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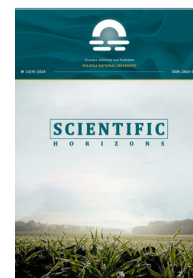
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Modelling of Piling Technology by Vibroimpact Device with Hydropulse Drive

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Abstract. Based on the analysis of the developed mathematical model, this paper proposes optimal operating modes of the vibroimpact device to ensure the intensification of driving construction piles. It is an original design of a modern, highly efficient device for driving construction piles, equipped with a compact, powerful hydraulic impulse drive unit. To develop a mathematical model of the construction pile driving technology, the following methods were used: mechanoreological phenomenology, hydrodynamics, and generalised laws of mechanics. Mathematical models of the dynamics of technological processes of driving construction piles with a vibroimpact device are improved based on a hydraulic impulse drive unit in the form of a spatial non-stationary formulation of the problem and integral equations of dynamic characteristics of the moving elements of the drive unit. The study obtained the distribution of the pressure and velocity of the working fluid in the hydraulic unit of the vibroimpact device, as well as changes in the kinematic parameters of the elements of the technological equipment based on the mathematical model developed by the finite volume method, using numerical modelling and high-performance computer systems. Optimal modes of operation of a hydraulic impulse drive of a vibroimpact device are proposed to provide an intensification of the construction pile driving technology. It was found that when low-frequency vibration is applied, the driving of construction piles is intensified. Application of a hydraulic impulse drive that is based on two-stage vibration excitation allowed implementing the vibroimpact modes of the device. The average pile driving speed with a vibroimpact interaction is five times higher compared to conventional driving methods

Keywords: impulse, impact, vibration, mathematical model, hydraulic drive, valve, construction pile



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INTRODUCTION

The growth of construction volumes is an essential sign of development as a separate construction industry, and of the country in general. Due to the large volume of housing and industrial construction, arises a need to build more structures in unfavourable soil conditions, which requires the use of special means, that often complicate and increase the cost of the construction process of facilities and buildings. The tendency to reduce these costs and the continuous advance of construction technology are leading to a wider introduction of pile foundations. That is why the problem of satisfying the increased requirements for reducing the cost of construction and increasing labour productivity by increasing the efficiency of the construction pile driving is relevant and acute (Bullock & William, 2001).

The main and the best way to intensify the pile driving is the use of vibroimpact loads (Glushak *et al.*, 1992). Vibroimpact (VI) load is created by a hammer equipped with a hydraulic impulse drive (HID) (Iskovych-Lototsky *et al.*, 2018a), the advantages of which include the intensification of the flow of several technological processes, ensuring optimal load parameters and high-quality results of technological processing (Fossen & Nijmeijer, 2012). However, the practices of using impact machines necessitated the solution of some issues, understanding which is impossible without analysing the effect of impact pulses on the object, as well as without analysing the dynamics of attachment equipment and the entire machine under the effect of loads from the working impact mechanism (Ivanchuk, 2020). Currently, mathematical modelling of physical processes is common (Guang & Min, 2005), using which allows deeply and fully exploring the influence of design and mode factors on the main characteristics of the construction equipment operation, to outline particular ways of their improvement while considerably reducing the amount of experimental research (Kang *et al.*, 2008). Despite the complexity of the calculations and the assumptions made in the mathematical description of the workflow, which can be clarified upon the accumulation of experimental data, the perspective of using such models for the development of effective structures of the VI attachments equipment for construction based on HID for pile driving, is evident (Ivanchuk, 2020).

The purpose of this article is to increase the efficiency of the theoretical study of the pile driving technology through the development of promising mathematical models of the physical processes of operating the hinged construction HID-based VI equipment. This will allow achieving several high-quality practical results: increasing the reliability of determining the operating characteristics upon designing a hydraulic drive; the possibility of developing systems with improved operational characteristics; reducing the time spent on development of certain technologies.

To achieve this purpose, the following *tasks* were solved:

- to improve the mathematical model of the pile driving technology based on a pre-made design of the developed hinged HID-based VI device;
- to determine the hydrodynamic parameters of the working fluid in the HID, which will allow analysing the operating modes of the hinged pile driving VI equipment;
- to determine the operating parameters of the HID to identify the efficient operating modes of the pile driving technology with a hinged VI device.

THEORETICAL OVERVIEW

The main apparatus of the mathematical modelling of VI processes includes exact methods of nonlinear mechanics based on adjustment solutions (Alessandro, 2016), describing adjacent intervals of movements of the executive body. These methods allowed thoroughly investigating the complex dynamic picture of the movements of several VI machines and revealing many of their inherent fundamental properties. However, the use of these methods for creation of mathematical models of a wide range of HID-based VI systems is laborious and limited by the scope of their application, especially when the dimension of the systems is increased, as well as when it is necessary to consider added nonlinear factors and the complication of the nature of disturbances from the action of non-periodic and random forces. A generally accepted approach for modelling VI systems is the asymptotic representation of solutions according to the degrees of a small parameter (Voskresenskiy, 2001) upon analysing the fundamental harmonic components of fluctuation. It is based on the selection of simpler ratios from their general mathematical description, while transitioning to spectral representations (Li *et al.*, 2019) and ideas of equivalent linearisation (Jörg *et al.*, 2010) but does not allow finding an acceptable mathematical model for vibration systems. This necessitates the development of new methods and approaches to create standard mathematical models of VI systems for a wide range of VI equipment.

The increase in speed, energy saturation, and compactness of the HID for VI machines are greatly influenced by the physical parameters of the energy carrier (working fluid) and the design parameters of the pressure pulse generator (PPG) (Iskovych-Lototsky *et al.*, 2018a), which provides control of the function of VI equipment. This leads to the development of mathematical models in the form of systems of differential equations of motion of the structural HID elements (Shatokhin *et al.*, 2019) based on an artificial dynamic model with the given coefficients for the oscillatory system. The practical implementation of the given approach is possible only for mathematical models mainly of low-dimensionality and describes the properties of objects in a narrow range of variation of operating

parameters such as the amplitude and oscillation frequency of the impulse drive elements. Available practices indicate that to overcome these difficulties, it is necessary to formulate a new objective of mathematical modelling of the pile driving technology by VI machines in a spatial non-stationary form. There is a need to develop new, more comprehensive and adequate mathematical models based on a system of differential equations in partial derivatives with coefficients in the form of integral functions of independent variables (Iskovych-Lototsky *et al.*, 2018b). At present, the possibility of replacing a physical experiment with a numerical one, involving the use of computer modelling methods, is still a relevant issue.

MATERIALS AND METHODS

This paper presents an original methodology of mathematical and computer modelling of the pile driving technology by HID-based VI systems, considering the specific features of this class of objects, to ensure high design efficiency of a corresponding type of technical systems:

1. Based on the design methodology of road-building machinery with HID, an effective design of a hinged VI device has been developed, wherein a two-stage pulsator valve is used as a vibration exciter (Iskovych-Lototsky *et al.*, 2018a; Iskovych-Lototsky *et al.*, 2018b).

2. Based on a systems-technical approach (Barkan, 1952; Manzhilevsky, 2019; Iskovych-Lototsky *et al.*, 2018b), structural and functional connections between the components of the hinged VI device were synthesised, which allowed determining the interconnection between the state parameters of the HID subsystems with the quality readings of the technological object.

3. Dynamic characteristics of the movable functional objects of the HID are described using linear inhomogeneous second-order differential equations, where the free term function is presented in the form of linearised functions of external forces, and the linearised coefficients express both elastic and dissipative force connections of the drive elements and rheological properties of the processing environment.

4. Mathematical model of the movement of functional objects of the HID is supplemented with a mathematical model of the working fluid movement, based on the system of nonlinear differential equations, in partial of the derivatives of Navier-Stokes and the continuity conditions for viscous fluids.

5. The developed mathematical model of the pile driving technology is implemented using separate software packages, which implement a corresponding numerical calculation method for solving mathematical models involving simulation modelling methods.

6. The analysis of the results of computer modelling of the dynamic processes of the HID-based VI system was carried out, which allowed determining the dynamic characteristics of the PPG function to determine the implementation of effective VI modes of operation.

RESULTS

For the pile driving technology (Barkan, 1952), a hinged HID-based VI device was developed, where a two-stage pulsator valve is used as a vibration exciter (Iskovych-Lototsky *et al.*, 2018a; Iskovych-Lototsky *et al.*, 2018b). The CAD software was used to develop a three-dimensional model of a mounted HID-based VI attachment (Fig. 1).

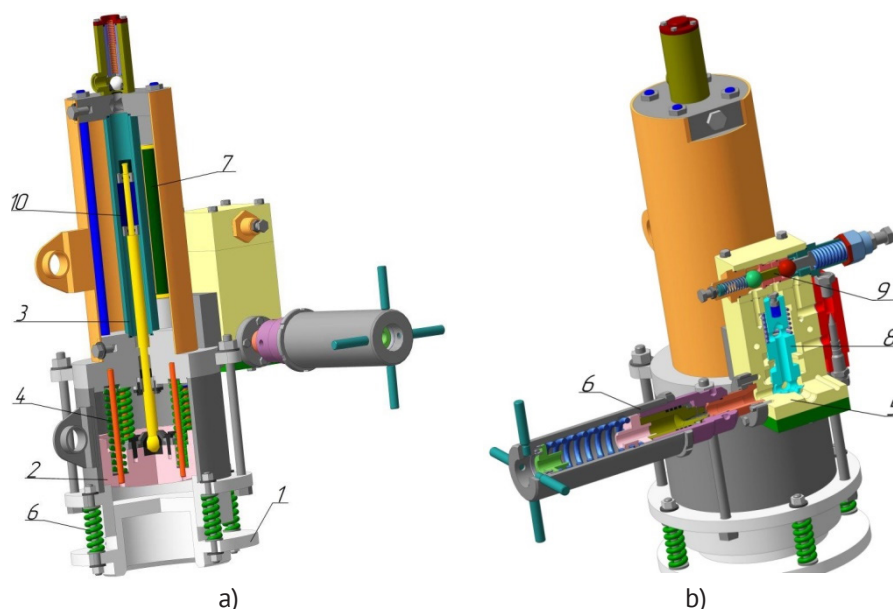


Figure 1. Hinged HID-based VI device: a – view of the section from the side of the executive HID body; b – sectional view from the HID side and the accumulator

Notes: 1 – headpiece; 2 – impact mass; 3 – executive hydraulic cylinder; 4 – elastic elements; 5 – HID; 6 – accumulator; 7 – drain line; 8 – second stage; 9 – servo valves; 10 – working body

The mounted inertial HID-based VI hammer (see Fig. 1) comprises a headpiece (1), which is connected to the pile and the impact mass (2), which is set in motion using the HID, which in turn consists of the executive hydraulic cylinder (3) and the HID (5). The HID is connected to the executive hydraulic cylinder (3) according to the "inlet" scheme (Manzhilevsky, 2019) through the hydraulic accumulator (6). This type of HID connection allows applying a power load to the working body (10) (plunger) of the executive hydraulic cylinder, which has the function of changing the impulse forces forms (Glushak *et al.*, 1992). The HID working cycle begins with filling the accumulator (6), and, accordingly, accumulating pressure in it up to a certain pre-set value p_1 , which is set by the control spring on the servo valves (9) of the HID (5). Having reached the specified pressure p_1 , the second stage valve (8) opens in the HID (5), which connects the cavity of the accumulator (6) with the working cavity of the executive hydraulic cylinder (3). A sharp increase in pressure in the working cavity of the executive hydraulic cylinder (3) forces the working body (10) (plunger) to move upward, which forces the inertial mass (2) to rise while compressing the elastic elements (4). The movement of the inertial mass (2) upward leads to

the accumulation of potential energy from the action of the forces of attraction and elastic forces. After a pressure drop p_2 in the HID system, the opening moment of which is determined according to the design parameters of the servovalves (9), the inertial mass (2) begins to move downward, which leads to shock interaction with the headgear (1), which in turn is transmitted to the count. In addition, when the pressure in the HID system drops to p_2 , the working cavity of the executive hydraulic cylinder (3) is combined with the drain line (7) using the second stage valve (8). In this case, the drain line (7) is connected to the supra-plunger cavity of the actuating hydraulic cylinder (3), additionally creating loads on the plunger (10) upon the downward stroke. This design solution gives added kinetic energy to the impact mass (2), which allows increasing the energy of the shock load on the headgear (1).

To develop a mathematical model of the pile driving technology with a mounted HID-based VI device, a three-component (flat multi-mass) inertial model with contacts between masses (Fig. 2) was developed, which allows simulating elastic-plastic deformations of the soil and the stress-strain state of a pile immersed in the soil.

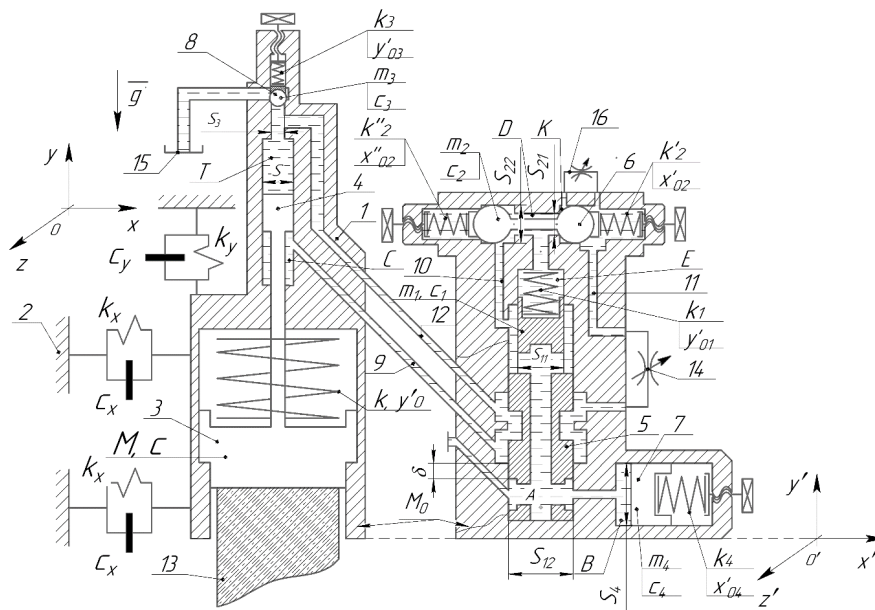


Figure 2. Calculation model of the technological process of driving the piles of the HID-based VI device

Notes: 1 – hull; 2 – boom vehicle; 3 – inertial mass; 4 – plunger; 5 – second stage valve; 6 – first stage valve; 7 – hydro accumulator spring; 8 – check valve spring; 9 – pressure head hydraulic lines; 10 – pressure channels; 11 – drain line; 12 – hydraulic lines; 13 – pile; 14 – throttle; 15 – hydraulic tank; 16 – first stage throttle; A – lower internal pressure chamber of the second stage valve; B – cavity of the hydro accumulator; C – pressure cavity of device 1; D – internal pressure chamber two-stage of the pulsator valve; E – upper internal pressure chamber of the second stage valve; T – drain cavity of device 1; K – internal pressure chamber of the first stage valve; c_1 – the coefficient of the forces of viscous friction between the walls of the body of the device 1 and the second stage valve 5; c_2 – the coefficient of the forces of viscous friction between the walls of the body of the device 1 and the first stage valve 6; c_3 – coefficient of viscous friction forces between the walls of the body of the device 1 and the check valve 8; c_4 – the coefficient of the forces of viscous friction between the walls of the body of the device 1 and the accumulator 4; c – coefficient of forces viscous friction between the walls of the body of the device 1 and the inertial mass 3; c_x, c_y – are the vertical and horizontal components of the coefficient of viscous friction forces of the damping component of the shock-absorbing unit connecting the HID device with the boom vehicle 2 (Manzhilevsky, 2019); k_1 – is the stiffness of the valve spring of the second stage 5; k'_2, k''_2 – is the stiffness of the valve spring of the first stage 6; k_3 – is the stiffness of the check valve spring 8; k_4 – is the stiffness of the accumulator spring 4; k – is the stiffness of the spring of the inertial mass 3; k_y, k_x – is the vertical and horizontal components of the stiffness of the elastic component of the shock-absorbing unit of the connecting HID device with the boom of the vehicle 2 (Thümmel, 2012); y'_{01} – is the pre-tension of the valve spring of the first stage 5; y'_{02} – is the pre-tension of the inertial mass spring 3; x'_{02}, x'_{03} – is the pre-tensioning of the valve springs of the first stage 6; y'_{03} – is the pre-tension of the check valve spring 8; x'_{04} – is the pre-tension of the hydro accumulator spring 7; M_0 – mass of the device; M – mass of the inertial mass 3; m_1 – mass of the second stage valve 5; m_2 – mass of the first stage valve stage 6; m_3 – mass of the check valve 8 with; m_4 – mass of the hydro accumulator 7; S_{11} – small surface of the second stage valve 5; S_{12} – external surface of the first stage valve 6; S_{21} – small surface of the first stage valve 6; S_{22} – external surface of the first stage valve 6; S_3 – surface area of the check valve surface 8; S_4 – inner surface of the hydro accumulator plunger 7; δ – is the second stage valve overlap 5; g – acceleration of gravity

The absolute $xOyz$ and the movable coordinate system $x'O'y'z'$ are introduced, which are rigidly connected to the hull (1) of the attachment with a mass M_0 , and the movable coordinate system $x''O''y''z''$ is rigidly connected to the submerged pile (13) (see Fig. 2). It is expedient to divide the pile driving technology with a hinged HID-based device into two periods: the period of accumulation of kinetic energy and the period of impact interaction of the inertial mass (3) by driving the pile (13) into the soil (Bingham *et al.*, 2000). The period

of accumulation of the kinetic energy of the HID by the device consists of the characteristic working movements of the locking elements (5-7) and the plunger (4). In turn, the period of impact interaction is characterised by viscoplastic deformations of the soil and the stress-strain state of the pile (13). Next, this study considers the working period of the accumulation of kinetic energy in more detail. The equations of motion for body (1) of a device (see Fig. 2) with mass M_0 is written as follows:

$$\begin{cases} -M_0\ddot{y} = -M_0g + k_1(y'_{01} + y'_1) + k_3(y'_{03} + y'_3) + k(y'_0 + y') - \\ - \iint_{\tilde{S}} p_{\tilde{S}}(t) dS_y + c_y\dot{y} + c_1\dot{y}'_1 + c_3\dot{y}'_3 + c\dot{y}' - N_{05y} - N_{08y} - N_{03y}; \\ -M_0\ddot{x} = - \iint_{\tilde{S}} p_{\tilde{S}}(t) dS_x + 2c_x\dot{x} + 2k_x x - k_2''(x''_{02} - x'_2) + \\ + k_2'(x'_{02} + x'_2) + k_4(x'_{04} + x'_4) + c_2\dot{x}_{02} - c_4\dot{x}'_4 - N_{06x} \end{cases} \quad (1)$$

where: $p_{\tilde{S}}(t)$ is the function of changing the pressure of the working fluid in the internal cavity hydraulic channels of the VI device; $\iint_{\tilde{S}} p_{\tilde{S}}(t) dS_y$, $\iint_{\tilde{S}} p_{\tilde{S}}(t) dS_x$ are the corresponding components of the forces, acting on the inner surface $\tilde{S}$ of the cavity of the hydraulic channels of the device; N_{05y} are the vertical components of the reaction forces of a cylindrical support of the second stage valve (5) on the body of the two-stage pulsator valve; N_{08y} are the

vertical components of the reaction forces of the check valve (8) on the conical support device body (1); N_{03y} is the reaction force of the inertial mass (3) into the device body (1); N_{06x} is the horizontal component of the reaction forces of the first stage valve (6) on the body of the device (1).

The equations of motion for the inertial mass (3) weighing Mg are as follows:

$$M\ddot{y}_0 = -Mg - k(y'_0 + y'_0) + \iint_S p_S(t) dS - c\dot{y}'_0 + N_{30y} \quad (2)$$

where $p_S(t)$ is the function of changing the pressure of the working fluid in the internal pressure C and drain T cavities of device (1); $\iint_S p_S(t) dS$ is the corresponding components of the forces, acting on the inner surfaces S of the plunger (4) of the inertial mass (3); N_{30y} is the

reaction force of the device body (1) to the inertial mass (3).

Since the first stage valve (6) along the y -axis moves together with the body of the hinged VI device (1), the equation of motion for the first stage valve (6) with mass m_2 along the x -axis is as follows:

$$m_2\ddot{x}_2 = k_2''(x''_{02} - x'_2) - k_2'(x'_{02} + x'_2) - c_2\dot{x}'_2 + \iint_{S_{22}-S_{21}} p_{S_{22}-S_{21}}(t) dS + N_{60x} \quad (3)$$

where $p_{S_{22}-S_{21}}(t)$ is the function of changing the pressure of the working fluid in the internal pressure chambers D and K two-stage of the pulsator valve; $\iint_{S_{22}-S_{21}} p_{S_{22}-S_{21}}(t) dS$ is the corresponding components of the forces acting on the inner surface of the $S_{22}-S_{21}$ of the

first stage valve (6); N_{60x} is the horizontal component of the reaction forces of the housing of device (1) on the first stage valve (6).

Equations of motion for the second stage valve (5) with mass m_1 :

$$m_1\ddot{y}_1 = -m_1g - k_1(y'_{01} + y'_1) - c_1\dot{y}'_1 + \iint_{(S_{12}-S_{11})} p_{(S_{12}-S_{11})}(t) dS + N_{50y} \quad (4)$$

where $p_{(S_{12}-S_{11})}(t)$ is the function of changing of the pressure of the working fluid in the internal pressure chambers A , E of a two-stage pulsator valve; $\iint_{(S_{12}-S_{11})} p_{(S_{12}-S_{11})}(t) dS$ are the corresponding components of the forces acting on the area $S_{12}-S_{11}$ of valve surface of the second stage valve (5); N_{50y} is the vertical components

of the reaction forces of the device body (1) on the lower cylindrical surface of the second stage valve (5). Since the plunger valve of the accumulator (7) on the y -axis is moving together with the hinged device (1), the equations of motion for the plunger of the hydro accumulator (7) with mass m_4 along the x -axis are as follows:

$$m_4\ddot{x}_4 = -k_4(x'_{04} + x'_4) - c_4\dot{x}'_4 + \iint_{S_4} p_{S_4}(t) dS \quad (5)$$

where $p_{S_{22}-S_{21}}(t)$ is the function of changing the pressure of the working fluid in the internal pressure chambers D and K of the two-stage pulsator valve; $\iint_{S_4} p_{S_4}(t)dS$ is the corresponding components of the

forces acting on the inner surface S_4 of the plunger of the hydro accumulator (7).

Equations of the motion for check valve (8) with mass m_3 are as follows:

$$m_3 \ddot{y}_3 = N_{80y} - k_3(y'_{03} + y'_3) - c_3 \dot{y}_3 + \iint_{S_3} p_{S_3}(t)dS - m_3 g \quad (6)$$

where $p_{S_3}(t)$ is the function of changing of the pressure of the working fluid in the internal pressure chambers A , E of the two-stage pulsator valve; $\iint_{S_3} p_{S_3}(t)dS$ are the corresponding components of the forces acting on the surface area S_3 of the surface of the check valve (8); N_{80y} are the vertical components of the reaction forces of the device body (1) on the conical surface of the check valve (8). In this case, it is a neglect of the inertial forces of the working fluid acting on the working parts of the HID as bearing an insignificant contribution to the changes of the device movement in general. To fully write down the mathematical model of the VI device

operation, it is necessary that the operation of the HID for the corresponding working phases of the two-stage of the pulsator valve is considered.

Pressure build-up phase. In this phase, the first stage valve (6) and second stage valve (5), as well as the inertial mass (3) with the check valve (8) are at rest. At this phase, the second stage valve (5) overlocks the pressure chambers A and C , causing a pressure build-up in the cavity B of the hydro accumulator (7). In this case, the cavity A is connected to the cavities D and E through the pressure channels (10). The initial conditions for this phase $0 \leq t \leq t_{sp}$ are as follows:

$$\begin{cases} \iint_{S_{22}-S_{21}} p_{S_{22}-S_{21}}(t)dS \leq k_2''x_{02}'' - k_2'x_{02}'; & \iint_{(S_{12}-S_{11})} p_{(S_{12}-S_{11})}(t)dS \leq k_1y'_{01}; & \iint_{S_3} p_{S_3}(t)dS \leq k_3y'_{03}; \\ \dot{y}_0(t) = \dot{y}_1(t) = \dot{y}_3(t) = 0; & \dot{x}_2(t) = 0; & 0 \leq x'_4(t) \leq x'_{4\max} \end{cases} \quad (7)$$

where $x'_{4\max}$ is the maximum stroke of the plunger of the hydro accumulator (7).

Pulsator valve actuation phase. In this phase, the first stage valve (6) opens, since the pressure in the cavity D acting on the working area $S_{22}-S_{21}$ got equated with the force adjustment of the springs $k_2''x_{02}'' - k_2'x_{02}'$. The first stage valve (6) leads to a pressure change in cavity E , causing a pressure drop across the working area $S_{12}-S_{11}$ of the second stage valve (5) and making it move upward.

For this phase $t_{sp} \leq t \leq t_w$, the initial conditions (Eq. 8) are written. The phase of the rise of the inertial mass (3). At this phase, there is a communication between the pressure cavities A , B , and the cavity C . At the same time, the internal cavities D , K , E are connected through the drain lines (11-12) and a throttle (14) with a drain cavity T . The combination of cavities A , B , and C through the piston (4) makes the inertial mass (3) move up:

$$\begin{cases} \iint_{S_{22}-S_{21}} p_{S_{22}-S_{21}}(t)dS \geq k_2''x_{02}'' - k_2'x_{02}'; & \iint_{(S_{12}-S_{11})} p_{(S_{12}-S_{11})}(t)dS \geq k_1y'_{01}; & \iint_{S_3} p_{S_3}(t)dS \leq k_3y'_{03}; \\ \dot{y}_0(t) = \dot{y}_3(t) = 0; & 0 \leq y'_1(t) \leq \delta; & N_{60x} = N_{06x} = 0; \\ N_{05y} = N_{50y} = 0; & 0 \leq x'_2(t) \leq x'_{2\max}; & 0 \leq x'_4(t) \leq x'_{4\max} \end{cases} \quad (8)$$

where $x'_{2\max}$ is the maximum stroke of the first stage valve (6), δ is the overlap of the second stage valve (5).

For this phase $t_w \leq t \leq t_{el}$, the following initial conditions are recorded as follows:

$$\begin{cases} \iint_{S_{22}-S_{21}} p_{S_{22}-S_{21}}(t)dS \geq k_2''x_{02}'' - k_2'x_{02}'; & \iint_{(S_{12}-S_{11})} p_{(S_{12}-S_{11})}(t)dS \geq k_1y'_{01}; \\ \iint_{S_3} p_{S_3}(t)dS \geq k_3y'_{03}; & \delta \leq y'_1(t) \leq y'_{1\max}; & 0 \leq x'_2(t) \leq x'_{2\max}; \\ 0 \leq x'_4(t) \leq x'_{4\max}; & 0 \leq y'_0(t) \leq y'_{0\max}; & 0 \leq y'_3(t) \leq y'_{3\max}; \\ N_{05y} = N_{50y} = 0; & N_{06x} = N_{60x} = 0; & N_{08y} = N_{80y} = 0; & N_{30y} = N_{03y} = 0 \end{cases} \quad (9)$$

where $y'_{1\max}$ is the maximum stroke of the second stage valve (5); $y'_{0\max}$ is the maximum stroke of inertial mass (3); $y'_{3\max}$ is the maximum stroke of the check valve (8).

The phase of lowering the inertial mass (3). At this phase, there is a drop in pressure in the cavity C and through the hydraulic lines (9, 12) the working fluid

flows into the drain cavity T and, accordingly, into the hydraulic tank (15). At the same time, the first stage valve (6) returns to the initial position and causes the overlap of the cavities K and D , while the throttle (16) allows the working fluid to drain into the drain line (11). After the overlap of the cavities K and D , the pressure in

the cavity E drops, which forces the second stage valve (5) to rotate to its original position.

For this phase $t_{el} \leq t \leq t_{ds}$, the initial conditions (10) are recorded as follows:

$$\left\{ \begin{array}{l} \iint_{S_{22}-S_{21}} p_{S_{22}-S_{21}}(t) dS \geq k_2'' x_{02}'' - k_2' x_{02}'; \quad \iint_{(S_{12}-S_{11})} p_{(S_{12}-S_{11})}(t) dS \geq k_1 y_{01}'; \\ \iint_{S_3} p_{S_3}(t) dS \geq k_3 y_{03}'; \quad 0 \leq y_1'(t) \leq y_{1\max}'; \quad 0 \leq x_2'(t) \leq x_{2\max}'; \\ 0 \leq x_4'(t) \leq x_{4\max}'; \quad 0 \leq y_0'(t) \leq y_{0\max}'; \quad 0 \leq y_3'(t) \leq y_{3\max}'; \\ N_{05y} = N_{50y} = 0; N_{06x} = N_{60x} = 0; N_{08y} = N_{80y} = 0; N_{30y} = N_{03y} = 0. \end{array} \right. \quad (10)$$

The period $t_{ds} \leq t \leq t_{dp}$ of the impact interaction of the inertial mass (3), the immersed pile (13) with the soil (17), is being considered (Fig. 3). Experimental studies of the immersion impact of piles (Manzhilevsky, 2019; Goncharevich, 1981) indicate that the immersion occurs as follows. During the inertial movement of the submerged pile (13), deformation of the soil occurs upon contact with the conical surface of the submerged pile with an inner cone angle α . Multiple frequency deformations of the contacting soil layer (17) contribute to the opening of internal cracks and to the accumulation of residual deformations, which, as a result, reduce the fracture stress and contribute to a relative displacement with subsequent compaction of the contacting soil layers (17) with the surface of the submerged pile (13). Considering the given regularities of the submersion process,

a phenomenological model of the soil layer has been developed, which is subjected to shock strain from the conical surface of the submerged pile (13) (see Fig. 3). The soil model is a trimass-elasto-viscoplastic rheological body (Israelashvili, 2004; Magnus *et al.*, 2008). Elastic deformations of the model are reproduced by radially distributed elastic elements with vertically and horizontally constituent stiffness coefficients k_y and k_x , and dampers with vertically and horizontally constituting viscous resistance coefficients c_x and c_y , respectively.

The process of deformation (destruction) and displacement of the two-mass model of the soil layer in projections on the x, y axis at the stage $t_{ds} \leq t \leq t_{ed}$ spring-viscous deformation of the soil layer is described by a system of differential equations as follows:

$$\left\{ \begin{array}{l} (1-\lambda)\xi m \ddot{x} = N_{13,17x} - c_x(\dot{x} - \dot{x}_0) - k_x(x - x_0); \\ (1-\lambda)\xi m \ddot{y} = N_{13,17y} - c_y(\dot{y} - \dot{y}_0) - k_y(y - y_0), \end{array} \right. \quad (11)$$

where $(1-\lambda)\xi m$ is the vibrational free mass of the soil layer 17; x, y are the mass movement $(1-\lambda)\xi m$ towards x, y axes; x_0, y_0 are the mass movement $\lambda\xi m$ towards the

axes x_0, y_0 ; $N_{13,17(x)}$ are the components of the reaction forces of pile 13 with soil 17 from the interaction with a conical surface $I-I$.

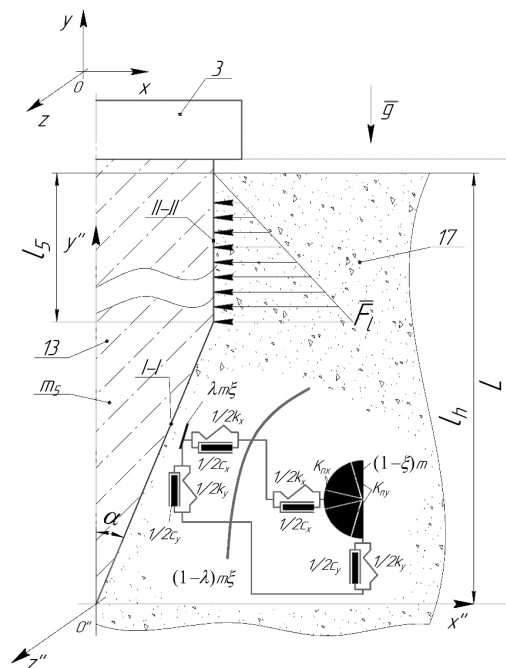


Figure 3. Dynamic model of the VI pile driving

Notes: 3 – inertial mass; 13 – pile; 17 – soil; I-I – conical pile surface; II-II – cylindrical submerged in the ground 17 pile surface 13; k_x, k_y – vertically and horizontally constituent stiffness coefficients, respectively; c_x, c_y – vertically and horizontally constituting viscous resistance coefficients, respectively; $(1-\lambda)\xi m$ – specific gravity of interacting soil 17; $(1-\lambda)\xi m$ – is the vibrational free mass of the soil layer 17; $\lambda\xi m$ – mass of the contacting soil layer 17; k_{ny}, k_{nx} – are the vertical and horizontal components of the deformation coefficients; m_s – mass of the pile 13; α – pile cone angle; l_h – submerged pile length; l – total pile length; l_s – length of the vertical part of the pile buried in the ground; F_l – specific reaction force of the soil 17 during the interaction of piles 13 along the vertical plane II-II; g – acceleration of gravity

Plastic deformations of the soil layer 17 at the stage $t_{ed} \leq t \leq t_{pd}$ are described by the following equations:

$$\begin{cases} (1-\lambda)\xi m\ddot{x} = N_{13,17x} - k_{nx}(x - x_{\kappa}^{ne}); \\ (1-\lambda)\xi m\ddot{y} = N_{13,17y} - k_{ny}(y - y_{\kappa}^{ne}) \end{cases} \quad (12)$$

where k_{ny} i k_{nx} are the vertical and horizontal components of the deformation coefficients.

Equations of motion for the y -axis of the pile 13 of the VI device with mass m_5 is as follows:

$$m_5\ddot{y} = m_5g + N_{3,13} - N_{17,13}(\sin\alpha + \mu_y) - \mu_y \int_0^{l_5} F_l dy \quad (13)$$

where $\int F_l dy$ is the reaction force of the soil (17) with interaction with the pile 13 along the vertical plane $II-II$; μ_y is the vertical component of the soil (17) and pile (13) friction coefficient; $N_{17,13}$ is the component of the reaction forces of the soil (17) from the interaction with the conical surface $I-I$ of the pile (13); $N_{3,13}$ is a compound of the

reaction forces of the inertial mass (3) with the upper base of the pile (13).

DISCUSSION

The mathematical model of the pile driving technology involving a HID-based VI device, which is represented by the systems of equations (1-13) and additionally by the Navier-Stokes hydrodynamics equations (Iskovych-Lototsky *et al.*, 2018b; Iskovich-Lototsky *et al.*, 2019), was implemented according to numerical modelling methods based on software systems FlowVision (Iskovich-Lototsky *et al.*, 2019), Matlab Simulink (Iskovych-Lototsky *et al.*, 2018a; Shatokhin *et al.*, 2019) powered by computing clusters of the V.M. Glushkov CS Institute of Cybernetics of the NAS (National Academy of Sciences) of Ukraine (Yarovyy *et al.*, 2012). The result of the calculation in the FlowVision software package was the distribution of pressure (Fig. 4, a) and velocity (Fig. 4, b) of the working fluid in the HID cavities.

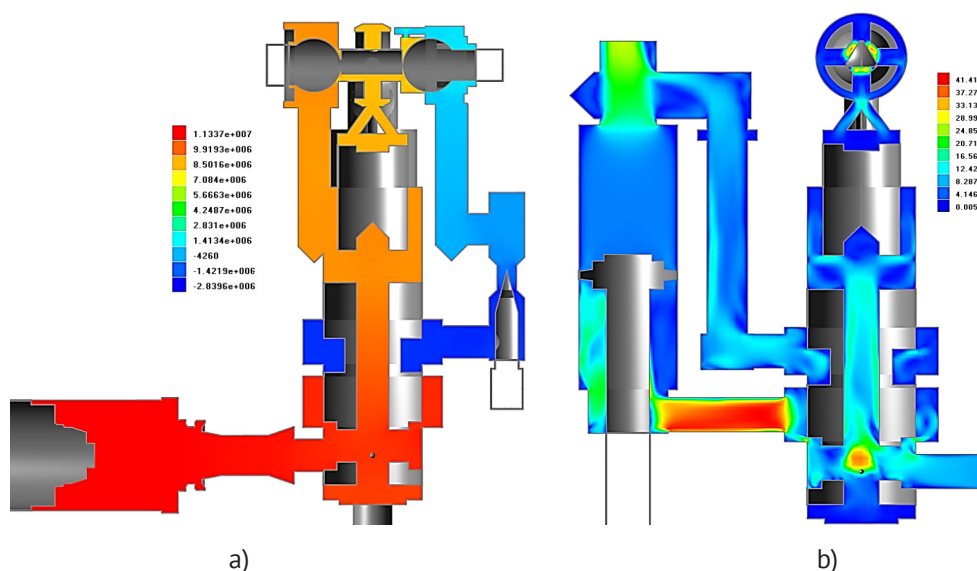


Figure 4. Distribution of pressure (a) and velocity (b) of the working fluid in the HID cavity of the VI device for pile driving

The numerical result of the calculation in the FlowVision software package is the diagrams of changes

in the integral value of pressure in various HID cavities of a VI device for pile driving (Fig. 5).

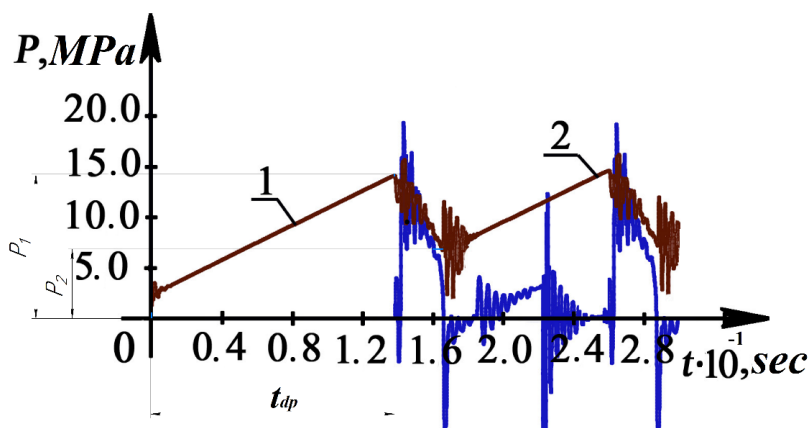


Figure 4. Diagrams of pressure changes in the HID cavities of the VI device for pile driving

Notes: 1 – change in pressure in the accumulator cavity; 2 – pressure change in the pressure chamber of the executive hydraulic cylinder; t_{dp} – charging time of the accumulator; p_1 – pressure pulsation amplitude in the executive hydraulic cylinder; p_2 – pressure pulsation amplitude in the accumulator

The next result of the calculation in the FlowVision software package is the diagrams of changes in the

movement of the moving elements of the HID of the VI device for pile driving (Fig. 6).

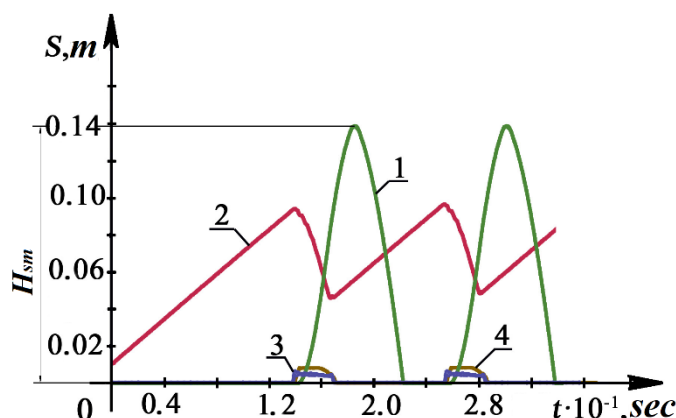


Figure 6. Diagrams of changes in the movement of movable elements of the HID of a VI device for pile driving

Notes: 1 – change in the displacement of the impact mass; 2 – change in the movement of the accumulator; 3 – change in the movement of the first stage valve; 4 – change in the movement of the second stage valve; H_{sm} – oscillation amplitude of the impact mass

Additionally, using the Matlab Simulink software package, motion diagrams (Fig. 7) of a pile submerged

into a soil layer of the “quartz sand” type were obtained during a two-time impact interaction.

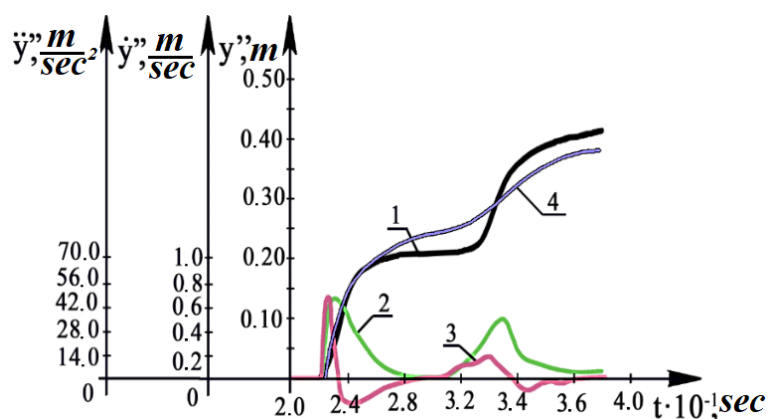


Figure 7. Diagrams of the change in the kinematic parameters of the pile submerged during operation of the HID-based VI device

Notes: 1 – change in the movement of the submerged pile; 2 – change in the speed of the pile submerged driven; 3 – change in the acceleration of the submerged pile; 4 – experimental data on changes in the displacement of a submerged pile (Manzhilevsky, 2019; Guo et al., 2014)

In the middle of the second stage valve cavity (8) (see Fig. 1), the pressure is considerably higher than in the lower cavity of the first stage valve (9), and this difference is approximately 1.5 MPa (see Fig. 4, a). This pressure difference creates an added force that allows the second stage valve (9) to move while connecting the pressure cavities of the accumulator 6 and the hydraulic cylinder (3). It is also noted that the pressure in the drain channel after the first stage valve (9) is 1.5 MPa is bigger than in the main drain channel (7) of the HID (see Fig. 4, a). This fact indicates the high efficiency of the throttle assembly (14) (see Fig. 1). The use of an added control stage in the form of the first stage valve allows considerably reducing the dimensions and, accordingly, the total weight of the control hydraulic equipment. Figure 4, b, demonstrates the extreme velocity values in the pressure channel (9) of the executive hydraulic

cylinder (3) (see Fig. 1), which is a consequence of the movement of the total flows of the working fluid from the hydraulic pump and accumulator. The second stage valve (8) is under an additional action of a dynamic force occurring from the velocity head in the middle of the valve itself at the conical chamfer. This added component of the hydrodynamic force requires an additional increase in the rigidity of the elastic element of the second stage valve (8) (see Fig. 1).

The analysis of the pressure diagrams (see Fig. 5) demonstrates that at the opening phase of the second stage valve (8) (see Fig. 1), the pressure in the pressure cavity of the actuating hydraulic cylinder (3) is 3 MPa more than the maximum pressure in the cavity of the accumulator (6), which is a consequence of hydraulic shock (Wilcox, 1994; Teng et al., 2010). In addition, during the second period of the pressure drop phase in the

pressure cavity of the actuating hydraulic cylinder (3), there is a certain pressure drop by 2 MPa compared to the nominal initial pressure in the hydraulic system. This phenomenon indicates the presence of weakness in the working fluid (Wilcox, 1994; Sevostianov *et al.*, 2021). The presence of pressure pulsations in the second phase of the pressure drop (see Fig. 5), both in the cavity of the accumulator (6) and in the pressure cavity of the actuating hydraulic cylinder (3) (see Fig. 1) indicates the presence of accumulated resonance phenomena in the working fluid and is the result of the presence of wave processes in a moving working fluid (Iskovych-Lototsky *et al.*, 2018a; Iskovych-Lototsky *et al.*, 2018b; Wilcox, 1994). The diagram in Figure 5 suggests that the amplitude of the pressure pulsations for the pressure cavity the accumulator $p_2=8$ MPa, and for the pressure chamber of the executive hydraulic cylinder $p_1=12$ MPa. The accumulator build-up time (energy storage time) is $t_{dp}=0.14$ s.

Analysis of the diagrams of changes in the displacement of the movable elements of the HID of the VI device for pile driving (see Fig. 6) allows determining the following operating parameters of the technological process:

- the oscillation amplitude of the impact mass of the HID $H_{sm}=140.6$ mm, the first stage valve – 6.0 mm; the second stage valve – 8.0 mm; hydroaccumulator – 48.2 mm;
- the frequency of operation (vibration) of the shock mass and, accordingly, the device itself is 5.8 Hz.

The diagram of the change in the kinematic parameters of the pile being driven during the operation of the HID-based VI device (see Fig. 7) suggests that the

depth of immersion during the first shock interaction, on average, is 0.23 m, and during the second – 0.18 m. Average pile sinking speed at the first impact interaction is 0.4 m/s, and at the second – 0.27 m/s. The average acceleration of pile sinking during the first impact interaction is 35.0 m/s², and at the second – 28.0 m/s². Average error in approximating the change in the displacement of a submerged pile in a soil medium of the “quartz sand” type in comparison with experimental data amounted to 10.40% (Manzhilevsky, 2019; Guo *et al.*, 2014), which allows considering the developed mathematical models highly adequate to real systems (Mori, 2017; Palm, 2007; Shabana, 2019; Spremann & Manoli, 2012).

CONCLUSIONS

As a result of the performed research, the authors of this paper offer a design of a VI device for pile driving with a highly efficient and compact HID based on two-stage pulsator valve. A mathematical model has been improved for studying the pile driving technology for a HID device based on the laws of hydrodynamics using mechanoreological phenomenology and generalised laws of mechanics.

Based on the developed mathematical model by finite volume methods using numerical modelling, working dependences were obtained to determine the main performance characteristics of the pile driving technology using the HID-based VI device. Comparative results of numerical modelling of the pile driving technology demonstrated the advantages of the chosen design approach and proved the efficiency of the developed HID design based on a two-stage pulsator valve.

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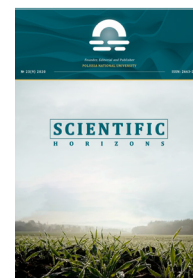
Моделювання технологічного процесу занурення паль віброударним пристроєм з гідроімпульсним приводом

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Анотація. Розроблена математична модель технологічного процесу роботи віброударного пристрою для занурення будівельних паль на базі гідроімпульсного приводу. На основі аналізу розробленої математичної моделі запропоновано оптимальні режими роботи вібраційного пристрою для забезпечення інтенсифікації процесу занурення паль. Запропоновано оригінальну конструкцію сучасного вискоефективного пристрою для занурення будівельних паль, який оснащено компактним потужним гідроімпульсним приводом. Для розробки математичної моделі технологічного процесу занурення паль використовувались методи механореологічної феноменології, гідродинаміки і узагальнені закони механіки. Удосконалено математичні моделі динаміки технологічних процесів занурення паль віброударним пристроєм на базі гідроімпульсного приводу, у формі просторово-нестационарної постановки задачі та інтегральних рівнянь динамічних характеристик рухомих елементів приводу. На основі розробленої математичної моделі методом кінцевих об'ємів, за допомогою чисельного моделювання і високопродуктивних обчислювальних комп'ютерних систем, отримано розподіл тиску і швидкості робочої рідини в гідроімпульсному приводі віброударного пристрою, а також зміни кінематичних параметрів елементів технологічного обладнання. Запропоновано оптимальні режими роботи гідроімпульсного приводу віброударного пристрою для забезпечення інтенсифікації технологічного процесу занурення паль. Встановлено, що при застосуванні низькочастотної вібрації відбувається інтенсифікація процесу занурення будівельних паль. Застосування гідроімпульсного приводу на основі двокаскадного віброзбудження дозволило реалізувати віброударний режим роботи пристрою. Середня швидкість занурення палі за ударно-вібраційної взаємодії у середньому в п'ять разів вища порівняно з традиційними методами занурення

Ключові слова: імпульс, удар, вібрації, математична модель, гідропривод, клапан, будівельна паля



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Features of Hepatopathy and Hematological Complications in Acute Spontaneous Babesiosis of Dogs

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Abstract. Spontaneous babesiosis in dogs is one of the most common and dangerous diseases. Erythrocyte damage conditioned upon pathogens is a trigger for hematological complications and lesions of the microcirculatory tract. The development of thrombotic processes leads to irreversible changes and subsequent death of the organism. The liver is one of the first organs to suffer damage. The aim of the study was to identify the links between the pathogenesis of hepatopathy and hematological disorders in the course of acute spontaneous babesiosis in dogs. To achieve this, two groups of dogs of 20 individuals were formed: the experimental group – animals with babesiosis, the control group – clinically healthy. It is established that acute spontaneous babesiosis occurs in successive forms – anemic and jaundice. Normochromic anemia, leukocytosis, thrombocytopenia were detected. Hemorheological parameters indicate increased aggregation of erythrocytes and platelets, which led to thrombotic conditions. The criterion of shock is established – decrease in the volume of circulating blood; its deficiency was 24% for anemic and 34% for jaundice. Also identified markers of disseminated intravascular coagulation syndrome (DIC) – hypofibrinogenemia, increased levels of soluble fibrin-monomer complexes, fibrin degradation products, including D-dimer. Changes in indicators increased as the disease progressed from anemic to jaundiced form. In the anemic form, shock is defined as subcompensated in moderate severity, and DIC syndrome – in the stage of consumption coagulopathy. Complications characterise a transitional state that is in unstable equilibrium. In the icteric form, shock is defined as decompensated severe, and DIC syndrome – in the stage of hypocoagulation. This condition is characterised as critical, with a pronounced tendency to irreversibility. Changes in liver parameters indicate hyperactivity of all indicator enzymes, hyperbilirubinemia and hyperuria. With the development of the disease, the rates increase significantly. DIC syndrome, shock and hepatopathy enter the vicious circle, in which hematological complications cause hepatopathy, and it, in turn, exacerbates them. Hepatopathy for babesiosis in dogs is considered a serious condition that requires intensive care

Keywords: shock, liver, DIC syndrome, hemorheology, hemodynamics, liver profile



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INTRODUCTION

Babesiosis of dogs is an urgent problem of veterinary medicine today. The disease is characterised by severe course and causes significant damage to the animal's body, even life-threatening. In the seasons of tick activity mortality can reach 50%. The causative agents of this disease are blood spores, representatives of the series *Piroplasmida* Wenyon 1936 (Bilić *et al.*, 2018; Vishwakarma & Nandini, 2019). Known large forms of pathogens – *Babesia canis* Piana et Galli-Valerio 1895; *B. vogeli* Reichenow 1977; *B. rossi* Nutall 1910. The first two species are cosmopolitan, and the latter is more typical of regions of Africa. These forms are characterised by a fairly large size of zoites at the stage of paired pear-shaped formations. The length of such zoites exceeds the radius of the erythrocyte, so the paired forms are placed at an acute angle to each other. In the form of odd generations, zoites are also quite large (Vishwakarma & Nandini, 2019; Dubova *et al.*, 2020).

Representatives of babesia of small forms are: *B. gibsoni* Patton 1910; *B. vulpes* Baneth, Florin-Christensen, Cardoso & Schnittger 2015; *B. conradae* sp. nov. – a recently discovered species of “small” babesia in the United States, *B. microti-like* sp. nov. (Bilić *et al.*, 2018; Azagi *et al.*, 2021; Hussain *et al.*, 2021). In small forms of babesia, paired pear-shaped zoites have a length shorter than the radius of the erythrocyte. In the form of odd formations, such zoites resemble small round inclusions, which are microscopically different from prokaryotes in the existing nucleus. Pathogen *B. equi* Laveran 1901 (syn. *Theileria equi*, *Nuttallia equi*) is an obligate parasite of horses, but cases of lesions in dogs have been identified, as confirmed by genetic identification of the pathogen (Vishwakarma & Nandini, 2019).

Babesia species are confined to certain vectors. Thus, for *B. canis* the relevant vector is *Dermacentor reticulatus*, which is common in Europe, in the temperate-continental climate zone. For *B. vogeli*, a specific vector is the brown dog mite *Rhipicephalus sanguineus*. It is widespread in the Mediterranean, prefers temperate climates, and is found in Central Europe and the British Isles (Bilić *et al.*, 2018; Vishwakarma & Nandini, 2019).

Small forms of pathogens (*B. gibsoni*, *B. microti-like*, *B. vulpis*) use mites of the genus *Ixodes* as a biological vector – *I. hexagonus* Leach 1815, *I. canisuga* Johnston 1849, sometimes *I. ricinus*. Small pathogens do not occur in Ukraine (Vishwakarma & Nandini, 2019; Dubova *et al.*, 2020).

Conditioned upon its landscape and climatic characteristics, the Polissya Lowland Zone of the Western part of the Eastern European Plain is the optimal territory for the formation of a stable epizootic center of dog babesiosis. The structure of the soils of this area, climatic conditions, the presence of appropriate biocenoses are ideal for creating an area of *Ixodes* mites – vectors of babesiosis. In the region, specific vectors of large forms of *Babesia* spp. *Dermacentor reticulatus* Fabricius 1794 (main vector) and *Ixodes ricinus* Linnaeus 1759 (Dubova *et al.*, 2020) are the causative agents of canine babesiosis.

Different types of pathogens have different virulence. There is information that among the largest forms of the most virulent species of *B. vogeli*. The virulence of small forms is still being studied (Bilić *et al.*, 2018; Vishwakarma & Nandini, 2019).

In the dog's body, the blood parasite begins its pathogenic effect after inoculation with the saliva of the tick. First of all, the pathogen destroys red blood cells. This leads to the development of hemolytic anemia, which results in most of the clinical symptoms of babesiosis (Azagi *et al.*, 2021; Ortiz *et al.*, 2020). Location *Babesia* spp. inside erythrocytes leads to the transformation of their discoid form, there are various pathological formations – spherocytes, planicites, fragments, etc. Such cells are actively destroyed in the liver and initially stimulate its hyperfunction, and then cause inflammatory and dystrophic processes (Bilić *et al.*, 2018; Wesley *et al.*, 2021). At the same time, the activated blood coagulation cascade causes thrombosis in the microcirculatory tract of the liver, which is accompanied by the appearance of ischemic areas. Necrobiotic and subsequent necrotic changes in hepatocytes exacerbate liver failure, increase hypoxic, acidotic and intoxication processes, both in the liver and in the body as a whole (Gil, 2019; Liu *et al.*, 2019).

Finding out the pathogenetic mechanisms and processes of hepatopathy in babesiosis, the sequence of their manifestation and consequences for the body is an urgent problem of veterinary therapy. In the absence of pathogenetic treatment aimed at stopping the development of complications and eliminating their consequences, the course of acute spontaneous babesiosis in terms of prognosis has a clear tendency to an unfavorable end.

Thus, given the special danger of the acute form of spontaneous babesiosis of dogs and the severity of concomitant complications, there is a need for the formation of scientifically sound protocols of pathogenetic therapy. This will allow to determine the priorities and sequence of treatment of drugs against the development of severe complications, such as shock and disseminated intravascular coagulation syndrome (DIC).

The purpose of the work is to establish the pathogenetic links between hepatopathy and disseminated intravascular coagulation syndrome in acute spontaneous babesiosis of dogs.

THEORETICAL REVIEW

The leading pathogenic factor of babesiosis is hypoxia due to erythrocyte damage. In addition, *Babesia* spp. have pyrogenic properties, have a toxic-allergic effect on the body and stimulate the emergence of immune complexes (Bilić *et al.*, 2018; Hussain *et al.*, 2021). The content of destroyed erythrocytes, and the damaged cells themselves are biologically active substances (Vishwakarma & Nandini, 2019). They react to the activation of immune processes: in particular, reactive changes in the vascular

endothelium, aimed at preventing the loss of circulating blood volume (Oaks *et al.*, 2020). The system of mononuclear phagocytes with the main function of protecting the body from invading agents is also activated. Indeed, mononuclear cells attack and destroy erythrocytes affected by babesia (Ortiz *et al.*, 2020; Roopali *et al.*, 2018). However, in the case of an autoimmune reaction, phagocytes will destroy completely healthy erythrocytes, which will greatly complicate the pathogenesis of the disease and the condition of the animal. Thus, as a result of a complex set of reactions in response to the invasion, all organs and systems are involved in the pathogenic process.

Previous studies have shown that the toxic-allergic effects of *Babesia spp.* causes severe complications that primarily affect the microcirculatory tract of organs (Ortiz *et al.*, 2020; Hildebrandt *et al.*, 2021; Rodeghiero *et al.*, 2019). The organs in which gas exchange and excretion of end products of metabolism (lungs and kidneys), and the liver, as the leading metabolic organ (Dubova *et al.*, 2020; Levi, 2018), suffer the most. In addition, pathogens spend some time of their prepatent period in liver cells and destroy them (Bilić *et al.*, 2018; Vishwakarma & Nandini, 2019). Thus, the pathogenesis of babesiosis is characterized by the development of complex multifactorial hepatopathy, which is exacerbated by the wrong circle of the axis of complication of the disease – DIC blood (Dubova *et al.*, 2020).

The destruction of erythrocytes causes the release of free biologically active hemoglobin and other substances into the blood plasma, which stimulates the activity of vascular endothelium and platelets (Roopali *et al.*, 2018; Gupta *et al.*, 2020). Their adhesive and aggregation properties increase, the process of activation of vascular-platelet hemostasis is started – the first link in the coagulation cascade (Quach & Li, 2020; Rayes *et al.*, 2018). Because the pathogenic stimulus *Babesia spp.* continues, the coagulation process becomes avalanche-like, further enhancing the primary vascular-platelet link of hemostasis (Tyutyumova *et al.*, 2019). Thus, a kind of vicious circle of activation of extensive blood clotting is created. DIC syndrome occurs throughout the bloodstream, and thrombosis and insufficiency of tissue perfusion develop at the level of microcirculation (Dubova *et al.*, 2020; Saini & Dunn, 2019).

Inadequate tissue perfusion, the development of hypoxia and ischemia is reflected in the processes of cellular metabolism. Necrobiotic and necrotic changes occur in cells. This condition is a pathomorphological criterion of shock. The development of shock changes at the level of organs shifts the local metabolism in the direction of toxic and hypoxic processes, which increases thrombosis and blood clotting. Thus, the next generalised vicious circle of shock is created (Dubova *et al.*, 2020; Liu *et al.*, 2019).

Conditioned upon the abundant blood supply network of the liver, thrombosis and shock changes, which initially occur only in hepatocytes, later spread to the liver lobes and the whole body. This leads to functional

liver failure. Thus, the liver becomes unable to detoxify the body and perform its synthetic functions (Thakur *et al.*, 2018).

Thus, the study of the pathogenesis of complications of acute spontaneous babesiosis and their relationship with the development of functional and organic changes in the liver is necessary to understand the nature of hepatopathy and develop scientifically sound schemes of pathogenetic therapy of sick dogs.

MATERIALS AND METHODS

The research was conducted during 2020-2021 on the basis of the educational-research-production clinic of veterinary medicine of Polissya National University, Zhytomyr, Ukraine.

The research was conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles of Animal Experimentation (Kyiv, 2001) and the Law of Ukraine "On the Protection of Animals from Cruelty". The compliance of the conducted research with the principles of bioethics and protection of animals from cruel treatment during scientific work was confirmed by the commission on bioethical examination of Polissya National University (Minutes No. 1 of 01/10/2022).

40 dogs of different breeds, age 2-4 years, body weight 20-30 kg were studied. 2 groups were formed – control and experimental, 20 animals in each, on the principle of pairs of analogues. The experimental group – dogs with acute spontaneous babesiosis. Control group – clinically healthy animals (no clinical signs, no history of contact with ticks, hematological and biochemical laboratory parameters were within the established standards).

Clinical and microscopic studies. Clinical studies of animals were performed by general methods. The diagnosis of babesiosis was established by light microscopy of thin fixed blood smears stained by the method of Pappenheim on the device V-Chromer® III (West Medica, Austria). Microscopy was performed using a KERN OBE digital microscope (KERN, Germany). Generic identification of the pathogen and group size determination was conducted. Species identification of the pathogen has not been established. The intensity of parasitemia was determined by the percentage of affected erythrocytes.

Hematological, hemorheological, hemodynamic studies. Venous blood samples taken from the right *vena subcutanea antebrachii* were examined. For this group of studies used Vacumed tubes with anticoagulant EDTA-K3, the concentration of which is 1.8 mg/ml.

The hemoglobin content, the amount of formed blood cells, the erythrocyte sedimentation rate (ESR) and the hematocrit were determined using an automatic hematology analyzer RT-7600 (Rayto, China). Spontaneous aggregation capacity of erythrocytes (spEAC) and platelets (spPAC) was determined by shaking a blood sample in a laboratory shaker S-3 MICROMed (MICROMed®, Ukraine) according to N. Tarasova (Barkagan & Momot, 2008).

Circulating blood volume (CBV) was calculated by diluting T-1824 blue Evans. Deficiency of blood volume was calculated by hematocrit using Moore's formula (1):

$$BLV = CBV_{norm} \times \frac{(Ht_{norm} - Ht_{research})}{Ht_{norm}} \quad (1)$$

where BLV – blood loss volume, ml; CBV – circulating blood volume normal, ml; Ht_{norm} – hematocrit value normal, L/L; $Ht_{research}$ – hematocrit value of the animals' research group, L/L.

Investigation of the coagulation link of hemostasis.

This series of studies was performed using a two-channel coagulometer RP-2202C (Rayto, China). Coagulogram parameters were studied: time of spontaneous coagulation of Lee-White blood, silicone time of blood coagulation, prothrombin time (prothrombin time) (PT), activated partial thromboplastin time [activated partial thromboplastin time] (APTT), and also determined the content of fibrinogen (Raves *et al.*, 2018; Saini & Dunn, 2019).

Determination of markers of DIC syndrome. The concentration of soluble fibrin-monomer complexes (SFMC) was measured colorimetrically by a quantitative variant of the orthophenanthroline method.

Fibrinogen/fibrin degradation products (FDP) and D-dimer were determined by enzyme-linked immunosorbent assay (Slater *et al.*, 2019).

Biochemical research. For this group of studies, whole blood samples were taken from EximLab vacuum tubes with coagulation activator and demarcation gel. Obtained serum, which served as material for the study.

The studies were performed using a semi-automatic biochemical analyzer Chem 7 (Erba Mannheim, India).

The concentration of total and conjugated bilirubin (Malloy-Edeline method), the activity of the enzymes alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) (Reitman-Frenkel method), gamma-glutamyltranspeptidase (GHT) method (phosphate method) (Bodansky), lactate dehydrogenase (LDH) (Bergmeier method). The concentration of urea was determined by the Rashkovan method.

Statistical analysis. Statistical processing of the obtained data was carried out using the application package SPSS Statistics 23.0 (IBM Company). ANOVA analysis of variance was performed to compare the variance caused by group differences with the variance caused by intragroup variability. The reliability of the obtained data was evaluated by Fisher's F-test at a confidence level of $p < 0.05$.

RESULTS AND DISCUSSION

The following clinical signs were observed in dogs with acute spontaneous babesiosis: pyretic fever, mucosal anemia, loss of appetite, intoxication syndrome (nausea, vomiting, abdominal pain). The primary acute process of development of the pathogen in the body developed without jaundice. Hemoglobinuria was observed in the period from the 1st to the 3rd day of the patent period.

Later in 3 days and later jaundice of mucous membranes and skin developed. The general condition of the animals deteriorated, the phenomena of intoxication intensified. Vomiting in the early stages was characterized by mucous and foamy masses, then there were impurities of bile, which soon turned reddish-brown.

In the area of the right hypochondrium palpation of the liver increased by 1.5-2 times. Manipulations were accompanied by a significant pain response of the animal. Subsequently, the dog went into a stupor-soporose state and coma. In the icteric form of acute spontaneous babesiosis, according to clinical statistics, mortality was about 40%.

Blood tests of dogs with acute spontaneous babesiosis were performed at different stages of the disease, which were defined as anemic (early) and jaundiced (late) forms. It was found that the changes found in sick animals are dynamically deteriorating and in the jaundiced form is more severe than in the anemic. The intensity of parasitemia remained throughout the observation period at the level recorded in the initial examination (Table 1).

Table 1. Hematological parameters in acute spontaneous babesiosis of dogs, $M \pm m$

Indicator	Experimental group (n=20)		Control group (n=20)
	Anemic form	Jaundiced form	
Hemoglobin, g/l	98.6±4.2***	80.2±5.2***	128.9±6.3
Erythrocyte sedimentation rate, mm/h	18.3±3.3***	26.7±4.6***	4.4±0.18
Erythrocytes, T / l	3.4±0.6***	3±0.6***	6.8±0.36
Leukocytes, G/l	19.2±3.1***	13.6±2.1	10.2±0.7
Platelets, G/l	201±12.2***	164.4±10.8***	312±26.3
Intensity of parasitemia, %	13.4±1.8	11.2±1.2	–

Note: Difference between investigated and control groups is significant ($p < 0.001$)

Anemia and progressive erythrocytopenia were noted in both forms of the disease ($p < 0.001$). Anemia is

normochromic in nature, and therefore has a hemolytic origin. In the anemic form of babesiosis hyperleukocytosis

was observed ($p < 0.001$). In the jaundiced form, a tendency to return the number of leukocytes towards the reference values was observed. The number of platelets decreased dynamically in the progression of the disease ($p < 0.001$), which was synchronized with the increase in spontaneous aggregation ability of the formed elements ($p < 0.001$).

ESR increased during the development of babesiosis ($p < 0.001$), and the number of platelets progressively decreased. Against the background of spPAC growth (Table 2), this picture indicates the release of platelets into blood clots and the area of the microcirculatory bed – thrombocytopenia “consumption”.

Table 2. Hemodynamic and hemorheological parameters in acute spontaneous babesiosis of dogs, $M \pm m$

Indicator	Experimental group (n=20)		Control group (n=20)
	Anemic form	Jaundiced form	
Hematocrit, l/l	0.34±0.07	0.27±0.08*	0.45±0.04
CBV, ml	3386±57.2***	2943±66.5***	4508±89.6
Specific CBV, ml/kg	81.6±4.8***	76.2±6.3***	122.5±7.1
Deficiency of blood volume, ml %	1100.3±96.3*** 24	1526±88.6*** 34	–
spEAC, %	37.3±5.6***	44.8±9.4***	8.4±1.8
spPAC, %	43.8±5.5***	61.2±7.3***	15.5±2.4
The index of wetting the vascular wall	0.72±0.04***	0.91±0.04***	0.31±0.02

Note: difference between investigated and control groups is significant: * $p < 0.05$; *** $p < 0.001$

In terms of hemodynamic and hemorheological parameters, progressive changes were observed in all parameters. CBV, respectively, and the specific CBV in the anemic form is significantly reduced relative to the control group. In the jaundiced form of the disease, all indicators are significantly lower ($p < 0.001$) and much lower than the parameters of the anemic form.

SpPAC and spEAC were significantly increased throughout the course of babesiosis ($p < 0.001$). Thus, there was a dynamic increase in the intensity of the shock process. It was expressed in the blockade of microcirculation conditioned upon the high aggregation capacity of blood cells (in particular, platelets) (Tyutyumova et al., 2019; Ho-Tin-Noé et al., 2018), including the loss of circulating blood conditioned upon the release of liquid component in interstitial edema explained by increased vascular wall permeability (Levi, 2018; Saini & Dunn, 2019). The calculated CBV deficiency ($p < 0.001$) determined the development of shock in acute spontaneous babesiosis (Dubova et al., 2020; Liu et al., 2019). In the anemic form,

the shock was characterised as subcompensated to moderate, and in the jaundiced form – severe with a persistent tendency to decompensation (Dubova et al., 2020).

Indicators of coagulation hemostasis, established in different forms of acute babesiosis in dogs, confirmed the presence of DIC (Levi, 2018). Significantly altered and dynamically progressive index of vascular wall wetting indirectly is an indicator of electrophysical properties of the vascular wall and determines the state of the endothelium, which may be a strong accumulation of platelets and erythrocytes (Saini & Dunn, 2019). This figure increases with the growth of spPAC, spEAC.

In the anemic form of babesiosis in coagulation tests, which characterize the activity of blood coagulation by external (PT) and internal (APTT) mechanism (Roshal & Gil, 2019), there were divergent changes: reducing the role of external mechanism and increasing the role of internal (Table 3). This determined the second, transitional phase of DIC syndrome – consumption coagulopathy (Levi, 2018; Saini & Dunn, 2019).

Table 3. Indicators of the hemostasis system in acute spontaneous babesiosis of dogs, $M \pm m$

Indicator	Experimental group (n=20)		Control group (n=20)
	Anemic form	Jaundiced form	
PT, s	22.7±0.3	32.4±0.6***	20.8±0.6
APTT, s	38.3±2.4	52.2±7.2	44±2.9
Fibrinogen, g/l	1.34±0.3***	0.83±0.13***	2.45±0.27
FDP, g/l	0.28±0.022***	0.32±0.043***	0.075±0.002
D-dimer, µg/l	0.42±0.08***	0.66±0.07***	0.15±0.02
SFMC, g/l	0.25±0.017***	0.51±0.03***	0.03±0.005

Note: difference between investigated and control groups is significant ($p < 0.001$)

In the jaundiced form of the disease in both indicators there was a tendency to hypocoagulation, which characterised the beginning of the third phase of the syndrome – hypocoagulation and fibrinolysis.

Criteria for the diagnosis of DIC are the appearance of the following markers: hypofibrinogenemia ($p < 0.001$), increase in FDP ($p < 0.001$), including D-dimer ($p < 0.001$), and SFMC ($p < 0.001$). Fibrinogen is blocked as a result of its “consumption” in blood clots. Conditioned upon fibrinogen deficiency and “consumption” of other coagulation factors, full-fledged fibrin cannot be formed.

Improper fibrinogen fermentation leads to increased levels of FDP, in particular D-dimer, and SFMC. Such markers are pathognomonic for DIC (Levi, 2018; Saini & Dunn, 2019). In the anemic form, these markers were reliable; in the jaundiced form, their numerical expression increased to the maximum. Thus, the degree of power of the DIC syndrome increased, the process tended to be irreversible.

Functional changes that occurred in the liver of sick dogs were clearly characterised by specific biochemical parameters of the “liver profile” (Table 4).

Table 4. Biochemical parameters of the liver profile in acute spontaneous babesiosis of dogs, $M \pm m$

Indicator	Experimental group (n=20)		Control group (n=20)
	Anemic form	Jaundiced form	
Total bilirubin, $\mu\text{mol/l}$	10.2 $\pm$ 1.2***	26.7 $\pm$ 4.2***	2.96 $\pm$ 0.7
Conjugated bilirubin, $\mu\text{mol/l}$	2.8 $\pm$ 0.4***	15.2 $\pm$ 3.7***	0.01 $\pm$ 0.002
AST, od / l	102 $\pm$ 6.4***	139.2 $\pm$ 7.4***	20.6 $\pm$ 1.8
ALAT, od/l	122.4 $\pm$ 8.4***	156.7 $\pm$ 9.2***	17.6 $\pm$ 1.7
GGT, od/l	28.5 $\pm$ 2.6***	74.8 $\pm$ 6.1***	5.2 $\pm$ 0.7
LDH, od/l	382.7 $\pm$ 3.1***	500.6 $\pm$ 40.6***	120.4 $\pm$ 11.3
LF, od/l	170.4 $\pm$ 13.8**	208 $\pm$ 11.2***	125 $\pm$ 8.3
Urea, mmol/l	15.3 $\pm$ 2.7***	26.3 $\pm$ 4.1***	4.9 $\pm$ 1.2

Note: difference between investigated and control groups is significant: ** $p < 0.01$; *** $p < 0.001$

In the anemic form of the disease there was a significant increase in the level of total and conjugated bilirubin ($p < 0.001$), hyperfermentation of transferases ($p < 0.001$), GGT, LDH ($p < 0.001$), LF ($p < 0.01$), uremia ($p < 0.001$). Thus, during the anemic form, functional disorders of the liver are pronounced and caused by the processes of destruction of hepatocytes. However, clinical signs and laboratory parameters indicated the development of subacute hepatitis. In this form of babesiosis, the functional state of the liver was within the reversibility of processes. Hyperactivity of indicator enzymes indicated the onset of insufficiency of bile production and bile excretion, and the tendency to develop toxic hepatitis.

In the jaundiced form of babesiosis, all indicators of the functional state of the liver significantly increased even in comparison with the anemic form. Increased activity of transaminases (7 and 9 times), GGT (14 times), LDH (4 times), LF (1.7 times), and hyperbilirubinemia (9 times), including an increase in the concentration of conjugated bilirubin (1.5 thousand times) indicated the course of reactive hepatitis of toxic nature, cholestasis, massive destruction of hepatocytes (Thakur *et al.*, 2018). Oversaturation of blood with bile pigments exacerbated intoxication and further stimulated the power of pathogenic secondary complications, such as shock and DIC (Dubova *et al.*, 2020; Liu *et al.*, 2019). Jaundice was hemolytic-parenchymal in nature (Levi, 2018).

The increase in urea levels determined the state of uremia, which aggravated the general intoxication

syndrome. At this stage, the liver was no longer able to perform detoxification functions, in particular, to dispose of hazardous and toxic to the animal residual nitrogen products (Ortiz *et al.*, 2020; Thakur *et al.*, 2018).

Liver damage in dogs with acute spontaneous babesiosis is primarily due to exposure *Babesia spp.*, which, according to the ideas accepted today in science, spends a certain period of its development in this body (Bilić *et al.*, 2018; Vishwakarma & Nandini, 2019). In addition, conditioned upon its functions, the liver becomes the center of pathogenetic events during the development of the disease (Vishwakarma & Nandini, 2019).

Summarising the results of our own research, we can note that the course of the pathological process of babesiosis in sick dogs becomes more powerful and is characterised first by anemic and then jaundiced form. One of the leading roles in the emergence of these forms is played by impaired liver function conditioned upon the development of severe processes with a persistent tendency to decompensation and irreversibility (Liu *et al.*, 2019; Saini & Dunn, 2019). The initial link of involvement in the pathogenic process of the vital organs of the body, including the liver, is the activation of the vascular-platelet link of hemostasis by the products of *Babesia spp.*, immune complexes and consequences of erythrocyte destruction (Dubova *et al.*, 2020; Ho-Tin-Noé *et al.*, 2018).

According to the results of the obtained hematological parameters, the pathogenetic mechanism of

changes can be expressed as follows. The systemic inflammatory response of the whole organism is reflected by leukocytosis as the patent period of babesiosis progresses (Ortiz *et al.*, 2020; Tyutyumova *et al.*, 2019). The increase in ESR also confirms the redistribution of plasma proteins in favor of the globulin fraction, which once again indicates the development of a systemic inflammatory process (Rayes *et al.*, 2018). Decreased white blood cell counts in the jaundice stage of the disease may indicate that the body develops cytopenia explained by hyperstimulation of the spleen and the occurrence of hypersplenism (Levi, 2018; Saini & Dunn, 2019).

Anemia with erythrocytopenia develops conditioned upon the activity of the pathogen and is the basis of the pathogenesis of the disease. Destruction of erythrocytes with the release of hemoglobin into the blood plasma causes insufficient oxygen supply to organs and tissues, causing metabolic disorders and the development of hypoxia (Wesley *et al.*, 2021; Rodeghiero *et al.*, 2019).

The main arena of pathological processes is the microcirculatory bed of organs, which first suffers from thrombosis conditioned upon increased adhesive-aggregation properties of platelets and then erythrocytes (Dubova *et al.*, 2020; Levi, 2018). Given the abundant network of hepatic capillaries, thrombosis in the microcirculatory tract becomes the morphological equivalent of shock at the organ level (Liu *et al.*, 2019). At this time, the process of blood coagulation, activated by external and internal mechanisms, is intensifying (Levi, 2018; Saini & Dunn, 2019). DIC syndrome occurs. According to the pathogenesis of severe complications, such as DIC and shock, these processes cause and reinforce each other, forming vicious circles at different levels of the body (Dubova *et al.*, 2020; Liu *et al.*, 2019).

The release of platelets into blood clots determines “consumption thrombocytopenia” – one of the leading factors in the development of DIC syndrome (Levi, 2018). Circulatory platelet deficiency damages the vascular endothelium explained by the lack of vascular wall nutrition factor (Gupta *et al.*, 2020; Ho-Tin-Noé *et al.*, 2018). Loss of vascular elasticity enhances the adhesion-aggregation capacity of platelets, causing the above-mentioned vicious circle of the process. The release of the liquid component of the blood into the surrounding interstitial space leads to a decrease in CBV, resulting in a lack of tissue perfusion, as well as efficient heart function. Hypoxia is exacerbated (Liu *et al.*, 2019).

Hepatocytes due to hypoxia lose their metabolic functions. Enzymes capable of transporting bilirubin and utilising endotoxins are gradually being lost. Intoxication of the body is increasing, which in turn increases the intensity of DIC and shock syndrome (Levi, 2018; Saini & Dunn, 2019).

Conditioned upon the sinusoidal structure of the

liver lobes, the body is able to resist vasoconstrictor effects, the formation of sludge and pooling as the main morphological criteria for shock, and thrombotic manifestations in the development of DIC (Liu *et al.*, 2019). However, if the etiological factor does not stop its action (babesia continues to multiply in the body), there is a depletion of the enzymatic capacity of the liver. There are organic changes, which are manifested in the death of hepatocytes with the formation of scars. The organ becomes a “shock liver”, developing acute liver failure, which often ends in death (Levi, 2018; Liu *et al.*, 2019).

Thus, hepatopathies in acute spontaneous babesiosis of dogs occur as a result of secondary complications – DIC and shock. Complications develop as a universal response to the activity of *Babesia spp.*, and their pathogenetic mechanisms cause hemodynamic and hemorheological disorders in the hepatic vascular bed. As a result of reduced tissue perfusion of the organ, reduction of CBV and the development of hypoxia, there is a violation of the functional activity of the liver, distortion of enzymatic processes, which increases the intensity of complications. This is how the vicious circle “shock-syndrome DIC-liver failure” is formed, in which there is a mutual strengthening and interdependence of these pathological processes. Hepatopathy in the anemic form of canine babesiosis manifests itself as subacute hepatitis and functional liver failure. In the jaundiced form – as acute reactive toxic hepatitis and severe acute liver failure, which is prognostically life-threatening for animals.

CONCLUSIONS

In the process of acute spontaneous babesiosis of dogs, hepatopathy develops, which is registered in the anemic form of the disease, and then progresses, causing a jaundiced form of the disease. Complications such as DIC and shock occur during babesiosis. In the anemic form, the shock is subcompensated, has a moderate degree, and DIC syndrome is in the stage of consumption coagulopathy. In the jaundiced form of severe shock acquires a steady tendency to irreversibility. DIC syndrome enters the stage of hypocoagulation/fibrinolysis.

Hepatopathy in the anemic form of the disease is in the form of subacute hepatitis and functional liver failure. In the jaundiced form develops acute reactive hepatitis of toxic nature, cholestasis, acute liver failure.

DIC syndrome, shock, hepatopathy are closely related and are involved in the vicious circles of increasing intensity of each other. The factor of activation of false circles is the DIC syndrome, which develops in response to the pathogenic influence of the pathogen.

Thrombotic lesions of the microcirculatory bed of the liver are a trigger for the development of shock and hepatocyte damage. In turn, pathological processes in the liver exacerbate the state of shock and DIC syndrome, closing the vicious circle.

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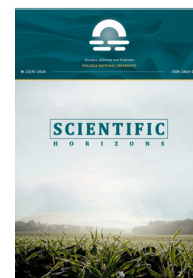
Особливості гепатопатії та гематологічних ускладнень за гострого спонтанного бабезіозу собак

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Анотація. Спонтанний бабезіоз собак належить до найбільш поширених і небезпечних захворювань. Ураження еритроцитів внаслідок впливу збудників є пусковим фактором гематологічних ускладнень і ураження мікроциркуляторного русла. Розвиток тромботичних процесів веде до незворотних змін та подальшої загибелі організму. Печінка є одним з перших органів, які потерпають від пошкоджень. Мета досліджень полягала у виявленні зв'язків патогенезу гепатопатії та гематологічних розладів за перебігу гострого спонтанного бабезіозу собак. Для її досягнення сформовано дві групи собак по 20 особин: дослідна група – тварини з бабезіозом, контрольна – клінічно здорові. Встановлено, що гострий спонтанний бабезіоз перебігає у послідовних формах – анемічній і жовтяничній. Виявлені анемія нормохромного типу, лейкоцитоз, тромбоцитопенія. Гемореологічні параметри вказують на посилену агрегацію еритроцитів і тромбоцитів, що зумовило тромботичний стан. Встановлено критерій шоку – зниження об'єму циркулюючої крові; його дефіцит склав 24 % за анемічної форми та 34 % за жовтяничної. Також визначені маркери синдрому дисемінованого внутрішньосудинного згортання (ДВЗ) крові – гіпофібриногенемія, збільшення рівня розчинних фібрин-мономерних комплексів, продуктів деградації фібрину, в тому числі Д-дімеру. Зміни показників наростали у міру переходу захворювання з анемічної у жовтяничну форму. За анемічної форми шок визначений як субкомпенсований середньої важкості, а ДВЗ-синдром – у стадії коагулопатії споживання. Ускладнення характеризують перехідний стан, який перебуває у нестійкій рівновазі. За жовтяничної форми шок визначений як декомпенсований важкий, а ДВЗ-синдром – у стадії гіпокоагуляції. Такий стан характеризується як критичний, з вираженою тенденцією до незворотності. Зміни показників печінки вказують на гіперактивність усіх індикаторних ферментів, гіпербілірубінемію та гіперуремію. З розвитком хвороби показники значно збільшуються. Синдром ДВЗ, шок і гепатопатія вступають у хибне коло, за якого гематологічні ускладнення викликають гепатопатію, а вона, зі свого боку, їх посилює. Гепатопатія за бабезіозу собак вважається важким станом, що вимагає проведення інтенсивної невідкладної терапії

Ключові слова: шок, печінка, синдром ДВЗ, гемореологія, гемодинаміка, печінковий профіль



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Treatment of Subclinical Mastitis of Cows with Probiotics

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Abstract. A large number of dairy cows in Ukrainian farms suffer from subclinical mastitis, which leads to significant economic losses in agriculture. Conditioned upon the lack of clinical manifestations it is difficult to detect, in particular, explained by insufficient information about the microbial composition of milk. The ban on the use of antibiotics for productive animals is forcing new safe and effective remedies. The aim of the study was to determine the therapeutic effect of *Bacillus megaterium* NCH 55 in subclinical mastitis of Holstein cows. Research materials – milk of cows with subclinical mastitis, isolates of microorganisms and *B. megaterium* NCH 55. Methods used: California test for mastitis; microscopic test to count the total number of somatic cells by the method of Prescott and Britt; bacterial method for the study of microorganisms; polymerase chain reaction to determine *Mycoplasma* spp. in milk; spectrophotometry; method VI Brillis to determine the adhesive properties of *Bacillus megaterium* NCH 55; determination of antagonistic properties of *B. megaterium* by diffusion into agar wells; the method of flow cytometry using the device “SomaCount Flow Cytometer”; physiological. The experiment was conducted in dairy farms of the North-Eastern region of Ukraine: LLC agrofirma “Lan”, LLC agrofirma “Vorozhbalatinvest”, LLC agrofirma “Vladana” in the period February-August 2021. Isolates of *S. aureus*, *S. agalactiae*, *E. coli* enterohemorrhagic, *E. coli*, *Candida*, *E. fecalis*, *S. epidermidis* and *Mycoplasma* spp. were detected in milk samples from cows with subclinical mastitis. Microscopic studies have shown that *Bacillus megaterium* NCH 55 are white gram-positive rods that have low adhesive properties and form spores. The greatest antagonism of *B. megaterium* is shown in relation to bacterial isolates in concentration of 1×10^9 , CFU/g. In 70% of cows that reached a productivity of more than 30 kg/day on the 30th day of research, milk parameters such as the number of somatic cells (CSC ≤ 400 thousand/cm³) and the number of mesophilic aerobic and facultative anaerobic microorganisms (kMAFANM) (≤ 100 thousand CFU/cm³) corresponded to the class “Extra”. The recovery time of animals with subclinical mastitis depended on the degree of damage to the breast and individual characteristics of the organism. Cows that did not reach a productivity of 30 kg/day continued treatment individually. The number of somatic cells in the milk of cows was ≤ 500 thousand/cm³ and kMAFANM ≤ 200 thousand CF/cm³.

Keywords: inflammation of the breast, *Bacillus megaterium*, mastitis pathogens, somatic cells, milk productivity



INTRODUCTION

Mastitis is a common and economically important disease of dairy cows and has a clinical or subclinical nature (Olde Riekerink *et al.*, 2008). Clinical mastitis leads to visible changes in milk and is easily diagnosed during a routine clinical examination (Sepúlveda-Varas *et al.*, 2016). Subclinical mastitis is not detected during a routine clinical examination, but is identified by identifying existing biomarkers of inflammation or causative agents of mastitis in glandular secretions (Sinha *et al.*, 2014). Mastitis can also be caused by mechanical damage to the udder during milking. Tire rubber loses strength, shape and elasticity during long-term technological operation and exposure to disinfectants (Paliy *et al.*, 2021a).

A. Paliy *et al.* (2021b) sought to improve milking equipment to reduce the risk of injury to cows. Reconstruction of milking equipment managed to increase milk hopes by 1.1 times, which allowed to gain an additional 132.5 kg. In addition, the fat content of milk increased, and the number of microorganisms decreased 2.2 times and mechanical particles – 4.6 times.

Epithelial cell models are used to study the level of mammary gland damage. In Ukraine, a culture for *in vitro* research has been created from the epithelial cells of Holstein cow exchange (Xu *et al.*, 2021).

Pathogens of mastitis can be transmitted from sick cows to subclinical mastitis in healthy animals, which is a cause for concern. Bacteriological culture of milk is the gold standard in the detection of subclinical mastitis (Anderson *et al.*, 1991). Unfortunately, the results of such a study are not available for at least 24-48 hours. This means that bacterial pathogens can spread among dairy cows before the results of the sowing are known. Testing cows for mastitis using the California test is constantly used in farms, because this express method takes about 5 minutes and is performed in a production environment (Barth, 2008). The degree of inflammation of the udder depends on the pathogen, the activity of its influence and the resistance of the cow's body.

S. Manyi-Loh *et al.* (2018) investigated that antibiotic resistance of bacteria associated with animal diseases can be pathogenic to humans, easily transmitted through food chains and spread in the environment through animal waste. They can cause complex, incurable and long-lasting infections in humans, leading to increased medical costs and sometimes death. Antibiotic resistance is so complex and difficult due to the irrational use of antibiotics in both clinical and agricultural conditions, low socioeconomic status, poor sanitation and hygiene, and irregular cultivation of zoonotic pathogens that are resistant to almost unused antibiotics. Manyi-Loh *et al.*, 2018).

Currently, a growing number of drug-resistant strains of bacterial pathogens are disrupting the effectiveness of existing treatments and increasing the incidence of new bacterial infections. These circumstances prompted researchers Ye. Karpun, V. Parchenko, T. Fotina,

D. Demianenko, A. Fotin, V. Nahorny, N. Nahorna (2021) to the search for new effective and at the same time low-toxic drugs that have innovative mechanisms of action. It was determined that triazole-3-thiols, which can be used as an alternative to antimicrobial agents, have bactericidal action against *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella pullorum*, *Salmonella typhimurium*, *Salmonella enteritidis*, *Escherichia vulgaris coli O 2 Clostridium perfringens* (Karpun *et al.*, 2021).

Researchers have also shown the effectiveness of destroying *Escherichia coli* (Zhao *et al.*, 2021) and *Salmonella enterica* (Wang *et al.*, 2019) using the antimicrobial peptide mastoparan. An option to solve the problem of antibiotic replacement may be a biological method of antagonism. The practice of using bacteriophages and probiotic microorganisms is known in veterinary medicine (Shively *et al.*, 2018).

Antibiotic treatment, even short-term (De Vlieghe *et al.*, 2012) is an effective measure for the prevention of mastitis for pregnant heifers. However, there is a negative impact on milk productivity. Also, the use of complex therapy based on antibiotics, vaccination and nanoparticles (Gomes & Henriques, 2016) had a significant effect in the treatment of mastitis. However, the widespread use of antibiotics leads to the formation of antibiotic-resistant biofilms in mastitis and reduced response to treatment (Babra *et al.*, 2013). In addition, most of the vaccines used in mastitis in cows have not shown a high level of protection and are very expensive from an economic standpoint (Côté-Gravel & Malouin, 2019).

An experiment on buffalo in patients with subclinical mastitis has shown that treatment of *Lactobacillus rhamnosus* in vivo has an anti-inflammatory effect, reducing the number of somatic cells and suggests new research in this area (Catozzi *et al.*, 2019). J. Gao *et al.* (2020) proved that oral intake of yeast and lactic acid bacteria reduced the content of somatic cells and improved the quality of cow's milk. Therefore, studies in this direction on the use of probiotic strains of microorganisms for the treatment of subclinical mastitis in cows are promising.

The aim of the study was to study the microbiological composition of Holstein cow's milk in subclinical mastitis and to determine the therapeutic effect of *Bacillus megaterium* NCH 55. The objectives of the study were: study of cows for subclinical mastitis, determination of the properties of *Bacillus megaterium* NCH 55, study of the therapeutic effect of use in cows with subclinical mastitis *Bacillus megaterium* NCH 55.

MATERIALS AND METHODS

The research was conducted in dairy farms of the North-Eastern region of Ukraine (Lan Agricultural Company LLC, Vorozhbalatinvest Agricultural Company LLC, Vladana Agricultural Company LLC) on Holstein cows in February-August 2021 in accordance with Directive 2010/63/EU (Hartung, 2010), which were approved by

the conclusion of the Commission on Ethics and Bioethics of the Faculty of Veterinary Medicine of Sumy National Agrarian University from 12/02/2021.

Study of cows for subclinical mastitis. A total of 50 cows were involved in the study. Milk samples were obtained from each quarter of the shift separately during maximum lactation in a separate sterile cup in the amount of 50 ml. The test for mastitis using the California test was performed on site in Petri dishes. Milk was mixed with the reagent, if a clot was obtained at the bottom of the cup, subclinical mastitis was diagnosed (Bhutto *et al.*, 2012). Milk smears were made in the laboratory. Dried at a temperature of 18–20°C and stained according to Romanowski-Gimza. Microscopic testing to count the total number of somatic cells was performed by the method of Prescott and Britt (Prescott & Breed, 1910) and to determine their species composition.

To study the microflora in cow's milk, the bacterial method was used and the composition of microorganisms and their number were determined. As an elective medium for intestinal bacteria: *Salmonella* and *Escherichia* used Endo agar on Petri dishes; presence and quantity of *Staphylococcus aureus* – on Chistovich's yellow-salt agar, presence and quantity of molds and yeasts – on Saburo nutrient agar. The presence of *Mycoplasma spp.* in milk was determined by polymerase chain reaction. The count of microorganisms was performed after cultivation on elective media, determined the number of colony-forming units in CFU/cm³ in accordance with DSTU 7357: 2013 "Milk and dairy products. Methods of microbiological control" (DSTU 7357:2013).

Determination of the properties of *Bacillus megaterium* NCH 55. Cultivation of *Bacillus megaterium* NCH 55 was performed on meat-peptone agar for 72 hours at 37°C. The color and shape of the colonies were determined macroscopically. CFU/g every 6 hours was determined in the meat-ash broth. The activity of cellulose and amylase enzymes for colonies of *Bacillus megaterium* NCH 55 was determined by mixing 0.5 ml of enzyme with 1 ml of 0.05 M citrate-phosphate buffer pH 4.8. This was followed by incubation at 50°C for 1 hour. The reaction was stopped by adding 3 ml of 3,5-dinitrosalicylic acid. The solution was then heated to 100°C for 5 minutes. After cooling, it was centrifuged at 3000 rpm for 15 minutes. The study was performed using a spectrophotometer at a wavelength of 575 nm (Andriani, 2015).

The adhesive properties of *Bacillus megaterium*

NCH 55 were investigated by the method of VI Brillis. Mean adhesion index (MAI), erythrocyte participation coefficient (EPC) and erythrocyte adhesion index (EAI) were determined. The calculation of these indicators was carried out according to formula (1):

$$EAI = MAI * 100 / EPC \quad (1)$$

Based on what we get MAI=1.70±0.09; EPC=84.25±2.53; EAI=2.00±0.11. Bacteria do not show adhesive properties at EAI≤1.77; IAM from 1.77 to 2.49 – low-adhesive, from 2.51 to 4.0 – medium-adhesive and >4.0 high-adhesive (Brillis *et al.*, 1986).

Determination of antagonistic properties of *B. megaterium*. The study was performed by diffusion into agar wells. The size of the growth retardation zone was determined by macroscopic method using a ruler in mm around different dilutions of *Bacillus megaterium* NCH 55 cultures: 1×10⁵, CFU/g; 1×10⁷, CFU/g and 1×10⁹, CFU/g (Garkavenko *et al.*, 2021). An appropriate concentration of probiotic strain of the microorganism *Bacillus megaterium* NCH 55 was poured into each well on the IPA with the corresponding isolate of the mastitis pathogen, incubation was performed for 24 hours at a temperature of 37°C. The demarcation zone around each well with a different degree of dilution of *Bacillus megaterium* was then determined.

Study of the therapeutic effect of use in cows with subclinical mastitis *Bacillus megaterium* NCH 55. The California test was used to detect cows with subclinical mastitis. A total of 50 heads were studied, of which 10 were patients and participated in a further experiment. The research period lasted from February to August 2021. The cows were of different ages and lactations, to prevent the study from being tied to these indicators. After determining the antagonistic properties of *Bacillus megaterium*, an effective concentration of 1×10⁹, CFU/g was established. Ten sick cows were given for 30 days together with concentrated feed *Bacillus megaterium* NCH 55 in the form of powder (spores) at a concentration of 1×10⁹, CFU/g 35 g/animal per day. During the six months of the experimental period, the clinical condition of the animals, productivity and signs of mastitis were evaluated. The number of somatic cells in milk was determined once a week using the SomaCount Flow Cytometer (flow cytometry method). DSTU 3662: 2018 (DSTU 3662:2018) was used in the research. Milk was considered high-quality if it met the requirements of the "Extra" class (Table 1).

Table 1. Criteria for raw milk in accordance with DSTU 3662:2018 Raw cow's milk. Specifications

Name of indicator, unit of measurement	Norm for varieties		
	Extra	Higher	First
Number of mesophilic aerobic and facultative anaerobic microorganisms (KMAFANM), thousand CFU/cm ³	≤100	≤300	≤500
The number of somatic cells, thousand/cm ³	≤400	≤400	≤500

Statistical analysis. Microsoft Excel 2010 was used to process the obtained data, as well as statistical analysis using the Fischer-Student method, taking into account statistical errors and probabilities of more than 95% ($p < 0.05$).

RESULTS AND DISCUSSION

The results of a study of cows for subclinical mastitis

The study began with the identification of cows with subclinical mastitis in the herd. Causes of mastitis in cows can be microorganisms and non-infectious factors. Violations of milking technology, metabolic diseases, udder injuries, postpartum stress give reason to consider mastitis a multifactorial disease (Holko *et al.*, 2019). In January 2021, the herd was monitored for subclinical

mastitis. The study involved 50 heads. 10 of them had subclinical mastitis, three of them were diagnosed with subclinical ketosis and delayed manure with subsequent development of endometritis. This whole set of symptoms suggests a polyetiological mechanism of mastitis in cows. Cows for subclinical mastitis were examined using the California test and smear microscopy (Figs. 1, 2).

It is known that the number of somatic cells during lactation should be within 400 thousand/cm³, according to DSTU 3662:2018 (2018). Somatic cells divide into lymphocytes, neutrophils and monocytes, which indicate the level of inflammation of the breast (Fig. 1, 2). Figure 1 shows the microscopy of the milk of a healthy cow. We observe a small number of epithelial cells, segmental neutrophils, single basophils and monocytes.

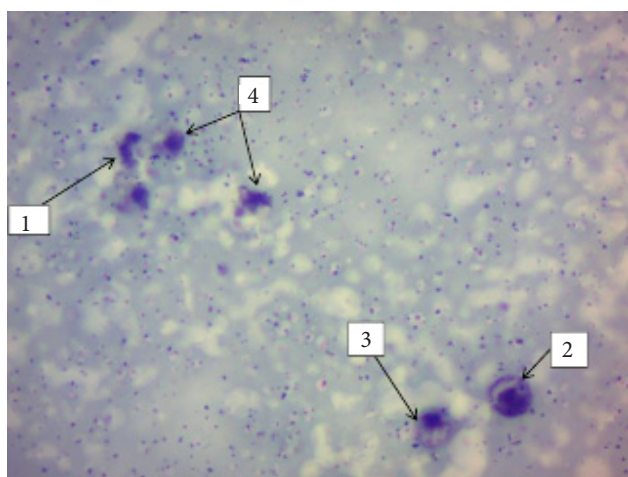


Figure 1. Light microscopy of healthy cow's milk (magnification $\times 400$) in the field of view:

- 1 – segmental neutrophil; 2 – monocyte; 3 – basophil; 4 – not differentiated (epithelial) somatic cells

Figure 2 shows a mass accumulation in the field of view of the microscope of epithelial cells, and a significant number of lymphocytes, basophils, segmental neutrophils and monocytes, which is characteristic of subclinical mastitis. Inflammation of the breast leads to a cellular reaction in which the number of somatic cells increases tenfold or more. Researchers have found that the amount of neutrophils in cow's milk can change constantly. Somatic cells (SC) are mainly represented by epithelial cells (60%) and a small number of granulocytes and lymphocytes (Shkromada *et al.*, 2019a). A similar trend was registered when determining the content of SC subpopulations in goat's milk. When the SC in milk increases to 2 million cells/ml, the number of neutrophils, macrophages and eosinophils increases (Fotina *et al.*, 2018). Therefore, the species composition of somatic cells may

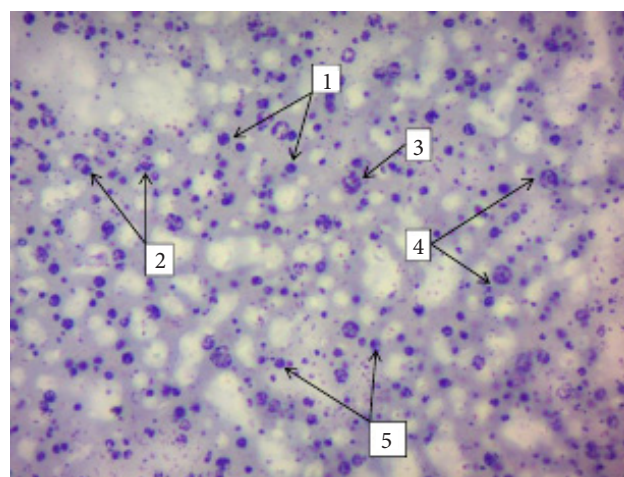


Figure 2. Light microscopy of cow's milk, with subclinical mastitis (magnification $\times 400$). Mass accumulation of somatic cells: 1 – lymphocyte; 2 – basophil; 3 – segmental neutrophil; 4 – monocyte; 5 – not differentiated (epithelial) somatic cells

vary depending on the degree of inflammation of the breast.

Without the provision of therapeutic care, undetected subclinical mastitis can progress to clinical. In addition, in subclinical mastitis, in addition to SC, microorganisms accumulate in milk. Calves develop diarrhea and rennet inflammation when drinking milk from sick cows. Even in the absence of pathogens in milk, an increase in the number of mesophilic aerobic and facultative anaerobic microorganisms (kMAFANM) more than 100 thousand. CFU/cm³ is an indicator of the safety of milk for consumption (DSTU 3662:2018).

Therefore, to determine the safety of milk, studies were conducted in dairy farms of Sumy region in order to identify the main pathogens that are one of the causes of mastitis in cows (Table 2).

Table 2. The results of a study of cow's milk for total bacterial contamination

Number of tested milk samples, pcs.	Characteristic	% of the total number of test samples of milk
53	<i>S.aureus</i>	22
56	<i>S. agalactiae</i>	15
40	<i>E. coli</i> enterohemorrhagic	12
37	<i>E. coli</i>	10
36	<i>Candida</i>	10
42	<i>E. fecalis</i>	9
39	<i>S. epidermidis</i>	8
51	<i>Mycoplasma spp.</i>	7

The obtained results indicate a significant number of staphylococci in milk, which are usually excreted in the microbial environment of livestock facilities. Researchers have shown that staphylococcus was the third most common microorganism and was positively correlated with subclinical mastitis during dry cows. Staphylococcus also has maximum resistance to ampicillin compared to other microorganisms (da Silva Duarte *et al.*, 2020). A similar level of resistance (44%) was recorded for *S. aureus* (n=50) isolated from cattle and pigs slaughtered in South African slaughterhouses (Tanih *et al.*, 2015). Studies in black-spotted cows have shown that *Staphylococcus aureus* was the cause of subclinical mastitis in 67-73% and *Streptococcus agalactiae* in 20% of all cases. It was found that by transmitting the causative agent of mastitis was rubber milking cups (Shkromada *et al.*, 2019b).

Therefore, the presence of only *S. aureus* and *S. agalactiae* in milk is sufficient for a cellular reaction in the form of mastitis. For dairy cows, subclinical mastitis is a major challenge.

Traditionally, antibiotics are used in farms to treat subclinical mastitis. Studies conducted by J. Burović (2020) in the Zenica region found the highest antimicrobial activity against mastitis pathogens to benzylpenicillin (56.3%) and oxytetracycline (46.2%). Florfenicol, cefoperazone, cephalexin and ceftiofur were also effective, but the microorganisms showed high resistance to trimethoprim-sulfamethoxazole, norfloxacin and tetracycline (Ribeiro *et al.*, 2015). However, an important drawback in the use of antibiotics is the ability to stay in milk for a long time and cause adverse effects in the consumer and antibiotic resistance (Kurjogi *et al.*, 2019). Unfortunately,

for a long time the milk has to be disposed of during the entire treatment period and after the last day from 14 to 28 days, depending on the pharmacokinetics of the drug. Therefore, the treatment strategy for non-severe forms of inflammation is better based on alternative methods.

The beginning of the inflammatory process in the urine was evidenced by the absence of characteristic symptoms of clinical mastitis. In most cases, the diagnosis of subclinical mastitis was established through regular testing and somatic cell count. This is especially important in those farms whose profitability depends on the quality of milk (grade). Mostly such farms produce milk of the "Extra" class, which has a number of requirements in accordance with the technical conditions of DSTU 3662:2018. Conditioned upon the fact that milk is sold collectively, some of the products from cows with subclinical mastitis can usually get to the total volume. However, when there is a large percentage of cows with subclinical mastitis on the farm, it is no longer possible to hide it. In addition to the number of somatic cells (NSC) in milk, the total number of microorganisms is counted. Of course, the requirements for products that are sold for consumer consumption should be as strict as possible. This encourages manufacturers to control the quality of products, namely the timely detection, treatment and prevention of mastitis.

The results of the study of the properties of *Bacillus megaterium* NCH 55

Microscopic examinations revealed that *Bacillus megaterium* NCH 55 had the appearance of smooth, slightly curved rods. They are gram-positive and able to form endospores (Fig. 3).

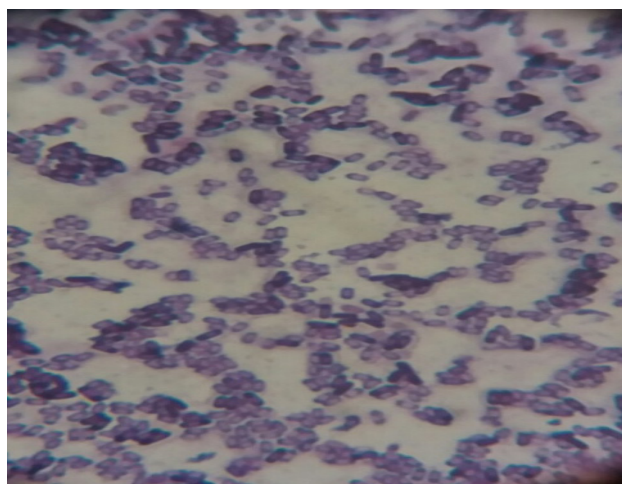


Figure 3. Light microscopy of *B. megaterium* (magnification $\times 4000$)

Spores can withstand temperatures up to $+80^{\circ}\text{C}$. Animals are given spores of the microorganism, which do not require special storage conditions. They mix well with food and easily get to the rumen without losing their properties. A study of the properties of *Bacillus megaterium* NCH 55 found that the bacterium uses lactose

and glucose as carbon sources. It has also been found that the bacterium does not produce indole, but is able to form urea. In addition, *Bacillus megaterium* NCH 55 produces the enzymes maltose, cellulase and amylase (Andriani *et al.*, 2017) (Table 2).

Table 2. Biochemical properties of *Bacillus megaterium* NCH 55

Indicators	<i>B. megaterium</i>
Glucose	+
Lactose	+
Mannitol	+
Maltose	+
Cellulase	+
Amylase	+
Indole	–
Urea	+
SPA	1.70±0.09
KUE	84.25±2.53
IAM	2.00±0.11

Source: (Brilis et al., 1986)

In the study of the properties of *Bacillus megaterium* NCH 55 (Fig. 4) it was found that the adhesion index of erythrocytes (IAM) was 2.00±0.11, which according to the Brilis classification (1986) is considered an indicator of low adhesion. The average adhesion (SPA) was 1.70±0.09 and the coefficient of participation of erythrocytes in the

adhesion process (CUE) was 84.25±2.53. The low adhesion of *Bacillus megaterium* NCH 55 is due to the inhibitory effect of the bacteriocin – megacin produced by it. It is a highly specific antimicrobial protein against a wide range of gram-negative bacteria, yeast, fungi and gram-positive microorganisms (Abriouel et al., 2011).

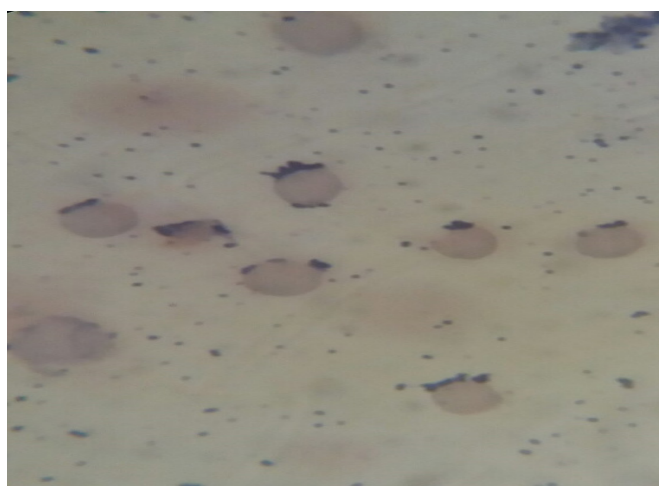


Figure 4. Light microscopy to determine the adhesive properties of *B. megaterium* on erythrocytes of cows (magnification × 1000)

The results of determining the antagonistic properties of *B. megaterium* NCH 55

Antagonistic properties of the probiotic strain of *Bacillus*

megaterium were determined in relation to microorganisms-isolates from cows with subclinical mastitis (Table 3).

Table 3. The results of determining the antagonistic properties of *B. megaterium* NCH 55, ($M \pm m$), $n=7$

Cultures of microorganisms isolated from cow's milk in subclinical mastitis	1×10 ⁵ , CFU/g <i>B. megaterium</i> NCH 55	Breeding culture	1×10 ⁹ , CFU/g <i>B. megaterium</i> NCH 55
		1×10 ⁷ , CFU/g <i>B. megaterium</i> NCH 55 Growth retardation zone, mm	
<i>S. agalactiae</i>	2.25±0.03	4.15±0.06	27.45±0.15*
<i>S. aureus</i>	2.53±0.07	6.20±0.09	26.56±0.14*
<i>S. epidermidis</i>	1.55±0.08	4.57±0.02	25.38±0.18*
<i>E. fecalis</i>	1.34±0.03	5.83±0.07	26.32±0.20*
<i>E. coli</i>	3.45±0.06	5.36±0.08	30.89±0.12*
<i>Mycoplasma spp.</i>	1.56±0.02	3.70±0.03	15.46±0.23*
<i>Candida</i>	1.34±0.04	5.64±0.09	30.32±0.11*

Note: * – $P \leq 0.05$ compared to 1×10⁵, CFU/g *B. megaterium*

According to the results of the studies in Table 3, it was found that at a dilution of 1×10^9 CFU/g *B. megaterium* NCH 55 showed maximum antagonistic properties in the form of a zone of growth retardation for all these microorganisms. The demarcation zone in Petri dishes with 1×10^9 CFU/g *B. megaterium* NCH 55 was higher compared to 1×10^5 CFU/g around *S. agalactiae* – by 12.2%; *S. aureus* – by 10.5%; *S. epidermidis* – by 16.3%; *E. fecalis* – by 19.6%; *E. coli* – by 8.9%; *Mycoplasma spp.* – by 9.9%; *Candida* – by 22.62%.

Similar results were obtained when prescribing antimicrobial activity of *B. megaterium* against *Candida albicans*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus sciuri*, *Micrococcus luteus* (Nguyen & Thu, 2015).

Usually properly selected antibiotics that have shown maximum bactericidal action against pathogens, have a large pronounced zone of growth retardation. However, for safety reasons, the use of a probiotic strain is more recommended.

The results of the study of the therapeutic effect of cows with subclinical mastitis *Bacillus megaterium* NCH 55

Cows with subclinical mastitis together with concentrated feed were given spores of *Bacillus megaterium* NCH 55 (1×10^9 CFU/g) at a dose of 35 g per animal for 30 days. During the experiment, the quality of milk obtained and milk productivity were determined (Table 4).

Table 4. Productivity of cows during treatment

Identification number	Date of birth	Last calving	No. of lactation	kg/day						
				February 2021	March 2021	April 2021	May 2021	June 2021	July 2021	August 2021
UA8014876120	06/06/19	06/12/21	1	–	–	–	–	14	26	34
kMAFAnM, thousand CFU/cm ³				–	–	–	–	560	250	75
NSC, thousand/cm ³				–	–	–	–	800	415	120
UA8010629706	03/11/14	01/05/21	4	37	21	25	28	27	26	35
kMAFAnM, thousand CFU/cm ³				65	120	180	210	300	150	59
NSC, thousand/cm ³				122	340	450	510	520	230	140
UA8013283674	11/20/17	01/16/21	2	35	36	37	36	29	22	35
kMAFAnM, thousand CFU/cm ³				70	110	120	180	300	150	68
NSC, thousand/cm ³				100	150	360	420	515	150	137
UA8013420982	03/18/18	06/21/20	1	15	15	15	14	15	15	28
kMAFAnM, thousand CFU/cm ³				420	450	510	530	780	460	257
NSC, thousand/cm ³				400	410	450	500	670	720	500
UA6100439832	08/04/13	01/04/21	6	24	21	37	30	32	20	25
kMAFAnM, thousand CFU/cm ³				150	180	100	240	350	240	210
NSC, thousand/cm ³				130	220	400	420	670	840	445
UA8012102570	04/17/16	06/07/21	4	–	–	–	–	20	26	35
kMAFAnM, thousand CFU/cm ³				–	–	–	–	350	100	65
NSC, thousand/cm ³				–	–	–	–	430	200	195
UA8013283631	11/01/17	03/26/21	2	–	22	23	25	22	20	29
kMAFAnM, thousand CFU/cm ³				–	110	150	300	450	300	280
The number of somatic cells, thousand/cm ³				–	350	400	670	800	630	340
UA8013283670	11/19/17	04/09/21	2	–	–	29	25	25	28	32
kMAFAnM, thousand CFU/cm ³				–	–	90	130	300	100	87
NSC, thousand/cm ³				–	–	190	200	420	250	200
UA8014655883	03/07/19	04/17/21	1	–	–	28	29	30	26	32
kMAFAnM, thousand CFU/cm ³				–	–	110	120	250	110	75
NSC, thousand/cm ³				–	–	180	270	350	260	156
UA8014703811	03/22/19	03/11/21	1	–	20	31	27	25	25	30
kMAFAnM, thousand CFU/cm ³				–	110	120	210	290	150	90
NSC, thousand/cm ³				–	320	550	670	800	400	180

In cows UA8014876120 in June, lactation was 14 kg/day, kMAFANM 560 thousand CFU/cm³ and NSC 800 thousand/cm³, indicating inflammation of the udder. In June, probiotic treatment was performed, with productivity increasing by 85.7%; kMAFANM decreased by 44.64% and NSC – by 51.87%. In August, the productivity of cows increased to 34 kg/day and the quality of milk corresponded to the “Extra” class.

In animals UA8010629706 at the beginning of the study productivity was 37 kg/day, but from March to June the month ranged from 21 to 28 kg/day, which was associated with a change in feed and an increase in kMAFANM by 250% and NSC – by 152.94%. The treatment period was 30 days in July. At the end of the experiment in August, the milk yield was 35 kg/day, the quality of milk corresponded to the “Extra” class. Cow UA8013283674 had a high productivity from February to May at the level of 35–37 kg/day, but the quality of milk deteriorated. In June, the indicators of kMAFANM increased by 428.57% and NSC – by 515%, which indicates the development of subclinical mastitis. After treatment based on *Bacillus megaterium*, productivity returned to baseline, milk quality complied with DSTU 3662:2018.

From February to June, the productivity of cows UA8013420982 was at the level of 14–15 kg/day with a gradual deterioration in milk quality. Probiotic therapy in July increased productivity by 86.6%; the content of kMAFANM decreased by 61.19% and NSC – by 125% compared to the beginning of the study. A similar pattern of subclinical mastitis was observed in animals UA6100439832. As a result of treatment in August, productivity indicators did not differ from the initial ones; the level of kMAFANM decreased by 140% and NSC – by 342.30%. The quality of milk corresponded to the “First” class.

In cows UA8013283631 during March and July the productivity fluctuated between 20–25 kg/day. The quality of the milk deteriorated in June, after which the animal received probiotic treatment. At the end of the experiment, productivity increased by 31.81%, decreased kMAFANM – by 62.84% and NSC – by 42.5%, which corresponds to the “Higher” class.

The animal UA8012102570 had low productivity and low milk quality. Therefore, in July, the cow received therapy based on *Bacillus megaterium*. At the end of the experiment in August, cow productivity increased by 75%; decreased kMAFANM – by 81.42% and NSC – by 45.34%, milk according to DSTU 3662:2018 corresponded to the “Extra” class.

In May and June, cows UA8013283670 decreased milk production and deteriorated milk quality. At the end of the experiment in August, productivity increased by 28%, decreased kMAFANM – by 29% and NSC – by 47.61%. In cows UA8014655883 in June, productivity was high but the quality of milk deteriorated. The productivity of the animal increased at the end of the experiment by 18.75%; at the same time kMAFANM decreased by 233.33%; and NSC – by 124.35%.

From May to June, the animal UA8014703811 gradually decreased milk productivity, as well as increased the number of microorganisms and somatic cells in milk. After treatment, cows increased productivity by 20%; kMAFANM decreased by 68.95% and NSC – by 77.5%.

During the study, there was an improvement in the general condition of the animals. Cows have increased appetite and gradually increased milk production. Also at the end of the experiment milk productivity was 40% – 34–35 kg/day; in 30% – 30–32 kg/day and 30% – 25–29 kg/day.

In 70% of cows that reached a productivity of more than 30 kg/day on the 30th day of the study, milk parameters such as somatic cell count (CSC ≤400 thousand/cm³) and kMAFANM (≤100 thousand CFU/cm³) corresponded to the “Extra” class.

Cows that did not reach a productivity of 30 kg/day continued treatment individually. The number of somatic cells in the milk of cows was ≤500 thousand/cm³ and kMAFANM ≤200 thousand CFU/cm³. Such milk is fit for consumption, but meets the technical conditions of the “Higher” or “First” class and is sold at a lower cost. We believe that the recovery period of animals with subclinical mastitis depended on the degree of damage to the breast and individual characteristics of the organism. The effect of probiotic drugs in animals is difficult to trace, because this effect is multi-vector.

Thus, researchers have proven that probiotics are not necessarily aimed only at restoring the microbial community, because, for example, some types of probiotics increase the resistance of animals to colonisation by pathogens (Ma et al., 2018), as well as their destruction. *Bacillus megaterium* is the largest of the *Bacillus* family. *Bacillus megaterium* multiplies to create a specific environment, improves the microflora of the rumen (Tytukh et al., 2021). In addition, *Bacillus megaterium* destroys pathogenic microorganisms through the production of highly specific antimicrobial protein – megacin.

CONCLUSIONS

1. It was found that *S. aureus* was isolated in 22% of experimental samples of milk from cows with subclinical mastitis. Somatic cells of the milk of a visual cow are represented mainly by epithelial cells, single segmental neutrophils, monocytes and basophils. In the milk of patients with subclinical mastitis of cows there is a ten-fold increase in epithelial cells, lymphocytes, basophils; neutrophils and monocytes.

2. Microscopic studies have shown that *Bacillus megaterium* NCH 55 – gram-positive rods that have the ability to form spores. The bacterium produces specific enzymes maltase, cellulase and amylase. The average adhesion was 1.70±0.09, the coefficient of participation of erythrocytes in the adhesion process – 84.25±2.53, erythrocyte adhesion index – 2.00±0.11, which according to the Brillis classification is considered an indicator of low adhesion, which is associated with the production of the antimicrobial peptide megacin.

3. It was found that in *B. megaterium* NCH 55 showed the ability to inhibit the growth of microorganisms (mastitis pathogens) in a dilution of 1×10^9 CFU/g.

4. Studies have shown that in the process of developing inflammation in the milk of cows simultaneously increases the number of somatic cells and microorganisms and reduces milk production. Also, the productivity of cows

decreases in the transitional winter-spring period due to changes in feed and climatic conditions. Treatment of patients with subclinical mastitis of cows with probiotic strain *B. megaterium* NCH 55 allowed increasing productivity in 70% of cows and improve the quality of milk to the "Extra" class.

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Лікування субклінічного маститу корів за допомогою пробіотиків

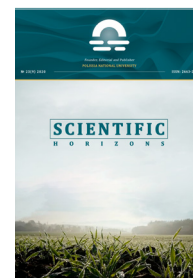
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Анотація. Велика кількість дійних корів у господарствах України страждає на субклінічний мастит, що призводить до значних економічних втрат у сільському господарстві. Через відсутність клінічних проявів його складно виявляти, зокрема, і через недостатню інформацію про мікробний склад молока. Заборона на використання антибіотиків для продуктивних тварин примушує до пошуку нових безпечних ефективних засобів. Метою дослідження було визначення лікувального ефекту *Bacillus megaterium* NCH 55 за субклінічного маститу корів породи голштин. Матеріали дослідження – молоко корів за субклінічного маститу, ізоляти мікроорганізмів та *B. megaterium* NCH 55. Використані методи: каліфорнійський тест на мастит; мікроскопічний тест для підрахунку загальної кількості соматичних клітин методом Прескотта і Бритта; бактеріальний метод для дослідження мікроорганізмів; полімеразна ланцюгова реакція для визначення *Mycoplasma spp.* в молоці; спектрофотометрію; методом В.І. Бриліса для визначення адгезивних властивостей *Bacillus megaterium* NCH 55; визначення антагоністичних властивостей *B. megaterium* методом дифузії в агарові лунки; метод проточної цитометрії за допомогою приладу «SomaCount Flow Cytometer»; фізіологічний. Експеримент проводився у молочних господарствах Північно-східного регіону України: ТОВ агрофірма «Лан», ТОВ агрофірма «Ворожбалатінвест», ТОВ агрофірма «Владана» у період лютий-серпень 2021 року. У зразках молока корів хворих на субклінічну форму маститу були виявлені ізоляти: *S. aureus*, *S. Agalactiae*, *E. coli* ентерогеморагічна, *E. coli*, *Candida*, *E. fecalis*, *S. Epidermidis* та *Mycoplasma spp.* Мікроскопічними дослідженнями встановлено, що *Bacillus megaterium* NCH 55 являють собою білі грампозитивні палички, які мають низькі адгезивні властивості та утворюють спори. Найбільший антагонізм *B. megaterium* проявляє стосовно бактеріальних ізолятів у концентрації 1×10^9 , КУО/г. У 70 % корів, які на 30 добу досліджень досягли продуктивності більше 30 кг/добу показники молока такі як кількість соматичних клітин (КСК ≤ 400 тис/см³) та кількість мезофільних аеробних і факультативно-анаеробних мікроорганізмів (кМАФАНМ) (≤ 100 тис. КУО/см³) відповідали класу «Екстра». Термін одужання тварин хворих на субклінічний мастит залежав від ступеня ураження молочної залози та індивідуальних особливостей організму. Коровам, які не досягли продуктивності 30 кг/добу продовжили лікування в індивідуальному порядку. При цьому у молоці корів кількість соматичних клітин була ≤ 500 тис/см³ та кМАФАНМ ≤ 200 тис. КУО/см³.

Ключові слова: запалення молочної залози, *Bacillus megaterium*, збудники маститу, соматичні клітини, молочна продуктивність



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Influence of Probiotic Microorganisms on Microbial Biofilms in Feeds

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Abstract. At different stages of feed production and storage, it is possible to contaminate both feed and their components with various pathogenic and opportunistic microorganisms that can cause infectious diseases not only among animals but also have epidemiological significance. The aim of the study was to isolate biofilm-forming strains of microorganisms from feed, as well as to study the inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus* against microbial biofilms. Identification and species affiliation of isolated bacterial field isolates were performed by cultural-morphological and biochemical properties. The formation of biofilms was studied by determining the ability of isolates of microbial associations and individual species of microorganisms to adhere to the surface of a 96-well polystyrene tablet according to the method of O'Toole & Kolter, 1998. Determination of microbial contamination of 50 industrial batches of feed from 4 pig farms in two regions of Ukraine (barley, compound feed SK-31 for rearing, SK-51 for fattening pigs, EXCELL starter for pigs 15%, shop prestarter, compound feed for lactating sows). In 11 experimental batches of barley (68.8%) and 13 batches of 3 types of feed (SK-31, SK-51, feed for lactating sows) identified associations with different microorganisms *Pasteurella multocida*, *Corynebacterium striatum*, *Bacillus subtilis*, *Leptothrix ochracea*, *Haemophilus parasuis* and yeast *Candida albicans*. The association of *Actinobacillus pleuropneumonia* bacteria with *B. subtilis* was identified in 2 batches (50%) of the shop prestarter. Moderate, by optical density, biofilm formation for associations of microorganisms *P. multocida* + *C. striatum* + *C. albicans* ($D_{620}=3.59$) and *P. multocida* + *L. ochracea* + *C. albicans* ($D_{620}=3.62$). Planktonic forms of *C. striatum* and *P. multocida* showed low film-forming activity at the level ($D_{620}\leq 1.51$). Inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus* (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*) was determined by isolated variations of microbial biofilms in 5 feed species, which displaced all biofilm-forming microorganisms except *H. parasuis*.

Keywords: biofilm-forming microorganisms, planktonic bacteria, microbial contamination of food, probiotic complex of bacteria of the genus *Bacillus*, inhibitory activity



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INTRODUCTION

According to the EU's (EU) livestock development strategy, the biosafety of feed production and animal feeding is one of the key factors in preventing epizootics and, consequently, microbial contamination of the human food chain (Gozdzielewska *et al.*, 2020). Therefore, in the EU the requirements for the sanitary quality of compound feeds are formulated almost stricter than for food products (Giraldo *et al.*, 2019; More, 2020).

The quality and safety of feed for farm animals depends on the correct composition and compatibility of nutrients, technology of their processing, and the degree of contamination by pathogenic microorganisms that are introduced into finished feed with raw materials (Paliy *et al.*, 2018; Przeniosło-Siwczyńska *et al.*, 2020). Pathogenic and opportunistic microorganisms in feed pose a threat to human and animal health and cause economic damage. Animal feed is most often affected by microorganisms: *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella*, *Clostridium perfringens*, *Neisseria* spp., *Pasteurella multocida*, *Actinobacillus pleuropneumonia*, fungi *Aspergillus niger* and *Aspergillus candidus*, *Candida albicans* (Monge *et al.*, 2012; Udhayavel *et al.*, 2017; Gosling *et al.*, 2021).

Unfortunately, feed production in Ukraine in terms of control of microbial contamination of raw materials and the final product is still regulated by old standards that do not take into account new scientific knowledge, in particular the existence of bacterial associations in the form of microbial biofilms. This is a significant problem, because today it is known that bacterial biofilms are a complex dynamic biological system for the protection of microorganisms and increase their resistance to antimicrobial drugs by 100-1000 times compared to planktonic cells (Tassinari *et al.*, 2019; Uruén *et al.*, 2020). Being in the attached state, bacteria in biofilms are protected from environmental factors and the action of antibacterial substances in the environment and the host organism during infection. Moreover, the ability of microorganisms to form biofilms (film-forming activity) is already considered a factor in their pathogenicity (Soll & Daniels, 2016; Goodwine *et al.*, 2019; Katongole *et al.*, 2020). Therefore, the study of the composition of microorganisms in feed and their biofilm-forming activity is an important area for preventing the development of associated animal diseases and, consequently, microbial contamination of the human food chain.

The aim of the study was to isolate biofilm-forming strains of microorganisms from feed with further study of the inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus* against microbial biofilms.

MATERIALS AND METHODS

Microbiological studies of feed were conducted in the laboratory for the study of pig diseases of the National Research Center "Institute of Experimental and Clinical Veterinary Medicine" (Kharkiv) according to modern

methods (Cullen *et al.*, 2021). Identification and species affiliation of isolated field isolates of bacteria were performed by cultural-morphological and biochemical properties (Goodfellow *et al.*, 2012).

Pasteurella multocida was cultured on Hottinger broth and agar, on blood agar with the addition of 5% sheep blood. According to the biochemical properties of *P. multocida*, D-glucose and fructose were catabolized with the formation of acid, were oxidase- and catalase-positive, reduced nitrate to nitrite; samples with methyl red and for the formation of acetoin (Foges-Proskauer), and lysinecarboxylase, arginine dihydrolase and gelatinase were negative.

Actinobacillus pleuropneumonia was isolated on meat-peptone agar (MPA) and 5% blood agar with the addition of 10% yeast extract. *A. pleuropneumonia* catabolized D-glucose and fructose to form acid, were positive for β -galactosidase, and negative for methyl red and indole.

Neisseria sicca was grown on meat-peptone broth (MBP), IPA and 5% blood agar. According to the biochemical properties of the bacterium *N. sicca* oxidase- and catalase-positive, formed carbonic anhydrase, reduced nitrite.

Yeast-like fungi *Candida albicans* were grown on wort agar, glucose-peptone medium. To determine the species of *C. albicans*, a test for the formation of germinal (embryonic) tubes was performed. The fungi *C. albicans* fermented glucose, maltose, sucrose, galactose, did not ferment lactose and raffinose.

Corynebacterium striatum was isolated on tellurite medium, blood and serum agar. *C. striatum* decomposed glucose, fructose, mannose, maltose, starch to acid; did not decompose lactose, sucrose. Urease was not isolated, nitrates were not reduced to nitrites.

Leptothrix ochracea was isolated on nutrient medium (Meus srl, manufactured by Piove di Sacco, Italy). Bacteria from glucose formed acid without gas, did not form protein and hydrogen sulfide, did not reduce nitrates and did not dilute gelatin, catalase-negative.

Bacillus subtilis grew on BCH, IPA, potato-peptone agar. Bacteria fermented sucrose, glucose, mannitol, salicin, esculin, fructose and did not ferment lactose, maltose, rhamnose, dulcitol, inositol, sorbitol, galactose, raffinose. Did not form indole, but formed hydrogen sulfide, hydrolyzed starch, casein, but did not hydrolyze tyrosine and hemolyzed sheep erythrocytes, reduced nitrates and produced catalase.

Haemophilus parasuis was cultured on 5% blood and chocolate agar. Bacteria formed indole, which did not produce urease and hemolysin, in addition, fermented with the development of xylose acid, salicin, glycine, diluting gelatin, exhibited catalase activity.

Biofilm development was studied by determining the ability of isolates of microbial associations and individual species of microorganisms to adhere to the surface of a 96-well polystyrene plate according to the

method of O'Toole & Kolter (1998). The microorganisms were cultured in meat-peptone broth (MPB) at a temperature of $(37 \pm 0.5)^\circ\text{C}$ for 48 hours.

According to the standard protocol, planktonic cells were removed from the wells of the plate and the microbial biofilms were stained with crystalline violet. To do this, 150.0 μl of distilled water and 20.0 μl of 1% crystal violet were added to the well and incubated for 45 min at room temperature. After washing three times with distilled water, 200.0 μl of 96% ethanol was added to the wells to extract the ink from the biofilm, and the optical density of the solution was measured on an ELISA reader at an optical wavelength of 620 nm (D_{620}). In the process of estimating the density of biofilms, film-forming microorganisms were used as an experiment, and a nutrient medium for the cultivation of biofilms was used as a control.

50 industrial batches of feed from 4 pig farms of two regions of Ukraine (barley, compound feed SK-31 for rearing, SK-51 for fattening pigs, shop prestarter, compound feed for lactating sows) were studied. Feed selection was carried out both in the feed shop and in the livestock room where the animals are kept, as well as directly from products obtained from feed mills.

Pathogenicity of isolated associations of microorganisms Dosis certa letalis (DCL) – from 5 types of feed was determined on 60 white outbred mice ($n=10$), weighing 18–20 g, age (8–9) – one month of age, kept in vivarium. The animals were adapted for 15 days to the conditions of detention. 5 experimental and one control group of animals of 10 individuals of the same age in each were formed.

White mice from 5 experimental groups were injected intraperitoneally for 24 hours with a certain association of bacteria at a concentration of 1 billion tons of food isolated from each type of food. Mice in the

control group were injected with 0.5 ml of saline. The criterion of avirulence was the absence of infectious pathology and death of mice within 10 days. Behavioral responses and physiological status of mice were monitored.

During the research on animals, manipulations were carried out in accordance with the existing documents regulating the organization of work with the use of animals in experiments and adherence to the principles of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (1986).

To study the inhibitory activity of the probiotic complex of bacteria of the genus *Bacillus*, namely *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens* (abbreviated *Bacillus spp.*) On isolated biofilm-forming associations of feed microorganisms, samples of contaminated feed with a period of 24 hours stirring at a temperature of $(37.0 \pm 0.5)^\circ\text{C}$ with a probiotic complex of bacteria of the genus *Bacillus*, taken in final concentrations of 100, 50 and 10 thousand spores per 1 ml of sterile saline, at a dose of 0.5 ml.

Probiotic complex of bacteria of the genus *Bacillus*, which includes 3 cultures of *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens* is used in poultry and pig breeding for aerosol disinfection, water purification, to stimulate and regulate digestive processes. It has antimicrobial activity associated with the ability to synthesize antibiotic-like substances with a broad spectrum of action, thereby suppressing pathogenic and opportunistic bacteria, fungal flora and stimulates the protective functions of animals and birds.

RESULTS AND DISCUSSION

According to the results of microbiological studies, feed of plant origin for pigs, regardless of the place of sampling, had a high level of microbial contamination, and their species composition was very diverse (Table 1).

Table 1. Microorganisms that were isolated from industrial batches of feed for pigs

Type of feed	In total, parties	Isolated microorganisms		Contaminated batches
		Feed breeding 10^{-5}	feed breeding $10^{-1.5}$	
Barley	16	<i>C. striatum</i>	<i>P. multocida</i> <i>C. striatum</i> <i>C. albicans</i>	11
Compound feed SK-31 for rearing piglets	7	<i>B. subtilis</i>	<i>P. multocida</i> <i>B. subtilis</i>	3
Compound feed SK-51 for fattening pigs	15	<i>P. multocida</i>	<i>P. multocida</i> <i>L. ochracea</i> <i>C. albicans</i>	6
Compound feed for lactating sows	8	–	<i>P. multocida</i> <i>N. sicca</i> <i>H. parasuis</i> <i>C. albicans</i>	4
Shop prestarter	4	<i>B. subtilis</i>	<i>A. pleuropneumonia</i> <i>B. subtilis</i>	2

All 50 batches of feed, which were studied at a dilution of 1:50 ($10^{-1.5}$), contained different types of microorganisms that formed stable associations, because during 3 consecutive passages of association with 2-3 microbial agents, in particular pathogenic for pigs, were not dissociated. At the same time, almost all feed samples (except for one batch of feed SK-51, samples of which were taken in the pigsty, as well as directly from the factory packages of the feed manufacturer), tested in accordance with current regulations in Ukraine at a dilution of 1:100000 (i.e. $10^{-5.0}$) in experiments on white laboratory mice did not have virulence properties (did not contain pathogens). During the entire observation period (10 days) after the introduction of a certain association of bacteria intraperitoneally, all animals remained viable, ate food well, no changes in the fur cover were observed. Mice were active, physiological discharges were

not disturbed, behavioral reactions were normal. No clinical signs of toxicosis were observed.

Field isolate of *P. multocida* isolated from compound feed SK-51 for fattening pigs caused the death of 60% of white mice (out of 10 infected white mice killed 6 individuals) by 2 day with signs of depression, refusal of food, lack of activity, indicating its virulence.

In 11 experimental batches of barley (68.8%) and 13 batches of 3 types of feed S-31, SC-51 and for lactating sows (43.3%) isolated *Pasteurella multocida* in association with *C. striatum*, *B. subtilis*, *L. ochracea*, *N. sicca*, *H. parasuis* and yeast fungi *C. albicans*.

Two batches (50%) of the shop prestarter were contaminated with the bacterial association of *A. pleuropneumonia* with *B. subtilis*. At the next stage of research, the ability of microorganisms isolated from feed to form biofilms was studied (Table 2).

Table 2. Estimation of density of biofilms of microorganisms isolated from different types of feed

Bacterial associations	Biofilm growth time, hours	Relative density of microbial biofilm (D_{620})	
		Experiment	Control
<i>P. multocida</i> <i>C. striatum</i> <i>C. albicans</i>	48	3.59±0.05	0.06±0.005
<i>P. multocida</i> <i>B. subtilis</i>	48	3.46±0.08	0.21±0.02
<i>P. multocida</i> <i>L. ochracea</i> <i>C. albicans</i>	24	3.62±0.19	0.05±0.003
<i>P. multocida</i> <i>N. sicca</i> <i>H. parasuis</i> <i>C. albicans</i>	48	3.52±0.51	1.47±0.12
<i>A. pleuropneumonia</i> <i>C. perfringens</i> <i>B. subtilis</i>	48	3.57±0.59	1.54±0.12
<i>P. multocida</i>	24	1.51±0.11	0.23±0.05
<i>B. subtilis</i>	24	2.86±0.35	0.22±0.06
	48	3.96±0.15	0.08±0.006

Note: the difference between the values of the indicators of experimental animals (group 1) is probable at $p \leq 0.05$ relative to the corresponding indicators in the control (group 2). Scale for assessing film-forming activity: Optical density of biofilms up to $\leq 2 \times OD$ – low; up to $\times 4 \times OD$ – moderate; $> 4 \times OD$ – expressed

The most pronounced, in terms of optical density, biofilm formation for associations of microorganisms *P. multocida* + *C. striatum* + *C. albicans*, $D_{620}=3.59$ and *P. multocida* + *L. Ochracea* + *C. albicans*, $D_{620}=3.62$. This indicates the important role of the yeast fungi *C. albicans* in the formation of polymicrobial biofilms – possibly as a “biofilm matrix” that serves as a framework for the planktonic forms to which they attach and hold (Lohse et al., 2018).

All field isolates with significant biofilm activity were isolated from a 1:50 feed extract dilution. Conversely, the vast majority of planktonic forms of these field isolates were isolated from feed extracts diluted

1:100,000, i.e. in accordance with the requirements of veterinary and sanitary control of feed in Ukraine.

Planktonic forms of *C. striatum* and *P. multocida* were formed at the level of $D_{620} \leq 1.51$ (low film-forming activity) and were found in feeds exclusively as part of polymicrobial biofilms. At the same time, field isolates of “feed” bacteria *B. subtilis* in all cases dominated in polymicrobial biofilms, the optical density of which was at the level of $D_{620}=2.86 \pm 0.35$ (moderate film-forming activity). These microbial associations with high biofilm-forming activity did not dissociate during successive 3-fold passage on agar media.

At the same time, during the passage on liquid

microbiological media, these associations dissociated into separate components. This phenomenon was especially evident in the presence of *Bacillus subtilis* in the association of forage microflora. This indicates the existence of biofilm-specific mechanisms of stability in the environment – at least for “solid-phase” polymicrobial biofilms compared to planktonic bacteria (“suspension forms”).

According to preliminary data from bacterioscopy, the level of film-forming activity of bacteria in microbial feed associations correlated with the phenomenon of “bacterial swarming”, which manifested itself in the development of associations of different microbial species in the form of “coacervates”: in smears 1). Most of these “coacervates” formed around the cells of fungi and yeast.

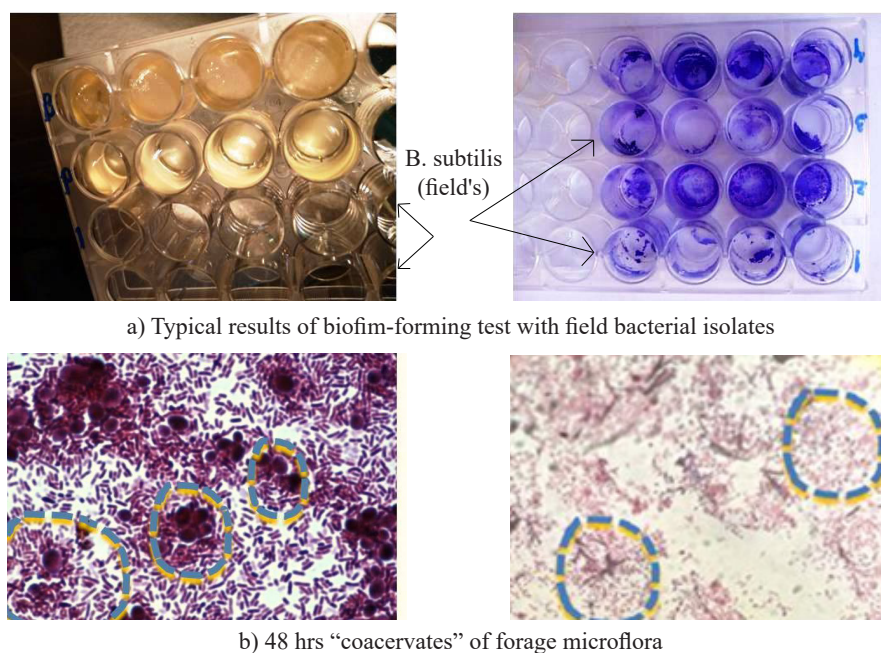


Figure 1. Bacterial film formation (a), including microscopy (b). Swarming of film-forming bacteria, with the development of “coacervates” (object magnification $\times 1100$)

The phenomenon of dissociation of forage microflora under the influence of *B. subtilis* in its composition motivated us to further study the mechanism of influence of probiotic bacteria on biofilm-forming associations of microflora isolated from feed (Table 1) using strains specially selected for use in probiotics – potential tool

for the “sanitation” of feed. The results of three series of treatment with a probiotic complex of bacteria of the genus *Bacillus* (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*) feed samples containing microbial biofilms are presented in Table 3.

Table 3. Associations of bacteria isolated from pig feed samples treated with a probiotic complex of bacteria of the genus *Bacillus*

Type of feed	Isolated microorganisms	Optical density at wavelength, D_{620}
Barley	<i>Bacillus</i> spp.	2.936 $\pm$ 0.42
Compound feed SK-31 for rearing piglets	<i>Bacillus</i> spp.	3.16 $\pm$ 0.51
Compound feed SK-51 for fattening pigs	<i>Bacillus</i> spp.	2.69 $\pm$ 0.27
Compound feed for lactating sows	<i>Bacillus</i> spp. <i>H. parasuis</i>	3.35 $\pm$ 0.59
Shop prestarter	<i>Bacillus</i> spp.	3.22 $\pm$ 0.23

It was found that the probiotic complex of bacteria of the genus *Bacillus* during exposure to feed samples displaced into biofilms of almost all species of isolated feed microflora. The exception was the biofilm-forming bacteria *Haemophilus parasuis* of the microbial association of one of the batches of compound feed for lactating

sows (their association with *P. multocida*, *N. sicca* and *C. albicans*). As shown in Table 3, under the conditions of the experiment (final concentration of spores 100 thousand), the biofilm of *Bacillus* spp. with an optical density in the range from $D_{620}=2.69$ to $D_{620}=3.22$ displaced from the feed microbial associations microorganism and

P. multocida, *N. sicca*, *C. perfringens* and fungi *C. albicans*. However, in this mode, they did not show competitive activity against the bacterium *H. parasuis*.

The obtained results forced us to look for an explanation of the peculiarities of the interaction of the probiotic complex of bacteria of the genus *Bacillus* with the causative agent of polyserositis/Glacier disease of pigs. For this purpose, comparative studies of the dynamics of film formation of the probiotic complex of bacteria of the genus *Bacillus* as such and in the presence of "feed" isolate of *H. parasuis* (polybacterial biofilms) were performed. Figure 2 presents data on the film-forming activity of these bacteria in a period of 10 days. It is obvious that *Bacillus* spp. under the same

conditions of cultivation, even at the peak of its growth (up to the 3rd day) by 13-15% lagged behind the growth intensity of the polybacterial biofilm *Bacillus* spp. + *H. parasuis*. Moreover, already on the 8th day after sowing the density of the biofilm of the probiotic complex of bacteria of the genus *Bacillus* in four replicates began to decrease significantly and on the 10th day the optical density reached values of $D_{620}=2.34$. At the same time, the polybacterial biofilm of *Bacillus* spp. + *H. parasuis* for 10 days, according to optical indicators, practically did not lose the volumes reached in 24-28 hours after sowing $D_{620}=3.55$.

Figure 3 presents data on the film-forming activity of the same bacteria, but in the period of 24 hours after sowing.

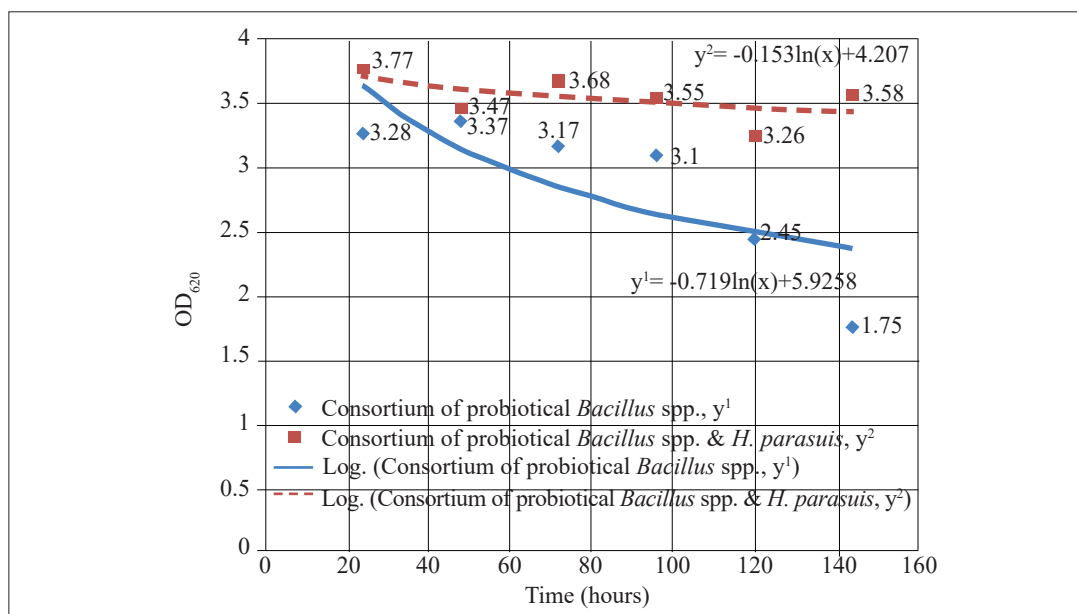


Figure 2. Comparison of the dynamics of film development of the probiotic complex of bacteria of the genus *Bacillus* separately and in association with *H. parasuis* for 10 days

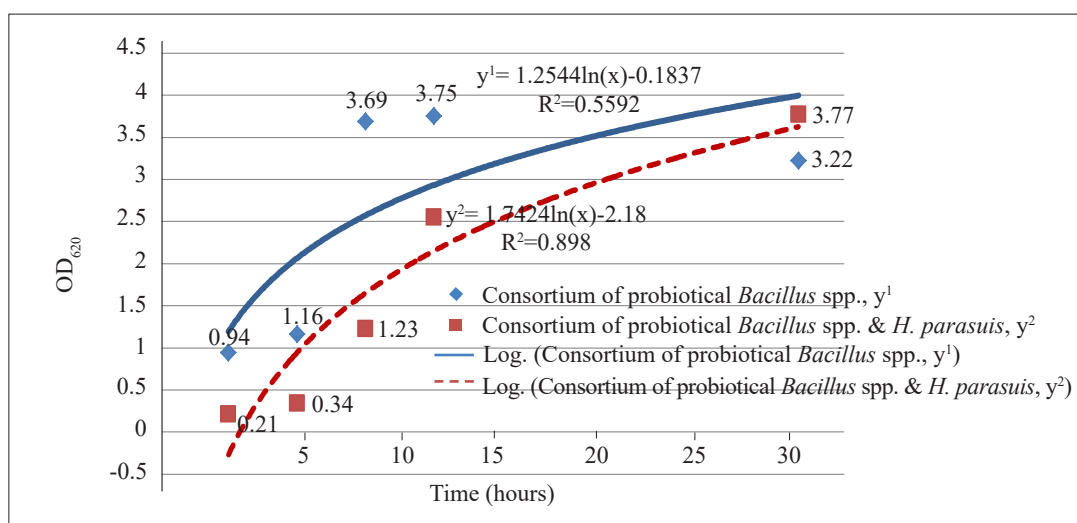


Figure 3. Comparison of the dynamics of film development of the probiotic complex of bacteria of the genus *Bacillus* separately and in association with *H. parasuis* on the first day

The obtained data indicate a significantly higher (more than 70%) growth rate of the probiotic complex of bacteria of the genus *Bacillus* under the same cultivation conditions during the first 18-20 hours after sowing, compared to its polybacterial biofilm *Bacillus* spp. + *H. parasuis*. Only after 24-26 hours of cultivation, judging by the obtained growth curve, the biofilms of these bacterial associations reached their maximum parameters according to the optical density of their samples in the test of control of film-forming activity (Fig. 2).

Therefore, it is established that the associations of bacteria that contaminate feed for pigs are characterized by high film-forming activity. This indicates the probable formation of microbial biofilms of different composition and physical properties on the surface of grain, granules and other feed components, as well as on the surfaces of containers, feeders, etc., which may contain and promote the survival of infectious agents dangerous to pigs (Abdullahi et al., 2016; Kukhtyn et al., 2019).

The results show that the current norms of feed contamination control in Ukraine do not provide an objective assessment, as they do not take into account the presence of strong microbial biofilms in feeds, from which it is almost impossible to isolate a reliable concentration of microflora so that it can be detected 10^{-5} . Obviously, this is beneficial to feed producers, but it poses a danger to pig farming and the human food chain.

A large number of opportunistic pathogens in the feed of decomposition are nutrients, which leads to metabolic disorders, beriberi, toxicosis, decreased overall resistance, productivity of animals (Vestby et al., 2020; Kim et al., 2021). 34% of feed samples received from pig farms had a high level of microbial contamination. This indicates technological violations during production in factories, and during their storage, in the absence of proper veterinary and sanitary control in their use in animal feed.

Even the saprophytic microflora of feed under favourable conditions for its reproduction transport or storage in critical concentrations can affect the consumption and value of feed – because it decomposes nutrients, and sometimes with the development of toxic products of catabolism (Ramírez-Castillo et al., 2018; Suarez et al., 2019; Burgos-Morales et al., 2021). The presence of pathogenic microorganisms in feed is usually associated with microbial contamination (MS) of raw materials. After all, the slightest contamination of feed materials, in particular premixes, with pathogenic microflora leads to favorable conditions for its reproduction to MH of ready-made feed in the feeder, with corresponding epizootic consequences (Makovcova et al., 2017; Guzmán-Soto et al., 2021). Prior to the discovery of a strategy to effectively protect bacteria from adverse environmental factors through the formation of microbial biofilms (Donlan, 2011), feed microbiology could not explain the mechanism of microbial contamination of feed (MSC) as a dynamic process. Therefore, modern criteria for the

assessment of MFC in the former USSR and now in Ukraine are based almost exclusively on data from biological control of feed by its producer, while microbial biofilms are formed mainly during transportation and storage (Magana et al., 2018; Muhammad et al., 2020; Zhang et al., 2020).

Bacterial biofilms are formed by the adhesion of microbial cells almost exclusively on the solid surfaces of polymers (both biological and abiological) with their inclusion in the polymer matrix (Yuan et al., 2020; Apiwatsiri et al., 2021; Skandalis et al., 2021). Under favourable conditions, microbial biofilms are formed in raw materials, feed, food, various organs and tissues in humans and animals, on the surface of equipment when food comes into contact with technological surfaces (Callaway et al., 2021; Jiang et al., 2021).

In the joint cultivation of opportunistic pathogens with bacteria of the genus *Bacillus*, the latter form antibiotic substances and thus have a bactericidal and bacteriostatic effect on microorganisms that form biofilms, namely yeast-like fungi *C. albicans*, *Escherichia coli*, *Streptococcus*, *Staphylococcus*, *Staphylococcus*, *Salmonella*, *Klebsiella*, *Proteus*, *Pseudomonas aeruginosa* (Bai et al., 2017). Different strains of bacteria of the genus *Bacillus* secrete different sets of antimicrobial substances. For example, one strain of *B. subtilis* secretes subtilin, which has antibiotic properties against gram-positive bacteria, another strain of *B. subtilis* secretes the antibiotic ericin S, which has the same spectrum of activity as subtilin (Stein et al., 2002). Antimicrobial peptides secreted by *B. subtilis* have a huge advantage over traditional antibiotics because they are close to antimicrobial peptides released by the animal and are part of its natural immunity (Teixeira et al., 2013). Similar substances have been identified in a large number of tissues and epithelial surfaces, including skin, eyes, ears, mouth, intestines, immune, nervous and urinary systems. Such as defensins, lysozyme, cathelicidin, dermidsidine, lectin, histatine and others (Wang, 2014). *B. subtilis* secrete similar substances, so resistance to them is rare, side effects are usually absent (Guariglia-Oropeza & Helmann, 2011). Lack of resistance to antimicrobial peptides of animals and *B. subtilis* is associated with the fact that their action is often aimed at the development of membrane pores, which leads to the death of bacteria. *B. subtilis* due to the release of antimicrobial substances inhibits the development of pathogenic microflora, which creates the conditions for filling the vacated niches and normal bacteria (Sumi et al., 2015).

Obtaining quality and safe animal feed is impossible without compliance with the basic veterinary and sanitary and technological aspects of production (Muckey et al., 2020; Paliy et al., 2021a). The main attention should be focused on the destruction of opportunistic, pathogenic microflora (Rodionova et al., 2021) and parasitic insects (Paliy et al., 2021b). Only a comprehensive approach to solving pressing problems of feed production and animal feeding will allow obtaining high quality livestock products.

CONCLUSIONS

In violation of the technology of feed production and storage of feed for farm animals, they can be contaminated with various microorganisms (*P. multocida*, *C. striatum*, *B. subtilis*, *L. ochracea*, *N. sicca*, *H. parasuis*, *C. albicans*, *A. pleuropneumonia*), which have epizootological and sanitary-hygienic significance. The ability of isolated microorganisms to form biofilms has been studied.

It is proved that the growth rate of the probiotic complex of bacteria of the genus *Bacillus* in biofilms (*Bacillus* spp.) Increases by the 8th day and gradually begins to decrease to the level of $D_{620} < 2.34$ on the 10th day, while the polybacterial biofilm of *Bacillus* spp. + *H. parasuis* maintains the intensity of growth up to the 10th day at the level of the index $D_{620} < 3.55$.

The probiotic complex of bacteria of the genus *Bacillus* (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*) is promising for the formation of microbial biofilms in feed for pigs, which do not allow to take root in most of the above species dangerous to the human food microflora. Indicators of the dynamics of the development of probiotic biofilms are of prognostic value for the analysis of antagonistic-symbiotic activity of bacteria – both in the first two days (hourly) and in a weekly period of time.

The prospect of further research is to develop an innovative scheme for storing feed for farm animals, taking into account microbiological risks depending on regional climatic conditions.

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Вплив пробіотичних мікроорганізмів на мікробні біоплівки у кормах

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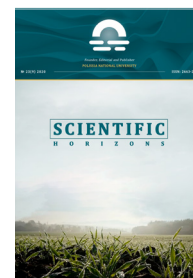
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Анотація. На різних етапах виробництва кормів та їх зберігання можливе обсіменіння як кормів, так і їх компонентів різними патогенними та умовно-патогенними мікроорганізмами, що можуть бути причиною виникнення інфекційних захворювань не тільки серед тварин, а й мати епідеміологічне значення. Метою роботи було виділення біоплівкоутворюючих штамів мікроорганізмів із кормів, а також вивчення інгібуючої активності пробіотичного комплексу бактерій роду *Bacillus* відносно мікробних біоплівок. Ідентифікацію та видову належність виділених польових ізолятів бактерій проводили за культурально-морфологічними та біохімічними властивостями. Утворення біоплівок вивчали за допомогою визначення здатності ізолятів мікробних асоціацій та окремих видів мікроорганізмів до адгезії на поверхні 96-лункового полістиролового планшета за методикою O'Toole & Kolter, 1998. Проведено визначення мікробної забрудненості 50 промислових партій кормів з 4-х свиногосподарств двох областей України (ячмінь, комбікорм СК-31 для дорошування, СК-51 для свиней відгодівлі, EXCELL стартер для свиней 15 %, цеховий предстартер, комбікорм для лактуючих свиноматок). У 11 дослідних партіях ячменя (68,8 %) та 13 партіях 3 видів комбікормів (СК-31, СК-51, комбікорм для лактуючих свиноматок) виділяли асоціації з різними мікроорганізмами *Pasteurella multocida*, *Corynebacterium striatum*, *Bacillus subtilis*, *Leptothrix ochracea*, *Neisseria sicca*, *Haemophilus parasuis* та дріжджоподібні гриби *Candida albicans*. У бавківсівах 2 партій (50 %) цехового предстартеру ідентифікували асоціацію бактерій *Actinobacillus pleuropneumonia* з *B. subtilis*. Встановлено помірне, за оптичною щільністю, біоплівкоутворення для асоціацій мікроорганізмів *P. multocida* + *C. striatum* + *C. albicans* ($D_{620}=3,59$) та *P. multocida* + *L. ochracea* + *C. albicans* ($D_{620}=3,62$). Низьку активність плівкоутворення демонстрували планктонні форми бактерій *C. striatum* та *P. multocida* на рівні ($D_{620}\leq 1,51$). Визначено інгібуючу активність пробіотичного комплексу бактерій роду *Bacillus* (*B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*) на виділені варіації мікробних біоплівок у 5 видах кормів, який витіснив усі біоплівкоутворюючі мікроорганізми, окрім *H. parasuis*.

Ключові слова: біоплівкоутворюючі мікроорганізми, планктонні бактерії, мікробна забрудненість корму, пробіотичний комплекс бактерій роду *Bacillus*, інгібуюча активність



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Yield of Marketable Seeds of Technical Hemp Depending on the Impact of Sowing Rates and Variety

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Abstract. At the present stage of ensuring the development of technology for growing non-narcotic hemp, the constant task is to study the response of new varieties to the development of agrophytocenosis at different parameters of plant density per unit area. The potential of monoecious non-narcotic varieties of hemp, bred at the Institute of Bast Cultures of the National Academy of Agrarian Sciences, in the technology of growing seeds in the western agricultural zones of Ukraine has been insufficiently studied. The aim of the research is to establish the dependence of commercial hemp seed yield on technical factor A, namely sowing rates based on their differentiation, including biological factor B, i.e. monocotyledonous hemp varieties in the organization of agrophytocenosis by wide sowing in Western Forest-Steppe. Data on the study of monoecious non-narcotic hemp of modern varieties were obtained on the basis of organised field experiment within experimental units under different sowing rates. Methods used to summarise the results of research: general, based on objectivity, provability, reproduction and mathematical and statistical – to process experimental data. The dependence of hemp seed yield on the variety, where the difference in data is on average up to 25% on the experiment. It is proved that the norms of sowing seeds by wide-row sowing method are an effective factor in ensuring the implementation of the productivity of hemp varieties in crops. The obtained results give grounds to claim that the differentiation of the seeding rate factor by the experimental step of 0.3 million units/ha provides an effective distribution of the obtained data into separate static groups. This allows identifying the best options for seeding rates to recommend production. Prospects for further research are an expanded study of Glesia cultivation of technical hemp at the same time on the yield of commercial seeds and fibrous products, i.e. for bilateral use directly in the Western Forest-Steppe of Ukraine

Keywords: row spacing, crop productivity, factors, monoecious hemp varieties, non-narcotic hemp, regularity of influence, efficiency



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INTRODUCTION

Hemp is one of the oldest and most famous diverse crops. It was grown all over the world as a fibrous crop, which was sown in large quantities in large areas (McPartland *et al.*, 2018; Struik *et al.*, 2000). But conditioned upon the high content of drugs, it was banned. However, since the 1990s in Ukraine, namely at the Institute of Bast Cultures of NAAS, a number of new varieties of technical hemp that do not contain drugs have been bred (Bazyl, 2019). This contributed to the restoration and significant spread of hemp cultivation, and varieties of Ukrainian selection began to be valued on the world market.

Hemp is a multifunctional crop, and it is this characteristic that has recently increased interest in its cultivation. Today it is a crop grown in more than 30 countries, including China, the United States, France, Italy, the Netherlands, Estonia and others. The revival of the hemp industry in the world is happening faster than in Ukraine.

Hemp is a universal crop, all parts of the plant – seeds, flowers, leaves, stems, roots – are used in the textile, pharmaceutical, aviation, paints and construction industries (Tsaliki *et al.*, 2021; Rehman *et al.*, 2021; Adesina *et al.*, 2020). It should be noted that hemp is a highly profitable crop, and the seeds and stems of which can be fully used and produce about 50,000 different products, including biocomposite, bioplastic, biofuels (Vyrovets *et al.*, 2021). This plant, according to scientists, could become an ecological raw material of the future. Industrial needs in hemp are of increasing interest around the world (Baraniecki *et al.*, 2022; Rahemi *et al.*, 2021).

The direction of use of the plant depends on the content of the psychoactive substance tetrahydrocannabinol (THC). Technical hemp is hemp in which the content of the psychoactive component of tetrahydrocannabinol (THC) does not exceed 0.3% (according to European legislation – 0.2%) (Boyko *et al.*, 2018; Vyrovets, 2015).

BRDO has prepared a whole study – the Green Paper on the regulation of the technical hemp market in Ukraine (Hruzinska *et al.*, 2020). It is noted that hemp contains about 200 active substances, of which 18 have been studied. Such substances include, in particular, CBD (cannabidiol) and THC (tetrahydrocannabinol), which are the most studied cannabinoids. According to Ukrainian legislation, hemp with a THC content of up to 0.08% belongs to the technical ones, and plants with a THC content exceeding this value are automatically considered narcotic (Hruzinska *et al.*, 2020). In Europe and America, the THC content is 0.2 and 0.3%, respectively.

Quite an interesting study was conducted by American scientists at Oregon State University. They investigated that certain cannabinoid compounds of cannabis prevent the pathogenic effects of coronavirus infection on the human body (van Breemen *et al.*, 2022). It is also emphasised that cannabinoid acids, which are

contained in hemp and many of its extracts, are safe for humans.

The main areas of cultivation of technical hemp include the need for commercial seeds and fibrous products. Recently, the technology of growing to ensure bilateral use is becoming widespread (Žuk-Gołaszewska & Gołaszewski, 2017; Zhang *et al.*, 2011; Primakov *et al.*, 2013).

Scientific publications state that monoecious hemp varieties are much better suited for seed production, while dioecious varieties are unsuitable conditioned upon their heterogeneity, excessive size and, as a result, difficulties with mechanical harvesting. But the potential of monoecious varieties for seed yield has been much less studied. It is known that in hemp, yield indicators, in addition to genotype, are strongly influenced by growing conditions, and often the end result depends on the seed propagation environment, where a particular variety was bred (Tang *et al.*, 2016; Baldini *et al.*, 2020).

Recently, in some countries, the technology is aimed at one-sided cultivation of hemp, ie only to obtain seeds for commercial purposes, for this purpose, low seeding rates are observed. The authors note that combine harvesters are used for harvesting (Kabanets & Kabanets, 2018; Kraszkiewicz *et al.*, 2019; Prade, 2011).

Hemp has attracted a lot of attention from the world market, including breeders and specialists in cultivation technology due to new opportunities for its wide use.

The cultivation of any agricultural crop is not without the production of seeds of this crop. In seed production the task of seed propagation, preservation of varietal purity on the basis of conformity of morphological features and formation of high sowing, and also productive properties of seed material is set. However, no less important task is the cultivation of technical hemp for seed to meet production needs, ie as raw materials for the relevant industries, namely food, pharmaceuticals and cosmetics. Accordingly, the cultivation of technical hemp for commercial seeds is an urgent issue that requires additional research, both in connection with the introduction of new monoecious non-narcotic varieties and in connection with the expansion of sown areas of these varieties within certain agricultural areas of Ukraine.

The volume of production of any plant products depends on many factors. They are primarily affected by market conditions and the need for a specific type of technical raw materials (Radishchuk, 2015). At the same time, not only the biological features of cultivated plants are important, but also the perfection of cultivation technology.

To obtain a high level of yield of hemp products, various agricultural techniques are introduced, aimed at creating optimal conditions for plant growth and development (Ferfuia *et al.*, 2021; Tang *et al.*, 2017). That is

why research to find new ways to increase crop yields, including industrial hemp, is an urgent task today.

Hemp cultivation requires a scientific basis in the management of plant growth and development. It is necessary to consider the favorable environmental conditions, soil type, length of daylight hours, temperature and water regimes. Important importance in this aspect is given to technological factors, in particular the use of mineral fertilizers, sowing dates, including seeding rates. Important importance should be given to the biological object, ie the variety of hemp, which is the main factor in technology. Modern varieties of hemp grown are quite different in terms of ensuring the maximum amount of final products, including commercial seeds and fiber. They react differently to technological factors. Most tests in the past have been limited to studies aimed at producing fibrous products.

The aim of the research was to establish the dependence of commercial hemp seed yield on technical factor A, namely seeding rates based on their differentiation, as well as biological factor B, ie monocotyledonous hemp variety in the organisation of agrophytocenosis by wide sowing method in western forest-steppe of Ukraine.

LITERATURE REVIEW

As a rule, the main purpose of growing any crop is to increase both quantitative and qualitative indicators of the resulting products. Breeding and seed production play a leading role in solving this problem. A characteristic feature of hemp is that they are grown for two important products – fiber and seeds, which, in turn, complicates the selection for simultaneous increase in yield and fiber and seeds (Mygal, 2015).

The main factor in increasing seed productivity is the increase in plant size, especially inflorescences. This is achieved through the use of sparse sowing, i.e. reducing the seeding rate (Mygal *et al.*, 2017).

Some scientists believe that the yield of straw (stems), fiber, seeds and the quality of technical hemp largely depend on many technological factors, in particular, seeding rates and sowing methods (Dmitriev *et al.*, 2001; Mediavilla, 2001; Keller *et al.*, 2001).

As a result of research by Kabanets & Kabanets (2016), it is noted the increase in sowing rates of hemp seeds contributes to the cultivation technology to improve the final results in terms of yield and fiber quality, but under such conditions the number of seeds per unit area becomes smaller. Conversely, it is noted that underestimation of sowing rates cause diametrically opposite results. In particular, the seed yield increases, but the yield per unit area of fibrous products naturally decreases. The authors emphasises that this pattern draws attention to the relevance of research on seeding rates, which would satisfy the farmer to receive in the technology of growing products of bilateral use. Particular attention is paid to this area of technology with the use

of new varieties (Kabanets & Kabanets, 106; Labanets & Kabanets, 2018).

P.A. Holoborodko & G.V. Dyshleva (1985) noted that when growing hemp for seed sowing should be carried out with a row spacing for varieties of Central Russian hemp 45 cm, southern – 60-70 cm and with a sowing rate of 1.5-2.0 million similar seeds (25-35 kg) per hectare.

Many studies have focused on the impact of fertilizer application rates, crop density on growth and yield of different types of hemp fibers (Tang *et al.*, 2017; Papastylianou *et al.*, 2018; Deng *et al.*, 2019). Appropriate sowing density allows efficient use of available resources such as light, moisture and nutrients by culture, significantly increasing the yield of hemp fibers (Tang *et al.*, 2017; Schafer & Honermeier, 2006).

The seeding rate depends, first of all, on the purposes of use of the grown production of technical hemp. When grown on seeds, crops are made sparse, because the plants need some space for their branching and the formation of full-fledged inflorescences (Tang *et al.*, 2017; Adesina *et al.*, 2020). However, information on the optimal sowing rate for the production of commercial hemp seeds is very limited and needs to be studied. The scientific knowledge about cannabis should be studied in more detail and updated to understand and recommend the best methods of plant productivity management.

MATERIALS AND METHODS

The research was performed during 2018-2020 at the Podolsk State University Higher Education Institution in the Western Forest-Steppe of Ukraine.

Agrochemical characteristics of soil of research sites.

Soil type – chernozem podzolic gley medium loam, in terms of physical and agrochemical properties is characterised as favorable for growing crops. The humus content is 3.2%, the supply of nutrients is as follows: alkaline nitrogen – 100 mg per 1 kg of soil, mobile phosphorus P_2O_5 – 176 mg per 1 kg of soil, exchangeable potassium K_2O_5 – 160 mg per 1 kg of soil. The reaction of the soil solution is close to neutral or neutral – pH of the salt extract 6.8-7.0 mg-eq./100 g of soil, low hydrolytic acidity 0.56-0.62 mg-eq./100 g of soil, the amount of absorbed bases 32-36 mg-eq./100 g of soil.

The humus content was determined by the method of Turin, alkaline hydrolyzed nitrogen – by the method of Cornfield, mobile phosphorus and exchangeable potassium – by the method of Chirikov, the amount of absorbed bases – by the method of Kappen-Gilkovitz, the reaction of soil solution pH.

Meteorological conditions during the research were favorable for the cultivation of technical hemp. The average monthly air temperature during the growing season in 2018, 2019 and 2020 was 17.1 in May, respectively; 14.3; 11.4°C, in June – 18.6; 21.2; 19.2°C, in July – 19.6; 18.8; 19.4°C and in August – 20.8; 20.1; 20.1°C. The amount of precipitation in 2018, 2019 and 2020,

respectively, was 22.1 in May; 155.3; 101.3 mm, in June – 128.7; 81.3; 148.3 mm, in July – 87.1; 59.9; 53.9 mm, in August – 26.5; 39.8; 14.1 mm.

The experiment is organised under the condition of formation of crops between rows 45 cm wide.

Technological factor A is included in the experiment – seed sowing rate 0.6; 0.9; 1.2; 1.5; 1.8 million units/ha.

In conducting field research as a biological object (factor B) involved monoecious varieties of hemp technical selection of the Institute of Bast Cultures NAAS of Ukraine – YUSO-31, Glyana and Glesia, which fully meet modern requirements for drug-free and productivity. In all studied varieties, the THC content corresponded to the norm established by law, which according to the “List of narcotic drugs, psychotropic substances and precursors” (Cabinet of Ministers of Ukraine, 2000), approved by the Cabinet of Ministers of Ukraine from 05/06/2000 No. 770 with the following changes, is 0.08%.

The YUSO-31 variety is a shining example of international recognition of the achievements of the Hemp Breeding Institute, which was included in the State Register of Plant Varieties of Ukraine in 1987, and in the registers of varieties of the European Union, Canada, Republic of Kazakhstan and other countries. The variety has been in international production for more than 30 years. Vegetation period – 110-115 days. Yield of straw – 6.5-8.0 t/ha, fiber – 2.0-2.4 t/ha, seeds – 1.2-1.4 t/ha. Fiber content – 28-30%, lignin – 0.01%, THC – 0.03-0.05%.

The Glyana variety was created using the YUSO-31 variety as a source material based on multiple family-group selection in the selection process using the method of halves. The task was aimed at stabilising the sex in the cannabis population, increasing productivity potential and reducing cannabinoid content (Kabanets & Kabanets, 2018). The characteristics of the variety also indicate: the duration of the growing season – 115-120 days, the yield of stems – 7.5-8.0 t/ha, seeds – 1.0-1.2 t/ha, plant height parameters – 2.5-3.0 m. Year of registration – 2007.

Gler variety was used as a starting material for the cultivation of Glacier variety in order to improve seed productivity (Kabanets & Kabanets, 2018). At the end of the vegetative development of plants, the height of the

variety reaches 2.5-3.0 m. The duration of the growing season is 115-120 days, the yield of stems – 7.5-8.0 t/ha, seeds – 1.5-2.0 t/ha. Year of registration – 2016.

Placement of technical hemp plots is systematised in tiers. Number of repetitions – four times. The total area of the plot is 60 m², the accounting area is 50 m².

The application rate of mineral fertilizers is N₉₀P₄₅K₄₅. Phosphorus and potassium fertilizers have been applied since autumn under the main tillage, nitrogen – in pre-sowing cultivation.

Accounting for the yield of marketable seeds of technical hemp varieties was carried out by the method of continuous threshing.

For the mathematical analysis of the obtained research results we used analysis of variance based on the multi-rank statistical criterion Duncan (Ermantaut *et al.*, 2017).

RESULTS AND DISCUSSION

V.H. Vyrovets *et al.* (2011) found that in the context of hemp seed yields, technical conditions are significantly influenced by growing conditions created by technological factors. These include seeding rates and row spacing. Many publications pay special attention to the fact that the potential of modern varieties of hemp in the cultivation of seeds are better realised in sparse crops. Reducing the seeding rate increases the yield of seeds and reduces the yield of fibrous products. The authors schematically characterise the dependence of seed and straw yields on seeding rates. Reveal patterns in changing the yield of one and the other type of hemp production from the seeding rate. This forces specialists to search for optimal sowing rates that would meet the requirements of production separately for seeds and separately for fibrous products, including for the bilateral use of both seed and fibrous products. As a result of the conducted researches which are directed on achievement of indicators of seed productivity of technical hemp, norms of sowing of seeds of 0.6 are involved; 0.9; 1.2; 1.5 and 1.8 million units/ha with a row spacing of 45 cm. Varieties of monoecious non-narcotic cannabis YUSO-31, Glyana and Glesia are included (Table 1). The maximum level of yield at the sowing rate of 0.6 million units/ha was provided by the variety Glesia – 1.737 t/ha.

Table 1. Yield of commercial hemp seed technical depending on the impact of sowing rates and varieties for row spacing 45 cm, t/ha (average for 2018-2020)

Seed sowing rates, million units/ha (factor A)	Varieties (factor B)			Average factor A
	YUSO-31	Glyana	Glacier	
0.6	1.271	1.506	1.737	1.505
0.9	1.219	1.465	1.662	1.449
1.2	1.178	1.422	1.572	1.391
1.5	1.143	1.381	1.513	1.345
1.8	1.107	1.344	1.479	1.310
Average factor B	1.184	1.424	1.592	1.400

In the conditions of the North-Eastern Forest-Steppe of Ukraine according to similar researches, the author of which is V.M. Kabanets (2017), set at the included variants of sowing rates 0.5; 1.0; 1.5; 2.0; 2.5 million units/ha, which is characterised by the best seed yield of 1.0 million units/ha, where the figure is 1.7 t/ha.

Analysis of the data based on the analysis of variance by Duncan's test for the YUSO-31 variety shows that during all the years of research, each seed sowing rate had a significant impact on changes in seed yield. In 2018, the maximum level of yield was at the rate of sowing 0.6 million units/ha and amounted to 1.255 t/ha.

The involved sowing rate of 0.9 million units/ha led to the formation of slightly lower productivity of crops, as a result of which the yield decreased significantly – by 60 kg (Table 2). Increasing the rate to 1.2 million units/ha again significantly reduced the seed yield in the variety YUSO-31, where the figure was 1.133 t/ha. A further increase in the seeding rate to 1.5 million units/ha did not provide an equivalent yield compared to the previous seeding rate, on the contrary, it caused a significant decrease. At the highest sowing rate of 1.8 million units/ha, the seed yield was the lowest and amounted to 1.067 t/ha.

Table 2. Dependence of yield of marketable hemp seeds of technical grade YUSO-31 on influence of sowing norms at width between rows of 45 cm by Duncan's criterion, t/ha

Sowing rate, million units/ha	Year			Average for three years	Homogeneous groups				
	2018	2019	2020		1	2	3	4	5
0.6	1.255	1.192	1.365	1.271	****				
0.9	1.195	1.161	1.300	1.219		****			
1.2	1.133	1.127	1.275	1.178			****		
1.5	1.100	1.080	1.250	1.143				****	
1.8	1.067	1.048	1.207	1.107					****

In 2019, the patterns of seed yield of the YUSO-31 variety remain the same as in 2018. The maximum productivity of technical hemp crops corresponds to the lowest seeding rate. Accordingly, the rate of 0.6 million units/ha in the study contributed to a significantly higher yield of seeds of the variety YUSO-31 – 1.192 t/ha. Each subsequent increase in seeding rate led to a significant decrease in seed yield. According to the norms of sowing seeds 0.9; 1.2; 1.5; 1.8 million units/ha, yield levels were set at 1.161; 1.127; 1.080 t/ha; 1.048 t/ha, where each norm provided a significant impact on the performance characteristic.

In 2020, the yield was slightly higher, but the distribution of indicators depending on the seeding rate remains the same as in previous years. The maximum level of seed productivity is set at sowing rates of 0.6 million units/ha – 1.365 t/ha. Each increase in seeding rate in the order indicated in the table led to a significant decrease in the yield of hemp seeds variety YUSO-31. According to the sowing rates of 1.8 million units/ha, the yield was the lowest and amounted to 1.207 t/ha. The difference

in data in yield levels between the highest and lowest seeding rates is 158 kg.

The analysis of data from the Duncan test for the Glan variety on the effect of sowing rates on the yield of technical hemp seeds does not differ from the pattern established for the YUSO-31 variety. In 2018, the maximum level of yield was also provided by the seed sowing rate of 0.6 million units/ha. The corresponding figure was 1.505 t/ha, which differs significantly from all other data obtained (Table 3). For each seeding rate 0.6; 0.9; 1.2; 1.5; 1.8 million units/ha according to the results of statistical calculations, the established seed yield indicators are allocated to separate statistical groups. This testifies to the regularity of the influence of each seed sowing rate with its increase on the development of significantly lower seed productivity of hemp crops of technical grade Glyana. The lowest value of seed yield was at the highest seeding rate. The range of changes in yields involved in sowing rates from 0.6 million units/ha to 1.8 million units/ha is in the range from 1.505 t/ha to 1.349 t/ha, respectively, the difference is 156 kg.

Table 3. Dependence of yield of marketable hemp seeds of technical grade Glyana on the influence of sowing rates at a row spacing of 45 cm according to the Duncan criterion, t/ha

Sowing rate, million units/ha	Year			Average for three years	Homogeneous groups				
	2018	2019	2020		1	2	3	4	5
0.6	1.505	1.420	1.592	1.506	****				
0.9	1.475	1.380	1.540	1.465		****			
1.2	1.435	1.332	1.500	1.422			****		
1.5	1.400	1.288	1.455	1.381				****	
1.8	1.349	1.253	1.431	1.344					****

In 2019, seed sowing rates provided a similar impact as in 2018. The best level of yield of 1.420 t/ha was set at the sowing rate of 0.6 million units/ha, at the sowing rate of 0.9 million units/ha was significantly lower and amounted to 1.380 t/ha. Increasing the seeding rate to 1.2 million units/ha led to a further significant decrease in crop productivity, with a yield of 1.332 t/ha. The seeding rate of 1.5 million units/ha caused a decrease in the yield of Glyana hemp to 1.288 t/ha, and the rate of 1.8 million units/ha – to 1.253 t/ha.

In 2020, the regularity of the impact of seed sowing rates on the yield of Glyana variety was the

same as in previous years. Each norm of sowing of seeds, as an effective sign corresponds to a certain value of the level of yield, which is allocated to a separate homogeneous group. According to the involved norms of sowing of seeds 0.6; 0.9; 1.2; 1.5; 1.8 million units/ha, the yield level of Glyana hemp was $1.592 > 1.540 > 1.500 > 1.455 > 1.431$ t/ha.

Regarding the yield of Glesia hemp depending on the influence of seed sowing norms, no differences in the regularity of statistical distribution of data were found, it was similar to Glyana and YUSO-31 varieties (Table 4).

Table 4. Dependence of yield of marketable hemp seeds of technical grade Glesia on the influence of sowing rates at a row spacing of 45 cm according to the Duncan criterion, t/ha

Sowing rate, million units/ha	Year			Average for three years	Homogeneous groups				
	2018	2019	2020		1	2	3	4	5
0.6	1.698	1.733	1.779	1.737	****				
0.9	1.655	1.605	1.727	1.662		****			
1.2	1.560	1.473	1.683	1.572			****		
1.5	1.506	1.404	1.628	1.513				****	
1.8	1.480	1.370	1.586	1.479					****

In 2018, the maximum seed yield of 1.698 t/ha was at the sowing rate of 0.6 million units/ha. Significantly lower yields were obtained when sowing seeds of 0.9 million units/ha – 1.655 t/ha, but higher compared to all other sowing rates. At the sowing rate of 1.2 million units/ha, the yield of 1.560 t/ha was significantly lower compared to the sowing rates of 0.9 million units/ha, and the yield at the sowing rate of 1.5 million units/ha was significantly lower compared to the data obtained by sowing rates of 1.2 million units/ha. At the sowing rate of 1.8 million units/ha, the yield was the lowest and amounted to 1.480 t/ha.

In 2019, similarly, the highest yield was formed at the rate of 0.6 million units/ha – 1.733 t/ha. Significantly lower hemp yield was at a higher rate of 0.9 million units/ha and amounted to 1.605 t/ha. Increasing the seed sowing rate to 1.2 million units/ha led to a further decrease in the productivity of Glesia hemp crops, as a result of which the seed yield decreased to 1.473 t/ha, or 132 kg compared to 0.9 million units/ha. The reduction of the seed sowing rate to 1.5 million units/ha and 1.8 million units/ha similarly caused a significant decrease in yield results, which confirms the obtained figures of

1.404 t/ha and 1.370 t/ha.

The test results for 2020 are characterised by similar patterns that were established in previous years. The maximum yield was achieved at the sowing rate of 0.6 million units/ha – 1.779 t/ha. Increasing the sowing rate to 0.9 million units/ha resulted in a decrease in yield by 52 kg. With a sowing rate of 1.2 million units/ha, the yield was even lower – 1.683 t/ha. A further increase in seeding rate led to a decrease in seed yield. Respectively, according to the norms of 1.5 million units/ha and 1.8 million units/ha, the indicators obtained for the Glacia variety were 1.628 t/ha and 1.586 t/ha.

Thus, the regularity of the influence of seed sowing norms on the yield of Glesia hemp is characterized by a significant compliance with each of them by the seed productivity of crops at a given width between rows in crops of 45 cm.

Regarding the comparison of yields of varieties according to Tables 2, 3, 4 Glesia variety is characterised by higher productivity. Statistical calculations based on the Duncan criterion show the predominance of the Glacia variety over the YUSO-31 and Glyana varieties (Table 5).

Table 5. Dependence of yield of commercial hemp seed technical on the influence of the variety at a row spacing of 45 cm according to the Duncan criterion, t/ha

Sowing rate, million units/ha	Year			Average for three years	Homogeneous groups		
	2018	2019	2020		1	2	3
Glacier	1.580	1.517	1.680	1.592	****		
Glyana	1.433	1.335	1.504	1.424		****	
YUSO-31	1.150	1.122	1.279	1.184			****

In 2018, the yield data of varieties according to the established indicators differ significantly compared to each other. The maximum productivity was provided by the variety Glesia, where the figure was 1.580 t/ha. Significantly lower seed yield was in the variety Glyana – 1.433 t/ha, the difference is 0.147 t/ha. Variety YUSO-31 is characterised by a yield of 1.150 t/ha, which is less than the data of the variety Glesia – by 0.430 t/ha.

A similar data distribution was in 2019. The maximum yield was formed by the cultivar Glesia – 1.517 t/ha, while in the cultivar Glyana the indicator was significantly lower, by 0.182 t/ha. YUSO-31 variety provided hemp seed yield at the level of 1.122 t/ha, which is 0.213 t/ha less than Glyan variety.

The same statistical distribution of data on the yield of varieties was in 2020. The highest yield of hemp seeds was shown by the variety Glesia, significantly inferior to the variety Glyana, and the variety YUSO-31 was inferior to the variety Glyana.

CONCLUSIONS

Efficiency in management of productivity of crops of hemp technical on a level of productivity of commodity seeds on the basis of application of the technological factor – norms of sowing of seeds and a biological factor – a variety of monoecious non-narcotic hemp in the conditions of the

Western Forest-Steppe of Ukraine is established.

Based on the data obtained as a result of statistical studies using the multi-rank Duncan criterion, it was found that the norms of sowing technical hemp seeds have a significant impact on the yield of commercial seeds of crops wide-row sowing for row spacing 45 cm. In accordance with seeding rates of 0.6; 0.9; 1.2; 1.5; 1.8 million units/ha on the average for years of researches on experiment the following indicators of productivity are established: 1.505 > 1.449 > 1.391 > 1.345 > 1.310 t/ha. With the increase in the seeding rate from 0.6 million units/ha to 0.9 million units/ha, the yield of commercial hemp seed decreased by 3.7%, at the seeding rate of 1.2 million units/ha compared to the previous sowing rates – by 4.0%, sowing rates of 1.5 million units/ha compared to the norm 1.2 million units/ha – by 3.3% and sowing rates of 1.8% compared to the norm 1.5 million units/ha – by 2.6%.

All varieties of monoecious technical hemp involved in the research provided the maximum realisation of biological potential in terms of marketability of marketable seeds in the development of crops at sowing rates of 0.6 million units/ha. Accordingly, according to this sowing rate, the varieties are characterized by the following yield indicators: Glesia – 1.737 t/ha, Glyana – 1.506 t/ha and YUSO-31 – 1.271 t/ha.

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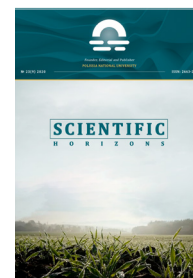
Урожайність товарного насіння конопель технічних залежно від впливу норм висіву та сорту

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Анотація. На сучасному етапі забезпечення розвитку технології вирощування ненаркотичних конопель постійне завдання полягає у необхідності дослідження реакції нових сортів на умови формування агрофітоценозу за різних параметрів густоти рослин на одиниці площі посіву. Потенціал однодомних ненаркотичних сортів коноплі, виведених в Інституті луб'яних культур Національної академії аграрних наук, у технології вирощування на насіння в умовах західних зон землеробства України недостатньо досліджено. Мета досліджень полягає у встановленні залежності урожайності товарного насіння посівів конопель технічних від технологічного фактора А, а саме норм висіву на основі їхньої диференціації, а також біологічного фактора В, тобто сорту однодомних конопель при організації агрофітоценозу за широкорядного способу сівби в умовах західного Лісостепу України. Дані щодо вивчення однодомних ненаркотичних конопель сучасних сортів отримані на підставі організованого польового досліді в межах експериментальних одиниць за умови різних норм висіву насіння. Застосовані методи для узагальнення результатів досліджень: загальнонаукові, в основі яких є об'єктивність, доказовість, відтворення та математично-статистичний – для обробки експериментальних даних. Встановлено залежність урожайності насіння коноплі від сорту, де різниця даних становить у середньому по досліді до 25 %. Доведено, що норми висіву насіння за широкорядного способу сівби є результативним фактором у забезпеченні реалізації продуктивності сортів коноплі в посівах. Отримані результати дають підстави стверджувати, що диференціація фактора норми висіву насіння за кроком експерименту 0,3 млн шт./га забезпечує результативний розподіл отриманих даних на окремі статичні групи. Це дає можливість виділити кращі варіанти норм висіву насіння з метою рекомендації виробництву. Перспективи подальшого дослідження полягають у розширеному вивченні вирощування сорту Глєсія коноплі технічної одночасно на урожайність товарного насіння та волокнистої продукції, тобто на двобічне використання безпосередньо в умовах західного Лісостепу України

Ключові слова: ширина міжрядь, продуктивність посівів, фактори, однодомні сорти конопель, ненаркотичні коноплі, закономірність впливу, ефективність



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Evaluation of Winter Barley Cultivars for Resistance to Leaf Fungal Diseases and Yield in the Conditions of the Western Forest-Steppe of Ukraine

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Abstract. Currently, the issue of creating varieties with complex resistance to the most common diseases of winter barley, considering soil and climatic conditions, is extremely important. This is primarily conditioned upon the tasks of protecting the environment from pesticide pollution and contributes to a significant improvement in the sanitary situation. Important in this context is the search for and evaluation of samples with effective disease resistance genes to use them as parental components in crosses. The aim of this study was to identify varieties of winter barley with a high level of yield and resistance to leaf fungal diseases in the western region of Ukraine. The study was conducted using field (assessment of disease development in winter barley varieties), laboratory, analytical and mathematical and statistical methods. The results of research of winter barley variety samples in the competitive variety test for resistance to leaf fungal diseases are highlighted. Varieties with resistance to pathogens of dark brown spot *Bipolaris sorokiniana* Shoem – Zbruch, Shyrokolysty, Liubomyr, Kormovyi, Dnister; and to the causative agents of rhinosporiosis *Rhynchosporium graminicola* Heinsen – Shyrokolysty, Liubomyr, Kormovyi, Dnister, Babylon, Babylon x HE 0.05%. The highest grain yields on average in 2017-2019 among the studied varieties of winter barley were Zbruch, Obroshynskyi x NE 0.05%, Dnister, Kormovyi, Shyrokolysty, 3.63; 3.42; 3.33; 3.33, 3.25 t/ha. It was found that the most valuable are the varieties: Shyrokolysty, Kormovyi, Dnister, which are characterised by a combination of high index of complex stability with productivity. The highest indicators of stress resistance were found in cultivars: Dnister (-0.07), Obroshynskyi x NE 0.05% (-0.12), Liubomyr (-0.13). High genotypic plasticity was observed in the varieties: Zbruch (3.84), Obroshynskyi x NE 0.05% (3.62) and Dnister (3.51 t/ha). Further research will focus on the development of rhinosporiosis and dark brown spots of winter barley depending on environmental factors in the western region of Ukraine

Keywords: yield, rhinosporiosis, dark brown spot, index of complex resistance, stress resistance, genotypic plasticity



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INTRODUCTION

In the grain balance of Ukraine, one of the main insurance crops is winter barley, which has a fairly high profitability, its cultivation requires minimal costs. The area under winter grain in Ukraine in 2019 was 7.2 million hectares, and in the Lviv region – 22.9 thousand hectares (Petrychenko & Likhochvor, 2020). The increase in the production of barley grain as a food and feed crop is explained by the modern concept of nutrition, which involves the use of a wide range of foods with high taste, the need to provide the human body with sufficient proteins, carbohydrates, minerals, essential amino acids, vitamins, etc. (Bilovus & Zayats, 2014; Lykhochvor & Matkovska, 2017; Mikhailenko, 2008).

Modern technologies of the selection process of such an important crop as winter barley, help to create new varieties with high yields at relatively low economic costs.

The experience of Ukrainian and world selection shows that in the process of creating varieties of winter barley in some cases the availability of source material is crucial, which combines productivity with adaptive traits (Gudzenko & Vasylykivsky, 2017; Demidov *et al.*, 2017; Anbessa *et al.*, 2010; Van Oosterom & Acevado, 1992).

Extensive adaptation of the variety to radically different growing conditions is a relatively rare case. In the history of selection and production, such unique generally recognised varieties were: wheat – Myronivska 808, barley – Wiener (Vasylykivskyi & Gudzenko, 2017; Hudzenko *et al.*, 2019). However, varieties with wide adaptability may be inferior to more locally adapted in specific conditions (Abessa *et al.*, 1992; Ceccarelli, 1996). This is especially true of conditions with strong pressure of environmental factors (drought, waterlogging, etc.) (Van Oosterom & Acevado, 1992; Von Korff *et al.*, 2008).

Data from a recent survey of 410 farmers and 114 consultants in Germany on the impact of recent climate change on plant productivity are almost identical. The increase in the frequency of droughts and dry winds, the increase in the incidence of heavy rains, and the mitigation of winters have been noted as the main effects of climate change. Based on empirical data, respondents are inclined to believe that special varieties are needed to adapt to these changes. Productivity, ecological stability, resistance to lodging and drought tolerance are noted as the main features of such varieties. There is a unanimous opinion on the need for continuous research on the ecological stability of the varieties created (Macholdt & Honermeier, 2016).

In connection with global climate change and warming, the selection of varieties of winter cereals for specific soil and climatic conditions with high winter hardiness and disease resistance is of particular importance (Levitin, 2012; Solonechnyi *et al.*, 2017).

One of the factors that negatively affects the yield is the impact of harmful pathogens. According to leading scientists (Solonechnyi *et al.*, 2017; Vasylykivsky

& Sabadyn, 2015; Lytvynenko & Volkodav, 2005), plant diseases are one of the main factors destabilising agricultural production. In most areas of Ukraine, fungal pathogens of winter barley reduce yields and worsen grain quality. Leaf fungal diseases, such as powdery mildew, rhiniosporiosis, reticulate, striped and dark brown spots, dwarf rust, root rot, etc., are the most harmful. Losses from widespread pathogens of these diseases range from 6.0 to 50.0% (Solodushko, 2013).

In the agroecosystem of winter barley there is a constant change in the virulence of pathogens. Damage to plants by pathogens depends not only on factors of plant resistance, but also on the state of the pathogen population and environmental conditions. The emergence and reproduction of new races of pathogens leads to the loss of resistance varieties. With the help of growing resistant varieties can significantly improve the ecological characteristics of agroecosystems, increase the profitability of plant production (Solonechnyi *et al.*, 2014).

Among the main causes of mass destruction of cereals, researchers call the narrowing of genetic diversity, insufficient use in the selection of introgression of resistance genes from wild plant species and the disappearance of local adapted varieties – sources of resistance to biotic and abiotic environmental factors (Solonechnyi *et al.*, 2014; Solonechnyi *et al.*, 2017; Blyzniuk *et al.*, 2019).

A genotype with complex resistance to dark brown spot pathogens and other diseases is rare, which is especially valuable for the selection of geneticists. Creation and introduction into production of resistant varieties of this culture to the action of various diseases (with a sign of complex resistance) is a priority for breeders and geneticists (Solonechnyi *et al.*, 2017; Blyzniuk *et al.*, 2019; Shishkin & Derova, 2014). It has already become clear that the use of sustainable varieties is an important environmental factor that provides a significant reduction in energy costs for crop production. In this regard, breeders and phytopathologists are faced with the need to create varieties that combine the same genotype traits of productivity and disease resistance. The negative impact of diseases is manifested in many ways and in different ways depending on the methods of spread, sources of infection, phase of culture development and weather conditions. To effectively control diseases, it is necessary to consider not only weather conditions, phases of crop development, but also have clear information about the etiology of the disease and the biology of pathogens.

Dark brown spot is the causative agent of *Bipolaris sorokiniana* Shoem. Prolonged wet and warm windy weather during the growing season causes damage to crops; precipitation; late sowing dates; application of nitrogen fertilizers only. The disease leads to a decrease in the assimilation surface area, premature drying of leaves and plants, reduced grain yield and deterioration of its sowing and technological qualities. With the intensive development of the disease, crop losses can be 30-40% (Sabadin, 2008; Bilovus & Marukhnyak, 2019).

Rhinosporiosis is the causative agent of *Rhynchosporium graminicola* Heinsen. Symptoms of the disease are manifested on both sides of the leaf and leaf sheaths in the form of gray-green watery, and later when drying – light spots with a dark brown border. Initially, the leaves of the lower tiers are infected, but with significant moisture, the disease spreads throughout the plant – to the flag leaf and ear. With the intensive development of the disease in the epiphytic years before the shortage of 25% or more (Bilovus & Marukhnyak, 2019). Thus, the creation of high-yielding varieties of winter barley with high fodder value is an urgent issue today, in particular in the Western Forest-Steppe of Ukraine.

The purpose of the work is to identify varieties of winter barley with a high level of yield and resistance to leaf fungal diseases in the Western region of Ukraine.

MATERIALS AND METHODS

The research was conducted in 2017-2019 in the conditions of selection and seed crop rotation of the laboratory of grain and fodder crops selection and in the laboratory conditions (plant protection laboratory) of the Institute of Agriculture of the Carpathian region of NAAS. The object of the study were 12 varieties of winter barley, various institutions-origins, which are included in the Register of plant varieties suitable for distribution in Ukraine. The soil of the experimental plot is gray forest surface gleyed, which is characterised by the following agrochemical indicators: humus content in the layer 0-20 cm (according to Tyurin) – 2.1%, saline pH – 5.8, lightly hydrolyzed nitrogen (according to Cornfield) – 112.7 mg/kg, mobile forms of phosphorus (according to Kirsanov) – 111.0 mg/kg, potassium (according to Kirsanov) – 109.0 mg/kg of soil. Agrotechnics of cultivation of winter barley cultivars is generally accepted for winter barley in the conditions of the western region of Ukraine. Predecessor – oil radish, background of mineral nutrition – $N_{60}P_{60}K_{60}$, agricultural machinery – generally accepted for growing winter barley in the research area. The estimated area of the site is 25 m², repetition – four times. Sowing was carried out with a selection seeder SKS-6-10 with the device of the central seeding, harvesting – the combine “Sampo-130”.

The intensity of disease of winter barley plants was determined on a 9-point scale (Babyants *et al.*, 1998):

- 9-8 – very high and high stability;
- 7-6 – stable;
- 5 – weak susceptibility;
- 4-3 – susceptibility;
- 2 – high susceptibility;

1 – very high susceptibility.

Phenological observations of the development of winter barley plants were carried out and the indicators of crop structure and technological qualities of grain varieties were determined according to the method of V.O. Yeshchenko (2014).

Records of diseases on winter barley were carried out in the phase of tube emergence, earing and milk ripeness according to generally accepted methods (Babyants *et al.*, 1998; Tribel *et al.*, 2001).

Individual resistance indices were calculated as the ratio of the average long-term value of resistance for an individual pest to the average for all samples studied. Indices of complex resilience expressed the average value of indices of individual resilience (Litun *et al.*, 2009).

According to the description of weather conditions for 2017-2019, the data of the Lviv Hydrogeological and Reclamation Station were used, the observation point was v. Obroshine. To determine the influence of climatic factors, in particular the amount of precipitation and temperature on the development of diseases used hydrothermal coefficient (SCC) in the period April-July (Lyashenko, 2014). When assessing the agro-climatic resources of this area, it was considered that SCC in the range of 1.0-1.5 characterises optimal humidity, greater than 1.5 – excessive, less than 1.0 – unstable, less than 0.5 – weak.

Statistical reliability of experimental data was performed using Microsoft Excel by determining the mean, minimum (m), maximum values (max) and range of variation. Mathematical processing of yield data was performed by the dispersion method (Ushkarenko *et al.*, 2013). In the comparative evaluation of the studied varieties, the indexing method was used, according to which the disease damage of plants in points was translated into an indicator of distance from the average value for all studied samples (resistance indices). The level of resistance to stress was determined by the difference between the minimum and maximum yield ($Y_2 - Y_1$). It is negative, and the greater its value, the greater the resistance to stress. The characteristic of varieties in terms of resistance to stress is complemented by the value $(Y_2 - Y_1)/2$, which expresses the degree of correspondence between the genotype of the variety and various environmental factors (Sych, 2005).

RESULTS AND DISCUSSION

With the beginning of the growing season in April 2017, precipitation was 16.1 mm less than normal, or 68% of normal (Table 1).

Table 1. SCC according to average monthly data for the period 2017-2019 (April-July)

Indicator	Period				
	April	May	June	July	For the whole period
2017					
Average air temperature (t_{avg}), °C	8.5	13.8	18.2	18.5	14.7
The amount of precipitation, mm	34.9	85.3	22.2	57.2	199.6
The sum of active temperatures ($t_{act} > 10$), °C	–	428	546	573	1547
SCC of Selyaninov	–	2.0	0.4	1.0	1.3
2018					
Average air temperature (t_{avg}), °C	13.7	16.9	18.3	19.2	17.0
The amount of precipitation, mm	21.6	69.0	153.5	116.0	360
The sum of active temperatures ($t_{act} > 10$), °C	411	524	549	595.2	2079.2
SCC of Selyaninov	0.52	1.3	2.8	1.9	1.73
2019					
Average air temperature (t_{avg}), °C	10.0	13.2	21.2	18.3	15.7
The amount of precipitation, mm	32.8	149.6	53.1	81.2	316.9
The sum of active temperatures ($t_{act} > 10$), °C	300	409	636	567	1912
SCC of Selyaninov	1.1	3.65	0.83	1.43	1.6

Thus, in April the temperature exceeded the norm by 1.1°C and amounted to 8.5°C, and in May an increase of 0.9°C was observed. The amount of precipitation in the third decade of May was 22 mm more than normal. In June-July there was a significant lack of precipitation. In June, only 22.2 mm fell – 24% to the norm, in July 57.2 – 56%. Even higher deviation from the norm was in June +1.9°C. The average monthly temperature in July was 18.5°C, which is 1°C higher than normal.

It should be noted that according to the calculations of the SCC on the average monthly data in 2017, the period from April to July was quite wet.

Because in April in the II and III decades there are no active air temperatures above 10°C, so the SCC for this month was not expected. It should be noted that May was excessively wet (SCC – 2.0), June – very dry (SCC – 0.4), and July – quite wet (SCC – 1.0). The third decade of May was favorable for the development of diseases such as dark brown spot, rhinosporiosis.

In April 2018, there was a sharp increase in air temperature to 13.7°C (+6.3°C above normal), in May to 16.9°C (+4.0°C above normal). The regime of soil moisture in these months did not meet the needs of plants. In June, there was a sharp change in the humidification regime (+60.5 mm to normal) with increasing air temperature (+2.0°C to normal), which led to the accelerated formation of reproductive organs of plants. The air temperature in July was 1.7°C higher than the perennial, and the amount of precipitation was 14.0 mm higher than normal.

Thus, the SCC calculations show that in addition to April, the growing season in May-July was quite wet. The month of April was very dry (SCC=–0.5), May was

quite wet (SCC=–1.3), and June and July were excessively wet. It should be noted that the weather conditions that were during the growing season of winter barley in 2018 contributed to the development of dark brown spot, rhinosporiosis. In 2019, April was characterised by warm and dry weather (air temperature was 2.6°C above normal and precipitation was 18.2 mm less than normal). The air temperature in May was 0.3°C above the norm, and the amount of precipitation was 64.6 mm higher than the norm (Table 1).

In summer, weather conditions differed. June was characterised by warm and relatively dry weather (air temperature was 4.9°C above normal, and precipitation was 39.9 mm less than normal). The air temperature in July was 0.8°C higher than the perennial, and the amount of precipitation was 20.8 mm less than normal. According to the results of SCC calculations in 2019 (Table 1), we can conclude that the period April-July was quite wet. April and July were quite wet (SCC=–1.1), May – excessively humid (SCC=–3.6), June – mild drought (SCC=–0.83).

It should be noted that weather conditions in the third decade of May and the first decade of June contributed to the development of diseases such as dark brown spot, rhinosporiosis. Thus, the weather conditions of 2017-2019 contributed to the maximum development of pathogens conditioned upon optimal and excessive humidity and the optimal air temperature.

Such conditions allowed to reliably assess the samples of winter barley for resistance to dark brown spot, rhinosporus. The development of dark brown spots, depending on the variety samples in 2017 was: 3.5-15.0%, in 2018 – 5.5-25.0%, in 2019 – 4.5-35.0%; rhinosporiosis – 2.5-25.5%; 5.5-35.0%; 3.5-28.5%.

Researchers S.V. Mikhailenko (2008), N.M. Shakhova & A.I. Shapovalov (2014), O.I. Borzykh (2015), O.V. Chaika & M.M. Klyuchevych (2009) and others argue that the timing of dark brown spots depends on the average air temperature and the amount of precipitation in May, which is confirmed by our research results. The problem of barley immunity in Ukraine has recently attracted the attention of many scientists in the field of crop production, including V.Ya. Sabadin (2008), V.M. Gudzenko (2013) and other. As a result of the work carried out in this direction, the sources of resistance to the main diseases of winter barley have been identified, including varieties and lines with high resistance to abiotic factors

have been created. Immunological evaluations were performed to determine the stability of winter barley cultivars with rhinosporiosis and dark brown leaf spot. As a result of the immunological evaluation (Table 2) marked varieties with high resistance to rhinosporiosis pathogen *Rhynchosporium graminicola* Heinsen (score 7), namely: Shyrokolistyi (St), Liubomyr, Kormovyi, Dnister, Babylon, Babylon x NE 0.05%. It should be noted that on average in 2017-2019 high resistance (score 8-7) to the causative agent of dark brown spot *Bipolaris sorokiniana* Shoem showed varieties: Zbruch, Shyrokolistyi (St), Liubomyr, Kormovyi, Dnister.

Table 2. Resistance of winter barley varieties against leaf diseases, average for 2017-2019

Variety sample	<i>Rhynchosporium graminicola</i> Heinsen		<i>Bipolaris sorokiniana</i> Shoem		Index of complex stability, ICS
	Score	Stability index, I	Score	Stability index, I	
Shyrokolistyi (St)	7	1.06	7	1.1	1.08
Zbruch	6	0.91	8	1.24	1.07
Liubomyr	7	1.06	7	1.1	1.08
Kormovyi	7	1.06	7	1.1	1.08
Dnister	7	1.06	7	1.1	1.08
N5 Ca (M6xM7)	6	0.91	6	0.93	0.92
ID No. 1453	6	0.91	5	0.78	0.84
Babylon	7	1.06	6	0.93	0.99
Babylon x NE 0.05%	7	1.06	6	0.93	0.99
Babylon x NE 0.05%	7	1.06	6	0.93	0.99
Obroshynskyi x NE 0.05%	6	0.91	6	0.93	0.92
Obroshynskyi x NE 0.05%	6	0.91	6	0.93	0.92
X*	6.6	0.99	6.41	1.0	0.99
min**	6.0	0.91	5.0	0.93	0.84
max***	7.0	1.06	8.0	1.24	1.08
R.	1.0	0.15	3.0	0.31	0.24

Note: x* – average, min ** – minimum value, max *** – maximum value, R **** – range (max-min)

According to the indices of resistance less affected by rhinosporiosis showed varieties: Shyrokolistyi, Liubomyr, Kormovyi, Dnister, Babylon, Babylon x NE 0.05%; dark brown spots: Shyrokolistyi, Zbruch, Liubomyr, Kormovyi, Dnister (Table 2).

The level of resistance of winter barley cultivars to pathogens was assessed by the index of complex resistance (ICS). The highest indicators of complex resistance to pathogens of two diseases were observed in cultivars: Shyrokolistyi, Liubomyr, Kormovyi, Dnister (ICS=1.08).

Variety as a genetic system responds specifically to external environmental factors. A distinctive feature of any variety is a set of properties that determine its

suitability for a particular area, and therefore the correct choice of variety is of paramount importance in the cultivation of cereals.

According to the results of research (Table 3) on average over the years of research varieties Zbruch (3.63 t/ha), Obroshynskyi x NE 0.05% (3.42), Kormovyi (3.33 t/ha), Dnister (3.33 t/ha), Shyrokolistyi (3.25 t/ha) had the highest grain yield. In the current climate change, an important indicator of varieties is their resistance to stress. The stress-resistance indicator has a negative sign and the smaller its value, the higher the stress-resistance of the variety. Dnister (-0.07), Obroshynskyi x NE 0.05% (-0.12), Liubomyr (-0.13) had the highest indicators of stress resistance.

Table 3. Adaptability and yield of winter barley cultivars, average for 2017-2019

Variety sample	Yield, t/ha			Yield, t/ha	Genotypic plasticity, t/ha
	Y ₂ (min)	Y ₁ (max)	X*		
Shyrokolystyi (St)	3.00	3.57	3.25	-0.57	3.29
Zbruch	3.71	3.97	3.63	-0.26	3.84
Liubomyr	3.13	3.26	3.04	-0.13	3.19
Kormovyi	3.03	3.81	3.33	-0.78	3.42
Dnister	3.48	3.55	3.33	-0.07	3.51
N5 Ca (M6 x M7)	3.28	3.48	3.20	-0.2	3.38
ID No. 1453	3.40	3.60	3.30	-0.2	3.50
Babylon	2.99	3.38	2.93	-0.39	3.18
Babylon x NE 0.05%	3.07	3.26	3.0	-0.19	3.16
Babylon x NE 0.05%	2.96	3.35	2.94	-0.39	3.15
Obroshynskiy x NE 0.05%	3.34	3.46	3.23	-0.12	3.40
Obroshynskiy x NE 0.05%	3.55	3.69	3.42	-0.14	3.62
X*	3.24	3.53	3.22	-0.29	3.39
min**	2.99	3.26	2.93	-0.07	3.15
max***	3.71	3.97	3.63	-0.78	3.84
R.	0.72	0.71	0.7	-0.71	0.69

Note: Y₂(min) – minimum grain yield, Y₁(max) – maximum grain yield, X* – average, min ** – minimum value, max *** – maximum value, R **** – range of variation (max-min)

The average yield of varieties in contrasting conditions (stress and non-stress) characterise their genotypic plasticity. The high value of this indicator indicates a high degree of correspondence between the genotype of the variety and environmental factors. The maximum relationship between genotype and environmental factors was observed in cultivars: Zbruch (3.84), Obroshynskiy x NE 0.05% (3.62) and Dnister (3.51).

According to generalised data, barley crops are damaged by more than 200 pests. At the same time, they have certain specific features regarding ecological and geographical origin. According to the results of our research, which coincide with the results of well-known researchers: V.M. Gudzenko & S.P. Vasylykivskiy (2017) O.A. Demidov et al. (2017), P.M. Solonechniy et al. (2017), V.Ya. Sabadin (2008) among the studied varieties of immune and highly resistant to several diseases at the same time is not identified. Given the above, to create varieties with group resistance, it is necessary to use in crossbreeding sources with high resistance to certain diseases, followed by selection of varieties with group resistance and productivity, considering soil and climatic conditions of the growing area.

CONCLUSIONS

According to the results of three-year research, winter barley varieties with resistance to dark brown spotting

were identified: Zbruch, Shyrokolystyi, Liubomyr, Kormovyi, Dnister; and to rhinosporiosis – Shyrokolystyi, Liubomyr, Kormovyi, Dnister, Babylon, Babylon x NE 0.05%.

On average, in 2017-2019, the highest grain yield among the studied varieties of winter barley was observed in the varieties: Zbruch, Obroshynskiy x NE 0.05%, Dnister, Kormovyi, Shyrokolystyi, respectively 3.63; 3.42; 3.33; 3.33, 3.25 t/ha. It was found that the most valuable are the varieties: Shyrokolystyi, Kormovyi, Dnister, which are characterised by a combination of high index of complex stability with productivity.

High resistance to stress was shown by Dnister (-0.07), Obroshynskiy x NE 0.05% (-0.12), Liubomyr (-0.13). High genotypic plasticity was observed in the varieties: Zbruch (3.84), Obroshynskiy x NE 0.05% (3.62) and Dnister (3.51 t/ha). According to the results of research, it is established that the most valuable varieties are Kormovyi, Dnister, Shyrokolystyi, which are characterised by a combination of high index of complex resistance to major diseases and productivity.

Isolation of genotypes with high productivity potential and high complex resistance to fungal diseases for the conditions of the Western Forest-Steppe of Ukraine with further use in practical breeding is an extremely relevant area of research for both breeding and industrial cultivation.

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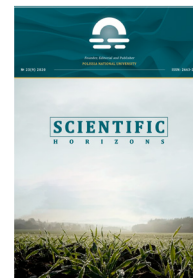
Оцінка сортотразків ячменю озимого за стійкістю до листових грибних хвороб та урожайністю в умовах Західного Лісостепу України

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Анотація. Нині надзвичайно актуальним постає питання створення сортів з комплексною стійкістю проти найбільш поширених хвороб ячменю озимого з врахуванням ґрунтово-кліматичних умов. Насамперед це пов'язано з завданнями охорони навколишнього середовища від забруднення пестицидами і сприяє суттєвому оздоровленню санітарної ситуації. Важливими в цьому контексті є пошук та оцінка зразків з ефективними генами стійкості до хвороб з метою використання їх як батьківських компонентів при схрещуваннях. Метою цього дослідження було виділити сортотразки ячменю озимого з високим рівнем урожайності та стійкості до листових грибних хвороб в умовах західного регіону України. Дослідження проведено з використанням польових (оцінка розвитку хвороб на сортах ячменю озимого), лабораторних, аналітичних і математично-статистичних методів. Висвітлено результати досліджень сортотразків ячменю озимого в конкурсному сортовипробуванні за стійкістю до листових грибних хвороб. Виділено сортотразки зі стійкістю до збудників темно-бурої плямистості *Bipolaris sorokiniana* Shoet – Збруч, Широколистий, Любомир, Кормовий, Дністер; та до збудників ринхоспоріозу *Rhynchosporium graminicola* Heinsen – Широколистий, Любомир, Кормовий, Дністер, Вавілон, Вавілон х НЕ 0,05 %. Найвищою врожайністю зерна в середньому за 2017–2019 рр. серед досліджуваних сортотразків ячменю озимого відзначились сорти Збруч, Оброшинський х НЕ 0,05 %, Дністер, Кормовий, Широколистий відповідно 3,63; 3,42; 3,33; 3,33, 3,25 т/га. Виявлено, що найбільш цінними є сортотразки: Широколистий, Кормовий, Дністер, для яких характерно поєднання високого індексу комплексної стійкості з продуктивністю. Найвищі показники стресостійкості було виявлено у сортотразків: Дністер (-0,07), Оброшинський х НЕ 0,05 % (-0,12), Любомир (-0,13). Високу генотипову пластичність відзначено у сортів: Збруч (3,84), Оброшинський х НЕ 0,05 % (3,62) і Дністер (3,51 т/га). Подальші дослідження будуть зосереджені на вивченні розвитку ринхоспоріозу та темно-бурої плямистості ячменю озимого залежно від екологічних чинників в умовах західного регіону України

Ключові слова: врожайність, ринхоспоріоз, темно-бура плямистість, індекс комплексної стійкості, стресостійкість, генотипова пластичність



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Dynamics of ^{137}Cs Accumulation by Cranberry on Sphagnum Bogs of Polissia of Ukraine

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Abstract. Oligotrophic and mesotrophic bogs are common ecosystems in Polissia of Ukraine. After the Chernobyl accident, these ecosystems were contaminated with radionuclides. A common berry plant in these bogs is the cranberry, which is widely used in food industry. The aim of research is to establish dynamics of migration of ^{137}Cs in cranberries during the last 30 years. The obtained results of monitoring studies are necessary for prognosis of radioactive contamination of cranberry. Investigation was conducted on stationary experimental plots (SEP) in Zhytomyr Polissia. ^{137}Cs specific activity was measured on spectrum analyzers with scintillation and semiconductor detectors. Obtained results testify about intensive ^{137}Cs migration on sphagnum bogs of Polissia of Ukraine. Results of investigation conducted after 34 years of Chernobyl accident showed low values of ^{137}Cs specific activity in cranberry (*Vaccinium oxycoccus* L.) on all stationary experimental plots. This index in 2020 in fresh berries hesitate from 871 ± 82.6 to 286 ± 16.9 Bq/kg (at the permissible level 500 Bq/kg), and in dry shoots – from 1492 (SEP 15) to 6197 Bq/kg (SEP 12). It was found that in the first period (1991-1996) rather significant decreasing of ^{137}Cs specific activity was observed in fresh berries of cranberry – from 3.8 times on SEP 11 to 2.2 times on SEP 12. In the next period (1996-2002) this decreasing was in the limits 1.4-2.9 times that is lower than in previous one. In the following period decreasing of ^{137}Cs specific activity in berries was significantly lower. Revealed regularities of decreasing of ^{137}Cs specific activity in berries during 1991-2020 also confirmed by decreasing of this index in the plant shoots. Total decreasing of this index in dry shoots on the whole period of investigation was: on SEP 11 – from 94590 ± 4236 to 6099 ± 366 Bq/kg (15.5 times), on SEP 12 – from 31800 ± 2325 to 6197 ± 242 Bq/kg (5.1 times), on SEP 15 – from 12120 ± 898 to 1492 ± 112 Bq/kg (8.1 times), on SEP 18 – from 14732 ± 1126 to 1854 ± 112 Bq/kg (7.9 times). Continuation of monitoring on stationary experimental plots will allow to predict levels of radioactive contamination of cranberry on the base of mathematical modeling

Keywords: specific activity, radionuclide, *Vaccinium oxycoccus* L., sphagnum, dry shoots, fresh berries



INTRODUCTION

Features of relief and complex of climatic conditions caused appearance in Polissya of Ukraine of oligotrophic and mesotrophic bogs, which, as is known, are characterized by specific conditions of moistening, flow of geochemical processes, and phytocenotic features. In these conditions, a phytogenic medium is created, in which its leading importance is its edificator – a sphagnum (different species), which has a large hygroscopic capacity. Water, on such bogs, is characterized by excessive acidity, low content of nutrients and oxygen. These circumstances, as well as the absence of bacterial decomposition of organics, lead to significant accumulation and formation of peat. At the same time, the fast-growing bogs play a significant role in maintaining the hydrological regime of the territories, water level in rivers, delay a considerable amount of dust and harmful substances (including radioactive). It's generally accepted that they reduce the amount of carbon dioxide in the atmosphere due to the concentration of a large amount of organics. In view of this, in the middle of the last century, radio-ecologists suggested that swamp ecosystems could play a role in the redistribution of radionuclides in significant areas (Mroz *et al.*, 2017).

After the Chornobyl disaster, researchers began to study the migration of some radioactive elements that came into the environment in different natural and artificial ecosystems and their accumulation in different species of plants that were the most representative or characteristic of the latter. For the study of migration of radionuclides on oligotrophic and mesotrophic bogs such plant sometimes was a cranberry (*Vaccinium oxycoccus* L.). Perhaps this was primarily due to the fact that the berries of this plant are widely used in food by locals for their own consumption and for sale. Cranberry is one of the important resource plants of Ukrainian Polissia and requires constant research of levels of radioactive contamination. In addition, it can serve as an indicative view for the sphagnum bogs of this region, as it has specific biological adaption, characterized by plants growing in these ecological conditions (Kaletnik *et al.*, 1990; Krasnov & Orlov, 2004).

The problems of ^{137}Cs biogeochemical migration in the ecosystems of oligotrophic bogs are focused on fragmented studies carried out mainly in the countries of Northern Europe, in particular, Sweden (Galán, 2006; Rosen *et al.*, 2009; Vinichuk *et al.*, 2010) and Finland (Vetikko *et al.*, 2010). In Ukraine, the laws of ^{137}Cs distribution in the systems of sphagnum bogs were studied in Polissia (Orlov & Dolin, 2010; Holovko, 2012; Holovko & Orlov, 2019; Maloshtan *et al.*, 2019). However, in the given publications the results of researches which scientists have received mostly during 1 year, less – 2-3 years are given.

The purpose of the research was to establish modern levels of radioactive contamination of the cranberry and to detect dynamics of ^{137}Cs specific activity in its berries and shoots during the last 30 years.

The scientific novelty of the research is that for the first time established dependence of dynamics of ^{137}Cs specific activity in berries and the shoots of cranberries during 30 years (1991-2020), which can be used for forecasting of their radioactive contamination in the next period.

LITERATURE REVIEW

In different periods since the Chernobyl disaster, researchers have studied the role of various food products in the radiation of the population in different regions of Polissia of Ukraine, including food products of forests (wild plant berries, edible mushrooms). Thus, scientists who carried out complex radiation-hygienic monitoring in some settlements of Rokytne Raion of Rivne Oblast in 2017 confirmed that wild plants of berry plants (including cranberry) continue to make a significant contribution to annual effective doses of radiation exposure to locals (Vasylenko *et al.*, 2018). Similar monitoring studies were conducted on the territory of the SOE "Polissya Forest Management", which is located in the western direction from the Chornobyl NPP (Chobotko *et al.*, 2018). In general, scientists have concluded that food products of forest origin (including wild berries) make a significant contribution to obtaining an additional dose of internal irradiation (Skrkal *et al.*, 2017), which is observed a significant increase in effective doses of internal irradiation during periods of wild berries and appearance of edible mushrooms. The authors of the above publications say about the necessity of continuation of monitoring of the contents of radio-cells in wild berry plants and mushrooms (Andric & Gajic-Kvascev, 2021). Radio-ecologists, who are currently studying the redistribution of radionuclides in the soil of forest-based automorphic and hydromorphic landscapes, confirm the generalization previously made by numerous researchers that in recent landscapes there is a more intensive migration of ^{137}Cs in soil and its inflow to plants (Imamura *et al.*, 2020). The researchers have developed models of migration of this radionuclide in the system "surface of the above-ground phytomass of plants – surface soil" of forest biogeocenosis (Almahaayni & Houska, 2020).

The researchers determined the contents of some chemical elements and technogenic radionuclides (^{137}Cs) in the cranberries on the sphagnum bogs of Northern Canada (Shotyk *et al.*, 2019), as well as southern Poland (Mroz *et al.*, 2017). The authors of the latest publication presented materials indicating a significant accumulation of radionuclide in the sphagnum mosses, the average concentrations of this radionuclide in these plants are almost 2 times higher than in other plants studied. These data are well correlated with materials that show the contents of potassium in the same plants. It has been found that this chemical element is contained in the sphagnum mosses in significant quantities (Krasnov & Orlov, 2004). It is known that ^{137}Cs is a chemical analogue of potassium and it probably explains its concentration in mosses.

In Poland, studies were carried out on the migration of ^{137}Cs in peat soils of oligotrophic swamp ecosystems, the intensity of the inflow of this radionuclide to plants (Boron *et al.*, 2001). Scientists have established that the observed radioactive element on the sphagnum bogs concentrated in the mosses and surface layers of the tirr (Gaca *et al.*, 2006). At the same time, plants that grow under these conditions, including the cranberry, contain a significant amount of radionuclide (Bunzk & Kracke, 1989). At present, periodic research on levels of radioactive contamination of plants of grass-shrub cover in the forests of the automorphic and hydromorphic landscapes (Malostan *et al.*, 2019) is continuing, but at a much smaller scale and do not deal with the cranberries.

In Ukraine at the beginning of 90-ies of the last century in the forest areas of Polissia of Ukraine were organized monitoring of migration of ^{137}Cs in forest biogeocoenosis and accumulation of this radioactive element of various species of berry plants. Studies on levels of radioactive contamination of the cranberry were carried out on mesotrophic sphagnum bogs. Scientists have studied biological and ecological peculiarities of this species (Orlov & Krasnov, 1998) quite fully, which allowed them to understand the mechanisms of radiation coming to vegetative mass of plants and berries. It is known that the shoots of the cranberry, on which the berries are located, placed on the surface of the sphagnum and can reach 1 m long. The annual rise of sphagnums in height leads to the deepening of a part of the shoot of cranberry in their thickness and formation on it additional roots. They are also responsible for the nutrition of the plant with the bog (surface) water. Since the root system of the cranberry is concentrated at a depth of 15-20 cm from the surface of the sphagnums, it becomes clear that the radiation coming to it will depend on its content in the water solution of the bog (Krasnov & Orlov, 2004). The researchers also found that the mineral nutrition of the cranberry is also due to the trophic connection with other components of this phytocenosis, first of all, with sphagnum mosses. The repartition of the cells is carried out by endophytic and mycorrhizic micromycetes, which combine marsh plants into a single biogeochemical cycle (Kurchenko *et al.*, 2013). The researchers studied the dynamics of ^{137}Cs in the shoots and the berries of the cranberries by the years, as well as its connection with the specific activity of the given radionuclide in various parts of the sphagnum cover (living soil cover, litter and tirr). In general, it is established that there is a gradual reduction of radioactive contamination of generative and vegetative organs of the cranberry with time (Krasnov & Orlov, 2004). In the last 10-15 years, only single publications on the results of the distribution of radionuclides in forest and marsh biogeocoenosis (Malostan *et al.*, 2019; Holiaka *et al.*, 2020) and fragmented data on radioactive contamination of the cranberry (Buzynnyi *et al.*, 2019) have been published.

The review of literature sources allows to make some generalizations and conclusions about the degree of study of intensity of ^{137}Cs migration in bogs of different type, distribution of ^{137}Cs in thickness of sphagnum mosses on oligotrophic and mesotrophic bogs and content of this radionuclide in the cranberry. Since the accident at the Chernobyl Nuclear Power Plant, a small number of studies have been conducted on the sphagnum bogs, due to the technical difficulty of sample selection, determination of the specific and total activity of radionuclide in living and died parts of the sphagnum mosses. The largest values of ^{137}Cs specific activity are marked in different, by depth, parts of the tirr (depending on the type of bog). Significant content of radionuclide in the berries of the cranberry at low levels of radioactive contamination of territories is also marked. It can be noted that in the last 10-15 years such research is not conducted.

MATERIALS AND METHODS

Research on ^{137}Cs accumulation in vegetative part and berries of the cranberry were carried out on stationary experimental plots (SEP) in Zhytomyr Polissia on the territories of SOE "Slovechne Forest Management" and SOE "Bilokorovyh Forest Management". They lasted from 1991 (the first year of observation) for 8 years annually, and in the next period till 2020 – in 5-7 years. SEP size of 100×100 m is laid in quite close ecological and phytocenosis conditions – on mesoligotrophic and oligotrophic bogs, but at different amounts of exposure dose of gamma-radiation. In 1991, it was fluctuating on SEP within 10-120 mCr·h⁻¹ at a height of 1 m from the surface of the bog, and in 2020 – it fluctuates within 5-40 mCr·h⁻¹.

Characteristics of forest range, as well as floristic composition and coenotic structure on SEP are close and differed only by the quantitative presence of certain types. On all SEP there was a cenosis – a pinewood cranberry-cotton grass-sphagnum. The stand composition is 10Sp, age – 60-80 years, completeness – 0.4, average height – 4 m, average diameter – 16 cm, type of forest conditions – wet pine forest. In the undergrowth there are single copies of pine of usual age from 3 to 8 years. The plant cover of the grass-shrub tier was 50-55%. There are cranberry (*Vaccinium oxycoccos* L.) – 15-20%, tussock cottongrass (*Eriophorum vaginatum* L.) – 20-25%, wild rosemary (*Ledum palstre* L.) – 5-8%, roundleaf sundew (*Drosera rotundifolia* L.) – 3-5%, bog bilberry (*Vaccinium uliginosum* L.) – 3-5%, bog-rosemary (*Andromeda polifolia* L.) – 1-3%. In the moss layer, which was solid and even with a design cover of up to 98%, dominated the flat-topped bogmoss (*Sphagnum fallax* Klinggr.) – 40%; Magellanic bogmoss (*Sphagnum magellanicum* Brid.) – 45%, met to a smaller extent the hair moss (*Polytrichum strictum* L.) – 5-10%.

At the end of August on each SEP were laid 3 accounting areas of size 1×1 m, on which samples of berries and shoots of the cranberry, as well as the living

part of the sphagnum, its tirm and the litter. The samples were dried (except the berries of cranberry) in the thermostats at temperature 105°C, chopped on the cutter of PRG-01T and PRP-01 and analyzed in the first 10 years on the spectrum analyzer LP-4900B "AFORA" with the semiconductor detector DGDK-100V3 and in the next years with the multichannel gamma-pulse-spectrum analyzer SEG-005-AKP with the scintillation counter BDEG-20-R1 and BDEG-20-R2. The relative error in measuring ^{137}Cs specific activity of did not exceed 15%. Mathematical processing was carried out by commonly accepted methods of variant statistics using the software MS Excel and Statistica.

RESULTS AND DISCUSSION

The results of the studies conducted in 2020 (34 years after the Chernobyl accident) show that the low rates of ^{137}Cs specific activity in the berries of the cranberry on

all stationary experimental plots (Table 1). This value ranged from 286 ± 16.9 Bq/kg to 871 ± 82.6 (at an acceptable level of ^{137}Cs – 500 Bq/kg). It is also necessary to indicate a small variation of this indicator within each stationary experimental plots: the variation coefficient within the SEP ranged from 7.6% (SEP 12) to 16.4% (SEP 11). Perhaps this is explained by the fairly uniform dispersion of radio radiation in the area of the bogs due to the low degree of density during the period of its arrival in the environment during the Chernobyl accident in comparison with forest ecosystems). This in part, is explained by the fact that in previous years of research the values of the variation coefficient were small: in 2002 – 7.1-18.1%, in 1991 – 5.7-8.5%.

The ^{137}Cs specific activity in air dry shoots of the cranberry in 2020 ranged from 1492 (SEP 15) to 6197 Bq/kg (SEP 12) (Table 2).

Table 1. Variation series statistics of definition ^{137}Cs specific activity (Bq/kg) in fresh berries of the cranberry in different years of research

Year of research	No. SEP	Statistics						
		M	$\pm m$	$\pm \sigma$	V, %	P, %	min	max
1991	11	13320	654.1	1131.6	8.5	4.9	12650	13990
	12	3626	120.4	208.3	5.7	3.3	3140	4112
	15	2072	99.8	172.7	8.3	4.8	1844	2300
	18	2320	95.3	164.9	7.1	4.1	2227	2413
2002	11	1334	72.5	125.5	9.4	5.4	1234	1433
	12	2150	151.1	261.4	12.2	7.0	1736	2564
	15	588	61.4	106.2	18.1	10.4	452	723
	18	491	20.2	34.9	7.1	4.1	461	520
2020	11	871	82.6	142.9	16.4	9.5	541	1201
	12	755	33.2	57.4	7.6	4.4	608	902
	15	286	16.9	29.2	10.2	5.9	187	385
	18	303	18.5	32.0	10.6	6.1	224	382

Note: M is the arithmetic mean value; m is the error of the arithmetic mean value; σ is the mean square deviation V is the coefficient of variation; P is the coefficient of value; min is the minimum value; max is the maximum value

Table 2. Variation series statistics of definition ^{137}Cs specific activity (Bq/kg) in dry berries of the cranberry in different years of research

Year of research	No. SEP	Statistics						
		M	$\pm m$	$\pm \sigma$	V, %	P, %	min	max
1991	11	94590	4236.2	7328.626	7.75	4.48	88287	100893
	12	31800	2325.1	4022.423	12.65	7.31	31037	32563
	15	12120	897.6	1552.848	12.81	7.41	11254	12986
	18	14732	1125.6	1947.288	13.22	7.64	11484	17980
2002	11	8847	414.7	717.4	8.10	4.70	7014	10680
	12	8647	672.4	1163.2	13.50	7.80	7745	9550
	15	2247	215.1	372.0	16.60	9.60	1923	2572
	18	2618	241.8	418.3	16.00	9.20	2179	3057
2020	11	6099	365.9	633	10.38	6.00	4798	7400
	12	6197	241.7	418	6.75	3.90	5034	7360
	15	1492	112.3	194	13.02	7.53	1364	1620
	18	1854	98.7	171	9.21	5.32	1352	2356

The materials obtained during the long period after the Chernobyl accident allow to compare the values of ^{137}Cs specific activity in berries and shoots and to draw conclusions about the rate of their reduction in different periods at each of the SEP.

For the first period of observation during 1991-1996 there is a significant decrease in ^{137}Cs specific activity in berries: from 3.8 times per SEP 11 to 2.2 times per SEP 12. In the next period (1996-2002) this decrease was within 2.9-1.4 times, less than in the previous one. In the next periods of decline in the value of the ^{137}Cs specific activity in berries was much smaller. Perhaps in the first 15 years since the arrival of radionuclide in the swamp ecosystems, there was a more intensive redistribution of ^{137}Cs between their components, which provided more flow of it to the cranberry. In general, during the observation period, the decrease in ^{137}Cs specific activity in berries

reached: on SEP 11 – 15.3 times (from 13320 ± 654 to 871 ± 83 Bq/kg), on SEP 12 – 4.8 times (from 3626 ± 120 to 755 ± 33 Bq/kg), on SEP 15 – 7.2 times (2072 ± 100 to 286 ± 17 Bq/kg), for SEP 18 to 7.7 times (2320 ± 95 to 303 ± 19 Bq/kg). On each of the SEP, this reduction is specific, which can be related to the peculiarities of the cenotic and hydrologic conditions of each experimental plots, the degree of development and the condition of the cranberry, etc.

The observed decrease in the index is most closely approximated by the exponential equation of the form $Y=e^x$, where Y is ^{137}Cs specific activity, Bq/kg; X is the number of years after the Chernobyl accident (Fig. 1 and 2):

On SEP 11: $Y=11123e^{-0.485x}$, $R^2=0.84$, $r=0.92$;

On SEP 12: $Y=3496.8e^{-0.288x}$, $R^2=0.85$, $r=0.92$;

On SEP 15: $Y=2218.9e^{-0.382x}$, $R^2=0.87$, $r=0.93$;

On SEP 18: $Y=1833.8e^{-0.352x}$, $R^2=0.82$, $r=0.91$.

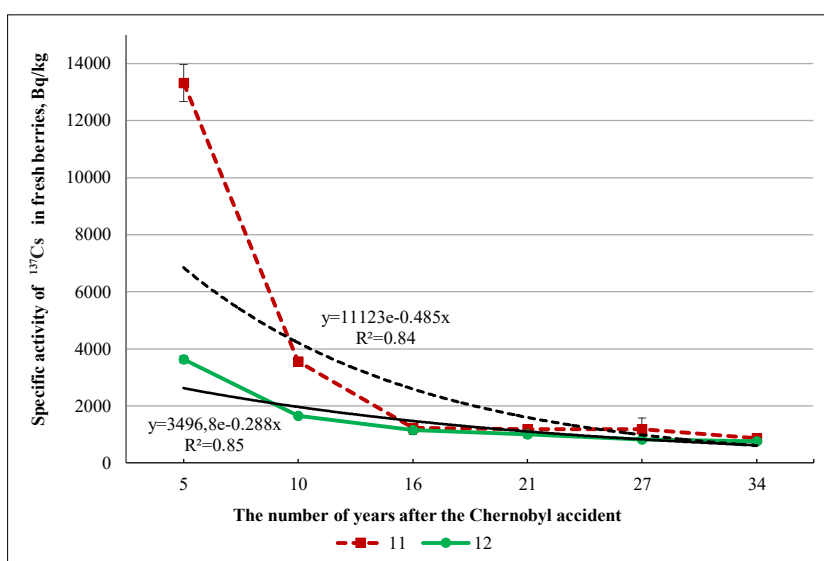


Figure 1. Change of ^{137}Cs specific activity in fresh berries of the cranberry during 1991-2020 (5-34 years after the Chernobyl accident) on SEP (11, 12)

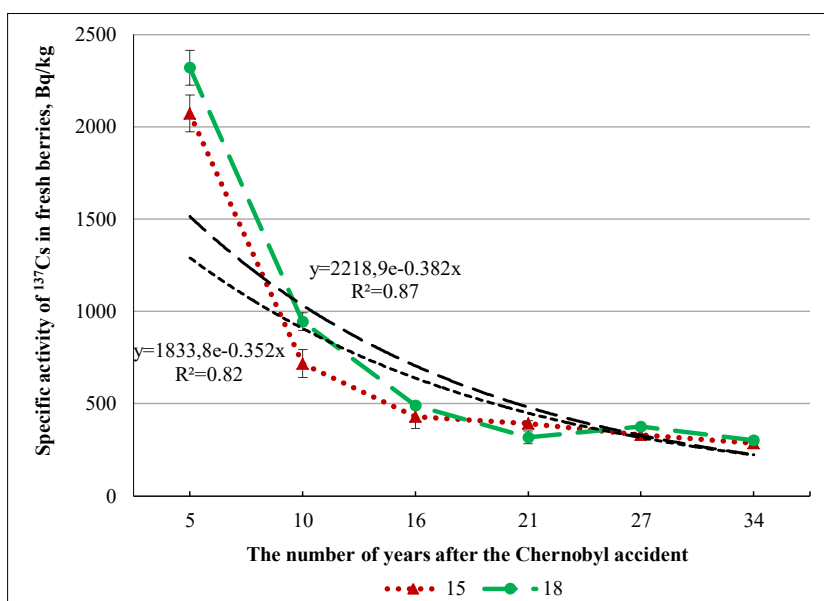


Figure 2. Change of ^{137}Cs specific activity in fresh berries of the cranberry during 1991-2020 (5-34 years after the Chernobyl accident) on SEP (15, 18)

Detected regularities in decreased ^{137}Cs specific activity in fresh berries of the cranberry during 1991-2020 are also confirmed by changes of the same indicator in the plant's shoots. Thus, the general decrease of ^{137}Cs specific activity in dry shoots of cranberry for the whole period of observation was: on SEP 11 – from 94590 ± 4236 to 6099 ± 366 Bq/kg (15.5 times), on SEP 12 – from 31800 ± 2325 to 6197 ± 242 Bq/kg (5.1 times), on SEP 15 – from 12120 ± 898 to 1492 ± 112 Bq/kg (8.1 times), on SEP 18 – from 14732 ± 1126 to 1854 ± 112 Bq/kg (7.9 times). At the same time, a more intensive decrease in the content of

radionuclide in the shoots also occurred in the first two periods of observation (1991-2002). The established decrease of ^{137}Cs specific activity in the shoots of cranberry are also most closely approximated by the exponential equation of the form $Y = e^x$, where Y is ^{137}Cs specific activity, Bq/kg; X is the number of years after the Chernobyl accident (Fig. 3 and 4):

On SEP 11: $Y = 12775e^{-0.382x}$, $R^2 = 0.83$, $r = 0.91$;

On SEP 12: $Y = 10551e^{-0.38x}$, $R^2 = 0.82$, $r = 0.91$;

On SEP 15: $Y = 79358e^{-0.49x}$, $R^2 = 0.85$, $r = 0.92$;

On SEP 18: $Y = 29703e^{-0.306x}$, $R^2 = 0.84$, $r = 0.92$.

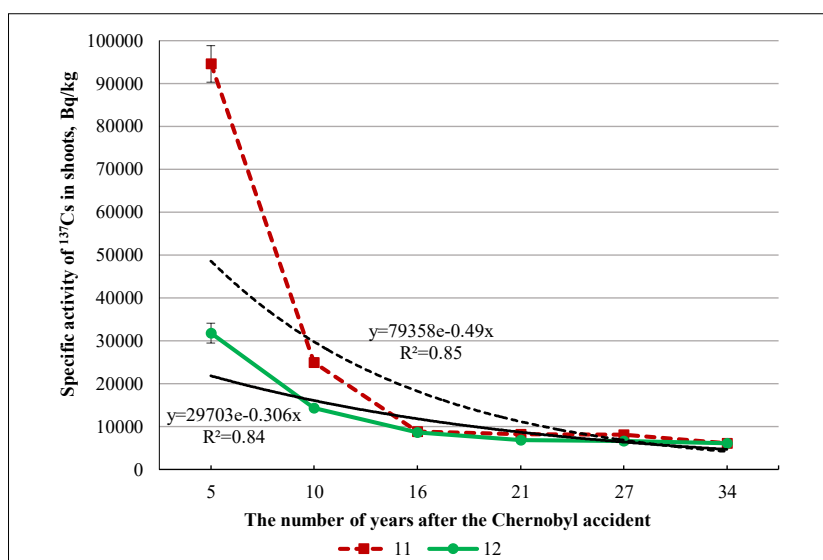


Figure 3. Change of ^{137}Cs specific activity in dry shoots of cranberry during 1991-2020 (5-34 years after the Chernobyl accident) on SEP (11, 12)

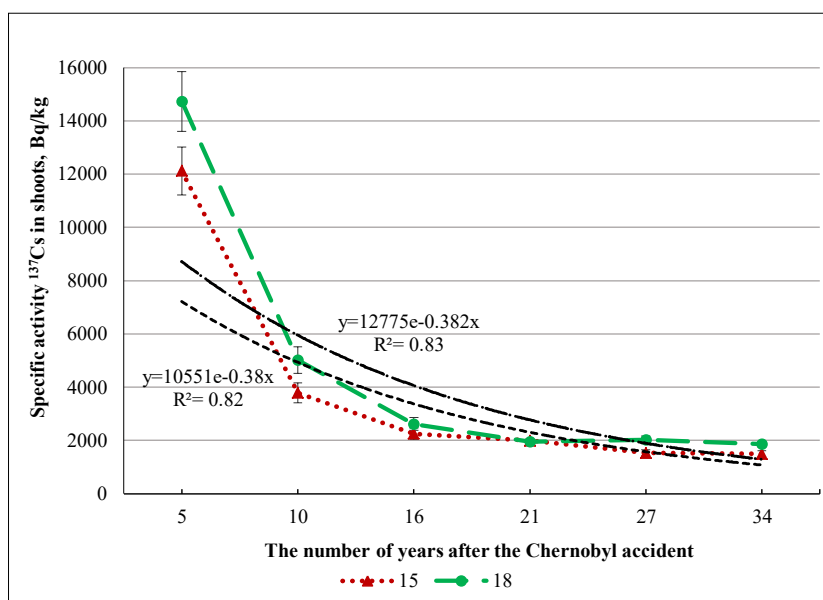


Figure 4. Change of specific activity ^{137}Cs in dry shoots of cranberry during 1991-2020 (5-34 years after the Chernobyl accident) on SEP (15, 18)

The materials received during such a long period of observation expand the idea of repartition of ^{137}Cs on oligotrophic and mesotrophic sphagnum bogs in time, which, on its part, will allow to work out the forecast methods of further migration of this radionuclide in the given ecological conditions and its inflow to various components. Given the importance of the bogs, which

was noted in the literature analysis, this is very important, because there is a need in forecasting the possible arrival of radioactive elements to neighboring, with bogs, ecosystems and groundwater. The literature sources did not reveal materials on levels of radioactive contamination of certain or other components of the sphagnum bogs in 30 years since the arrival of radionuclides. Data

on ^{137}Cs in the shoots and berries of the cranberry were published by the authors of this publication in previous years (Krasnov & Orlov, 2004). This allows to identify received regularities as priority. At the same time, according to the authors, in the course of the further researches it is necessary to pay some attention to the following question: to develop the methodology of determination of density of radioactive contamination of substrate that will allow to find dependencies between content of radionuclide in plants and substrate on which they grow; to study migration of ^{137}Cs in deeper layers of soil.

CONCLUSIONS

Reduction of ^{137}Cs specific activity in berries and shoots of cranberry after the Chernobyl accident has been established. During 1991-2020 this indicator decreased: in the berries of plants in 15.3 (SEP 11) – 4.8 (SEP 12) times, in the shoots – in 15.5 (SEP 11) – 5.1 (SEP 12) times. The

rate of reduction of radionuclide content in vegetative and generative organs of cranberry was more intensive in the first 12 years of observation (1991-2002) and significantly slowed down in the next period until 2020 inclusive. The decrease of ^{137}Cs specific activity in the cranberry (dry shoots and fresh berries) over the years is described by the exponential equation of the form $Y=e^x$. The revealed regularities of the dynamics of the studied indicators allow to create the forecast models of radioactive contamination of the cranberry.

At present, ^{137}Cs continue to be supplied to the berries and shoots of cranberry, but the intensity of this process has considerably decreased compared to the initial period. These circumstances require a review of the existing regulation of the berrying of this plant taking into account the current radiation situation on specific areas of the sphagnum bogs.

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Динаміка накопичення ¹³⁷Cs журавлиною болотною на сфагнових болотах Полісся України

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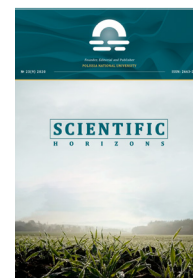
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Анотація. Оліготрофні і мезотрофні болота є поширеними екосистемами у Поліссі України. Після аварії на Чорнобильській АЕС дані екосистеми були забруднені радіонуклідами. Поширеною ягідною рослиною на цих болотах є журавлина болотна, що широко використовується у харчовій промисловості. Метою досліджень є встановлення динаміки питомої активності ¹³⁷Cs у журавлині болотній впродовж останніх 30 років. Отримані результати моніторингових досліджень є необхідним для прогнозування радіоактивного забруднення журавлини. Дослідження проводились на постійних пробних площах (ППП) у Житомирському Поліссі. Питому активність ¹³⁷Cs вимірювали за допомогою спектроаналізаторів, з використанням сцинтиляційних і напівпровідникових детекторів. Отримані результати свідчать, що спостерігається інтенсивна міграція ¹³⁷Cs у сфагнових болотах Полісся України. Результати досліджень, проведених через 34 роки після аварії на ЧАЕС, демонструють, що нині відмічаються невисокі величини питомої активності ¹³⁷Cs у журавлині болотній (*Vaccinium oxycoccus* L.) на всіх пробних площах. Даний показник у 2020 р у свіжих ягодах коливався від 871±82,6 до 286±16,9 Бк/кг (при допустимих рівнях вмісту ¹³⁷Cs – 500 Бк/кг), а у сухих пагонах – від 1492 (ППП 15) до 6197 Бк/кг (ППП 12). Виявлено, що за перший період досліджень протягом 1991–1996 рр. спостерігається досить значне зниження питомої активності ¹³⁷Cs в ягодах: від 3,8 разів на ППП 11 до 2,2 разів на ППП 12. У наступний період спостережень (1996–2002 рр.) це зниження було у межах 2,9–1,4 разів, що менше ніж у попередній. У подальший період зниження величини питомої активності ¹³⁷Cs в ягодах було значно меншим. Виявлені закономірності у зниженні питомої активності ¹³⁷Cs в ягодах журавлини болотної протягом 1991–2020 рр. підтверджуються змінами цього показника у її пагонах. Загальне зниження цього показника у пагонах за період спостережень склало: на ППП 11 – від 94590±4236 до 6099±366 Бк/кг (15,5 разів), на ППП 12 – від 31800±2325 до 6197±242 Бк/кг (5,1 рази), на ППП 15 – від 12120±898 до 1492±112 Бк/кг (8,1 рази), на ППП 18 – від 14732±1126 до 1854±112 Бк/кг (7,9 разів). Продовження моніторингу на постійних пробних площах дозволить прогнозувати рівні радіоактивного забруднення журавлини на основі математичного моделювання

Ключові слова: питома активність, радіонуклід, *Vaccinium oxycoccus* L., сфагн, сухі пагони, свіжі ягоди



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Collective Bargaining and Payment Regulation at the Regional Level: Assessment and Prospects (on the Example of Ukraine)

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Abstract. In the conditions of constant challenges and threats in the realization of citizens' right to work, collective bargaining becomes almost the only effective tool that allows to reconcile the interests of different actors and consolidate their joint efforts. The purpose of the study is a comparative analysis of the level of development of collective bargaining in different regions of Ukraine and identify challenges and prospects for the development of this institute. The authors proposed a methodology that allows quantitative and qualitative assessment of collective bargaining regulation of wages based on a number of indicators and a comparative analysis of its level of development in different regions. The evaluation confirmed the hypothesis of low activity of social partners and inability to act mobile in the new socio-economic ecosystem. The results showed the underdevelopment of wage regulation through collective bargaining in all regions, which increased distrust in the institution of social partnership and showed the inability of partners to create conditions for decent work. Indicators of the effectiveness of collective bargaining and agreements typical of countries with economies in transition (Ukraine), and the root causes of low quality social dialogue at the regional level are highlighted. Challenges inherent not only in Ukraine but also in most European countries allowed the authors to identify areas for improving collective bargaining, which include expanding the social partners, adapting the content of agreements to the strategic goals of the region and its sustainable development. The identified general trends conditioned upon the challenges of the digital economy and the new economic ecosystem have highlighted the need to rethink the role and content of collective bargaining. The practical value of the study lies in the possibility of applying the developed methodology by social partners, local administrations, local communities to assess social dialogue at the regional level, study and disseminate best practices of wage policy on the basis of social partnership

Keywords: collective bargaining, wages, social dialogue, decent work, evaluation, regional agreements



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INTRODUCTION

The ambiguous transformations taking place in the labor market require overcoming the consequences of the pandemic and require an adequate response from the social partners, considering the requirements of the new economic reality. In such conditions, the requirements for the responsibility of all market actors are only intensifying and the need to ensure decent work and preserve and/or achieve gradual sustainable development is growing. The historical experience of many countries shows that the most suitable in conditions of uncertainty is the institution of social dialogue, which is based on collective bargaining (Erickson *et al.*, 2002; Doellgast *et al.*, 2018; Hayter & Jelle, 2018; Volkova & Kuznetsova, 2018; Kerkhofs, 2019).

At the same time, various researchers emphasise the weakening of social dialogue in the regulation of social and labor relations in European countries: Portugal (Tavora & Gonzalez, 2016); Spain (Rodríguez *et al.*, 2016); Slovenia (Stanojević *et al.*, 2016); Germany (Addison *et al.*, 2017). This trend is primarily explained by the weak organisational capacities of the state and especially of the social partners (Mailand & Due, 2004).

It is important to note that the institution of social dialogue is constantly changing and adapting to the challenges of economic and social reality. For example, during the pandemic, it became a safeguard against the destruction of many jobs, reduced the negative impact on workers' incomes (Molina, 2021; Tørrer, 2021). However, the developed practical recommendations may not be universal for all countries.

Despite the trend towards decentralisation of collective bargaining (Amable, 2016; Rodríguez *et al.*, 2016), the emphasis of dialogue in the field of working time regulation, ensuring decent wages and safe working conditions, granting vacations, etc. is growing.

These processes are inherent in Ukraine, but they have a number of features conditioned upon legislation and current practice. In particular, social dialogue is implemented at four levels: national, sectoral, regional (territorial) and industrial (Law of Ukraine No. 3356-XII "On Collective Agreements and Contracts", 1993). At the same time, if collective bargaining at the first two and production levels is a fairly common practice, the territorial one has become a conglomerate of sectoral agreements and social responsibility agreements of regional companies. But after the introduction of administrative reform and decentralization reform in the country, the problems and shortcomings of contractual regulation of social and labour relations at the territorial level have worsened. They were especially evident in the field of wages. After all, to date, the regulation of wages in Ukraine is a strategic task, as it allows to create favorable conditions for the reproduction of the labor force, its size depends on the level of well-being of employees and their families. Therefore, to develop the relevant territories, which should be accompanied by effective socio-economic

policies of communities, including the creation of opportunities for productive employment, safe working conditions and decent wages, it is important to assess the potential of collective bargaining, identifying problem areas, challenges and its further prospects.

Unfortunately, in Ukraine a comprehensive assessment of collective bargaining has not been conducted. Therefore, the results of comparative analysis of the level of development of collective bargaining in different regions can be an important source of information for local governments to make appropriate decisions and disseminate best practices and experience in the regulation of social and labour relations.

Thus, *the aim of the study is to conduct a comparative analysis of the level of collective bargaining in different regions of Ukraine and identify challenges and prospects for the development of this institute, which is based on the author's methodology for assessing the level of collective bargaining at the territorial level. The main hypothesis put forward by the authors is a stable correlation between the activity of social partners and the effectiveness of collective bargaining and agreements at the regional level.*

LITERATURE REVIEW

The scientific field of research on the development of social and labor relations is inevitably associated with social dialogue and collective bargaining. Considerable attention is paid to the national and regional levels of negotiations, in particular, the latter is associated with potential opportunities to adapt the labour market to the challenges of digitalisation through the acquisition of relevant skills and decent work. After analysing data from many countries, sectors of the economy, firms, the link between different conditions of collective bargaining with employment, wage inequality and productivity is undeniable (Global Deal *et al.*, 2020). For many years, social dialogue has been proving its effectiveness as a tool for influencing the dynamics of economic growth and stability. (Grimshaw *et al.*, 2017). At the same time, the authors note that the institute of social dialogue faces new challenges: changing the employment structure, economic crisis, reducing employee involvement in trade unions, decentralisation of collective labor relations (Gumbrell-McCormick & Hyman, 2013). Unfortunately, these challenges are systemic and have their origins in the 1990s (Jobert, 2005). In particular, anti-crisis decisions of some governments (aimed at weakening the collective bargaining regulation of social and labour relations) exposed the desire of organisational management to narrow the range of tasks to be negotiated and identified practices of partial implementation of agreements (Tavora & Gonzalez, 2016) became a precondition for weakening trust in the trade union and the institution of social dialogue. No less important challenge was precarious work, which at the same time actualised the

monitoring potential of social dialogue (Grimshaw *et al.*, 2016). Therefore, the search for solutions to these problems requires the institution of collective bargaining regulation of social and labor relations adaptation and greater inclusion, finding new approaches to solving major social problems, especially in areas such as digitalisation and new technology, demographic change, green economy and sustainable development; and it is essential to increase and acquire competencies (Eurofound, 2020).

Studying the impact of collective bargaining on wages, scientists note an unconditional direct relationship. For example, Research of Villanueva (2015) proves the positive impact of extending collective bargaining agreements to all employees in an industry on ensuring common working conditions, limiting wage inequality by setting occupation-specific minimum wages, and reducing gender wage gaps, mainly at the bottom of the wage distribution. At the same time, as a rule, the evaluation of the effectiveness of collective bargaining is proposed according to a formalised indicator – the degree of collective bargaining (International Labour Organisation, 2018), which is explained by the assumption: readiness to participate in collective bargaining reflects the potential including wages. However, some researchers argue that while trade union representatives appear open to the responsibilities placed upon them, there is little evidence to suggest that management has responded (Stuart & Lucio, 2002). It is also proposed to study the range of indicators of the effectiveness of collective bargaining regulation through the prism of assessing social dialogue in general. Therefore, the indicators of settlement of labour remuneration policy issues during collective bargaining on the conclusion of a sectoral agreement in accordance with the legislation, number of social partners' obligations for remuneration, the share of the provisions of the sectoral agreement on labor remuneration fulfilled by the social parties (Tsymbaliuk & Shkoda, 2022), allow to

find out the activity of social partners in the implementation and enforcement of workers' rights. The use of the wage potential indicator in the context of the implementation of social dialogue (Danylevych & Poplavska, 2020) allows assessing the level of employee satisfaction with wages and the effectiveness of trade unions.

Note that, for the most part, assessing collective bargaining regulation, researchers focus on setting a minimum wage (Dustmann *et al.*, 2019; Ellguth, 2014; Bellmann *et al.*, 2018). However, such a position is justified to guarantee basic standards in the field of wages, but other guarantees in wages, such as income indexation, compensation for work in hazardous conditions, etc. no less important, especially since in an unstable economy, the challenges of a pandemic, these components are becoming increasingly important. Assessing collective bargaining regulation through the prism of decent work significantly expands the range of indicators and indicators for analysis (Tsymbaliuk *et al.*, 2019). Considering the concept of "labor 4.0" (Kolot *et al.*, 2020) allows assessing the compliance of wages with the costs, the nature of labour. At the same time, we should not forget about the changes that have taken place in recent years in the collective bargaining of social and labour relations, especially after the financial crisis. As noted by B.D. Müller and P. Hans-Wolfgang (2017), they have become significantly different across countries and these differences will only increase. Therefore, the assessment of collective bargaining should be carried out in accordance with the country's goals in the field of labour and the peculiarities of the regulation of social and labor sphere.

MATERIALS AND METHODS

To analyse and assess the level of development of collective bargaining regulation at the territorial level is proposed a set of indicators with established standards (Table 1).

Table 1. Indicators for assessing the level of development of collective bargaining

No.	Indicator	Standard
1	Existence of a territorial agreement	Yes
2	Existence in the territorial agreement of guarantees and norms concerning the regional subsistence level (regional minimum wage)	Yes
3	Availability in the territorial agreement of guarantees and norms on the implementation of payments financed from the local budget	Yes
4	Number of commitments of social partners	→ max
5	Ratio of the number of obligations of the social partners to pay in accordance with the current territorial agreement compared to the number of obligations under the previous territorial agreement	→ max (≥1)
6	Ratio of the number of provisions of the territorial agreement on wages that contain specific obligations to the total number of obligations of the social partners on wages, %	→ max (100)
7	Ratio of the tariff rate of the worker of the 1st category and the minimum wage (subsistence level) in accordance with the territorial agreement, %	→ max (≥110)
8	Existence in the territorial agreement of provisions on introduction of systems of participation of the personnel in profit and (or) in the share capital at the enterprises of the region	Yes
9	Existence in the territorial agreement of provisions on introduction of social packages and programmes of social insurance at the enterprises of the region	Yes
10	Presence in the territorial agreement of provisions on observance of gender equality in payment	Yes

Table 1, Continued

No.	Indicator	Standard
11	Level of coverage of employees by collective agreements in the study region, %	→ max (100)
12	Real wage growth rates in the region, %	→ max (≥ 110)
13	Ratio of average wages in the region with the average wage in the country	→ max (≥ 1)
14	Share of employees receiving wages below the subsistence level, of the total number of employees in the region, %	→ min (0)
15	Share of workers receiving wages below the poverty line in the region, %	→ min (0)
16	Share of basic wages in the wage bill in the region, %	→ max (> 60)
17	Wage arrears in the region, UAH	→ min (0)
18	Ratio of women's wages to men's wages, %	→ max (100)

Source: compiled by the author

An expert survey method was used to verify the reliability of the indicators. The experts were scientists and specialists in the field of social and labour relations

and wages. The number of experts who took part in the survey was 13. Table 2 shows the main characteristics of experts.

Table 2. Characteristics of experts in the field of social and labour relations and wages

Feature	Persons	In % to the total number of experts
<i>Professional group</i>		
• Teachers	8	62
• Scientists	3	23
• FPU payroll specialists	2	15
<i>Total</i>	<i>13</i>	<i>100</i>
<i>Academic status</i>		
• Professor	3	23
• Docent	6	46
• No	4	31
<i>Total</i>	<i>13</i>	<i>100</i>
<i>Degree</i>		
• PHD	5	39
• PhD	6	46
• No	2	15
<i>Total</i>	<i>13</i>	<i>100</i>
<i>Work experience</i>		
• From 10 to 20 years	6	46
• More than 250	7	54
<i>Total</i>	<i>13</i>	<i>100</i>

Source: compiled by the author

The survey was conducted in the form of a questionnaire. Experts were asked to establish the significance (weight) of indicators on the following scale: 0 points – the indicator is not important; 1-2 points – the indicator is almost not important; 3-4 points – the indicator is important; 5-6 points – the indicator is very important; 7 points – the main (key) indicator.

To assess the level of development of collective bargaining regulation at the territorial level, methodological principles for determining individual indices and complex indicators have been developed. Different methodological approaches to the calculation of unit indices (I_j)

for different indicators depending on their specifics, units of measurement and standards are proposed.

The unit indices (I_j) for the indicators for which the established standards have the wording “yes” are determined in the following order:

– if the actual value of a certain indicator does not meet the established standard, the unit index (I_j) of this indicator is 0;

– if the actual value of a certain indicator meets the established standard, the unit index (I_j) of this indicator is equal to 1.

For the rest of the indicators, the unit indices (I_j) are

determined on the basis of the standardisation procedure:

– according to the indicators “the share of workers receiving wages below the subsistence level (below the poverty line), the total number of workers in the region” and “wage arrears in the region” according to formula (1):

$$I_i = \frac{[X_{actual} - X_{max}]}{[X_{min} - X_{max}]} \quad (1)$$

– for other indicators according to formula (2):

$$I_i = \frac{[X_{actual} - X_{min}]}{[X_{max} - X_{min}]} \quad (2)$$

where X_{actual} is the actual value achieved in a certain region, according to a separate indicator; X_{min} – the lowest value for a single indicator among the regions studied; X_{max} – the highest value for a single indicator among the regions studied.

The complex indicator of the level of development of collective bargaining regulation at the territorial level (K) is determined by the formula (3):

$$K = \frac{\sum_{i=1}^n I_i}{n} \quad (3)$$

where n is the number of indicators.

The value of the complex indicator can be in the range from 0 to 1.

Ranges of values of the level of development of collective bargaining regulation of wages at the territorial level:

$$\text{If } K \in \begin{Bmatrix} [0.8; 1] \\ [0.6; 0.8] \\ [0.4; 0.6] \\ [0.2; 0.4] \\ [0; 0.2] \end{Bmatrix}, K \in \begin{Bmatrix} \text{High level} \\ \text{Level above average} \\ \text{Average level} \\ \text{Level under average} \\ \text{Low level} \end{Bmatrix}$$

Comparative analysis of the level of development of collective bargaining regulation of wages was carried out on the example of the following regions: Vinnytsia, Dnipropetrovsk, Ivano-Frankivsk, Luhansk, Lviv, Mykolaiv, Sumy, Kharkiv, Kherson and Chernihiv.

Criteria for forming a sample of regions:

– representation in the study of different parts of Ukraine: Vinnytsia and Dnipropetrovsk regions belong to the central region, Ivano-Frankivsk and Lviv – to the western region, Luhansk and Kharkiv – to the east, Sumy and Chernihiv – to the north and Mykolaiv and Kherson – to the south;

– the presence of concluded territorial collective agreements.

It is important to pay attention to some limitations of the study. Thus, one of the important indicators of wage policy evaluation is the indicators that characterise wage differentiation, in particular the Gini coefficient and the decile coefficient. However, conditioned upon the lack of statistical information on these indicators by region, they were not considered when calculating a comprehensive indicator of the level of development

of collective bargaining. An important indicator that characterises the level of development of social partnership at the territorial level is the level of fulfillment of their obligations by the social partners. Conditioned upon the lack of information on the extent to which the social partners comply with the provisions of territorial agreements, this indicator is also not considered when calculating a comprehensive indicator of the level of development of collective bargaining in the studied regions.

When studying the indicators that characterise the level and dynamics of wages, it is advisable to analyse not the arithmetic mean of the indicators, and the median and modal values. However, such information is not published by the State Statistics Service of Ukraine, which was a limiting factor in our study. During the development of the methodology for studying the development of collective bargaining regulation of wages, we accepted that the defined indicators are equivalent. At the same time, it would be expedient to consider the significance of the formed indicators using the method of expert survey, which can serve as a further direction of research and improvement of the methodology.

It is important to note that the methodology of research on the development of collective bargaining can change, adapt depending on the level of research, goals and objectives, information base, target priorities of socio-economic development of individual countries, regions and even enterprises.

RESULTS AND DISCUSSION

Analysis of territorial agreements in the field of wages of the studied regions

The main indicator of assessing the level of development of collective bargaining regulation is *the presence of a territorial agreement*. Analysis of territorial agreements (Territorial Agreement between Vinnytsia Regional State Administration, Vinnytsia Regional Council, Vinnytsia Regional Association of Employers' Organisations and the Joint Representative Body of Trade Unions of the Vinnytsia Region for 2016-2020, 2016; Territorial Agreement between Dnipropetrovsk Regional State Administration, Dnipropetrovsk Regional Council, Federation of Employers' Organisations of the Dnipropetrovsk Region and the Joint Representative Body of Trade Unions of the Dnipropetrovsk Region for 2017-2020, 2017; Territorial Agreement between the Ivano-Frankivsk Regional State Administration, the Ivano-Frankivsk Regional Association of Employers' Organisations and the Council of Trade Unions of the Ivano-Frankivsk Region for 2018-2020, 2018 (Territorial Agreement between..., 2018).

Territorial Agreement between the Luhansk Regional State Administration, the Federation of Trade Unions of the Luhansk Region and the Association of Employers' Organizations of the Luhansk Region and the Federation of Trade Unions of the Luhansk Region for 2019-2020, 2018; Territorial Agreement between the

Lviv Regional State Administration, the Lviv Regional Association of Employers' Organisations and the Trade Unions of the Lviv Region for 2018-2020, 2018; Territorial Agreement between the Mykolayiv Regional State Administration, the Mykolayiv Regional Organisation of Employers "Industrialists and Entrepreneurs of the Mykolayiv Region" and the Mykolayiv Regional Council of Trade Unions 2017-2020 on the regulation of socio-economic issues, 2017; Territorial Agreement on cooperation between the Sumy regional association of employers' organizations and the Sumy regional council of trade unions for 2019-2021, 2019; Territorial Agreement between the Kharkiv Regional State Administration, the Joint Representative Bodies of the Parties of Employers and Trade Unions of the Kharkiv Region for 2016-2018, 2016; Territorial Agreement between the Kherson Regional State Administration, the Association of Employers' Organizations of the Kherson Region, the Kherson Regional Intersectoral Council of Trade Unions on the regulation of basic principles and norms of socio-economic policy and labor relations in the Kherson region for 2019 -2021, 2019; Territorial Agreement on the regulation of basic socio-economic principles and labor relations for 2017-2020 between the authorities – Chernihiv Regional State Administration, Chernihiv Regional Council, employers and entrepreneurs – Regional Association of Employers' Organisations, Joint Representative Body of Employers and Employers of Chernihiv – Joint representative body of representative trade unions at the territorial level, 2017, showed that some of them (in Vinnytsia, Dnipropetrovsk, Luhansk, Lviv, Kharkiv and Chernihiv regions) provide that they are regulations that can be considered positive from in terms of determining the legal nature of these agreements. At the same time, the norms and provisions of territorial agreements do not apply to enterprises that did not participate in the negotiation process and the signing

of agreements in accordance with Art. 9 of the Law of Ukraine "On Collective Agreements and Contracts" (Law of Ukraine No. 3356-XII "On Collective Agreements and Contracts", 1993). In this regard, despite the recognition of territorial agreements by the social partners as normative acts, the latter cannot be considered as such, as their norms and provisions are not mandatory for all enterprises located in the territory of the region.

Unlike collective agreements at other levels, Ukrainian law does not regulate a clear list of issues to be negotiated at the territorial level, and limits their subject matter to the regulation of social guarantees, benefits and compensations. The legislation contains a general wording: agreements at the territorial level regulate the norms of social protection of employees of enterprises, include higher than the general agreement social guarantees, compensations and benefits (Article 8 of the Law of Ukraine "On Collective Bargaining and Agreements"). Analysis of the content of territorial agreements showed that they regulate a wider range of issues than required by law. Comparison of the number of obligations of the parties to territorial agreements in the field of wages of the studied regions are given in Table 3. According to the data in Table 3, the largest number of obligations of social partners in the field of remuneration are contained in territorial agreements in Vinnytsia and Dnipropetrovsk regions. If we analyse the number of obligations in terms of specific subjects of social and labor relations (local executive bodies, employers and trade unions), the most successful practices of social partnership at the territorial level include the Territorial Agreement in Vinnytsia region for 2016-2020. The Territorial Agreement in Sumy Oblast for 2019-2021 does not contain obligations of local executive bodies, and the Territorial Agreement in Lviv Oblast for 2018-2020 is limited to joint agreements, which negatively characterises the level of social partnership development.

Table 3. Comparison of the number of obligations of the parties to territorial agreements in the field of wages of the studied regions

Deals	Number of joint commitments / parties agreed	Number of obligations of local executive bodies	Number of obligations of the employers	Number of obligations of the trade unions	Total number of obligations of the parties	Ratio of the number of obligations compared to the previous agreement, %
1. Territorial agreement in Vinnytsia region						
– for 2011-2015	8	8	8	6	30	–
– for 2016-2020	11	13	8	10	42	1.4
2. Territorial agreement in the Dnipropetrovsk region						
– for 2011-2013	12	4	3	4	23	–
– for 2017-2020	20	5	7	6	38	1.65

Table 3, Continued

Deals	Number of joint commitments / parties agreed	Number of obligations of local executive bodies	Number of obligations of the employers	Number of obligations of the trade unions	Total number of obligations of the parties	Ratio of the number of obligations compared to the previous agreement, %
3. Territorial agreement in Ivano-Frankivsk region						
– in 2016	4	5	3	4	16	–
– for 2018-2020	16	8	4	7	35	2.19
4. Territorial agreement in Luhansk region						
– for 2013-2015	3	1	1	4	9	–
– for 2019-2020	14	5	7	4	30	3.33
5. Territorial agreement in the Lviv region						
– for 2015-2017	17		0	0	17	–
– for 2018-2020	18	0	0	0	18	1.06
6. Territorial agreement in the Mykolaiv region						
– for 2012-2015	12	6	6	4	28	–
– for 2017-2020	15	3	4	4	26	0.93
7. Territorial agreement in the Sumy region						
– for 2017-2018	11	11	5	9	36	–
– for 2019-2021	11	0	5	8	24	0.67
8. Territorial agreement in the Kharkiv region						
– for 2013-2015	6	2	1	7	16	–
– for 2016-2018	6	2	1	7	16	1.0
9. Territorial agreement in the Kherson region						
– for 2017-2018	10	9	6	6	31	–
– for 2019-2021	8	7	6	6	27	0.87
10. Territorial agreement in the Chernihiv region						
– for 2011-2015	7	9	7	7	38	–
– for 2017-2020	6	8	6	8	28	0.74

Source: compiled according to data (Territorial Agreement in the Vinnytsia Region, 2011; Territorial Agreement in the Vinnytsia Region, 2016; Territorial Agreement in the Dnipropetrovsk Region, 2011; Territorial Agreement in the Dnipropetrovsk Region, 2017; Territorial agreement in the Ivano-Frankivsk Region, 2016; Territorial agreement in the Ivano-Frankivsk Region, 2018; Territorial agreement in the Luhansk Region, 2013; Territorial agreement in the Luhansk Region, 2018; Territorial agreement in the Lviv Region, 2015; Territorial agreement in the Lviv Region, 2018; Territorial agreement in the Mykolayiv Region, 2012; Territorial agreement in the Mykolayiv Region, 2017; Territorial agreement in the Sumy Region, 2017; Territorial agreement in the Sumy Region, 2019; Territorial agreement in the Kharkiv Region, 2013; Territorial agreement in the Kharkiv Region, 2016; Territorial agreement in the Kherson Region, 2017; Territorial agreement in the Kherson Region, 2019; Territorial agreement in the Chernihiv Region, 2011; Territorial agreement in the Chernihiv Region, 2017)

If we compare the number of commitments contained in the current territorial agreements with the commitments provided for in previous agreements, the positive dynamics can be seen in the social dialogue in Ivano-Frankivsk and Luhansk regions. The negative trend in reducing the number of obligations of the social partners is typical for territorial agreements in Mykolaiv,

Sumy, Kherson and Chernihiv regions. Comparison of individual norms of territorial agreements in the field of wages of the studied regions are given in Table 4. According to the results of a comparative analysis of different norms on wages, most provisions of territorial agreements call for the regulation of relevant norms by sectoral agreements and collective agreements.

Table 4. Comparison of individual norms of territorial agreements in the field of remuneration of the studied regions

Deals	The growth rate of average wages	Minimum guarantees in the remuneration of non-budgetary employees	Inter-qualification and inter-job pay ratios	The share of basic wages in the payroll	Norms on non-discrimination in pay
Territorial agreement in Vinnytsia region for 2016-2020	Determined by the development programme of the region (p. 2.1). Not lower than the growth rate determined by sectoral and general agreements (p. 2.2) Higher than the inflation rate (p. 2.3)	Defined in collective agreements (p. 2.27)	Defined in collective agreements (p. 2.4)	Not less than 70%, defined in collective agreements (p. 2.27)	–
Territorial agreement in the Dnipropetrovsk region for 2017-2020	Not less than provided by the programme of development of the region (item 5.2). Not lower than at the state level (p. 5.3)	Defined in sectoral agreements and collective agreements in accordance with the law (p. 5.4)	Defined in collective agreements (p. 5.5, 5.7)	Not less than 65% are defined in collective agreements (item 5.29)	–
Territorial agreement in Ivano-Frankivsk region for 2018-2020	Not lower than at the state level (p. 3.1). In accordance with the provisions of sectoral agreements (p. 3.2)	Defined in collective agreements in accordance with sectoral agreements. Not less than 110% of the subsistence level (p. 3.3)	–	Defined in collective agreements, not less than specified in the sectoral agreement (p. 3.31)	Observance of gender equality in remuneration of labour (items 3.8, 3.17)
Territorial agreement in Luhansk region for 2019-2020	Not less than provided by the programme of development of the region (item 5.2). Not lower than at the state level (p. 5.3)	Defined in sectoral agreements and collective agreements in accordance with the law (p. 5.4)	Defined in collective agreements (p. 5.5, 5.7)	It is recommended to provide for collective agreements to increase the share of basic wages (p. 5.5)	–
Territorial agreement in the Lviv region for 2018-2020	–	–	–	–	–
Territorial agreement in the Mykolaiv region for 2017-2020	Higher than the inflation rate (p. 2.1)	Defined in collective agreements in accordance with sectoral agreements (p. 2.24)	Defined in sectoral agreements (p. 2.2)	Defined in collective agreements, not less than specified in the sectoral agreement (p. 2.25)	Observance of gender equality in remuneration of labour (item 2.11)
Territorial agreement in Sumy region for 2019-2021	Higher than the inflation rate in enterprises with an average PA below 1.5 MZP (p. 2.1.1). In accordance with the provisions of sectoral agreements at enterprises with an average PA higher than 3 minimum wage (p. 2.1.2)	Defined in sectoral agreements. Not less than 115% of the MRM at enterprises where sectoral agreements have not been concluded (items 2.1.3, 2.1.12). Defined in collective agreements (p. 2.1.4)	Defined in collective agreements (p. 2.1.4)	Increase the share to 70% by the end of 2021 (p. 2.1.9)	–

Table 4, Continued

Deals	The growth rate of average wages	Minimum guarantees in the remuneration of non-budgetary employees	Inter-qualification and inter-job pay ratios	The share of basic wages in the payroll	Norms on non-discrimination in pay
Territorial agreement in the Kharkiv region for 2016-2018	Determined by the programme of development of the region and collective agreements (p. 3.1.2).	Defined in sectoral agreements (p.3.1.4)	–	–	–
Territorial agreement in the Kherson region for 2019-2021	According to the growth rate of production and productivity (p. 2.9)	–	–	–	–
Territorial agreement in the Chernihiv region for 2017-2020	Not lower than at the state level (item 5.1)	Defined in collective agreements (clause 5.2)	Defined in collective agreements (clause 5.2)	–	–

Source: compiled according to data (Territorial Agreement in the Vinnytsia Region, 2016; Territorial Agreement in the Dnipropetrovsk Region, 2017; Territorial Agreement in the Ivano-Frankivsk Region, 2018; Territorial Agreement in the Luhansk Region, 2018; Territorial Agreement in the Lviv Region, 2018; Territorial agreement in the Mykolayiv Region, 2017; Territorial agreement in the Sumy Region, 2019; Territorial agreement in the Kharkiv Region, 2016; Territorial agreement in the Kherson Region, 2019; Territorial agreement in the Chernihiv Region, 2017)

The practice of developing regional development programmes and determining the growth rates of average wages at enterprises in the region (Vinnytsia, Dnipropetrovsk, Luhansk and Kharkiv regions) is positive. Inflation-oriented practices and state-level wage growth are common in determining wage growth rates. The practice of regulating by territorial agreements the tariff rate of a 1st category worker and the share of the basic salary in the wage fund should be recognized as positive. Despite some positive practices, based on the analysis of the structure and content of territorial agreements in terms of partners' obligations to pay, it was concluded that agreements at this level can not claim recognition of their territorial regulations. This conclusion is based on the following provisions:

- the norms do not take into account regional peculiarities, in particular do not determine the size of the regional subsistence level and the regional minimum wage;
- there are no guarantees and norms on the implementation of payments financed from the local budget (in addition to those provided by law or in amounts exceeding those specified by law);
- most norms and provisions duplicate legislative norms and norms provided by the general agreement, etc.

A significant number of provisions of territorial agreements are declarative (non-specific) and do not contain specific mechanisms for the implementation of the obligations undertaken by the parties, there is a lack of personification of responsible persons. A comparative analysis of current and previous territorial agreements showed that the rules of current agreements of different regions practically duplicate the provisions of previous agreements. Most agreements, which are characterised by an increase in the number of obligations of the social partners (Table 3), have such an increase conditioned upon general (non-specific) obligations, and recommendations for the inclusion of relevant rules by collective agreements of enterprises in the region.

The ratio of the number of provisions of territorial agreements on wages, which contain specific obligations (figures, amounts, interest, documents, procedures, programmes), and the number of general obligations of the social partners on wages is shown in Figure 1. As evidenced by the data of Figure 1, the most successful in this indicator is the practice of regulating wages on the basis of social partnership in Ivano-Frankivsk and Sumy regions, where 71% of the obligations of the social partners are specific. Among the least successful is the practice of wage regulation in Chernihiv region, where only 29% of commitments are specific.

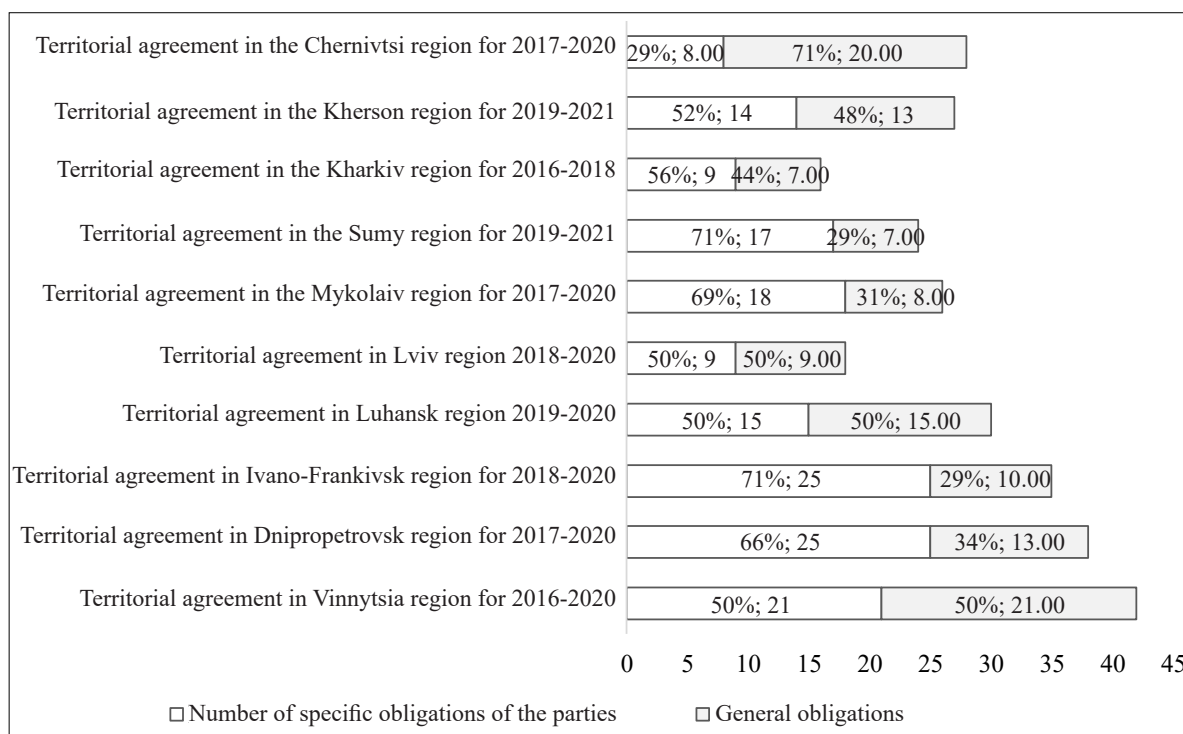


Figure 1. Correlation between the number of provisions of territorial wage agreements that contain specific obligations and the number of general obligations of the social partners on wages

Source: compiled according to data (Territorial Agreement in the Vinnytsia Region, 2016; Territorial Agreement in the Dnipropetrovsk Region, 2017; Territorial Agreement in the Ivano-Frankivsk Region, 2018; Territorial Agreement in the Luhansk Region, 2018; Territorial Agreement in the Lviv Region, 2018; Territorial agreement in the Mykolayiv Region, 2017; Territorial agreement in the Sumy Region, 2019; Territorial agreement in the Kharkiv Region, 2016; Territorial agreement in the Kherson Region, 2019; Territorial agreement in the Chernihiv Region, 2017)

The ratio of the tariff rate of the 1st category worker and the minimum wage (subsistence level) is regulated only by territorial agreements in Ivano-Frankivsk and Sumy regions: respectively not less than 110 and 115% of the subsistence level/minimum wage that meets and exceeds the established standard. Territorial agreements in Lviv and Kherson oblasts do not regulate this relationship. Other territorial agreements stipulate that the amount of the tariff rate of a 1st category worker should be regulated by sectoral agreements and collective agreements. Progressive are the rules for employee participation in the development, use and distribution of enterprise profits. Such norms include agreements in Ivano-Frankivsk, Chernihiv and Kherson regions. However, the agreements of the studied regions do not contain obligations and (or) recommendations for the implementation of social packages, social insurance programmes (medical, private pension, life, accidents, etc.) at enterprises in the region, which negatively characterises the level of collective bargaining wages at the territorial level. The experience of including norms on observance of gender equality in remuneration of labor by territorial agreements in Ivano-Frankivsk and Mykolayiv oblasts is positive.

One of the main indicators of the assessment of collective bargaining regulation of wages is the extent to which the social partners comply with the provisions of

the territorial agreement. In Ukraine, there is no practice of publishing reports on the implementation of agreements by social partners. It is not possible to check the level of implementation of agreements of a general nature. Fulfillment of part of the obligations should be investigated separately at each enterprise and institution in the region. However, the unequivocal conclusion can be drawn that not all commitments of the social partners have been fulfilled. For example, all agreements in the surveyed regions contain partners' obligations to repay wage arrears. However, the analysis of the data of the State Statistics Service of Ukraine shows that in all regions of Ukraine there are arrears of wages. Conditioned upon the lack of complete information on the extent to which the social partners comply with the provisions of territorial agreements, this indicator will not be taken into account when calculating a comprehensive indicator of the level of collective bargaining in the studied regions.

The next indicator is the level of coverage of employees by collective agreements. The analysis of this indicator in different regions (Fig. 2) showed that Sumy and Kharkiv regions are leaders in this indicator, which indicates a high level of development of collective bargaining regulation at the local level. The lowest level of coverage of employees by collective agreements in the Lviv region.

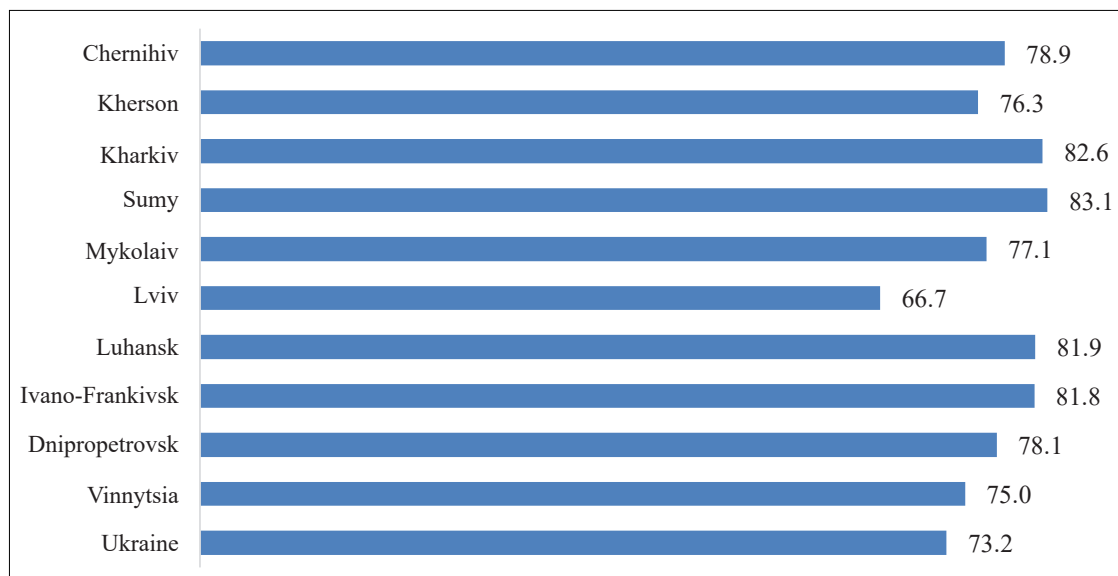


Figure 2. The level of coverage of employees by collective agreements in the studied regions in 2019, %

Source: compiled according to data (State Statistics Service of Ukraine, 2020)

Analysis of the size and dynamics of wages

Among the indicators that characterize the level of development of collective bargaining regulation at the territorial level, the *size and dynamics of real wages* occupy a prominent place. These indicators reflect the result of the practical implementation of the agreements of the main subjects of collective bargaining, structure, timeliness of payment of wages, non-discrimination and

ensuring gender equality in pay. The amount of nominal and real wages in the studied regions are given in Table 5. According to the data in Table 5, the highest growth rates of real wages in 2019 among the studied regions were observed in Mykolayiv region, the lowest – in Lviv.

The ratio of average wages in the studied regions with the average wage in Ukraine in 2018-2019 is shown in Figure 3.

Table 5. The amount of nominal and real wages in the studied regions

Region	Nominal salary, UAH		Price index until 2019, %	Real salary in 2019, UAH	Real wage growth rates in 2019, %
	2018	2019			
Ukraine	8865	10497	104.1	10083.57	13.7
Vinnitsya Region	7801	9299	102.4	9081.05	16.4
Dnipropetrovsk Region	8862	10751	103.7	10367.41	17.0
Ivano-Frankivsk Region	7551	8817	104.5	8437.32	11.7
Luhansk Region	7365	8731	104.3	8371.05	13.7
Lviv Region	8001	9271	104.2	8897.31	11.2
Mykolayiv Region	8160	9976	103.8	9610.79	17.8
Sumy Region	7324	8579	104.8	8186.07	11.8
Kharkiv Region	7657	9081	104.8	8665.08	13.2
Kherson Region	7058	8187	103.3	7925.46	12.3
Chernihiv Region	6995	8206	104.3	7867.69	12.5

Source: compiled according to data (State Statistics Service of Ukraine, 2020)

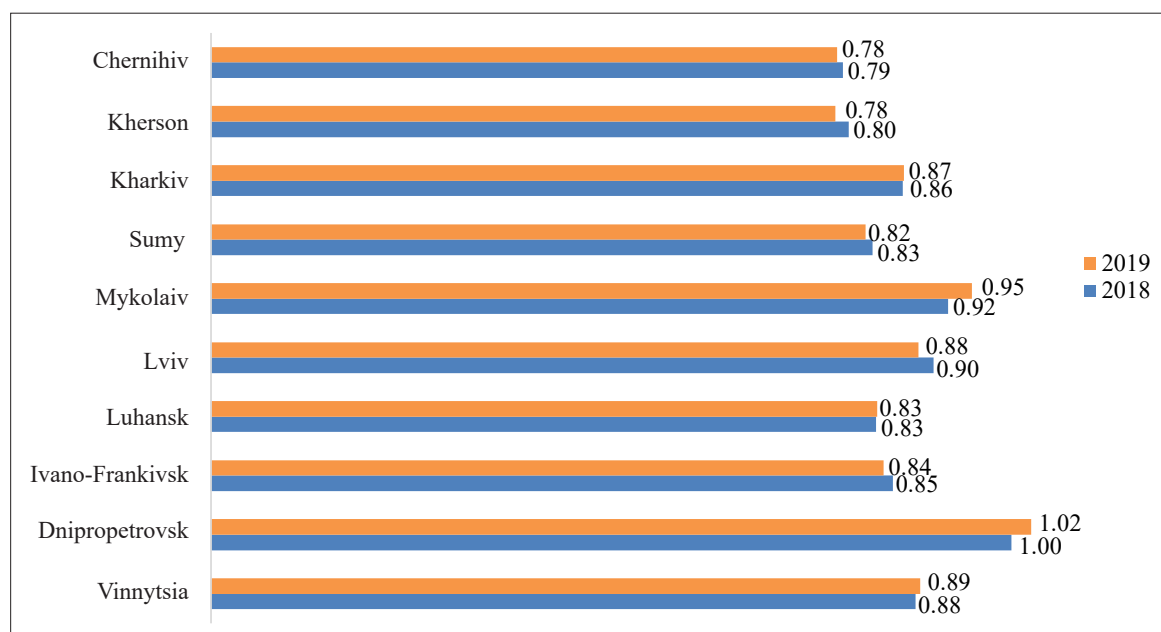


Figure 3. The ratio of average wages in the studied regions with the average wage in Ukraine in 2018-2019

Source: compiled according to data (State Statistics Service of Ukraine, 2020)

The highest is the ratio of average wages in the region with the average wage in Ukraine in Dnipropetrovsk region, the lowest – in Kherson and Chernihiv regions. Moreover, during 2018-2019, this indicator in some regions had a positive trend, and in others – negative. So, the lag of a salary in the Mykolaiv region in comparison with a salary in economy of Ukraine considerably decreased and increased – in the Lviv and Kherson regions. The next indicator for assessing wage policy is *the share of workers who receive wages below the subsistence level in the total number of workers in the region*. According to the Ministry of Social Policy of Ukraine, the amount of the actual subsistence level for able-bodied persons, considering the amount of mandatory payments as of the end of 2018 in Ukraine was 3884.9 UAH (The actual subsistence level..., 2020). According to the State Statistics Service of Ukraine (2020) in 2018 in terms of surveyed regions in 2018 the average per capita equivalent total income below the actual subsistence level received: 26.3% of the population of Vinnytsia region, 18.8% – Dnipropetrovsk, 23.3% – Ivano-Frankivsk, 27.3% – Luhansk, 17.5% – Lviv, 31.9% – Mykolaivska, 37.5% – Sumy, 32.1% – Kharkiv, 42.5% – Kherson, 21% – Chernihiv regions. According to the data, the worst situation is in the Kherson region. The lowest is the share of the population with an average per capita equivalent total income below the actual subsistence level in Lviv region, which may indicate a slight differentiation of household income.

The next indicator of the assessment of collective bargaining regulation is *the share of workers in the region who received wages below the poverty line*. According to

World Bank experts, the absolute poverty line for Central and Eastern Europe is 4.3 US dollars per day, i.e. 130 dollars. US per month. The hryvnia equivalent (at the NBU exchange rate) of the absolute poverty line in Ukraine at the end of 2018 was 3,599.48 UAH, and at the end of 2019 – UAH 3,079.21. According to the State Statistics Service of Ukraine, in 2018 part of the population received an average per capita equivalent total income less than the statutory subsistence level (UAH 1,853): 0.4% of the population of Vinnytsia region, 2.6% – Dnipropetrovsk, 0.1% – Ivano-Frankivsk, 1.5% – Luhansk, 0.5% – Lviv, 0.6% – Mykolaiv, 0.8% – Sumy, 0.4% – Kharkiv, 2.6% – Kherson, 1.3% – Chernihiv region. Since the statutory subsistence level is less than the hryvnia equivalent of the absolute poverty line, it can be concluded that some workers in the surveyed regions received wages below the poverty line. The presence of workers with incomes below the subsistence level and the absolute poverty line negatively characterizes wage policy, as a significant part of the population lacks the means to reproduce their workforce, professional, personal and cultural development.

Regarding the share of basic wages in wages (Table 6), in all surveyed regions in 2019 this figure was low – less than 60%. This indicator was the highest in Kharkiv region, the lowest – in Dnipropetrovsk and Ivano-Frankivsk regions. Conditioned upon the fact that the basic salary is a constant component of income, the structure of wages does not contribute to meeting the needs of workers in protection and stability, which belong to the basic needs. Thus, the share of basic wages negatively characterises the wage policy of the studied regions.

Table 6. The share of basic wages in wages in the studied regions in 2019

Region	The share of basic wages in wages, %
Vinnitsya Region	57.3
Dnipropetrovsk Region	55.5
Ivano-Frankivsk Region	55.7
Luhansk Region	56.1
Lviv Region	58.9
Mykolayiv Region	56.0
Sumy Region	57.2
Kharkiv Region	59.1
Kherson Region	57.3
Chernihiv Region	59.5

Source: compiled according to data (State Statistics Service of Ukraine, 2020)

One of the main indicators is *wage arrears*. The dynamics of this indicator in the studied regions in 2011-2020 is shown in Figure 4. Wage arrears occur in

all regions studied, and in some of them it has tended to increase in recent years. This primarily applies to Luhansk, Kharkiv, Dnipropetrovsk and Sumy regions.

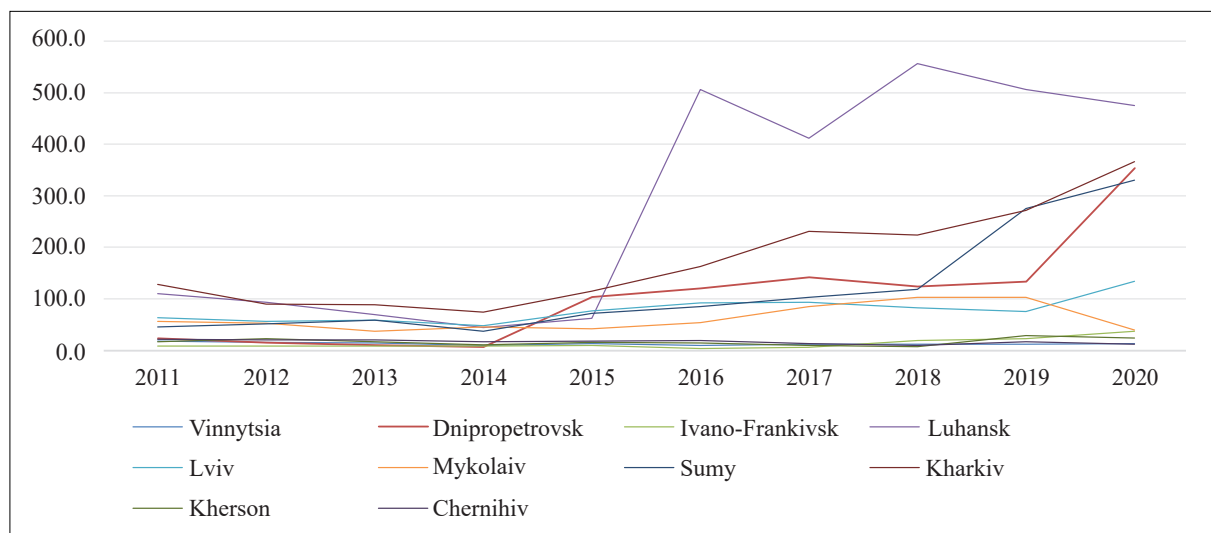


Figure 4. Dynamics of arrears of wages in the studied regions on 01.01 of the corresponding year in 2011-2020, UAH million

Source: compiled according to data (State Statistics Service of Ukraine, 2020)

The presence of arrears of wages negatively characterizes the level of development of collective bargaining regulation of wages in the studied regions, as it does not allow employees to meet a variety of basic needs. Thus, the indicators that characterise the level and dynamics of wages in the studied regions, debt and wage structure negatively characterise the level of development of collective bargaining. Low wages negatively affect the motivation of employees, causes loss of interest in professional activities as a way of earning income, provokes illegal employment. This leads to the outflow of human capital abroad.

An important indicator of assessing the level of

development of collective bargaining is the *ratio of women's wages to men's wages*, as ensuring gender equality and non-discrimination is one of the priorities of socio-economic development of the state and regions. As evidenced by the data of Figure 5, the highest rate was in Kherson and Lviv oblasts (91 and 86.1%, respectively): in the regions with the lowest wages among men. The lowest ratio is in Dnipropetrovsk and Mykolaiv oblasts (71.4 and 74.7%, respectively): in the regions with the highest wages. The presence of significant gender gaps in workers' incomes negatively characterizes wage policy in terms of creating equal conditions and opportunities and does not meet the basic principles of decent work.

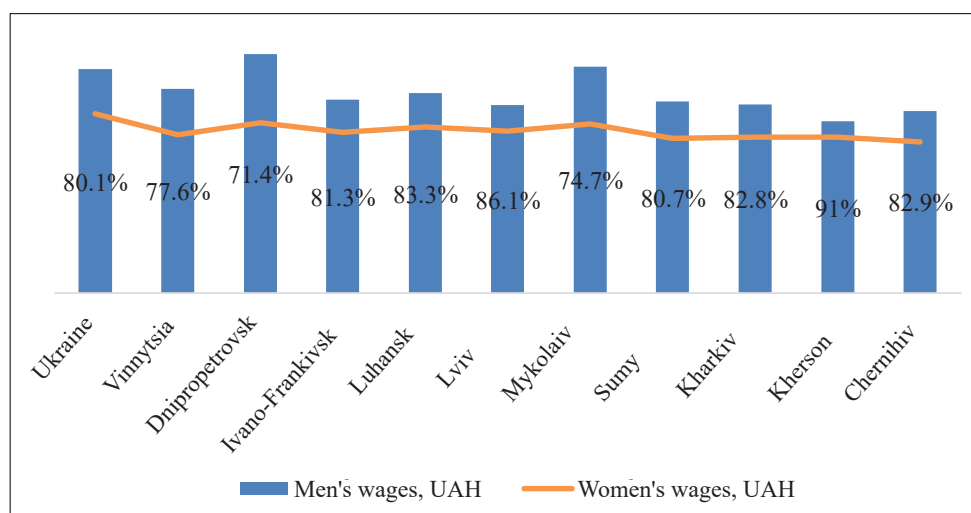


Figure 5. The ratio of women's wages to men's wages in the studied regions of Ukraine in the second quarter of 2020
Source: compiled according to data (State Statistics Service of Ukraine, 2020)

Analysis of a complex indicator of the level of development of collective bargaining regulation

The initial information for calculating the complex indicator of the level of development of collective bargaining regulation of wages is given in Table 7.

The calculation of the complex indicator of the level of development of collective bargaining regulation of wages in the studied regions is given in Table 8. Clustering of regions according to the level of development of collective bargaining regulation of wages is shown in Fig. 6.

Table 7. Initial information for calculating a comprehensive indicator of the level of development of collective bargaining

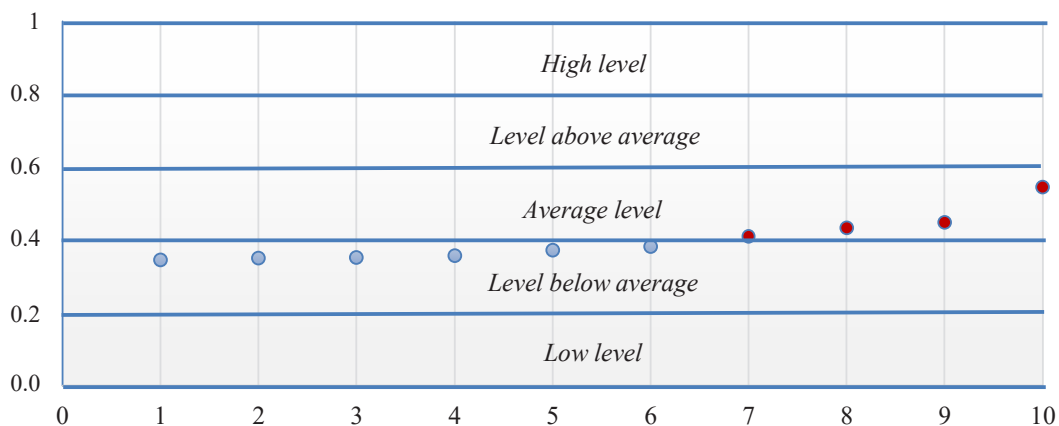
Indicator	Standard	X_{max}	X_{min}
1	Yes	–	–
2	Yes	–	–
3	Yes	–	–
4	→ max	42	16
5	→ max (≥ 1)	3.33	0.67
6	→ max (100)	71	29
7	→ max (≥ 110)	115	110
8	Yes	–	–
9	Yes	–	–
10	Yes	–	–
11	→ max (100)	83.1	66.7
12	→ max (≥ 110)	17.8	11.2
13	→ max (≥ 1)	1.02	0.78
14	→ min (0)	42.5	17.5
15	→ min (0)	2.6	+0.1
16	→ max (> 60)	59.5	55.5
17	→ min (0)	10 million EUR	10 million EUR
18	→ max (100)	91	71.4

Source: compiled by the author

Table 8. Calculation of a comprehensive indicator of the level of development of collective bargaining regulation of wages in the studied regions

Indicator	Single index									
	Vinnitsia Region	Dnipropetrovsk Region	Ivano-Frankivsk Region	Luhansk Region	Lviv Region	Mykolaiv Region	Sumy Region	Kharkiv Region	Kherson Region	Chernihiv Region
1	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
2	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
4	1.0000	0.8462	0.7308	0.5385	0.0769	0.3846	0.3077	0.0000	0.4231	0.4615
5	0.2744	0.3684	0.5714	1.0000	0.1466	0.0977	0.0000	0.1241	0.0752	0.0263
6	0.5000	0.8810	1.0000	0.5000	0.5000	0.9524	1.0000	0.6429	0.5476	0.0000
7	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000
8	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.0000	1.0000
9	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
10	0.0000	0.0000	1.0000	0.0000	0.0000	1.0000	0.0000	0.0000	0.0000	0.0000
11	0.5061	0.6951	0.9207	0.9268	0.0000	0.6341	1.0000	0.9695	0.5854	0.7439
12	0.7879	0.8788	0.0758	0.3788	0.0000	1.0000	0.0909	0.3030	0.1667	0.1970
13	0.4583	1.0000	0.2500	0.2083	0.4167	0.7083	0.1667	0.3750	0.0000	0.0000
14	0.6480	0.9480	0.7680	0.6080	1.0000	0.4240	0.2000	0.4160	0.0000	0.8600
15	0.8800	0.0000	1.0000	0.4400	0.8400	0.8000	0.7200	0.8800	0.0000	0.5200
16	0.4500	0.0000	0.0500	0.1500	0.8500	0.1250	0.4250	0.9000	0.4500	1.0000
17	0.9970	0.2623	0.9475	0.0000	0.7368	0.9419	0.3139	0.2349	0.9760	1.0000
18	0.3163	0.0000	0.5051	0.6071	0.7500	0.0000	0.4745	0.5816	1.0000	0.5867
Comprehensive indicator	0.4343	0.3822	0.5455	0.3532	0.3509	0.4482	0.3722	0.3571	0.3458	0.4109

Source: compiled by the author

**Figure 6.** Clustering of regions according to the level of development of collective bargaining regulation

Note: 1 – Kherson, 2 – Lviv, 3 – Luhansk, 4 – Kharkiv, 5 – Sumy, 6 – Dnipropetrovsk, 7 – Chernihiv, 8 – Vinnitsia, 9 – Mykolaiv, 10 – Ivano-Frankivsk

Source: compiled by the author

The results of the assessment of the level of development of collective bargaining regulation of wages showed that none of the surveyed regions fell into the cluster of regions with high and above average levels. Ivano-Frankivsk, Mykolaiv, Vinnytsia and Chernihiv regions were included in the cluster with an average level. The rest of the studied regions were in the cluster below average. The situation in the Kherson region is the least favourable in terms of the level of development of collective bargaining.

Among the oblasts that received the highest indicators of the level of development of collective bargaining regulation are representatives of different regions of Ukraine: Ivano-Frankivsk belongs to the western region, Mykolaiv – to the south, Vinnytsia – to the central and Chernihiv – to the north. This indicates that in Ukraine there are no clearly defined regions (Western, Eastern, Central, Southern or Northern) with the most favourable wage conditions and a high level of development of social partnership in terms of their regulation.

Interestingly, none of the studied regions from the eastern region was included in the oblasts that received the highest indicators of the level of development of collective bargaining regulation. Although traditionally for a long period of time in the eastern region of Ukraine the highest indicators of wages were observed. Such results are primarily related to the unsatisfactory political situation in the region, the occupation of some territories and hostilities in some parts of the eastern region, which negatively affected the socio-economic development of these regions.

Other Ukrainian scientists have reached similar conclusions. Studying depressed labour markets, V.Ya. Brych & O.V. Borysiak (2016) included the labour market of the eastern region of Ukraine as a result of hostilities and the emergence of internally displaced persons, which led to lower employment and incomes. The authors of another study (Burda & Chebotaryova, 2021) also singled out military action and occupation of some territories as the main adverse factor in the socio-economic development of the Donetsk economic region, which led to a decrease in the economically active population.

Each of the studied regions received at least one indicator of a single index at the level of the maximum value (1.0000). Most regions have maximum values of individual indices for 2-3 indicators. This indicates that each of the surveyed regions has its own best practices for the development and implementation of wage policies based on social partnership, which can be disseminated in other regions. In addition, all regions received zero values of single indices on at least three indicators, which indicates the presence of bottlenecks in wage policy. Policy makers and social partners (local governments, employers' organisations, trade unions) should focus primarily on the indicators with the lowest, including zero values of unit indices.

The results of the evaluation allow drawing an important conclusion that despite the unity of the legal space, which determines the movement in one direction, common traditions and forms of negotiations in different regions of the country, different priorities and tasks in regulating wages. The only feature for all agreements at the regional level is the inclusion of commitments of the social partners on wage growth rates and ignoring the requirement to publish the results of the analysis of the implementation of agreements (as a rule, performance is not evaluated by the negotiators). To some extent, this is the result of socio-economic policies pursued by the relevant regional administrations and a reflection of the general economic crisis in the country. At the same time, the data show the inability of the majority of social partners to expand the range of problems and update it in line with the challenges of the new socio-economic reality, in particular the sustainable development goals (overcoming poverty, promoting decent work).

The results of the evaluation showed a decrease in the activity of the social partners in the negotiation process, which correlates with the European trend. A study (Mailand & Due, 2004) of social dialogue in Poland, the Czech Republic, and Hungary found that social dialogue in these countries did not meet the minimum efficiency criteria and could not counteract the significant decline in real wages. The results of the analysis of the role of trade unions in social dialogue indicate a decrease in the coverage of employees by collective agreements and a weakening of trade unions in most EU countries, including Eastern Europe and Greece (Kjellberg, 2021). The results of the study of social and labour relations of Ukrainian researchers (Kolot & Pavlovskaya, 2013; Lanchenko, 2018; Motsna, 2019) also indicate the ineffectiveness of collective bargaining and social partnership, including in the regulation of wages.

Thus, the typical challenges facing the vast majority of European countries are relevant for Ukraine: unwillingness of the social partners to interact in a decentralised collective bargaining, low motivation to expand the areas of responsibility of partners (inclusion of important tasks for the region), lack of awareness of regional social partners (on regional levels) on world practices. The specific feature of the Ukrainian regional segment of collective bargaining is low opportunities (resources) and awareness of the use of digital platforms in current activities and the traditional focus on the national level of negotiations, and "removal" from responsibility.

Recommendations for the development of social dialogue and social partnership

Collective bargaining remains a powerful tool for the region's development, as only the ability of the social partners to find a compromise solution can alleviate economic and social unrest. First of all, the effective

use of this tool to regulate social and labor relations is associated with rethinking the goals of collective bargaining in accordance with the challenges of the new volatile economy with the development of a relevant legislative field that would expand the rights and obligations of social partners, including at the regional level. In particular, in Ukraine it is extremely important that the agreement becomes a real normative act, non-compliance with the provisions of which leads to legal consequences. The recommendations are common to all countries:

- development of the content of collective agreements on the principle of “responsibility to stakeholders” (the content of the collective agreement at the regional level should consider trends and characteristics of the region, so include provisions to address pressing issues; at the same time, the setting of tasks should be carried out on the basis of project management, so the establishment of regional standards in wages implies their realism and validity);

- harmonisation of priorities of goals and objectives and their actualization in accordance with forecast calculations, trends in the socio-economic environment (social and economic tasks should be balanced and coordinated; priority should be social tasks that affect the economic development of the region, in particular, it may be justification of the minimum wage, the actualisation of tasks includes the inclusion of provisions for the elimination of socio-economic inequality, for example, the elimination of gender discrimination in wages, ensuring an independent audit of the effectiveness of agreements);

- ensuring transparency and inclusiveness of the negotiation process (the agreement should be a comprehensive document, so the provisions on wages should be correlated with others and be as transparent as possible; all market actors should be involved in the negotiation process, including NGOs and the scientific community, expand social consolidation dialogue and social responsibility).

CONCLUSIONS

From now on, collective bargaining has become almost the only tool that allows us to respond flexibly and quickly to changes in the socio-economic environment. At the same time, the collective bargaining itself should

change the format and expand the circle of social partners, involving other stakeholders – market players. Such transformations will allow updating the content of the agreements, updating them in accordance with the strategic goals of the region, which is in the outline of sustainable development goals.

The results of the study of the level of development of collective bargaining regulated wages and identified best practices that can be implemented by police makers and social partners (local governments, employers' organisations, trade unions) at the regional level, at the level of local communities. Among the bottlenecks that negatively characterise the level of social partnership development and regional wage policy are low wages, unsatisfactory structure of labor incomes, including low share of basic wages, the presence of arrears of wages. This has a negative impact on employee satisfaction with wages, interest in professional implementation, and as a consequence on work results. The presence of significant gender pay gaps does not contribute to equal conditions and opportunities, and causes a sense of injustice.

Among the positive practices that should be considered by police makers and social partners, we highlight the practice of developing regional development programmes and determining the growth rate of average wages in enterprises in the region. Progressive practices of collective bargaining regulate wages include regulations on the tariff rate of the 1st category worker, the share of basic wages in the wage fund, employee participation in the formation, use and distribution of enterprise profits, gender equality in wages.

Summing up, we add that the proposed method considers the specific phenomena that exist in the social and labour sphere in Ukraine. As it covers the basic and partial components of the evaluation, it can be adapted to the conditions of other countries. In particular, the basic components (indicators) are common and are found in countries where territorial agreements are a practice of social dialogue. However, one of the indicators (the ratio of the wage rate of the 1st category worker and the minimum wage or subsistence level) and the partial components to be taken into account in other countries must be calibrated according to the standards and laws and practices of that country. However, the vast majority of indicators can be useful for assessing the effectiveness of collective bargaining in other countries.

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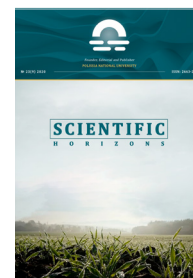
Колективні переговори та регулювання оплати на регіональному рівні: оцінка та перспективи (на прикладі України)

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Анотація. В умовах постійних викликів і загроз у реалізації громадянами свого права на працю колективно-договірне регулювання стає чи не єдиним ефективним інструментом, який дозволяє узгодити інтереси різних суб'єктів і консолідувати їх спільні зусилля. Метою дослідження є порівняльний аналіз рівня розвитку колективно-договірного регулювання оплати праці у різних регіонах України та визначення викликів і перспектив розвитку цього інституту. Автори запропонували методику, яка дозволяє кількісно та якісно оцінити колективно-договірне регулювання оплати праці на основі низки індикаторів та здійснити порівняльний аналіз рівня його розвитку у різних регіонах. Проведене оцінювання підтвердило гіпотезу про низьку активність соціальних партнерів і неспроможність мобільно діяти в умовах нової соціально-економічної екосистеми. Результати показали нерозвиненість регулювання оплати праці шляхом колективних переговорів у всіх регіонах, що посилює недовіру до інституту соціального партнерства та свідчило про нездатність партнерів створити умови для гідної праці. Виокремлено показники ефективності колективних переговорів і угод, характерних для країн з перехідною економікою (Україна), а також першопричини низької якості соціального діалогу на регіональному рівні. Виклики, притаманні не лише Україні, а й більшості європейських країн, дозволили авторам визначити напрями вдосконалення колективних переговорів, які передбачають розширення кола соціальних партнерів, адаптацію змісту угод до стратегічних цілей регіону та його сталого розвитку. Виявлені загальні тренди, що зумовлені викликами цифрової економіки та нової економічної екосистеми, актуалізували потребу переосмислення ролі та змісту колективних переговорів. Практична цінність дослідження полягає в можливості застосування розробленої методики соціальними партнерами, місцевими адміністраціями, територіальними громадами для оцінки соціального діалогу на регіональному рівні, вивчення та дисемінації кращих практик формування політики оплати праці на основі соціального партнерства.

Ключові слова: колективно-договірне регулювання, оплата праці, соціальний діалог, гідна праця, оцінювання, регіональні угоди



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Comparative Analysis of Ukrainian and European Accounting Standards

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Abstract. The relevance of the study is substantiated by the need to study the peculiarities of the implementation of accounting standards in Ukraine. This research is focused on the comparison of Ukrainian, American and European accounting standards in a number of key elements. The research aims to study problems, perspectives and factors that influence the implementation of international financial accounting standards, conduct analysis and make recommendations. The comparison method is the main method for investigating this problem, which allowed comparing National Accounting Standards, International Accounting Standards and the American system of accounting United States Generally Accepted Accounting Principles. The design of this research is observational and theoretical in structure, which is a prerequisite for detailed study of the theoretical grounds of international and Ukrainian accounting standards, their analysis and comparison. For the processing of information obtained through the method of comparison, the following methods of scientific knowledge were used: the method of analysis, method of synthesis, deduction and induction, the method of classification, as well as the method of analysis of scientific sources, studies, regulations and laws. The article presents the results of comparative analysis of Ukrainian, American and European accounting standards in a number of key elements with the aim of studying the problems, perspectives and factors that influence the implementation of International Financial Reporting Standards. The study includes recommendations for the direction of development and expansion of the National Accounting Standards. Materials of the article are of practical value for accountants, lecturers and students of the specialty "Accounting and Audit", public servants

Keywords: stocks evaluation, regulation of agricultural business transformation accounting, regulations, international accounting standards, accounting standards comparison



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INTRODUCTION

Globalisation has contributed to increased economic changes and the internationalisation of companies and markets, which has enhanced the demand for harmonisation of accounting standards. The adoption of International Financial Reporting Standards (IFRS) (2013) around the world is a reflection of the move towards global accounting standards (International Accounting Standards – IAS (2020)), which are vitally important trends indicating an increase in the sophistication of the accounting system. Standardisation is a requirement that establishes a set of strict rules that are the same in different economies, whether they are related or not (Gruda, 2016; Garzella *et al.*, 2020). Historically, financial accounting standards have been developed independently by each country. Sometimes standards are set by governments and in some cases even by accounting specialists or independent accounting committees (Jakupi & Statovci, 2017). Over the past two decades, the accounting literature has increasingly focused on the value relevance of accounting information. Since the announcement of the implementation of IFRS (2013), there has been an increase in the literature that binds the value relevance of accounting information with the implementation of IFRS (2013) (Bhatia & Mulenga, 2019). Accounting practitioners in the public sector should be aware of the importance of reviewing the specific contexts of the developing countries, including the government structure, communication channels, informal networks and communication flows, before the reforms are rolled out. When these contextual elements are not emphasised, reforms tend to be delayed and encounter difficulties (Ashraf *et al.*, 2019; Adhikari *et al.*, 2021).

Accounting theory has logically explained and reinforced each accounting practice, which obtains systematic principles and methodologies other than additions to accounting and also guides the formulation of new practices and methods. Accounting is considered a digital language and periodic disclosure of financial information minimises its asymmetry. Even if such information is good or bad for the company's reputation, uneven, biased, flexible and incomplete, it is still considered valuable for its users. The literature documents numerous advantages of high-quality accounting information, ability to explain different aspects of business, impact on cost changes, increased comparability and transparency, enhanced reliability and relevance of value, improved and reliable information on profits, a significant reduction in management level, with revenue smoothing, reduced audit fees and tax burden, as well as asymmetric tax information (Deb, 2019; Okanova *et al.*, 2021; Polo-Garrido & Melia-Marti, 2021). Public administration and accounting systems have changed dramatically over the past decades. In line with the paradigm of new public management and economic rationality, financial information systems have been upgraded to improve accountability and transparency. Innovative efforts in accounting are

related to the implementation of cash-based accounting in the public sector.

An accrual-based accounting method is expected to improve the transparency of financial information and intergenerational fairness as well as cost-efficiency. Increased transparency, in turn, leads to reduced corruption and opportunistic behaviour of politicians. Although more and more countries in Europe and around the world are implementing accrual-accounting or at least a modified accrual-accounting at the administrative level and recent research shows that accrual-accounting is expected to grow even faster, some European countries are still using cash-based accounting or at least modified cash-based accounting, and national accounting practices and standards still vary across countries (Frintrup *et al.*, 2020). The recent move towards standards for fair value measurement beyond financial assets and obligations calls for more empirical evidence of fair value assessment, particularly of intangible assets. Not subjected to the monitoring fair value estimates are linked to the underlying economics but are also distanced from true values due to the presence of incentives for management accountability (Zhang & Zhang, 2017). In accordance with the Accounting Standards for Enterprises – Basic Standards. These Standards have been developed to regulate the recognition and assessment of the accounting elements, as well as the financial reporting presentation, which is controlled by the initial implementation of the Accounting Standards for Enterprises (Riccardi, 2016; Ilchuk *et al.*, 2019).

Thus, this study is aimed at comparing Ukrainian, American and European accounting standards in several key elements to identify problems, prospects and factors that affect the implementation of International Financial Reporting Standards.

MATERIALS AND METHODS

The design of this study has a review-theoretical structure, which is a prerequisite for a detailed study of the chosen theoretical fundament of international and Ukrainian accounting standards, their analysis and comparison. The method of comparison was the main method for researching the specifics of the implementation of accounting standards. This method was used to compare the National Accounting Standards (NAS) (2020) with the International Accounting Standards (2020) according to the following items: the scope of application; the composition of the assets defined by the standards as stocks; evaluation of stocks upon receipt; evaluation of stocks on disposal; valuation of stocks at the balance sheet date; allowable disclosure information about stocks in the notes to the financial statements (Accounting Research Bulletin..., 1953; 1955).

The differences in valuation of stocks in the financial reports between the National Accounting Standards (2020), International Accounting Standards (2020) and an American Accounting Standard – United States Generally

Accepted Accounting Principles (US GAAP) (2021) were also compared. The comparison was carried out based on the following characteristics: identification; expenses included in the prime cost; evaluation; methods of accounting of stocks loss; comparison of legislation regulating the transformation of agricultural activities were carried out through such indicators: capacity to change; management of changes. To process the obtained information through the method of comparing the different methods of scientific knowledge were used, including method of analysis, method of synthesis, deduction and induction, method of classification, as well as the method of analysis of scientific sources, studies, regulations and laws. Through a detailed analysis of the information obtained by means of comparisons, several key principles were formulated that distinguish international and Ukrainian accounting standards. This helped to deepen the understanding of legal, economic, cultural, ethnographic, territorial, etc. differences between Ukraine on the one side and the United States of America (USA) and European countries at the other.

Synthesis of this information helped to understand the greater tendency of National Accounting Standards (2020) to the International Accounting Standards (2020) than to the American system of US GAAP (2021), which led to the recommendation that in order to better understand the accounting information under IAS (2020) to add additional lines on the nature, liquidity, materiality of assets and their functions within the company, the amounts, nature, terms and conditions of repayment of

obligations, as well as the uniform assessment of assets and obligations. The conclusions that were made were based on the provisions of the accounting standards, the deduction method was applied. The deduction method was used in this work with the aim to justify the assumption that the necessity of production expenses accounting becomes especially urgent in recent years, biological conversion and calculation of the prime cost of animal products following International Financial Reporting Standards (2013) and management system. The method of classification plays an important role in this study, as all the tables presented were created using this method. The classification method was applied to divide the principles and characteristics of the reviewed accounting standards according to the categories. In the course of this study, an analysis of legal documents, standards and regulations, scientific research on accounting standards and the implementation of these standards was also carried out.

RESULTS AND DISCUSSION

The main prerequisite for the performance of business activities of the majority of enterprises is sufficient volume and effective use of production stocks. Among the objects of accounting in the enterprise, stocks take a significant place, because the speed of turnover of invested capital and profit of the enterprise depend on their efficient use. Table 1 provides a comparison of the definition of "Stocks" according to the National Accounting Standards (2020) and the International Accounting Standards (2020).

Table 1. Characteristics of NAS 9 "Stocks" and IAS 2 "Stocks"

NAS 9 "Stocks"	IAS 2 "Stocks"
Scope of application	
The norms of this standard do not apply to the following categories: unfinished production; current biological assets; financial assets	This Standard applies to all stocks, with the exception of the following: unfinished production; financial instruments; current biological assets, that are the result of agricultural activities and the production of agricultural products at the point of harvesting
Assets defined by standards as stocks	
– Raw, basic and auxiliary materials, components and other inventory items intended for production, works, services, distribution, transfer, production, its maintenance and administrative purposes; – Finished goods; – Low-value and perishable items; – Goods	– Goods retained for sale in the course of the business; – Manufactured for sale; – Main or supplementary materials for use in the production process or in the delivery of services
Evaluation of stocks upon arrival	
In accordance with the prime cost	In accordance with the prime cost or net realisable value (the lower of the two)
Evaluation of stocks during the loss period	
– The method of identifying the prime cost of a particular stock unit; – Evaluation of the average prime cost; – For the prime cost of first arrived goods (FIFO); – Standard costs; – Sale price	– Standard expenses method; – Retail price method

Continued, Table 1

NAS 9 "Stocks"	IAS 2 "Stocks"
Evaluation of stocks upon balance sheet date	
Stocks are recorded in the accounting and reporting at the lower out of two estimates: primary cost or net realisable value in the accounting and reporting system	Stocks are recorded in the accounts and reported at the net realisable value
Permissible information for disclosure of stocks in the notes to the financial statements	
Balance value of stocks transferred for processing, to the commission or to the outpost	The amount of any reversal of any partial disposal accepted as a decrease in the amount of stock, considered as the expenses of the period

Note: FIFO – "first-in-first-out"

Sources: What is the procedure for recognizing inventories as assets? Which accounting accounts reflect recognized inventories? (2003)

In the USA stocks accounting is carried out through US GAAP (2021) and is regulated by Accounting Research Bulletin No. 43 "Restatement and revision of Accounting research bulletins" (ARB No. 43) (1953), chapter 4 "Stocks Assessment", which describes the general principles used in assessing these stocks. Statement of Financial Accounting Standards No. 151 "Inventory Costs an amendment of ARB No. 43, Chapter 4" (2004), is now used for the stocks accounting. Compared to IFRS (2013), US GAAP (2021) is clearer and more ambiguous. In addition, accounting

terminology varies considerably. For example, in the British version, it is "stocks", while in the American version it is "inventories". Revealing the real financial state of the company depends on a reliable and correct evaluation. Table 2 shows certain differences in the evaluation of stocks in accordance with the requirements of generally accepted accounting principles of the USA (US GAAP (2021)), International Standards of Financial Reporting (2013) and NAS (2020).

Table 2. Characteristics of NAS 9 "Stocks" and IAS 2 "Stocks"

No.	Indicator	NAS	IFRS	US GAAP
1	Definition	Stocks are assets held by an enterprise/institution for the purpose of further sale, and held for use in the production of goods, work and the provision of services, as well as the management of the enterprise/institution	Stocks are assets held for realisation in the normal course of business. Stocks are held in the production process for such sale or are available in the form of main or auxiliary materials for use in the production process or the provision of services	Stocks are used for sale during one typical business cycle or one production cycle
2	Costs included in prime cost	The amount to be paid by the purchaser to the supplier according to the contract (after deducting indirect taxes), the amount of import tax, the amount of indirect taxes imposed on the enterprise in connection with the procurement of non-refundable stocks, transport-supply expenses and other costs directly connected with the acquisition of stocks and their pre-sale preparation	The price paid to suppliers, import duties and other taxes (other than those refundable), transportation costs, loading, unloading and other costs directly related to the purchase of finished goods, materials and services, expenses on processing (treatment), other costs incurred during the delivery of supplies and bringing them to a condition suitable for obtaining economic benefits by the economic entity	The amount of the invoice including purchase allowances, freight costs, the amount of insurance for the goods on the road, appropriate taxes and tariffs
3	Evaluation	According to the identified prime cost of the respective stock unit, average cost, the prime cost of first arrived goods (FIFO), standard expenses, selling price	At the prime cost of lower out of two estimates: initial (actual) price or market price	At lower out of the two estimates: initial (actual) price or the market price
4	Methods of accounting for the loss of stocks	The identified prime cost of a given stock unit is assessed by average prime cost, the prime cost of first arrived goods (FIFO), standard expenses assessment, or selling price	Using the specific identification method, the average prime cost method, the prime cost of first arrived goods (FIFO), the standard expenses method, and the retail prices method	At the prime cost of stock unit, the average prime cost, the prime cost of first purchased CC (FIFO method), the standard prime cost, the selling price, the prime cost of latest purchased a CC (LIFO method)

Note: LIFO – "last-in-first-out"; CC – Cost Centers

Source: National Regulation (Standard)... (2017); International Financial Reporting... (2021)

Purchased stocks are accounted at their actual value. This includes the interest of loan funds taken to acquire stocks. More specific provisions are listed in US GAAP (2021): the primary estimate includes the actual costs for purchasing after deducting commercial but taking into account also administrative and general expenses directly related to purchasing. When accounting under the contract completion method, this part of administrative and general costs is included in direct contract costs, and if the calculation is performed under a standard contract, it is included in indirect costs. Under the completed-contract method, revenues are recognised only when most of the work under the contract is completed (Accounting Research Bulletin..., 1955). US GAAP (2021) also permits to include in the actual cost of stocks the cost of interest incurred after recognition of the stocks, but up to the time when the stocks have been brought to a state of usability or disposal. According to p. 30 NAS 9 "Stocks", the first cost of stock, which is produced by the own efforts of the company, is composed of the expenses of raw materials spent directly on the production of stocks, as well as the labour costs of workers directly involved in the production of these stocks, with appropriate deductions on US GAAP (2021) also require consideration of social policies (National Accounting Standards, 2020). However, stocks at actual cost do not allow for inclusion in the prime cost of excess materials used, excess expenses associated with equipment downtime, transportation, storage and preparation for transportation (Bulatov *et al.*, 2020).

In accordance with ARB No. 43 (1953), the allocation of fixed production expenses is based on normal operating capacities. In this case, the normal level of utilization of equipment is defined as the level expected to be achieved over a given period under normal circumstances, taking into account the losses in productivity related to the planned maintenance of production capacity. Therefore, in order to easily distinguish within the transformation process the overnormative part of constant production costs, costs of transportation, storage of inventories, equipment downtime and consumed materials, it is better to report them in the accounting in separate sub-accounts. To determine the amount of constant total production costs, which are included in the cost of stocks, it is necessary to know the actual and normal level of production. If the actual level is lower than the normal level, the operating expenses per unit of stock are not increased.

US GAAP (2021) standards make it possible to identify, evaluate and improve the areas of application of accounting principles; reduce costs and complexity while preserving or improving the usefulness of the information provided to users of financial reports. In addition, the evaluation of stocks under ASC 330 "Stocks" is extremely challenging as it provides several potential outputs. To simplify the evaluation of stocks, the United States Financial Accounting Standards Board (FASB)

proposes to value stocks at a lower cost: first or net realisable value. Net realisable value is defined in the Main Dictionary as "estimated sale price in the normal course of business, except for reasonably anticipated expenses on completion, export and transportation", taking into account the replacement cost of stocks and the net realizable value of stocks less approximately normal profit margins. This proposition more closely aligns the measurement of stocks in US GAAP (2021) with the measurement of stocks in International Financial Reporting Standards (2013). IFRS 2 "Stocks" requires inventories to be valued at the lower cost, first or net realisable value. Net realisable value is defined in IFRS 2 as "the estimated selling price in the normal course of business with deducting the estimated costs of completion and the approximate costs necessary to accomplish the sale" (International Financial Reporting..., 2013), which is similar to the US GAAP (2021) definition. Hence, the main differences between IFRS (2013) and US GAAP (2021) regarding the presentation of stocks in reporting are as follows:

1. Prime cost definition. In IFRS, the prime cost of stocks is determined using the formula FIFO ("first-in-first-out") or the average cost. The LIFO method ("last-in-first-out") is not allowed under IFRS. In US GAAP, the prime cost of stocks is determined using the formula of FIFO, lower cost or LIFO.

2. Stocks evaluation. Before 2017, IFRS and US GAAP measured the value of stocks at the balance sheet date after their initial recognition in different ways. Under US GAAP, stocks were valued at the balance sheet date at the lower out of two values: prime cost or market price. Under IFRS stocks were valued at the lower of prime cost or net realizable value. US GAAP allows all methods, accounting at sales prices less costs of realization (section 4, p. 16, ARB No. 43 (1953)). This method can be used for the stock accounting listed on an active market, in particular for precious metals with a fixed monetary valuation, the cost of sales of which is not significant (section 4, p. 9, ARB No. 43 (1953)). Another evaluation method in US GAAP may be standard cost accounting; this method is used in p. 21 IAS 2 (International Accounting Standards, 2020). However, it can only be used if the standard cost approximately reflects the cost of stocks calculated by one of the above-mentioned methods. In this case, it is necessary to disclose the method of determining. Starting from 2017, the value of stocks as of the balance sheet date is determined as the lowest value of the prime cost of or net realizable value of sales in both accounting methods.

3. US GAAP is a national accounting standards that exists in a particular country. It differs from IFRS in that it is very detailed and has several proprietary accounting rules. There is a general trend for all countries to converge US GAAP and IFRS standards.

The integration of Ukrainian agricultural producers into the world economy, participation of Ukrainian

producers in the competition on the international market and implementation of progressive market mechanisms of state management have led to the reform of the accounting system and required the improvement of economic information as well as bringing the national accounting and reporting system into compliance with international standards (Trusova *et al.*, 2021). Thus, the adoption and implementation of National Accounting Standard 30 “Biological Assets” (National Accounting Standards, 2020) was a necessary regulatory and methodological document for agricultural enterprises due to the specifics of their activities. The activity of agribusiness enterprises is related to the production and realisation of agricultural products of agriculture and livestock, and the main part of the income is generated from the sale of these products. Thus, the accounting of income from the primary recognition and realisation of biological assets requires a detailed study. Today, the regulatory framework for agricultural activities in Ukraine is regulated by the NAS 30 “Biological Assets” (National Accounting Standards, 2020) and Methodical recommendations on accounting of biological assets (Order of the..., 2006), and by international standards – IAS 41 “Agriculture” (International Accounting Standards, 2020).

In recent years, in order to provide information on various layers of accounting, control and management of agricultural activities, the need to bring the prime costs of goods into line with international standards has become particularly urgent, as well as biological reorganization and calculation of the prime cost of animal products in accordance with International Financial

Reporting Standards (2013) and management system. The practical application of the provisions of IAS 41 “Agriculture” is of particular relevance for agricultural enterprises (International Accounting Standards, 2020). For comparison, authors should review the main criteria of regulatory acts on agricultural business accounting: NAS 30 “Biological assets” (National Accounting Standards, 2020), Methodological recommendations on the accounting of biological assets (Order of the..., 2006) and IAS 41 “Agriculture” (International Accounting Standards, 2020). The main criteria of IAS (2020) differ from criteria NAS 30 (National Accounting Standards, 2020) and Methodological recommendations on the accounting of biological assets (Order of the..., 2006). Moreover, analyzing NAS 30 (National Accounting Standards, 2020) and IAS 41 (International Accounting Standards, 2020), authors see different definitions of “agricultural activities”. Thus, according to NAS 30 (National Accounting Standards, 2020), agricultural activity is “the process of managing biological transformations to obtain agricultural products and/or additional biological assets”. According to IAS 41 (International Accounting Standards, 2020) agricultural activity is “the management by the economic entities of biological transformation and collection of the crop of biological assets for sale or for processing into agricultural products or additional biological assets”. Thus, both Ukrainian and international legislation have inconsistencies even in the term “agricultural activities”. However, it should be noted that despite the different types of agricultural activities: gardening, animal husbandry, poultry farming and others, there are also general rules (Table 3).

Table 3. Comparison of regulations governing agricultural transformation accounting

Criterion	NAS 30 “Biological assets”	IAS 41 “Agriculture”
The capacity to change*	Reflects processes of change in biological assets in their qualitative and quantitative characteristics	Quantitative and qualitative changes in biological activities are characterized by growth, degeneration, production and reproduction processes
Change management**	The management of changes in biological assets is aimed at the production of agricultural products and/or other biological assets	Biological transformation is managed through the economic entities

Note: * – live animals and plants capable of biological transformation; ** – change management differentiates agricultural activity from other activities

Source: International Accounting Standard 2... (2012)

The main difference between IFRS (2013) and US GAAP (2021) regarding the accounting of biological assets in the reports is the biological assets accounting. Biological assets (live animals and plants, excluding fruit crops) in an enterprise may choose one of two models of biological asset accounting: primary (historical) cost accounting model; calculating the fair value model while not including the cost of sale. If the enterprise applies the first model in its accounting policy, it will have to test biological assets on devaluation by analogy with other capital assets every month. If the enterprise chooses a different accounting model, the number of changes

in the fair value of biological assets in subsequent periods will be reported in the Profit and Loss Report for the period to which it relates, in accordance with IAS 41 (International Accounting Standards, 2020). US GAAP (2021) “Agriculture” provides only one model for accounting for biological assets: at initial recognition and as of each reporting date, biological assets must be fairly valued based on sell expenses, unless the fair value cannot be estimated. All changes in fair value are recorded in the Profit and Loss Report in the period in which they occur. Thus, the existence of gaps in both IFRS (2013) and US GAAP (2021) systems leads to their convergence.

Under US GAAP (2021) items in “Balance Sheet (Financial State Report)” Form No. 1 must be ranked by the degree of decrease in liquidity of assets, from the most to the least liquid. International standards, unlike United States standards, do not regulate the order of liquidity but require it, items in the Balance Sheet may be in both a decreasing and increasing order of liquidity. According to international standards, the balance may be both classified and unclassified, but the assets and the obligation of repayment period less or more than 12 months must always be filed separately. International standards define current assets and liabilities (which differs from the definition in American standards), as well as formulating requirements for the minimum amount of information that is submitted directly to the Balance Sheet. The separate filing of these items in the Balance Sheet is due to their essence and significance for the users. The choice of the optimal method of evaluation of stocks by the enterprise will contribute to an increase in total assets, attracting investments, which will result in obtaining more profits and generally improving the financial condition of the enterprise.

The research of specific features of the accounting standards implementation was aimed at analysing the international experience of developed countries and comparing the main accounting standards. When comparing the main items to be disclosed in the balance sheet under NAS 1 (National Accounting Standards, 2020) and the basic information to be provided in the Financial State Report under IAS 1 (International Accounting Standards, 2020), it should be noted that there are differences in the Ukrainian version that do not comply with the approach to compiling financial statements under international standards. Thus, in accordance with IAS (2020) for a better understanding of information on the results of the analysis, additional lines can be added regarding nature, liquidity, the essence of assets and their functions within the company; amounts, character, terms and conditions of repayment of liabilities; uniform assessment of assets and liabilities.

CONCLUSIONS

Therefore, it can be concluded that the process of improving the structure of the Balance Sheet (Financial State Report) and updating its items in Ukraine is carried out in accordance with IAS. Ignoring US GAAP is justified, as the USA differs greatly from Ukraine both for its economic level of development, and geopolitical, economic and social parameters. In the author's opinion, the changes in the structure of the Balance Sheet (Financial State Report) and the financial reporting, in general, should include the experience of the European Union, as the European integration processes are now gaining more and more momentum. The convergence of IAS and US GAAP standards is a strategic issue for the future. Based on the purpose of the study, it was found that the fundamental difference between the most widespread accounting and reporting systems in the world (IFRS and US GAAP) is that US standards are based on rules, while European (international) standards are based on principles. Having analysed stock accounting, it can be concluded that US GAAP is more detailed and specific and obtains numerous guidelines; IFRS provides a general approach, offering alternative models for the use and presentation of the value of inventories in financial reports.

In a detailed study of the topic, the authors noted the features of the process of gradual convergence of IFRS and US GAAP. The process of their convergence has led to the overlapping of principles and rules for the financial reporting process, which is certainly a progressive step on the path of development and implementation of global world standards (US GAAP) in Ukraine. The adoption of new high-quality accounting standards in Ukraine appears promising. The adoption of such standards will help both align Ukrainian legislation with international norms and facilitate financial reporting by companies in certain sectors. For the development of a comprehensive system of accounting standards, further research and comparison of Ukrainian and American experience in production inventory accounting are required.

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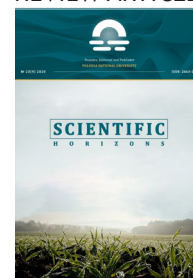
Порівняльний аналіз українських та європейських стандартів бухгалтерського обліку

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Анотація. Актуальність дослідження зумовлена необхідністю вивчення питання особливостей запровадження стандартів бухгалтерського обліку в Україні. Це дослідження спрямоване на порівняння українських, американських і європейських стандартів бухгалтерського обліку за ключовими елементами з метою вивчення проблем, перспектив і чинників, що впливають на впровадження міжнародних стандартів фінансової звітності, проведення аналізу, надання рекомендацій. Головним методом у дослідженні цієї проблеми є метод порівняння, який дозволив провести зіставлення Національного Положення (Стандарту) Бухгалтерського Обліку, Міжнародного Стандарту Бухгалтерського Обліку та американської системи обліку US GAAP. Дизайн цього дослідження має оглядово-теоретичну структуру, що є передумовою для детального вивчення обраного теоретичного підґрунтя міжнародних та українських стандартів бухгалтерського обліку, їх аналізу та порівняння. Для обробки інформації, отриманої за допомогою методу порівняння було використано такі методи наукового пізнання: метод аналізу, метод синтезу, дедукцію та індукцію, метод класифікації, а також метод аналізу наукових джерел, досліджень, постанов і законів. В статті подано результати проведеного порівняльного аналізу українських, американських і європейських стандартів бухгалтерського обліку у ряді ключових елементів з метою вивчення проблем, перспектив і чинників, які впливають на впровадження міжнародних стандартів фінансової звітності, надано рекомендації щодо напрямку розвитку і розширення Національного Положення (Стандарту) Бухгалтерського Обліку. Матеріали статті становлять практичну цінність для бухгалтерів, викладачів і студентів спеціальності «Облік і аудит», державних службовців

Ключові слова: оцінка запасів, регулювання обліку трансформації сільськогосподарської діяльності, нормативні акти, міжнародні стандарти бухгалтерського обліку, порівняння стандартів обліку



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Concept and Measurement of the Food System Sustainability: A Bibliometric Research

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Abstract. In view of climate change and population growth, making food system sustainable is a global concern. A lot of policies regulate this issue, but recent data show that global (and national) food systems are still far from the sustainability. Improvement of existing policies in the field of food system regulation requires a clear understanding of the food system sustainability concept itself: specification of its traits, attributes, measurement indicators, and goals. These issues are reflected in a lot of research papers, but none of these articles summarise the major trends, content, and features of the food system sustainability concept's evolution. This study for the first time summarises history and contents of research in the field of concept and measurement of food system sustainability through bibliometric analysis of Scopus indexed papers for 1991-2022. The growth of scientific interest in this area, led by researchers from the USA, Italy, and France, was found to be wave-like with peaks following the global food crises appearance patterns. The results of this study show that modern perception of the concept (since 2018) is complicated and intertwined in the notions of systems and system thinking, sustainability, and life cycle assessment, contrasting to the previous views (1991-2018) focused on food production system and food security. In general, a little attention is given to social and economic aspects of food system sustainability, in contrast to environmental, food, and agri-food issues. The elaboration of one-size-fits-all policy and solutions favouring food systems sustainability is suggested to be unlikely due to the complexity of the concept's perception. Governance of food system sustainability should go in line with concrete institutional, economic, social, and natural environments that need to be comprehensively studied in a future. Studies from developing countries are of special interest in this context

Keywords: sustainable food, concept evolution, assessment, bibliometrix, biblioshiny



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INTRODUCTION

Food security and global food system sustainability are the main challenges of modern development on the background of climate change, natural disasters, and pandemic crisis (Pachapur *et al.*, 2020). Although the FAO defined a sustainable food system in 2018 as “a food system that delivers food security and nutrition for all in such a way that the economic, social, and environmental bases to generate food security and nutrition for future generations are not compromised” (FAO, 2018), society still lacks the clear vision of ways, steps, and tools to guarantee the food systems sustainability locally and globally (Loboguerrero *et al.*, 2020; Towards a sustainable food system, 2020; von Braun *et al.*, 2021; Mishenin *et al.*, 2021). Food security and global food system sustainability are the main challenges of modern development on the background of climate change, natural disasters, and pandemic crisis (Pachapur *et al.*, 2020). Although the FAO defined a sustainable food system in 2018 as “a food system that delivers food security and nutrition for all in such a way that the economic, social, and environmental bases to generate food security and nutrition for future generations are not compromised” (FAO, 2018), society still lacks the clear vision of ways, steps, and tools to guarantee the food systems sustainability locally and globally (Loboguerrero *et al.*, 2020; Towards a sustainable food system, 2020; von Braun *et al.*, 2021; Mishenin *et al.*, 2021). According to FAO and World Bank estimates, about 11% of people globally are undernourished, which renders the achievement of “zero hunger” sustainable development target until 2030 impossible (FAO, 2019; World Bank, 2020). Despite the sufficiency of resources available to feed up to 10 billion people, current hunger problem exists precisely because of unfair resources’ allocation (World Bank, 2020). Food system vulnerability also deepens due to climate changes affecting agricultural productivity and income of rural people. Extension of farmlands (especially in developing countries) – to meet the challenges of productivity decrease and additional food demand caused by population growth – jeopardises functioning of ecosystems and exacerbates the climate problem (Koblianska *et al.*, 2021). In another vein, developed and rapidly developing countries also adversely affect the global food system sustainability due to change in diet and consumer behaviour following unhealthy and unsustainable patterns (increase of meat consumption, eating out, excessive food consumption, and food wasting). For instance, about 38% of energy consumed by global food chain is lost with food wastes (FAO, 2017; Klymchuk *et al.*, 2020). Meeting the food system sustainability challenge is eminently complicated for developing countries exporting food. Ukraine is a good case here to illustrate the adverse effects of prominent agriculture development. Despite the large amount of food exports feeding the world, Ukrainians (rural habitants above all) lack basic food in the diet: meat and dairy products, fish, vegetables, and fruits (Mishenin *et al.*, 2021). Number of people suffering from hunger and undernourishment amounts up

to 1.1 million (FSIN, 2020). Additionally, an environmental price of food produced in Ukraine, measured via environmental footprint, increases year by year (Koblianska *et al.*, 2021; Sineviciene *et al.*, 2021). The latter is true for other agriculture-dependent regions in the world: land-use change and forestry sectors, in contrast to developed world pathway, cause a considerable increase of carbon dioxide emissions in these countries (WRI, 2021). All this and expected resource scarcity (until 2050) will significantly intensify competition, destructive land use practices, and conflicts, endangering welfare of the most vulnerable, agriculture-dependent people around the world. In view of this, the food system sustainability transition is rather a matter of humanity survival than debatable issue. However, society needs a constructive, multi-stakeholder, interdisciplinary discussion on technological, social, cultural, economic, political, international aspects of future food system development path.

The current vision of further food systems development embraces transformations towards the sustainability driven by science and innovations. In this context, not only technological but also social, political, and institutional innovations ruled by values, perceptions, and conceptions of the food system sustainability come to the fore (Koblianska *et al.*, 2020; von Braun *et al.*, 2021). The value underlying the concept of food system sustainability also influences means of measurement and so, implementation actions that need to be harmonised (Schader *et al.*, 2014; Alrøe *et al.*, 2017; Leźnicki, 2021). This requires documenting knowledge and experience from different disciplines and intellectual traditions to reflect the challenges and critical threats to the sustainability of food system and to define a suitable framework for research, policy, and actions (von Braun *et al.*, 2021; El Bilali *et al.*, 2021). In view of this, a study of the complete set of interpretations of food system sustainability as a concept and its evolution is an effective way to shed light on the opportunities, strengths, and weaknesses of ensuring the systemic and coordinated actions towards the food system sustainability on innovative basis. *The purpose of this article* is to cover the evolution of the food system sustainability concept and explore insights via bibliometric research to support further fruitful scholar and policy discussions in this field.

LITERATURE REVIEW

The food system sustainability concept is quite broad and covers various phenomena. To illustrate the complexity and multifaceted nature of the concept, one should point out, for instance, the range of topics covered by the special issue “Towards more sustainable food systems” of the International Journal of Environmental Research and Public Health: 1) food security at the local level; 2) manifestation and potential impact of food production environment transformations; 3) food consumption environmental impacts and specific measures to deal with; 4) ways to support expedient food supply system transformations

(Kusch-Brandt, 2020). Despite the growing number of issues covered and studies on the food system sustainability, the society is still far from the desired changes (Loboguerrero *et al.*, 2020) and scholars point the need to explore the variety of values, perceptions, and approaches related to food systems sustainability aiming to ensure coordinated and cooperative actions towards further food system transformations (Alrøe *et al.*, 2017; Loboguerrero *et al.*, 2020; Kusch-Brandt, 2020). Within this framework, the bibliometric studies, providing insights on research field development and trends, are of growing interest.

Recent studies explore scientific efforts and results concerning the issues of sustainable consumption (Maria Claudia *et al.*, 2019; Nornajihah *et al.*, 2021), sustainable agri-food systems (El Bilali *et al.*, 2021) and their modelling (Monasterolo *et al.*, 2016), halal food system sustainability (Rejeb *et al.*, 2021), sustainability of urban food systems (Zhong *et al.*, 2021) and concrete food supply chain (Both *et al.*, 2021), etc.

Results of bibliometric analysis of Scopus indexed publications on sustainable consumption for 1974-2019 (2352 documents) made by N.H. Nornajihah *et al.* (2021) evidence the USA dominance in the number of articles published, the University of Göttingen (Germany) leadership in scientific productivity, and the most relevance of the Sustainability Switzerland as a publishing source in this field. Authors also identify four clusters of sustainable consumption psychosocial factors: social norms, attitude, environmental concern, and perceived value (Nornajihah *et al.*, 2021). Studying the food consumption sustainability, Maria Claudia *et al.*, (2019) found consumer and consumption patterns to be the thematic core of the Web of Science covered sample of publications for 1975-2018 with abstracts including "sustainable food consumption" (103 publications) (Maria Claudia *et al.*, 2019).

Agri-food systems are the closest to sustainability issues strand of food systems. Utilising the data from the Web of Science (1289 documents), H. El Bilali *et al.* (2021) analysed studies on the sustainability of agri-food systems and their relationships to environmental, economic, social, and political aspects of sustainable development in early January 2020. Authors noted a growing scientific interest concerning the sustainability of agri-food systems as confirmed by an exponential increase in the number of publications. Scholars from developed countries are found to be the most productive and relevant in this field. Research results testify a lack of emphasis on the social, economic, and political aspects of sustainable agri-food systems development, while environmental aspects are well explored (El Bilali *et al.*, 2021).

Modelling of agricultural and agri-food systems functioning constitutes an essential aspect of the food systems sustainability concept and studies. Monasterolo *et al.* (2016), applying citation and co-citations analysis, bibliographic coupling, and other classic bibliometric techniques, explored records from ISI Web of Science for 1970 to 2016 on Agricultural Modelling and Agricultural

Complex Systems Modelling topics and found the growing trend to use a comprehensive system approach to the analysis of agri-food systems in the framework of classical modelling techniques. Results of research show the need to account for the complexity of agricultural systems when developing the conceptual and methodological innovations aimed at changing global food policy towards sustainable development and inclusive agri-food systems (Monasterolo *et al.*, 2016).

A. Rejeb *et al.* (2021), exploring the halal food systems sustainability studies (74 journal articles), also noted the growing interest in this topic for the last two decades, but, in contrast to (El Bilali *et al.*, 2021), found the primary focus on economic and social aspects of halal food systems sustainability with a little attention paid to environmental issues (Rejeb *et al.*, 2021).

Within the corpus of urban food systems sustainability research covered by the Web of Science Core Collection (5,360 publications), Q. Zhong *et al.* (2021) found the dominance of environmental issues. Scholars noticed the growing scientific interest in this research field fuelled by urbanisation, social, and environmental changes, but a lack of studies concerning the urban food system resilience, trade-offs between resilience and sustainability, and comparative research. Issues of food security, food consumption, and food waste form the core of the urban food system sustainability research area (Zhong *et al.*, 2021).

As A.V.R. Both *et al.* (2021) mention, sustainability concern becomes a growing trend in organisations involved in food value chains (in particular, rice value chain) since 2016, driving research in this field (Both *et al.*, 2021).

One should point out that within the food system sustainability research field, studies on cellular agriculture (Nyika *et al.*, 2021), human rights and social justice (Oktaviani *et al.*, 2021), food security and climate change (Sweileh, 2020) are also of growing interest.

The abovementioned bibliometric studies explore various aspects of the complex issue of food system sustainability. However, there is a lack of a comprehensive research concerning the concept and measurement of food system sustainability. This study aims to fill this knowledge gap and, through bibliometric analysis, to elaborate on the contributors, evolution, modern visions, and growing trends in studies focused on conceptualisation and measurement of sustainability of food system.

MATERIALS AND METHODS

This study utilises the Scopus collection to form the sample of scientific records for bibliometric analysis. The Scopus provides a comprehensive database of publications analysing citations over a longer period compared to the ISI Web of Science Core Collection (Aria *et al.*, 2020; Nornajihah *et al.*, 2021). The search query was the following: TITLE (sustainab* W/3 *food* W/3 system*) AND (KEY (concept* OR theor* OR design* OR method* OR metric* OR assess* OR indicator OR measur* OR index*

OR frame* OR content); only articles, conference papers, reviews, and book chapters included; only English-language; all available years. The list of search results contained 107 documents as of October 19, 2021. The initial screen and analysis of records were made to eliminate the possible duplicated and inconsistent records: 1 document was excluded due to duplication, 9 documents – due to mismatching the request (not relevant content), 11 documents – due to absence of abstracts (2) and authors' keywords (9). The final sample amounts to 86 documents obtained from 52 sources for 1991-2022.

Bibliometric and network analysis is an effective way to illustrate general trends in the research field allowing to identify key schools of thoughts, academic interrelationships, research productivity through performance analysis, co-word analysis, bibliographic coupling, and factorial analysis. The analysis of networks reveals the social, intellectual, and conceptual structures of the studied field. Intellectual structures are exposed through bibliographic coupling that is an alternative to co-citation criterion for evaluating relationships. Coupling allows visualising links between publications revealing the common ideas. Conceptual structures are explored through the mapping of research space using co-word networks and factorial analysis. This allows to interpret the knowledge base embodied in the analysed collection and to explore the diverse topics within the research field (Aria *et al.*, 2020). The analysis was performed with the use of R {bibliometrix} package and

Biblioshiny App (R Core Team, 2014; Aria & Cuccurullo, 2017). Bibliometrix package is a tool to quantify the scientometric and bibliometric indicators, to analyse knowledge structures (intellectual, social, conceptual) utilising R programming language. In this research {bibliometrix} was applied through RStudio that is user-friendly integrated development environment (IDE) for data science (RStudio Team, 2020). The Bibliometrix package allows to run a Biblioshiny App – an easy-to-use web-based extension to perform a science mapping analysis comprising nice graphics (for example, to visualise indicators of different levels via three fields plot) (Aria & Cuccurullo, 2017).

RESULTS AND DISCUSSION

Food system sustainability research: Evolution and geographical patterns

Table 1 summarises the main data about the bibliographic collection, which mainly comprise journal articles (77%). The number of authors studying the food system sustainability concept and measurement amounts to 391 persons; the average number of documents per author is 0.22. Multi-authored publications predominate with an average size of research group of about five persons. However, the share of single-authored publications is also significant and amounts to 9%, that is every 11th publication in this collection is single-authored. The average time from publication to citation of the article is a little more than 4 years.

Table 1. Descriptive statistics for bibliographic collection

Description	Results
MAIN INFORMATION ABOUT DATA	
Timespan	1991-2022
Sources (Journals, Books, etc)	52
Documents	86
Average years from publication	4.2
Average citations per documents	16.12
Average citations per year per document	2.626
References	7021
DOCUMENT TYPES	
Article	66
Book chapter	2
Conference paper	11
Review	7
DOCUMENT CONTENTS	
Keywords plus (ID)	620
Author's keywords (DE)	398
AUTHORS	
Authors	391
Author appearances	414
Authors of single-authored documents	7
Authors of multi-authored documents	384
AUTHORS COLLABORATION	
Single-authored documents	8
Documents per Author	0.22
Authors per Document	4.55
Co-Authors per Documents	4.81
Collaboration Index	4.92

Source: authors' development via Bibliometrix

Analysing the quantitative parameters of research field development, one should note the wave-like growing number of publications and citations (Fig. 1).

Figure 1 illustrates periods of scientific interest increase and some spikes: 2003, 2008-2009, 2013-2017 (with peaks in 2014, 2017), 2020. A decline in number of research is followed by an increase in 2019 and a peak

in 2020. Change of an average number of citations per year also comprises severe peaks: 2003 (an average annual number of citations amounts to 4.87 and to 87.67 per article), 2008 (1.5 and 19.5, respectively), 2017 (7.93 and 31.73), 2019 (3.0 and 6.0) and 2021 (with the average number of citations per article of 0.23).

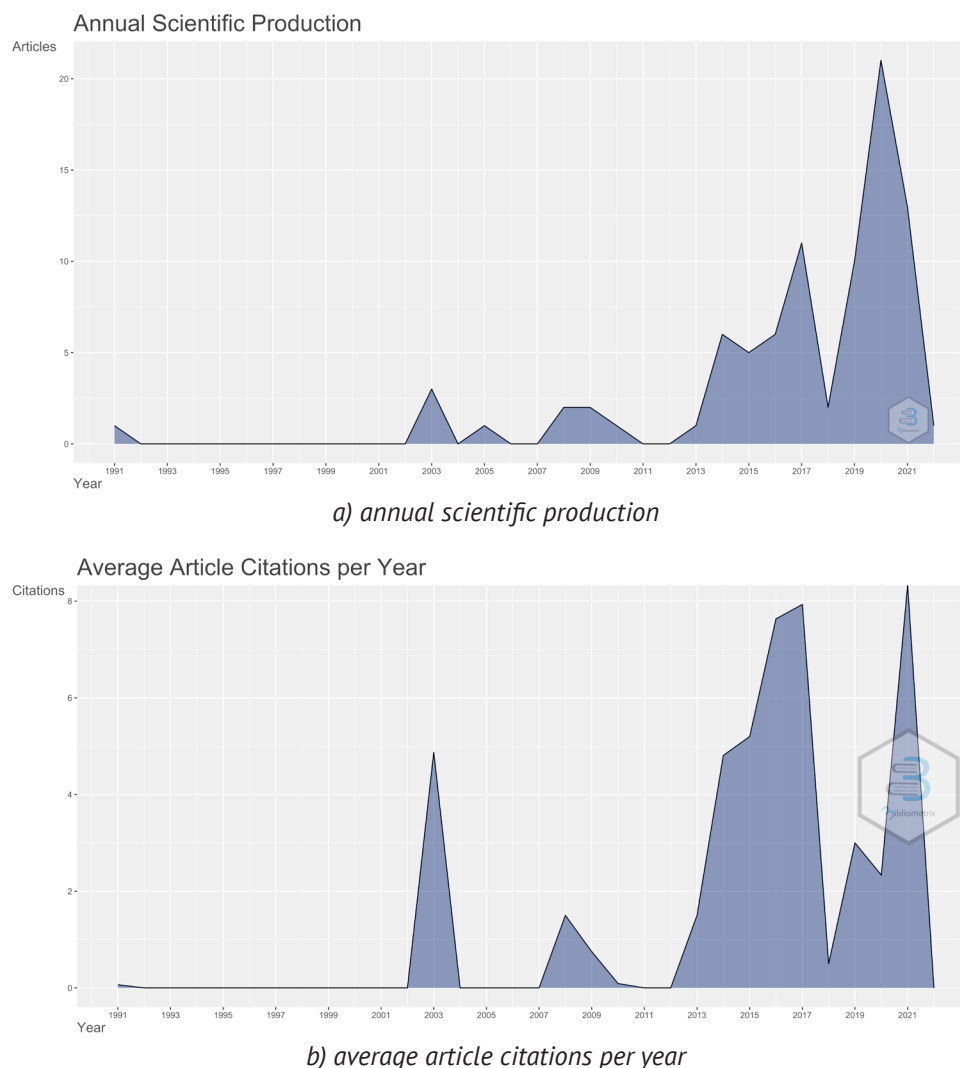


Figure 1. Historical development of research field

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

In 1991 only one article was published and no publications on food system sustainability concept and measurement were covered by Scopus over the decade to follow. 2003 was marked by the food crisis in central and eastern Africa caused by the drought. Moreover, the decline of wheat production in the USA and respective depletion of food stock destined to aids led to the prices increase. As a result, the FAO reported about 39 countries "facing severe food shortages" that year (Reliefweb, 2003). This could be the reason for an increase of scientific interest and publications on food systems sustainability concept and measurement. This is true also for 2008 crisis with the largest world food prices surge for the last

30 years. Issues of food availability, security, and abilities to overcome the crisis were put at the forefront of food policies at different scales (Hultman, 2008), pushing the research activity. Then, due to food price fluctuations (2011-2012) and a critical decline in global food stocks in 2012 (below 1974 level) expectations of the possible onset of the food crisis in 2013 (Vidal, 2012) were reasonable. This could be judged as a factor leading to an increase of scientific publications in the analysed field.

In 2015-2016, the United Nations Organisation approved the Agenda 2030 putting the sustainability of food system at the centre of 17 sustainable development goals. In December 2016, Barilla released the food

sustainability index (Barilla Center for Food..., 2021) allowing to quantify and to illustrate the scale of the problem. In 2018, the FAO formulated an official definition of sustainable food system. Those events could serve core drivers of significant increase in the number of publications concerning the concept and metrics of food system sustainability since 2015.

The bibliographic collection analysed comprises

publications made by authors from 45 countries in total (Fig. 2). Scientists from the United States, Italy, and France make the greatest contribution to the scholarly elaboration of the concept and metrics of food system sustainability (frequencies amount to 40, 36, 27 documents authored, respectively). The Netherlands (18), Canada (16), UK (15), Australia (14), Germany (12), China (9), Brazil, New Zealand, and Spain (7 for every) are a little less productive.

Country Scientific Production

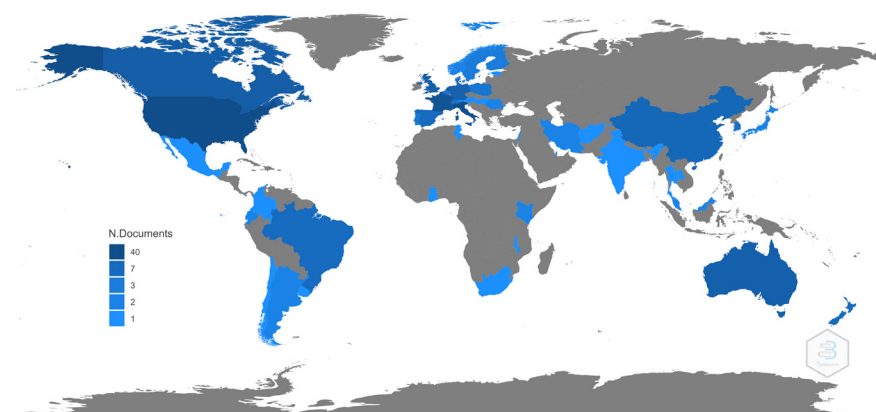


Figure 2. Country scientific production for 1991-2022

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

The United States, Italy, and France also represent the most three cited countries, which is logical; Switzerland is the fourth in the ranking of cited countries (Fig. 3),

although it ranks only 15th in terms of productivity with only five articles in the collection.

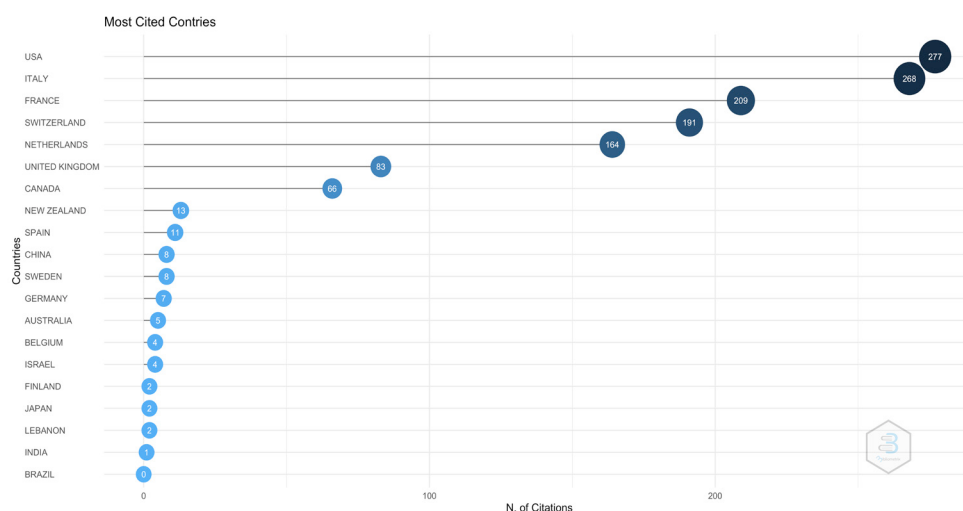


Figure 3. Most cited countries

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

With respect to the corresponding author, the USA, Italy, and France also are leading countries by number of papers published (12, 11, 7, respectively). The UK and the Netherlands (6 documents each) follow those countries and complete the top five countries in the field of food system sustainability and metrics research.

Among the countries with a high productivity, the

UK, Switzerland, Italy, the Netherlands, and France are the most engaged in international scientific cooperation, according to the collaboration index (0.833, 0.667, 0.545, 0.5, 0.286, respectively). Although the collaboration indices for publications made by corresponding authors from Sweden, India, and New Zealand are the highest and amount to one each, the number of articles authored

by researchers from these countries is small (2, 1, 1, respectively).

To illustrate the scientific relationships between

countries, the countries collaboration network was built using Louvain clustering and an association strengths normalisation approach (Fig. 4).

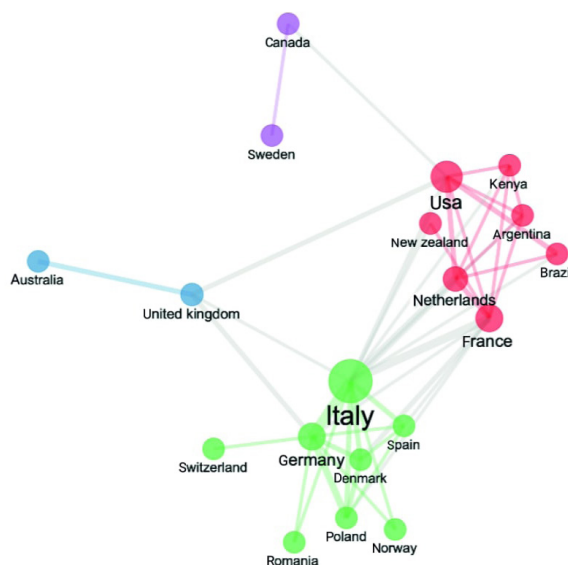


Figure 4. Countries collaboration network

Source: authors' development via Bibliometrix (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

Figure 4 illustrates the existence of four clusters of international cooperation in the field of conceptualisation and measurement of food system sustainability research. There are two dominant clusters: 1) European, which is led by Italy and unites mainly researchers from European countries (and Spain); 2) American, led by the USA and France, connecting scientists from Argentina, Brazil, Kenya, the Netherlands, and New Zealand.

The most relevant organisations (by number of publications), according to the corresponding author's

affiliation are the following: University Paris-Saclay (8), University of Sydney and Wageningen University and Research (5 articles each), Acadia University, UN Food and Agriculture Organisation, University of California, and University of Copenhagen (4 articles each), Asia University, Cornell University, University of Manchester, Montpellier University, University of Minnesota, University of Naples Parthenope, Wageningen University (3 articles each). Thematic avenues of research (top 20) by countries and organisations are given in Figure 5.

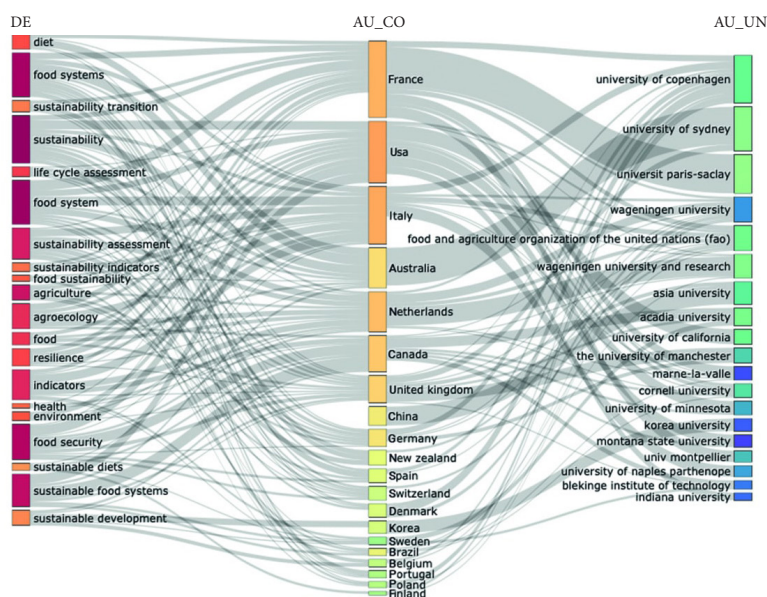


Figure 5. Three fields plot: Author's Keywords-Countries-Affiliations

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

The Sustainability (Switzerland) journal (16 documents), the Journal of cleaner production (5 papers), the Frontiers in Sustainable Food Systems journal, the Journal of Hunger and environmental nutrition (4 articles each), the Agricultural Systems journal, and the Nutrients journal (4 papers each) are the most relevant sources for publications covered by the sample. This goes in line with previous research (Sweileh, 2020) concerning the

leading role of Sustainability (Switzerland) as the platform to present research on food system sustainability issues.

Main concepts and trends in food system sustainability research

The most productive authors by number of documents fractionalised are ranked in Figure 6.

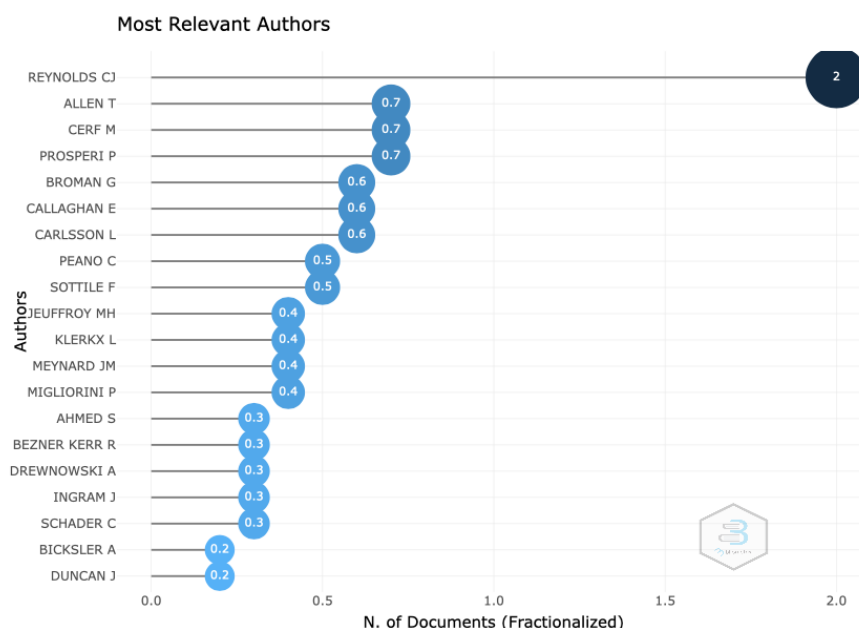


Figure 6. Most relevant authors

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

The major of these scientists has launched research on food system sustainability conceptualisation

and measurement in 2016-2017 and continue to explore this topic (Fig. 7).

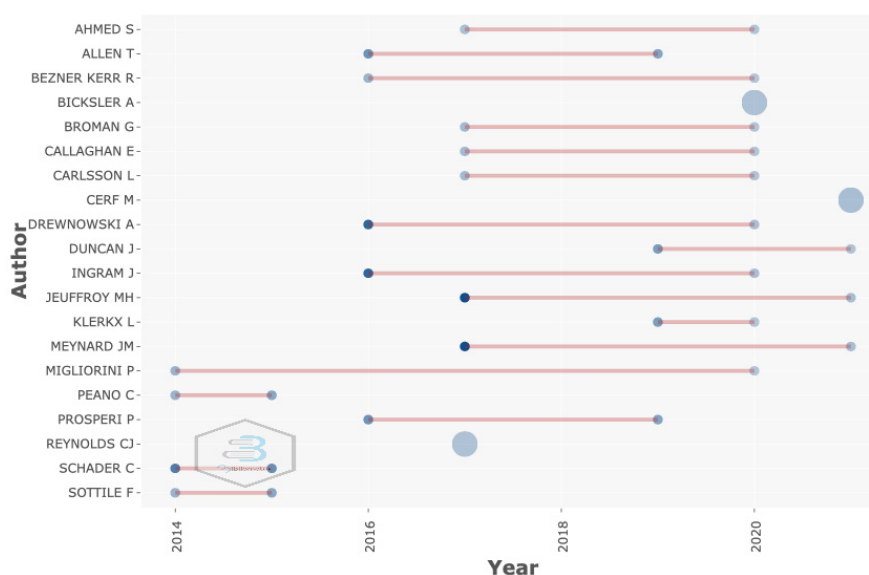


Figure 7. Top-authors' production over the time

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

T. Allen & P. Prosperi (2016) started to explore the concept and metrics of food system sustainability in 2016 with the study of sustainable food system (SFS) modelling (Allen & Prosperi, 2016), and then develop indicators to measure SFS applying the Delphi approach (Allen *et al.*, 2019). Carlsson, Callaghan, and Broman (2017) began with the research on planning and evaluating of community development favourable for SFS (Carlsson *et al.*, 2017), and further pursue a study of the community impact on the SFS development with respect to the food habits and culture formation (Carlsson *et al.*, 2020). Starting with an innovations' design supporting the agri-food systems sustainable transformations (Meynard *et al.*, 2017), Meynard, Jeufroy, and Cerf focus on research organisation promoting innovations' development and implementation for sustainability of agri-food systems (Brun *et al.*, 2021). Drewnowski and Ingram (2016) investigated indicators to measure the SFS in respect of nutrition security (Gustafson, 2016) and develop healthy diets conducive to the food system

sustainability (Drewnowski *et al.*, 2020). Ahmed (2017, 2020) focuses on the educational aspect of movement towards the SFS: practice-oriented student's learning (Ahmed *et al.*, 2017) and joint development of adaptive knowledge outcomes to make education contributory to the SFS transformation (Ebel *et al.*, 2020).

Recent papers (2019-2021) focus on agri-environmental measures ensuring the SFS development (Mottet *et al.*, 2020; Barrios *et al.*, 2020), diversity of food systems and their characteristics in terms of sustainability (Gaitán-Cremaschi *et al.*, 2019), food system resilience concerning crises (Bisoffi *et al.*, 2021), food system and agricultural policies changes in the context of digitisation (Lajoie-O'Malley *et al.*, 2020).

Word cloud (Fig. 8) shows the most frequent author's keywords (Fig. 8b): "food system", "food security", "indicators", "agriculture". Measured by the logarithmic occurrence (Fig. 8a), keywords like "agriculture", "indicators", "agroecology", "resilience", and "food" constitute the corpus of terms with the growing use.

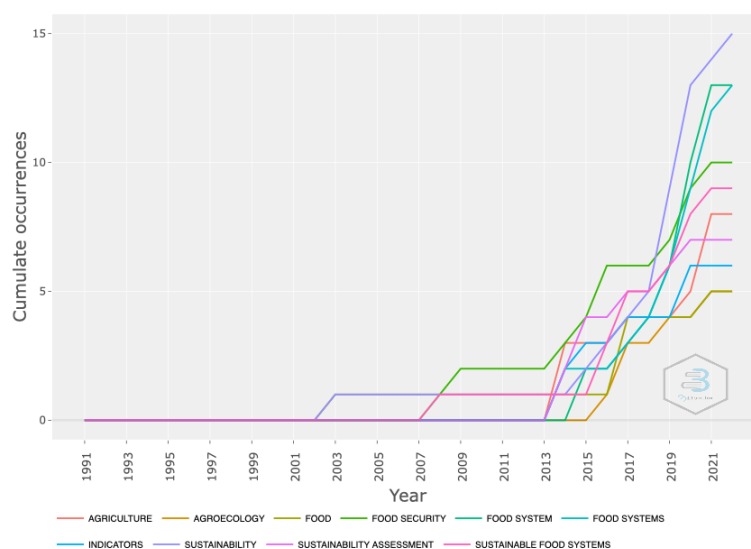


Figure 8. Word cloud of author's keywords

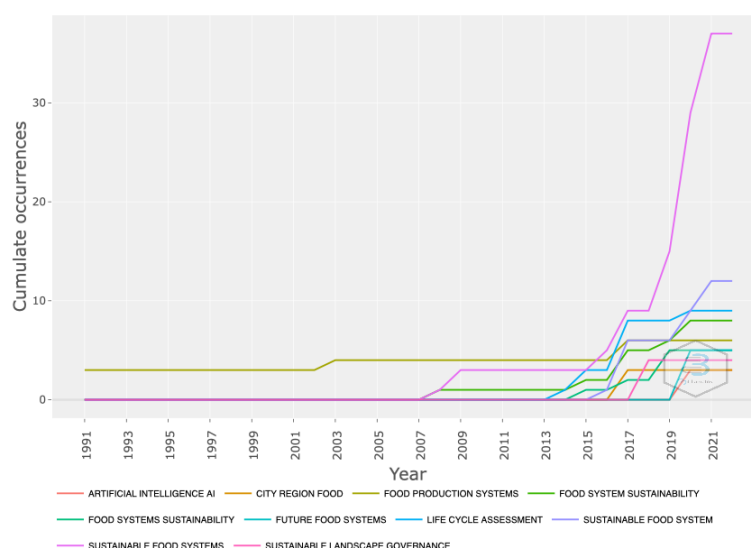
Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

Word growth analysis allows to identify growing and new trends in the research field. Cumulate occurrence of author's keywords (Fig. 9a) shows changes in scientific elaboration of SFS concept and measurement for 2002-2021: the term "sustainability" was leading in 2002-2008 period, then the "food security" concern became dominant since 2008 until 2018, when the "sustainability" came to the fore again. Current SFS conception embraces a lot of views with the predominance of "sustainability" and "food system (systems)" context leading to the failure of "food security" significance. Keywords "agriculture" and "agroecology" complete a semantic variety of phrases constituting the SFS concept (other than terms used in the search query).

Cumulate occurrence of abstract trigrams (Fig. 9b) provides more comprehensive insights of research contents. The phrase "food production systems" dominated in the analysed area over 25 years since 1991. In 2016 the trigram "sustainable food systems" received higher rates and is demonstrating an exponential growth of occurrence since that. According to abstract trigrams, modern view of the SFS concept and measurement is embodied mainly in "sustainable food system (systems)", "life cycle assessment", "food system sustainability", followed by the growing trends like "future food systems", "sustainable landscape governance", "city region food", and "artificial intelligence".



a) authors' keywords (top 10)



b) abstracts trigrams (top 10)

Figure 9. Word growth measured by cumulative occurrence

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

To show common visions and variety of approaches to the SFS concept and measurement, documents were coupled by references and clustered (Fig. 10). The following threshold values and settings were used in the analysis: number of documents – 250; minimum cluster frequency – 5; impact measurement – a global citation ranking; cluster title – three author keywords. Cluster entitled “sustainability, agri-food products, agri-food systems” is the most relevant in terms of global citations (impact value of 5.03), while cluster “sustainability, food security, agriculture” is the least (1.61). Despite the common keyword used (sustainability), identified clusters disclose different views on the concept and measurement of the food system sustainability, putting emphasis on food security and food system (green-coloured cluster), food security and agriculture (blue-coloured), agri-food products and systems (purple-coloured). Cluster with keywords “food system, food systems, sustainable food systems” embraces

documents with the most frequent references, while documents in the cluster with the highest impact reference the least frequent sources.

Words co-occurrence network graph shows the insights of SFS concept and measurement studies (Fig. 11). Build by abstracts unigrams with Louvain clustering, association strength normalisation, and Fruchterman and Reinghold shaping approach, Figure 11 illustrates four contextual clusters with the dominance of block combining issues of systematicity, sustainability, food, environmental aspects, approach, and frameworks. Another, slightly less relevant cluster embraces terms like development, research, policy, change, challenges, and agriculture. The word “production” is largely accompanied by analysis, consumption, and current state. Sustainability assessment studies comprises indicators, data, levels, results, and knowledge. Changes in the research subject are well illustrated with the thematic evolution map (Fig. 12).

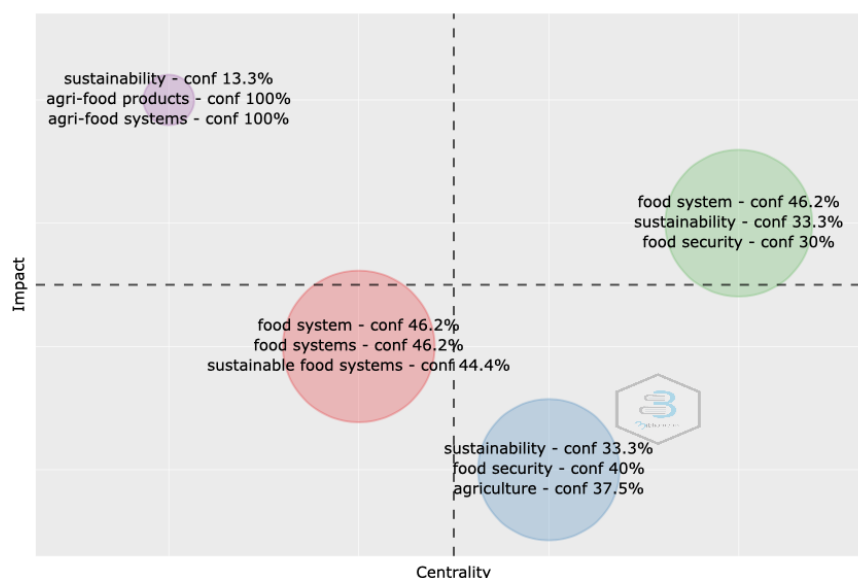


Figure 10. Clusters of documents coupled by references

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

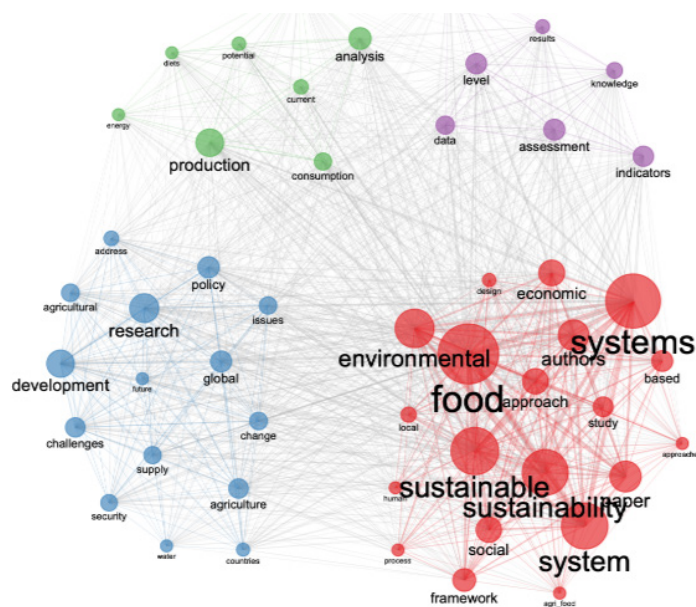


Figure 11. Abstracts' unigrams co-occurrence network

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

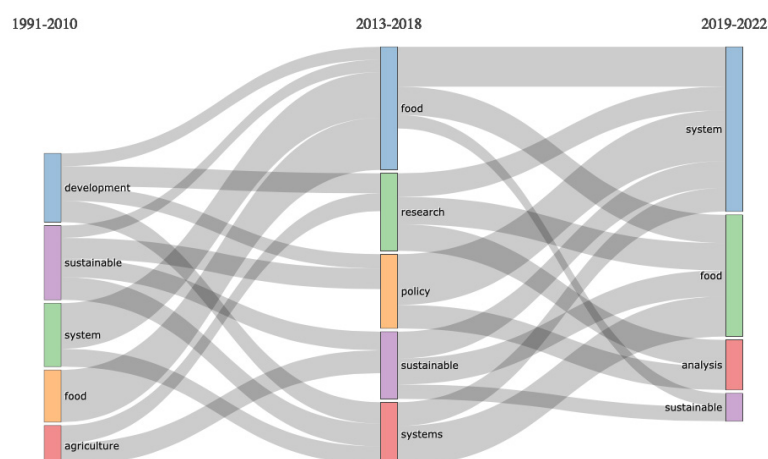


Figure 12. Thematic evolution map by abstracts' unigrams

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

Previously identified periods of scientific interest growth (Fig. 1) were used to set up historical stages of research field (the SFS concept and measurement) development. The following settings were applied: the inclusion index weighted by word co-occurrence with the threshold value of minimum 0.1, the minimal number of words – 250, and minimum cluster frequency of 20. Applying abstract unigrams approach allows revealing the broader context compared to keywords. The results obtained show the dominance of system and food concerns in recent studies (2019-2022), while studies of 1991-2010 were focused on development, sustainable, system, food, and agriculture issues. Period of 2013-2018 is characterised by the balancing of research, policy, sustainable, system aspects with a slight dominance of food issue.

Concept map gives a measure of relatedness among single notions within the research field – the closer the

terms are on the concept map, the more similar they are in use. Conceptual map of abstracts' bigrams was constructed by multiple correspondence analysis (MCA) approach with the following threshold values: number of terms – 50; number of documents – 5 (Fig. 13). Upon doing that, two clusters of scientific thoughts were specified: 1) cluster dominated by issues of sustainability assessment (the growing trend) centred around food supply, agri-food systems, supply chains, food security, climate change; 2) cluster comprising research focused on environmental impacts, food consumption, sustainable diets, and life cycle assessment. Commenting on the relatedness of the concepts covered, one should emphasise the relations between “local level” and “nutrition security”; “sustainability” and “system thinking”; “future research”, “climate change” and “agri-food systems”; “agricultural production” and “environmental sustainability”.

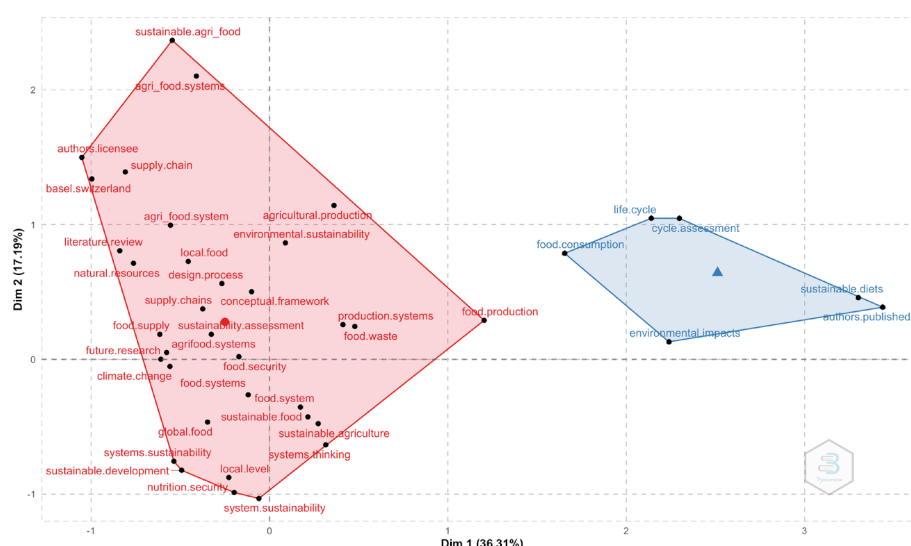


Figure 13. Conceptual structure map: MCA approach by abstracts' bigrams

Source: authors' development via Biblioshiny app (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

Finally, to show research contents it is desirable to analyse the most globally cited documents (Table 2). The most cited papers focus on the complexity of food systems that causes difficulties in conducting sustainability assessments by means of life cycle assessment (LCA) (Heller & Keoleian, 2003; Notarnicola *et al.*, 2017), explore

the research priorities in assessment of food system sustainability (Soussana, 2014), investigate organisational issues promoting the innovation-based food system sustainable development (Berti & Mulligan, 2016; Meynard *et al.*, 2017).

Table 2. Top 10 global cited documents (by total citations per year)

Paper	Total Citations (TC)	TC per Year
Notarnicola, B., Sala, S., Anton, A., McLaren, S.J., Saouter, E., & Sonesson, U. (2017). The role of life cycle assessment in supporting sustainable agri-food systems: A review of the challenges. <i>Journal of Cleaner Production</i> , 140, 399-409. doi: 10.1016/j.jclepro.2016.06.071	197	39.4
Meynard, J.-M., Jeuffroy, M.-H., Le Bail, M., Lefèvre, A., Magrini, M.-B., & Michon, C. (2017). Designing coupled innovations for the sustainability transition of agrifood systems. <i>Agricultural Systems</i> , 157, 330-339. doi: 10.1016/j.agsy.2016.08.002	92	18.4
Schader, C., Muller, A., El-Hage Scialabba, N., Hecht, J., Isensee, A., Erb, K.-H., Smith, P., Makkar, H.P.S., Klocke, P., Leiber, F., Schwegler, P., Stolze, M., & Niggli, U. (2015). Impacts of feeding less food-competing feedstuffs to livestock on global food system sustainability. <i>Journal of the Royal Society Interface</i> , 12(113). doi: 10.1098/rsif.2015.0891	104	14.86

Table 2, Continued

Paper	Total Citations (TC)	TC per Year
Gustafson, D., Gutman, A., Leet, W., Drewnowski, A., Fanzo, J., & Ingram, J. (2016). Seven food system metrics of sustainable nutrition security. <i>Sustainability</i> , 8(3). doi: 10.3390/su8030196	88	14.67
Berti, G., & Mulligan, C. (2016). Competitiveness of small farms and innovative food supply chains: The role of food hubs in creating sustainable regional and local food systems. <i>Sustainability</i> , 8(7). doi: 10.3390/su8070616	74	12.33
Schader, C., Grenz, J., Meier, M. S., & Stolze, M. (2014). Scope and precision of sustainability assessment approaches to food systems. <i>Ecology and Society</i> , 19(3). doi: 10.5751/ES-06866-190342	87	10.86
Di Vaio, A., Boccia, F., Landriani, L., & Palladino, R. (2020). Artificial intelligence in the agri-food system: Rethinking sustainable business models in the COVID-19 scenario. <i>Sustainability</i> , 12(12). doi: 10.3390/SU12124851	16	8
Heller, M. C., & Keoleian, G. A. (2003). Assessing the sustainability of the US food system: A life cycle perspective. <i>Agricultural Systems</i> , 76(3), 1007-1041. doi: 10.1016/S0308-521X(02)00027-6	149	7.84
Allen, T., & Prosperi, P. (2016). Modeling Sustainable Food Systems. <i>Environmental Management</i> , 57(5), 956-975. doi: 10.1007/s00267-016-0664-8	46	7.67
Soussana, J.-F. (2014). Research priorities for sustainable agri-food systems and life cycle assessment. <i>Journal of Cleaner Production</i> , 73, 19-23. doi: 10.1016/j.jclepro.2014.02.061	52	6.5

Source: authors' development via Bibliometrix (R Core Team, 2014; Aria & Cuccurullo, 2017; RStudio Team, 2020)

Scholars point out and discuss the main problems, caused by food systems complexity, concerning the LCA sustainability assessment: the right choice of a unit of analysis; difficulties to incorporate and assess the alternative and multifunctional value of biological resources and ecosystems; measuring and modelling of emissions; the need to assess consumer behaviour within the LCA (Notarnicola *et al.*, 2017). M.C. Heller & G.A. Keoleian (2003) suggest developing indicators to measure environmental, economic, and social impacts along the whole food production value chain, note the need to change consumer behaviour to ensure the sustainability of food systems (Heller & Keoleian, 2003). J.-F. Soussana (2014) proposes to improve the LCA methodology through inclusion of natural capital and public health concerns to assess the food system sustainability (Soussana, 2014). C. Schader *et al.* (2014) and D. Gustafson *et al.* (2016) investigate other approaches to measure the food system sustainability including that measuring the nutritional adequacy and safety (Gustafson *et al.*, 2016). However, LCA-focused studies predominant in the field of SFS measurement research.

Specification of the main SFS attributes, a search for trade-offs, overcoming suboptimality in decision-making concerning the composition and functioning of the SFS (Allen & Prosperi, 2016); an introduction of innovations to support sustainable transformation of food system based on a system thinking and network development (Meynard *et al.*, 2017); organisational changes supporting better introduction of new technologies for the sustainability of food systems (Berti & Mulligan, 2016), in particular, digitisation (Di Vaio *et al.*, 2020) – these are the main issues covered by the most cited papers.

CONCLUSIONS

Results of the study show that research on the concept and measurement of food system sustainability has a long

history starting in 1991, when the first paper was published (indexed by Scopus). Since then, a scientific interest to this topic has been growing wave-like, with peaks following the global food crises existence pattern and admission of the problem by the global institutions like FAO. Despite the wave-like course, there is a tenfold increase in the number of publications for 2013-2020. The United States, Italy, and France are leading countries in this research area by the number of published and cited documents. Among them, Italy dominates in international collaboration: the number of publications engaging authors from different countries even exceeds the number of domestic authored publications. There are distinguished two main geographical clusters of scientific collaboration: European, led by Italy, and American, guided by the USA and France. The top three relevant organisations consist of University Paris-Saclay (France), University of Sydney (the USA), and Wageningen University and Research (the Netherlands). Sustainability (Switzerland) was found to be the most relevant source for publishing research on the concept and measurement of food system sustainability.

Although the most frequent authors' keywords include "food system", "food security", "agriculture", terms "indicators", "agroecology", "food", "resilience" are of growing use. Modern perception of the SFS concept and measurement (since 2018) is intertwined in the notions of systems and system thinking, sustainability, and life cycle assessment. The comprehensive vision of sustainable food system seemingly goes behind the food production and embraces the system-wide perspective. The results of factorial analysis (conceptual structure map) confirm this thesis to a certain extent: studies focused on food supply, agri-food systems, supply chains, food security, climate change are dominant and close to the growing trend of sustainability assessment. Conceptual mapping discloses a variety of visions and understandings that are relevant

to the SFS and allows suggesting the complex and multifaceted nature of the concept. However, food, environmental aspects, agriculture, agri-food systems, and life cycle assessment are still the main terms constituting the core of research on the concept and measurement of SFS with a little attention given to social and economic aspects of food system sustainability.

Due to the complexity of the SFS connotation, the elaboration of one-size-fits-all policy and solutions are unlikely. In view of this, further research on the concept, measurement, and governance of food system sustainability should go in line with concrete institutional, economic, social, and natural environment. Studies from developing countries are of special interest in this case.

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Концепція та вимірювання сталості продовольчої системи: бібліометричне дослідження

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Анотація. В умовах змін клімату та зростання населення, забезпечення сталості продовольчої системи є глобальною проблемою. Багато політик регулюють це питання, але останні дані показують, що глобальна (і національні) продовольчі системи ще далекі від сталого стану. Удосконалення існуючої політики у сфері регулювання продовольчої системи вимагає чіткого розуміння самої концепції сталого розвитку продовольчої системи: уточнення її ознак, атрибутів, показників для оцінки та цілей. Ці питання відображені у багатьох наукових роботах, але жодна з наявних не підсумовує основні тенденції, зміст та особливості еволюції концепції сталого розвитку продовольчої системи. У цьому дослідженні вперше узагальнено історію та зміст досліджень щодо концепції та вимірювання сталості продовольчої системи шляхом бібліометричного аналізу робіт у Scopus за 1991–2022 роки. Встановлено хвилеподібне зростання наукового інтересу до цієї теми, де лідерами є дослідники із США, Італії та Франції, з піками, що збігаються із часом виникнення глобальних продовольчих криз. Результати цього дослідження показують, що сучасне сприйняття концепції (з 2018 р.) є складним і відображено у тісно пов'язаних поняттях системності та системного мислення, стійкості та оцінки життєвого циклу, на відміну від попередніх поглядів (1991–2018 рр.), що концентрувались на системі виробництва продовольства та продовольчій безпеці. Загалом, мало уваги приділяється соціальним та економічним аспектам сталості продовольчої системи, на відміну від екологічних, продовольчих та агропродовольчих питань. Підкреслено, що розробка універсальної політики та рішень, що сприяють стійкості харчових систем, є малоімовірною через складність сприйняття концепції. Управління сталим розвитком продовольчої системи має відповідати конкретним інституційним, економічним, соціальним і природним умовам, які потребують всебічного вивчення в майбутньому. Особливий інтерес у цьому контексті представляють дослідження умов країн, що розвиваються

Ключові слова: стале продовольство, еволюція концепції, оцінка, bibliometrix, biblioshiny

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