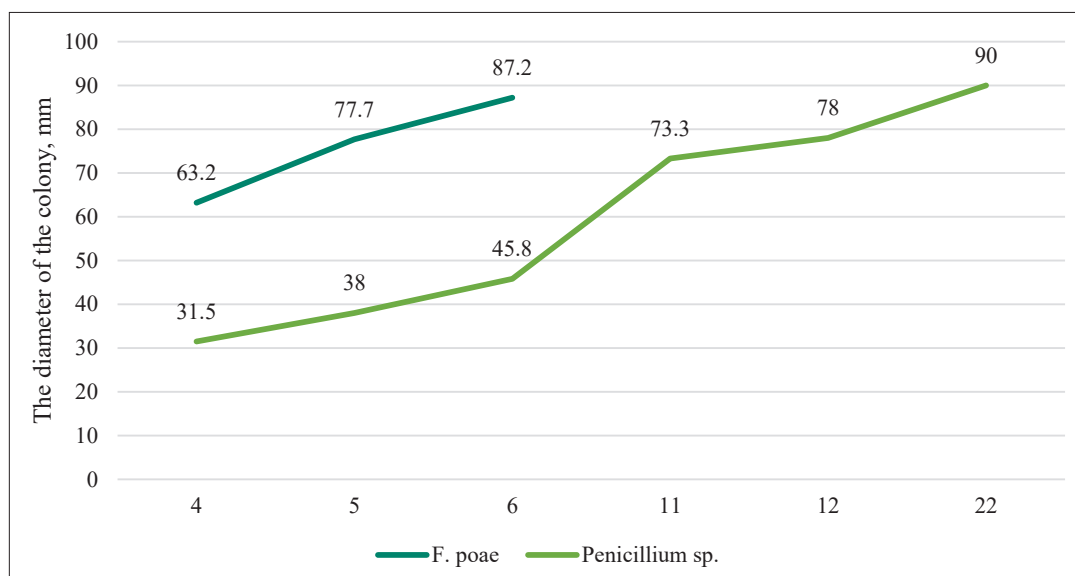
**Table 2.** Comparison of growth of *F. graminearum*, *A. pullulans*, and *N. oryzae* (2018)

Fungal species	The diameter of the colony, mm				
	Day 3	Day 6	Day 7	Day 8	Day 13
<i>F. graminearum</i>	21x21	55x52	65x62	77x73	90x90
<i>A. pullulans</i>	23x13	57x30	53x36	57x39	59x48
<i>N. oryzae</i>	46x43	90x90	90x90	90x90	90x90
LSD <sub>05</sub>	3.5	3.9	6.3	4.9	4.7

*N. oryzae* filled the entire Petri dish on Day 6. This species proved to be the most aggressive, having the fastest growth of its colony. *A. pullulans* lagged behind the other two fungi.

The study of the growth of *Penicillium* fungi was complicated by the fact that over time, several colonies formed on the medium. Thus, out of five replicates

of one colony on Day 4 of observation, 3 colonies were formed in one of the replicates, on Day 6 there were already two replicates with several colonies, on Day 11-3 replicates. Therefore, the data was analysed from only two replicates. The specific features of growth of a fairly common *Fusarium* species – *F. poae* (Fig. 2) were studied.



**Figure 2.** Linear growth of *F. poae* and *Penicillium* on PGA (2018) (LSD<sub>05</sub>,6=9.3)

*F. poae* fungi demonstrated rapid colony growth: on Day 6, they filled almost the entire diameter of the Petri dish. Isolates of these fungi from wheat seeds in Poland on Days 4 and 7 of cultivation had a growth rate of 5.4-10.3 mm/day (Lukanowski *et al.*, 2008). On Day 4, the isolate under study had a speed of 15.8, and on Day 6-14.5 mm/day in the diameter of the colony.

The *Penicillium* fungi were inferior to the growth of the *Fusarium* fungi, but in the initial stages of development grew faster than *Alternaria* fungi. However, after Day 12 of cultivation, the rate of development of the

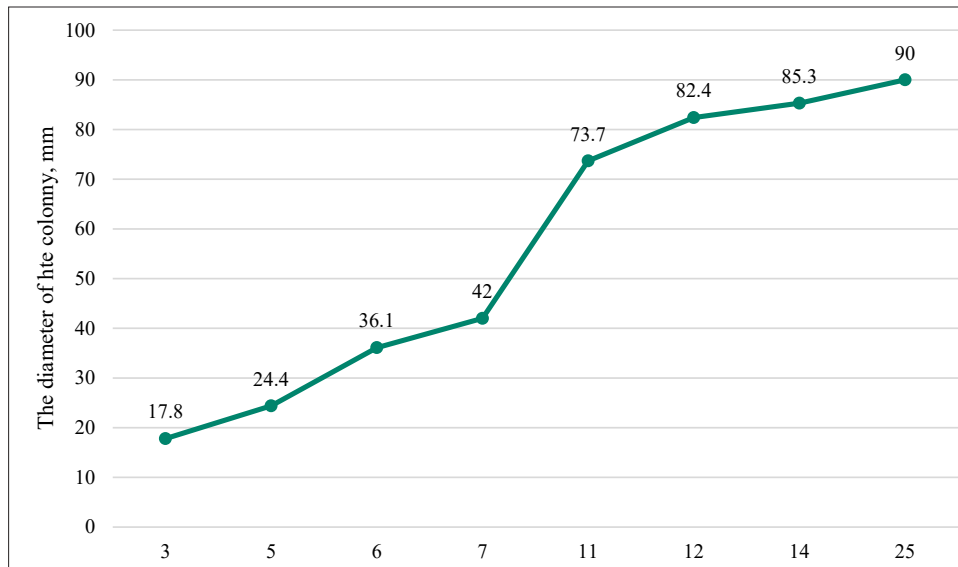
fungi decreased. Only on Day 22 the fungi completely filled the diameter of the Petri dish.

Comparison of the growth rate of endophytic and phytopathogenic isolates of *F. poae*, *Alternaria alternata* and *Penicillium funiculosum* Thom on PGA arranged them in the above order. That is, the *Fusarium* fungi formed the fastest growing colonies. Phytopathogenic isolates had higher growth rates than endophytic ones (Kurichenko *et al.*, 2015).

*T. roseum* began to be observed in grain batches at the beginning of this study. Moreover, its presence was

different: from isolated cases to a recurrence in the mycoflora of wheat seeds in 2020. These fungi behaved quite aggressively towards other fungi when they actively germinated from seed. Sometimes they co-existed with other

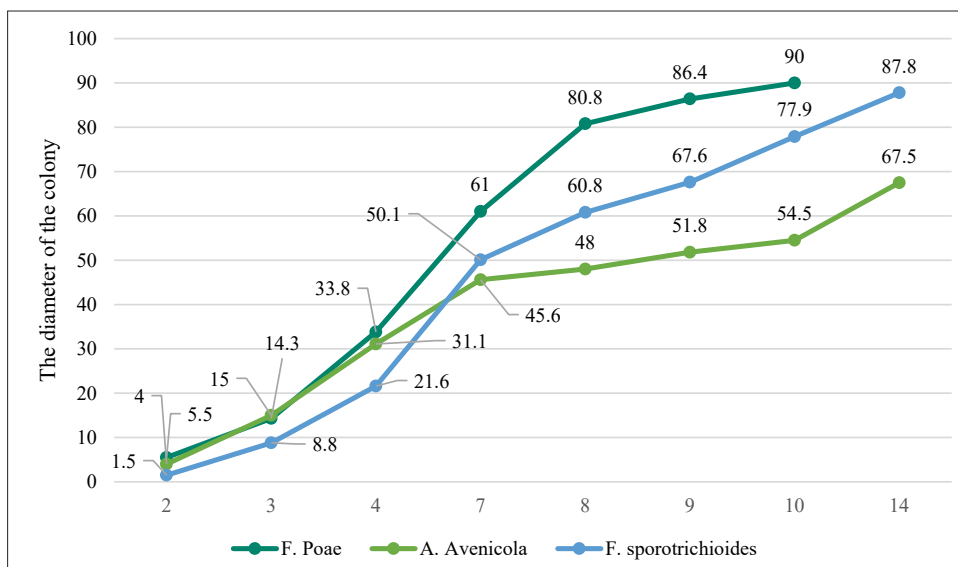
fungi (mostly *Alternaria*), and it was even difficult to spot. Therefore, it was necessary to investigate their growth on the medium (fourfold repetition) (Fig. 3).



**Figure 3.** The diameter of *T. roseum* (growing at 22°C) (2018)

First, the gradual linear growth of the fungal colony was noted, which lasted for 12 days of their cultivation. They grew the fastest on Days 5-6 – 11.7 mm. After Day 12, the growth of the fungal colony started to slow down. Observations of the colony growth showed that it hardly grew. Only on Day 25 *T. roseum* completely

filled the entire Petri dish. Isolates grown from winter wheat seeds harvested in 2019 were investigated in two experiments of single cultivation. First, the growth characteristics of the three following species were compared: *F. poae*, *F. sporotrichioides*, and *A. avenicola* (repeatability – four times) (Fig. 4).



**Figure 4.** Linear growth of *F. poae*, *F. sporotrichioides*, and *A. avenicola* (2019) ( $LSD_{05}7=4.5$ ,  $LSD_{05}14=3.2$ )

Isolates of *F. poae* grown from seeds of the 2019 harvest were less aggressive than their respective isolates of 2018 (Fig. 2). It took them 10 days to fill the entire surface of the medium. *Alternaria* species initially lagged behind *Fusarium* fungi, but from Day 7 it overtook the growth of the colony of *F. sporotrichioides*. Of the three

species under study, the most aggressive was *F. poae*.

Simultaneous study of seven varied species of seeds mycobiota allowed isolating new fungi with rapid colony growth and confirm the high rate of already identified aggressive species (Table 3) (repeatability – three times).

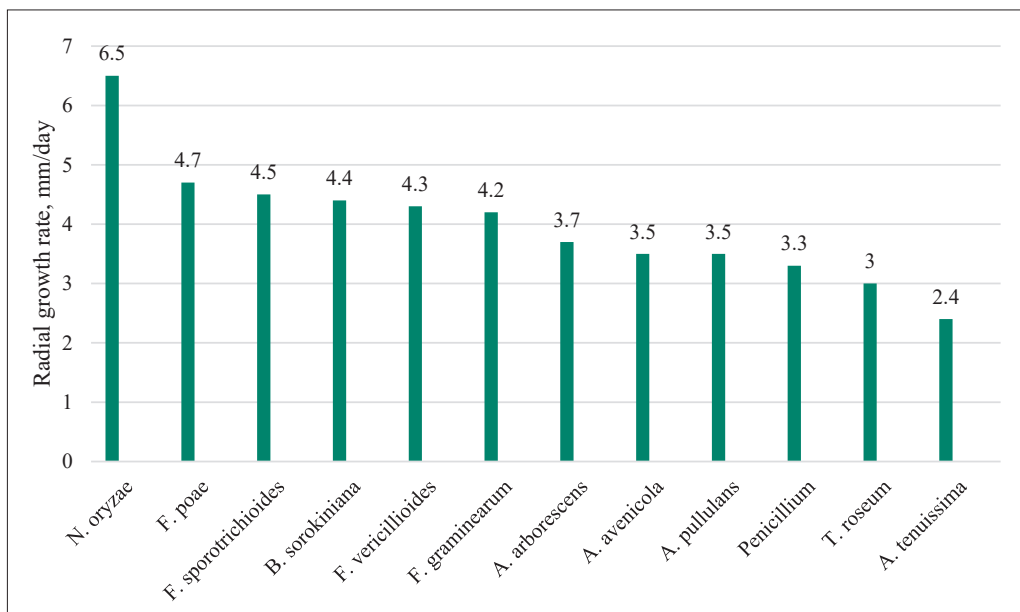
**Table 3.** Comparison of the growth of fungal colonies of winter wheat seeds mycobiota (2019)

Fungal species	The diameter of the colony for a certain day of cultivation, mm						
	Day 4	Day 6	Day 7	Day 11	Day 14	Day 19	Day 22
<i>N. oryzae</i>	43.7	84.7	90	-/-	-/-	-/-	-/-
<i>F. sporotrichioides</i>	43.2	69.2	77.7	90	-/-	-/-	-/-
<i>F. poae</i>	36.2	54.8	62.8	90	-/-	-/-	-/-
<i>F. verticillioides</i>	35.3	52.7	60	84.7	90	-/-	-/-
<i>A. avenicola</i>	24.5	43.5	47.2	80.7	90	-/-	-/-
<i>A. arborescens</i>	30.7	45.3	53.2	76.2	83.3	90	-/-
<i>B. sorokiniana</i>	37.7	53	61.5	75.3	77.2	83.5	87.5
HIP <sub>05</sub>	2.5	3.4	3.1	2.6	Did not count		

In 2019, the fastest development of *N. oryzae* was confirmed. In 2018, isolates of these fungi were more aggressive; they completely filled the Petri dish on Day 6. Different ability of colonies of isolates of one species – *F. sporotrichioides* – was noted. If in the first experiment this species lagged behind the *Alternaria* fungi, then in the second – they were the second most aggressive species, even surpassing the fairly fast species of *F. poae*. The last fungi had a lower growth rate both compared to the first experiment and the previous year of the study.

*F. verticillioides* predominated in the development of *Alternaria* fungi. *A. avenicola* grew faster from *Alternaria* species. *B. sorokiniana* developed seven days faster than *Alternaria* fungi and *F. verticillioides*, and from Day 11 their growth rate was minimal. Isolates of *B. sorokiniana* from barley seeds in Argentina had an average growth rate per PGA of 9.9 mm/day (Dominguez *et al.*, 2020). The isolates under study had a colony growth rate of 8.8 mm/day on the seventh day.

Since mycobiota were analysed on Day 7, the radial growth rate during this period was calculated (Fig. 5).

**Figure 5.** Radial growth rate of representatives of wheat seeds mycobiota (2017-2019)

The area of variation of the indicator was 2.4–6.5 mm/day. *N. oryzae* had the maximum speed. Radial growth of *Fusarium* sp. was similar to *B. sorokiniana*. The next block in speed was *Alternaria* sp. and *A. pullulans*. *Penicillium* and *T. roseum* gave way to them. *A. tenuissima* had the lowest radial speed.

## CONCLUSIONS

Single cultivation fungi of wheat seeds mycobiota on PGA medium showed different growth rates of colonies of isolates of different years, from different samples, but allowed distributing fungi by growth rate, i.e., aggressiveness. *N. oryzae* had the fastest development of colonies, followed by *Fusarium* sp. (*F. poae*,

*F. sporotrichioides*, *F. verticillioides*, *F. graminearum*), and *B. sorokiniana* (only in the first seven days), which were inferior to *A. arborescens* and *A. avenicola*. *A. pullulans* developed at the level of *Alternaria* sp. The *Penicillium* fungi had average growth rates in the first week, but their growth rate gradually decreased. *T. roseum* developed similarly but had maximum time to fill the Petri dish. *A. tenuissima* showed the lowest radial growth rate. The growth of colonies on the PGA did not affect the specific features of the isolation of fungi from the seeds of winter wheat. Data on the isolation of fungi from mycobiota correlate with their presence inside the seeds and were not determined by their development on agar medium.

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## Лінійний ріст представників мікобіоти насіння пшениці

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**Анотація.** Гриби комплексу насіння пшениці взаємодіють з рослиною на різних етапах її розвитку та між собою. Можливо, у результаті конкуренції краще виділяються гриби з найвищою швидкістю росту. Метою досліджень було порівняти ріст колоній на поживному середовищі для градації грибних родів та видів з насіння пшениці за агресивністю. Ці дані допоможуть зробити висновки про результативність мікоекспертизи насіння пшениці озимої. Аналіз грибного комплексу провели на картопляно-глюкозному агарі. Семиденні культури грибів висіяли у центр чашок Петрі. Визначили лінійний ріст колоній грибів на КГА з додаванням гентаміцину. Було вивчено особливості розвитку 12 представників мікобіоти насіння з Північного Сходу України врожаїв 2017–2019 рр. За аналізу грибного комплексу встановили домінування *Alternaria* sp. та незначне виділення *Fusarium* sp. Перше порівняння лінійного росту *Fusarium graminearum*, *F. poae* та *Alternaria tenuissima* у 2017 р. показало, що фузарієві колонії швидше ростуть на середовищі. У 2018 р. детально вивчили особливості росту *A. arborescens*, який швидко зайняв домінуюче положення у мікофлорі насіння пшениці, та малопоширеного *Trichothecium roseum*. За порівняння росту швидкоростучого *F. graminearum* з поширеним *Aureobasidium pullulans* та агресивним *Nigrospora oryzae* встановили найшвидший розвиток третього та найповільніший другого виду. *F. poae* заповнив чашку Петрі на шосту добу, *Penicillium* – на 22-гу. У 2019 р. у першому досліді за порівняння *F. poae*, *F. sporotrichioides* та *A. avenicola* другий вид мав найгірші показники росту. У другому досліді він став другим за швидкістю розвитку колонії при дослідженні росту семи видів. Ізоляти *N. oryzae* у 2018 р. були агресивнішими, ніж у 2019 р. Ріст колоній на КГА не вплинув на виділення грибів з насіння пшениці озимої. За загального домінування *Alternaria* sp. найвищу радіальну швидкість мав *N. oryzae*. Швидко розвивались *Fusarium* sp. з (*F. poae*, *F. sporotrichioides*, *F. verticilliioides* і *F. graminearum*) та *B. sorokiniana*, *A. arborescens* та *A. avenicola* росли на рівні з *A. pullulans*, *Penicillium* та *T. roseum* за швидкістю відставали від інших грибів і найдовше заповнювали чашки Петрі. *A. tenuissima* мав найменшу радіальну швидкість росту

**Ключові слова:** грибний комплекс насіння, ріст колоній, картопляно-глюкозний агар, пшениця озима

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## Preliminary Results of Evaluation of Collection Samples of Meadow Timothy as a Valuable Source Material for Breeding

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**Abstract.** Meadow Timothy is one of the most common perennial forage grasses for haymaking and pasture use, as well as one of the components of legume-cereal grass mixtures. High productivity of Meadow Timothy can only be provided by new modern varieties, for the creation of which it is of foremost importance to use source material of various ecological and geographical origin in breeding programmes. For this, it is necessary to evaluate it for a complex of economic and breeding-valuable characteristics, which was the purpose of this study. The methodological framework included field and laboratory methods of research, which was conducted during 2020-2021 in the experimental field of the pre-Carpathian Department of Scientific Research of the Institute of Agriculture of the Carpathian Region of the National Academy of Sciences. The study investigated 16 collection samples of local selection obtained as a result of individual and mass selection, as well as one hybrid population. Standard – Daryna variety. According to two-year data, the highest plant height was found in samples MS 1496 – 116.0 cm and IS 1612 – 114.6 cm, foliage – MS 1510 – 68.2%, IS 1512 – 60.4%, IS 1612 – 65.0%, MS 1816 – 61.3 %, green mass yield – IS 1512 – 4.05 kg/m<sup>2</sup>, IS 1612 – 4.10 kg/m<sup>2</sup>, MS 1816 – 4.07 kg/m<sup>2</sup>, MS 1510 – 4.00 kg/m<sup>2</sup>, dry matter – IS 1612 – 0.888 kg/m<sup>2</sup>, MS 1816 – 0.861 kg/m<sup>2</sup>, seed productivity – IS 1512 – 28.0 g/m<sup>2</sup>, IS 1608 – 27.0 g/m<sup>2</sup>, HP – 26.5 g/m<sup>2</sup>, IS 1506 – 26.0 g/m<sup>2</sup>, panicle length – IS 1608 – 15.0 cm, HP – 15.1 cm, number and weight of seeds from one panicle – IS 1608, IS 1509, IS 1506, IS 1610, weight of 1,000 seeds – IS 1512 – 0.68 g, IS 1608 – 0.65 g, IS 1509 – 0.63 g, crude protein content – IS 1612 – 14.4%, IS 1512, IS 1954 – 14.1%, HP – 14.0%, fibre – IS 1512 – 30.0%, MS 1823, IS 1612 – 29.8%, MS 1602 – 29.6%. All samples had high winter hardiness and resistance to diseases. The study will continue in 2022. Based on three-year data, the best source material will be used in the further scheme of the breeding process

**Keywords:** variety, attribute, collection nursery, productivity, feed value



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## INTRODUCTION

The main prerequisite for strengthening the feed base of animal husbandry is to improve and expand the area of cultivated pastures and hayfields by increasing the efficiency of field grass sowing. In the herbage of natural hayfields and pastures of the western regions of Ukraine, especially in the Carpathians and the Eastern Carpathian Foothills, the most common are grasses. They make up 60-70% of the total herbage, often forming pure groupings. Perennial grasses have a high feed value. In highly productive natural and sown meadows, they predominate in the herbage and produce high yields of hay and pasture feed from early spring to late autumn, are resistant to trampling, and grow well after mowing and grazing (Tarariko *et al.*, 2018; Petrychenko *et al.*, 2020). Among the many common types of perennial grasses (shive, *Bromus inermis*, orchard grass, perennial ryegrass, bulbous oat grass), Meadow Timothy occupies a prominent place.

Meadow Timothy (*Phleum pratense* L.) is a plant from the family of cereals (Poaceae) with a height of 80-100 cm or more. It was introduced into culture from natural flora at the beginning of the 18<sup>th</sup> century. This is one of the key top-soil, non-dense, medium-life grasses of meadow and field grass sowing in the forest-steppe and polissia zones of Ukraine. As a crop, the plant is grown in Europe, introduced to North America and Australia.

It belongs to cereals of temperate cold climate, has high winter hardiness, is quite moisture-loving, and tolerates high acidity of the soil solution. It grows in most types of soils, except sandy ones (Kokhan *et al.*, 2018; Jorgensen *et al.*, 2020). This is a plant of spring and spring-winter development type. In spring, it develops rapidly, but blooms later than other forage grasses. The duration of the vegetating season of Meadow Timothy is 85-130 days. Its first hay harvest is formed in 40-45 days, and the second – in 50-60 days. Inflorescence – spike-shaped panicle (sultan) of cylindrical shape with a length of 10-20 cm. The weight of 1,000 seeds is 0.4-0.8 g. The plant is high yielding. Among all cereals, it occupies one of the first places in terms of yield and nutritional value. Thus, the yield of its green mass is 330-380 cwt/ha, hay – 60-120 cwt/ha, dry matter – 80-90 cwt/ha. Seed productivity is 4-6 cwt/ha. 100 kg of Timothy hay contains 3 kg of digested protein and corresponds to 45 feed units, while 100 kg of green mass – 1.5-1.7 kg and 20-25 feed units, respectively. The most foliose Timothy species have the best feed value (the mass of leaves is 60-65% of the total mass). It grows well after mowing and grazing, so it is used for hay and grazing, and is well eaten by all animals. It is best to mow it for hay in the end-earring – beginning of flowering phase. After flowering, the stems of Timothy begin to quickly become rough, resulting in a decrease in its feed value. Thus, during spring regrowth, the protein content in the green mass is 15.5%, in the phase of the early earing – 13.9%, at the beginning of flowering – 9.3%, and at full flowering – 8.1%. When used for hay, two mowing operations are

obtained per year. The herbage lasts 3-5 years or more but gives the highest yield in the second or third year of use (Shtakal, 2020; Khomyak *et al.*, 2020).

Meadow Timothy is a plant of haymaking and pasture use. It is used as decorative and lawn grass, but its main purpose is forage. This is one of the most valuable components in the grass mixture with meadow clover, burclover, sainfoins, birdsfoot deervetch. According to studies, the stands of these Timothy grasses are 20-25% more productive than their pure crops (Figurin & Kislitsyna, 2020; Pomerleau-Lacasse *et al.*, 2019; Lauzon *et al.*, 2019). In field crop rotations mixed with clover, it is used for 2-3 years, and in haymaking and pasture – 5-6 years (Kapsamun *et al.*, 2021). Thus, it is one of the main components of grass mixtures in improving meadows and creating long-term cultivated pastures in Ukraine.

According to O.V. Zakharchuk *et al.* (2020), in modern technologies of crop production, namely in feed production, the greatest increase in yield is provided by the variety. The role of the variety in shaping the size and quality of the crop is constantly growing and currently ranges from 20% to 40% or more. A modern variety should be focused not only on a certain level of provision, but also on ensuring that its main adaptability parameters correspond to a wide range of environmental factors of a particular agroclimatic growing zone (Demydas *et al.*, 2018). The State Register of Plant Varieties Suitable for Distribution in Ukraine for 2022 (as of January 27, 2022) includes 11 varieties of Meadow Timothy: Vytava, Summergrass, Presto, ATURO, Charivna horianka, Lishka, FRVL-1, Vyshhorodska, Pidhyrianka, Milena, Daryna (Ministry of Agrarian Policy..., 2022).

Highly productive varieties of Meadow Timothy were created in Poland (Brudzinska), France (Alpade), Lithuania (Jauniai, Dainai, Obeliai). In Ukraine, this crop is bred by such scientific institutions as the Scientific Research Centre "Institute of Agriculture of the National Academy of Sciences" (Chabany village), the Institute of Fodder and Agriculture of the National Academy of Sciences of Podillia (Vinnytsia), the Sarnen Research Station of the Institute of Water Problems and Land Reclamation of the National Academy of Sciences (Rivne Oblast). In the west of Ukraine, the leading role belongs to scientists of the Institute of Agriculture of the Carpathian Region of the National Academy of Sciences (Institute of Water Problems and Reclamation, n.d.; Perehrym, 2021).

Breeding is a complex biotechnological process. It is based on the use of the available and new methods for creating genetic diversity, evaluating it and selecting the desired genotypes, which combine as many traits, qualities, and properties as possible that should be inherent in the future new variety. To search for such genotypes, large volumes of source material are studied and analysed, and the selected samples are included in the further breeding process. The correct choice of source material and its use in breeding is of significant importance. This is the critical stage of the

breeding process, which determines the final result of the breeder's work.

The source material in breeding is samples that the breeder uses in their practical work to create new varieties. These can be wild forms, local varieties, populations, and samples of the world collection, hybrid populations, self-pollinating lines, artificial mutants and polyploid forms, introduced samples. When mobilising the source material, preference should be given to local samples and populations. They are adapted to the climatic conditions of the growing region and are characterised by high yields (Mazur *et al.*, 2020; Baystruk-Hlodan *et al.*, 2020).

Proceeding from the above, the main task of this study is to investigate the collection samples of Meadow Timothy to select the best numbers, which, according to a complex of economically valuable characteristics,

can be included in the further breeding process as a source material for creating new varieties.

## MATERIALS AND METHODS

To evaluate the source material, a collection nursery of Meadow Timothy was laid in 2019. The size of the registered land area is 1 m<sup>2</sup>. The material for the study was 16 samples originating from Ukraine. These are samples of local selection, bred as a result of individual and mass selection, as well as one hybrid population. As a standard, the Daryna variety of Meadow Timothy is taken. This is a variety selected by the Institute of Agriculture of the Carpathian Region of the National Academy of Sciences, which in 2018 was listed in the State Register of Plant Varieties Suitable for Distribution in Ukraine. The standard was seeded every four samples (Table 1).

**Table 1.** The samples of Meadow Timothy under study in the collection nursery (sowing in 2019)

No. national catalogue	Institution registration No.	Sample name	Country origin
UJ 1100101 (standard)	PFZ 00906	Daryna	UKR, LVV
	PFZ 02089	IS 1506 (individual selection from No. 1506)	UKR, LVV
	PFZ 02090	IS 1509 (individual selection from No. 1509)	UKR, LVV
	PFZ 02092	IS 1512 (individual selection from No. 1512)	UKR, LVV
	PFZ 02094	MS 1602 (mass selection from No. 1602)	UKR, LVV
	PFZ 02177	IS 1610 (individual selection from No. 1610)	UKR, LVV
	PFZ 02178	IS 1608 (individual selection from No. 1608)	UKR, LVV
	PFZ 02179	IS 1602 (individual selection from No. 1602)	UKR, LVV
	PFZ 02180	IS 1612 (individual selection from No. 1612)	UKR, LVV
	PFZ 02181	MS 1816 (mass selection from No. 1816)	UKR, LVV
	PFZ 02182	MS 1954 (mass selection from No. 1954)	UKR, LVV
	PFZ 02183	MS 1510 (mass selection from No. 1510)	UKR, LVV
	PFZ 02184	HP (hybrid population No. 1954 × No. 1942)	UKR, LVV
	PFZ 02185	MS 1823 (mass selection from No. 1823)	UKR, LVV
	PFZ 02096	IS 1814 (individual selection from No. 1814)	UKR, LVV
	PFZ 02085	MS 1496 (mass selection from No. 1496)	UKR, LVV
	PFZ 02091	IS 1511 (individual selection from No. 1511)	UKR, LVV

**Note:** UKR – Ukraine; LVV – Lviv Oblast.

The study was conducted on the experimental basis of the pre-Carpathian Department of Scientific Research of the Institute of Agriculture of the Carpathian Region of the National Academy of Sciences in the sub-region of the middle Eastern Carpathian Foothills (200-400 m a.s.l.) of the Drohobych District of the Lviv Oblast (village of Lishnia) on on soddy medium-podzolic surface-gleyed medium-acid loamy soils formed on diluvial deposits with the following main agrochemical indicators of fertility: humus content in the arable (0-20cm) layer (according to Tiurin) – 1.22%, pH of salt extract (Potentiometric method) – 4.6, hydrolytic acidity (according to Kappen-Gilkowitz) – 4.23 mg-eq per 100 g of soil, Hr (sum of absorbed bases) – 11.8 mg-eq per 100 g of soil, mobile forms of phosphorus (according to Kirsanov) – 118 mg, exchange potassium (according

to Kirsanov) – 82 mg, easily hydrolysed nitrogen (according to Cornfield) – 108 mg per 1 kg of soil.

Preparation and cultivation of the soil for sowing the collection of Meadow Timothy was generally accepted for the zone of the Eastern Carpathian Foothills of Ukraine. Its predecessor is perennial legumes. Laying of experimental plots was carried out manually, in the summer period of sowing in a coverless way. In spring, Meadow Timothy plants were fed with mineral fertiliser in the form of ammonium nitrate, row spacing was loosened, and weeds were cleared. Laying of the collection nursery and research in it was carried out according to the generally accepted requirements for the methodology of field experiment according to B.A. Dospekhov (1985) and methodological guidelines for the selection of perennial grasses (Kosopalov *et al.*, 2012).

The main characteristics used to evaluate collection samples were plant height and foliage, winter hardiness, productivity, feed value, and resistance to diseases. Structural analysis of plants was performed according to such economically valuable characteristics as the length of the panicle, the number of seeds in the panicle, the mass of seeds from the panicle, and the mass of 1,000 seeds. During the growing season, phenological observations were made on the growth and development of Meadow Timothy plants.

Accounting of the green mass yield was performed in the phase of full earing (two hay harvests) by harvesting and weighing the grass from the entire accounting area of the site. The yield of dry matter was determined by test sheaves weighing 1 kg selected after harvesting and drying to a constant weight.

Seeds were harvested in the full ripeness phase

separately from each site by threshing, wiping, cleaning, and weighing it.

To assess the feed value of Meadow Timothy samples, the following factors were determined: crude protein – according to the Keldahl method, crude fibre – according to the Hennenberg-Stoman method.

The study results were mathematically processed according to the method of variance analysis on a personal computer using the Agrostat software and information complex.

## RESULTS AND DISCUSSION

Weather conditions of the growing season in the years of research (2020-2021) were typical for the zone of the Eastern Carpathian Foothills and differed from each other, which enabled an objective assessment of collection samples according to the parameters under study (Table 2).

**Table 2.** Meteorological indicators in the years of research (according to the Drohobych weather station)

Months of the year	Mean monthly air temperature, °C			Amount of precipitation per month, mm		
	Years		Average many-year	Years		Average many-year
	2020	2021		2020	2021	
March	4.9	2.8	1.8	37.9	42.3	38.0
April	8.9	6.6	7.9	22.5	39.8	53.0
May	11.2	13.4	13.2	169.0	52.7	97.0
June	18.4	18.2	16.2	131.5	80.2	119.0
July	19.0	21.5	17.6	87.4	64.4	110.0
August	19.7	17.7	17.0	31.4	128.8	92.0
During the vegetating season (March-August)	13.7	13.4	12.3	479.3	408.2	509.0

In the conditions of the Eastern Carpathian Foothills region, the vegetation of Meadow Timothy, depending on weather conditions, begins from the middle of the second to the end of the third decade of March. Thus, in 2020, spring regrowth of Timothy plants began on March 16, in 2021 – on March 29. At the end of the winter period, a density of 220-460 plants/m<sup>2</sup> was observed in the crops of Meadow Timothy (first year of use), 231-505 plants/m<sup>2</sup> (second year of use).

The duration of the growing season of the samples of Meadow Timothy under study from the beginning of spring regrowth to full seed ripeness differed in two years of study and amounted to 135-140 days in 2020, 128-131 days in 2021. On average, for two years, it took 62-75 days for plants to form the first hay harvest (the period from the beginning of spring regrowth to the beginning of earing). Based on the obtained data on the duration of the growing season, all samples of the collection nursery were conditionally divided into three ripeness groups: early-maturing, medium-maturing, and late-maturing. The early-maturing group (the period from the beginning of spring regrowth to full ripeness of seeds is 125-128 days) includes 5 samples, medium-maturing (129-133 days) – 8 samples, and late-maturing (134-140 days) – 3 samples.

To identify the most promising collection samples, they were studied according to indicators that mainly affect the formation of fodder and seed productivity of plants.

In all collection samples under study, plant growth occurred intensively before the earing phase – the beginning of flowering, and later almost stopped. The study of the height of plants of perennial grasses, including Meadow Timothy, is of immense importance in breeding work, as it is one of the indirect indicators of the yield of green mass. Furthermore, the height of plants is one of the criteria for determining the timing of hay harvesting. In the studies conducted by the author, the height of plants of the first mowing in the phase of full earing on average for two years of use ranged from 94.0 to 116.0 cm, with a height of the standard variety of 104.7 cm. According to the height of plants, the collection samples were conditionally divided into low-growing samples with a plant height of 94.0 to 98.5 cm (25%), medium-sized – 100.8-109.4 cm (50%), and tall – 110.0-116.0 cm (25%). The tallest samples in the first and second years of use were MS1496-116.0 cm and IS1612-114.6 cm.

An important breeding feature that determines the quality and nutritional value of the feed mass is the foliage of plants. Meadow Timothy is a foliose cereal. The

yield of the green mass of perennial grasses depends on the degree of foliage: the higher the foliage, the higher the yield.

According to two-year data, samples of Meadow Timothy MS 1510-68.2%, IS 1512-60.4%, IS 1612-65.0%, MS 1816-61.3% had the best foliage. According to this feature, the collection of Meadow Timothy was divided into samples with a low level of foliage – 30.5-38.6% (four samples), samples with an average level of foliage – 41.4-55.1% (six samples) and samples with high foliage – 58.8-68.2% (six samples).

The yield of green mass is the feature that is of key practical importance and towards which the perennial grasses are bred. This is one of the crucial criteria for

sampling in productivity breeding. The size of the crop depends on various indicators (the ratio of the number of vegetative and generative shoots, plant height, foliage, etc.). The results of the conducted studies show that on average for two years of study (2020-2021), the yield of green mass for two hay harvests ranges from 3.05 to 4.10 kg/m<sup>2</sup> (at the LSD<sub>05</sub> (least significant difference) 0.21-0.28 kg/m<sup>2</sup>). The yield of green mass of the standard was 3.51 kg/m<sup>2</sup>. Therewith, 11 samples exceeded the standard by 0.23-0.59 kg/m<sup>2</sup>, or by 8-17%. Samples of IS 1512-4.05 kg/m<sup>2</sup> had the highest yield of green mass, IS 1612-4.10 kg/m<sup>2</sup>, MS 1816-4.07 kg/m<sup>2</sup>, MS 1510-4.00 kg/m<sup>2</sup>. They exceeded the Daryna variety standard by 14-17% (Table 3).

**Table 3.** Productivity and its structural elements of Meadow Timothy samples in the collection nursery (average for 2020-2021)

Sample name	Plant height, cm	Foliage, %	Green mass harvest			Duration of the vegetation period, days:
			kg/m <sup>2</sup>	% to St	± to St	
Daryna (St)	104.7	47.7	3.51	100	–	128
IS 1506	108.3	41.4	3.05	87	-0.46	129
IS 1509	105.2	58.8	3.82	109	+0.31	129
IS 1512	107.8	60.4	4.05	115	+0.54	140
MS 1602	110.0	55.1	3.92	112	+0.41	126
IS 1610	96.2	30.5	3.31	94	-0.20	128
IS 1608	102.2	45.5	3.80	108	+0.29	132
IS 1602	94.0	38.6	3.25	92	-0.26	138
IS 1612	114.6	65.0	4.10	117	+0.59	133
MS 1816	111.8	61.3	4.07	116	+0.56	125
MS 1954	100.8	42.6	3.74	106	+0.23	134
MS 1510	108.0	68.2	4.00	114	+0.49	129
GP	104.2	56.0	3.90	111	+0.39	126
MS 1823	109.4	50.1	3.83	109	+0.32	133
IS 1814	98.5	35.5	3.21	91	-0.30	133
MS 1496	116.0	42.0	3.79	108	+0.28	125
IS 1511	94.3	38.1	3.15	89	-0.36	130
LSD <sub>05</sub> 2020			0.21			
2021			0.28			

One of the elements of seed productivity of Meadow Timothy plants is the length of the panicle. On average, for two years it was the largest in the IS 1608 sample – 15.0 cm and the hybrid population sample – 15.1 cm, while in the standard Daryna variety – 12.7 cm. According to the number of seeds in one panicle, samples of IS 1512 were distinguished – 608 pcs, IS 1608-615 pcs, hybrid population – 603 pcs, IS 1509-620 pcs. The mass of seeds from one panicle was the highest in the following samples: IS 1506-0.70 g,

IS 1610-0.68 g, MS 1823-0.63 g, IS 1509-0.62 g. Samples IS 1506, IS 1509, IS 1512, IS 1608, hybrid population, MS 1496 had the largest seeds with a weight of 1,000 seeds of 0.60-0.70 g.

In terms of seed productivity, on average, 11 collection samples exceeded the standard by 1-28 % over two years of use. The highest seed yield was provided by samples of IS 1512-28.0 g/m<sup>2</sup>, IS 1608-27.0 g/m<sup>2</sup>, hybrid population – 26.5 g/m<sup>2</sup>, IS 1506-26.0 g/m<sup>2</sup>, which is 4.2-6.2 g/m<sup>2</sup>, or 19-28 % more than the standard (Table 4).

**Table 4.** Seed productivity and crop structure of Meadow Timothy samples in the collection nursery (average for 2020-2021)

Sample name	Panicle length, cm	Number of seeds in a panicle, pcs	Mass of seeds from panicles, g	Weight of 1000 seeds, g	g/m <sup>2</sup>	Seed harvest	
						% to St	± to St
Daryna (St)	12.7	529	0.35	0.47	21.8	100	–
IS1506	12.5	564	0.70	0.60	26.0	119	+4.2
IS1509	13.5	620	0.62	0.63	25.0	115	+3.2
IS1512	14.4	608	0.54	0.68	28.0	128	+6.2
MS1602	13.3	548	0.48	0.55	23.7	109	+1.9
IS1610	10.6	524	0.68	0.58	22.4	103	+0.6
IS1608	15.0	615	0.60	0.65	27.0	124	+5.2
IS1602	10.8	508	0.28	0.42	20.0	92	-1.8
IS1612	11.2	510	0.30	0.44	21.6	99	-0.2
MS1816	11.8	528	0.33	0.43	22.1	101	+0.3
MS1954	12.6	530	0.51	0.55	23.0	105	+1.2
MS1510	9.5	502	0.32	0.41	20.8	95	-1.0
GP	15.1	603	0.58	0.70	26.5	121	+4.7
MS1823	11.8	560	0.63	0.50	24.6	113	+2.8
IS1814	10.2	520	0.24	0.42	20.2	93	-1.6
MS1496	13.1	575	0.58	0.61	25.1	115	+3.3
IS1511	10.0	505	0.38	0.46	21.1	97	-0.7
LSD <sub>05</sub> 2020					0.16		
2021					1.12		

In terms of dry matter yield, on average, 10 collection samples exceeded the standard by 0.066-0.174 kg/m<sup>2</sup> over two years of use, or by 9-24%. The highest yield of dry matter – 0.888 kg/m<sup>2</sup> was provided by sample ID 1612, which is 0.174 kg/m<sup>2</sup> more than the standard. Such collectible samples that exceeded the standard for dry matter yield also deserve attention: MS1816 – by 0.147 kg/m<sup>2</sup>, or by 20%, MS1510 – by 0.134 kg/m<sup>2</sup>, or by 19%, IS1512, MS1823 – by 0.120 and 0.125 kg/m<sup>2</sup>, or by 17%.

Feed value is of significant importance, since the quality of feed determines its overall suitability.

Analysis of feed value data from Meadow Timothy samples shows that over an average of two years, the sample IS1612 had the highest content of crude protein in dry matter – 14.4%, the hybrid population – 14.0%, IS1512 and MS1954-14.1%. The highest fibre content was found in samples IS1512-30.1%, MS1602-29.6%, IS1612, MS1823-29.8%. The feed value of Meadow Timothy, as well as other perennial grasses, is explained by the fact that it has many vegetative shoots. These shoots contain more leaves than generative shoots. In addition, the leaves contain more nutrients (Table 5).

**Table 5.** Nutritional value of Meadow Timothy samples in the collection nursery (first harvest of the haymaking method of use, average for 2020-2021)

Sample name	Dry matter yield			Content in dry matter, %	
	kg/m <sup>2</sup>	% to St	± to St	Crude protein	Fibre
Daryna (St)	0.714	100	–	10.8	28.4
IS1506	0.602	84	-0.112	10.1	26.4
IS1509	0.704	98	-0.010	13.6	28.0
IS1512	0.834	117	+0.120	14.1	30.1
MS1602	0.810	113	+0.096	13.8	29.6
IS1610	0.662	93	-0.052	10.2	28.3
IS1608	0.814	114	+0.100	10.4	27.6
IS1602	0.637	89	-0.077	10.8	27.7
IS1612	0.888	124	+0.174	14.4	29.8

Table 5, Continued

Sample name	Dry matter yield			Content in dry matter, %	
	kg/m <sup>2</sup>	% to St	± to St	Crude protein	Fibre
MS 1954	0.824	115	+0.110	14.1	29.1
MS 1510	0.848	119	+0.134	13.2	29.4
GP	0.801	112	+0.087	14.0	28.1
MS 1823	0.839	117	+0.125	12.4	29.8
IS 1814	0.629	88	-0.085	10.6	28.4
MS 1496	0.780	109	+0.066	11.7	27.2
IS 1511	0.631	88	-0.083	10.0	26.8
LSD <sub>05</sub> 2020	0.04				
2021	0.06				

Meadow Timothy is a winter-resistant and cold-resistant crop. According to winter hardiness, the collection samples under study were divided into three groups: high winter resistance (85-98% of plants survived), medium winter resistance (71-84%), and low winter resistance (45-70%). Samples of MS 1602-96%, IS 1608-94%, IS 1612-98%, MS 1496-90%, IS 1814-92% had high winter resistance of plants (9 points) for two years of use.

The most harmful and widespread diseases of Meadow Timothy are stem and leaf rust, powdery mildew. Due to unstable weather conditions, the yield of crops in some years from diseases can decrease by up to 80%. Therefore, it is essential in breeding to evaluate samples for the most common diseases. According to the author's observations, no damage to collection samples by these diseases was observed during the years of research. The resistance of Meadow Timothy plants to major diseases was 9 points.

Evaluation of collectible samples of Meadow Timothy will also continue in 2022. Based on the results of three-year studies, the best samples in terms of economically valuable characteristics will be selected for further breeding work.

The expediency of using raw materials of various ecological and geographical origin in the breeding of Meadow Timothy is evidenced by the data of other researchers. Thus, during 2019-2020, 28 collectible samples of Meadow Timothy originating from Ukraine, Russia, Belarus, and Estonia were investigated at the Ustimovska Experimental Crop Production Station in the southern part of the forest-steppe of Ukraine. Promising

samples were selected, which exceeded the yield standard, were distinguished by economically valuable features (plant height, foliage, weight of 1,000 seeds, duration of the growing season) and can later be used as a starting material in the selection of varieties of this crop. It was found that the best source material for breeding Meadow Timothy is wild forms, as well as individual breeding varieties (Kocherha & Rohovyi, 2020).

## CONCLUSIONS

The collection of samples of Meadow Timothy is a valuable source of variety of source material for creating new varieties. According to preliminary data of the assessment of samples of Meadow Timothy in the collection nursery during 2020-2021, promising samples were identified that exceed the Daryna variety standard for the main economically valuable characteristics with the following productivity indicators: plant height – 105.2-116.0 cm (9 samples), foliage – 50.1-68.2% (8 samples), green mass yield – 3.74-4.10 kg/m<sup>2</sup> (11 samples), dry matter yield – 0.780-0.888 kg/m<sup>2</sup> (11 samples), panicle length – 13.5-15.0 cm (6 samples), number of seeds in the panicle – 530-620 pcs (9 samples), panicle seed weight – 0.38-0.68 g (11 samples), 1,000 seed weight – 0.48-0.70 g (10 samples), seed yield – 22.4-27.0 g/m<sup>2</sup> (10 samples), crude protein content – 11.7-14.4% (10 samples), fibre content – 29.1-30.1% (6 samples). All the samples under study had high resistance to winter and diseases. The study of the collection material will continue next year. Based on the results of a three-year evaluation, the best samples will be selected to attract them to further breeding work with Meadow Timothy.

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## Попередні результати оцінки колекційних зразків тимофіївки лучної як цінного вихідного матеріалу для селекції

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**Анотація.** Серед найбільш поширених багаторічних кормових трав сінокісно-пасовищного використання, а також одним із компонентів бобово-злакових травосумішок є тимофіївка лучна. Високу продуктивність тимофіївки лучної можуть забезпечити тільки нові сучасні сорти, для створення яких велике значення має використання в селекційних програмах вихідного матеріалу різного еколого-географічного походження. Для цього необхідно провести оцінку його за комплексом господарських та селекційно-цінних ознак, що і було метою нашої роботи. Методологічну основу становили польові та лабораторні методи дослідження, яке проводили протягом 2020–2021 рр. на дослідному полі Передкарпатського відділу наукових досліджень Інституту сільського господарства Карпатського регіону НААН. Вивчалось 16 колекційних зразків місцевої селекції, отриманих в результаті індивідуального та масового добору, а також одна гібридна популяція. Стандарт – сорт Дарина. За дворічними даними найбільшу висоту рослин мали зразки МД 1496 – 116,0 см та ІД 1612 – 114,6 см, облиственість – МД 1510 – 68,2 %, ІД 1512 – 60,4 %, ІД 1612 – 65,0 %, МД 1816 – 61,3 %, врожай зеленої маси – ІД 1512 – 4,05 кг/м<sup>2</sup>, ІД 1612 – 4,10 кг/м<sup>2</sup>, МД 1816 – 4,07 кг/м<sup>2</sup>, МД 1510 – 4,00 кг/м<sup>2</sup>, сухої речовини – ІД 1612 – 0,888 кг/м<sup>2</sup>, МД 1816 – 0,861 кг/м<sup>2</sup>, насінневу продуктивність – ІД 1512 – 28,0 г/м<sup>2</sup>, ІД 1608 – 27,0 г/м<sup>2</sup>, ГП – 26,5 г/м<sup>2</sup>, ІД 1506 – 26,0 г/м<sup>2</sup>, довжину волоті – ІД 1608 – 15,0 см, ГП – 15,1 см, кількість і маса насіння з однієї волоті – ІД 1608, ІД 1509, ІД 1506, ІД 1610, маса 1000 насінин – ІД 1512 – 0,68 г, ІД 1608 – 0,65 г, ІД 1509 – 0,63 г, вміст сирого протеїну – ІД 1612 – 14,4 %, ІД 1512, ІД 1954 – 14,1 %, ГП – 14,0 %, клітковини – ІД 1512 – 30,0 %, МД 1823, ІД 1612 – 29,8 %, МД 1602 – 29,6 %. Всі зразки мали високу зимостійкість, стійкість до захворювань. Дослідження буде продовжено в 2022 році. На основі трирічних даних кращий вихідний матеріал буде залучено в подальшу схему селекційного процесу

**Ключові слова:** сорт, ознака, колекційний розсадник, продуктивність, кормова цінність



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## Influence of Foliar Top-Dressing on the Yield of Soybean Varieties

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**Abstract.** The use of complex microfertilisers on a chelated basis in agricultural technologies of the main crops is limited due to the lack of clear recommendations on the norm, methods, and timing of their use in particular production conditions and the levels of expected yield increase. Based on the rather specific mechanism of action of drugs, these recommendations are adjusted by investigating the level of reaction of plants and crops in particular zonal and weather conditions. The purpose of this study was to establish the reaction of soybean varieties to foliar top-dressing with complex Vuksal Microplant microfertiliser. Scientific research was conducted according to the field method during 2019-2021. According to the scheme of the experiment, the following varieties were investigated: Krynytsia, ES Hladiator, Melodiia, Korona, Feieriia, Etiud, Sava, Orfei, Everest, which are classified as early-maturing. The crop was fertilised according to the following variants:  $N_{15}P_{30}K_{40}$ ;  $N_{15}P_{30}K_{40}+1$  Vuksal Microplant top-dressing and  $N_{15}P_{30}K_{40}+2$  Vuksal Microplant top-dressing. According to the tasks of experimental studies, the field germination rate of seeds was identified by calculating the density of plants in the phase of full germination for all repetitions of the experiment; phenological observations were made in variants of the experiment using the method of variety testing of agricultural crops; the leaf surface area was determined according to the clear-cutting method and the yield was established according to the weight method using direct combining of each site. Statistical processing of experimental data was performed using the Microsoft Excel and Statistica 10.0 application software package. A variant of the fertiliser system was established, which provides a substantial impact on soybean yield and a variety that formed stable productivity over years with changing weather conditions. Based on the results of the study, it is recommended to grow the Etiud soybean variety in production crops with culture fertilisation according to the system of applying macroelements at the rate of  $N_{15}P_{30}K_{40}$  and performing two top-dressings with the Vuksal Microplant complex fertiliser on a chelate basis at the rate of 2 l/ha. The first spraying should be carried out in the phase of 2 ternate leaves (BBCH 13-14), and the second in the phase of bean formation (BBCH 70-71)

**Keywords:** legumes, cultivation technology, fertiliser system, microfertilisers



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## INTRODUCTION

Normal growth and development and formation of the soybean grain crop is possible only involving secondary fertilising components (Vozhegova *et al.*, 2016). Studies conducted in various soil and climatic zones of Ukraine have determined that not all secondary fertilising components and not all soils need to be applied under agricultural crops (Gamajunova *et al.*, 2021). Microelements increase the yield of soybean grains, provided that they are applied on soils that are poor in fertility and content on the corresponding elements (Vozhegova *et al.*, 2018).

The relevance of the subject under study lies in the fact that important achievements in biology over the past century are proven facts of the need for trace elements for the active life of plant, animal, and human bodies. Considerable attention of the scientific community around the world is paid to establishing the role of secondary fertilising components in plant life. Microfertilisers have a positive effect on the processes of organogenesis of soybean plants.

Scientists confirm that the agrochemical and physiological role of microfertilisers is multifaceted (Shevnikov & Shevnikov, 2020). They improve the metabolic processes of substances in plants, activate their synthesising functions and contribute to the optimal course of physiological and biological processes (Tafij *et al.*, 2016). They have a positive effect on the process of chlorophyll synthesis and improve the intensity of photosynthesis (Kots *et al.*, 2022). The action of secondary fertilising components contributes to the resistance of plants to fungal and bacterial diseases (Pospelova *et al.*, 2021). It affects the increase in tolerance of such unfavourable environmental conditions as lack of productive moisture in the soil, short-term decrease or increase in air temperature, and other biotic factors (Gamayunova *et al.*, 2020). The most effective measures to influence the productivity of soybean varieties are the protection of crops from harmful organisms, the use of irrigation, a balanced fertiliser system, biologics, and regulators.

The purpose of the study was to establish the yield level of modern soybean varieties depending on the fertiliser system.

To fulfil the stated purpose, the following tasks were identified:

- to calculate the density of plants in the germination phase and determine the field germination rate of soybean seeds depending on the variety;
- to conduct phenological observations of the onset of growth and development phases of soybean varieties and record the duration of the entire vegetation season;
- to determine the leaf surface area of soybean plants depending on the fertiliser system;
- to determine the influence of the properties of varieties and the fertiliser system on the yield of soybean seeds.

## MATERIALS AND METHODS

Scientific research was conducted during 2019–2021 in the conditions of the central forest-steppe of Ukraine.

The object under study was varieties of the early-maturing group: Krynysia, ES Hladiator, Melodiia, Korona, Feieria, Etiud, Sava, Orfei, Everest.

In the field experiment, the system of fertilisation of soybean varieties was used according to the following variants:

1.  $N_{15}P_{30}K_{40}$ ;
2.  $N_{15}P_{30}K_{40}$  + 1 top-dressing with Vuksal Microplant;
3.  $N_{15}P_{30}K_{40}$  + 2 top-dressings with Vuksal Microplant.

Mineral fertilisers for soybeans were applied in the norm –  $N_{15}P_{30}K_{40}$ . During the main tillage, 30 kg of active agent/ha of phosphorus and 40 kg of active agent/ha of potassium were added. For this purpose, 150 kg/ha of physical weight of simple granular superphosphate and 100 kg/ha of physical weight of potassium salt were used. During sowing, a seeder applied 15 kg of active agent/ha of full mineral fertiliser in the form of ammonium nitrate phosphate fertiliser, in the norm of 100 kg/ha of physical weight of fertiliser. For foliar top-dressing, Vuksal Microplant was used – a mineral fertiliser, the components of which are chelated complexes (%): N – 5;  $K_2O$  – 10; MgO – 3;  $SO_3$ –13; B – 0.3; Cu – 0.5; Fe – 1; Mn – 1.5; Mo – 0.01; Zn – 1.

On the variants where 1 top-dressing was used, the crops were sprayed with a working solution in the phase of 2 ternate soybean leaves (BBCH 13–14) with 2 l/ha of Vuksal Microplant.

On the variants where 2 top-dressings were used, soybean crops were sprayed with a working solution in the geminate leaf phase (BBCH 13–14) with 2 l/ha of Vuksal Microplant and in the bean formation phase (BBCH 70–71) – with the same fertiliser at 2 l/ha.

In total, 27 variants were investigated in the experiment: nine varieties (Factor A) and three variants of the fertiliser system (Factor B). The experiment repeatability – three times. Site placement – randomised (Yeshchenko *et al.*, 2005). The area of the experimental plot was 36 m<sup>2</sup>, accounting area – 25 m<sup>2</sup>. Soybeans were sown in the usual drills with a row spacing of 15 cm. According to the scheme of the experiment, the cultivation technology in the variants was the same, only the fertiliser system under study differed.

The main type of soil of the experimental sites is typical heavy loamy chernozem. Humus content in the soil at a depth of 0–20 cm was 3.8–4.5%; easily hydrolysed nitrogen (according to Tiurin) – 8.6–12.2 mg/100 g of soil;  $P_2O_5$  (according to Chyrykov) – 15.8–20.1 mg/100 g of soil;  $K_2O$  (according to Maslova) – 10.3–12.1/100 g of soil. During the three years of research, weather conditions had deviations compared to the long-term average. In terms of humidity and temperature conditions, the best conditions for soybeans were during the growing season of 2019 and 2021, but the increased air temperature combined with the drought, due to the lack of precipitation during the second half of July and throughout August, limited the synthesis of organic matter, which adversely affected the development of crop productivity. The worst weather conditions were recorded during 2020, especially the lack of moisture was characteristic.

## RESULTS AND DISCUSSION

Scientists have found that upon working to increase the adaptive potential of soybean varieties, it is possible to increase the annual collections of vegetable protein and oil by 10-15% or more (Vozhegova *et al.*, 2019).

Plants use only a fraction of the mineral elements introduced into the soil (Arbačauskas *et al.*, 2021). Thus, for most brands of mineral fertilisers, the average utilisation rates of the active agent range from 40-60% nitrogen, phosphorus 10-20%, potassium 20-40% (Taratenko *et al.*, 2021). Furthermore, the level of nutrient absorption depends on the structural parameters and quality of the soil, as well as on the development of the plant's root system (Hanhur *et al.*, 2020). According to the data provided in most reference books (Shepilova *et al.*, 2021) the formation of one hundredweight of soybean seeds requires 4.5-9.5 kg of nitrogen, 1.5-3 kg of phosphorus, 3.5-6 kg of potassium. Rather wide limits

of variation of coefficients indicate the presence of factors that contribute to or, conversely, reduce the level of assimilation of soil minerals (Punchyshyn *et al.*, 2019).

One of the first tasks of the study was to establish the field germination of seeds by counting plants in the phase of full soybean germination.

According to the results of calculating the number of plants by variants in the phase of full germination, it was found that the germination rate of soybean seeds was influenced by weather conditions of the year and biological characteristics of varieties (Table 1). Depending on the conditions of the year, the best field germination of seeds was in 2019, on average for variants. Depending on the varieties, the highest density of plants in the full germination phase was in the Etiud variety. Field germination of seeds of this variety, on average, was 91.2%.

**Table 1.** Field germination rate of soybean seeds, % (2019-2021)

Item No.	Experiment variants	N <sub>15</sub> P <sub>30</sub> K <sub>40</sub>	N <sub>15</sub> P <sub>30</sub> K <sub>40</sub> + 1 top-dressing with Vuksal Microplant	N <sub>15</sub> P <sub>30</sub> K <sub>40</sub> + 2 top-dressing with Vuksal Microplant
1	Krynytsia	75.1	77.8	76.3
2	ES Hladiator	84.3	87.7	85.1
3	Melodiia	82.1	84.5	83.6
4	Korona	85.6	87.1	85.9
5	Feieriia	86.1	87.2	86.5
6	Etiud	90.3	92.5	90.9
7	Sava	80.5	82.6	80.9
8	Orfei	81.4	83.1	82.3
9	Everest	83.4	85.3	84.1

The duration of the growing season is an indicator that describes the conditions of crop formation of field crops.

As for the duration of the soybean growing season, it is not a constant value. It varies for several reasons, primarily the temperature of the soil and air, the intensity and duration of lighting, the level and nature of moisturising (Vozhegova *et al.*, 2020). The level of response depends on the specific features of the genotype, dosage, and ratio of these factors (Gamayunova & Panfilova, 2020).

A critical review of scientific sources on the influence of abiotic and biotic factors on the duration of soybean vegetation indicates considerable differences in opinions on their role and place in changing the duration of vegetation. Thus, M. Galytska *et al.* (2021) emphasise that the intensity of plant organogenesis mainly depends on the temperature regime of the environment, and the water regime affects only the duration of certain interphase periods. Namely, for the following periods:

sowing – germination and flowering – maturation. The complex influence of factors on the development of agricultural plants is indicated by O.V. Tryhub *et al.* (2020), noting that the duration of each of the phases of ontogenesis mainly depends on the level of accumulation of organic compounds at apical growth points. The data on the close correlation between the duration of the soybean growing season, the intensity and spectral composition of sunlight are quite convincing (Miladinov *et al.*, 2020).

According to the results of phenological observations, it was found that in all variants of the experiment, the longest growing season of soybeans was in the Melodiia variety (Table 2). The soybean fertiliser system had differing effects on the formation of vegetative and generative organs and the maturation of the crop in particular. The use of foliar top-dressing with complex Vuksal Microplant microfertiliser affected the lengthening of the growing season from 2 to 7 days, on average, according to the experiment.

**Table 2.** Duration of the growing season of soybean plants, days (2019-2021)

Item No.	Experiment variants	$N_{15}P_{30}K_{40}$	$N_{15}P_{30}K_{40} +$ 1 top-dressing with Vuksal Microplant	$N_{15}P_{30}K_{40} +$ 2 top-dressing with Vuksal Microplant
1	Krynytsia	110	114	116
2	ES Hladiator	109	112	115
3	Melodiia	110	113	117
4	Korona	108	110	116
5	Feieriia	105	107	108
6	Etiud	101	103	105
7	Sava	109	114	115
8	Orfei	106	108	109
9	Everest	102	105	106

Spraying of crops with Vuksal Microplant micro-fertiliser twice during the growing season lengthened the growing season by 3-8 days, compared to variants where foliar top-dressing of plants was not carried out at all.

The foliar top-dressing factor had an accumulative effect, which provided a gradual increase in the difference between the indicators of vegetative development of plants from juvenile to generative stages of soybean organogenesis. The same conclusions were obtained in the study by S.Y. Kots *et al.* (2022) but using other solutions for foliar top-dressing.

A substantial difference between the control and the experimental variants in terms of leaf surface area was recorded starting from the "budding" phase. Such a mechanism of variation in the indicators of vegetative

development of plants, according to the variants of the experiment using microfertilisers for top-dressing, indicates the physiological reaction of a certain variety, which expands the agrotechnical possibilities of increasing the photosynthetic apparatus of plants.

The development of the assimilation surface of soybean plants, within the framework of the experiment, was influenced by the weather conditions of the year, the characteristics of the variety and the complex use of macro- and microfertilisers with different effects on the physiological and biochemical processes in soybean plants (Table 3). According to the results of the experiment, the maximum leaf surface area is 0.905 m<sup>2</sup>/ the plant was formed in the Etiud variety with the fertiliser system of culture  $N_{15}P_{30}K_{40} + 2$  top-dressings with Vuksal Microplant.

**Table 3.** Leaf surface area in the soybean seed filling phase, m<sup>2</sup>/ plant (2019-2021)

Item No.	Experiment variants	$N_{15}P_{30}K_{40}$	$N_{15}P_{30}K_{40} +$ 1 top-dressing with Vuksal Microplant	$N_{15}P_{30}K_{40} +$ 2 top-dressing with Vuksal Microplant
1	Krynytsia	0.695	0.701	0.713
2	ES Hladiator	0.792	0.794	0.799
3	Melodiia	0.732	0.735	0.738
4	Korona	0.806	0.809	0.845
5	Feieriia	0.85	0.851	0.858
6	Etiud	0.898	0.903	0.905
7	Sava	0.733	0.739	0.751
8	Orfei	0.781	0.784	0.79
9	Everest	0.771	0.788	0.811

The results of phenological observations, measurements and calculations during the field experiment indicate a fairly high level of reaction of soybean plants to the use of microfertilisers for foliar top-dressing during the growing season of the crop. However, in agronomy, the effectiveness of the elements of field crop cultivation technology under study can be analysed only based on the main indicator, namely the yield of the main products.

The most favourable weather conditions for the formation of soybean yields were in 2019. The yield of varieties differed substantially (Table 4). The maximum yield of soybean seeds of 3.11 t/ha was obtained from crops of the Etiud variety on the variant of a combination of mineral fertilisers application in the norm  $N_{15}P_{30}K_{40}$  and two foliar top-dressings with the Vuksal Microplant complex microfertiliser on a chelated basis.

**Table 4.** Soybean yield depending on the variety and fertiliser system, t/ha (2019-2021)

Item No.	Experiment variants	N <sub>15</sub> P <sub>30</sub> K <sub>40</sub>	N <sub>15</sub> P <sub>30</sub> K <sub>40</sub> +	N <sub>15</sub> P <sub>30</sub> K <sub>40</sub> +
			1 top-dressing with Vuksal Microplant	2 top-dressing with Vuksal Microplant
1	Krynytsia	2.19	2.22	2.31
2	ES Hladiator	2.47	2.48	2.58
3	Melodiia	2.45	2.49	2.53
4	Korona	2.69	2.73	2.8
5	Feieriia	2.78	2.81	2.87
6	Etiud	2.99	3.02	3.11
7	Sava	2.46	2.51	2.59
8	Orfei	2.6	2.62	2.67
9	Everest	2.62	2.64	2.69
Least significant difference <sub>0.05</sub> t/ha		A <sub>(variety)</sub> – 0.03; B <sub>(fertiliser system)</sub> – 0.01		

According to the results of the studies by Z. Miladinov *et al.* (2020) it was also found that foliar spraying with fertiliser solutions had a considerable impact on soybean yields. And the year factor, namely the availability of moisture, affected the productivity of the crop and the effectiveness of top-dressing solutions.

## CONCLUSIONS

It is established that production conditions necessitate the use of several varieties with differing biological characteristics, ratio to environmental factors, protein content, oil content, sensitivity to fertilisers, resistance to diseases, and crop density. It should also be considered that even in agroclimatic zones, where varieties can be grown with a longer vegetating season, it is necessary to select genotypes described by different maturation periods. This approach will reduce the impact of possible adverse abiotic factors (rainy summers, low air temperatures), simplifying the optimisation of sowing and harvesting terms. Agrotechnical elements of cultivation technology in modern conditions do not

sufficiently contribute to the realisation of the genetic potential of modern soybean morphobiotypes in terms of productivity indicators, which is associated with the low compliance of agricultural measures with the ecological and biological features of intensive varieties. Proceeding from this, there is a problem of improving the elements of cultivation technology to adapt them to the biological characteristics of soybeans, which contributes to the maximum use of its yield potential. The most effective measures to influence the productivity of soybean varieties are the use of a balanced fertiliser system. Therefore, for the central forest steppe zone of Ukraine, the authors of this study recommend growing the Etiud soybean variety using the fertiliser system N<sub>15</sub>P<sub>30</sub>K<sub>40</sub> + 2 top-dressings with Vuksal Microplant, with the norm of 2 l/ha. The first top-dressing should be carried out in the phase of 2 trifoliolate leaves (BBCH 13-14), the second in the phase of bean formation (BBCH 70-71).

*Prospects for further research* lie in the study of the complex application of elements of technology for growing soybean varieties of different ripeness groups.

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## Вплив позакореневого підживлення на врожайність сортів сої

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**Анотація.** Застосування комплексних мікродобрив на хелатній основі у агротехнологіях основних культур обмежене через відсутність чітких рекомендацій щодо норми, способів та строків їх використання у конкретних виробничих умовах і рівнів очікуваної прибавки врожаю. Виходячи із досить специфічного механізму дії препаратів, коригування цих рекомендацій проводиться шляхом вивчення рівня реакції рослин і посівів у цілому в конкретних зональних та погодних умовах. Метою досліджень було встановити реакцію сортів сої на позакореневе підживлення комплексним мікродобривом Вуксал Мікроплант. Наукові дослідження проводили польовим методом упродовж 2019–2021 років. За схемою досліджу вивчали сорти: Криниця, ЕС Гладіатор, Мелодія, Корона, Феєрія, Етюд, Сава, Орфей, Еверест, які класифікують як ранньостиглі. Удобрення культури проводили за такими варіантами:  $N_{15}P_{30}K_{40}$ ;  $N_{15}P_{30}K_{40}+1$  підживлення Вуксал Мікроплант та  $N_{15}P_{30}K_{40}+2$  підживлення Вуксал Мікроплант. Згідно з завданнями експериментальних досліджень було визначено польову схожість насіння, шляхом підрахунку густоти рослин у фазі повних сходів по всіх повтореннях досліджу; проведено фенологічні спостереження у варіантах досліджу за методикою сортовипробування сільськогосподарських культур; визначено площу листової поверхні методом «висічок» та встановлено рівень урожайності ваговим методом за допомогою прямого комбайнування кожної ділянки. Статистичну обробку експериментальних даних проведено з використанням пакету прикладних програм Microsoft Excel і Statistica 10.0. Установлено варіант системи удобрення, що забезпечує істотний вплив на врожайність сої та сорт, який формував стабільну продуктивність по роках з мінливими погодними умовами. За результатами досліджень рекомендовано у виробничих посівах вирощувати сорт сої Етюд із удобренням культури за системою внесення макроелементів у нормі  $N_{15}P_{30}K_{40}$  та проведення двох підживлень комплексним добривом на хелатній основі Вуксал Мікроплант у нормі 2 л/га. Перше обприскування потрібно проводити у фазі 2-х трійчастих листків (BBCH 13-14), а друге у фазі формування бобів (BBCH 70-71)

**Ключові слова:** зернобобові, технологія вирощування, система удобрення, мікродобрива



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## Environmental Sustainability and Perception of Safety of Vaccine in the COVID-19 Pandemic

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**Abstract.** The study on environmental sustainability and perception of safety of vaccination in the context of the COVID-19 pandemic has been relevant for a long time and has been caused by people's concerns and fears about the consequences of the vaccination procedure. This paper provides a detailed analysis of approaches to determining environmental sustainability and emphasises the urgent need to maintain human health in a pandemic. Thus, the purpose of this study is to empirically investigate the readiness for vaccination against COVID-19 as a condition of environmental sustainability. The subject of the study is the environmental factors of readiness for vaccination against COVID-19. A set of methods and techniques were used to achieve this purpose, namely theoretical research methods, surveys, associative method, content analysis, methods of mathematical statistics using SPSS 23.0 and ArcGis. The paper presents the results of an empirical study of environmental sustainability and perception of safety of vaccination during pandemic, such as a comparative analysis of the sense of security of supporters and opponents of vaccination. The results of an empirical study of environmental sustainability and perception of safety of vaccination in a pandemic is provided. It was established that there are age and gender differences between proponents and opponents of vaccination. It was determined that subjects who were wary of vaccination did not consider COVID-19 to be a source of personal threat. The results of associations for the word-stimulus "danger" were analysed. There were differences in deep subconscious beliefs of danger in those who plan to be vaccinated and those who question vaccination. It was determined that the proponents of vaccination consider the general unavoidable external circumstances as a danger, and its opponents consider the very COVID-19 vaccination as such. Statistically significant differences were found between the sense of security in different areas of life in those who consider COVID-19 a danger and those for whom COVID-19 is not a source of concern. A prognostic portrait of a resident of Ukraine who is ready and willing to be vaccinated is presented. The obtained empirical results are of scientific value for researching the psychological characteristics of individual attitudes towards safe environmental sustainability and can be used in the development and implementation of programmes to work with people suffering from internal feelings of danger for their health caused by external circumstances

**Keywords:** areas of life, health, readiness for vaccination, factors of readiness for vaccination, personality psychotype, the sense of security



## INTRODUCTION

With the current global pandemic, total uncertainty and exacerbation of the environmental crisis, the problem of environmental sustainability is most relevant. The study of sustainability is gaining wide research-to-practice application for the development of environmental sustainability and safety. Environmental sustainability is defined as the responsibility to conserve natural resources and protect global ecosystems to maintain health and well-being now and in the future (Corvalan *et al.*, 2005).

Since the key element of environmental sustainability is its long-term nature, environmental sustainability should be defined as meeting current needs without harming future generations, their ability to meet their needs as the US Environmental Protection Agency defines them. According to the UN World Commission on Environment and Development, the main point of environmental sustainability is to provide future generations with available natural resources, and their standard of living should not be lower than the current one (United Nations Environment Programme..., 2020). Along with this, according to International Union for Conservation of Nature, environmental sustainability is also defined as the ability to improve the quality of human life; stabilisation of the modern conflicting relationship between the two global systems of the Earth: human culture and the living world (International Union for Conservation of Nature, 2020).

Thus, the phenomenon of ecological sustainability is so relevant that it requires humanity to intervene at once to stabilise the destructive impact of humans on the environment and preserve, and even restore the current level of the Eco world. This view of the situation is supported by quantitative indicators of the level of environmental sustainability, namely the Environmental Performance Index (EPI) (Wendling *et al.*, 2020). Thus, the EPI score for Ukraine was 49.5 points in 2020, while the health score was 49 points, which ranked the country 60<sup>th</sup> and 69<sup>th</sup> out of 180 countries according to their environmental performance (Wendling *et al.*, 2020). The index of ecological efficiency is derived from the index of ecological sustainability and defines the main categories of national policy and ranks countries according to their ecological achievements (Wendling *et al.*, 2020). The EPI classifies 180 countries according to the impact of the environment on human health and ecosystem life using 32 performance indicators. The indicators defined by the Index of Ecological Groups, which reduce the burden of the environment on human health, are of priority scientific interest for this study (Wendling *et al.*, 2020) as a basic condition for ecological sustainability.

The World Health Organisation (WHO) defines health as a state of complete physical, spiritual, and social well-being, and not merely the absence of disease or infirmity (Preamble to the Constitution..., 1946). Mental health is a state of well-being in which everyone can best realise their potential, cope with life's stresses, work productively, and make an effective contribution to the life of their community (Ostafin *et al.*, 2021; Order of

the Cabinet of Ministers of Ukraine No. 1018-r..., 2017). Awareness and realisation of life self-determination, responsibility for one's life and health, being in a state of well-being, ability to adequately respond to uncertainty and threats, show empathy for oneself and the world (Zhuravlova & Chebykin, 2021), ability to overcome life stresses and post-traumatic growth (Jayawickreme *et al.*, 2021) are the main indicators of human health.

The era of the global COVID-19 pandemic has provoked a new wave of threats and worries, fears and expectations, anxiety and uncertainty, which is another damage caused by this virus. The situation of long-term and constant psychological stress and the decrease in psychological security are caused by the deterioration of key areas of human life (social, economic, communication) in its different age periods. It has been established that the COVID-19 pandemic has adversely affected the income of young people (including students) and their families (Guadalupe-Lanas *et al.*, 2021); increased feelings of danger, anxiety about personal health and poor communication among the elderly (Raycheva, 2021); the level of feeling of well-being, comfort, and stability of the environment among people aged 15 to 59 has considerably decreased (Wei *et al.*, 2021).

Instead, a positive attitude, adequate response, and the ability to timely receive qualified medical care is the key to preventing and overcoming COVID-19 in a mild form. Thus, a healthy lifestyle, an adequate attitude towards threats, and to COVID-19 vaccination against it are indicators of reducing the burden on the environment for human health and, consequently, environmental sustainability. The global pandemic, its consequences, and the situation of complete uncertainty have clearly indicated the importance and priority of developing the measures of environmental sustainability. The latter is catalysed by conditions endowed with environmental significance and preventing conscious inclusion in life (Rayne, 2013). Given the substantial amount of research on psychological safety (Bedny, 2021; Knowles & Olatunji, 2021; Ornell *et al.*, 2020), the effects of human exposure to COVID-19 (Ahorsu *et al.*, 2020; Ornell *et al.*, 2020; Palgi *et al.*, 2021), readiness of people for vaccination (Lackner & Wang, 2021; Paul *et al.*, 2021), the problem of environmental sustainability and safety in a pandemic remains unexplored.

Critical analysis of earlier research suggests that environmental sustainability is determined by the current state (psychological, physical) of the individual, followed by their readiness to act. Given the understanding of psychological attitude as a specific state of the person that expresses readiness to engage in certain activities aimed at meeting current needs and determines their psychophysiological organisation in a particular situation (Uznadze D.N., 1997), it can be said that readiness for vaccination is determined by a personal attitude towards vaccine against COVID-19. Accordingly, a realistic adequate response to a pandemic situation, an understanding of the likely consequences for human life and health and, as a result, an adequate response

and the possibility of obtaining qualified medical care in case of an illness is a constructive approach to vaccination. This understanding of readiness for vaccination against COVID-19 indicates the environmental sustainability of the individual. The latter is expressed by the individual's willingness to save their life and health through vaccination.

*The purpose of the study* was to empirically investigate the readiness for vaccination against COVID-19 as a condition for environmental sustainability. The subjects of the study are the environmental factors of readiness for vaccination against COVID-19. The main hypothesis of the study was the assumption that readiness to be vaccinated is influenced by subjective and objective factors.

## MATERIALS AND METHODS

The empirical study involved 568 people aged 12 to 70 years old (112 men and 462 women). The average age of respondents was 32.0 years, including women – 32.3 years, men – 30.7 years. The study was conducted from the beginning of October 2020 to the end of January 2021. To conduct the study, authors used the social network Facebook, which is the first-ranking social network in terms of the number of users in Ukraine. Several theoretical (analysis of Ukrainian and English-written sources, generalisation of concepts and approaches, systematisation, comparison) and empirical (surveys, collection of related information, associative method, content analysis, methods of mathematical statistics using SPSS 23.0 and ArcGis) methods were used in this study. The former ones were used to analyse the psychological characteristics of environmental sustainability and perception of safety of vaccine in a pandemic. The empirical methods were utilised for the study of age, gender, and psychological differences in the context of such factors as environmental sustainability and safety. To investigate the above phenomena, the authors developed an online questionnaire, the purpose of which is the remote systematic study of the factors of readiness of Ukrainians to be vaccinated against COVID-19. The questionnaire comprises several content-related blocks:

- collection of general information: place of residence, age, and sex of respondents;
- study of security: self-assessment of personal security during the pandemic in various spheres of life (physical, sexual, gender, family, territorial, financial, religious, national, political, social, food, business);
- research of personal psychological constructs: psychodiagnostics of personal characteristics such as

tendency to sociopathy, Machiavellianism, and narcissism using the “Dark Core” method and semantic thesauri using the method of free associations;

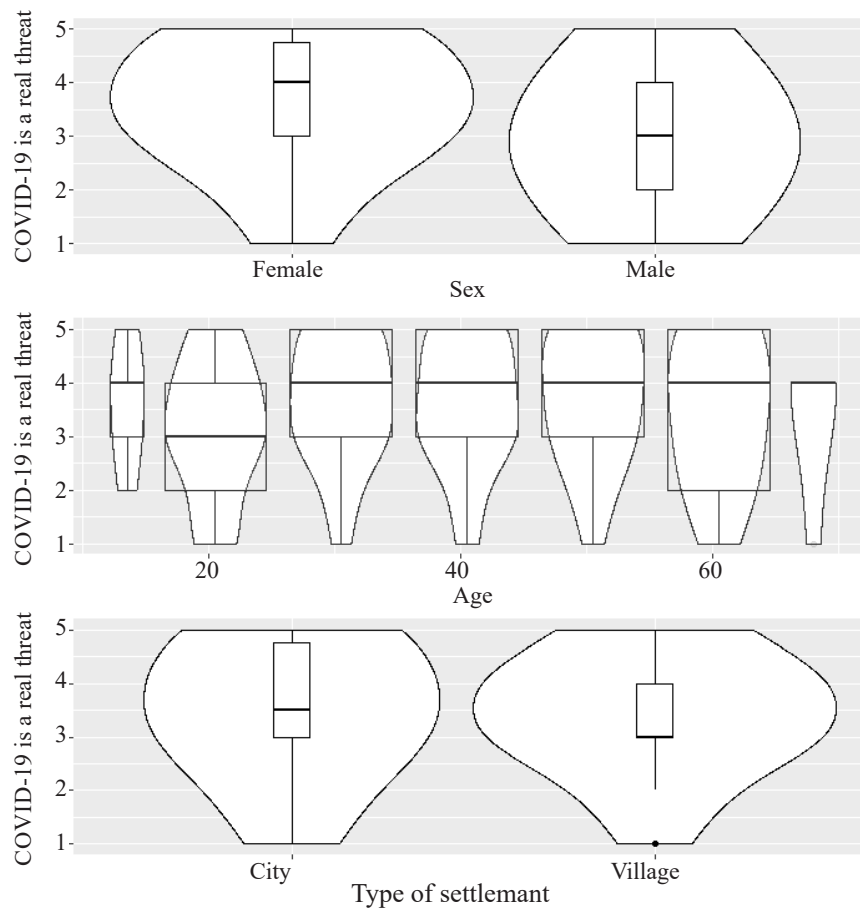
- determine attitudes towards COVID-19: measures of awareness of the COVID-19 pandemic as a source of danger and readiness for vaccination.

The study was conducted via the distribution of the questionnaire on social networks, which are described by the diversity of users by gender, age, social status, and place of residence. The general information collection unit allowed determining the age, gender, and urban diversity of the respondents. The research unit about the feeling of security allowed establishing the level of subjective feeling of protection experienced by Ukrainians in the fight against a pandemic in every sphere of life by asking “Assess the level of your personal safety in the following spheres of life on a five-point scale” (1 – minimum security, 5 – maximum security).

Such scaling has demonstrated its effectiveness in the study of the attitude towards the vaccine and the readiness to get vaccinated (Palgi *et al.*, 2021). The unit of research of personal psychological constructs was realised through the use of the standardised and validated method called “Dark Core” (or “Dark Triad”, “Dark core of personality”), which allows establishing not only borderline variants of the norm, but also variants of the norm (Jones & Paulhus, 2011; Furnham *et al.*, 2013). The “Dark Triad” is a technique for measuring three psychological personality traits: non-clinical narcissism and non-clinical psychopathy and Machiavellianism as a single complex of personality traits. The questionnaire contained 12 questions, with each question evaluated on a five-point scale. The questionnaire involves the identification of one of the personality types or a tendency towards it. The block of attitudes towards COVID-19 allowed establishing the specific features of the subjects' behaviour (“If I have an important meeting, I will attend it, even with cold symptoms”), awareness of COVID-19 as a source of danger (“COVID-19 is a threat to me personally”), readiness for the vaccination procedure (“I must be vaccinated against COVID-19 when the vaccine is available”). Respondents' answers were evaluated on a five-point scale (disagree – 1 point, rather disagree – 2 points, partially agree – 3 points, rather agree – 4 points, completely agree – 5 points).

## RESULTS AND DISCUSSION

It was found that there were gender, age, and demographic differences in the assessment of the level of threat caused by the COVID-19 pandemic (Fig. 1).



**Figure 1.** Dispersion of COVID-19 threat estimates according to gender, age, and demographics

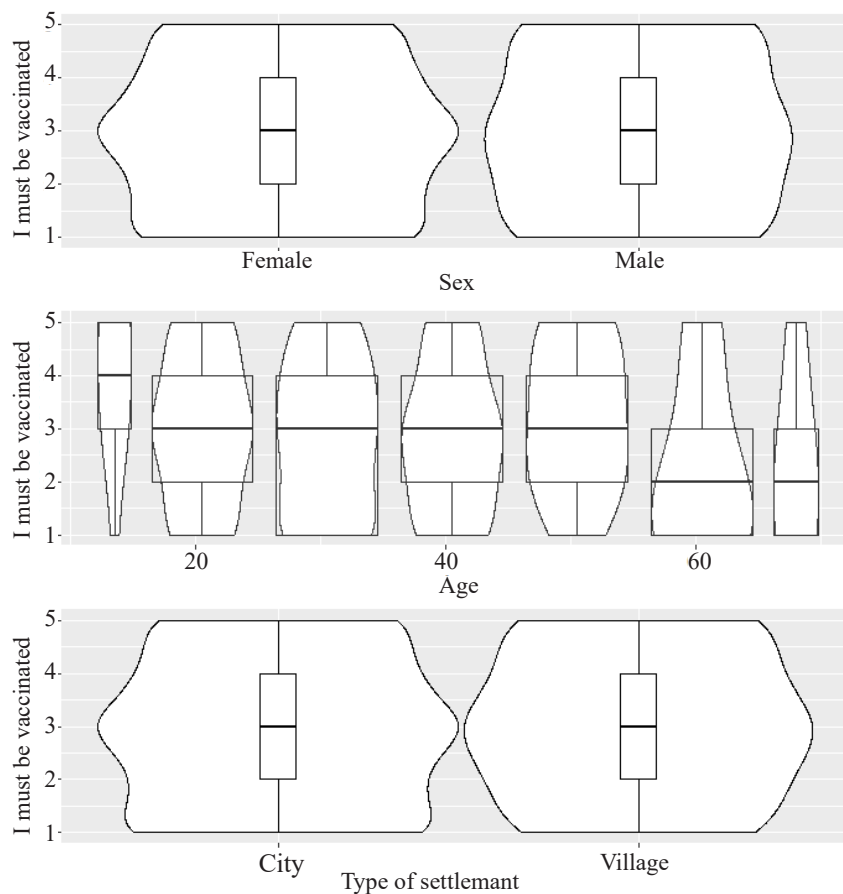
Subjective factors such as the sense of imminent threat of COVID-19 and the assessment of personal safety in key areas of life (sexual, family, national, and food) determined the readiness for vaccination. Objective factors were gender, age, demographics. Men and young people in Ukraine expressed a higher level of readiness to be vaccinated compared to women, older adults, and the elderly, respectively. The elevated level of urbanisation also determined a person's readiness for vaccination.

Women were more likely than men to consider COVID-19 a personal threat. Men did commonly demonstrate the fear of the virus. Demographic features of the variance of pandemic threat estimates turned out to be gender equivalent. Respondents living in rural areas responded to the pandemic according to the "female" type, and those living in cities – according to the "male" type, but urban residents, compared to rural ones, more frequently understood the threat of COVID-19. Most of the subjects worried about COVID-19 were aged between 25 and 45. Younger and older respondents were less serious (more cynical) and did not consider COVID-19 a substantial threat. Between the ages of 45 and 55, the number of respondents considering COVID-19 a personal threat was low.

The influence of a person's subjective sense of security in various spheres of life on their fear of a pandemic was also investigated. Interesting results were found upon comparing the supporters and opponents

of vaccination against COVID-19. Positive links were found between respondents' perception of security in the family and religious spheres and the threat of a pandemic (respectively,  $t\text{-value}=1,980$ ;  $p\text{-value}\leq 0.05$  and  $t\text{-value}=2,024$ ;  $p\leq 0.03$ ). In the gender and business spheres, a negative correlation was found between the respondents' measures of security in these areas and the threat of a pandemic (respectively,  $t\text{-value}= -3,426$ ;  $p\leq 0.000$  and  $t\text{-value}= -1,078$ ;  $p\leq 0.03$ ). The safer respondents felt in family and religious life, the less likely they were to worry about COVID-19. And the more vulnerable they considered the sexual and business spheres of their lives to be, the more threatening COVID-19 was for them.

Interesting results were found upon comparing indicators of supporters and opponents of vaccination against COVID-19. Notably, a considerable part of the age groups under study did not trust vaccination and unequivocally refused it. 21.39% of respondents stated they would not be vaccinated and 17.74% – that they most likely would not be vaccinated. Only 18.43% of respondents said that they should and would be vaccinated, and 16.87% were likely to be vaccinated. There were no considerable gender differences among proponents and opponents of vaccination (variance is homogeneous): most people show moderate readiness for vaccination. However, there were more respondents among women who had not yet decided on vaccination than men. There were age-specific features in readiness for being vaccinated (Fig. 2).



**Figure 2.** Dispersion of readiness for vaccination according to gender, age, and demographic characteristics

Young people were ready to be vaccinated, while people of older age and the elderly (55 years and older) were wary of vaccination.

Subjective and objective factors of readiness for vaccination were determined based on the results of the linear regression analysis. One of the dominant factors of such readiness was the feeling of imminent threat of being defeated by COVID-19 ( $t$ -value=8,617;  $p \leq 0.000$ ), as well as the subjective assessment of safety in sexual ( $t$ -value= -2,096;  $p \leq 0.04$ ), family ( $t$ -value=1,595;  $p \leq 0.1$ ), national ( $t$ -value= -1,501;  $p \leq 0.1$ ), and food ( $t$ -value=1,419;  $p \leq 0.1$ ) spheres. Indicators of a sense of security in the physical, gender (sexual), territorial, financial, religious, political, social, and business spheres of life were not related to the fear of being vaccinated against COVID-19. To objectify the subjective factors of readiness for vaccination, the authors analysed the difference between assessments of feelings

of security in different areas of life by supporters (strongly agree – 5 points) and opponents (disagree – 1 point) of vaccination (Table 1). Proponents of vaccination included respondents who rated their readiness for vaccination with a maximum score (5), and opponents – those who rated their readiness very low (1). Subjects who did not want to be vaccinated considered themselves safe in the business sphere, and their sense of security in other areas of their lives did not affect their readiness for vaccination. Respondents who showed a clear willingness to be vaccinated felt insecure in sexual sphere and secure in sexual (gender) and national spheres of life. Similar to the study by C.A. Latkin, *et al.* (2021), the authors of this paper found that in Ukraine a considerable proportion of subjects did not trust the vaccination against COVID-19. This study also found that study participants who did not trust the vaccines had trust issues in certain areas of their lives.

**Table 1.** The influence of a sense of security in various spheres of life on the readiness to be vaccinated by supporters and opponents of vaccination

No. n/a	Areas of life	T-test	
		(Do not want to be vaccinated)	(Want to be vaccinated)
1	Physical	9,910e-01	8,800-02
2	Sexual	-1,393e+00	-1,716+00*
3	Gender	7,900e-01	1,515+00*
4	Family	4,300e-02	-1,319+00
5	Territorial	-7,790e-01	1,267+00



of the study of objective factors of readiness for vaccination were ambiguous. Young people were ready to be vaccinated, unlike older people. There was an inversely proportional relationship between the age of the subjects and their desire to be vaccinated ( $t$ -value =  $-2.828$ ;  $p \leq 0.005$ ). On the other hand, the level of urbanisation did not affect the desire to be vaccinated. The generalisation of the research results allowed building a portrait of a resident of Ukraine who is ready and willing to be vaccinated ( $R=0.165$ ;  $p \leq 0.000$ ). This is a man under 45 ( $p \leq 0.001$ ), who considers COVID-19 a personal threat ( $p \leq 0.000$ ), is financially secure ( $p \leq 0.05$ ), but feels vulnerable in the social sphere ( $p \leq 0.1$ ). The research hypothesis was partially proved. The authors consider the prospects for further research in the investigation of the dynamics of psychological security under quarantine restrictions and the COVID-19 pandemic.

## CONCLUSIONS

1. A healthy lifestyle and an adequate attitude towards situations of uncertainty form the basis for the development of environmental sustainability. Safety conditions endowed with ecological content are indicators of the level of this sustainability. Threats, namely the COVID-19 pandemic, the uncertainty of the vaccination situation are among the factors of physical and mental stress on human health and, consequently, its environmental sustainability.

2. There are gender and age characteristics of the subjective experience of feelings of danger and instability in the face of current threats to humanity (COVID-19). Women are more likely than men to consider COVID-19

a personal threat. However, both men and women are equally moderately sceptical of vaccination. Young and middle-aged adults are most concerned about COVID-19, and young people and the elderly are not inclined to consider COVID-19 as a primary threat. A subjective factor in an attitude towards COVID-19 is a sense of security in the family and religious spheres, and the perception of a pandemic as a personal threat depends on a sense of danger in the gender and business spheres.

3. Subjective factors of readiness for vaccination are the feeling of imminent threat of defeat by COVID-19, assessment of the degree of danger in the sexual, family, national, business, religious, food spheres, and propensity to narcissism. The objective factors of readiness for vaccination are the age and sex of the individual.

4. The semantic features of proponents and opponents of vaccination from COVID-19 represented a subjective-existential factor of readiness for vaccination such as dominance in the hierarchy of deep associations of objective global phenomena.

5. In Ukraine, financially well-off men under the age of 45 who consider COVID-19 a personal threat and feel vulnerable in the social sphere are most ready for vaccination.

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## Екологічна стійкість та сприймання безпечності вакцини в умовах пандемії COVID-19

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**Анотація.** Дослідження екологічної стійкості та сприймання безпечності вакцини в умовах пандемії COVID-19 зберігає свою актуальність уже тривалий час і спричинене хвилюваннями та побоюваннями людей щодо наслідків процедури вакцинації. У статті здійснено детальний аналіз підходів до визначення екологічної стійкості та наголошується на нагальній потребі підтримувати здоров'я людини в умовах пандемії. Таким чином, метою дослідження є емпіричне вивчення готовності до вакцинації проти COVID-19 як умови екологічної стійкості. Предметом дослідження є екологічні фактори готовності до вакцинації проти COVID-19. Для реалізації поставленої мети було використано комплекс методів та методик, зокрема, теоретичні методи дослідження, опитування, асоціативний метод, контент-аналіз, методи математичної статистики з використанням SPSS 23.0 та ArcGis. Подано результати емпіричного дослідження екологічної стійкості та сприймання безпечності вакцинації в умовах пандемії. Встановлено, що існують вікові та статеві відмінності прихильників та противників вакцинації у почутті захищеності. Визначено, що досліджувані, які з осторогою ставляться до вакцинації не вважають COVID-19 джерелом особистої загрози. Проаналізовано результати асоціацій на слово-стимул «небезпека». Виявлені відмінності в підсвідомих уявленнях про небезпеку у тих хто планує вакцинуватися та тих, хто ставить вакцинацію під сумнів. Визначено, що прихильники вакцинації небезпекою вважають загальні неминучі зовнішні обставини, а її противники – COVID-19. Знайдено статистично значущі відмінності між почуттям захищеності в різних сферах життя в осіб, які вважають COVID-19 небезпекою, та тими для кого COVID-19 не є джерелом хвилювань. Подано прогностичний портрет жителя України, який готовий вакцинуватись. Отримані емпіричні результати складають наукову цінність у вивченні психологічних особливостей індивідуального ставлення до безпечної екологічної стійкості та можуть бути використані при розробці та впровадженні програм роботи з людьми, які потерпають від внутрішнього відчуття небезпеки щодо власного здоров'я, яке зумовлене зовнішніми обставинами

**Ключові слова:** сфери життя, здоров'я, готовність до вакцинації, фактори готовності до вакцинації, психотип особистості, почуття захищеності



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## Foreign Economic Priorities for the Development of Agro-Food Enterprises in European Integration Business Partnership

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**Abstract.** Agro-food production is a source of the national food security, the basis for increasing its export potential, meeting domestic demand for agricultural products and food. The relevance of the subject under study is that Ukraine is currently developing the European vector of foreign economic activity. The purpose of this study is to substantiate a set of indicators used to assess the export potential of agro-food enterprises in the European integration business partnership both qualitatively and quantitatively. The authors used general scientific methods (analysis, synthesis), integrated approach, model of the hierarchy of factors influencing the integration business environment on the export potential of enterprises. Permissible tariff quotas for the volume of duty-free exports of crop products, livestock, and processed goods for Ukraine have been allocated. The volume of concentration of production of export-oriented agricultural crops in the enterprises of agro-food production of Ukraine was analysed. It was established that the bulk of agro-food exports are agricultural raw materials, including cereals and oilseeds, vegetable (sunflower) oil. The study estimated the efficiency of corn exports on average per agro-food enterprise in Ukraine under the conditions of currency risks. It was determined that in 2020 the total export costs for 1 tonne amounted to 186.18 USD. Furthermore, it was found that the profit received as a result of export activity for 1 tonne was 76.90 USD. The authors identified the parameters of the regression equation and their estimation in the dependence model of Ukrainian internal prices on export prices for agro-food products on the world market. The gravitational model of the integrated level of the export potential of Ukrainian agro-food enterprises was proposed to stimulate foreign trade on the world market and ensure a stable business partnership with European Union member states

**Keywords:** export potential, agricultural products, market, trade, price



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## INTRODUCTION

Agro-food production demonstrates the stability of development. Strengthening the position of any country in the world market of agricultural products and food, in terms of exports of its key types, is ensured by the factors of agricultural development in the conclusion of the Association Agreement between European Union (EU) countries and the creation of a Free Trade Area (European Commission, 2021). Prospects for the gradual development of the EU market allow building an effective policy on the implementation of foreign economic priorities for its expansion. Creating preconditions for mechanisms of interaction between the state and business allows focusing on measures of national agricultural policy to improve the efficiency of agricultural market instruments. This contributes to the free and transparent movement of agricultural products, the smoothing of price fluctuations, the functioning of agricultural logistics as a factor of increasing the profitability of enterprises by reducing total production costs.

The multilevel hierarchical system of national support for the basic and promising sectors of agriculture is aimed at harmonising agro-food production in the European integration business partnership. Based on market control instruments over agricultural quality in the internal market it strengthens international competition, reduces restrictions and barriers to exchange, and increases competitiveness and effective interaction between producers and consumers. Aspects of ensuring the foreign economic activity (FEA) of agro-food production are considered by many scientists who investigated these issues through the interaction of functioning institutional entities and structural components of the institutional system that regulate their international economic interactions and relationships. N. Cuckovic, *et al.* (2013) investigated the factors affecting regional competitiveness and ways for its measurement on example of Croatia. In Latvia, this aspect was investigated by I. Judrupa (2021). A study on regional competitiveness in Latin America was conducted by S. Catalán (2021). In Ukraine, this issue was studied by Yu.H. Kozak *et al.* (2016), and A.V. Zavhorodnii (2019). V. Januškaite & L. Užiene (2018) paid attention to intellectual capital and its importance for regional competitiveness. O. Romanko *et al.* (2019) studied the importance of innovation and investment activities. Brás *et al.* (2021) considered the Entrepreneurial University construct. A. Bilbao-Terol *et al.* (2019) and V. Charles & T. Sei (2019) created their original approaches to measuring regional competitiveness.

Priority areas for the development of foreign economic relations in the agricultural sector of the economy, with an emphasis on the transformation of the market model of the country and the modernisation of the criteria of foreign economic activity of economic entities were studied (Bakhchivandzhy *et al.*, 2013). A.V. Kliuchnyk (2015) characterised state and non-state regulation of foreign economic activity including agricultural sector. The connection between export orientation of the country and its competitiveness were investigated

by M.O. Lepekha & H.M. Svyrydenko (2017). N.V. Trusova *et al.* (2021) developed an approach to assessing the investment infrastructure of agri-food entities, which allowed forecasting the volume of agri-food exports. N.H. Varshavska (2016) studied the European standards of agro-food production with a focus on basic socially oriented market relations. I.M. Demchak *et al.* (2019) investigated the state of the agricultural products and food market in Ukraine, having summarised information on the volume and dynamics of its exports and imports. V.S. Ivanchenkov (2014) explained the concept of innovation in the canning industry and highlighted areas of further research on this issue. M. Moraliyska, (2018) considered the EU cohesion policy, the changes it had caused, its impact on the economy, as well as the European standards of agro-food production. Yu.M. Umantsiv & O.I. Miniailo (2018) described the specific features of the national economy development in the context of globalisation.

*The purpose of this study* was to substantiate a set of indicators used to assess the export potential of agro-food enterprises and increase their competitiveness in foreign markets to harmonise European integration business partnership in international trade and consumption of agricultural products and promote export-oriented internal market.

## MATERIALS AND METHODS

Foreign economic priorities for agro-food enterprises, with the heterogeneous nature of the main structural elements of export potential require a special approach to its quantification. The synergy of levels of harmonisation of business partnership in the European integration space, interpreted by qualitative and quantitative criteria of the mathematical apparatus, is built on the universal principles of economic analysis, with methodological positions of an integrated approach. From the standpoint of creating an environment for the effective functioning of foreign economic activity subjects, it is advisable to investigate them through the lens of international integration relations and their development level, as well as factors of national regulation, stimulation, and control of foreign economic relations, investment attractiveness (Nelep, 2011).

Analysis of the interaction of formal and informal contradictions in business partnerships and relationships to harmonise the transformation of the integrated business environment at the stage of investigating important causal links between structural elements of the export potential of agro-food enterprises is provided for socio-economic variables at various levels: international, national, sectoral, regional, and micro-level. The system of ensuring foreign economic activity of agro-food enterprises in the expansion of the European integration process forms the basic business relations of business partnership based on norms and rules establishing certain restrictions and opportunities (exchange, processing, distribution, storage, transportation, and

consumption). In general, the national system of foreign economic activity support in the European vector is unformed and has several destructive elements (Rodrik, 2001). Therefore, it is proposed to assess the cumulative impact of factors of export-oriented internal market on the coefficient of impact of factors of European integration business environment on the export potential of agro-food enterprises, which is calculated according to formula (1) (Ryneyska, 2018):

$$C_{MAC} = \frac{\pm A_1 B_1 \pm A_2 B_2 \pm \dots \pm A_n B_n}{[\max A_i B_i] \cdot n} = \frac{\sum_{i=1}^n (\pm A_i B_i)}{[\max A_i B_i] \cdot n} \quad (1)$$

where  $C_{MAC}$  is the coefficient of influence;  $A$  is the expert assessment of the importance of factors of export-oriented internal market;  $B$  is the expert assessment of the impact of factors of the European integration business environment;  $n$  is the number of influencing factors.

Using the method of correlation analysis to assess the impact of factors of the European business environment on the export potential of agro-food enterprises

$$\begin{cases} a_0 m + a_1 \sum_{j=1}^m x_{1j} + a_2 \sum_{j=1}^m x_{2j} + \dots + a_n \sum_{j=1}^m x_{nj} = \sum_{j=1}^m y_j, \\ a_0 \sum_{j=1}^m x_{1j} + a_1 \sum_{j=1}^m x_{1j}^2 + a_2 \sum_{j=1}^m x_{1j} x_{2j} + \dots + a_n \sum_{j=1}^m x_{1j} x_{nj} = \sum_{j=1}^m x_{1j} y_j, \\ \dots \\ a_0 \sum_{j=1}^m x_{nj} + a_1 \sum_{j=1}^m x_{nj} x_{1j} + a_2 \sum_{j=1}^m x_{1j} x_{2j} + \dots + a_n \sum_{j=1}^m x_{nj}^2 = \sum_{j=1}^m x_{nj} y_j \end{cases} \quad (3)$$

Dynamic changes in foreign economic relations lead to the need for permanent coordination of European integration interests of all business partners with various levels of stimulating levers of export-oriented support in the internal market of agro-food production (Kosach, 2017). This process is influenced by such factors as the specific features of economic activity of entities, the volume and complexity of tasks, the level of socio-economic development of society (territory), which depends on the ability of socio-political system to ensure its stability in

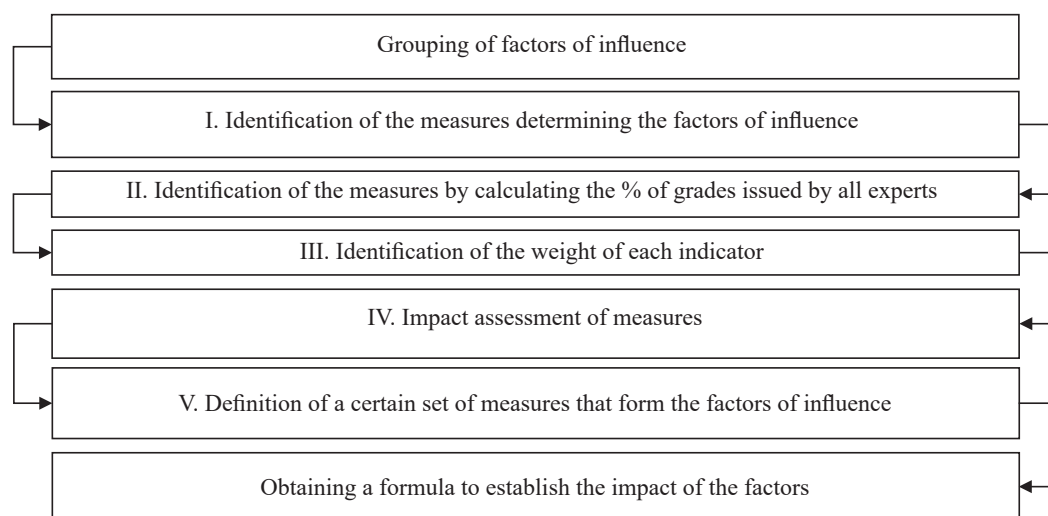
allows establishing the most significant of them (Zhuk & Bilianskyi, 2020; Ryneyska, 2018). A prominent issue in this case is the choice of the analytical form for the function  $f$ , which connects the available factors with the effective feature-function. This feature must reflect the factual correlation between the indicators and factors under study. Empirical substantiation of the type of function by graphical analysis of relationships for multifactor models is not suitable. Considering that any function of many variables can be reduced to a linear form by logarithmising or substituting variables, then in practice the multiple regression equation (2) is given in a linear form (Kosach, 2017):

$$\hat{Y} = a_0 + a_1 X_1 + a_2 X_2 + \dots + a_n X_n \quad (2)$$

where:  $a_0, a_1, \dots, a_n$  are the parameters of the equation to be determined.

If for each factor, including the resultant attribute  $n$  values  $y_i, X_{1i}, X_{2i}, \dots, X_{ni}, i = 1, 2, \dots, m$ , are known, then the regression equation (3) is obtained as follows:

European integration (Karasova, 2015). Several unresolved methodological issues on harmonisation of foreign economic priorities of cooperation between agro-food enterprises as an innovative process of balancing the goals and interests of all subjects of foreign economic relations in time and space, allows building models of key factors of European integration business environment. Accordingly, based on the method of hierarchies, an alternative multi-level structure of incentives for export-oriented internal market is built as follows (Fig. 1).



**Figure 1.** Algorithm for stimulating export-oriented internal market

**Source:** developed by the authors

The next step is to identify the key measures of the factors of the European integration business environment on the export potential of agro-food enterprises with subsequent calculation of the percentage of points (4) (Kosach, 2017):

$$B_{gi} = \frac{\sum_{k=1}^n a_k}{\sum_{k=1}^n \max a_k} = \frac{\sum_{k=1}^n a_k}{50} \quad (4)$$

where:  $B_{gi}$  is the percentage of experts' estimates of the indicator  $g_i$ ;  $a_k$  is the assessment by the  $k^{\text{th}}$  expert of the  $i^{\text{th}}$  indicator;  $k$  is the total number of experts;  $\max a_k$  is the maximum possible value of the indicator  $a_k$  in this scale.

The weight of each indicator is determined as the

ratio of the value of each indicator to the sum of all estimates (5) (Kosach, 2017):

$$g_i = \frac{B_{gi}}{\sum_{i=1}^m B_{gi}} \quad (5)$$

where:  $m$  is the total number of factors assessed by experts.

Further assessment of the export potential of agro-food enterprises requires the development of a model of the hierarchy of factors of influence. For the convenience of further modelling, the notation for each of the elements will be supplemented with a mnemonic name (Table 1).

The binary matrix A is formed in the form of a table with information rows and columns (Table 2).

**Table 1.** Mathematical interpretation of the model of the hierarchy of factors influencing the European integration business environment on the export potential of agro-food enterprises

Mathematical notation	The name of the factor influencing the European business environment on agro-food enterprises	Mnemonic name
$Z_1$	Factors influencing monetary interests	<i>F</i>
$Z_2$	Factors influencing the foreign economic interests of agro-food enterprises	<i>FEIE</i>
$Z_3$	Factors influencing foreign economic relations	<i>FER</i>
$Z_4$	Relations with international institutions	<i>RII</i>
$Z_5$	Factors influencing the correlation between internal and external European integration business environment of FEA agro-food enterprises	<i>IEI</i>
$Z_6$	Factors influencing the economic interests of staff in the development and promotion of innovative products for export	<i>EISdpIPE</i>
$Z_7$	Factors influencing environmental interests	<i>EI</i>
$Z_8$	Factors influencing production interests	<i>PI</i>

Source: developed by the authors

**Table 2.** Binary matrix (A) of elements that determine the factors of influence

	$F_1$	$F_2$	$F_3$	$F_4$	$F_5$	$F_6$	$F_7$	$F_8$
$F_1$	x	x	x	x	x	x	x	x
$F_2$	x	x	x					x
$F_3$								x
$F_4$	x	x	x	x	x	x	x	x
$F_5$	x	x	x	x	x	x	x	x
$F_6$			x	x	x	x	x	x
$F_7$	x	x	x	x			x	x
$F_8$	x	x	x	x	x	x	x	x

Source: developed by the authors

Based on the matrix A, a binary matrix of reachability (I+A) is constructed, where I is a unit matrix that satisfies the following condition (6) (Kosach, 2017):

$$(I + A)^{k-1} \leq (I + A)^k = (I + A)^{k+1} \quad (6)$$

The combined results of the reachability matrix (I + A) are summarised in Table 3. The vertex is reached if  $z_j$  has a vector of motion from vertex  $z_i$ , if on the graph there is a vector of motion from vertex  $Z_i$  to vertex  $Z_j$ .

**Table 3.** Matrix of reachability (I+A) of elements

	$F_1$	$F_2$	$F_3$	$F_4$	$F_5$	$F_6$	$F_7$	$F_n$
$F_1$	x	x	x	x	x	x	x	x
$F_2$	x		x		x			x
$F_3$		x	x	x	x	x	x	
$F_4$	x	x	x	x	x	x	x	x
$F_5$	x	x	x		x	x	x	
$F_6$	x		x	x				x
$F_7$		x	x	x	x	x	x	x
$F_n$	x	x	x	x	x	x	x	

**Source:** developed by the authors

For further modelling, a subset of similar vertices  $S(Z_j)$  and analogous vertices ( $Z_i$ ) is formed, which are formed before vertex  $Z_j$ , provided that it reaches its vertex. The set of previous vertices forms a subset  $P(Z_i)$ , that is satisfied by the condition (7) (Bilianskyi, 2020):

$$R(z_i) = S(z_i) \cap P(z_i) \quad (7)$$

The set of actions performed will determine the first level of the hierarchy of elements. Based on the previous matrix, the consolidated results of the levels of the model of the hierarchy of elements are built (Table 4).

**Table 4.** The combined results of the levels of importance of the elements

$i$	$S(z_i)$	$P(z_i)$	$S(z_i) \cap P(z_i)$
1	1, 2, 3, 4, 5, 6, 7, n	1, 2, 3, 4, 5, 6, n	1, 2, 3, 4, 5, 6, n
2	1, 2, 4, 5, 6, 7, n	1, 2, 4, 5, 6, 7, n	1, 2, 4, 5, 6, 7, n
3	1, 3, 4, 5, 6, 7, n	1, 3, 4, 5, 6, n	1, 3, 4, 5, 6, n
4	1, 2, 3, 4, 5, 6, n	1, 2, 3, 4, 5, 6, n	1, 2, 3, 4, 5, 6, n
5	1, 2, 3, 4, 5, 6, 7, n	1, 2, 3, 4, 5, 6, 7, n	1, 2, 3, 4, 5, 6, 7, n
6	1, 2, 3, 4, 5, 6, n	1, 2, 3, 4, 5, 6, 7, n	1, 2, 3, 4, 5, 6, n
7	1, 2, 5, 7, n	2, 3, 5, 6, 7, n	2, 6, 7, n
n	1, 2, 3, 4, 5, 6, 7, n	1, 2, 3, 4, 5, 6, n	1, 2, 3, 4, 5, 6, n

**Source:** developed by the authors

The emergence of a particular element at a certain level significantly depends on the correlations established between them, given in the original graphic image, the change of which in number and nature will lead to a modification of the resulting model (8) (Yemelianenko et al., 2019):

$$F = \begin{cases} f_1(x_1, x_2, \dots, x_n; y_1, y_2, \dots, y_n) \\ f_2(x_1, x_2, \dots, x_n; y_1, y_2, \dots, y_n) \\ f_n(x_1, x_2, \dots, x_n; y_1, y_2, \dots, y_n) \end{cases} \quad (8)$$

where:  $F$  is the total functional dependence;  $f_i$  is the conversion function between effective parameters with their fixed number ( $i = 1, z$ );  $x_j, y_j$  are the groups of parameters that can be functionally independent if the elements of one group  $x_j$  or  $y_j$  are always functionally interdependent ( $j = 1, n$ ).

Economic substantiation of priority directions of the development of foreign economic relations on the world market and maintenance of European integration business partnership of agro-food enterprises is carried

out based on the analysis of group of efficiency indicators (Verbytska, 2013): absolute indicators; relative indicators; structure indicators; efficiency indicators. When analysing the priority areas of foreign economic relations in the world market and ensuring European integration business partnership of agro-food enterprises, the chosen criterion of efficiency should be considered, it should meet the methodological principles of generalising indicators (Didukh, 2020), be proportional, and meet its economic content. The presented data allows calculating intermediate and integrated economic indicators; build factor models to identify the impact of individual factors on the generalised indicator, etc. They are used to identify deviations in foreign economic activity of agro-food enterprises, reserves of increasing production, namely export-oriented ones and other parameters requiring management decisions.

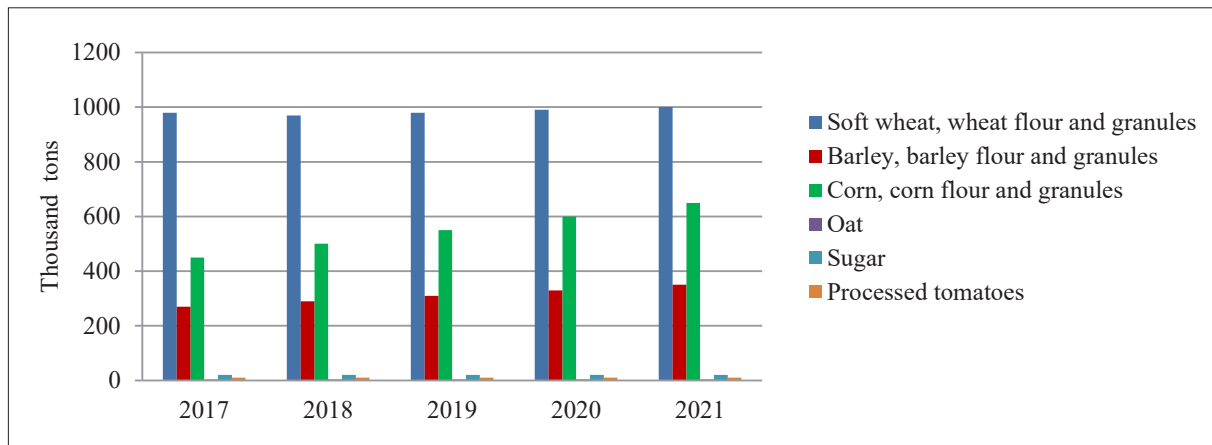
## RESULTS AND DISCUSSION

The procedure for the creation of a Deep and Comprehensive Free Trade Area (DCFTA) with the EU is part of the Association Agreement between the European Union

and its Member States, on the one part, and Ukraine, on the other part (2014) and lasts from 2016 to 2026. Mutual access to markets, including agricultural products, which are based on mandatory trade rules, expand; there are processes of convergence of the European integration business environment with various levels of stimulation of the national export-oriented internal market for agro-food products according to international rules and regulations. This is manifested in substantial adaptation of Ukrainian legislation, in the reduction of import and export duties on imports of Ukrainian products to EU countries (providing access to markets without customs restrictions under tariff quotas).

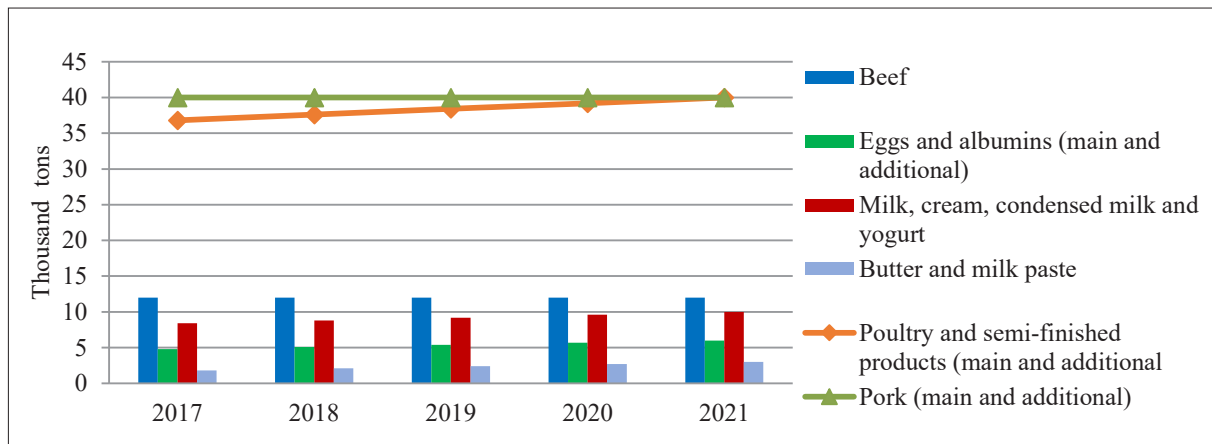
The tariff quota for trade with the EU countries is abolished in export to their territory 83.1% of tariff

lines and 35.2% of tariff lines when importing European products into the territory of Ukraine. Therewith, transitional periods from 1 to 7 years and duty-free tariff quotas have been introduced for agro-food products (Kvasha, 2013). That is, agricultural enterprises are given the opportunity to adapt to highly competitive European markets. Trade in these products is insignificant compared to other products of the national economy, as agricultural production in the EU is supported from the national budget. Thus, according to the agreement with the EU, Ukrainian exporters can import agricultural products of Ukrainian origin into the EU countries without paying import duties within the established tariff quotas. The dynamics of allowable volumes of duty-free exports and their increase is presented in Figure 2; 3.



**Figure 2.** Dynamics of allocated tariff quotas for the volume of duty-free exports of crop products and products of their processing for Ukraine for 2017-2021, thousand tonnes

**Source:** compiled by the authors according to data State Statistics Service of Ukraine (2021)



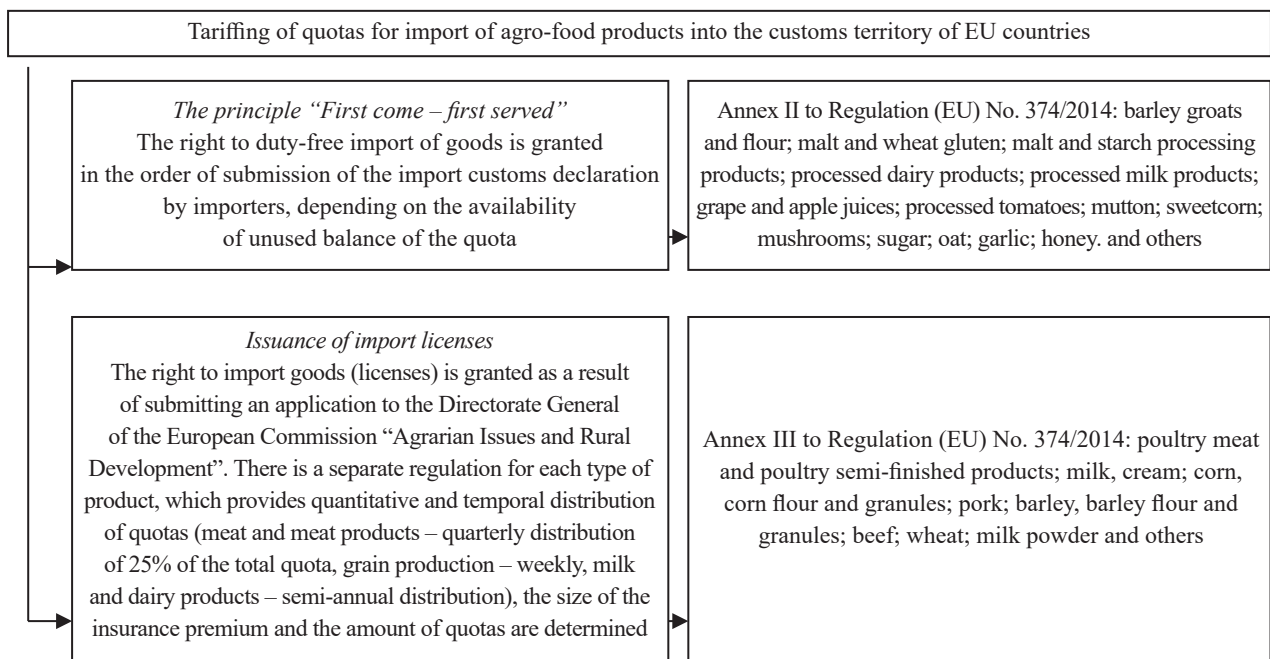
**Figure 3.** Dynamics of allocated tariff quotas for the volume of duty-free exports of livestock products and goods for Ukraine for 2017-2021, thousand tonnes

**Source:** compiled by the authors according to data State Statistics Service of Ukraine (2021)

It is established that the list of products comprises 36 items including beef, pork, lamb, poultry, milk, cream, butter, yogurt, cereals, and processed products, honey, sugar, eggs, and other foods. For some types of agricultural products, added quotas are allocated, which are introduced when the basic tariff quota is exhausted,

namely for wheat, corn, barley groats, honey, grape juice, barley flour.

The distribution of quotas is based on two principles "first come – first served" or through a system of import licenses. The order of distribution and the list of goods subject to such distribution are presented in Figure 4.



**Figure 4.** Tariffing of quotas on imports of agro-food products to EU countries

**Source:** compiled by the authors according to data from Diia Business (2021); Regulation (EU) No. 374/2014 of the European Parliament and of the Council "On the Reduction or Elimination of Customs Duties on Goods Originating in Ukraine" (2014)

To reduce barriers to trade in agro-food products by Ukrainian exporters, which may arise due to differences in technical regulations and standards for conformity assessment and other quality requirements, harmonisation of national norms with EU standards and metrology, accreditation, conformity assessment and market surveillance is envisaged. Thus, trade business partnership

with EU countries creates a positive image in international markets for Ukrainian agro-food enterprises. According to the analysis of foreign trade of agro-food products (for the period of January–August 2021 compared to January–August 2020) in Ukraine exports and imports of goods of all kinds increased as follows: imports +20.5%, exports +9.5%, balance +5.0% (Table 5).

**Table 5.** Ukraine's Foreign Trade in Agricultural Goods with EU, January-September 2021

	Exports		Imports		Balance
	Billion USD	In % to January–September 2020	Billion USD	In % to January–September 2020	
Austria	0.709	181.3	0.592	148.8	0.117
Belgium	0.498	130.4	0.478	129.1	0.020
Bulgaria	0.615	177.8	0.394	195.2	0.221
Greece	0.136	101.6	0.323	143.0	-0.187
Denmark	0.212	134.5	0.226	150.5	-0.014
Estonia	0.121	156.1	0.127	93.5	-0.007
Ireland	0.053	70.6	0.165	106.2	-0.112
Spain	1.013	113.9	0.657	126.7	0.356
Italy	2.495	187.0	1.783	126.8	0.713
Cyprus	0.032	140.1	0.020	212.8	0.012
Latvia	0.216	129.4	0.146	137.8	0.070
Lithuania	0.426	138.8	0.917	148.3	-0.490
Luxembourg	0.011	86.0	0.015	121.0	-0.004
Malta	0.027	397.7	0.113	1308.6	-0.085
Netherlands	1.583	126.3	0.723	133.7	0.860
Germany	2.034	137.4	4.521	116.5	-2.486
Poland	3.989	171.3	3.542	121.2	0.447

Table 5, Continued

	Exports		Imports		Balance
	Billion USD	In % to January–September 2020	Billion USD	In % to January–September 2020	
Portugal	0.234	156.9	0.066	146.9	0.169
Romania	1.129	148.5	0.588	132.3	0.541
Slovakia	0.788	235.6	0.631	81.1	0.158
Slovenia	0.057	197.9	0.205	118.6	-0.147
Hungary	0.117	129.9	1.224	126.7	-0.049
Finland	0.063	142.2	0.220	122.6	-0.157
France	0.581	151.6	1.277	122.8	-0.696
Croatia	0.027	126.7	0.047	119.4	-0.021
Czech Republic	1.125	201.4	1.007	156.2	0.118
Sweden	0.075	147.2	0.468	163.8	-0.393

**Note:** USD – United States dollar

**Source:** compiled by the authors according to data V. Kruglyak (2021)

In 2020, agro-food enterprises concentrated 85% of the gross harvest of corn, 80% of wheat, 58% of barley, 99% of rapeseed, 92% soybeans and 86% of sunflower seeds. Therewith, a considerable concentration of production was formed by such crops as wheat (40.5% of

production volumes are concentrated in 4.3% of agro-food enterprises with sown area over 1000 ha), corn (52.8% of production volumes – in 5.2% of enterprises), sunflower (40.4% of production volumes – in 4.7% of enterprises), soybean (36.4% – in 2.5% of enterprises) (Table 6).

**Table 6. Concentration of production of the main export-oriented crops in the enterprises of agro-food production of Ukraine, 2020**

Products	Groups of enterprises by sown area, ha	Share in total production, %	Share of enterprises in total, %	Yield, c/ha
Wheat	under 1,000 ha	59.5	95.7	37.4
	over 1,000 ha	40.5	4.3	40.1
Corn	under 1,000 ha	47.2	94.8	79.6
	over 1,000 ha	52.8	5.2	94.9
Barley	under 1,000 ha	88.7	99.5	31.7
	over 1,000 ha	11.3	0.5	38.0
Sunflower seeds	under 1,000 ha	59.6	95.3	23.6
	over 1,000 ha	40.4	4.7	24.8
Rapeseed	under 1,000 ha	79.2	97.2	26.7
	over 1,000 ha	20.8	2.8	25.9
Soybeans	under 1,000 ha	63.8	97.5	25.3
	over 1,000 ha	36.4	2.5	28.6

**Source:** compiled by the authors according to data V. Kruglyak (2021)

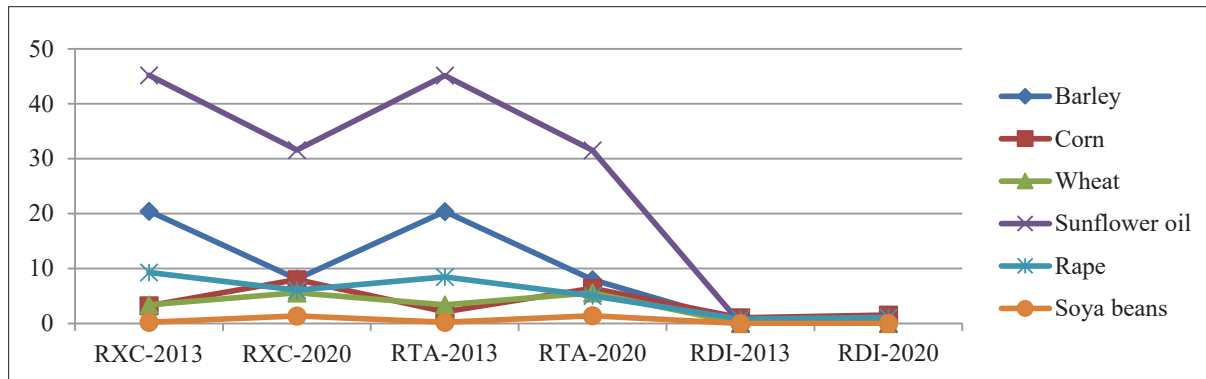
The main factor in the formation of a considerable export potential of agro-food products constitutes a significant concentration of export-oriented products in large enterprises with sown grain and oilseeds over 1,000 hectares, which allows forming large consignments (2.5-8 thousand tonnes). In the commodity structure in agricultural products in January–July 2021, the most exported from Ukraine were soybean oil – 25.2%, corn – 23.8%, and wheat – 13.9%. Asian countries occupy the largest niche in the export of Ukrainian agro-food products – over 50%. During the period of January–August 2021, Ukraine exported the most to Asian countries – by 7.57 billion USD, EU – by 4.09 billion USD, Africa – by

1.91 billion USD, Russia, Belarus, Moldova – by 0.84 billion USD, America – by 0.16 billion USD. The top five countries in terms of exports of Ukrainian agro-food products include China (2.7 billion USD), India (1.06 billion USD), the Netherlands (1.02 billion USD), Egypt (0.8 billion USD) and Turkey (0.6 billion USD). The largest share of imports to Ukraine from the EU is 48.8% (2.34 billion USD), from Asia – 20% (0.96 billion USD) and from America – 13.3% (0.64 billion USD). The United States imports the most frozen fish to Ukraine – 4.9%, soybean seeds – 4.2 and other food products – 4.4% (Kruglyak, 2021).

Given the openness of markets and significant exports of certain types of agro-food products, the price

situation in the internal market depends on the world market. In turn, this determines the specific features of price formation and cash inflows for agro-food producers, which are directly or indirectly (through traders) sent for export. The competitiveness of export-oriented agro-food products of Ukraine (wheat, barley, corn, sunflower oil, rapeseed, soybeans) on the world market is determined using

relative indicators of competitiveness, including the index of relative export competitiveness (RXC), index of relative dependence on imports (RDI), index of relative trade advantages (RTA) (Novikova & Tkachuk, 2011; Bukhtiarov, 2014). Figure 5 shows the indices of identified benefits for certain types of Ukrainian agro-food products and products of their processing for 2013-2020.



**Figure 5.** Indices of identified advantages for certain types of agro-food products and goods of their processing in Ukraine on the world market

**Source:** M.V. Novikova and N.Yu. Tkachuk (2011); O.S. Bukhtiarov (2014)

Thus, for the period under study, the RTA index shows that the most competitive products are sunflower oil, which in 2020 had a relative trade advantage in size 31.6, barley – 8.0, and corn – 6.4. However, for 2013-2020, there is a declining trend of RTA index, namely for barley – a decrease of 2.5 times, for rapeseed – by 40.6%, for sunflower oil – by 1.5 times. There is a clear tendency to increase the share of products that are raw materials or have a low degree of processing products, in the production of which there is an elevated level of mechanisation and automation involving significant land resources. In particular, the share of foreign exchange earnings from grain exports during 2013-2020 increased by 15 percentage points, oilseeds – by 6 percentage points. While the share of exports of finished food, milk, and dairy products decreased by 17% and 10%, respectively. There is a positive trend in the export of sunflower oil, the share of vegetable oils in the

structure of exports increased by 8% points. Thus, today agro-food exports are still focused on raw materials.

The foreign economic activity of agro-food enterprises faces a considerable impact on the efficiency of export and import operations of currency risks, which affect the pricing policy and increase the cost of production (State Service of..., 2021). Y. Zhang & Y. Zong (2018) studied the specific features of agricultural products export in Jiangsu Province. A. Nagurney *et al.* (2019) have developed their original model of the agricultural supply chain, which considers tariff quotas. J.Ch. Bureau *et al.* (2019) reviewed the tariffs applied by different countries for agricultural products and found that more opportunities should be used to protect imports in the agricultural sector. In this study, the average financial losses and benefits of the agro-food enterprise in the export of corn, given the appreciation and change of the national currency against the USD (Table 7) were determined.

**Table 7.** Estimation of efficiency of corn exports on average per one agro-food enterprise in Ukraine under the conditions of currency risks

Indicators	2013	2020	Subject to currency risks	
			With a devaluation of 1.2 times	With a devaluation of 1.2 times
The cost of mineral fertilisers per 1 tonne, USD	20.03	282.87	339.44	282.87
Costs for fuels and lubricants per 1 tonne, USD	12.02	269.82	323.78	269.82
Total cost of 1 tonne, USD	148.44	375.84	430.96	375.84
Price subject to delivery FOB, USD for 1 ton without VAT	229	638.92	743.71	638.92
<b>Export costs</b>				
Transportation of products to the elevator	8.14	12.73	12.73	12.73
Reception, finishing of products on the elevator	6.01	7.35	7.35	7.35
Loading into railway transport	5.63	11.27	11.27	11.27
Transportation by rail	23.03	50.07	50.07	50.07

Table 7, Continued

Indicators	2013	2020	Subject to currency risks	
			With a devaluation of 1.2 times	With a devaluation of 1.2 times
Certificate of quality and quantity of an independent surveyor	1.00	27.58	28.7	27.58
Freight forwarding services in the port, customs clearance	2.25	28.75	34.07	28.75
Transshipment of products	17.02	45.93	55.07	45.93
Fumigation	0.75	2.50	3.00	2.50
Total export costs	63.83	186.18	202.26	186.18
The profit received as a result of export activity for 1 tonne, USD	16.7	76.90	110.49	76.90

**Note:** FOB – Free on board; VAT – value added tax

**Source:** calculated by the authors

G. Sacchi (2018) explored the importance of prices and food policies for the development of enterprises and the economy. An increase in the prevalence of ethical consumption behaviour was found as well. An analysis of the dairy sector was conducted, identifying rising prices for dairy products over the past 10 years and explaining

the reasons for this trend (Reviron & Python, 2018). This study allowed stating that the effectiveness of changes in the European business environment in the fluctuations of price policy in the export-oriented internal market of agro-food products is proposed to assess by several indicators presented in Table 8.

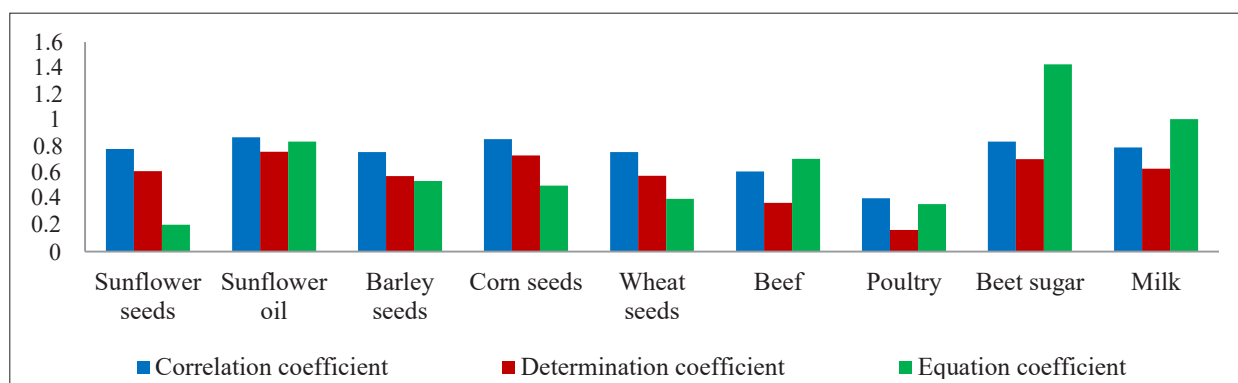
**Table 8.** Indicators of efficiency of foreign economic activity of agro-food production enterprises of Ukraine at change of the European integration business environment and stimulation of export-oriented business partnership in the internal market

Indicators	2010	2015	2020
The cost of agricultural exports per employee in agriculture, USD	3262.3	6392.87	8335.7
Including cereals	1119.9	3111.6	5874.4
Oilseeds	429.3	645.9	865.2
Fats and oils	585.7	2365.7	3874.4
The cost of agricultural imports per capita, USD	139.8	242.7	277.1
Foreign direct investment in agriculture per 1 employee, USD	142.6	355.7	297.8
The cost of agricultural exports per 1 ha of agricultural land, USD	596.5	1514.6	2023.2
Costs to produce cereals per 1 ha of harvested area, USD	323.3	613.7	805.4
Costs to produce oilseeds per 1 ha of harvested area, USD	336.7	648.6	868.3

**Source:** compiled by the authors according to data State Statistics Service of Ukraine (2021)

As a result of the research, a correlation-regression model of dependence of internal prices in Ukraine (Y) and prices of agro-food products on the world market (X) was built (Kvasha *et al.*, 2014). The value of the Student's distribution (t) and Fisher's (F) was used, considering the

corresponding degrees of freedom, probability for t (0.95) and significance level for F (0.01 – for crop products and 0.05 – for livestock products). A significant influence of factors on all constructed models of regression coefficients was established (Fig. 6).



**Figure 6.** Parameters of the regression equation and their estimation in the model dependence of domestic prices in Ukraine (Y) of export prices for agro-food products on the world market (X)

**Source:** calculated by the authors

Correlation-regression models of the impact of world price on the internal prices of agro-food products.

Sunflower seeds –  $Y=415.2+0.204X1$ .

Sunflower oil –  $Y=753.1+0.841X1$ .

Barley seeds –  $Y=502.7+0.540X1$ .

Corn seeds –  $Y=644.7+0.504X1$ .

Wheat seeds –  $Y=599.3+0.403X1$ .

Beef –  $Y= -2.346+0.708X1$ .

Poultry –  $Y=1.783+0.363X1$ .

Beet sugar –  $Y=2.544+1.429X1$ .

Milk –  $Y= -3.82+1.411X1$ .

Thus, the greatest influence of the world market is observed on such types of products as sunflower oil (76.3%), corn seeds (73.5%), beet sugar (70.5%), and milk (63.4%). The coefficient of elasticity obtained as a result of model construction shows by what percentage (from the average) the average prices of the internal market for the studied types of agro-food products will change when the price situation of the respective market changes by 1%. The greatest elasticity was observed in milk prices. Thus, the increase in prices in the EU countries on 1% led to the corresponding price trends in the internal market on 1.17%. Changes in

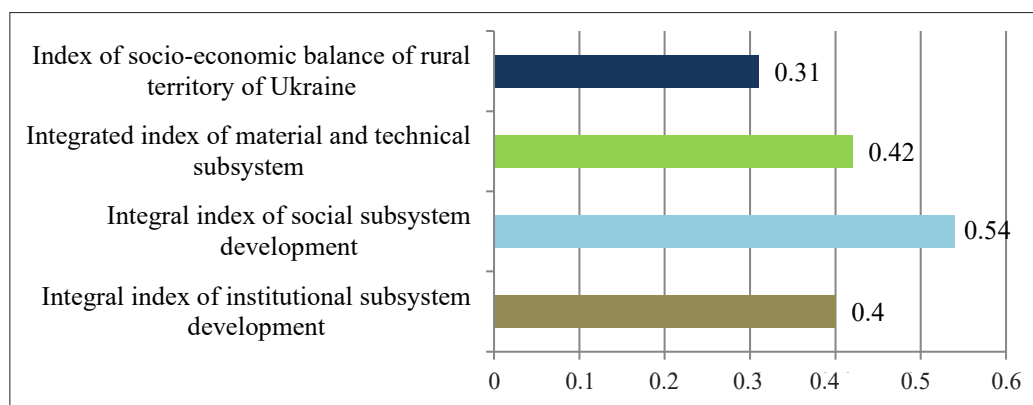
world sugar prices by 1% were reflected in the internal market for 0.92%.

S.-H. Huang & S.-T. Ji (2018), upon investigating the possibilities of expanding the export of agro-food products developed their original programme to strengthen the export infrastructure, which has demonstrated its effectiveness. There are studies, the results of which allow determining and comparing the profitability of enterprises according to different business models (Lutsiak et al., 2021). According to the findings of the study on the export activity of agro-food enterprises in the European market, based on the standard gravitational model of foreign trade, the gravitational model of trade with the European Union was built, considering conditions of formation of stable export potential. Using indicators characterising the state of these subsystems and multilevel stimulation of export-oriented internal market, an integrated index was calculated, which allows assessing the internal and external European business environment to ensure business partnership with EU member states and defining the foreign economic priorities of harmonisation of interests of the enterprises of agro-food production (Fig. 7). Modified classical gravitational model is as follows (9):

$$E^p_{ij} = a_0(GPAP_i) \times a_1(GPAP_j) \times a_2(N_{ij}) \times a_3(d_{ij}) \times a_4(I_{sv}) \times a_5(I_{sz}) \times a_6(B_j) \times a_7(SP_{ij}) \times a_8 + q + T_j \quad (9)$$

where  $E^p_{ij}$  is the export of products;  $GPAP_i$  is the gross production of agricultural products in Ukraine (weighted average for 5 years);  $GPAP_j$  is the gross production of agricultural products in EU countries;  $N_{ij}$  is the population;  $d_{ij}$  is the distance between Ukraine and EU countries;  $I_{sv}$  is the internal institutional system;  $I_{sz}$  is the institutional system of the external environment;  $B_j$  is

the restrictions on the export of agricultural products (quotas, licenses, certificates, etc.);  $SP_{ij}$  is the system of support and preferences for agricultural exports in countries;  $q$  is the accidental error;  $T_j$  is the expiration date of agricultural products, including the validity of licenses;  $a_0$  is the free member of the equation;  $a_1 \dots a_8$  are the coefficients of elasticity.



**Figure 7.** Gravitational model of the integrated level of export potential of agro-food enterprises of Ukraine to stimulate foreign trade in the world market and ensure stable business partnerships with EU member states

**Source:** calculated by the authors

Export potential as a system of interaction of its elements allows harmonising the economic interests of enterprises-exporters of agro-food production and stimulating export-oriented internal market to high added value products, as well as creating conditions for production efficiency.

## CONCLUSIONS

Thus, to determine identify the promising areas for the increase in exports of agro-food products, especially

to the EU market, it is required to improve the foreign economic function of agricultural sector. The vectors in expanding the European integration process include the development of farming; the improvement of the financial infrastructure of the agro-food sector and development of effective financial institutions; the improvement of the investment climate and stimulation of vertical and horizontal integration processes, including international ones. The progress of export potential of agro-food enterprises should be considered as a holistic system of creating competitive advantages and focusing

products on foreign consumers, given that the results of export potential are appropriated not by agricultural enterprises, but by entities, exporters, and intermediaries.

The progress of export potential should be based on a strategy of increasing export opportunities. The systematic interaction of the components of the export potential allows harmonising the economic interests of agro-food enterprises and subjects of circulation in the export of products. The European integration necessitates the formation of high purchasing power

in this market segment with a prominent level of control over export-import operations, establishing appropriate requirements, norms and rules for raw materials and finished products for exporters. Along with the opportunities for foreign economic activity of agro-food enterprises in the EU market, there is a need to ensure the liberalisation of foreign trade between European countries. This increases competition due to the entry of European agro-food producers into the internal market of any country.

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## **Зовнішньоекономічні пріоритети розвитку агропродовольчих підприємств в умовах євроінтеграційного ділового партнерства**

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**Анотація.** Агропродовольче виробництво є джерелом продовольчої безпеки країни, основою нарощування її експортного потенціалу, задоволення внутрішнього попиту на сільськогосподарську продукцію та продовольство. Актуальність теми дослідження полягає в тому, що на даний момент Україна розвиває європейський вектор зовнішньоекономічної діяльності. Метою даного дослідження є обґрунтування комплексу показників, які використовуються для якісної та кількісної оцінки експортного потенціалу агропродовольчих підприємств євроінтеграційного ділового партнерства. Авторами використано загальнонаукові методи (аналіз, синтез), комплексний підхід, модель ієрархії факторів впливу інтеграційного бізнес-середовища на експортний потенціал підприємств. Визначено допустимі тарифні квоти на обсяги безмитного експорту продукції рослинництва, тваринництва та продуктів переробки для України. Проаналізовано обсяги концентрації виробництва експортоорієнтованих сільськогосподарських культур на підприємствах агрохарчового виробництва України. Встановлено, що основну частину агропродовольчого експорту становить сільськогосподарська сировина, зокрема зернові та олійні культури, рослинна (соняшникова) олія. У дослідженні оцінено ефективність експорту кукурудзи в середньому на одне агропродовольче підприємство України в умовах валютних ризиків. Визначено, що у 2020 році загальні витрати на експорт за 1 т склали 186,18 дол. Крім того, встановлено, що прибуток, отриманий в результаті експортної діяльності за 1 т склав 76,90 дол. Визначено параметри рівняння регресії та їх оцінку в моделі залежності внутрішніх цін України від експортних цін на агропродовольчу продукцію на світовому ринку. Для стимулювання зовнішньої торгівлі на світовому ринку та забезпечення стабільного ділового партнерства з країнами Європейського Союзу запропоновано гравітаційну модель інтегрованого рівня експортного потенціалу українських агропродовольчих підприємств

**Ключові слова:** експортний потенціал, сільськогосподарська продукція, ринок, торгівля, ціна

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## Mining and Industrial Landscapes of Podillia as Potential Structural Elements of the Regional Eco-Network

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**Abstract.** The current state of the environment caused by irrational nature management necessitates the search for new approaches in the field of restoration and protection of biotic and landscape diversity. The new strategy for its protection is an ecological network that is being developed in Ukraine according to European requirements at the national, regional, and local levels. An integral part of the national eco-network is the territory of Podillia, for which an important topical issue is increasing the productivity of ecosystems and stabilising the ecological balance. One of the ways to optimise the regional eco-network of Podillia is to search for promising territories and include them in structural elements that will ensure its spatial integrity and representativeness. The most common objects of mining and industrial landscapes are quarry-dump complexes that are original in their origin, structure, conditions, natural properties, spatial location, features of the geological structure, the nature of the biotic-landscape structure, and economic development. Therefore, the purpose of this study is to identify and describe the specific features of mining and industrial landscapes that require a set of measures for renaturalisation (reclamation, restoration of natural vegetation, reintroduction, etc.) within the latitudinal Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor of the regional eco-network of Podillia. General scientific (analysis, synthesis), laboratory and field methods, monitoring, comparison and statistical processing methods were used in this study. Factors of influence for the restoration of disturbed ecosystems of mining and industrial landscapes of Podillia were determined on the example of the Andriikovetskyi sand quarry and dump complex. It is established that edaphic conditions, elemental composition and organic matter content in the newly formed substrate, atypical relief, which differs sharply from the natural one, have the greatest influence. With the implementation of a set of measures for renaturalisation, the proposed quarry-dump complex can become a centre of zonal biodiversity as a renewable site – as a structural element of the regional eco-network

**Keywords:** restoration areas, quarries, mining, environmental protection measures, reclamation, renaturalisation, self-regeneration



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## INTRODUCTION

Intensive human use of natural resources and anthropogenic burden associated with irrational use of natural resources contribute to the partial or complete destruction of biodiversity in certain areas of the environment. The creation of nature reserves for the preservation of entire ecosystems and landscape complexes constitutes an effective conservation strategy since apart from the function of protecting and enriching biota, such territories can perform a complex soil protection, water regulation, wind protection role, and positively influence the climatic features of the respective region (Yuhlichek & Vyhovska, 2011). According to the State Cadastre of the Nature Reserve Fund (Ministry of Environmental Protection and Natural Resources of Ukraine, 2022), as of 01/01/2021, there were 8,633 territories and objects of the nature reserve fund in Ukraine, with a total area of more than 41,000 km<sup>2</sup>. In percentage terms, the reserve indicator (the ratio of the factual area of the nature reserve fund to the total area of the state) is only 6.8%, with a norm of 12-15% and an optimal indicator of 18-20%. This is an extremely low indicator of protected areas compared to pan-European standards, where it is 15.33% (the area of protected land per 1 person in Ukraine is 1,054 m<sup>2</sup>, in Europe – 2,220 m<sup>2</sup>), and insufficient to maintain the overall ecological balance and ensure proper protection of species. In this regard, there is an urgent need to optimise the system of protection of biotic and landscape diversity to ensure the conservation and restoration of ecosystems (Mudrak & Mudrak, 2020).

A modern integral concept on the way to sustainable development is the creation of a comprehensive multifunctional environmental protection system – an ecological network. The pan-European ecological network, as a single spatial system comprising natural and semi-natural territories, was defined as the main vector for implementing the pan-European strategy for Biodiversity and Landscape Conservation (Pan-European strategy for Biodiversity and Landscape Conservation, 1995), adopted at the conference “Environment for Europe” (Sofia, 1995). Most countries in the European community have made the transition from conservation strategies for biotic diversity units to the creation of national ecological networks (NENs) (Mudrak *et al.*, 2018). Considering European regulatory practices (Smyrnova *et al.*, 2021), protection and use of nature reserves, development of the national eco-network in Ukraine is carried out according to the basic requirements of the functioning of the pan-European eco-network at the national, regional, and local levels. An integral part of the ecological network of Ukraine is Podillia, which makes up 10.1% of its territory, for which the problems of preserving biotic and landscape diversity, increasing ecosystem productivity and stabilising the ecological balance of the region are a critical issue (Mudrak, 2012).

According to the Law of Ukraine “On the Ecological Network” (2018), the eco-network is a single territorial system formed to improve conditions for the development and restoration of the environment, increase the

natural resource potential, preserve landscape and biodiversity, places of settlement and growth of valuable species of the animal and plant world, gene pool, animal migration routes through the association of territories and objects of the nature reserve fund, territories of agricultural land that have special value and, according to the laws and international obligations of Ukraine, are subject to special protection. The process of transition from local protection to the creation of a system of protected areas has several obstacles, namely the emergence of new forms of ownership, licensing of land, forest, and water use, and the land market (Mudrak, 2012). Land, forest, and environmental legislation requires amendments and adoption of new legislative provisions regarding the priority of reserving natural territories over all other land purposes (Hrytsku & Danilova, 2018).

According to the functional principles and approaches of the pan-European strategy for preserving biotic and landscape diversity, as well as regulatory support for Ukraine, the structural elements of the national eco-network include key, connecting, buffer, and renewable territories. Key territories ensure the preservation of the most valuable and typical components of landscape and biodiversity for a given region. Connecting territories (eco-corridors) join key territories, ensure the migration of animals and the exchange of genetic material. Buffer territories protect key and connecting territories from external influences. Restored territories ensure the formation of the spatial integrity of the eco-network, for which priority measures must be taken to restore the primary natural state. Potential territories for research are territories that require additional measures to restore the natural state (renaturalisation, reclamation, repatriation, afforestation, regrassing, etc.) – restored territories that, as part of the eco-network, ensure the formation of its spatial integrity and achievement of an ecological balance (Mudrak & Mudrak, 2020; Law of Ukraine 1864-IV “On Ecological Network of Ukraine”, 2018). With the successful implementation of restoration measures, such facilities constitute a potential reserve of biodiversity for further transformation and inclusion as structural elements of the regional eco-network. For the territory of Podillia, this is an urgent issue, which is still understudied.

Therefore, the purpose of this study is to determine the features of the functioning of mining and industrial landscapes covering an area of more than 650 km<sup>2</sup> of the region and require restoration measures to perform further environmental protection functions as restored territories as structural elements of the eco-network. As the object of this study, the authors chose the Andriikovetskyi quarry and dump complex of mining and industrial landscapes within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor of the Central Podillia (Mudrak, 2012).

## LITERATURE REVIEW

The first basic principles of creating an ecological network of Ukraine, proposals for the development of its

structure, criteria for identifying the main elements of the ecological network were proposed by Yu.R. Sheliah-Sosonko (1999); Ya.I. Movchan (2001) considered the main functions and principles of developing an eco-network, legal and economic aspects. Yu.R. Sheliah-Sosonko *et al.* (2005), based on the main principles of building an eco-network and analysing biodiversity, proposed a variant of the territorial location of eco-corridors at the national level. Theoretical justification and scientific and methodological development of the foundations for the development of the ecological network of Ukraine is an essential stage for analysing the territorial structure and developing a policy of rational use of natural resources in the regions, namely Podillia. It is relevant to investigate the applied aspects of the regional eco-network development since the natural complex of Podillia has a special local and state ecological and environmental significance. P.L. Tsaryk (2005) identified geographical aspects of the eco-network development in the Ternopil Oblast with justification for the creation of new protected areas; T.L. Andriienko (2006) defined and characterised the territories of the nature reserve fund of the Khmelnytskyi Oblast; in the future, these territories were fully included in the ecological network, which was developed and scientifically justified by L.S. Yuhlichek & T.V. Vyhovska (2012), indicating the place of the Khmelnytskyi Oblast eco-network in national and transnational eco-networks; O.V. Mudrak (2012) focused on the development of scientific justifications for the effective introduction of elements of the ecological framework of the regional eco-network of Podillia in the general scheme of planning the territory of Ukraine, which were formed based on complex studies of flora, defined and described main elements of the eco-network and representative natural and artificial territories. H.V. Mudrak (2018) identified the main stages of development and conditions for the effective functioning of the eco-network within the Eastern Podillia; Yu.Yu. Ovchynnykova (2019) evaluated the natural cores of the eco-network according to the main criteria for their development; Ye.D. Tkach & V.I. Shavrina (2019), N.S. Kovka (2020) identified the role of eco-network elements in the spectrum of conservation and protection of biodiversity; N.S. Kovka (2019) noted representative territories for further inclusion in eco-networks; the study by O.V. Mudrak *et al.* (2021) justifies the creation of new nature reserves in the context of expanding the eco-network.

Quarry-dump complexes for mineral development are promising in terms of research and use as reserve territories for further inclusion and expansion of the eco-network. The search for methods for their renaturalisation depends on several individual conditions, as evidenced by studies of ecosystem reproduction at sulphur (Nazarovets *et al.*, 2017), basalt (Savchuk & Vyhovskiy, 2019), clay (Bonchkovskiy & Bezsmertna, 2020), granite (Savosko *et al.*, 2019) extraction sites, etc. The results of foreign studies confirm the importance of developing holistic approaches with the correlation between biotic and abiotic factors. Spontaneous succession can make

a valuable contribution to nature protection, eliminating the need for expensive reclamation methods, but mineral properties, pH level, and low nutrient content of unstable substrate, disturbed hydrological regime, steep slopes are unfavourable conditions, as a result, vegetation in these areas rarely appears, the slopes remain bare, and a cascade effect can be observed. It is possible to preserve the newly formed topography as a secondary or replacement habitat for species, but these areas may become an ecological trap (Salguiero *et al.*, 2020). To stabilise soil conditions, it is proposed to introduce engineering solutions for strengthening or constructing new artificial slopes (Wang *et al.*, 2018), implementation of identification of water erosion processes by remote sensing (Padro *et al.*, 2022) and filling with additional materials to protect groundwater (Semeraro *et al.*, 2019), improvement of the organic component by sewage sludge (Carabassa *et al.*, 2020) or carrying out transplanting of soil and plant litter from undisturbed areas (Benetkova *et al.*, 2020). In the case of a slow succession, it is advisable to perform a biological stage of reclamation, provided that the ecological plant species inherent in the region are selected (Sampaio *et al.*, 2021). Indicators of the success of recovery activities are the level of biodiversity: settlement of atypical (invasive) species, decrease in species richness (Salgueiro *et al.*, 2020; Sampaio *et al.*, 2021; Gentili *et al.*, 2020), atypical ratios of trophic levels (Twerd *et al.*, 2021) compared to surrounding natural areas indicate incorrectly selected renaturalisation methods and strategies.

## MATERIALS AND METHODS

During the study, the authors applied such methods as general scientific (analysis, synthesis), laboratory, statistical, field, comparative, monitoring. Based on the processed information sources, potential renewable territories of the regional eco-network of the Central Podillia are identified, which are located within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor and are depicted using Adobe Illustrator and SketchBook graphic software.

Research within the territory of the selected quarry and dump complex, with an area of 0.015 km<sup>2</sup>, were conducted during 2019-2021. The quarry-dump sand complex as a renewable territory of the natural eco-corridor of the regional eco-set was described according to the generally accepted methodology (Mudrak, 2012) and data was included on its location (geographical and administrative affiliation in the eco-corridor), configuration, length, width, area, status in the eco-set, the degree of violations, natural conditions, formed relief, typical groupings, the degree of naturalness of the territory, data on succession transformations, features of the settlement of species, the level of biodiversity.

Since the primary settlement and reproduction of vegetation cover depends on many factors and most relate to geological, orographic, and edaphic conditions (mechanical and physical properties of the geological rock layer, exposure to dumps, instability of substrates, the presence of steep slopes, etc.), the authors identified the conditions within the object under study. To

investigate the trends and rates of change in the area, the dynamics of their development are tracked using images in different years of existence. The terrain in the conditions of the quarry-dump complex was investigated using remote sensing of the earth (aerial photography and analysis of data from artificial Earth satellites). Materials of aerospace surveys provide information about the features of the structure and dynamics of mining and industrial landscapes and can be used to monitor spent territories, determine the effectiveness of the stages of reclamation work, the spread of hazardous phenomena, etc.

Using satellite data, one can calculate the NDVI (Normalised Difference Vegetation Index) – one of the most common and widespread indices for solving problems that use quantitative assessment of vegetation cover. Using this indicator, the authors of this study analysed the features of the development and distribution of diverse types of vegetation within the quarry during the active growing season. NDVI data was systematised and analysed based on the Crop monitoring system (EOS, 2022).

To analyse the territory for the presence of necessary elements in the substrate, such agrochemical studies were carried out as determination of active acidity and hydrolytic acidity (DSTU 7862:2015, 2016B; DSTU 7537:2014, 2015); determination of the sum of absorbed bases (calcium and magnesium ions) in water extract (DSTU 7945:2015, 2016C); determination of humus content according to the Tiurin method (DSTU 7828:2015, 2016A); content of nutrients: nitrogen (DSTU 4729:2007, 2008), phosphorus and potassium according to the Chyrykov method (DSTU 4115-2002, 2003). The growth and development of vegetation cover can be affected by such a limiting factor as the content of heavy metals in the substrate: the content of cadmium (DSTU 4770.3:2007, 2009B), lead (DSTU 4770.9:2007, 2009d), copper (DSTU 4770.6:2007, 2009c), zinc (DSTU 4770.2:2007, 2009a) was determined. To conduct these studies, samples were taken according to the method of a combined sample from 3 localities: sample No. 1 – the selected soil sample of agricultural land that surrounds the territory of the quarry-dump complex comprehensively, on which sunflower and corn are grown; sample No. 2 – the selected soil sample, near agricultural land as a sample of the reference undisturbed zonal soil; sample No. 3 – a sample of the substrate directly from the site of the sandy quarry-dump complex, on which there was no settlement of species.

## RESULTS AND DISCUSSION

Within Podillia there are all the necessary conditions and resources for the development of a regional eco-network: nature reserves and objects of national and local significance, water bodies, forest ecosystems, recreation areas, resort territories, remnants of semi-natural and natural vegetation, a considerable part of which belongs to agro-landscapes with single- and perennial plantings. Podillia is described by a unique geological and geomorphological structure and favourable weather, climatic

and soil conditions. Within the region under study, the climate is described as temperate continental with a long warm period, and critically low temperatures are not recorded in winter. The average annual temperature ranges from 8.9°C to 8.1°C, and precipitation ranges from 570 mm/year to 600 mm/year. All these conditions contributed to the specific mosaic spread of the soil cover and the development of various landscape complexes. However, the proportion of undisturbed soils is decreasing due to the increased exploitation of natural resources due to human activities and the lack of proper renaturalisation measures. Agricultural and mining activities have a considerable anthropogenic impact on the natural landscapes of Podillia.

An essential resource in the development of a regional eco-network is the use of mining and industrial landscapes, which currently occupy over 650 km<sup>2</sup> of lands in Podillia (Mudrak, 2012). Within the framework of Podillia, the study of recovery areas of local and regional eco-networks is at the initial stage (Yatsentiuk *et al.*, 2020), studies conducted in this area include the identification of potential renaturalisation zones with subsequent planning of environmental measures. The criteria for selecting such sites have not yet been fully developed, but there are two main ones – the criteria for conditional compliance and the criteria for real opportunities. In the first case, potential lands are evaluated according to criteria that are assigned to the main structural elements of the eco-network – natural cores, buffer zones, and ecological corridors. The territory is considered to meet the established criteria after proper renaturalisation measures are carried out: restoration of natural vegetation, reintroduction, population settlement, changes in the size and configuration of the territory, etc. In the second case, they assess the factual state and feasibility of renaturalisation: territories that are proposed as restored territories can fully meet the criteria for conditional compliance of structural elements of the eco-network. Next, the authors of this study consider the state of such sites within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor.

Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor is a connecting territory at the local level and includes the valleys of the Buzhok, Pivdennyi Buh, Vovk, and Smotrych rivers and has a total length of 172 km. The eco-corridor connects the Verkhnopobuzke, Horodotske, and Tovtrynske natural cores, connects the Yuzhnobuh national long-term eco-corridor with the Dniester transnational eco-corridor and is part of the latitudinal Halytsko-Slobozhanskyi eco-corridor (Yuhlichek & Vyhovska, 2011). There are 26 nature reserves and objects located along the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor. Part of the territory is deeply eroded, and considerable areas are occupied by agricultural land. Within the borders of this eco-corridor are mineral deposits that are currently under development, and anthropogenically disturbed lands after the cessation of production, which require further measures to restore and stabilise the ecological balance. A considerable part of the land affected by mining is accounted for by localised deposits of minerals such as clay, loam, limestone, and sand. Quarries in the region are unique

in their origin, structure, conditions, natural properties, spatial location, features of the geological structure, the nature of the biotic-landscape structure, and economic

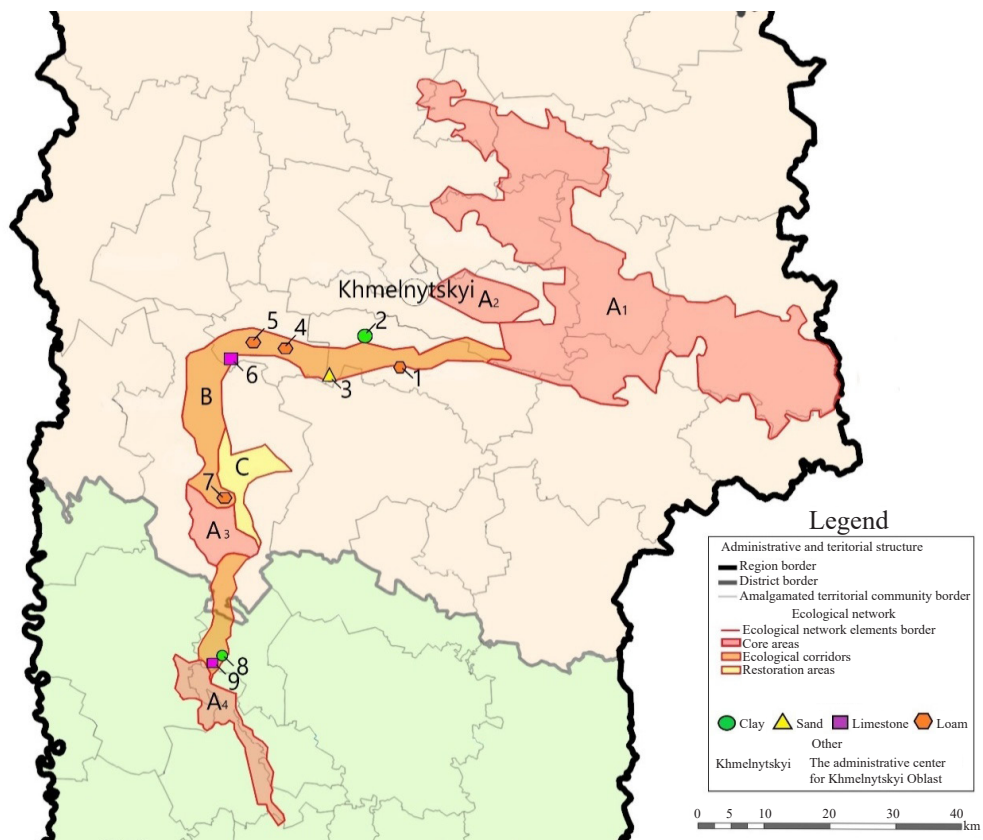
development. The list of identified areas of non-metallic minerals according to the State Enterprise "Derzhheoinform" (2022) is given in Table 1.

**Table 1.** List of mineral deposits identified within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor

Item No.	Item name	Area, km <sup>2</sup>	Type of minerals	Development status
1	Perehinka-Pivnich	0.161	Productive part – loam, sole – clay	Not being developed
2	Nyzhchevovkivetske	0.128	Productive part – clay	Not being developed
3	Andriikovetske	0.015	Productive part – sand, sole – clay	Not being developed
4	Hvardiiske	0.0583	Productive part – loam, sole – clay	Not being developed
5	Dobrohorshchanske	0.057	Productive part – loam, sole – sand	Being developed
6	Zhuchkovetske	0.054	Superface – clay, productive part – limestone, sole – sand.	Not being developed
7	Horodotske	0.142	Productive part – loam, sole – clay	Being developed
8	Pivdenosmotrytske	–	Productive part – clay, sole – clay + loam	Not being developed
9	Smotrytske	0.3704	Superface – clay, productive part – limestone, sole – clay	Being developed

Within the territory, there are also promising areas for the development of fuel and energy raw materials – peat, upon the development of which over 26 thousand hectares of productive land in the region of the Andriikovetske and Heletynske deposits are annually disturbed. Andriikovetske deposit is promising for further development, while Heletynske is limed. Within peat deposits and quarries, the process of natural self-regeneration is long, the productivity of phytocenoses is low and

depends on environmental conditions and other factors. Such sites should be restored according to landscape and ecological principles that will consider natural factors and contribute to the development of highly productive phytocenoses, terminating adverse impacts, etc. A schematic representation of the location of quarries within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor is presented in Figure 1.



**Figure 1.** Location of mineral deposits within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor

**Note:** eco-network elements: A1 – Verkhnepobuzke natural core, A2 – Davydkovetske natural core, A3 – Horodotske natural core, A4 – Chernetsko-Kolubaivske sub-core of the local eco-network, B – Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor, C – Zhyshchynetska renewable territory; deposits: 1 – Perehinka-Pivnich, 2 – Nyzhchevovkivetske, 3 – Andriikovetske, 4 – Hvardiiske, 5 – Dobrohorshchanske, 6 – Zhuchkovetske, 7 – Horodotske, 8 – Pivdenosmotrytske, 9 – Smotrytske)

After the cessation of mining, one of the stages necessary for the design of measures for recultivation and restoration of the natural potential of territories is the identification of these sites in geospatial space and determining the level of anthropogenic impact according to types of violations within the devastated lands. The current state of mining sites was assessed using aerospace and aerial photographs. According to the obtained satellite data, within the quarries that are not currently being developed, the emergence of vegetation

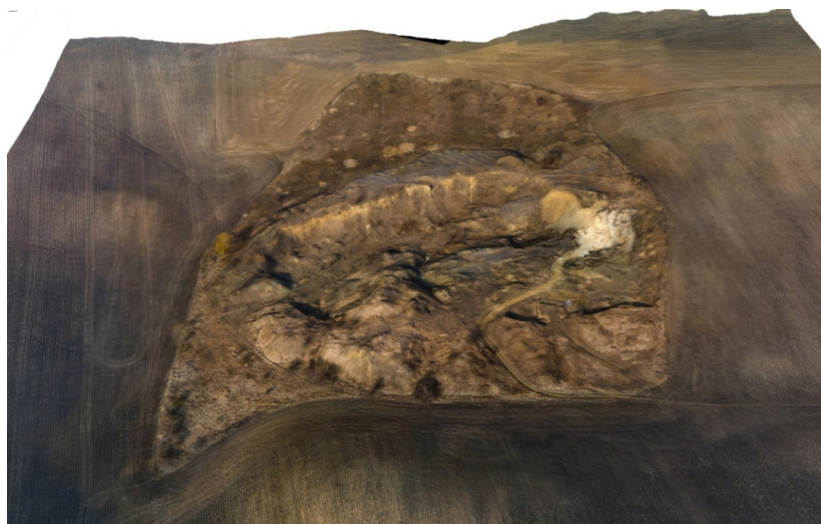
cover is recorded, which is an essential factor in soil formation: elements and humus compounds accumulate, the water regime changes, and the anthropogenic substrate is transformed into soil. Under favourable conditions, the process of self-growth passes from the initial successive stage of pioneer development by individual foci to the development of complex stable phytocenoses. Satellite data (as of 2021) of the detected devastated areas are presented in Figure 2.



**Figure 2.** State of mining and industrial landscapes according to satellite images

Other studies were conducted to characterise the Andriikovetske sand quarry and dump complex as a recovery area, predict the further state and plan measures for its renaturalisation. The climatic conditions within the quarry under study are favourable for vegetation development, but the ability of sandy substrates to provide plants with the necessary amount of moisture is minimal (precipitation quickly seeps down, the capillarity of sand is very low, and the reverse rise of water is practically not observed). According to the results of route studies, a variation in species diversity through

different ecotones was revealed. A similar trend was identified as a result of studies of other quarry-dump complexes: the authors A. Bonchkovskiy & O. Bezsmertna (2020) indicated that the main influence on the dispersal of species is exerted by factors such as topography and lithological substrate, which determine the heterochrony of succession; a considerable influence of topography on vegetation development is noted by L.K. Savchuk (2020). The general view and terrain of the quarry is presented in Figure 3.

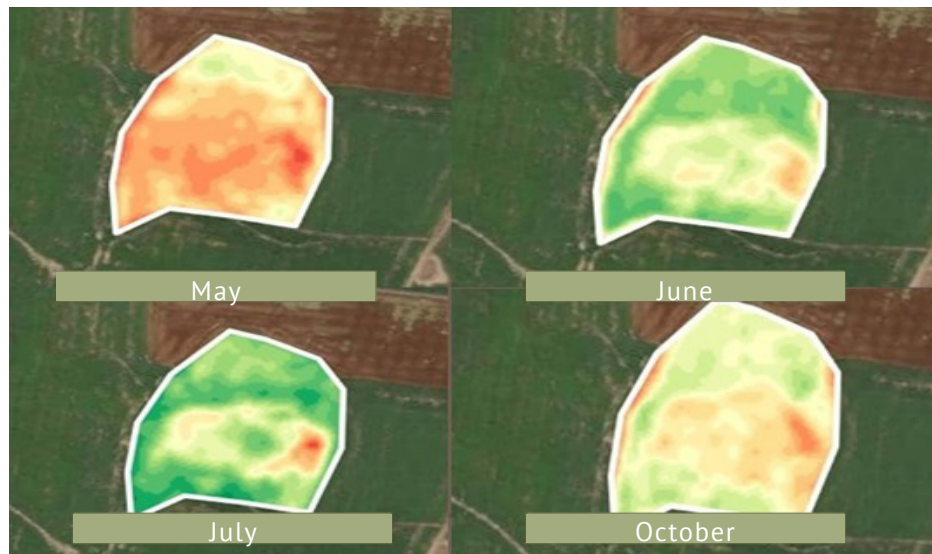


**Figure 3.** General view and terrain

Studies of quarry-dump complexes confirm that self-regeneration can occur in various scenarios, depending on the type of quarry and the degree of impact: according to the results of L.K. Savhuk & I.V. Vyhovsky (2019), succession stages can occur immediately after the completion of anthropogenic impact with the development of zonal flora and rare inclusions; based on the results of U.R. Nazarovets *et al.* (2017), the development of stable phytocenoses is carried out gradually, provided that erosion processes weaken, the hydrological regime stabilises, and biogenic elements accumulate moderately in the substrate. V.M. Savosko *et al.* (2019) identified a distinctive trend: the data obtained indicate that

the specific conditions of devastated lands at mining sites can become foci of synanthropisation with the localisation of invasively dangerous species. In such cases, it is advisable to engage in the technical and biological stage of reclamation to stabilise the conditions.

Using the NDVI indicator, the authors analysed the features of vegetation development and distribution within the quarry-dump complex under study during the active vegetation season (from May to October). Red and light-yellow colours indicate the absence of vegetation or the distribution of segetal-ruderal species. A graphical representation of the NDVI index is presented in Figure 4.



**Figure 4.** Data on changes in the NDVI index during the year

The growth and development of vegetation on the territory of the quarry-dump complex also depends on the content of nutrients in the substrate. This is confirmed by the data obtained by A. Bonchkovskyi & O. Bezsmertna (2020): the authors found that the abnormally rapid colonisation of the quarry under study with vegetation occurred due to the opening of the rock horizon with a relatively high content of nutrients in it. Their total content indicates a corresponding indicator of potential fertility. In

conditions of deficiency and lack of nutrients, the normal functioning of phytocenoses is impossible, since they take part in such essential processes as the growth of vegetative mass, the development of organic compounds, photosynthesis, root system growth, regulation of water balance, transport of substances, resistance to diseases and pests, etc. The results of the agrochemical study to determine the influence of the elemental composition of the substrate are presented in Table 2.

**Table 2.** Results of agrochemical study of the Andriikovetske sand quarry and dump complex

Sample No.	Acidity, mmol/100 g		Sum of absorbed bases (Ca+Mg), mg-eq/100 g of soil	Humus, %	Content of nutrients, mg/kg of soil		
	Hr	pH			N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
1	0.91	6.7	32.4	2.68	114.8	90	227
2	4.92	5.0	16.4	1.88	95.2	138	198
3	0.43	7.1	45.0	0.3	28.0	135	270

The content of heavy metals in the substrate can not only affect succession transformations, but also the choice of further measures for renaturalisation: for their detection in the soil, it is necessary to select biological agents that will perform a dual function – structuring

and improving the substrate performance and removing heavy metals, but this method has several disadvantages, namely the problem of further disposal of the extracted metals. The results of the study are presented in Table 3.

**Table 3.** Heavy metal content in samples of the Andriikovestke sand quarry and dump complex

Sample No.	Level of heavy metal contamination, mg/kg			
	Cu	Zn	Cd	Pb
1	0.16	0.34	0.08	0.91
2	0.28	0.39	0.06	0.96
3	1.37	0.70	0.28	0.45
MPC, mg/kg	3.0	23.00	0.7	6.0

None of the declared heavy metals exceeds the maximum permissible concentrations, which means that this factor can be excluded as affecting the development of phytocenoses.

Based on the conducted research, the characteristics of the quarry-dump complex as a recovery territory of the regional eco-network are formed as follows:

*Ecosystem restoration site* – Andriikovestke sand quarry and dump complex.

*Administrative regulations.* Andriikovestke sand quarry, which is located 2 km south of the village of Andriikivtsi of the Rozsoshanska Rural Territorial Community of the Khmelnytskyi District of the Khmelnytskyi Oblast (geographical coordinates 49°18'21.1"N 26°48'10.7"E).

*Area* – 0.015 km<sup>2</sup>.

*Typical characteristics.* Official development was discontinued in 2015. The territory of the Andriikovestke quarry and dump complex is located in a group of landscapes of the Central Podolsk type in the Vovchko-Buzhotsky natural area. The deposit is part of the deposits of the Sarmatian tier – the lower geological tier of the upper Miocene of the Neogene period. Violations that were caused by mining processes are marked by the formed anthropogenic accumulative relief: the inclined parts of the quarry are steep, uneven, in the northern and western parts from 70° to 80°, gentler in the southern part – up to 40°. The territory is described by sharp elevation differences in comparison with the surrounding areas of agricultural land. The deepest point is located in the north-eastern part of the quarry and is 328 m above sea level, the highest point is 340 m above sea level. The territory is described by rock displacements from slopes, dumps, the presence of wind and partially water erosion, uneven distribution of moisture, and surface overheating (various ecotopes). In one of the inclined ledges there is a nesting of the Common swift (*Apus apus*). Such edaphic conditions at the level of low availability of humus compounds on the sandy substrate and the lack of proper stages of mining reclamation are the main factors influencing the development of stable phytocenoses. There is a variation in the species diversity of plant communities, the ecological and cenotic structure is dominated by meadow-steppe, meadow, segetal/ruderal plant types, which indicates the beginning of the formation of zonal vegetation, but the stages of succession are heterogeneous. Pioneer settlements of vegetation are described by the occurrence of such species as coltsfoot (*Tussilago farfara* L.), wild carrots (*Daucus carota* L.), common meadow-grass (*Poa pratensis* L.) in small areas, with a flatter surface,

where the sand layer is smaller and reaches the clay sole. Within the eastern (productive) part of the quarry and the inner part of the slopes, there is no/practically no vegetation cover, minor coverage includes the occurrence of segetal vegetation (there are such species as annual fleabane (*Erigeron annuus* L. Pers), couch grass (*Elymus repens* (L.) Gould), shepherd's purse (*Capsella bursa-pastoris* L.), etc.). The occurrence of the black poplar species (*Populus nigra* L) is observed in all parts of the quarry-dump complex, but individuals have certain differences in phenotype, which is associated with uneven growing conditions. Renaturalisation measures aimed at stabilising edaphic conditions will contribute to the uniform settlement of species within the quarry, the accumulation of humus compounds and necessary elements for the development of stable zonal phytocenoses. During further restoration of vegetation cover, the territory of the quarry-dump complex will represent the diversity of meadow, meadow-steppe ecosystems within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor.

## CONCLUSIONS

The increased anthropogenic impact on the environment necessitates the prediction of possible threats to biotic, especially floral and phytocenotic diversity. Urgent tasks of scientific research are to identify valuable promising areas for further conservation and protection of nature, especially those that can become valuable hotbeds of biodiversity. Ecosystems that require special attention and, while reducing the anthropogenic impact, are promising for the further development and functioning of the eco-network are those formed at the mining sites. Such areas were identified within the Buzhotsko-Buzko-Vovksko-Smotrytskyi eco-corridor, and they require the search for optimal ways for renaturalisation for further improvement and expansion of the regional eco-network. An example of the restoration of disturbed ecosystems of mining and industrial landscapes of Podillia is the Andriikovestke sand quarry-dump complex, where the main influence on the development of phytocenoses is carried out by geological, orographic, and edaphic conditions. Therefore, the authors of this paper recommend the following reclamation measures with a mandatory engineering stage: waterlogging, strengthening slopes, removing the unproductive substrate layer, and filling with potentially fertile rocks. This will further contribute to the rapid spread of zonal biodiversity and bring the conditions of the quarry-dump complex closer to natural ones.

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## Гірничо-промислові ландшафти Поділля як потенційні структурні елементи регіональної екомережі

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**Анотація.** Сучасний стан навколишнього середовища, спричинений нераціональним природокористуванням, потребує пошуку нових підходів у сфері відновлення та охорони біотичного і ландшафтного різноманіття. Новою стратегією його охорони є екологічна мережа, яка розвивається в Україні відповідно до європейських вимог на національному, регіональному і локальному рівнях. Невід'ємною складовою національної екомережі є територія Поділля, для якої важливим актуальним питанням є підвищення продуктивності екосистем та стабілізація екологічної рівноваги. Одним із шляхів оптимізації регіональної екомережі Поділля є пошук перспективних територій та їх включення до структурних елементів, які забезпечуватимуть її просторову цілісність та репрезентативність. Найпоширенішими об'єктами гірничо-промислових ландшафтів є кар'єрно-відвальні комплекси, оригінальні за своїм походженням, структурою, умовами, природними властивостями, просторовим розташуванням, особливістю геологічної будови, характером біотично-ландшафтної структури, господарським освоєнням. Тому метою дослідження є ідентифікація та характеристика особливостей гірничо-промислових ландшафтів, які потребують проведення комплексу заходів з ренатуралізації (рекультивациі, відновлення природної рослинності, реінтродукції тощо) в межах широтного Бужоцько-Бузько-Вовксько-Смотрицького екокоридору регіональної екомережі Поділля. Під час дослідження використовувались загальнонаукові (аналіз, синтез), лабораторні та польові методи, моніторинг, порівняння та методи статистичної обробки. Фактори впливу для відновлення порушених екосистем гірничо-промислових ландшафтів Поділля було визначено на прикладі Андрійковецького піщаного кар'єрно-відвального комплексу. Встановлено, що найбільший вплив мають едафічні умови, елементний склад і вміст органіки в новоутвореному субстраті, нетиповий рельєфом, який різко відрізняється від природного. За проведення комплексу заходів з ренатуралізації, запропонований кар'єрно-відвальний комплекс може стати осередком зонального біорізноманіття в якості відновлюваної ділянки – як структурного елемента регіональної екомережі

**Ключові слова:** відновні території, кар'єри, видобування, природоохоронні заходи, рекультивациа, ренатуралізація, самовідновлення

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