Long-term dynamics of humus content under different technologies of soil tillage

O. V. Demydenko, P. I. Boyko, V. A. Velychko

Study of the process of preparing feeding mixtures using the composite mixer

M. I. Chernovol, M. O. Sviren, R. V. Kisiliiov

Biological evaluation of the rationality of soil usage in agriculture

S. Yu. Bulyhin, O. L. Tonkha

Experimental research on vibrational digging-up of sugar beet

V. V. Adamchuk, V. M. Bulgakov, I. V. Holovach, Ye. I. Ignatiev

Optimization of fertility indices of podzolic soils via cultivation of phytomeliorants

Yu. L. Tsapko, A. I. Ohorodnia

Genetic variation determination and interbreed differentiation of two ukrainian dairy cattle breeds using microsatellite loci of DNA

A. V. Shelyov, K. V. Kopylov, S. S. Kramarenko, O. S. Kramarenko

Improvement of methods of estimating the change in the ecological state of soils under the influence of external loads

A. S. Kholodna, K. O. Desiatnyk

Zonal pathogen complex of sunflower in the left bank Forest-Steppe of Ukraine

I. Yu. Borovskaya, V. P. Petrenkova

Impact of cryopreservation on lipid composition of sperm cells of male sterlets (Acipenser ruthenus L.)

L. P. Drahan, S. P. Veselsky, Yu. P. Rud, L. P. Buchatsky
Державне видавництво «Аграрна наука» Національної академії аграрних наук України з 2018 року є членом Міжнародної асоціації видавців наукової літератури (PILA) та бере участь у проекті CrossRef на правах резидента.

CrossRef є незалежною асоціацією, заснованою та керованою видавцями. Основним завданням CrossRef є організація доступу користувачів до первинних публікацій, що містять науковий контент, та сприяння колективній роботі видавців.

Ідентифікатор цифрового об’єкта (DOI) – це система ідентифікації об’єктів контенту в цифровому середовищі. Система DOI забезпечує структуру для постійної ідентифікації, управління інтелектуальним контентом і метаданими, зв’язок користувачів із постачальниками контенту.

Державне видавництво «Аграрна наука» НААН надає послуги присвоєння унікальних номерів – індексів DOI, які на сьогоднішній день стали однією з умов для включення видання у міжнародні наукометричні бази, такі як Scopus та Web of Science, і включення до Переліку наукових фахових видань України.

Для участі у програмі CrossRef, будь ласка, зв’яжіться з видавництвом «Аграрна наука» НААН: agrovisnyk@ukr.net; macropas@gmail.com; тел. +380 44 2574081
LONG-TERM DYNAMICS OF HUMUS CONTENT UNDER DIFFERENT TECHNOLOGIES OF SOIL TILLAGE

O. V. Demydenko, P. I. Boyko, V. A. Velychko

1 Cherkasy State Agricultural Experimental Station, NSC “Institute of Agriculture”, NAAS of Ukraine, 13, Dokuchaiev Str., Kholodnianske village, Smila District, Cherkasy Region, Ukraine, 20731; e-mail: smilachiapv@ukr.net

2 NSC “Institute of Agriculture” NAAS, 2-b, Mashynobudivnykiv Str., Chabany village, Kyiv-Sviatoshyn District, Kyiv Region, e-mail: iznaan@ukr.net

3 NSC Institute for Soil Science and Agrochemistry Research named after O. Sokolovsky, 4, Chaikovskoho Str., Kharkiv, Ukraine 61024; e-mail: agrovisnyk@ukr.net

Received on February 02, 2018

The continuous agronomic experiment revealed long-term dynamics of the total humus content and presented a forecast of the change in humus content in typical low humus chernozem under different technologies of tillage till 2050. Aim. To determine the rates of humus accumulation dynamics and mineralization of total humus and to develop the forecast of the change in its content within a continuous agronomic experiment under long-standing application of different technologies of soil tillage to typical low humus chernozem of the Left-Bank Forest-Steppe of Ukraine. Methods. Field, laboratory-analytical, mathematical and statistical. Results. The application of different technologies of tillage to typical low humus chernozem for 42 years resulted only in the delay in dehumification processes and some stabilization of humus mineralization, but it did not promote its preservation and extended restoration to the initial level as of the start of the experiment. The increase in the total humus content for simple and extended restoration of humus in the centennial cycle equaled 20–25 t and 30–33 t per 1 ha respectively. To ensure the increase in content and reserves of humus for 42 years, it is necessary to introduce 10–12 t of humus per 1 ha for simple restoration of total humus content and 14–15 t per 1 ha – for extended restoration annually. Conclusions. Simple restoration of humus in typical chernozem may be stated after achieving its actual (2017) content of at least 90 % from the content as of the beginning of the centennial cycle (92 years), which ensures maximal approximation to the non-decreasing cycle of humus dynamics trends in the centennial cycle. If the humus content is ensured in the actual measurement for the level, exceeding 90 % from the initial content, and dynamics trends are growing, one may state the success of achieving the state of extended restoration of humus. The obtained state of simple and extended accumulation of humus is ensured by the positivity of the trends of humus increase during the continuous experiment (42 years) with simultaneous decrease in dehumification process in the centennial cycle (92 years) which is impossible to neutralize completely.

Keywords: typical chernozem, organic matter, humus, trend, straw, manure, mineral fertilizers, humification, soil tillage.

DOI: 10.15407/agrisp5.01.003

INTRODUCTION

Humus content is the most important index of fertility and agroecological state of chernozems. The organic matter defines its structural-aggregate condition, its physical, chemical, and exchange properties, and is a considerable source of nutrients [1–8]. Humus ensures the resistance of chernozem to external impact, thus promoting one of the overall functions – its biogenicity [9–13]. Due to intense processes of humus mineraliza-
tion under the impact of anthropogenic burden, chernozem acquires the features of agrophysical degradation [14]. Numerous studies demonstrate active manifestation of dehumification during the tillage of chernozems and their further usage in agriculture [15]. The application of fertilizers and different systems of tillage and crop rotations is a powerful factor, impacting the parameters of the humus state of chernozems and the implementation of their potential fertility via its effective form [15, 16]. However, the fertilizers satisfy the needs related to the decrease in humus reserves [13] and humus balance in chernozems while cultivating some crops only by 50–60 %.

Modern studies of humus content dynamics are related to solving rather complicated methodological problems [17], caused, first of all, by the spectrum of chernozem humus, which changes the actual picture of humus dynamics considerably. Therefore, the most significant way of solving these issues is a continuous agronomic experiment – the most realistic way of studying the processes of accumulation and mineralization of humus, forecasting the dynamics for the nearest perspective and working out the agrotechnical technologies of regulating it in chernozems.

The mathematical and statistical models have become widely spread recently [18]. It should be noted that the drawback of the suggested models lies in the fact that they do not give an absolutely accurate forecast, the dynamics of humus accumulation or decomposition is a very complicated biochemical process, depending on many factors, which have not been accounted for in the model, and any change in the crop rotation (a set of crops or crop rotation structure, soil tillage, kind of organic fertilizers and their dosage) may lead to the change in a humus state of chernozem. Therefore, periodic study of humus state of chernozems with further correction of the change in coefficients of prognostic equations of humus accumulation or mineralization is required [2, 15]. The work [19] describes the impact of fertilizers on humus dynamics. A relevant task of preserving the fertility of chernozem is forecasting the change in total humus of typical chernozem under long-term application of different technologies of tillage.

The aim of this work is to determine the rates of humus accumulation dynamics and mineralization of total humus and to develop the forecast of the change in its content within a continuous agronomic experiment up to 2050 at the longstanding application of different technologies of soil tillage to typical low humus chernozem of the Left-Bank Forest-Steppe of Ukraine.

MATERIALS AND METHODS

The studies were conducted in conditions of the central part of the Left-Bank Forest-Steppe of Ukraine during a continuous agronomic experiment in the Drabiv experimental field of the Cherkasy State Agricultural Experimental Station, NSC “Institute of Agriculture”, NAAS of Ukraine. The experiment was located on typical low-humus heavy-clay light loamy chernozem with the humus content of 3.8–4.2 %, the content of mobile phosphorus – 12–14 mg per 100 g of soil, and that of mobile potassium – 8–10 mg per 100 g of soil, \( \text{pH}_c = 6.8–7.0 \). The study involved two five-field crop rotations: perennial grasses–winter wheat–sugar beet–corn–barley with some sown grasses (crop rotations: 60 % – grains, 20 % – technical crops, perennial grasses – 20 %). Fertilization system (1995–2017): no fertilizers and N\(_{31-65}\), P\(_{31-82}\), K\(_{41-82}\) per 1 ha of crop rotation + 6–7 t/ha of by-products. Until 1995 manure was introduced in the amount of 6 t/ha with the similar dose of mineral fertilizers. The technologies of soil tillage in five-field crop rotations: ploughing of different depth for 22–25 cm; subsurface tillage for 22–25 cm; and surface tillage for 10–12 cm. The content of total humus was determined according to I. V. Turin in the modification of M. V. Simakov (DSTU 4289:2004). The content of humus in the humus horizon as of 1925 was studied using the research materials of the Drabiv experimental station, prepared by Kh. G. Zinovieva [20]. Three factors were considered while analyzing the centennial cycle of total humus dynamics: a type of organic fertilizers, a technology of tillage and a crop rotation with perennial grasses.

Forecasting of the change in the humus state of chernozem under different technologies of tillage was conducted for each separate technology of soil tillage. Thus, the impact factor was deemed to be the number of years after 1925 \( (t_1 = 92) \) and after the start of the experiment – 1975 \( (t_2 = 42 \text{ years}) \). The logarithmic parametric function was selected as a functional dependency: \( y = \alpha \pm \beta \ln x \), where: \( y \) – unknown parameter (humus content); \( \alpha \) – coefficient of parametric function; \( \beta \) – coefficient related to the explanatory factor; \( \ln \) – natural logarithm; \( x \) – quantitative characteristic \( (t – \text{time}) \). The results of field studies were statistically processed by the dispersion analysis method (B. O. Dospelkov, 1985) using Statistica-8 software.

RESULTS OF INVESTIGATIONS

As of 1925, the humus content in chernozems of the Drabiv experimental field was 6.49 % while keeping the fallow in the 0–20 cm layer, and 5.12 % – in the...
LONG-TERM DYNAMICS OF HUMUS CONTENT UNDER DIFFERENT TECHNOLOGIES OF SOIL TILLAGE

Fig. 1. The dynamics of the change in total humus content depending on the tillage technology of typical chernozem: in 1925–2017 (a) and in 1975–2017 (b)

1. $y = -0.59 \ln(x) + 4.67$
   $R^2 = 0.85$

2. $y = -0.45 \ln(x) + 4.57$
   $R^2 = 0.67$

3. $y = -0.34 \ln(x) + 4.48$
   $R^2 = 0.41$

1. $y = -0.15 \ln(x) + 4.02$
   $R^2 = 0.61$

2. $y = -0.046 \ln(x) + 3.94$
   $R^2 = 0.41$

3. $y = 0.013 \ln(x) + 3.85$
   $R^2 = 0.39$
humus horizon (0–40 cm). There were 4.86 % and 4.56 % in the variant of intense use of chernozem under tillage respectively (Fig. 1, a).

The decrease in humus content relative to the fallow was 0.63 % and 0.16 %. In 1975 the content of total humus as of the start of the continuous experiment was 3.82–3.96 % (0–20 cm) and 3.65–3.79 % (0–40 cm).

In 50 years (1925–1975), typical chernozems lost 28–30 % and 21–23 % of humus compared to the content in the fallow, and in case of the intense use of chernozem – 19–21 % and 18–20 % in the soil layers of 0–20 cm and 0–40 cm respectively.

In 1975 the experiment was started with the purpose of continuous study of the impact of different technologies of chernozem tillage on the change in the humus state (Fig. 1, b). Until 1995, the gradient of change in the humus content compared to 1925 fluctuated considerably either decreasing or increasing regardless of

Table 1. The dynamics of the change in the total humus under the impact of different technologies of tillage of typical low-humus chernozem in 1925–2017

<table>
<thead>
<tr>
<th>Years</th>
<th>Technologies of soil tillage</th>
<th>Period after start of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ploughing 0–20 cm</td>
<td>0–40 cm</td>
</tr>
<tr>
<td>1925</td>
<td>4.86</td>
<td>4.56</td>
</tr>
<tr>
<td>*1975</td>
<td>3.96</td>
<td>3.79</td>
</tr>
<tr>
<td>1985</td>
<td>3.91</td>
<td>3.87</td>
</tr>
<tr>
<td>1995</td>
<td>3.98</td>
<td>3.85</td>
</tr>
<tr>
<td>2010</td>
<td>3.85</td>
<td>3.78</td>
</tr>
<tr>
<td>2015</td>
<td>3.66</td>
<td>3.62</td>
</tr>
<tr>
<td>2017</td>
<td>3.45</td>
<td>3.41</td>
</tr>
</tbody>
</table>

Gradient of the change in humus content compared to 1925, %

<table>
<thead>
<tr>
<th>Years</th>
<th>Period after start of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>–</td>
</tr>
<tr>
<td>1985</td>
<td>–1.00</td>
</tr>
<tr>
<td>1995</td>
<td>–1.00</td>
</tr>
<tr>
<td>2010</td>
<td>–0.77</td>
</tr>
<tr>
<td>2015</td>
<td>–0.77</td>
</tr>
<tr>
<td>2017</td>
<td>–0.77</td>
</tr>
</tbody>
</table>

Average estimated mineralization/formation of humus for 92 years, % a year

<table>
<thead>
<tr>
<th>Period after start of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>–0.0153</td>
</tr>
<tr>
<td>–0.0125</td>
</tr>
<tr>
<td>–0.0121</td>
</tr>
<tr>
<td>–0.009</td>
</tr>
<tr>
<td>–0.010</td>
</tr>
</tbody>
</table>

± humus compared to 1975

<table>
<thead>
<tr>
<th>Period after start of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>–1.51</td>
</tr>
<tr>
<td>–1.38</td>
</tr>
<tr>
<td>–1.11</td>
</tr>
<tr>
<td>–1.10</td>
</tr>
<tr>
<td>1.21</td>
</tr>
<tr>
<td>0.03</td>
</tr>
</tbody>
</table>

Average estimated mineralization/formation of humus for 42 years, % a year

<table>
<thead>
<tr>
<th>Period after start of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>–0.0121</td>
</tr>
<tr>
<td>–0.0091</td>
</tr>
<tr>
<td>–0.003</td>
</tr>
<tr>
<td>–0.0026</td>
</tr>
<tr>
<td>0.005</td>
</tr>
<tr>
<td>+0.0007</td>
</tr>
</tbody>
</table>

± humus from ploughing (control)

<table>
<thead>
<tr>
<th>Period after start of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>–0.30</td>
</tr>
<tr>
<td>+0.24</td>
</tr>
<tr>
<td>+0.68</td>
</tr>
<tr>
<td>+0.27</td>
</tr>
</tbody>
</table>

Note. *1975 – year of starting the experiment.
the technology of tillage, which is related to the period of introducing the manure in the amount of 30 t/ha for sugar beet or 6 t/ha of the crop rotation (Table 1). Typical chernozem passed from medium-humus gradation into low-humus gradation. Likely this is due to the fact that the dehumification passed to relatively quasi-equilibrium state with the formation of humus at a certain level of organics intake into soil and the stability of the fertilization system.

From 1995 till 2017, when by-products were introduced instead of manure, the gradient of humus content under ploughing was 0.13–0.21 % (0–20 cm) and 0.07–0.21 % (0–40 cm), while under systematic application of subsurface tillage in the 0–20 cm soil layer the humus content decreased by 0.07–0.10 %, and by 0.04–0.24 % – in the 0–40 cm of chernozem layer. The regularity, found for the subsurface tillage, was even more manifested under surface tillage.

It was determined that regardless of the technology of tillage, there was further dehumification of chernozem, and the decrease in humus content depended on the intensity of tillage. The average estimated annual mineralization of humus for 52 years was in agreement with the found regularity and had the lowest values under surface tillage (0.009–0.01 % per year). Under ploughing, the gradient of change in the humus content in 1975 was negative (−0.51 and −0.38 %); under subsurface tillage the gradient of humus decrease was 3.8–4.6 times lower than that for ploughing, and the surface tillage demonstrated the increase in the humus content by +0.21 and +0.03 %.

Under ploughing, the average estimated mineralization of humus for 42 years was the highest and decreased 3.5–4 times under subsurface tillage; and under surface tillage the annual increase in the humus content was +0.005 and +0.0007 % relative to soil layers. The logarithmic equations of dynamics trends for the total humus for 92 years had a declining character with the reliable level of approximation (R² > 0.4) regardless of the technology of chernozem tillage, and the regression coefficients (Cr) for the variable x(t) were negative. Under subsurface tillage, Cr decreased 1.31–1.75 times in the 0–20 soil layer, and 1.43 times in the humus horizon (0–40 cm) which testifies to the decrease in the mineralization rate per one time unit. The estimation of logarithmic equations and trends of declining dynamics of the total humus during the period of studying the efficiency of different technologies of tillage (1975–2017) demonstrated the reliability of the approximation of trends (R² > 0.40). Under ploughing in the 0–20 cm chernozem layer, the regression coefficient for the variable x(t) was 3.26 times higher compared to the regression coefficients of the trends for the subsurface tillage, and under surface tillage the regression coefficient for the variable x was positive and 11–12 times higher, which testifies to the increasing character of the humus accumulation trend.

In the humus horizon (0–40 cm), the trends of humus dynamics had a declining character regardless of the technology of tillage, but under ploughing the value of Cr was 5.5–11 times higher compared to the subsurface and surface technologies of tillage, which testifies to the decrease in the rate of humus mineralization in the latter two cases.

While preserving and restoring the humus in chernozem, a relevant task is to forecast the changes in the humus content under long-term application of different technologies of soil tillage, which is complicated and possible only in conditions of a continuous agronomic experiment. The parametric logarithmic functions were used to estimate the theoretical (forecast) content of total humus in typical chernozem under different variants of chernozem tillage till 2050. The values, obtained about the anticipated humus content, were used to estimate the theoretical (forecast) humus balance relative to 1975 (Fig. 2, a).

It was established that under systematic ploughing the humus content will decrease in the most intense way compared to subsurface and surface technologies. It is on the highest level in the 0–20 cm chernozem layer. Under ploughing, the regression coefficients for the variable x(t) in the regression equations are 1.35–1.55 times higher compared to the subsurface and surface technologies of tillage. This regularity is less expressed in the humus horizon, but the regression coefficients at x(t) for subsurface tillage are 1.18–1.23 times lower. In all the cases, the regression coefficients were negative, which testified to the declining rate of humus accumulation. By the declining trend, the humus content in its absolute manifestation in the 0–20 cm soil layer would be 2.35 % (2025) and 2.11 % (2050) for systematic ploughing; 2.85 % and 2.65 % – for subsurface tillage; 3.15 % and 2.66 % – for surface tillage. The change in the total humus content in the humus horizon (0–40 cm) is subject to the found regularity for the 0–20 cm chernozem layer in accordance to the years of forecasting (2025 and 2050) and the technologies of chernozem tillage (Table 1).

The estimation of the humus balance demonstrated that regardless of the technology of tillage, in 2017
Fig. 2. The estimated rates of the decrease in the total humus content (A) and the anticipated balance (B) of humus depending on different technologies of typical chernozem tillage for 1925–2050.
the humus balance was negative, and its deficiency decreased from ploughing (−0.19–0.21 %) to the surface tillage (−0.15–0.17 %). Under subsurface tillage the value of the humus balance had interim values: −0.16–0.19 %. Compared to 1975, the estimated humus balance till 2050 has an increasing trend, but regardless of the technology of soil tillage, the humus balance acquires a negative value, and the increase in the intensity of soil tillage (ploughing) increases the deficiency of humus balance, which rises, compared to the subsurface and surface technologies of tillage, 1.55–3.31 times and 1.19–1.31 times higher in the 0–20 cm and 0–40 cm soil layer (Fig. 2, b).

The increasing character of trends of humus balance is determined by a high rate of humus accumulation in 1975–1995, when manure was introduced as an organic fertilizer (6–7 t/ha). When manure was replaced with by-products, the rate of the increase in the deficiency of humus balance rose actively, by 2050 they are estimated to reach negative values at the level of −0.29 % for subsurface tillage and the values, exceeding −0.40 %, for systematic ploughing. In general, the estimated trends in humus balance by 2050 are of increasing character, but they are not likely to reach the zero value. By the regression coefficients, the rate of deficiency increase in the humus balance is 1.85–2.0 times more intense for ploughing compared to the subsurface tillage.

Regardless of the fact that for 42 years of experiment, the humus content was not preserved at the initial level (1975), the application of subsurface and surface technologies of tillage promoted the increase in its content in humus horizon compared to the ploughing. In 2017 the content of total humus was determined in different variants of soil tillage. It was determined that the application of subsurface technologies of soil tillage led to considerable slowing down of the dehumification processes and resulted in some stabilization of the humus content but did not promote its extended restoration. The increase in the content of total humus was determined only compared to ploughing and the control, where the fertilizers were not introduced. The stabilization of humus content took place in the declining trend compared to the control point of the humus content both in 1925 and in 1975, when the experiment was started. The extended restoration of humus took place while keeping the fallow (+0.022 % a year): for 42 years of keeping the fallow, humus was restored by 95–97 % compared to 1925, and while keeping the fallow for more than 54 years – by 113 % (Fig. 3).

There is a logical question – which rate of humus accumulation and increase in humus balance should be ensured to reach the edge of transition from the declining trend to the increasing one? The evaluation demonstrated (Table 2) that in 2017 it was necessary to ensure 4.41–4.43 % humus content in the 0–20 cm of soil layer and 4.18–4.23 % – in the humus horizon. It was determined that to reach the level of transition to the increasing trend of humus accumulation, one should reach the 4.65–4.67 % humus content in the 0–40 cm chernozem layer for ploughing and 4.39–4.41 % – for subsurface tillage. Therefore, it is necessary to ensure the increase in the humus content by 11.6–11.9 % and 16.4–17.9 % for ploughing; 12.8–12.9 % and 15.8–20.9 % for subsurface tillage and 13.3–13.4 % and 19.7 % for surface tillage, relative to the level of restoration of fertility and soil layers. In case of such provision of the humus content, the average estimated mineralization of humus
### Table 2. The estimated dynamics of the total humus under the impact of different technologies of tillage of typical low humus chernozem in 1925–2017

<table>
<thead>
<tr>
<th>Years</th>
<th>Technologies of soil tillage</th>
<th>Content of humus, %</th>
<th>Gradient of decline/increase in humus compared to 1925, %</th>
<th>Average estimated mineralization/formation of humus for 92 years, % a year</th>
<th>For stable transition to the positive trend of increasing – extended restoration of humus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ploughing</td>
<td>subsurface</td>
<td>surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–20 cm</td>
<td>0–40 cm</td>
<td>0–20 cm</td>
<td>0–40 cm</td>
<td>0–20 cm</td>
</tr>
<tr>
<td>1925</td>
<td>4.86</td>
<td>4.56</td>
<td>4.86</td>
<td>4.56</td>
<td>4.86</td>
</tr>
<tr>
<td>*1975</td>
<td>3.96</td>
<td>3.79</td>
<td>3.93</td>
<td>3.75</td>
<td>3.89</td>
</tr>
<tr>
<td>2017</td>
<td>4.43</td>
<td>4.23</td>
<td>4.45</td>
<td>4.22</td>
<td>4.41</td>
</tr>
</tbody>
</table>

To reach the positive trend of increasing – simple restoration of humus

<table>
<thead>
<tr>
<th>Years</th>
<th>Technologies of soil tillage</th>
<th>Content of humus, %</th>
<th>Gradient of decline/increase in humus compared to 1925, %</th>
<th>Average estimated mineralization/formation of humus for 92 years, % a year</th>
<th>For stable transition to the positive trend of increasing – extended restoration of humus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ploughing</td>
<td>subsurface</td>
<td>surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0–20 cm</td>
<td>0–40 cm</td>
<td>0–20 cm</td>
<td>0–40 cm</td>
<td>0–20 cm</td>
</tr>
<tr>
<td>1975</td>
<td>–0.90</td>
<td>–0.77</td>
<td>–0.93</td>
<td>–0.81</td>
<td>–0.97</td>
</tr>
<tr>
<td>2017</td>
<td>+0.05</td>
<td>+0.05</td>
<td>+0.06</td>
<td>+0.05</td>
<td>+0.07</td>
</tr>
</tbody>
</table>

Gradient of decline/increase in humus as of 1925

<table>
<thead>
<tr>
<th></th>
<th>± humus as of 1925</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>–1.43</td>
<td>–0.33</td>
<td>–1.41</td>
<td>–0.34</td>
<td>–0.45</td>
</tr>
<tr>
<td>2017</td>
<td>–1.43</td>
<td>–0.33</td>
<td>–1.41</td>
<td>–0.34</td>
<td>–0.45</td>
</tr>
</tbody>
</table>

Average estimated mineralization/formation of humus for 92 years, % a year

<table>
<thead>
<tr>
<th></th>
<th>± humus compared to 1975</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>–0.0047</td>
<td>–0.0036</td>
<td>–0.0045</td>
<td>–0.0037</td>
<td>–0.0049</td>
</tr>
<tr>
<td>2017</td>
<td>±0.47</td>
<td>+0.44</td>
<td>+0.51</td>
<td>+0.47</td>
<td>+0.52</td>
</tr>
</tbody>
</table>

Average estimated mineralization/formation of humus for 42 years, % a year

<table>
<thead>
<tr>
<th></th>
<th>± humus compared to 1975</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1975</td>
<td>+0.0112</td>
<td>+0.0105</td>
<td>+0.0121</td>
<td>+0.0112</td>
<td>+0.0124</td>
</tr>
<tr>
<td>2017</td>
<td>±0.71</td>
<td>+0.62</td>
<td>+0.81</td>
<td>+0.60</td>
<td>+0.76</td>
</tr>
</tbody>
</table>

Note. *1975 – year of starting the experiment.
LONG-TERM DYNAMICS OF HUMUS CONTENT UNDER DIFFERENT TECHNOLOGIES OF SOIL TILLAGE

Table 3. Statistical parameters and fractal dimensionality of the trends of time series of total humus depending on the technology of chernozem tillage by actual and estimated models of development

<table>
<thead>
<tr>
<th>Depth, cm</th>
<th>Equation of trend exponent</th>
<th>*R²</th>
<th>Coef. var, %</th>
<th>**0.46 Coef. var, %</th>
<th>***Iₚ</th>
<th>****FR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ploughing – 75–100 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.70e⁻⁰.⁴⁵ₜ</td>
<td>0.79</td>
<td>36.1</td>
<td>16.6</td>
<td>-0.045</td>
<td>1.05</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.41e⁻⁰.⁳₅ₜ</td>
<td>0.73</td>
<td>15.7</td>
<td>7.23</td>
<td>-0.035</td>
<td>1.04</td>
</tr>
<tr>
<td>Subsurface tillage – 42 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.51e⁻⁰.₂₈ₜ</td>
<td>0.51</td>
<td>23.8</td>
<td>10.9</td>
<td>-0.028</td>
<td>1.03</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.25e⁻⁰.₂₁ₜ</td>
<td>0.49</td>
<td>19.6</td>
<td>9.00</td>
<td>-0.021</td>
<td>1.02</td>
</tr>
<tr>
<td>Surface tillage – 2 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.28e⁻⁰.₁₄ₜ</td>
<td>0.41</td>
<td>28.1</td>
<td>12.9</td>
<td>-0.014</td>
<td>1.01</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.08e⁻⁰.₁₆ₜ</td>
<td>0.39</td>
<td>17.9</td>
<td>8.22</td>
<td>-0.016</td>
<td>1.02</td>
</tr>
<tr>
<td>Ploughing – 75–100 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=5.61e⁻⁰.⁰₉₈ₜ</td>
<td>0.87</td>
<td>35.0</td>
<td>16.1</td>
<td>-0.098</td>
<td>1.10</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.55e⁻⁰.₇₂₉ₜ</td>
<td>0.41</td>
<td>20.0</td>
<td>9.20</td>
<td>-0.072</td>
<td>1.07</td>
</tr>
<tr>
<td>Subsurface tillage – 42 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=5.09e⁻⁰.⁶₁₈ₜ</td>
<td>0.56</td>
<td>25.0</td>
<td>11.5</td>
<td>-0.061</td>
<td>1.06</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.59e⁻⁰.₄₆ₙₜ</td>
<td>0.56</td>
<td>19.0</td>
<td>8.74</td>
<td>-0.046</td>
<td>1.05</td>
</tr>
<tr>
<td>Surface tillage – 42 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.67e⁻⁰.₄₇ₙₜ</td>
<td>0.44</td>
<td>27.0</td>
<td>12.4</td>
<td>-0.047</td>
<td>1.05</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.44e⁻⁰.₄₀ₙₜ</td>
<td>0.47</td>
<td>16.0</td>
<td>7.36</td>
<td>-0.040</td>
<td>1.04</td>
</tr>
<tr>
<td>Indices by the model forecasting of simple restoration of fertility, 1925–2050</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ploughing – 75–100 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.2₈e⁺⁰.₃⁹ₙₜ</td>
<td>0.29</td>
<td>–</td>
<td>–</td>
<td>0.0998</td>
<td>1.01</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.0₇e⁺⁰.₉₁₉ₜ</td>
<td>0.25</td>
<td>–</td>
<td>–</td>
<td>0.0991</td>
<td>1.01</td>
</tr>
<tr>
<td>Subsurface tillage – 42 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.2₁e⁺⁰.₁₇ₙₜ</td>
<td>0.23</td>
<td>–</td>
<td>–</td>
<td>0.017</td>
<td>1.02</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.0₅e⁺⁰.₀₉₅ₙₜ</td>
<td>0.22</td>
<td>–</td>
<td>–</td>
<td>0.0095</td>
<td>1.01</td>
</tr>
<tr>
<td>Surface tillage – 42 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>Y=4.₂₀e⁺⁰.₀₁₉ₙₜ</td>
<td>0.22</td>
<td>–</td>
<td>–</td>
<td>0.021</td>
<td>1.02</td>
</tr>
<tr>
<td>0–40</td>
<td>Y=4.₀₅e⁺⁰.₁₁₆ₙₜ</td>
<td>0.19</td>
<td>–</td>
<td>–</td>
<td>0.016</td>
<td>1.02</td>
</tr>
</tbody>
</table>

Note. *R² – value of approximation reliability; **0.46*Coef.var,% – approximation curve deviation; ***Iₚ - indicator of fractality; ****FR – fractal dimensionality: (FR = 1 – |Iₚ|), [23].
in the 92-year-long cycle should be 0.0036–0.0049 %, and the increase in the humus content in the 42-year-old cycle – 0.0012–0.0124 % a year.

The increasing trend of humus accumulation in the 92-year-long cycle is ensured at the background of average estimated mineralization of humus in the range of 0.0016–0.0021 %, which is 2.25–2.33 times lower than the variant of approximation to the conditions of the increasing trend and 7.3–7.5 times lower than the actual mineralization for 1975–2017.

The forecast and estimation of the behavior of the dynamic soil system include the evaluation and assessment of fractal dimensionality of time series of integral soil parameters, which may involve the dynamics of total humus and fertility of chernozems in agroecosystems. The determination of fractal dimensionality of time series of linear systems is based on the change in the curve length depending on the scale. If the curve is estimated as a fractal one, the length of the curve will increase with the decrease of the scale in an exponential way [21–24].

The analysis of statistical parameters and fractal dimensionality of the trends of time series of humus in the centennial cycle depending on the technology of soil tillage and according to actual and prognostic indices demonstrated (Table 3) that regardless of the technology of chernozem tillage, the equations of exponential trends have a declining character at the reliable value of approximation ($R^2 = 0.41–0.79$) and the reliable deviation of the approximation curve ($0.46 \times $ Coef. var, % $= 7.23–16.6$): the regression coefficients ($-b$) for the variable ($a* e^{-bx}$) have a negative value. Under soil-protective technologies of chernozem tillage, the values of the regression coefficients were 1.6–1.7 times lower (subsurface tillage) and 2.19–3.31 times lower at the surface tillage, which demonstrated a slower character of dehumification in the centennial cycle.

The determination of the fractality indicator ($I_f$) as a degree of approximating exponential function, giving low and positive values of the argument ($x$) allows for a conclusion about the stable process of the declining humus state of chernozem regardless of the technology of tillage, introduction of different kinds of organic and mineral fertilizers.

The estimation of fractal dimensionality (FR) demonstrated [23] that FR values correspond to the stability interval of the declining development of the soil system (FR=1.01–1.41) regardless of the technology of soil tillage. If the value of FR $< 1.5$, the impact of factors, forming the declining trend of the humus state of chernozem, will take place steadily in future without any changes and the applied agrotechnical measures (introduction of fertilizers, type of crop rotation, technology of soil tillage, sowing of perennial grasses) will not be able to correct the declining dynamics of humus in the centennial cycles. It is only possible to have the correcting impact, capable of restraining the progressing dehumification of chernozems in agroecosystems to some degree (Table 3).

The value of FR indicates stable dehumification of chernozem after its removal from the quasi-equilibrium state of the virgin field of long-term fallow, thus, one should accept the thought that the process of chernozem dehumification in the agroecosystem is a permanent and time-wise steady process, a necessary “evil” which makes it possible to grow crops in the agroecosystems.

The equations of trends ($y=ae^{-bx}$) of the declining dynamics of humus content, depending on time under different technologies of tillage, both actual and anticipated ones, differed in the value of the free member ($a = 4.25–5.61$) and the degree index ($b=-0.014+-0.061$), which impacted the value of $I_f$. However, the value of FR was in a narrow range of values (FR = 1.01–1.07), which testified to the proximity of the declining dynamics of humus regardless of the technology of chernozem tillage in the centennial cycle (Table 3).

The simulated trends of time series of total humus, which correspond to the approximation to the simple restoration of fertility in the centennial cycle, are achieved in case when the regression coefficients in the exponential equations with the variable ($a* e^{-bx}$) are positive. Under soil-protective technologies, the values of regression coefficients increase 1.9–2.3 times compared to ploughing. Here $I_f$ acquires positive values regardless of the technologies of soil tillage, and under the impact of agrotechnical measures chernozem as a system comes to the stable process of increasing humus-formation in time (Table 3).

The estimates demonstrated that the increase in the content of total humus, which would be required for simple and extended restoration of humus in the centennial cycle, equaled 25–30 t per 1 ha and 35–40 t per 1 ha respectively. To ensure this increase in the content and reserves of humus for 42 years, it is necessary to introduce 15–18 t of manure per 1 ha for simple restoration of total humus content and 18–20 t per 1 ha – for extended restoration annually. In case of replacing manure with by-products, the standardized dose of ma-
nure (coefficient 3.5) by the yield of straw is 15–18 t per 1 ha and 18–20 t per 1 ha annually, which is actually impossible in modern industrial conditions.

Discussion of results. Achieving the state of simple and extended accumulation of humus is ensured by the positivity of the trends of humus increase during the continuous experiment (42 years) with simultaneous decrease in dehumification process in the centennial cycle (92 years) which is impossible to neutralize completely. Under the impact of the increasing economic activity of humans involving the use of chernozems, there is a stage in the centennial cycle when its development is directed towards mitigating elementary processes of soil formation, which is related to the development of the processes of dehumification and agrophysical degradation, and, as a result, the decrease in the level of natural and efficient fertility of chernozems in the conditions of natural-anthropogenic soil formation.

The intense use of chernozem (ploughing for over 75–100 years) promotes the decrease in the content of total humus the most, but a sharp decrease in the humus content takes place only during the first 25–30 years after ploughing the fallow and virgin fields. With further “improvement” of chernozems, the processes of humus formation-decomposition get stabilized, whereas with high culture of arable farming the mineralization of humus is somewhat restrained, but here the type of humus formation remains extensive and declining by its trend in the centennial cycle.

Among different components of organic matter of chernozem, detritus is paid special attention, as its amount is directly related to the increase in the content and reserves of total humus [25]. Detritus is capable of accumulating newly formed humus substances and peculiar humic substances (PHS) on its surface, which testifies to its relevant role in improving the agrophysical properties of chernozem. Under ploughing for over 75–100 years, the content of detritus was 28–30 % from the total reserves of humus, and its highest amount was formed in places of localization of afterharvest, root remains and manure, i.e. in the lower part of the humus horizon. Under long-term (over 40 years) subsurface tillage, the reserves of detritus in the humus horizon were 1.16–1.4 times higher compared to the long-term ploughing, and the most considerable accumulation of detritus took place in the 0–20 cm soil layer, where the total reserves of detritus increased up to 35–38 % from the reserves of the total humus, which is confirmed by the data of other researchers, proving that the content of detritus in the total humus is from 35 % to 40 %, and exceeds 40 % in the virgin analogues of chernozems [26].

Detritus is the least stable component of the organic matter, the amount of which in the intensively tilled chernozems decreases due to the impairment of the mutual compensation of the conditions of transforming organic residues and forming humus, which is related to the change in the quantitative and qualitative composition of humus. Under systematic implementation of soil-protective technologies, there is acceleration of the processes of soil formation – the humification coefficients of organic matter increase by 12–15 % compared to ploughing. Their highest number was formed for subsurface tillage at different depths: total reserves in the 0–20 cm soil layer were 2.8–3.3 times higher, and in the variant of subsurface tillage for 10–12 cm – 2.6–2.7 times higher compared to the systematic ploughing.

In case of tillage minimization, there was differentiation of the content of active forms of humus in the humus horizon of chernozem, whereas under ploughing the differentiation was manifested inconsiderably. Under subsurface tillage for 10–12 cm, the reserves of active forms of humus in the 0–20 cm soil layer reached 58–60 % of the total reserves in the 0–40 cm layer, whereas under ploughing – 40–44 %, and under subsurface tillage at different depth – 52–53 %. The redistribution of active forms of humus in the humus horizon of chernozem was remarkable for all the variants of chernozem tillage, and the variants differed in the degree of differentiation manifestation within the humus horizon. The restoration of differentiation by the content of active humus is a natural process of soil formation in agroecosystems and it should be stimulated by the soil-protective system of tillage instead of being ruined by ploughing.

The change in the ratio of humus components in chernozem under the impact of soil-protective systems of tillage is related to the increase in the reserves of total humus in the humus horizon of chernozem. Under systematic ploughing, with the introduction of a sufficient amount of mineral and organic fertilizers, the reserves of humus were smaller compared to the variants, where the subsurface tillage was conducted. Taking into account the fact that in all the variants PHS were in the same amount, the increase in the reserves of total humus took place due to the accumulation of detritus, and the ratio of PHS reserves and the detritus reserves under surface tillage was smaller (1.83–1.91 to 1), whereas under subsurface tillage at diffe-
rent depth and ploughing the ratio was getting broader. While keeping the fallow, the ratio of the reserves of PHS and the reserves of detritus was stable at the level of 1.65–1.75 to 1.

The systematic implementation of soil-protective technologies in the crop rotation, based on subsurface tillage and covering the surface with manure, afterharvest and root remains, somehow restores the chernozem process of soil formation, which is manifested in the accumulation of detritus, active (newly formed) forms of humus in the organic matter and in the increase of total reserves of humus. The intensive cultivation (tillage) of chernozem promoted the increase in the content of active forms of humus due to biological transformation of some passive humus into its active form: humus got renewed due to the mineralization of PHS, which was related to the decrease in its content and reserves in the centennial cycle. The application of the soil protective tillage of chernozem for 42 years resulted only in the delay in dehumification processes and some stabilization of humus mineralization, but it did not promote its preservation and extended restoration to the initial level as of the moment of starting the experiment and in the centennial cycle. The increase in the humus content took place compared to ploughing and control variant without fertilizers and with their introduction, but it is not enough for the restoration of humus in the centennial cycle. The application of soil restoring measures (subsurface tillage, fertilizers, manure, straw, grasses) should be viewed as stabilizing measures in the centennial process of chernozem dehumification.

CONCLUSIONS

The estimation of the humus state of typical low humus chernozem was used to determine that under different systems of tillage the level of humus supply changes the most in the first years after the beginning of the mentioned actions, then the humus content stabilizes and changes very slowly time-wise due to the transition of humus formation processes into the quasi-equilibrium state with degradation phenomena.

The application of different technologies of tillage to typical low humus chernozem for 42 years resulted only in the delay in dehumification processes and some stabilization of humus mineralization, but it did not promote its preservation and extended restoration to the initial level as of the moment of starting the experiment. The increase in the humus content under different technologies of tillage took place compared to ploughing and the control variant without any fertilizers.

To ensure simple and extended restoration of humus in the centennial cycle, it is necessary to introduce 10–12 t of manure per 1 ha for simple restoration of total humus content and 14–15 t per 1 ha – for extended restoration annually. In case of replacing manure with by-products, the standardized dose of manure (coefficient 3.5) by the yield of straw is 10–12 t per 1 ha and 14–16 t per 1 ha annually, which is actually impossible in industrial conditions.

Simple restoration of humus in typical chernozem may be stated after achieving its actual (2017) content of at least 90 % from the content as of the beginning of the centennial cycle (92 years), which ensures maximal approximation to the non-decreasing cycle of humus dynamics trends in the centennial cycle. If the humus content is ensured in the actual measurement for the level, exceeding 90 % from the initial content, and dynamics trends are growing, one may state achieving the state of extended restoration of humus.
вало мінералізацію гумусу, але не сприяло його збереженню та розширеному відтворенню до початкового рівня на момент закладки досліду. Зростання вмісту загального гумусу для простого і розширеного відтворення гумусу у віковому циклі рівноцінне: 20–25 та 30–33 т на 1га відповідно. Для забезпечення зростання вмісту і запасу гумусу на протязі 42 років необхідно щорічно вносити 10–12 т на 1 га для простого і 14–15 т на 1 га розширеного відтворення загального вмісту гумусу щорічно

**Висновки.** Просте відтворення гумусу чорнозему типового можна констатувати при досягненні його реального (2017 р.) умісту не менше 90 % від умісту з початку відліку вікового циклу (92 роки), що забезпечує максимальне наближення до неспадного циклу трендів динаміки гумусу у віковому циклі. Якщо уміст гумусу забезпечується у реальному вимірі на рівні більшому за 90 % від початкового вмісту, а тренди динамики набувають зростаючого характеру, то можна констатувати досягнення стану розширеного відтворення гумусу. Досягнення стану простого і розширеного накопичення гумусу забезпечується досягненням трендів зростання гумусу за час проведення стаціонарного досліду (42 роки) при одночасному зниженні процесу дегуміфікації у віковому циклі (92 роки), яке повністю зі зневеливати неможливо.

**Ключові слова:** чорнозем типовий, органічна речовина, гумус, тренд, солома, гній, мінеральні добрива, гуміфікація, обробіток ґрунту.

**Многолетняя динамика содержания гумуса при различных технологиях обработки почвы**

A. V. Демиденко 1, П. И. Бойко 2, В. А. Величко 3

1 Черкасская государственная сельскохозяйственная опытная станция
2 Ул. Докучаева, 13, с. Холодянское, Смейлского р-он, Черкаской обл., Украина, 20731;
email: smilachiapv@ukr.net
3 ННЦ «Институт земледелия НАН»
Ул. Машиностроителей, 2-б, пгт Чабаны, Киево-
Святошинский р-н, Киевская обл.
email: iznaan@ukr.net
4 ННЦ «Институт почвоведения и агрохимии
имени А.Н. Соколовского», ул.Чайковского, 4,
Харьков, Украина, 61024;
email: agrovisnyk@ukr.net

**Цель.** Определить темпы динамики накопления и мінерализации общего гумуса и разработать прогноз изменения его содержания в долгосрочном стационарном полевом опыте при длительном применении различных способов обработки чернозема типичного малогумусного левобережной Лесостепи Украины.

**Методы.** Полевой, лабораторно-аналитический, математический и статистический.

**Результаты.** Применение различных способов обработки чернозема типичного малогумусного в течение 42 лет привело лишь к задержке процессов дегумификации и в определенной степени стабилизировало минерализацию гумуса, но не способствовало его сохранению и расширенному воспроизводству к исходному уровню на момент закладки опыта. Рост содержания общего гумуса для простого и расширенного воспроизводства гумуса в вековом цикле равноценны 20–25 т и 30–33 т на 1 га соответственно. Для обеспечения установленных прибавок гумуса необходимо ежегодно вносить навоза 10–12 т на 1 га для простого и 14–15 т на 1 га расширенного воспроизводства общего содержания гумуса ежегодно.

**Выводы.** Простое воспроизводство гумуса чернозема типичного можно определить, как достижение его реального (2017 г.) содержимого не менее 90 % от содержимого начала отсчета векового цикла (92 года), что обеспечивает максимальное приближение к возрастаному тренду динамики гумуса в возрастном цикле. Если содержание гумуса обеспечивается в реальном измерении на уровне, превышающем 90 % от первоначального содержания, а тренды динамики приобретают возрастной характер, то можно констатировать достижение состояния расширенного воспроизводства гумуса. Достижение состояния простого и расширенного накопления гумуса обеспечивается положительностью трендов роста гумуса за время проведения стационарного опыта (42 года) при одновременном снижении процесса дегумификации в возрастном цикле (92 года), которую полностью нивелировать невозможно.

**Ключевые слова:** чернозем типичный, органическое вещество, гумус, тренд, солома, гній, минеральные удобрення, гумификация, обработка почвы.

**REFERENCES**

9. Bertolini P, Tian SP. Low-temperature biology and pa-
STUDY OF THE PROCESS OF PREPARING FEEDING MIXTURES USING THE COMPOSITE MIXER

M. I. Chernovol, M. O. Sviren, R. V. Kisiliov

Central Ukrainian National Technical University
8, pr. Universytetsky, Kropyvnytsky, Ukraine, 25030

e-mail: rektor@kntu.kr.ua, kaf_sgm_kntu@ukr.net, ruslan_vik@ukr.net

Received on November 27, 2017

Aim. Enhancing the quality and improving the technological process of mixing feeds using the new construction of the mixer and substantiating its rational parameters. Methods. Mathematical modeling theories, fundamentals of using machinery in animal breeding. Results. The estimated model of the functioning of a constructive-technological scheme of a composite mixer and the mathematical model of the dynamic interaction of mixer paddles and the solid mass of feeds were elaborated. It was established that the technological efficiency of preparing the homogeneous mixture depends on physical and mechanic properties of its components, the impact and interaction between the form and geometric parameters of the attacking surface of the paddles, the slope angle, the setting increment and working modes of the mixer. Conclusions. The results of the studies confirm the possibility of enhancing the efficiency of the technology of preparing completely balanced feeding mixtures for cattle via the intensification of the mixing process using the construction of the composite belt-paddle mixer, the elaboration of theoretical fundamentals of the interaction of feed components with the working bodies and substantiating their main constructive and technological parameters.

Keywords: feed mixer, feeds, animal breeding, paddle mixer, zootechnic requirements.

DOI: 10.15407/agrisp5.01.017
An improved mixer with a combined scheme of the flow of raw materials using multi-section helical, line and flat paddles is suggested for elimination of current drawbacks of traditional mixers (Fig. 1).

To expand the mass, to intensify the process and to enhance the dynamics of mixing the components in the microvolumes, the helical and flat paddles were additionally equipped with radial paddles.

The process of mixing feeds using the improved mixer is done as follows. The corresponding doses of the components of the feeding mixture are loaded in layers into the tank using the composite transporter with gradual leveling of the raw material using the long line helical paddles with fingers and then are supplied into the multi-section mixer with flat paddles (Fig. 2). The paddles of the upper range with the right slope angle separate the mixture portion along the width of the paddle and transport it in radial, circular, and axial directions towards the right end of the mixer, and the second range, with the left slope angle – towards the left end of the mixer, creating a large microvolume mass of the mixture with the discrete content of the shares of the mixture components along with the radial fingers. Here the shares of each mixture component enter the area of interaction of complicated movements, crossings, and collisions, and are periodically transported from one flow to the other which ensures the intense mass exchange and accelerates the process of feed mixing.

The translocation of a feed mixture along the surface of paddles with different slope angle in the zone
of inertial (free) motion is made in the mode of increased dynamics of the process and the increased number of collisions and crossings in the radial and axial directions which is determined by the form and sizes of the attaching paddle, their setting increment, the slope angle and kinematic modes of the work of paddles (Fig. 3).

The determination of kinematics of the motion of a mixture share was conducted with the consideration of the friction forces and the slope angle for paddles [10–12]. In case of friction, depending on the slope angle of the paddle towards the shaft axis a the translocation of the material point of the mixture component in the axial direction will occur while the paddle moves by the value of (Fig. 4):

\[ h_x = S \cdot \frac{\cos \alpha \cos (\alpha + \varphi)}{\cos \varphi}, \]  

lags behind in the axial direction by the value of:

\[ Z_y = S \cdot \frac{\sin \alpha \sin (\alpha + \varphi)}{\cos \varphi}, \]  

lags behind in the circular direction:

\[ \lambda = S \cdot \frac{\cos \alpha \sin (\alpha + \varphi)}{\cos \varphi}, \]  

where \( \alpha \) – the slope angle of the paddle; 
\( \varphi \) – the angle of the particle friction along the paddle surface; 
\( S \) – projection of the paddle width.

The axial velocity of the translocation of the mixture share is defined using the equation:

\[ \dot{h}_x = S \cdot (1-\mu) = S \cdot \left[1 - \frac{\sin \alpha \cdot (\sin \alpha + \varphi)}{\cos \varphi}\right], \]  

where \( \mu \) – coefficient of the axial lagging behind of the shares depending on the angles \( \alpha \) and \( \varphi \).

The analysis of equations (1), (2) and (4) demonstrates that the translocation of mixture shares in the axial direction and the axial velocity of their translocation depend on the slope angle of the paddles towards the shaft axis of the mixer \( \alpha \), the friction angle for the mixture along the paddle surface and the coefficient of axial lagging behind of the material shares of the mixture \( \mu \) (Fig. 5, 6). With the increase in the angle \( \alpha \) on condition of constant coefficient of friction \( f \) the axial lagging behind of the translocation of mixture shares decreases, and with the constant slope angle of the paddle \( \alpha \) on condition of increasing the coefficient of friction \( f \), there is also an increase in...
against the relative movement of shares along the paddle. The friction force $F$ is divided into the circular and axial components:

$$F'_c = F \cdot \sin \alpha = f \cdot P_H \cdot \sin \alpha,$$

$$F'_a = F \cdot \cos \alpha = f \cdot P_H \cdot \cos \alpha.$$  

Taking the received vectors into consideration by the movement directions, we receive the circular and axial efforts:

$$P_p = P_p' + F'_p = P_H \cdot (\cos \alpha + f \cdot \sin \alpha),$$

$$P_o = P_o' + F'_o = P_H \cdot (\sin \alpha - f \cdot \cos \alpha).$$

In case of incomplete filling of the mixer tank the normal component $P_H$ is defined using the formula:

$$P_H = 9.81 \gamma \cdot h_{\text{ave}} \cdot F_1 \cdot \tan(45 + \frac{\phi}{2}),$$

where $h_{\text{ave}}$ – average depth of the largest depression on the paddle, m;

$F_1$ – projection of the paddle area on the direction of the rotation of the mixer, m$^2$;

$\phi$ – internal friction angle, degrees;

$\gamma$ – bulk weight of the mixture, kg/cc.

The required power of the drive of the mixer paddles is defined using the equation, kilowatt:

$$N_p = \frac{1}{1000} (P_{p_r} + P_{p_o}) \cdot Z_p,$$

where $Z_p$ – the number of paddles, which are simultaneously submerged into the feeding mixture.

Therefore, the total power of the mixer drive is defined as follows:

$$N_m = N_{hp} + N_{fp} + N_{hp}' + N_{fp}' + N_f + N_{horp} +$$

$$+ N_{fr} + N_{hor.p},$$

where $N_{hp}, N_{fp}, N_{hp}', N_{fp}', N_f, N_{horp}, N_{fr}, N_{hor.p}, N_{m}$ – power losses on the drive regarding the helical and flat paddles, frames of helical and flat paddles, radial fingers, horizontal pipes, friction of the mixer from the body and bearings of the shaft, kilowatt.

$$N_{hp} = \frac{P_{p_r} + P_{p_o}}{10^3} \cdot Z_p$$

(12)

The power on the drive of helical and flat paddles:

where $P_{p_r}$, $P_{p_o}$ – circular and axial effort, H; $\vartheta_p, \vartheta_o$ – circular and axial velocity of the mixture movement, m/s; $Z_p$ – number of simultaneously submerged paddles.

The power on the drive of frames of helical and flat paddles

$$N_{fr} = \frac{M_{fr} \cdot Z_p \cdot \vartheta_o}{10^6}$$

(13)
where $M_p$ – rotational moment from the force of resistance of the frame, $H \cdot m$.

$$M_p = \frac{g \cdot l \cdot R}{2} \cdot h_{ave} \cdot \alpha \cdot \gamma \cdot \tan \varphi, \ H \cdot m, \ (14)$$

where $l$ – frame length, m; $\frac{R}{2}$ – ratio of the frame length to the resistance force, m; $h_{ave}$ – average depth of submerging the frame into the feed mass, m; $\alpha$ – frame width, m; $\varphi$ – angle of feed slope, degrees.

The power on the drive of radial fingers, kilowatt:

$$N_r = \frac{M_a \cdot Z_r \cdot \omega}{10^3}, \ (15)$$

where $M_a$ – rotational moment from the force of resistance of the finger, $H \cdot m$.

$$M_a = P_f \cdot l_f \cdot d_f \cdot R_f, \ (16)$$

where $P_f$ – the relative resistance of the mixture, $H/m^2$; $l_f$ – finger length, m; $d_f$ – finger diameter, m; $R_f$ – average radius of rotation for the fingers, m.

The power on the drive of horizontal pipes, kilowatt:

$$N_{hor} = \frac{M_p \cdot Z_p \cdot \omega}{10^3}, \ (17)$$

where $M_p$ – rotational moment from the resistance force of the horizontal pipe, $H/m$.

$$M_p = P_f \cdot L \cdot d_p \cdot R_p \ (18)$$

CONCLUSIONS

The usage of the composite belt-paddle mixer can enhance the efficiency of the technology of preparing completely balanced feeding mixtures for cattle. The suggested construction of the mixer ensures the homogeneity of the mixture $V_0 = 95…98 \%$ and required technological efficiency and reliability of performing the process with minimal energy losses which corresponds to the active zootechnic requirements ($V_0 = 90…92 \%$) to the homogeneity of preparing complete mixtures for cattle.

Исследование процесса приготовления кормовых смесей комбинированным смесителем

М. И. Черновол, Н. А. Свиринь, Р. В. Кисилев
Центральноукраинский национальный технический университет
Пр. Университетский, 8, Кропивницкий, Украина, 25030,
e-mail: rektor@kntu.kr.ua, kaf_sgm_kntu@ukr.net, ruslan_vik@ukr.net

Цель. Повышение качества и совершенствования технологического процесса смешивания кормов за счет применения новой конструкции смесителя и обоснование его рациональных параметров. Методы. теории математического моделирования, основ машино-использования в животноводстве. Результаты. Разработана расчетная модель функционирования конструктивно-технологической схемы комбинированного смесителя и математическая модель динамического взаимодействия лопаток мешалки с кормовым монолитом. Установлено, что технологическая эффективность приготовления однородной смеси зависит от физико-механических свойств ее компонентов, влияния и взаимодействия формы и геометрических параметров атакующей поверхности лопаток, угла наклона, шага
установки и режимов работы смесителя. **Выводы.** Результаты проведенных исследований подтверждают возможность повышения эффективности технологии приготовления полноценных сбалансированных кормосмесей для КРС путем интенсификации процесса смешивания с применением конструкции комбинированного ленточно-лопастного смесителя, разработкой теоретических основ взаимодействия компонентов корма с его рабочими органами и обоснованием их основных конструктивных и технологических параметров.

**Ключевые слова:** смеситель кормов, корма, животноводство, лопастная мешалка, кормосмеси, зоотехнические требования.

**REFERENCES**

INTRODUCTION

The soil cover of Ukraine has been studied rather well, but the intense process of soil degradation has not been stopped. Approximately a third of arable lands is eroded, about 20 % of organic substances are lost, almost half of all the soils have excessive density, the reserves of nutrients, especially phosphorus and potassium, are decreasing. The main reason of the mentioned problems is underestimation of the actual danger of soil degradation for future generations, lack of understanding of the fact that the fertility of soils is the actual value of arable lands, which requires technological measures, aimed at its preservation [1, 2]. According to the data of the UN Food and Agriculture Organization (FAO), the agricultural activity has resulted in considerable degradation processes. Soil degradation is a relevant problem for Ukraine, directly impacting the fertility of soil and the quality of agricultural products, which brings about economic loss (over USD 6 billion a year) [3]. The solution of problems, related to the management of land resources, requires the biological monitoring of the rationality of using agrotechnologies.

Microbial cenosis is remarkable for high sensitivity to the changes in the technologies of cultivating crops [4]. A relevant role of soil microorganisms is conditioned by their participation in the formation of soil fertility and, in particular, in the transformation of nutrients in soil (nitrogen and carbon, first and foremost) [5]. Fertilizers (organic and mineral) and soil tillage influence the number and the ratio of different physiological groups of soil microorganisms. The impact of mineral fertilizers on soil biota is of low specificity; it depends on the chemical nature and the dose of introduction [6]. For instance, it was demonstrated in the works [7–10] that the introduction of a considerable amount of mineral fertilizers leads to qualitative and quantitative changes in the microbial complex of typical chernozem, which is accompanied with the simplification of trophic relations and the decrease in biodiversity. The introduction of organic fertilizers on the background of moderate mineral fertilizers under the ecological and biological systems of agriculture leads to the increase in the number of microorganisms by 23 % and 7 % respectively.
as they consume organic nitrogen, and to the decrease of the groups, consuming the mineral nitrogen, in the biological system by up to 36 % [8]. Under these systems, there are changes in the agrobiocenosis towards the decrease in oligotrophicity due to the formation of a smaller number of substances in the soil, which are notable for the latter stages of organic matter mineralization, and the increase in pedotrophs, utilizing the water-soluble fractions of organic matter [8, 9]. In conditions of excessive technological burden, chernozem soils start degrading with acceleration. It is especially true for less stable meadow-chernozem soils. Intense application of modern crop rotations on three fields brought about sharp deterioration of their condition. The problem is getting more pressing. Living matter is a clear reference point of determining the gradient of the soil-formation process.

The aim of the studies was to determine the impact of different fertilization systems in a crop rotation on the number of the main groups of microorganisms, which participate in the transformation of nitrogen and carbon compounds.

MATERIALS AND METHODS

The biological evaluation of the 60-year-long application of mineral fertilizers in the 10-field crop rotation was conducted in the industrial division of the National University of Life and Environmental Sciences of Ukraine (ID NULES of Ukraine “Agricultural Experimental Station” in conditions of a continuous agronomic experiment of the Chair of Agrochemistry and Quality of Plant Products named after O. I. Dushechkin, which was started in 1956 in a 10-field crop rotation: clover, winter wheat, sugar beet, corn for silo, spring wheat, green peas, winter wheat, sugar beet, corn for grain, barley with clover. The experimental field is located in the Right-Bank Forest-Steppe zone and belongs to the Bila Tserkva agrosoil district. The relief of the area is flat (Kyiv plateau), the relief of the territory is an undulate flat. In addition, the fallow is left in the form of protection belts as absolute control.

The soil of the experimental plot is meadow-chernozem, carbonate, low-humus, heavy loamy, medium clay on forest-like clay. The amount of total humus in soil of the experimental plots was 4.09–4.50 %, and on the fallow – 5.50 %. The following fertilization variants were studied in the experiment: 1) no fertilizers (control); 2) phosphate fertilizers; 3) phosphate and potassic fertilizers; 4) a single dose of NPK; 5) one-and-a-half dose of NPK. The system of soil tillage was traditional, presupposing moldboard plowing, and there was surface tillage for winter wheat after green peas. The studies of soil were conducted in the 0–10 and 10–20 cm layers of soil in May. The selection and keeping of the soil for the study of aerobic microbiological processes in laboratory conditions was performed in accordance with DSTU ISO 10381-6-2001. A comprehensive approach was selected for the evaluation of the processes of microbial transformation of carbon-containing compounds in typical chernozem, which presupposed three stages of implementing the studies: 1 – estimating the agrochemical state of soil; 2 – investigating the quantitative and qualitative composition of the microbial complex, including microorganisms, participating in the transformation of carbon compounds in soil; 3 – determining the biological activity of the microbiota of the meadow-chernozem soil and the direction of the processes of microbial transformation of carbon-containing compounds. The selection of indices for the evaluation of microbial transformation of carbon compounds in soil was based on the following criteria: informative value and high sensitivity of indices, probability of results, high rate of the determination method, application of novel experience of foreign countries and compliance with current regulatory standards in force [6]. Meat-and-peptone agar (MPA) was used to study the total number of microorganisms, decomposing the organic substances; starch-ammonium medium (SAM) – to study the microorganisms, assimilating mineral nitrogen forms. The number of microorganisms, synthesizing melanin, was studied in Czapek’s medium at pH = 5.0; the ones, decomposing humates – in the medium with potassium humate; pedotrophs – in the soil agar.

The methods of registering the colonies of microorganisms in soil and the composition of media were studied according to D. G. Zviagintsev [8]. The results of the number of microorganisms were presented with the number of forming units in 1 g of absolutely dry soil (CFU/g of soil), taking into account the coefficient of soil humidity [8]. The direction of microbial processes in soil was determined via the evaluation of the following indices: the coefficient of mineralization-immobilization of nitrogen (evaluated by the ratio of the number of microorganisms, consuming mineral and organic nitrogen) [9]; the coefficient of pedotrophicity (the ratio of the number of pedotrophic microorganisms to MPA) [8]; the coefficient of microbial transformation of organic matter of soil (the ratio of the number of microorganisms to MPA and SAM to the mineral-
lization coefficient) [8]. The statistical processing and mathematical analysis of the results of studies were conducted using MS Excel 10.0, STATISTICA 7.0 and QIIME 1.7.0 programs.

THE RESULTS OF INVESTIGATIONS

Nitrogen cycle consists of the process of its microbial fixation from the atmosphere and the introduction of the bound nitrogen into a small biological circulation with the destruction of nitrogen-containing organic compounds to ammonium (ammonification), oxidation of ammonium to nitrates (nitrification), subsequent reduction to free nitrogen (denitrification), which is released into the atmosphere again. Microorganisms play the main role in the transformation of nitrogen in soil [5].

In our studies the number of ammonifying and amylolytic microorganisms (Table 1) in the meadow-chernozem carbonate soil depended on the variant of fertilization and decreased with depth. For instance, the highest number of microorganisms, decomposing the organic forms of nitrogen in all the variants of the studies, was obtained in the root-containing layer of 0–10 cm, with further decrease by 14–87 % in the 10–20 cm layer. A similar regularity was obtained for the number of amylolytic microorganisms, here their decrease in the 10–20 cm layer compared to 0–10 cm layer was 10–67 %. The highest number of ammonifying microorganisms was obtained in the variant with 1.0 dose of NPK which is related to the saturation of soil with nitrogen. While evaluating the degree of saturation by the number of ammonifiers using the method of D. G. Zviagintsev [7], one should note that all the fertilization variants are characterized as poor ones, except for 1.0 dose of NPK – a very rich one.

Therefore, the situation with humus accumulation is not very good in all the fertilization variants, except for 1.0 dose of NPK. The highest number of microorganisms, using mineral forms of nitrogen in the meadow-chernozem soil, was obtained using PK, 1.5 dose of NPK and the fallow. It indicates the intensity of the mineralization processes using the abovementioned variants of fertilization.

In the opinion of Yu. P. Moskalevska, M. V. Patyka [6], H.O. Iutynska [9], I. D. Prymak [10], the processes of mineralization of humic substances are conditioned by biochemical activity of the specific microflora – humate-decomposing ones, including the microorganisms, capable of using carbon of the cyclic and heterocyclic bonds, which are most resistant to decomposition. Other researchers deny the specialization of microorganisms in humus decomposition and refer these processes to the activity of all the soil microorganisms [8]. According to the data of H. O. Iutynska

Table 1. The number of ammonifying and amylolytic microorganisms in the meadow-chernozem soil in the crop rotation with sugar beet after winter wheat*

<table>
<thead>
<tr>
<th>Fertilization variant</th>
<th>Soil layer, cm</th>
<th>Ammonifying microorganisms (mln CFU/g of soil)</th>
<th>Amylolytic microorganisms (mln CFU/g of soil)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizers (control)</td>
<td>0–10</td>
<td>2.54 ± 0.18</td>
<td>6.32 ± 0.50</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>1.76 ± 0.11</td>
<td>4.32 ± 0.34</td>
</tr>
<tr>
<td>P</td>
<td>0–10</td>
<td>3.25 ± 0.27</td>
<td>10.6 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>2.81 ± 0.24</td>
<td>6.16 ± 0.26</td>
</tr>
<tr>
<td>PK</td>
<td>0–10</td>
<td>3.03 ± 0.23</td>
<td>15.25 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>2.76 ± 0.17</td>
<td>11.16 ± 0.64</td>
</tr>
<tr>
<td>1.0 dose of NPK</td>
<td>0–10</td>
<td>26.02 ± 1.51</td>
<td>9.74 ± 0.37</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>14.23 ± 1.50</td>
<td>7.56 ± 0.17</td>
</tr>
<tr>
<td>1.5 dose of NPK</td>
<td>0–10</td>
<td>2.39 ± 0.24</td>
<td>11.12 ± 1.63</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>1.94 ± 0.28</td>
<td>10.11 ± 0.74</td>
</tr>
<tr>
<td>Fallow</td>
<td>0–10</td>
<td>4.59 ± 0.31</td>
<td>14.42 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>3.64 ± 0.34</td>
<td>9.71 ± 0.41</td>
</tr>
</tbody>
</table>

* ID NULES of Ukraine “Agronomic Experimental Station”
the decomposition of peripheral chains of humus molecules involves the participation of pedotrophic microorganisms, while deep destruction is performed by humate-decomposing ones. The number of pedotrophs, utilizing water-soluble fractions of organic matter and microorganisms, conducting deep destruction of nuclear aromatic components of humus, is presented in Table 2.

The results of investigations demonstrated that the highest number of pedotrophic microorganisms was observed in case of a single dose of mineral fertilizers, and the lowest one – in case of one and a half dose. The highest values in terms of the number of humate-decomposing microorganisms were obtained while using phosphate and potassic fertilizers both in 0–10 and 10–20 cm layers.

Table 2. The number of pedotrophic and humate-decomposing microorganisms depending on the fertilization variants in the meadow-chernozem carbonate soil

<table>
<thead>
<tr>
<th>Fertilization variant</th>
<th>Soil layer, cm</th>
<th>Pedotrophic microorganisms, mln CFU/g</th>
<th>Humate-decomposing microorganisms, mln CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizers (control)</td>
<td>0–10</td>
<td>10.79 ± 1.87</td>
<td>2.39 ± 0.07</td>
</tr>
<tr>
<td>10–20</td>
<td>7.12 ± 0.63</td>
<td>1.19 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>0–10</td>
<td>15.34 ± 2.13</td>
<td>1.88 ± 0.12</td>
</tr>
<tr>
<td>10–20</td>
<td>9.12 ± 1.52</td>
<td>1.23 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>PK</td>
<td>0–10</td>
<td>12.34 ± 1.56</td>
<td>3.28 ± 0.06</td>
</tr>
<tr>
<td>10–20</td>
<td>8.56 ± 0.46</td>
<td>2.74 ± 0.36</td>
<td></td>
</tr>
<tr>
<td>NPK 1.0</td>
<td>0–10</td>
<td>33.3 ± 2.89</td>
<td>2.63 ± 0.2</td>
</tr>
<tr>
<td>10–20</td>
<td>18.43 ± 1.13</td>
<td>1.19 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>NPK 1.5</td>
<td>0–10</td>
<td>4.11 ± 0.24</td>
<td>2.11 ± 0.08</td>
</tr>
<tr>
<td>10–20</td>
<td>3.87 ± 0.17</td>
<td>1.81 ± 0.08</td>
<td></td>
</tr>
<tr>
<td>Fallow</td>
<td>0–10</td>
<td>12.70 ± 1.17</td>
<td>2.65 ± 0.22</td>
</tr>
<tr>
<td>10–20</td>
<td>8.17 ± 0.41</td>
<td>1.46 ± 0.18</td>
<td></td>
</tr>
</tbody>
</table>
The coefficient of pedotrophicity indicates the degree of consumption of organic matter (Fig. 1). According to this index in the control and in case of the introduction of phosphate (variant P), phosphate and potassic (PK) fertilizers (Cp. = 3.10–4.72), there is development of autochthonous microbiota in the meadow-chernozem soil and the mineralization processes from the total fund are increased.

The most optimal conditions, according to the trend line, are seen in case of one and a half dose of mineral fertilizers. In terms of the number of humate-decomposing microorganisms, the lowest values and the best conditions were obtained under clover. The worst conditions were in the field under sugar beet No. 2, which had 2.1–5.2 times more humate-decomposing microorganisms compared to the field with clover.

The number of micromycetes in the meadow-chernozem carbonate soil depending on the fertilization is presented in Fig. 2.

The highest number of micromycetes in the 0–20 cm soil layer was obtained at the one-and-a-half dose of mineral fertilizers, with other values in the descending order: fallow – 1.0 NPK – PK – control – P.

**CONCLUSIONS**

The optimal conditions for humus accumulation and nitrogen transformation are created on the fallow and

<table>
<thead>
<tr>
<th>Fertilization variant</th>
<th>Soil layer, cm</th>
<th>Pedotrophic microorganisms, mln CFU/g</th>
<th>Humate-decomposing microorganisms, mln CFU/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fertilizers (control), under sugar beet No. 2</td>
<td>0–10</td>
<td>10.79 ± 1.87</td>
<td>2.39 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>7.12 ± 0.63</td>
<td>1.19 ± 0.08</td>
</tr>
<tr>
<td>No fertilizers (control) under clover</td>
<td>0–10</td>
<td>13.73 ± 1.02</td>
<td>0.61 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>9.11 ± 0.74</td>
<td>0.42 ± 0.04</td>
</tr>
<tr>
<td>1.5 NPK, under clover</td>
<td>0–10</td>
<td>18.5 ± 0.84</td>
<td>0.76 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>8.44 ± 0.51</td>
<td>0.58 ± 0.04</td>
</tr>
<tr>
<td>No fertilizers (control), under sugar beet No. 1</td>
<td>0–10</td>
<td>14.46 ± 1.45</td>
<td>1.16 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>10–20</td>
<td>9.70 ± 0.64</td>
<td>0.81 ± 0.07</td>
</tr>
</tbody>
</table>
in the variant with complete mineral fertilization. The use of one-and-a-half dose of fertilizers enhanced the mineralized processes in soil 1.2–1.4 times compared to the single dose. The highest number of pedotrophic microorganisms was observed in case of a single dose of mineral fertilizers, and the lowest one – in case of one-and-a-half dose. The highest value in terms of the number of humate-decomposing microorganisms were obtained while using phosphate and potassic fertilizers both in 0–10 and 10-20 cm layers. Compared to clover, 2.1–5.2 times more humate-decomposing microorganisms are formed in the field under sugar beet No. 2. The application of the 10-field crop rotation with legumes (clover) and complete mineral fertilizers for 60 years ensured non-degradation development of the meadow-chernozem soil at the quasi-stable level.

Біологічна експертиза раціональності використання грунту в агрокультурі

С. Ю. Булигін, О. Л. Тонха,
Національний університет біоресурсів і природокористування України, 03041, Київ,
Вул. Г. Оборони 15,
e-mail: Oksana16095@gmail.com, s.bulygin@ukr.net

Мета. Провести біологічну експертизу 60-ти річного застосування різних варіантів удобрений щодо чисельності мікроорганізмів, які трансформують сполуки нітрогену і карбону. 

Методи. Мікробіологічні – визначення чисельності мікроорганізмів, які трансформують органічні сполуки та нітроген, статистичні.

Результати. Встановлено, що на лучно-чорноземних карбонатних грунтах застосування звісімін з бобовими культурами та варіанту з повним мінеральним удобрением формує збалансований склад мікробного ценозу і найбільш оптимальні умови для накопичення гумусу і трансформації азоту.

Висновки. Мікробіологічний моніторинг показав, що бездеградаційного розвитку лучно-чорноземного ґрунту на квазистабільному рівні можна досягти за умови застосування 10-пільної звісімін з бобовими культурами (клевером) та повного мінерального удобрения.

Ключові слова: амоніфікатори, амілолітичні мікроорганизми, педотрофі, гуматролюючі мікроорганізми, повне мінеральне удобрение.

Біологічна експертиза раціональності використання почв в агрокультуре

С. Ю. Булигін, О. Л. Тонха
Национальный университет биоресурсов и природопользования Украины, 03041, Киев,
Ул. Г. Оборони 15,
e-mail: Oksana16095@gmail.com, s.bulygin@ukr.net

Цель. Провести биологическую экспертизу 60-летнего применения различных вариантов удобрения на численность микроорганизмов, которые трансформируют соединения азота и углерода.

Методы. Микробиологические – определение численности микроорганизмов, которые трансформируют органические соединения и азот, статистические.

Результаты. Установлено, что на лугово-черноземной почве введение севооборота с бобовыми культурами и применение полного минерального удобрения формируют сбалансированный состав микробного ценоза и наиболее оптимальные условия для накопления гумуса и трансформации азота.

Выводы. Микробиологический мониторинг показал, что бездеградационного развития лугово-черноземной почвы на квазистабильном уровне возможно достигнуть при условии ведения 10-польного севооборота с бобовыми культурами (клевером) и полного минерального удобрения.

Ключевые слова: аммонификаторы, амилолитические микроорганизмы, педотрофы, гуматразлагающие микроорганизмы, полное минеральное удобрение.

REFERENCES

1. World Development Indicators, Data Bank, 2015; http://databank.worldbank.org/data


INTRODUCTION

The conditions, required for the technological process of beet harvesting, are ensuring the performance, decreasing the energy losses and increasing the quality of the harvested products. As for beet harvesters, the required condition of ensuring the quality of performing the technological process is avoiding the damage to crop roots while they are dug up, first and foremost.

Therefore, there is a need to investigate the process of vibrational digging-up and to use its results to determine kinematic and constructive parameters of the digging-up working tools on condition of avoiding damage to the crop roots.

The technological process of vibrational digging-up of sugar beet has spread in many sugar beet-sowing countries. Many years of using this process demonstrated a number of its advantages compared against other methods of digging-up. Therefore, this technological process requires further detailed analytical and experimental research.
EXPERIMENTAL RESEARCH ON VIBRATIONAL DIGGING-UP OF SUGAR BEET

Experimental research which will lay the foundation for the elaboration of improved vibrational digging-up working tools.

Substantial theoretical research on the process of vibrational digging-up of crop roots was done in the works [1–4], but only the works [5] and [6] present some experimental results of the impact interaction of pendulum impact testing machine and a crop root. The analysis of scientific literature [7–10] demonstrated that quality indices of digging-up sugar beet from soil are paid considerable attention in Europe. As stated above, there have been scarce experimental researches on the process of vibrational digging-up of sugar beet from soil, therefore, this topic is urgent for sugar beet-growing industry, as this research can allow improving the most energy-consuming technological process.

The aim of the research is the experimental determination of rational parameters and modes of the vibrational digging-up working tool to ensure the required quality of conducting the technological process of digging-up sugar beet from soil.

MATERIALS AND METHODS

A new model of the vibrational digging-up working tool was designed for experimental research on the process of vibrational digging-up of sugar beet from soil which was deemed to ensure quality extraction of crop roots from dry and solid ground. The construction design of the vibrational digging-up machine is protected with the Patent of Ukraine for an invention [11] Fig. 1.

The digging-up machine consists of digging-up blades (1), installed on the ends of the mounts (2), which are connected via suspension brackets (3) to the drive mechanism (4) of the mentioned blades (1) to obtain the vibrational movement. The mechanism (4) has a device, which can be used to set (regulate) the frequency and amplitude of the vibrational movements of blades in a wide range of values (the frequency is regulated from 8.5 to 20.3 Hz, the amplitude – from 8 to 24 mm). The suspension bracket (3) of the mounts (2) was equipped with an additional hinge which allows for free movements of coupled mounts (2) in a small range in the longitudinal-transversal plane. This ensures the automatic installation of blades (1) during the translational movement of the vibrational digging-up machine.

The general view of the designed vibrational digging-up working tool is presented in Fig. 2.

Fig. 1. The construction and technological scheme of the vibrational digging-up machine: 1 – a digging-up blade; 2 – mounts; 3 – mechanism of regulating the distance between blades; 4 – vibrational drive mechanism with the mechanism of regulating the amplitude and the frequency of blade vibrations; 5 – guide pins

Fig. 2. The general view of the vibrational digging-up working tool: a – computerized 3D model; b – a photograph A towed four-row beet harvester was produced for laboratory-field experimental research of the vibrational digging-up working tool under different parameters and working modes of the vibrational working tool (Fig. 3)
The experimental device (Fig. 3) consists of the frame (11), bearing on posterior (2) supporting and front (3) copying wheels. The front part of the frame (11) has the installed vibrational digging-up working tools (4), formed by digging-up blades (8), set on the mounts (9). The posterior necked part of blades (8) has a beater (5) with a 4-blade beater transporter (6) behind it. The vibrational digging-up working tools (4) are connected to the drive mechanism (7) for oscillatory movements with a wide range of amplitudes and frequencies.

To determine the energy-force characteristics, a tenzometric traction link was attached to the device for simultaneous measurement of the horizontal and vertical components of the traction effort on the towed device with a wheeled tractor (1). Foil tenzometric sensors were installed on the mounts (9) to determine the ef-
EXPERIMENTAL RESEARCH ON VIBRATIONAL DIGGING-UP OF SUGAR BEET

forts of the interaction between the blades (8) and soil. The drive of all the working tools of the experimental device was ensured by the power take-off shaft of the wheeled tractor (1), class 1.4. To define the angular velocity, the steering torque and the power, transmitted to the working tools, an electric joint dynamometer was installed between the power take-off shaft of the tractor (1) and the drive shaft of the working tools of the experimental device. The general view of the experimental device during field experimental research and that of the vibrational digging-up working tools under investigation is presented in Fig. 4. A movable tenzometric station, installed on a vehicle, was moving along the laboratory field device during the experimental research.

During the work of the experimental device, the digging-up blade (8) takes a complicated load, estimated by the value of the bending motion of its mount (9) with the installed tenzometric sensors. A track-measuring wheel is attached to the frame to determine the velocity of the experimental device.

The registration of tenzometric indices of the investigated parameters within the energetic estimation of the work of vibrational digging-up machines was conducted using the movable tenzometric laboratory ChEK-1 (based on UAZ vehicle) which was moving along the experimental device during the experiments (Fig. 4, a). This laboratory allows measuring, registering and demonstrating the average values of power and velocity parameters via six independent measuring channels immediately after the experiments.

The cleaning and transporting working tools of the beet harvester were disconnected from the drive mechanism. A device for stripping the tape to collect the sugar beet, dug up from soil, in order to estimate the quality of their extraction in the field work was installed behind the vibrational digging-up machines. The running depth of the digging-up machine in soil was controlled using the measuring device.

The determination of the agrotechnical indices of the experimental field was conducted according to the general method and the method of the Ukrainian Research Institute of Forecasting and Testing of Equipment and Technologies named after Leonid Pogorilyi [12–15].

To determine the indices of agrotechnical evaluation, three standard plots, 20 m long and 2.7 m wide, i.e. having the width of six rows, were indicated along the sugar beet field.

The thickness of weeds in the plot was determined by laying the rectangular frame of 90×111 cm (the area of 1 m²) on two adjacent rows in five repeats along the diagonal of the plot. All the weeds were counted within the rectangular frame and separately in the 20-cm-wide stripe of the row zone.

Prior to the experimental research, according to the method of determining the quality of beet harvesters [5] the following physical and mechanic properties of crop roots were determined: maximal diameter of a crop root; root length; weight of one root; distance between roots in the row; width of interrow distances; height of crop roots relative to the soil surface; deviation of crop roots from the relative axis line of the row.

The results of experimental research were processed according to the known method of statistical processing of experimental data [12, 14, 16] with further pre-
Field testing of the beet harvester was conducted using the method of a multifactor experiment, which was described in [12–15].

The analysis of scientific literature, theoretical studies and previous testing of the machine were used to determine the rational ranges of factor changes, which have the most considerable impact on the quality of digging up crop roots from soil. Therefore, the velocity of the experimental device was set in the range of 1.3…2.55 m/s, the running depth of the digging up blades in soil fluctuated in the range of 0.06…0.12 m, the frequency of the working tool oscillations – 8.5…20.3 Hz. The listed factors are independent, thus it is possible to change their values regardless from one another.

A complete three-factor experiment on investigating the impact of the mentioned factors on the quality indices of work was conducted with the corresponding standard matrix.

The impact of three factors on the quality indices of work was described using the results of processing the data of experimental research in regression equations in the form of a polynomial of degree 2:

\[ Y = b_0 + b_1 X_1 + b_{11} X_1^2 + b_2 X_2 + b_{22} X_2^2 + b_3 X_3 + b_{33} X_3^2 + b_{12} X_1 X_2 + b_{13} X_1 X_3 + b_{23} X_2 X_3, \]  
(1)

where \( b_0, b_1, b_{11}, b_2, b_{22}, b_3, b_{33}, b_{12}, b_{23}, b_{13} \) – regression coefficients.

After the matrix of experiment planning was realized on the experimental device, the coefficients for variables were defined using Statistica 6 program.

RESULTS AND DISCUSSION

During the experiment the agricultural background of the experimental plot had the following parameters: soil type – heavy clay loam, soil solidity – 3.8…4.0 MPa; soil humidity – 6…8%; sugar beet density – 150 thousand of plants per hectare; average sizes of crop roots: diameter – 0.094 m, length – 0.24 m, weight – 0.9 kg.

The results of experimental research demonstrated that the increase in the frequency of working tool oscillations leads to the decrease in the loss of crop roots with a slight increase in the degree of crop roots in most cases.

The equation of the regression of the dependence between the losses of crop roots \( (Y_1) \) and the frequency of working tool oscillations \( (X_1) \), running depth of the working tools in soil \( (X_2) \) and the velocity of the translational movement of the vibrational digging-up machine \( (X_3) \) is as follows:

\[ Y_1 = 12.751–0.365 X_1 + 0.004 X_1^2 + 175.545 X_2 + 0.912 X_1 X_2 + 884.748 X_2^2–5.551 X_2 X_3 + 0.216 X_3^2, \]  
(2)

with the squared correlation coefficient (squared multiple correlation) \( R^2 = 0.789 \); multiple correlation coefficient \( R = 0.888 \); standard deviation \( S_r = 0.508 \). For this type of function, regression coefficients are insignificant for factors \( X_3 \) and \( X_1 X_3 \).

The obtained model was used in the Statistica 6 application to build the surface of the response of crop root loss due to the frequency of oscillations of the working tool and its running depth in the soil for the velocity values of the translational movement of the digging-up machine 1.3; 1.75; 2.1; 2.55 m/s and their two-dimensional cross-sections were obtained (Fig. 5).

It was also important to investigate the dependence of the crop root losses on the solidity and humidity of soil during the vibrational digging-up. The beet harvester was tested at the frequency of the working tool oscillations of 8.5 Hz. The study of the loss of sugar beet losses depending on the velocity of the translational movement \( (X_1) \) and the running depth of the working tools \( (X_2) \) was also studied under different working conditions.

The following regression equation was obtained for the soil solidity of 3.8 MPa and its humidity of 8.0%:

\[ Y_4 = 0.401 + 9.242 X_1 + 131.572 X_2–71.088 X_1 X_2 + 1015.235 X_2^2, \]  
(3)

at \( R^2 = 0.950; R = 0.975; S_r = 0.454 \).

The model obtained was used to build the response surface and its two-dimensional cross section (Fig. 5).

As seen from the obtained charts (Fig. 5), the losses increase with the increase in the velocity of the translational movement of the digging-up machine and decrease with the increase in the running depth in soil. This is explained by the fact that the higher velocity of the translational movement of the digging-up machine is, the fewer crop roots are taken by the working tool (the frequency of 8.5 Hz provides for this capture less with the increase in the translational velocity), the more crop roots remain either not captured or broken in the tail part. It is clear that the smaller the running depth of the digging-up machine in soil is, the higher is the level of breaking the tail part of crop roots or absence of their capture, which allows for higher losses. When
the digging-up machine moves at a higher depth, the impact of the translational velocity on the value of the crop root losses decreases due to breaking the tail part at a higher depth, thus this loss is smaller in percentage and less dependent on the velocity of the translational movement of the digging-up machine. The loss is minimal for the running depth of 0.11 m in soil.

The following regression equation was obtained for the soil solidity of 2.0 MPa and its humidity of 20.0%:

$$Y_5 = -7.75 + 231.582X_1 + 3.301X_1^2 - 94.891X_1X_2 - 682.32X_2^2,$$

at $R^2 = 0.869$; $R = 0.932$; $S_r = 0.674$.

The graphic presentation of the losses of crop roots depending on the velocity of the translational movement of the digging-up machine and its running depth in soil under these conditions is presented in Fig. 6.

As seen from the presented chart (Fig. 6), in case of the running depth of the working tool in soil of 0.06...0.09 m the losses increase with the rise in the velocity of the translational movement of the digging-up machine, and do not depend considerably on the velocity in case of the running depth of 0.10...0.12 m. The reasons are the same as for the previous case. The losses also decrease with the increase in the running depth of the working tool in soil and become minimal at the running depth of 0.12 m in soil.

The obtained experimental data and charts (Fig. 6) demonstrate that the losses for soil with the solidity of 3.8 MPa and the humidity of 8 % are 0.3...6.2 %; for soil with the solidity of 2.0 MPa and humidity of 20 % the losses of crop roots are in the range of 0.3...5.8 %. Therefore, the change in the status of soil in the range of 2.0...3.8 MPa and the humidity of 20...8 % does not have any considerable impact on the value of crop root losses.

The analysis of the data of the statistical processing of the experiment results demonstrated that there is a value of the vibration frequency and running depth in soil of the vibrational digging-up working tool for each value of the translational velocity of the vibrational working tool which corresponds to minimal losses and damage to crop roots. It was also established that the degree of crop root damage depends on the conditions of performing the technological process of vibrational digging-up (solidity and humidity of soil). The weight of damaged crop roots increases with the increase in solidity and decrease in the humidity of soil.
ADAMCHUK et al.

The experimental research demonstrated that the dirtiness of crop roots, extracted by the vibrational digging-up working tools of the new design is under 1%.

The performance of the beet harvester with the installed new vibrational digging-up working tools under reasonable rational modes of its work was determined by its functioning and duration of stops for repairs, and its efficiency was evaluated by the agrotechnical indices of harvesting sugar beet.

The results of experimental research demonstrated that it is reasonable to use the design of the vibrational digging-up working tool which would ensure the frequency of oscillations of 10...18 Hz and the running depth of 0.08...0.10 m in the range of velocities of the translational movement of the digging-up machine 1.3...2.1 m/s. The mentioned kinematic parameters of work ensure qualitative implementation of the technological process of the vibrational digging-up of sugar beet which corresponds to current agrotechnical requirements regarding losses and damage of crop roots.

The agrotechnical indices of the field plot, where experimental research was conducted to define energy parameters, are presented in the Table.

The investigation on the energy parameters of beet harvester in field conditions was conducted by reading the values of tensometric sensors under different working modes of the machine and different parameters and working modes of the vibrational digging-up tools. The graphic curves of energy-power characteristics of the vibrational digging-up working tool depending on the velocity of its movement are presented in Fig. 7.

**Fig. 6.** The square area of the response (a) and the two-dimensional cross-section of the square area of the response (b) of the loss of crop roots due to the velocity of the translational movement of the digging-up machine and its running depth in soil (at the frequency of the working tool oscillations of 8.5 Hz; soil solidity of 2.0 MPa; soil humidity of 20.0 %)

**Fig. 7.** The energy-power characteristics of the vibrational digging-up working tool (at the frequency of blade oscillations of 8.5 Hz and the running depth in soil of 0.09 m): 1 - towing force; 2 - moment on the power take-off shaft; 3 - thrust power; 4 - power on the drive mechanism of the vibrational digging-up working tool.
EXPERIMENTAL RESEARCH ON VIBRATIONAL DIGGING-UP OF SUGAR BEET

The analysis of the graphic curves demonstrated a shift in the towing force $P$ – curve 1 – from 6.6 to 7.8 kN in the investigated range of velocities (from 0.6 to 1.4 m/s). Therefore, one may consider that the change in the velocity of the experimental device conditions the increase in the towing force in a small range. The change of the rotational moment on the power take-off shaft $M$ – curve 2 occurs in the range from 50 to 70 newton-meter.

The agrotechnical indices of the experimental field plot

<table>
<thead>
<tr>
<th>Indices</th>
<th>According to the data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic of a crop: deviation of crop roots from the theoretical axis of the row, %:</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No data</td>
</tr>
<tr>
<td>$\pm$ 10</td>
<td>The same</td>
</tr>
<tr>
<td>$\pm$ 20</td>
<td>-- // --</td>
</tr>
<tr>
<td>$\pm$ 30</td>
<td>-- // --</td>
</tr>
<tr>
<td>$\pm$ 40</td>
<td>-- // --</td>
</tr>
<tr>
<td>mm and more location of crop root heads relative to the level of soil surface, %:</td>
<td></td>
</tr>
<tr>
<td>– over $-30$ mm</td>
<td>No data</td>
</tr>
<tr>
<td>– from $-20$ to $-30$ incl.</td>
<td>The same</td>
</tr>
<tr>
<td>– from 0 to $-20$ incl.</td>
<td>-- // --</td>
</tr>
<tr>
<td>– from 0 to $+20$ incl.</td>
<td>-- // --</td>
</tr>
<tr>
<td>– over $+20$ to $+40$ mm incl.</td>
<td>-- // --</td>
</tr>
<tr>
<td>– over $+40$ to $+60$ mm incl.</td>
<td>-- // --</td>
</tr>
<tr>
<td>– over $+60$ to $+80$ mm incl.</td>
<td>-- // --</td>
</tr>
<tr>
<td>– over $+80$ mm</td>
<td>-- // --</td>
</tr>
<tr>
<td>Density of plants, thousands of plants/ha</td>
<td>-- // --</td>
</tr>
<tr>
<td>Biological performance of crop roots, t/ha</td>
<td>70.0</td>
</tr>
<tr>
<td>Biological performance of tops, t/ha</td>
<td>20.00</td>
</tr>
<tr>
<td>The state of tops on crop roots by the form of leaf location, %</td>
<td></td>
</tr>
<tr>
<td>– rosette</td>
<td>No data</td>
</tr>
<tr>
<td>– semi-rosette</td>
<td>The same</td>
</tr>
<tr>
<td>– cone</td>
<td>-- // --</td>
</tr>
<tr>
<td>Soil type and name by the mechanic composition</td>
<td></td>
</tr>
<tr>
<td>Relief</td>
<td>-- // --</td>
</tr>
<tr>
<td>Microrelief</td>
<td>Deep low-humus chernozem</td>
</tr>
<tr>
<td>Soil humidity, %</td>
<td></td>
</tr>
<tr>
<td>0–10 cm</td>
<td>20.0…23.0</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>No data</td>
</tr>
<tr>
<td>20–30 cm</td>
<td>The same</td>
</tr>
<tr>
<td>Soil solidity, MPa</td>
<td></td>
</tr>
<tr>
<td>0–10 cm</td>
<td>-- // --</td>
</tr>
<tr>
<td>10–20 cm</td>
<td>-- // --</td>
</tr>
<tr>
<td>20–30 cm</td>
<td>-- // --</td>
</tr>
<tr>
<td>Field weedingness:</td>
<td></td>
</tr>
<tr>
<td>weeds, plants/m2 up to 100 cm high</td>
<td>Not exceeding 5.0</td>
</tr>
<tr>
<td>Predecessor and previous soil tillage</td>
<td>No data</td>
</tr>
</tbody>
</table>
As indicated in [4], when the vibrational digging-up tool is used, the tractive resistance decreases 2.5…3.5 times compared to the resistance of the passive disk digger. Here, the resistance of the vibrational digging-up machine increases less intensively with the increase in the velocity of the translational movement compared to that of the passive disk digger, and even more so — that of the passive blade digger, which is proven by the experiment results.

When the towing force $N$ – curve 3, is calculated along with the power on the drive mechanism of the vibrational digging-up working tool $N_{pto}$ – curve 4, the graphic curves are built to demonstrate that $N$ and $N_{pto}$ of the vibrational digging-up working tool change from 4.0 to 7.0 kW.

We built separate graphic curves for the dependence of the power, required for the drive mechanism of the oscillations of the vibrational digging-up working tool, on the velocity of the movement and the running depth of the digging-up blades in soil (Fig. 8) and on the velocity of movement and frequency of oscillations (Fig. 9).

As seen from the curves (Fig. 8), the lowest power, used for the drive of the vibrational digging-up working tools to the oscillatory movements, is present at the frequency of 8.5 Hz and the running depth of 0.06 m in soil.

However, considering that the minimal losses and damage of crop roots take place at the running depth of the digging-up machine of 0.09 m in soil, it is more rational to have the running depth of the digging-up machine of 0.08…0.10 m and the frequency of oscillations of the working tool of 10…18 Hz.

CONCLUSIONS

The experimental research established that the losses of crop roots decrease with the increase in the oscillation frequency of the working tool, and the increase in the velocity of the translational movement leads to their increase. Here, in the range of velocities of the translational movement of the digging-up machine of 1.3…2.55 m/s, minimal losses of crop roots are observed at the running depth of the digging-up machine of 0.09 m, and these losses increase for lower and higher running depth of the digging-up machine.

It was established that at soil solidity of 4.0 MPa and humidity of 8.0% under the running depth of 0.09 m in soil and the range of velocities of the translational movement of 1.3…2.55 m/s, the frequency of oscillations of the working tool of 8.5 Hz does not correspond to the agrotechnical requirements to the loss of crop roots (the losses are 2.7 %, 1.5 % is acceptable), and, vice versa, the frequencies of 15.7 and 20.3 Hz meet the requirements (the losses are 0.5 and 0.4 % respectively).

It was experimentally established that at the soil solidity of 4.0 MPa and humidity of 8.0 %, and the change in oscillations of the working tool in the range of 8.5…20.3 Hz, the damage of beet roots is of changeable nature, but the impact of the change in frequencies on the damage of crop roots is insignificant. There is minimal damage to the crop roots at the running depth of the working tool of 0.09…0.10 m. The damages to crop roots increase with the increase in the velocity.
of the translational movement of the digging-up machine. For instance, at the velocity of the translational movement of the digging-up machine of 1.3 m/s the mass of the damaged crop roots is 8.0…9.8%; at the velocity of 1.75 m/s – 8.1…9.8%; at the velocity of 2.1 m/s – 8.2…10.3%; at the velocity of 2.55 m/s – 10.5…12.8%.

The velocity of the translational movement of the digging-up machine in the range of 1.3…2.1 m/s meets the agrotechnical requirements in terms of damage to crop roots (not more than 10% are acceptable), but the velocity of 2.55 m/s does not meet these requirements.

It was found that the mass of the damaged crop roots depends on the solidity and humidity of soil considerably. For instance, at the solidity of 2 MPa and humidity of 18% it is in the range of 3.0…6.2%, and at the solidity of 4 MPa and humidity of 8% – in the range of 8.0…13.0%.

The application of the vibrational digging-up working tool allows achieving the 2.5…3.5-fold decrease in the relative energy consumption of harvesting compared to the application of the passive disk digger, and even more so – the passive blade digger. It was established that the change in the velocity of the translational movement of the digger conditions the increase in the towing effort in a small range, and the change in the rotational moment on the power take-off shaft at the change in the velocity of the translational movement in the range of 0.5…1.4 m/s is in the range from 50 to 70 newton-meter.

The smallest power, used for the drive of the vibrational digging-up working tools (providing oscillatory movements), corresponds to the frequency of oscillations of the working tool of 8.5 Hz and the running depth of 0.06 m in soil. Considering that the minimal losses and damage of crop roots take place at the running depth of the digging-up machine of 0.09 m in soil, it is more rational to have the running depth of the digging-up machine of 0.08…0.10 m and the frequency of oscillations of the working tool of 10…18 Hz.

It was found that the kinematic working parameters of the vibrational digging-up working tool, ensuring complete extraction of the crop root from soil on condition of avoiding the break of the top part of the root during the impact interaction, which were obtained theoretically, meet the agrotechnical requirements in terms of losses and damage to crop roots completely.

It was established that it is reasonable to use the design of the vibrational digging-up working tool which would ensure the frequency of oscillations of 10…18 Hz and the running depth of 0.08…0.10 m in the range of velocities of the translational movement of the digging-up machine 1.3…2.1 m/s at the length of the posterior part of the working plant passage of 0.15…0.20 m.
датчиков на разных режимах роботи установки та режимах роботи вибраційних викопуючих органів. Висновки. Встановлено, що для кожного значення постійної швидкості руху вібраційного копача існує відповідне значення частоти коливань та глибини ходу в ґрунті вібраційного викопуючого робочого органу, якому відповідають мінімальні втрати та пошкодження корнеплодів. Також встановлено, що ступінь пошкодження корнеплодів залежить від умов виконання технологічного процесу вібраційного викопування (твердості і вологості ґрунту), причому з ростом твердості та зменшенням вологості ґрунту маса пошкоджених корнеплодів збільшується.

Ключові слова: вібраційне викопування, польовий експеримент, цукровий буряк, коренеплід, пошкодження, втрати.

**Експериментальне ісследование вибрационного выкапывания корнеплодов сахарной свеклы**

В. В. Адамчук 1, В. М. Булгаков 2, И. В. Головач 3, Е. И. Игнатьев 3

e-mail: vbulgakov@meta.ua

1 Национальный научный центр “Институт механизации и электрификации сельского хозяйства” НААН Украины
Ул. Вокзальная, 11, Глеваха-1, Васильковский р-н, Киевская обл., Украина, 08631
2 Национальный университет биоресурсов и природопользования Украины
Ул. Героев Обороны, 15, Киев, Украина, 03041
3 Таврийский государственный агротехнологический университет
Пр. Б. Хмельницкого, 18, Мелитополь, Запорожская обл., Украина, 72310

Технологический процесс вибрационного выкапывания корнеплодов свеклы сахарной получил распространение во многих сельскохозяйственных странах мира. Опыт использования этого процесса показал, что он имеет ряд преимуществ по сравнению с другими способами выкапывания, но экспериментальных исследований вибрационного выкапывания корнеплодов сахарной свеклы из почвы очень мало, поэтому данная тематика актуальна для отрасли свеклосовства. Цель. Экспериментальное определение рациональных параметров и режимов вибрационного выкапывающего рабочего органа для обеспечения требуемого качества выполнения технологического процесса выкапывания корнеплодов сахарной свеклы из почвы. Методы. Применены методы планирования многофакторного эксперимента, польовых исследований, статистической обработки и регрессионного анализа исследовательских данных с построением графических зависимостей. Результаты. Разработана новая конструкция вибрационного выкапывающего органа для вибрационного выкапывания корнеплодов сахарной свеклы из почвы. На основе принятой программы и методики были проведены экспериментальные исследования влияния основных конструкционных и технологических параметров вибрационного выкапывающего рабочего органа корнеуборочной машины на показатели качества выполнения технологического процесса уборки корнеплодов сахарной свеклы. В польевых условиях исследованы энергетические параметры новых вибрационных выкапывающих рабочих органов путем регистрации показателей температурных датчиков на разных режимах работы установки и различных параметрах и режимах работы вибрационных выкапывающих органов. Выводы. Установлено, что для каждого значения поступательной скорости движения вибрационного выкапывающего рабочего органа существует соответствующее значение частоты колебаний и глубины хода в почве вибрационного выкапывающего рабочего органа, которому соответствуют минимальные потери и повреждения корнеплодов. Также установлено, что степень повреждения корнеплодов зависит от условий выполнения технологического процесса вибрационного выкапывания (твердости и влажности почвы), причем с ростом твердости и уменьшением влажности почвы масса поврежденных корнеплодов увеличивается.

Ключевые слова: вибрационное выкапывание, полевой эксперимент, сахарная свекла, корнеплод, повреждения, потери.

REFERENCES
7. Kenter C., Hoffmann C. Changes in the processing quality of sugar beet (Beta vulgaris L.) during long-term stor-
EXPERIMENTAL RESEARCH ON VIBRATIONAL DIGGING-UP OF SUGAR BEET

INTRODUCTION

Podzolic soils are rather specific natural formations with a genetically inherent multivector character of soil-forming processes. In case of combining multidirectional podzolic-forming and accumulative processes, specific soil genesis of podzolic soils is the reason, conditioning their “problematic nature” in the aspect of resistance to degradation under the impact of increasing natural and anthropogenic burdens. First of all, climatic changes, occurring with impressive rates recently, should be referred to natural burdens, negatively impacting the soil-forming processes in podzolic soils. These changes are accompanied by contrast weather...
cataclysms – rapid change of temperature, continuous periods without any rains, and, vice versa, intense precipitations. The latter conditions the increase in transpirational ability of soils, promotes washing-out colloids, saturated alkali and nutrients for plants, destabilization of acid-alkaline state, deterioration of buffer properties, development of erosion, etc. [1–3]. A considerable impact on the development of degradation processes is also made by anthropogenic actions, which, first and foremost, include soil tillage with heavy machinery, decrease in the volumes of introducing organic fertilizers, insufficient introduction of mineral fertilizers and meliorants, especially calcium-containing ones. Starting with 1990, the average annual introduction of organic fertilizers in Ukraine decreased catastrophically from 12 t/ha to current 0.4–0.1 t/ha [4, 5]. Along with the prevailing podzolic component, the abovementioned negative factors promote the development of a number of degradation processes and phenomena in the old-arable soils, such as overconsolidation, loss of agronomically valuable structural aggregates, destabilization of acid-alkaline state, deterioration of buffer properties, development of erosion, etc. For instance, the rates of expanding the areas of podzolic soils with increased acidity are 0.4–0.5 % a year.

Due to the negative effect of the mentioned factors, the podzolic soils with high fertility transformed into soils with medium level of fertility, and their properties keep deteriorating. As a result, the Forest-Steppe of Ukraine, a place with common podzolic soils, has about 1.8 million ha soils with increased acidity [5]. It is the podzolic soils of the Forest-Steppe zone, where the deterioration of physical, chemical and aqueous-physical properties of soils is felt especially bitterly. The result of degradation of these soils is the decline in the yield of crops due to the acidity of soil, deterioration of physical-chemical and physical properties, and water-air regime, etc. The abovementioned degradation phenomena cause huge losses for farmers and the state in general. The actual losses of agricultural products only due to overconsolidation of soils amount to hundreds of thousands of dollars annually. The annual shortfall in the production of the main crops due to negative impact of soil acidity is about 1 million 350 thousand tons of grain units [5].

It is dangerous to leave degradation processes in podzolic soils often undetected, thus, their results are evident only in the state of decay, when common preventive measures are not capable of counteracting the degradation. Therefore, it is easier to prevent these processes than to eliminate their consequences.

The decisions of the UN Conference on Environment and Development (1992, Rio de Janeiro) stated that protection and rational use of soils should be the central component of the state policy.

At present, special relevance is attributed to the measures of increasing the fertility of soils and maintaining ecological equilibrium in agroecosystems via the application of biological factors [6]. In this aspect a hopeful direction of solving the problem of keeping the podzolic soils from degradation is phytomelioration as a “soft” ecology-safe measure of restoring the fertility of soils via natural phytopotential of crops. The main value of phytomelioration is ensuring the change in the direction of the soil-forming process from the eluvial trend to the cumulative one. In addition, due to the absence of finances for fertilizers and meliorants, this is an additional reserve of economy of energetic and material resources with the simultaneous orientation to protecting the ecological safety of the environment. Phytomelioration allows stepping aside from template technologies, commonly used to increase the fertility of podzolic soils, which are a relevant component of the soil cover of Ukraine (about 7.8 million ha of agricultural land) and a valuable component of biosphere in general, and coming to the new stage of developing balanced use of soil.

It should be noted that scientific literature highlights the issue of phytomelioration impact on the change in the properties of alkali soils rather well [7–10]. Phytophobiological melioration presupposes correct selection of crops, capable of both growing and developing on halogenous soils, and promoting the formation of the negative salt balance in them. They are also one of the ways of fighting toxic burden on soils.

Regardless of rather multidirectional subject matter of studies on the application of phytomeliorative measures to improve the properties of soil both in Ukraine and abroad [11–14], this problem has not been studied sufficiently in the aspect of complex approach and introduction into practice with the purpose of preserving and restoring fertility of podzolic soils proper.

MATERIALS AND METHODS

The studies were conducted at the National Scientific Center “Institute for Soil Science and Agrochemistry Research named after O. N. Sokolovsky” for 2013–2017. The object of studies was podzolic heavy loamy chernozem on forest-like clay loam (Kharkiv district, State enterprise “Experimental Farm Hrakivske”).
Podzolic chernozem is heavy loamy by its granulometric composition, it contains 42.2 % physical clay. It has the following physical-chemical indices in the arable layer:

- $\text{pH}_{\text{salt}}$: 4.7, hydrolytic acidity – 4.3 mmol/100 g of soil, the sum of absorbed alkali – 21.9 mg-eq/100 g of soil. Calcium prevails in the composition of exchange cations (18.3 mg-eq/100 g of soil) and magnesium (3.1 mg-eq/100 g of soil).

Prior to starting the field experiment on small plots, podzolic chernozem had the following characteristics by agrochemical indices: the content of mineral nitrogen was 12.4 mg/kg, mobile phosphorus – 35.8 mg/kg of soil, movable potassium – 78.3 mg/kg of soil. The content of total humus was 2.5 % which characterizes the studied soil as low humus soil.

Soil section was laid in the variant with alfalfa (Fig. 1): He (0–30 cm) – humic weakly eluviated (arable), dark gray with silica powdering $\text{SiO}_2$, dusty-loamy, dry, low density, with a plow sole at the depth of 18 cm. Nut-like structure is observed starting from the depth of 20 cm. The whole horizon is densely packed with the roots of alfalfa, the transition is gradual by its structure and color;

Hpi (30–59 cm) – upper transitional, weakly eluviated, gray with brown shade, weak glossiness of aggregates is observed, nutty-loamy, dry, dense, with worm holes, less packed with roots, the transition is gradual by the color and structure;

Phi (59–75 cm) – low transitional, weakly eluviated, dark brown, with colloid glossiness, nut-prismatic, cold, heavily dense, weakly packed with roots, the transition is gradual;

P(h)i (75–105 cm) – transitional to the source rock, eluviated, brown, heavily dense, transition to the next horizon along the line of carbonate deposits;

P$k$ (105 cm and deeper) – the formation is forest-like clay, dark tan color, fine grain structure, carbonates in the form of mycelium, frequent mole runs, filled with humus material, some roots are observed.

The field experiment was started by the scheme, including the following variants: control, alfalfa, sainfoin, soy, lupine, mustard, Sudan grass. Annual phytomeliorants were grown in the following rotation: soy → Sudan grass → lupine → mustard; control: barley → corn → millet. The crops of varieties, introduced to the State register of varieties of plants, suitable for cultivation in Ukraine, were grown in the experiment.

The technology of cultivating crops is common for the Left-Bank Forest-Steppe of Ukraine. Two years prior to starting the field experiment and during the latter, fertilizers and meliorants were not introduced, which allowed singling out phytomeliorant properties of each selected crop proper. In addition, the studies investigated direct phytomeliorant action of crops (without covering with plow) which is absolutely different from sideration.

Field studies were performed by the method of B. O. Dospekhov, the selection of soil samples and their preliminary processing were done in accordance to DSTU 4287:2004 and DSTU ISO 11464:2007. The following indices were determined in the selected samples: acid-alkali buffer of soil (DSTU 4456:2005); density of soil composition per dry mass (DSTU ISO 11272:2001); structural and aggregate composition of soil using sieve method in the modification of M. I. Savinov (DSTU 4744:2007); the content of organic matter (DSTU 4289:2004); the content of mineral forms of nitrogen (N-nitrate and N-ammonium) by the modified method of NSC “ISSAR named after O.N. Sokolovsky” (DSTU 4729:2007); determination of calcium ions activity by the potentiometric method (DSTU 4725:2008); the content of exchange calcium and magnesium by the method of Schollenberg in the modification of NSC “ISSAR named after O.N. So-
In our opinion, this fact is conditioned by the capability of the root system of perennial grasses to have biological translocation of calcium compounds from the lower layers of soil and even from the calcareous rock of forest-like clay into the upper layers. It was established that with the mass of alfalfa roots of 9.7 t/ha and sainfoin of 9.9 t/ha, the 0–100 cm layer of podzolic chernozem contains 148.6 and 109.2 kg/ha CaO respectively. Further on, after partial dying-off of the root system, calcium compounds, accumulated in the roots of alfalfa, along with the plant remains enrich the root-containing layer of soil with calcium. This meliorative specificity of perennial grasses promotes replenishing the soil with active calcium, thus ensuring the optimization of lime potential (pH-0.5pCa), the optimal values of which for podzolic chernozem are in the range of 4.8–5.2 in accordance to the current gradation [17]. The latter affects both the acid-alkaline balance and the regulation of physical and chemical processes in soil.

It was established that after three years of phytomelioration the soil in variants with perennial grasses is characterized with higher buffer against acid-alkaline load compared to the control variant. The latter is confirmed with considerable area of buffer volumes both in alkali (BVal) and acid (BVac) intervals of loads. It should be noted that the mentioned indices of buffer capacity, along with the coefficient of buffer asymmetry and total estimated index of buffer capacity, are quantitative indices of buffer capacity of soils [17]. Positive phytomeliorative action of alfalfa on acid-alkaline buffer capability of podzolic chernozem after three years of cultivation is reflected on the chart of pH-buffer capacity (Fig. 2), where considerable areas of buffer volumes are observed both in alkali (positive) and acid (negative) intervals of loads.

After three years of cultivating alfalfa and sainfoin, BVal in the 0–20 cm layer was 38.74 and 33.83 points respectively, with the control of 36.12 points, BVac increased to 22.42 and 18.96 points, with the control of 17.78 points.

The total estimated index of buffer capacity and the buffer volume of the acid part decreased along the profile in the 20–40 and 40–60 cm soil layers, whereas the buffer volume of the alkali part increased along the profile.

### Table 1. The change in pH of the activity of calcium ions and lime potential of podzolic chernozem (0–20 cm layer) after three years of phyto-improvement

<table>
<thead>
<tr>
<th>Variant</th>
<th>pH</th>
<th>Lime potential, pH-0.5pCa</th>
<th>Calcium activity, aCa, mmol/dm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.8</td>
<td>4.2</td>
<td>1.3</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>6.5</td>
<td>5.5</td>
<td>21.9</td>
</tr>
<tr>
<td>Sainfoin</td>
<td>6.2</td>
<td>5.1</td>
<td>16.6</td>
</tr>
</tbody>
</table>
the buffer asymmetry in the 20–40 cm layer are better compared to the depth of 40–60 cm. In our opinion, this is due to the fact that the 20–40 cm layer is characterized with higher acidity and lower activity of calcium, compared to the lower layer. A similar tendency was established in the variant with cultivating sainfoin.

The statistical analysis of the results obtained allows stating that the very increased activity of calcium is one of buffer mechanisms of soil against acidification. The coefficient of correlation between these indices was 0.87, which testifies to a high level of interrelation between them.

The main indices, testifying to the aggravation of the qualitative state of soils and the development of degradation processes, include the decrease in the amount of organic matter, which is conditioned by extremely low volumes of introducing organic fertilizers due to the decay of animal breeding in Ukraine [5]. The studies established the tendency to the increase in the content of organic matter in the 0–20 cm layer due to phytocultivation, which is especially relevant in the context of catastrophic decrease in the introduction, and sometimes even complete absence of organic fertilizers. It was determined that the highest content of organic matter was observed after cultivation of perennial grasses for three years (from 1.83–1.90 % of organic matter under perennial grasses, the control being 1.51 %), which is reasonable considering the accumulation of the root mass of grasses (9.7–9.9 t/ha in the 0–100 cm layer) in soil. It proves the relevance of phytomelioration to ensure extended restoration of organic matter even in conditions of the absence of organic fertilizers.

The efficiency of phytomelioration in improving the physical properties of soils, including their structure, was proven. The highest number (89.3 %) of valuable structural components (10–0.25 mm) in the 0–20 cm soil layer under alfalfa was accompanied with the decrease in the dusty fraction down to 0.8 % at 4.4 % for the control. On the contrary, the smallest number of loamy aggregates was noted in the variant with sainfoin in the 0–20 cm layer (2.8 %). In the variant of cultivating Sudan grass for one year, the percentage of loamy aggregates was notably decreased down to 5.0–6.6 % at 11.2–16.4 % for the control. The differentiated impact of phytomeliorants of the soil structure, determined by us, allows correcting the direction of structure formation via the selection of crops, depending on the fraction, prevalent in the structure composition of soil – loamy or dusty. For instance, the decrease in the number of loamy aggregates is achieved due to the mechanic action of root systems of Sudan grass and perennial grasses. The decrease in the dusty fraction of soils takes place while cultivating alfalfa and sainfoin – via biotranslocation of calcium compounds as a relevant factor of structure formation. A similar impact is manifested by mustard – due to the structure-forming action of the root exudate.
OPTIMIZATION OF FERTILITY INDICES OF PODZOLIC SOILS VIA CULTIVATION OF PHYTOMELIORANTS

The sum of valuable structural components in the arable layer was increased by 11–18% depending on the year of studies and the predecessor. In addition, the selection of phytomeliorants with well-developed root systems has a positive effect on the processes of structure formation in lower horizons of soils. It should be noted that downwards along the soil profile the highest number of valuable structural aggregates was also observed in the variants with perennial grasses—after three years of phyto-improvement it was about 87–88% in the 0–20 cm layer and 84–85% in the 40–60 cm layer with the control being 73–75%.

A relevant index of structural-aggregate composition of soil is the sum of aggregates of 1–3 mm in diameter, which are characterized as “agronomically most valuable”, as this is the fraction with the highest resistance to the ruining action of water and wind erosion (Fig. 3).

As seen from the diagram (Fig. 3) the percentage share of this index compared to the control variants is rather high for all the investigated phytomeliorants (after three years of phyto-improvement). The highest content of 1–3 mm fraction is noted under alfalfa and sainfoin, which is practically twice higher compared to the same index for the control.

In our opinion, a relevant reason of the formation of solid structure of podzolic chernozem may lie in the fact that the soil under perennial grasses has a plant cover for a longer period of time, which protects it from being ruined with precipitation, as the plant cover of the fields with crops receives the impact of raindrops.

In addition, our studies prove that during the cultivation of perennial grasses, the process of structure-formation of soils also depends on the content of calcium compounds in their root mass. Thus, during the seasonal dying-off of roots, the soil solution receives a considerable amount of active calcium, which takes a direct part in the formation of an agronomically valuable structure.

It was established that due to phytomeliorant measures the saturation of soil-colloid absorbing complex with calcium ensures intense improvement of water resistance of aggregates for two years. The coefficient of water resistance increases from 0.53 in the control to 0.57–0.95 under phytomeliorant crops.

It was proven that the decrease in the density of soil composition may also be achieved via the selection of crops with well-developed root system. In our studies this crop was Sudan grass which, within one year of cultivation, decreased the density of composition of podzolic chernozem in the 0–60 cm layer (Table 2) to the optimal values of density of the composition of soil for most crops of the Forest-Steppe zone [3].

Based on the results obtained, one may assume that in case of accurate selection of phytomeliorants with the consideration of their physiological specificities

**Table 2. The impact of the cultivation of Sudan grass on the density of soil composition**

<table>
<thead>
<tr>
<th>Depth, cm</th>
<th>Density of composition, g/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial value</td>
</tr>
<tr>
<td>0–20</td>
<td>1.31</td>
</tr>
<tr>
<td>20–40</td>
<td>1.34</td>
</tr>
<tr>
<td>40–60</td>
<td>1.39</td>
</tr>
</tbody>
</table>
and meliorative possibilities, the danger of excessive density, dusting and formation of loams decreases. In addition, the studies demonstrate a close correlation (a reverse linear dependence) between the density of composition and content of organic matter in podzolic chernozem while cultivating Sudan grass \( r = 0.64 \).

At the same time, phyto-improvement also has a positive impact on the accumulation of macroelements in soil, required for balanced growth and development of crops, for instance, nitrogen, the deficiency of which inhibits the growth of crops, especially during the initial period of their ontogenesis. It was determined that the cultivation of alfalfa, sainfoin, lupine and soy promotes the increase in the level of supplying the soil with nitrogen from the low level to the medium one. The latter testifies to the ability of these crops to provide for both its own need of nitrogen, and that of the crops of the following years, which is a relevant economic factor.

The improvement of the main indices of soil fertility using phytomeliorative measures led to the increase in the yield: corn – in the first year of aftereffect up to 4.4–5.8 t/ha with the control being 3.3 t/ha; barley – the second year of aftereffect up to 1.4–2.2 t/ha with the control being 1.3 t/ha. Here the qualitative properties of grain are improved as well, in particular, there is an increase in the nitrogen content in corn grain up to 0.46–0.84 % per dry mass with 0.45 % in the control; in barley grain – up to 0.55–1.52 % with 0.44 % in the control. There is also an increase in the calcium content in corn grain – up to 0.73–1.14 % per dry mass with 0.72 % in the control; in barley grain – up to 0.57–1.31 % with 0.43 % in the control.

Therefore, the application of phytomeliorant measures allows ensuring high functional resistance of podzolic soils which gives a possibility to increase their performance, to improve the agroecological state of environment in the territory of their spreading and to save 30–60 % of finances depending on the cultivation of specific crops-phytomeliorants. The estimation of the economic effect of the application of phytomeliorative measures with the consideration of the pricing policy during the years of studies (including two years of aftereffect) proves their high economical character: a conventional profit from the cultivation of crops-phytomeliorants is 1590–3630 UAH/ha.

CONCLUSIONS.

Phytomeliorative technology of cultivating podzolic soils via the use of natural potential of crops ensures the balanced use of soils with simultaneous orientation towards preservation of resources, ecological safety and supplying the population with stable yield of crops with high quality of products.

It was determined that the cultivation of perennial grasses for three years is reasonable to ensure optimal indices of fertility of podzolic chernozem with the purpose of optimizing the acid-alkali state and pH-buffer capacity. The optimization of agrophysical parameters of soil is achieved while cultivating perennial grasses, mustard, and Sudan grass. It was proven that the cultivation of Sudan grass for one year allows achieving optimal indices of the density of podzolic chernozem composition for most crops. The prolonged action of phytomeliorative measures was established. High efficiency of this agromeasure and adaptive properties of phytomeliorants to soil and climatic specificities allow stating the possibility of using the phytomelioration not only in the Forest-Steppe of Ukraine, but also in other zones of podzolic soils.

**Optimіzаtiоn показників родючості опідзолених грунтів шляхом вирощування фітомеліорантів**

Ю. Л. Чапко, А. І. Огородня

e-mail: yaroshevich26@i.ua

Національний науковий центр “Інститут грунтознавства та агрохімії імені О. Н. Соколовського”

Вул. Чайковська, 4, Харків, Україна, 61024

**Мета.** Виявлення та оцінювання впливу фітопотенціалу культурних рослин на показники родючості опідзолених грунтів, головні лімітуючі урожайність сільськогосподарських культур, а також встановлення економічної ефективності вирощування різних за фізіологічними особливостями фітомеліорантів. **Методи.** Польовий, лабораторний, математико-статистичний. **Результати.** На основі комплексних чотирьох років досліджень встановлено, що оптимізація фізико-хімічних властивостей опідзолених грунтів досягається шляхом застосування фітомеліоративних заходів. Ці заходи, в першу чергу, спрямовані на запобігання їх декальцінації, тобто обумовлюють акумуляцію активного іона Са\(^2\), який сприяє покращенню характеристик грунту внаслідок прогресуючого розвитку акумулятивного грунтоґенезу. Встановлено, що при масі коріння люцерни 9,7 т/га та еспарцету 9,9 т/га, у шарі 0–100 см чорнозему опідзоленого зосереджується, відповідно 148,6 та 109,2 кг/га СаO. Збагачення кореневмісного шару грунту кальцій-сполуками сприяє поповненню грунту активним кальцієм, а отже забезпечує оптимізацію вапняного потенціалу (рН-0,5pСa). Останнє впливає як на кислотно-основну рівновагу, так на регуляцію фізико-хімічних процесів у грунті. Встановлено, що після трьох років проведення
фитомелиорацию грунтов на вариантах с багаторічними травами характеризуется вищою буферністю проти кислотно-луужних навантажень, порівняно з варіантом контролю, що підтверджується показниками кислотно-основної буферності. Відповідно до вищезазначеного, можна стверджувати, що саме підвищення активності кальцію є одним з буферних механізмів грунту проти підкислення. Доведено ефективність фітомеліорациї щодо покращення фізичних властивостей грунтів і, зокрема, грунтової структури за рахунок зменшення кількості пилуватої і брилуватої фракції. Встановлено, що насичення грунтово-колоїдного поглинального комплексу кальцієм, завдяки фітомеліоративним заходам, забезпечує інтенсивне покращення водостійкості агрегатів протягом двох років. Доведено, що оптимальних значень щільності складення грунту для більшості сільськогосподарських культур зон Лісостепу можна досягти при вирощуванні садінської трави впродовж одного року. Покращення основних показників родючості грунту завдяки фітомеліоративним заходам призвело до підвищення урожайності кукурудзи та ячменю та покращення якісних характеристик зерна.

Висновки. Фітомеліоративний спосіб окультування опідзолених грунтів забезпечує виход на збалансоване використання грунтів з одночасною орієнтацією на ресурсозбереження, екологічну безпеку та забезпечення населення сталими врожаями сільськогосподарських культур з високою якістю продукції.

Ключові слова: окультування, фітомеліоративні заходи, опідзолені грунти, кислотно-основний стан, структурно-агрегатний склад, щільність складення.

Оптимизация показателей плодородия оподзоленных почв путем выращивания фитомелиорантов
Ю. Л. Цапко, А. И. Огородняя

e-mail: yaroshevich26@gmail.com

Национальный научный центр «Институт почвоведения и агрохимии имени А. Н. Соколовского»,
Ул. Чайковская, 4, г. Харьков, Украина, 61024

Цель. Выявление и оценка влияния фитопотенциала культурных растений на главное, лимитирующее урожайность сельскохозяйственных культур, показатели плодородия оподзоленных почв, а также установление экономической эффективности выращивания различных по физиологическим особенностям фитомелиорантов.

Методы. Полевой, лабораторный, математический, статистический. Результаты. На основе комплексных четырехлетних (2013–2017 гг.) исследований установлено, что оптимизация физико-химических свойств оподзоленных почв достигается путем применения фитомелиоративных мероприятий, которые, в первую очередь, направлены на предотвращение их декальцинации, то есть обусловливают аккумуляцию активного иона Ca²⁺, который способствует улучшению характеристики почвы, вследствие прогрессирующего развития аккумулятивного генезиса почвы. Установлено, что при массе корней люцерны 9,7 г/га и эспарцета 9,9 г/га в слое 0–100 см чернозема оподзоленного сосредоточивается, соответственно, 148,6 и 109,2 кг/га CaO. Обогащение кореобитаемого слоя почвы соединениями кальция способствует пополнению почвы активным кальцием, а, следовательно, обеспечивает оптимизацию известкового потенциала (pH-0,5Ca). Последнее влияет как на кислотно-основное равновесие, так и в целом на регуляцию физико-химических процессов в почве. Установлено, что после трех лет проведения фитомелиорации почва на вариантах с многолетними травами характеризуется высокой стойкостью к кислотно-щелочным нагрузкам, по сравнению с контрольным вариантом, что подтверждается показателями кислотно-щелочной буферности. Согласно вышеприведенному, можно утверждать, что именно повышение активности кальция является одним из буферных механизмов почвы против подкисления. Доказана эффективность фитомелиорации в улучшении физических свойств почв и, в частности почвенной структуры, за счет уменьшения количества пылевидной и глибистой фракций. Определено, что насыщение почвенно-коллоидного поглотительного комплекса кальцием, в результате фитомелиоративных мероприятий, обеспечивает интенсивное улучшение водостойкости агрегатов в течение двух лет. Доказано, что оптимальных значений плотности сложения почвы, для большинства сельскохозяйственных культур зоны Лесостепи, можно достичь при выращивании садинской травы течение одного года. Улучшение основных показателей плодородия почвы фитомелиоративными мероприятиями привело к повышению урожайности кукурузы и ячменя, улучшению качественных характеристик зерна.

Выводы. Фитомелиоративное направление окультурирования оподзоленных почв обеспечивает выход на сбалансированное использование почв, с одновременной ориентацией на ресурсосбережение, экологическую безопасность и обеспечение населения по- стоянными урожаями сельскохозяйственных культур с высоким качеством продукции.

Ключевые слова: окультурирование, фитомелиоративные мероприятия, оподзоленные почвы, кислотно-основное состояние, структурно-агрегатный состав, плотность сложения.

REFERENCES
2. Nael M, Khademi H, Mohammadi J. Evaluation of Soil Degradation in Different Rangeland Management Sys-
INTRODUCTION

The estimation of interbreed differentiation of the most numerous cattle breeds in Ukraine is one of the most relevant elements of breeding in domestic animal farming and the preservation of local breeds. One of the most conclusive directions of estimating this characteristic is the analysis of genetic diversity.

DNA microsatellites – highly polymorphic multilocus genetic systems – are highly informative markers of the degree of genetic differentiation for the populations of domestic animals [1, 2, 4]. Almost all the microsatellites are located in qualitative trait loci (QT) or are related to genes, connected to reproduction processes [3].

Microsatellites of DNA have been used for the analysis of genetic diversity of many cattle breeds including Northern breeds [5], breeds of Central [6] and Eastern Europe [7], breeds of India [8], Africa [9], Korea [10] and Southern-Eastern Asia [4], including those of Indonesia [11].

The most common cattle breeds in Ukraine are Ukrainian Red-and-Motley (URM) and Ukrainian Black-and-White (UBW) breeds. The main aim of this study was to analyze the genetic structure of the mentioned breeds and to determine the degree of their affinity and differentiation.

To this end, an analysis was made of allelic and genotypic polymorphism of microsatellites.

MATERIALS AND METHODS

The study was conducted using a population (n = 88 heads) of Ukrainian Red-and-Motley dairy cattle breed (URM; n = 45 heads) and Ukrainian Black-and-White dairy cattle breed (UBW; n = 43 heads), kept at the Voronkiv farm in Boryspil district, Kyiv region.

Blood was sampled under sterile conditions from the jugular vein using double-ended needles Venoject
and vacuum tubes and holders Venosafe (Terumo, Belgium) following the standard method in accordance to the manufacturer’s recommendations.

DNA isolation from blood samples was conducted using the DNA-sorb-B kit (Amplisense, Russia) according to the manufacturer’s recommendations. The microsatellite analysis was performed using 10 loci (Table 1), recommended by the International Society for Animal Genetics (ISAG).

The polymerase chain reaction (PCR) was conducted using an ABI 2720 Thermal Cycler (Applied Biosystems, CA, USA). The reaction mixture for PCR was prepared according to the protocol, recommended by the manufacturer of the test-system (StockMarc, Cattle Bovine Genotyping Kit (Applied Biosystems, CA, USA). The amplified DNA was separated by the method of capillary gel electrophoresis on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, CA, USA). Data registration and mapping (genotyping) was performed using programs Run 3130 Data Collection v.3.0 and GeneMapper 3.7 of Applied Biosystems, CA, USA.

The frequencies of alleles and the genotypes were estimated, including one-time (N1), two-times (N2) observed and unique ones (Nunik). The comparison of breeds in terms of frequencies of alleles and genotypes was performed using the criterion \(\chi^2\) of K. Pearson (by Monte Carlo method for low frequencies) [12] using special software for population-genetic analysis – GenAIEx [13], BOTTLENECK [14], PopGene [15] and NetEstimator [16].

**RESULTS AND DISCUSSION**

The analysis of the allelic diversity of 10 microsatellite loci demonstrated that the highest average number of the allelic variants was found in the population of URM breed – 9.5 alleles/locus, whereas the same index for UBW breed was 9.2 alleles/locus. The values of the effective number of alleles were 6.757 and 6.023 alleles/locus, respectively. In our study each breed was characterized by the highest number of alleles in three microsatellite loci out of the 10 investigated ones, namely, URM – in loci TGLA126, BM2113 and SPS115, and URM – in loci INRA23, BM1824 and TGLA227. The differences between breeds did not exceed two alleles. In the remaining loci, the number of allelic variants was the same for the two breeds.

The broadest allelic spectrum was observed for the breed with the highest number of alleles. Loci with a similar number of allelic variants had the same allelic spectrum, except for locus ETH3 that had a wider one. The obtained estimates of the criterion \(\chi^2\) of K. Pearson allow stating highly reliable (P < 0.001) differences in the distribution of the investigated population by the allelic frequencies in all the investigated loci.

Unique alleles were registered in seven out of ten investigated microsatellite loci, seven (30 %) for URM breed and five (22 %) – for UBW. A quite similar distribution was found for the number of loci with

<table>
<thead>
<tr>
<th>Locus</th>
<th>URM</th>
<th>UBW</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>number of alleles</td>
<td>inc. rare ones</td>
</tr>
<tr>
<td>TGLA126</td>
<td>7.45</td>
<td>0.14</td>
</tr>
<tr>
<td>TGLA122</td>
<td>10.39</td>
<td>1.43</td>
</tr>
<tr>
<td>INRA23</td>
<td>10.50</td>
<td>0.20</td>
</tr>
<tr>
<td>ETH3</td>
<td>6.80</td>
<td>1.00</td>
</tr>
<tr>
<td>ETH225</td>
<td>7.96</td>
<td>0.002</td>
</tr>
<tr>
<td>BM1824</td>
<td>9.78</td>
<td>0.05</td>
</tr>
<tr>
<td>TGLA227</td>
<td>11.67</td>
<td>1.85</td>
</tr>
<tr>
<td>BM2113</td>
<td>10.82</td>
<td>1.45</td>
</tr>
<tr>
<td>ETH10</td>
<td>6.98</td>
<td>0</td>
</tr>
<tr>
<td>SPS115</td>
<td>8.55</td>
<td>1.84</td>
</tr>
<tr>
<td>Mean</td>
<td>9.09 ± 0.555</td>
<td>0.80 ± 0.251</td>
</tr>
</tbody>
</table>

**Table 1.** The number of alleles, including rare ones, calculated using the rarefaction-method (n = 25) for 10 microsatellite loci of two cattle breeds, Ukrainian Red-and-Motley (URM) and Ukrainian Black-and-White (UBW)
unique alleles: the highest number (five, or 33%) was found for URM breed, and the lowest number (four, or 27%) – for UBW. In the populations of URM and UBW, two unique alleles were found in two and one loci (TGLA227, SPS115 and TGLA227), respectively. It should be noted that only in loci TGLA122 and TGLA227 unique alleles were found in both investigated breeds. Unique alleles in loci ETH3, BM2113 and SPS115 were recorded for URM breed, and loci INRA23 and BM1824 – for UBW. In general, the frequency of unique alleles in URM breed (0.049) was almost twice higher than for UBW breed (0.026).

Two main models were used to analyze the character of the distribution of alleles of microsatellite DNA by specific loci – the infinite alleles model (IAM) [17, 18, 19] and the stepwise mutation model (SMM) [20, 21].

SMM was more adequate for both investigated breeds with the approximation of the level of allelic diversity in all the investigated microsatellite loci without exceptions, compared to IAM model (P < 0.0001). The latter exaggerates the actual values greatly both in general and for each breed, in particular (Fig. 1).

Taking into account our limited number of samples and limited number of loci studies where direct comparison of the obtained results is impossible, we used the rarefaction procedure. The number of alleles (calculated per 25 randomly selected diploid animals) varied greatly both for animals of different breeds and for different loci (Table 1). In general, URM breed had a higher total of alleles and more unique alleles were found than in UBW breed. The highest level of allelic diversity, however, was observed for UBW in locus TGLA227 (13.40 alleles), and the lowest – for the same breed in locus TGLA126 (5.83 alleles). The highest number of unique alleles (2.45) was also found for UBW in locus TGLA227, and the lowest – for the same breed in the locus SPS115 (0.01). The estimates, made using the rarefaction-procedure, prove the absence of unique alleles in both populations for locus ETH10, and as for UBW – for locus ETH225 as well.

The highest number of genotype variants was found in URM, viz. four microsatellite loci out of ten investigated ones (INRA23, ETH225, TGLA227, BM2113), while for UBW this was found only for three loci – TGLA122, ETH3 and BM1824. The number of genotype variants in loci TGLA126, ETH10 and SPS115 was the same for representatives of both breeds.

The analysis of the distribution character of the found genotype frequencies allows stating that URM and UBW breeds demonstrate a high level of similarity by this index (Table 2). There is also a high demonstrated level of polymorphism by the number and character of distribution of the frequencies of the established genotypes, which is proven by the value of the approximating model (the selected exponential function) and the degree of its adequacy and determination coefficient (Fig. 2).

In general, the number of rare genotypes in the representatives of the investigated breeds fluctuated from 11 (in locus TGLA126) to 45 (in locus TGLA227) (Table 2). The tendency of frequency distribution (the same loci were the most and the least polymorphic, etc.) was...
found for the total number of genotypes, which were observed only once – from five (in locus TGLA126) to 33 (in locus TGLA227). The lowest number of genotypes, observed only twice each time (four genotypic variants), were found in locus SPS115, and the highest (15) – in locus INRA23. The lowest number of rare genotypes (two variants) determined for the representatives of URM in locus ETH3, and for UBW – in the locus ETH225. The highest number of rare genotypes (10 variants) was found for URM (in locus TGLA122), and as for UBW, this index was eight variants (in locus BM1824). The lowest number of rare genotypes (two variants) determined for the representatives of URM in locus ETH3, and for UBW – in the locus ETH225.

The analysis of the frequencies of unique genotypes showed that the lowest number of such genotypes (four genotype variants) was found in the loci TGLA126 and BM1824 in animals of both breeds and in the loci ETH3 and ETH225 – in UBW, and the highest (20 variants) – in locus BM2113 in URM. The intrabreed analysis demonstrated that the average number of unique genotypes per locus in URM was 9.0 and fluctuated from four (in loci TGLA126 and BM1824) to 20 (in locus BM2113). For UBW the average was 8.1 and fluctuated from four (in loci TGLA126, ETH3, ETH225 and BM1824) to 17 (in locus TGLA227). It is noteworthy that no differences were found between the breeds in the ratio of three out of ten investigated microsatellite loci, and there was only one genotype variant found in three more.

In general, the analysis of genetic variation in the two Ukrainian breeds of Bos taurus species demonstrated that all the investigated microsatellite loci were characterized by a high number of incongruous genotype variants, for instance, TGLA126<sup>116/116</sup>, TGLA126<sup>116/118</sup>, TGLA126<sup>116/120</sup>, TGLA126<sup>118/118</sup>, TGLA126<sup>118/120</sup>, etc. Their total number in the animals of this species fluctuated from 21 (in loci TGLA126) to 53 (in locus TGLA227). The highest level of genotype polymorphism was found in loci INRA23 and TGLA227 in URM (32 variants) and UBW (31 variant). Locus ETH3 was found to be the least polymorphic in URM (15 variants), and for UBW it was TGLA126 (16 variants).

According to the nonparametric method of A. Chao, the use of which ensures more accurate comparison of the populations (groups of animals) of different size [22] in general, the distribution for the number of genotypes in the animals of different breeds is incongruous. For instance, in loci TGLA126, INRA23, ETH3, ETH225, TGLA227, ETH10 and SPS115 no reliable difference was found between the breeds, and in locus BM2113 in URM, the potential number of genotypes was much higher than that for the representatives of the other breed.

The parametric rarefaction-procedure was also used to estimate the “possible genotype diversity”. The ob-

### Table 2. The total number of genotypes, determined, rare and unique genotypes for 10 microsatellite loci of two Ukrainian cattle breeds

<table>
<thead>
<tr>
<th>Locus</th>
<th>UR</th>
<th>URM</th>
<th>UR</th>
<th>URM</th>
<th>UR</th>
<th>URM</th>
<th>UR</th>
<th>URM</th>
<th>UR</th>
<th>URM</th>
<th>UR</th>
<th>URM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na</td>
<td>N1</td>
<td>Na</td>
<td>N1</td>
<td>Na</td>
<td>N1</td>
<td>Na</td>
<td>N1</td>
<td>Na</td>
<td>N1</td>
<td>Na</td>
<td>N1</td>
</tr>
<tr>
<td>TGLA126</td>
<td>16</td>
<td>16</td>
<td>6</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGLA122</td>
<td>21</td>
<td>25</td>
<td>6</td>
<td>14</td>
<td>10</td>
<td>6</td>
<td>11</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INRA23</td>
<td>32</td>
<td>31</td>
<td>23</td>
<td>22</td>
<td>7</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETH3</td>
<td>15</td>
<td>18</td>
<td>6</td>
<td>8</td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETH225</td>
<td>25</td>
<td>19</td>
<td>14</td>
<td>12</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM1824</td>
<td>24</td>
<td>25</td>
<td>15</td>
<td>13</td>
<td>3</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGLA227</td>
<td>32</td>
<td>31</td>
<td>24</td>
<td>22</td>
<td>5</td>
<td>7</td>
<td>14</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM2113</td>
<td>31</td>
<td>20</td>
<td>23</td>
<td>8</td>
<td>4</td>
<td>7</td>
<td>20</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETH10</td>
<td>22</td>
<td>22</td>
<td>10</td>
<td>12</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SPS115</td>
<td>22</td>
<td>22</td>
<td>11</td>
<td>12</td>
<td>3</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>24</td>
<td>22.9</td>
<td>13.8</td>
<td>13.1</td>
<td>5</td>
<td>5.3</td>
<td>9</td>
<td>8.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Fig. 2. Character of distribution for genotype frequencies in the investigated cattle breeds in 10 loci of microsatellite DNA, where $y$ – approximating model (selected exponential function) and its adequacy level, $R^2$ – determination coefficient.
tained data allow for the assumption that by the majority of loci both URM and UBW demonstrate high similarity in the distribution of genotype frequencies. For loci TGLA126, INRA23, TGLA227, ETH10 and SPS115 there was an observed tendency, similar to the general one, i.e. the distribution of frequencies in URM and UBW breeds was practically identical, in locus BM1824 it was very similar, and only for four loci (TGLA122, ETH3, ETH225 and BM2113) the investigated animals demonstrated breed-specific character of the distribution of genotype frequencies in locus TGLA126.

CONCLUSIONS

Our analysis of the presented data allows the assumption that the investigated breeds (populations) demonstrated high level of identity with a simultaneous reliable level of genetic differentiation. This is confirmed with the observed differences by the character of distribution of frequencies of the found allelic variants (profiles of allelic polymorphism) (P < 0.001) and by the distribution of genotypes, which is proven by the estimates of the approximating model (the model of the exponential function) and the degree of its adequacy and determination coefficient.

High level of similarity between the investigated breeds, determined by the results of estimating «potential genotype diversity», obtained on the basis of the rarefaction-procedure, allows stating that by the prevailing majority of loci the animals of both breeds demonstrate high similarity regarding the distribution of genotype frequencies. For both breeds, the character of variability in the investigated microsatellite loci for both the ratio of observed alleles and the character of their distribution corresponds to the stepwise mutation model (SMM).

The results of estimating the indices of allelic (the highest number of found alleles, the number and distribution of breed-specific alleles) and genotypic variability (the number of found rare and breed-specific genotypes), confirmed by nonparametric methods (rarefaction-method and the method of A. Chao) demonstrate high level of genetic differentiation of the investigated breeds.

**Визначення генетичної варіативності та міжпородна диференціація двох українських молочних порід великої рогатої худоби за мікросателітними локусами ДНК**

A. V. Shelyov 1, K. V. Kopylov 1,  
С. S. Kramarenko 2, O. S. Kramarenko 2

1 Інститут розвидення і генетики тварин НААН,  
Вул. Погребняка 1, с. Чубинське, Київська область,  
Україна, 08321  
2 Миколаївський національний аграрний університет,  
Вул. Георгія Гонгадзе 9, м. Миколаїв, Україна, 54020

**Мета.** Метою нашої роботи було дослідження міжпородної диференціації українських червоно- та чорно-рібної порід молочної худоби за результатами аналізу альтернацій генотипового поліморфізму мікросателітів ДНК. **Методи.** Генотипування 88 зразків ДНК двох найчисленніших в Україні молочних порід великої рогатої худоби – української червоно-рібної молочної та української чорно-рібної молочної – проводили за 10 локусами, рекомендованими Міжнародним товариством з генетики тварин (ISAG). Було здійснено аналіз альтернацій генотипового поліморфізму із застосуванням параметричних та непараметричних методів. **Результати.** Вперше в Україні викладено результати аналізу альтернацій генотипового поліморфізму популяцій українських червоно- та чорно-рібної молочної худоби за використання 10 мікросателітних локусів ДНК. Показано, що мікросателіти ДНК, як високо-поліморфні генетичні системи, є надзвичайно інформативними маркерами генетичних процесів, що мають місце в популяціях свійських тварин. **Висновки.** До складу пород демонструють достовірний рівень генетичної диференціації й, одночасно, високий рівень подібності, що, безумовно, пояснюється однаковими цілями та методами селекційної роботи з ними. **Ключові слова:** велика рогата худоба, ДНК-маркери, мікросателіти, біорізноманіття, популяційна генетика.
88 образцов ДНК двух наиболее многочисленных в Украине молочных пород крупного рогатого скота – украинской красно-пестрой молочной и украинской черно-пестрой молочной. Был проведен анализ аллельного и генотипического полиморфизма с применением параметрических и непараметрических методов. Результаты. В работе изложены результаты анализа аллельного и генотипического полиморфизма популяций украинского красно-пестрого и черно-пестрого молочного скота с использованием 10 микросателлитных локусов ДНК. Показано, что микросателлиты ДНК, как высоко полиморфные мультилокусные генетические системы, являются сверхинформативными маркерами генетических процессов в популяциях домашних животных. Выводы. Исследованные популяции украинских молочных пород крупного рогатого скота показывают достоверный уровень генетической дифференциации и, одновременно, высокий уровень идентичности, что, несомненно, объясняется схожими целями и методами селекционной работы с ними.

Ключевые слова: крупный рогатый скот, ДНК-маркеры, микросателлиты, биоразнообразие, генетика популяций.

REFERENCES


INTRODUCTION

Due to the desire to obtain maximal yields, there has been an increase in anthropogenic load on soils, related to the use of physiologically acid fertilizers, pesticides, herbicides, chemical meliorants of different quality degree as well as the use of green manure crops as “substitutes” of manure, which lead to the acidity of soils as a result of fast mineralization of their fresh and highly labile organic matter. At the same time, the soils are prone to stress due to global climate changes, as the expected increase in the soil temperature enhances the dissociation of organic acids in soil, the intensity of the impact of soil biota on the mineralization of organic matter and additional formation of acid-forming oxides of nitrogen, sulphate and carbon. Therefore, at present some of the main diagnostics criteria of the functional

© A. S. KHOLODNA, K. O. DESIATNYK, 2018
stability of soils should be its operational efficiency and ability to reflect the “health” of soil [1].

The traditional system of the agrochemical and agro-ecological diagnostics of the state of soils is mostly based on the complex of such agrotechnical and physical-chemical indices as pH level, hydrolytic acidity, the sum of absorbed bases, the degree of saturation with bases, the content of mobile aluminum, the content of heavy metals, biological indices and, less frequently, physical indices. The determination of the content of any substances in soil using traditional analytical methods is a labor- and time-consuming process as it involves selecting and transporting samples and their laboratory and chemical analyses.

We proposed a convenient resource-saving and, more relevantly, rather cheap development – the operational diagnostics of the functional stability of soils under the impact of natural and anthropogenic loads. The diagnostics is made in several stages: potentiometric studies in-situ, simulating the loads (natural and anthropogenic), diagnostics of changes in soil via bioindication using the organisms, sensitive to the environmental changes, and determination of acid-base buffer ability of soils. This method ensures complex estimation of the efficiency of soil functioning [1].

It should be noted that the coefficient of functional stability of soil (K f.st.) as an integral index of physical-chemical (buffer ability and activity of calcium) and biological (activity of protease enzyme) properties of soil was proposed for the first time. This is a substantial factor, taking into consideration the soil as a living biological body which can be presented not via separate components but only via their specific combination [1].

As buffer properties of soils play a relevant role in the stabilization of soil fertility and in the estimation of evolutionary direction of the fertility potential of soils, this property was selected by us as one of the components with the greatest impact on the soil stability. The buffer capacity regarding a specific element of fertility is a reliable criterion of its stability and an actual cumulative index for the estimation of the functioning of the nutrition regime by a specific element of fertility.

The acid-base state of soil is one of key indices of its fertility. The acid-base balance of soils is directly connected to the content of alkaline and alkaline-earth cations as the main antagonists of hydrogen and other elements, which have their impact on the formation of pH level. It is the balance of these elements in soil that defines the vector of direction of soil processes towards acidification or, vice versa, alkalization of the soil medium.

The value of active or potential acidity or alkalinity is often not an objective characteristic of the acid-base function of soils. It is more important in the theoretical and practical sense to have clear determination of the level of susceptibility of soils to the acidification or, vice versa, alkalization. The application of the recent achievements of soil studies towards the use of theoretical and practical provisions of the buffer ability of soils allows eliminating these drawbacks [2].

The example of diagnostics and optimization of the acid-base status of a specific type of soils is presented in the works [3–7].

Talking about the soil stability, it is impossible to omit calcium which is a relevant component of soil stability as a natural body. It is also one of relevant macroelements of the nutrition of plants, a factor of soil structuring and humus stabilization, and also a regulator of soil acidity. Therefore, the stability of reserves of soil calcium and the optimization of processes of its accumulation-dissipation in soil is an urgent issue. The latter is especially urgent in modern conditions of climatic instability due to the accumulation of carbon dioxide in the atmosphere, and thus – to the hazard of shifts in the carbonate equilibrium.

Soil processes involving calcium are characterized via a group of potentials, among which a prominent place is given to the lime potential pH – 0.5pCa or the potential of hydrogen-calcium exchange.

The methods, based on determining the hydrolytic acidity, saline and water pH in soils, prevail among many current methods of diagnostics and optimization of the acid-base balance in soils.

Soil microorganisms are the pioneers of the soil-forming process. They determine the biological activity of soils, their fertility and ecological status to a large degree. However, at present their ability of serving as indicators of “soil health” is becoming more urgent. It is microorganisms that are some of the first to react to the slightest environmental changes, therefore, their reactions to different pollutants, more often – heavy metals [8, 9], or fluctuations in the existence conditions are very valuable for ecological monitoring, including soil monitoring.

As for microbiota, a considerable role is played by enzymes – organic catalysts of protein nature, accumu-
lated in soil in the process of activity of living organisms. Due to enzymes, there are processes of humus accumulation and restoration in soils, the trophic and sanitary functions are activated, etc. However, natural catalysts are often inhibited due to the following factors: the application of intensified soil tillage [10, 11]; the pollution of soil with pesticides [12]; the formation of specific natural compounds such as tannins [13] and terpenes [14] in soils under forest plants and microcystins in the soils of lacustrine deposits [15]; the pollution of environment [16], etc.

The enzymes take a considerable share of soil fauna, therefore, the improvement of their living conditions is one of the most important tasks of ecological soil studies in current conditions of intensifying agriculture. In particular, the use of the scientifically grounded system of tillage and fertilization of soil [10, 17] promotes the increase in populations of different microorganisms. However, due to popularization of resource-saving methods, there are new ways of optimizing the microbial regime of soils in the world. One of these methods is covering oak (and other kinds) of biochar with the arable layer of soil [18, 19]. It was proven that this leads to the increase in the amount of total organic carbon and microbiological activity, and, as a result, in soil quality.

The degradation processes are of dual nature – natural and anthropogenic. A new issue of soil studies is the recultivation and restoration of disturbed soils of urbanized areas. Recently, there has frequently been a new term in the literature – “urban soils” – anthropogenically modified [20] soils of urban territories, the artificial profile of which has a surface layer of up to 50 cm, created by humans by molding, mixing, burying materials (substrates) of purely urbanogenic origin [21]. Therefore, the functions and properties of such soils are subject to considerable disturbance, especially in terms of functioning of their biological component.

We were interested in the studies on ecologically sensitive soils of urbanized areas (urban soils) with the purpose of activating their biological component and, as a result, partial self-restoration. The cultivation of several kinds of energy crops was suggested as they have a positive influence on the status of soil fauna and the activity of protease enzyme [22, 23] not only on urban soils but also on a number of other types of soils. It is noteworthy that such energy crops as giant miscanthus (Miscanthus Giganteus) and willow (Salix) are also decorative plants, which is especially valuable while cultivating them in urbanized zones.

Protease activity is one of integral indices of the total biological activity of soils, a potential ability of soils to decompose proteins and peptides [24]. Protease takes part in the mobilization and circulation of nitrogen. The higher the content of mobile nitrogen and other elements of nutrition in soil is, the more active the process of cellulose oxidation is. The cellulose-decomposing microorganisms ferment the fiber, synthesize and partially release amino acids into the medium.

Protease is considered to be one of the most important enzymes in the soils of chernozem type, but one should not underestimate its positive action in other soil types as well. The determination of protease activity in acid and forest soils, the soils which suffered from degradation processes is an efficient method of soil-ecological monitoring. The degraded soils present a wide field for studies in the sphere of ecological soil studies, as they are the first to be subject to recultivation, which are ecologically safe due to their susceptibility [25].

The biological restoration and maintaining the fertility of soils, both degraded ones and those with undisturbed structure, is impossible without the consideration of microbiological, and thus enzymatic component. The special place in our work is given to protease as an enzyme, taking part in the transformation of nitrogenous compounds. The determination of protease activity was paid much attention in the previous century, but even after many years of studies there are many gaps in the literature regarding the response of protease to foreign factors [26]. Current methods of estimating the activity of this enzyme are characterized by much labor and the application of rather a large amount of reagents and devices. In addition, the deviations of these analyses are rather substantial.

We have improved Mishustin’s method of determining protease activity and elaborated an accurate expression-mechanism of determining a quantitative index of protease activity of soil, which ensures considerable facilitation and acceleration of current methods and obtaining objective data about the soil quality. It is also important that this method is easily applied in practice with just a required minimum of materials. Therefore, the suggested method has considerable advantages compared to current ones.

It would be reasonable to determine the advantages of the methods, suggested by us, with current ways of determining the abovementioned indices.

First of all, the determination of any soil indices directly in the field is surely a faster and less expensive
method, because it does not require time and material resources for the preparation and transportation of samples, the acquisition of reagents, etc. Therefore, the advantage of the potentiometric method of studying the acid-base status of soils is obvious.

Up till now there have been several methods of determining the biological activity of soils, but all of them are rather complicated in terms of implementing, and the process of obtaining results is very time-consuming. The following methods have proven to be most commonly used: the method, based on the application of cellulose standards [27]; the method using the intensity of flax linen decomposition [28]; the method of determining the proteolytic activity of soils using photopaper or photofilm [29].

Taking into consideration the fact that in modern world the preference is given not only to quality but also to the rate of obtaining the results, the following method of estimating changes in the ecological status of soils will ensure considerable acceleration of the ecological monitoring of soils of different genesis and, as a result, the restoration of their physical and biological properties.

MATERIALS AND METHODS

Potentiometric, biological methods and the methods of determining acid-base buffer capacity were used during the studies.

The estimation of acid-base status of soils is done efficiently via direct potentiometric determination using ion-selective electrodes, as it allows estimating directly in soil without taking a sample, and it does not require any processing of soil material in the laboratory conditions except for diluting with water.

The activity of a substance in soil, determined potentiometrically, is active content of this substance in the aqueous phase in the field soil, or in conditions, maximally close to the field soil.

The activity is usually measured in milligram-equivalents per one liter of soil solution: \( a, \text{[mg-equiv/l]} \), or presented in the form of a logarithmic index, similar to pH: \( p_a = - \log a \). To transfer to milligrams per liter, one should multiply the value of \( a \) by atomic or molecular mass of the investigated element, referred to the unit of its valency (equivalent mass). To transfer to the content in soil, one should consider the content of aqueous phase in it (humidity) [2].

As for exchange cations of calcium (\( \text{Ca}^{++} \)), the activity should be interpreted as active content of mobile water-soluble form.

Any potentiometric estimation is efficient for direct study of the dynamics in the mobile substance in soil as well as for the determination of the spatial variability (diversity) of this content – for instance, to find “spots” in order to correct the norms of fertilizers in the fields which is especially relevant in the practice of directed agriculture [2].

At present, there is a common method of potentiometric determination of pH. Calcium may be determined according to DSTU 4725:2008.

The method of determining the acid-base buffer of soil is based on estimating the change in pH of the soil suspension due to the addition of increasing doses of acid and alkali. It is determined according to DSTU 4456:2005. The results are presented in a graphic form as a dependence of pH the dose of additive (ad). The obtained curve of pH-buffer capacity is the basis for normative forecast of the needs of chemical melioration of some soils.

The acid part of the buffer capacity for acid soils was taken by us as negative part of pH-buffer capacity and the alkali part – as a positive part. The main indices of pH-buffer capacity of soils are as follows:

- buffer capacity of soils in the alkali part (interval) of loads (\( BC_{Al} \));
- buffer capacity of soils in the acid part (interval) of loads (\( BC_{Ac} \));
- the coefficient of buffer asymmetry (CBA) – the ratio of the difference and sum of the abovementioned capacities
  \[ \frac{BC_{Al} - BC_{Ac}}{BC_{Al} + BC_{Ac}} \]
- the total estimation index of buffer capacity (TEIBC), which includes the sum of buffer capacities with the consideration of the asymmetry coefficient, determined by the formula:
  \[ (BC_{Al} + BC_{Ac})(1 - |CBA|) \]

The lower the asymmetry coefficient is, the higher is the rate of the reverse processes or the rate of self-regulation of genetically inherent acid-base balance of soils.

The diagnostics and optimization of the acid-base status of specific soil was conducted in the following way:

- determining optimal values of pH of the soil solution for crops;
– building the chart of pH-buffer capacity for specific soil, distinguishing the optimal pH zones on the chart;
– determining the optimal pH level of soil within a specific crop rotation, reaching which requires the estimation of the meliorant dose;
– calculating and introducing the corresponding dose of the meliorant into soil to reach the given rate.

The estimation of the enzymatic activity by the indices of protease activity was performed by the modified photoautography method on the basis of the laboratory of fertility of hydromorphic and acid soils of NSC ISSAR named after O. N. Sokolovsky. The abovementioned technology is based on Mishustin’s method of determining protease activity [28].

Photofilms were used as application material. We have improved the method of estimating the protease activity using the graphic editor Adobe Photoshop.

The estimation of proteolytic activity requires the following items: soil samples (50 g – the mass of one sample); Petri dishes; unexposed film; distilled water.

A piece of film is placed on the bottom of a Petri dish (films of 2.5 cm × 7.5 cm were used in our study) with the gelatin layer upwards. Maximally homogenized soil with the weight of 50 g is placed onto the film. One of our tasks was to determine the rate of complete decomposition of the gelatin layer of the film, therefore, we moistened soil samples with distilled water up to 80 % from the complete water capacity of soil.

The films were extracted 3–5 days later, depending on the type of soil. For further estimation of the quantitative value of protease activity, the films are carefully washed with distilled water, dried and fixed to carton, preferably of black or yellow color (the most convenient colors for further work in the graphic editor). The obtained films are scanned. The film with non-decomposed gelatin is taken as the control.

The scanned image is opened using the graphic editor Adobe Photoshop. A graphic document is created with the sizes of 2.5 × 7.5 cm with transparent background and the resolution of 78.74 pixels per one centimeter (px/cm). The area with the photofilm is outlined on the scanned image, copied and pasted into a previously created file, then scaled.

Using the instrument “Select → Color range”, select the color of the background (which is the decomposed layer of gelatin) with an eyedropper, here the value of the command “the range” should be 200 for maximal accuracy. Then, the instrument “Histogram” should be opened, which indicates in pixels the areas of the whole image (in our case this is 116427 pixels), and of the decomposed gelatin layer – i.e. the immediate background of the image. Using the ratio of the areas of variants with the decomposed gelatin and the control sample, we receive the value of the total biological activity of soil in percentage.

The final stage of operational diagnostics of the functional activity of soils is the actual calculation of the coefficient of their functional stability. Due to the fact that the acid-base buffer capacity of soil is an integral index, reflecting the changes therein (fluctuations in pH and pCa) and soil capacity to counteract the external load, this index should be one of the main ones to determine the soil stability.

However, it is impossible to have objective estimation of the soil reaction to the exogenous impact, neglecting the living component of soil. Therefore, we proposed the following variants of the formula of calculating the coefficient of functional stability of soils (K f.st.), based on their genetic features:

– for turf-podzolic soils – K f.st. = 0.4 × B + 0.6 × P;
– for gray forest soils – K f.st. = 0.6 × B + 0.4 × P;
– for podzolic chernozem – K f.st. = 0.7 × B + 0.3 × P;
– for meadow soils – K f.st. = 0.5 × B + 0.5 × P;

where B – the ratio of the value of the total estimation index of buffer capacity (TEIBC) of the investigated soil under the impact of anthropogenic or natural loads and the value of TEIBC of the same soil without any loads; P – the ratio of the value of protease activity of the investigated soil under the impact of the anthropogenic or natural loads and the value of protease activity of the same soil without any loads.

Taking into consideration the fact that turf-podzolic soils have the smallest buffer capacity, its role in maintaining the functional stability of soils will be less significant compared to gray forest and chernozem soils. Meadow soils are distinguished by high buffer capacity and considerable biological diversity, i.e. these indices have equal impact on their properties. Therefore, we suggested the abovementioned ratio of the index of the buffer capacity – TEIBC and the biological index PA, the combination of which is the coefficient of the functional stability of soils.
RESULTS AND DISCUSSION

It is noteworthy that the results of further estimations will depend on the quality of scanned image and the color of the background. Further visualization requires editing the image in the graphic editor, as shown in Figure 1.

Below is the algorithm of calculating the proteolytic activity for images with the resolution of 78.74 px/cm and the films with the size of 2.5 x 7.5 cm:

- the area of one square centimeter in pixels: S = 78.74 x 78.74 = 6200 px;
- the area of the decomposed gelatin layer in square centimeters: Sg = Sg(px)/6200;
- proteolytic activity in %: PA = Sg/Sfilm x 100.

Based on the data obtained, the analysis of different kinds of soils demonstrated that it is possible to compare the results of the control and the obtained variants of the experiment in laboratory conditions with the indicated moisture capacity as early as 3–4 days later. Table 1 presents the data of observations of the process of decomposition of the gelatin layer of the film in soils on the third day of the experiment. According to the results obtained, it is possible to check the sufficiency of the process duration of 3 days as on the 4th–5th day the gelatin layer is almost completely destroyed on all the films.

In addition, this method may be used to determine the rate of protease action in different soils under various tillage conditions and under different loads, as shown in the charts below (Fig. 2–3).

Table 2 demonstrates the change in the coefficient of functional stability of soils under the impact of anthropogenic loads on the soils of agricultural purpose, and the change in the coefficient on the soils (including degraded ones) where crops are not cultivated. In addition, we calculated the coefficient of functional stability of soils while cultivating energy crops.

Table 2 demonstrates that the highest stability is notable for chernozem. Turf-podzolic soils are more inclined to losing functional stability under the impact of external loads.

CONCLUSIONS

Current methods of estimating the protease activity of soils were optimized which allowed reproducing this
Improvement of Methods of Estimating the Change in the Ecological State of Soils

In modern conditions of intense increase in the anthropogenic load on environment, some of the main criteria of diagnostics of the functional stability of soils should be its operational ability, as many types of soils are susceptible to changes due to external loads, thus, there is an urgent need for timely revelation of these changes with the purpose of efficient management of their fertility.

The algorithm of estimating the functional stability of soils was suggested for the first time. It includes a number of simple stages which do not require much labor or cost and allow obtaining the data regarding specific type of soil in a short period of time. The stages are as follows:

- diagnostics of acid-base status of soils using ion-selective methods and determining the rates of activity of calcium ion and pH of soil;
- simulating the loads (natural and anthropogenic ones) on soils;
- diagnostics of changes in the biological activity of soils using the operational method of determining the protease activity in it;
- determining the changes in the acid-base buffer capacity of soil under the impact of loads;
- calculating the coefficient of functional stability of soils.

**Fig. 2.** The rate of gelatin decomposition on the photofilm (turf-podzolic soils)

**Fig. 3.** The rate of gelatin decomposition on the photofilm (chernozem soils)
KHOLODNA et al.

Table 2. The functional stability of soils with different acid-base balance under the impact of anthropogenic loads

<table>
<thead>
<tr>
<th>Variant</th>
<th>TEIBC, points</th>
<th>PA, %</th>
<th>K f.st.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Turf-podzolic soils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.3</td>
<td>37.0</td>
<td>1.00</td>
</tr>
<tr>
<td>NPK</td>
<td>11.4</td>
<td>25.0</td>
<td>0.74</td>
</tr>
<tr>
<td>Wet lime</td>
<td>15.0</td>
<td>36.0</td>
<td>1.03</td>
</tr>
<tr>
<td>Green manure crops</td>
<td>11.8</td>
<td>20.0</td>
<td>0.67</td>
</tr>
<tr>
<td>Energy crops</td>
<td>12.1</td>
<td>39.0</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>Gray forest soils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>16.4</td>
<td>42.0</td>
<td>1.00</td>
</tr>
<tr>
<td>NPK</td>
<td>14.2</td>
<td>36.0</td>
<td>0.86</td>
</tr>
<tr>
<td>Wet lime</td>
<td>18.0</td>
<td>30.0</td>
<td>0.94</td>
</tr>
<tr>
<td>Green manure crops</td>
<td>16.0</td>
<td>38.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Energy crops</td>
<td>16.2</td>
<td>40.0</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Chernozem soils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30.2</td>
<td>50.0</td>
<td>1.00</td>
</tr>
<tr>
<td>NPK</td>
<td>28.2</td>
<td>45.0</td>
<td>0.92</td>
</tr>
<tr>
<td>Wet lime</td>
<td>29.0</td>
<td>39.0</td>
<td>0.91</td>
</tr>
<tr>
<td>Green manure crops</td>
<td>30.0</td>
<td>58.0</td>
<td>1.04</td>
</tr>
<tr>
<td>Energy crops</td>
<td>30.1</td>
<td>63.0</td>
<td>1.10</td>
</tr>
<tr>
<td><strong>Meadow soils</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>38.7</td>
<td>63.0</td>
<td>1.00</td>
</tr>
<tr>
<td>NPK</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Wet lime</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Green manure crops</td>
<td>36.4</td>
<td>61.0</td>
<td>0.95</td>
</tr>
<tr>
<td>Energy crops</td>
<td>38.0</td>
<td>65.0</td>
<td>1.01</td>
</tr>
</tbody>
</table>

The proposed operational diagnostics ensures complex demonstration of the efficiency of functioning of the soil, and thus fast elaboration of management events to improve the indices of soil with the purpose of receiving high yields of crops.

Due to the abovementioned method, we calculated the coefficients of functional stability of soils of different genesis. The results obtained confirm high stability of chernozem and meadow soils. The abovementioned K f.st. for turf-podzolic and other sensitive soils under different loads may serve as an instrument of improving their properties.

Удосконалення методів діагностики зміни екологічного стану ґрунту під впливом зовнішніх навантажень

A. С. Холодна, К. О. Десятник
E-mail: lonakalt@gmail.com
Національний науковий центр

The Institute of Soil Science and Agrochemistry
Im. O. N. Sokolovsky Institute of Soil Science and Agrochemistry
Vul. Chaykivska, 4, Kharkiv, Ukraine, 61024

Мета. Оптимізація та спрощення вже існуючих методів екологічної діагностики ґрунтів різної генези під впливом різноманітних зовнішніх навантажень. Методи. Лабораторно-модельні (фізико-хімічний, біологічний): визначення кислотно-основної буферності ґрунту (ДСТУ 4456:2005); визначення активності іонів кальцію (ДСТУ 4725:2008); визначення активності протеаз за модифікованим методом Мішустіна. Результати. Запропоновані нами методи значно пришвидшують екологічне діагностування ґрунтів, незважаючи на їх походження та зовнішні чинники. Завдяки цьому спрошується моніторинг ґрунтових ресурсів та виявляються фактори, що негативно, чи позитивно, впливають на перебіг ґрунтових процесів. Дані методи покладені в основу «Методології оперативної діагностики впливу природних та антропогенних навантажень на функціональну стійкість кислих ґрунтів» та можуть бути
Improvement of methods of estimating the change in the ecological state of soils

Використані не тільки науковцями в спеціальних наукових установах, але і виробничиках (за умов наявності необхідного мінімуму приладової бази). 

Висновки. На базі лабораторії родючості гідроморфних та кислих грунтів Національного наукового центру Інститут грунтознавства та агрохімії ім. О.Н. Соколовського НААН України був розроблений модифікований метод фотоавтографії для визначення ферментативної активності за показниками активності протеази. В основу способу покладено метод визначення протеазної активності за Мішустиным. Крім того, завдяки цьому методу можна визначити швидкість дії протеаз в окремих грунтах при різних умовах обробітку та при різних навантаженнях. Діагностика кислотно-основного стану грунтів іон-селективними методами із визначенням рівнів активності іона кальцію та рН грунту, моделювання навантажень (природних та антропогенних) на грунти, визначення зміни кислотно-основної буферності грунту під впливом навантажень та визначення біологічної активності через активність протеази за досить короткий термін дає змогу точно визначити функціональну стійкість того чи іншого грунту з метою її подальшого підвищення/підтримання. В якості фінального етапу проведення діагностування нами запропоновано формулу виведення коефіцієнту функціональної стійкості грунтів (К ф.ст.), виходячи з їх генетичних особливостей. Дуже важливим є той факт, що дана розробка не потребує високих фінансових затрат, проте дає максимально повну картину стану грунту в поточний період.

Ключові слова: функціональна стійкість, буферна здатність, активність протеази, коефіцієнт функціональної стійкості.

Усовершенствование методов диагностики изменения экологического состояния почв под влиянием внешних нагрузок

А. С. Холодная, К. А. Десятник
e-mail: lonakalt@gmail.com
Национальный научный центр Институт почвоведения и агрохими
им. А. Н. Соколовского НААН Украины
Ул. Чайковская, 4, Харьков, Украина, 61024

Цель. Оптимизация и упрощение существующих методов экологической диагностики почв различных генезиса под влиянием разнообразных внешних нагрузок. 

Методы. Лабораторно-модельные (физико-химические, биологический): определение кислотно-основной буферности почвы (ДСТУ 4456:2005); определение активности ионов кальция (ДСТУ 4725:2008); определение активности протеазы по модифицированному методу Мишустина. 

Результаты. Предложенные нами методы значительно ускоряют экологическую диагностику почв, не беря во внимание их происхождение и внешние факторы. Благодаря этому упрощается мониторинг почвенных ресурсов и выявляются факторы, которые негативно или положительно влияют на ход почвенных процессов. Данные методы положены в основу «Методологии оперативной диагностики влияния природных и антропогенных нагрузок на функциональную устойчивость кислых почв» и могут быть использованы не только учеными в специальных научных учреждениях, но и производственниками (при наличии необходимого минимума приборной базы). 

Выводы. На базе лаборатории плодородия гидроморфных и кислых почв ННЦ «ИГА имени А. Н. Соколовского» был разработан модифицированный метод фотоавтографии для определения ферментативной активности по показателям активности протеазы. В основу способа положен метод определения протеазной активности по Мишустину. Кроме того, благодаря этому методу можно определить скорость действия протеаз в отдельных почвах при различных условиях обработки и при различных нагрузках. Діагностика кислотно-основного состояния почв ион-селективными методами с определением уровней активности ионов кальция и рН почвы, моделирование нагрузок (природных и антропогенных) на почвы, определение изменения кислотно-основной буферности почв под воздействием нагрузок и определение экологической активности по активности протеаз в достаточно короткие сроки, позволяет точно определить функциональную устойчивость почв и в дальнейшем повышения/поддержания. В качестве финального этапа проведения диагностики нами предложено формулу вывода коеффициента функциональной устойчивости почв (К ф.уст.), исходя из их генетических особенностей. Важен факт, что данная разработка не требует высоких финансовых затрат, однако дает максимально полную картину состояния почвы в текущий период.

Ключевые слова: функциональная устойчивость, буферность, активность протеазы, коеффициент функциональной устойчивости.

REFERENCES

5. Zayceva TF. Buffer capacity of soils and the issues of di-


INTRODUCTION

Sunflower is the main raw material in the world for oil for food (OFF) in terms of production volume, and at present and in the near future it is one of the strategic crops of the country. The upward trend in the global seed production persists, and Ukraine remains the main country-producer of seeds as per FAO data. A dynamic increase in the acreage under sunflower is attributed to the high profitability of its production. Sunflower cultivation is one of the major sources of income for agricultural enterprises of different forms of ownership [1]. The analysis of sunflower cultivation in Ukraine indicates that its acreage grew threefold from 1990 to 2014 – from 1,636 to 5,257,000 hectares. Over this period, the production of sunflower seeds has grown sevenfold since 1994 – from 1,569,000 tons of seeds to the record value of 11,051,000 tons in 2013. During these 25 years, the yield was 0.89–2.17 tons per hectare [2]. Agrocenoses are transformed as a result of the concurrent impact of anthropogenic activity and weather conditions [3].
Due to the wide introduction of short crop rotations in production, which are more repaid than scientifically rationalized 9-field ones, and the narrowing assortment of cultivated crops, the general trends of changes in the phytopathogen complex composition of field crops have been revealed recently [4]. In Ukraine, crop rotations are focused on three major issues: cereals, oil crops and fodder crops. The simplification of crop rotations ignoring the traditionally established principles and regulations for crop rotating leads to the threatening spread of specialized weeds, pests and diseases, despite the increasing use of protective chemicals [5]. An enhancement in low pathogenic causative agents (polyphages), common to most cultivated crops [6], was recorded. There is an upward trend for charcoal rot – a disease that is adapted to affect any cultivated crop due to the drastic accumulation of long-lasting agents in soil [7].

The collection of information on the number and status of populations of harmful organisms to assess the phytosanitary condition of a field/a region is a primary measure in the integrated plant protection concept. This information serves as a basis to justify the application of chemicals, to determine the phytopathogen composition, as well as to assess the populations and their variability in multi-year observations [8, 9].

PURPOSE AND METHODS OF RESEARCH

The purpose of our research was to determine the phytopathogen complex of sunflower and its variability under the influence of hydrothermal conditions during the vegetative period as a factor that adapts to hybrids and, under favorable conditions, may limit the potential yield capacity to a large extent.

The phytosanitary monitoring of breeding crops of the scientific crop rotation of the Plant Production Institute named after V.Ya. Yuriev of NAAS in 2007–2016 determined the variability degrees for the most common sunflower diseases in the Left Bank Forest-Steppe of Ukraine, namely its eastern part. The prevalence of downy mildew was evaluated from a multi-year study of starting material in a disease nursery of the Laboratory of Plant Immunity against Diseases and pests of the Plant Production Institute n. a. V.Ya. Yuriev NAAS.

The diseases were assessed visually via direct examination of plants and via visual determination of the effect intensity by specific symptoms of each disease. To evaluate the development intensity of diseases, we examined calathidiums for gray mold, the middle part of stems for stem canker, and the bottom part of stems for charcoal rot. The plants with sporulation of the downy mildew pathogen were accounted in relation to the total number of affected plants.

Disease assessments included the prevalence or affection degree, percentages of affected plants [10], the disease development intensity, which was calculated from the affected surface area on plant organs [11]. To assess the disease intensity, a disease-specific eye scale was used [12]. Judging by the corresponding surface area of the affected tissue (organ), plants received a certain score. By the number of plants in each score, the weighted average area of the affected surface of an accession was calculated as a percentage [13].

To characterize the weather conditions during the vegetation period, the data of Kharkiv Regional Center of Hydrometeorology on air temperature and precipitation amount in 2007–2016 were used. The hydrothermal coefficient (HTC) is presented for the sunflower vegetative period and by the developmental phases [8].

The data were statistically processed using Microsoft Excel; cluster analysis was performed with Statistics 6.1 software.

RESULTS

The pathogen complex composition, ratios of different pathogens, and intensities of their development were heterogeneous during the study years.

Phomopsis blight (also called stem canker) (Phomopsis/Diaporthe helianthi Munt.-Cvet. Et al.), gray mold (Botrytis cinerea Pers.), dry rot (Rhizopus sp.), charcoal rot (Sclerotium bataticola Taub), and downy mildew (Plasmopara helianthi Novot. f. helianthi) were the most common diseases on sunflower in the Left Bank Forest-Steppe of Ukraine in 2007–2016.

Investigating the dependence of the disease development on hydrothermal conditions, we observed the ambiguity in their manifestation. It is well-known that increased humidity contributes to the ingress of infection in plants and to pathogen development, while unfavorable conditions generally limit the development of fungal diseases. However, the cases of the intensive disease development during arid periods can be due to condensed moisture, for example, morning dew, which emerges from the difference in night and daytime temperatures. If this occurs, the disease development depends on the moisture time on the leaf surface, the high humidity period length after precipitation, features of plant architectonics, and the plant density, where a microclimate, which is favorable for fungal spore germination, is formed. The rise in
In the eastern forest-steppe region of Ukraine, grey mold is associated with moderate temperatures and high air humidity. If prolonged moistening of soil and air coincides with a phase of the plant development that is crucial for pathogen affection, the downy mildew pathogen spreads rapidly at the initial stages of the diffuse affection development. Regular, though short precipitation, sufficient for the pathogenic affection and preservation of aero-genic inoculum from drying out, is enough for secondary infection. Stem canker development reaches high levels even in a dry month, if the previous ones were optimal and waterlogged.

Downy mildew in the experimental plots of the disease nursery sown with non-dressed seeds ranged from 18.0% to 83.0% of affected plants in the years, when the first half of the sunflower vegetation period was cool and waterlogged: in 2008–2009, 2011–2012, and 2014–2015 (see Figure). The weather conditions in 2007–2016 were noticeable for significant variability and fluctuations in the hydrothermal coefficient (HTC) from 0.57 in 2009 to 1.1 in 2014. However, despite the overwhelming majority of years, which were characterized as arid by the HTC values, the peak prevalence of rots and stem canker amounted to 100.0%. Therefore, to illustrate the year-by-year variability of diseases, we used the averages.

Only in 2008 and 2010, the disease prevalence was lower than that in the other years – 55.0% and 27.0%, respectively. These very years had higher average monthly air temperature during the sunflower vegetation period than the multi-year average values by 0.4°C – 4.6°C. In 2007 and 2014, the prevalence was 72.0–100.0% of the affected plants. Such values are rated as an epiphytoty.

The wholesale outbreaks of grey mold on sunflower calathidiums were noted every second year during the period of 2007–2016, namely in 2007, 2009, 2011, 2013, and 2015–2016, with fluctuations from 8.0% of the affected plants in 2007 to 58.0% in 2011.

Due to oversaturation of crop rotations with sunflower, under hot conditions during the seed ripening, the epiphytotinous (maximum) level of charcoal rot was reported: 83.0% of the affected plants in 2015 and 100.0% in 2012. Concerning the averages, an upward tendency in the disease prevalence was observed: from mild degree (average = 10.0% of the affected plants) in 2012 to severe degree (average = 65.0%) in 2016.

The spread of charcoal rot was similar to that of dry rot. In 2012, the prevalence amounted to 85.0%, reaching the maximum of 100.0% in 2014. The weather conditions in 2015 were anomalous, as there was no rainfall for 3 months (August-October), when sun-
flower seeds ripened, which limited the average disease prevalence to 27.0%. The prevalence of dry rot on sunflower calathidiums ranged within 3.0–37.0% that year.

Since the development of stem canker is tightly linked to the hydrothermal conditions of a year, we think that it is most fully characterized by a qualitative index of damage – the disease development intensity, which is determined from the weighted average area of affected stem surface. In its turn, to describe this index, the analysis of the HTC is required not only for the entire vegetation period of sunflower, but also for each month of vegetation, which almost coincides with the developmental phase lengths of the crop. Thus, the optimal water availability (HTC = 1.0–1.5) for sunflower and favorable conditions for the plant infection or stem canker development were in June of 2007 and 2015, (HTC = 1.1–1.2), May and September of 2010 (HTC = 1.33 and 1.45, respectively), July of 2013 and 2016 (HTC = 1.2–1.34), August of 2012 (HTC = 1.3), and September of 2007 (see Table).

The moisture level in May of 2016 (HTC = 3.14), June of 2011 and 2014, July of 2011 and 2015 (HTC = 1.6–1.8) was excessive (HTC > 1.5).

Over the ten-year period, the low average weighted values of the disease development intensity (2.0–5.0%) and peak ones (21.0–31.0%) were recorded in arid August of 2008 and 2010. In the other years, as the HTC in May increased, the average and peak values of the disease intensity grew from 13.0% and 50.0% to 33.0% and 80.0%, respectively. The peak values in most years (7 of 10) indicate significant levels of disease development intensity.

Gray mold was not widespread, as the arid weather conditions of the beginning of autumn in 2008, 2010, 2012, and 2014 restricted the disease development and its symptoms were only found on a few plants. The comparison of the average and maximum values of the disease development intensity in 5 years showed significant fluctuations from 6.0% to 32.0% of the affected calathidium area, respectively, and from 43.0% to 100.0%, respectively.

Thus, high degrees of gray mold manifestation on sunflower calathidiums of 43.0–100.0% were noted every second year in 6 of 10 years.

Conjugate frequencies of occurrence of the five most damaging sunflower diseases (dry rot, charcoal rot, gray mold, stem canker, downy mildew) were established during the ten-year research (2007–2016). Cluster analysis grouped the diseases in two core clusters.

According to the paired incidence of diseases, depending on the weather, dry and charcoal rots, which occurred in arid conditions, were incorporated in clus-

### Monthly Fluctuations in the HTC during the Sunflower Vegetation Period

<table>
<thead>
<tr>
<th>Year</th>
<th>May “sowing – leaf formation”</th>
<th>June “calathidium formation”</th>
<th>July “anthesis – seed setting”</th>
<th>August “seed filling”</th>
<th>September “physiological – technical ripeness of seeds”</th>
<th>Average HTC over the vegetation period of sunflower</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>0.7</td>
<td>1.2</td>
<td>0.5</td>
<td>0.5</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>2008</td>
<td>0.9</td>
<td>0.7</td>
<td>0.7</td>
<td>0.4</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>2009</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
<td>0.2</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>2010</td>
<td>0.8</td>
<td>0.3</td>
<td>0.8</td>
<td>0.2</td>
<td>2.5</td>
<td>0.9</td>
</tr>
<tr>
<td>2011</td>
<td>0.5</td>
<td>1.9</td>
<td>1.8</td>
<td>0.2</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>2012</td>
<td>0.8</td>
<td>0.5</td>
<td>0.2</td>
<td>1.3</td>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>2013</td>
<td>0.6</td>
<td>0.6</td>
<td>1.2</td>
<td>0.8</td>
<td>3.3</td>
<td>1.3</td>
</tr>
<tr>
<td>2014</td>
<td>0.9</td>
<td>2.5</td>
<td>0.6</td>
<td>0.8</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>2015</td>
<td>0.7</td>
<td>1.1</td>
<td>1.6</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
</tr>
<tr>
<td>2016</td>
<td>3.14</td>
<td>0.89</td>
<td>1.34</td>
<td>0.93</td>
<td>0.33</td>
<td>1.33</td>
</tr>
<tr>
<td>Average multi-year HTC</td>
<td>0.99</td>
<td>1.02</td>
<td>0.96</td>
<td>0.88</td>
<td>0.97</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Note. HTC ranking: < 0.5 – low (drought); 0.5–0.9 – insufficient (unstable); 1.0–1.5 – optimal; > 1.5 – excessive.
ter I; downy mildew and gray mold, which were associated with high wetness during a certain period of sunflower development formed cluster II. The Euclidean distance separates stem canker from the other diseases, as it occurs annually and, therefore, does not appear to clearly depend on any particular conditions.

CONCLUSIONS

Thus, the results of the phytosanitary monitoring of crops in the Left Bank Forest-Steppe of Ukraine determined the composition of the phytopathogen complex of sunflower. The prevalence of stem canker, gray mold, dry and charcoal rots, and downy mildew was evaluated. The development intensities of stem canker and gray mold were established. Establishing the conjugate frequencies of the five most damaging diseases of sunflower in the Eastern Forest-Steppe of Ukraine (dry rot, charcoal rot, gray mold, stem canker, downy mildew) during the 10-year-long research, depending on the weather conditions, we incorporated dry and charcoal rots, which occurred in arid conditions, in cluster I, while downy mildew and gray mold, which are associated with high wetness during a certain period of sunflower development, were included in cluster II. The Euclidean distance separates stem canker from the other diseases, as it occurs annually and, therefore, does not appear to clearly depend on any particular conditions.

Zonal Pathogen Complex of Sunflower in the Left Bank Forest-Steppe of Ukraine

I. Yu. Borovska, V. P. Petrenkova
e-mail: irinaborovska000@gmail.com

Institut roslinництва im. В. Я. Юр’єва НААН Україна, Харків, Пр-т Московський, 142

Мета. Визначення фітопатогенного комплексу соняшнику і його внутрішньовидової мінливості під впливом погодних умов вегетаційного періоду культури. Методи. В ході фітосанітарного моніторингу селекційних посівів наукової сівозміни Інституту рослинництва ім. В. Я. Юр'єва НААН в 2007–2016 рр. оцінено ступінь поширеності, інтенсивність розвитку, мінливості хвороб соняшнику в умовах Лівобережного Лісостепу України. Гідротермічний коефіцієнт (ГТК) представлений за вегетаційний період соняшнику і по фазам розвитку культури. Результати. При визнанні частоти прояву п'яти найбільш поширених і шкідливих хвороб соняшнику в умовах східної частини Лісостепу України в 2007–2016 рр. в залежності від погодних умов року, виявлено, що в посушилих умовах сполученням проявом характеризувалися суха (Rhizopus sp.) і вугільна (Sclerotium bataticola Taub) гнилі, за високого рівня вологозабезпеченості в певний період розвитку соняшнику – несправжня борошниста роса і сіра гниль (Botrytis cinerea Pers.). Фомопсис (Phomopsis/Diaporthe helianthi Munt. – Cvet. et al.), який проявляється щорічно, на відміну від інших хвороб не має чіткої залежності від будь-яких погодних умов. Висновки.За результатами фітосанітарного моніторингу посівів в умовах Лівобережного Лісостепу України визначено склад фітопатогенного комплексу соняшнику. Встановлено ступінь поширеності фомопсису, сірої, сухої, вугільної гнилей, несправжньої борошнистої роси і інтенсивність розвитку фомопсису та сірої гнилі. Визначено сполучену частоту їх прояву за період десятирічних досліджень в залежності від погодних умов року.

Ключові слова: соняшник, хвороби, патогенний комплекс, поширеність, інтенсивність розвитку, епіфітотія
Лесостепи Украины определен состав фитопатогенных комплекса подсолнечника. Установлена степень распространённости фомopsis, серой, сухой, угольной гнилей, ложной мучнистой росы и интенсивности развития фомopsis и серой гнили. Определена сопряженная частота их проявления за период десятилетних исследований в зависимости от погодных условий года.

Ключевые слова: подсолнечник, болезни, патогенный комплекс, распространенность, интенсивность развития, эпифитотия

REFERENCES
INTRODUCTION

One of the most urgent issues of fisheries in Ukraine is enhancing the efficiency of restoring populations of aborigine, unique, endangered and economically valuable species of fish. In order to achieve this, there is a definite need of full scale growing of these species under artificial conditions and the development of aquaculture as an agro-industrial sector using low-cost and efficient technologies. A popular trend to improve and increase or save gene pools of fish populations is artificial fertilization. One of the decisive factors in the efficiency of artificial fertilization is improving the methods of long-term preservation of fish sperm at low temperatures (cryopreservation).

Moreover, the presence of genetically representative collections of fish genomes from female populations in sturgeon fish-breeding complexes from natural populations in a cryobank ensures the preservation of genetic biodiversity of these species for the environment and industry.

The technology of preserving and using the frozen sperm of rare and endangered fish has long been worked out [1, 2]. But many of its negative impacts on sperm have not been fully overcome yet. Freezing without cryoprotectant compounds added to the freezing medium causes considerable structural and biological defects of sperm cells. These defects lead to the impairment of permeability of plasmatic membranes and the release of some enzymes and other relevant metabolites of cellular exchange from sperm cells with an often considerable decrease in fertility of sperm [3].

ISSN: 2312-3370, Agricultural Science and Practice, 2018, Vol. 5, No. 1

UDC 57.08:636:31

IMPACT OF CRYOPRESERVATION ON LIPID COMPOSITION OF SPERM CELLS OF MALE STERLETS (ACIPENSER RUTHENUS L.)

L. P. Drahan ¹, S. P. Veselsky ², Yu. P. Rud ¹, L. P. Buchatsky ¹, ²

¹ Institute of Fisheries, NAAS, 135, Obukhivska Str., Kyiv, Ukraine 03164
² Taras Shevchenko National University of Kyiv, 60, Volodymyrska Str., Kyiv, Ukraine, 01033

e-mail: dragan_l@ukr.net,

Received on January 31, 2018

Aim. To investigate the impact of low temperatures on the lipid composition of reproductive cells of male sterlets (Acipenser ruthenus L.) in a deep-freezing environment. Methods. The determination of sperm quality (color, consistence, concentration, and motility of spermatozoa) was estimated by common biochemical methods and light microscopy using standard equipment. Thin-layer chromatography was used to reveal five fractions of neutral lipids in the sperm of sterlet (Acipenser ruthenus L.) from three different river regions viz. Danube, Dnipro, and Volga. All lipid fractions (phospholipids, cholesterol, free fatty acids, triacylglycerol and ethers of cholesterol) showed a mean lower percentage (70, 12, 10, 5 and 3 %, respectively) for the three sterlet populations. Motility was also severely impaired (with a mean c. 50 %). Results. The impact of low temperatures on the lipid composition of reproductive cells of male sterlets (Acipenser ruthenus L.) in a deep-freezing environment leads to substantial changes therein. Conclusions. The impact of low temperatures on the lipid composition of reproductive cells of male sterlets (Acipenser ruthenus L) in cryopreservation environment leads to impairments of the phospholipid bilayer of their membranes. It was established that during the thawing of frozen sperm cells which preserved their viability after cryopreservation their motility and capability of fertilizing an ovum were somewhat restored with slowing down of sperm cell motility.

Keywords: lipids, cryoprotecting environment, cryobank.

DOI: 10.15407/agrisp5.01.075
The efficiency of the technology of cryopreserving sperm of male sterlets and its impact on the lipid composition of this sperm is presently not well known.

Our study tries to fill this gap, possibly allowing determination of new ways to enhance the viability and biological full-value of sterlet sperm cells during short- and long-term storage in deep-frozen state.

**MATERIALS AND METHODS**

The samples originated from the sperm of sterlet (*Acipenser ruthenus* L.), obtained from Danube, Dniipro, and Volga populations during the spawning campaign.

Ten samples were taken from each river population, deep-frozen and studied at the specialized fishery of Vilkove, Odesa Sturgeon Complex LLC and the Biotechnology laboratory of the Institute of Fisheries, NAAS.

Sperm quality was determined visually using a light microscope Biolam D-13 (Russia). Sperm appearance (color, consistency) was determined as well as its concentration using Goryaev’s chamber (equivalent of a haemacytometer), and their lifetime using a stop-watch.

Freezing/thawing of sterlet sperm samples was conducted by the methods, commonly used in cryobiology [4]. Cryopreservation and defrosting of sperm was conducted in glass ampules according to the method, described by Lunev in 2009 [5], using methyl alcohol instead of the traditional dimethyl sulfoxide. This method was implemented via diluting (1:1) the sperm prior to freezing with a cryoprotecting solution, containing saccharose 14.6 mM, potassium chloride 13.4 mM, and methanol 3.73 M with subsequent liquid nitrogen vapor freezing. The diluted sperm was frozen in two stages: the first one – from 5 °С till – 15 °С with the rate of 2–3 °С min-1; the second one – 15–70 °С with the rate of 15÷20 °С min-1, with subsequent slow submerging into nitrogen till 196 °С. Changes in temperature in the samples were registered by a chromel-copel thermocouple using the multimeter EC890G (MAXTECH company, Canada) with 0.2 °С precision. To thaw the ampules, the latter were transferred to a water bath kept at 38–40 °С, stirring the water constantly to enhance heat exchange until the liquid phase and controlling the temperature in the thawed samples to be in the range of 18–23 °С. The thawed sperm was activated with pond water. The preservation of sperm was checked in each sample at least three times with the determination of mean values. Motility of sperm cells at different stages during the preservation process was determined visually with 20-fold magnification. Total lipids in the ejaculate were separated by micro-thin-layer chromatography. The lipids from sterlet ejaculate were extracted with a chloroform-methanol mixture (2 : 1). The separation of lipids into fractions was performed on the plates with a thin silica gel layer (LS 5/40, Czech Republic) in the following system of solvents : hexane: diethyl ether : glacial acetic acid (85 : 15 : 1). Chromatograms of specific fractions of lipids were developed in iodine vapor using the method of Maksymenko [6]. Lipid extraction was performed by the method of Folch [7]. Protein concentration was determined according to the Lowry method [8]. The adaptive capacity of sperm was estimated via determination of the percentage of spermatozoa survival immediately prior to and after cryopreservation [9]. Statistical analysis was performed using Student’s t-criterion and the package of applications for processing medical and biological information – Statistica 6.0 (StatSoft, Inc., USA) for Windows [10].

**RESULTS AND DISCUSSION**

![Table 1. Characteristics of sterlet sperm (*Acipenser ruthenus* L.) prior to and after cryopreservation (*M* ± *m*, *n* = 5)](image)

<table>
<thead>
<tr>
<th>Sterlet populations</th>
<th>Sperm concentration mlн/mm³</th>
<th>Duration of motility of native sperm cells, min</th>
<th>Duration of motility of thawed sperm cells, min</th>
<th>Percentage of sperm cells, preserving their motility before and after freezing in liquid with cryoprotectants, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>native</td>
</tr>
<tr>
<td>Danube</td>
<td>0.93 ± 0.018</td>
<td>8–10</td>
<td>4–6</td>
<td>98–100</td>
</tr>
<tr>
<td>Dnipro</td>
<td>0.74 ± 0.026</td>
<td>5–7</td>
<td>2–5</td>
<td>99–100</td>
</tr>
<tr>
<td>Volga</td>
<td>0.99 ± 0.023</td>
<td>7–10</td>
<td>3–6</td>
<td>99–100</td>
</tr>
</tbody>
</table>

*P* ≤ 0.05; (*M* ± *m*, *n* = 5) from each population.
50 %) deterioration after freezing/thawing, even with a cryoprotectant added (Table 1).

The number of mobile gametes after thawing decreased to 54, 48 and 56 % respectively. The fact that the decrease was not more than 60 % may be explained by the presence of a cryoprotectant such as methanol, in our freezing medium [11, 12]. The mechanisms of energy exchange regulation are known for their highest sensitivity to cooling and freezing/thawing sperm. Such experimental manipulations with sperm cells of animals lead to changes in the organization and integrity of membranes of plasmatic and acrosomal membranes, the release of enzymes from cells, damage to membrane structures of mitochondria and impairing the functioning of the respiration chain – separating respiration and phosphorylation and increasing the production of damaging free oxygen radicals [13, 14].

Structural defects of the membrane may serve as a target point for many agents – osmotic forces, lyotropic ions, catalysts, toxins, temperature and other factors, leading to secondary biochemical changes [13]. Therefore, the following stage in our research was to determine the lipid composition of sperm of sterlet from Danube, Dnipro, and Volga populations before and after freezing.

In our micro-thin-layer chromatography the following five fractions were recovered and visualized: phospholipids, cholesterol, free fatty acids, triacylglycerol and ethers of cholesterol. These compounds are the main structural elements of biological membranes, determining the main physical, chemical and functional characteristics of membranes of sperm cells [17].

The lipid profile of native sperm of different populations of sterlet (Danube, Dnipro, and Volga populations) did not show qualitative differences, except for the content of cholesterol and its ethers in sterlet from the Dnipro population.

Our study shows that there were impairments of the phospholipid bilayer of sperm membranes during freezing/thawing. Phospholipids decreased to 34 % in sterlet of the Danube population, to 35 % in those of the Dnipro population, and to 49 % in those of the Volga population as compared to their native sperm (Table 2).

The main factor, controlling the fluidity and permeability of the cellular membrane, is cholesterol. When the content of cholesterol is increased, the bilayer of lipids becomes less fluidal on the external surfaces and more fluidal on the internal, hydrophobic layer. It regulates the permeability of cellular membranes, dependent on temperature, and is capable of partial removal of the membrane-destabilizing action of lipids in the cooling process [18]. As the ratio of cholesterol and phospholipids in the sperm cells of fish is low and cholesterol is asymmetrically located, it is more present in the external layer of the membrane than in the internal one. Therefore, the internal layer of lipids is rather liable to cold shock [11, 19].

Our experiment showed that the content of cholesterol decreased from 59.6, 32.1 and 70.4 mg/100 ml in the native ejaculate to 23, 11, and 33 % for frozen sperm cells of the Danube, Dnipro, and Volga populations respectively (Table 2).

It is known that cholesterol and phospholipids, as structural components of biomembranes, form complexes of different stoichiometry in them, and the ratio of cholesterol and phospholipids (Gyorgyi’s coefficient) is one of the main indices for the degree of viscosity (fluidity) or rigidity of biological membranes [13, 14]. Gyorgyi’s coefficient in the sperm of Danube and Volga sterlet was 0.14 and in Dnipro sterlet it was

Table 2. The content of total lipids in sterlet sperm (Acipenser ruthenus L.) prior to and after cryopreservation

<table>
<thead>
<tr>
<th>Indices, mg/100 ml of ejaculate</th>
<th>Danube sterlet</th>
<th>Dnipro sterlet</th>
<th>Volga sterlet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>native</td>
<td>cryopreserved</td>
<td>native</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>272.0 ± 0.09</td>
<td>179.6 ± 1.28</td>
<td>181.9 ± 0.06</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>59.6 ± 0.04</td>
<td>25.9 ± 0.05</td>
<td>32.1 ± 0.003</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>33.1 ± 0.02</td>
<td>29.9 ± 0.07</td>
<td>39.9 ± 0.005</td>
</tr>
<tr>
<td>Tryacylglycerol</td>
<td>17.3 ± 0.07</td>
<td>13.7 ± 0.006</td>
<td>18.2 ± 0.002</td>
</tr>
<tr>
<td>Cholesterol ethers</td>
<td>20.4 ± 0.006</td>
<td>8.9 ± 0.003</td>
<td>10.5 ± 0.004</td>
</tr>
</tbody>
</table>

P ≤ 0.05; (M ± m, n = 5) from each population.
0.25. A low ratio of cholesterol and phospholipids in the ejaculate may lead to the impairment of permeability of the cellular membranes and destructive and functional changes in sperm which, at the end, leads to the decrease in resistance and reproductive capability of sperm cells [23, 24].

As for phospholipids and cholesterol, the concentration of ethers of cholesterol was lower in sperm after cryopreservation as compared to the native sperm. It is especially true for sterlet of the Volga population, where it was 9% less compared to the native state.

The changes in the ratio of the contents of free and etherized cholesterol were also determined. In the sperm of sterlet of Danube, Dnipro, and Volga populations the proportion between the content of free and etherized cholesterol was 29, 36, and 35% lower compared to the indices of the control ejaculate. The obtained data demonstrate that the level of etherized cholesterol in the sperm of sterlet of Dnipro and Volga populations was 7% and 6% higher respectively as compared to the Danube population.

Our experiment established furthermore that the level of free fatty acids in the sperm of the three populations of sterlet was 11, 14.5, and 25% lower as compared to the sperm which was not subject to cryopreservation.

It should be noted regarding the determined regularities of the content of free fatty acids that their special composition of cellular membranes of the sperm of sturgeons with prevailing essential shares of unsaturated fatty acids, which are predecessors of eicosanoids (prostaglandins, thromboxanes, and leukotrienes) and play the role of bioregulators of many physiological processes in the cell [15, 23], impacts the cryoresistance of the cells of these objects and requires principally novel approaches to elaborating more efficient types of cryoprotectors or membrane stabilizers.

It was established during the experiment that the concentration of tryacylglycerol in the fish ejaculate was lower compared to the native sperm of Danube sterlet by 12%, Dnipro sterlet by 13%, and Volga sterlet by 22%.

The obtained results indicate the important role that lipids of the plasmatic membrane of sperm cells may have in the resistance to cooling and the observed changes are in good agreement with the findings of other researchers [11, 13, 14]. It is known that the sensitivity of fish sperm cells to cold shock changes depending on time and temperature, absence or presence of cryoprotectants and the type of cryoprotectant. Decisive factors are the stage of maturity of sperm cells and the degree of ejaculate dilution [2, 14, 26]. Further studies of interactions between sperm cells and their environment during cryopreservation, under different temperature conditions and the addition of other cryoprotectants, are instrumental to have a deeper understanding of the ways of preventing or decreasing negative changes, caused by cryopreservation processes.

CONCLUSIONS

The impact of low temperatures on the lipid composition of reproductive cells of male sterlets (Acipenser ruthenus L.) in a deep-freezing environment leads to substantial changes therein as it does in other fish species. It was established that during the thawing of frozen sperm cells which preserved their viability after deep freezing, their lipid composition and motility were severely impaired.

Вплив кріоконсервації на ліпідний склад репродуктивних клітин самців стерляді (Acipenser ruthenus L.)

Л. П. Драган 1, С. П. Весельський 2, Ю. П. Рудь 1, Л. П. Бучацький 1, 2

e-mail: dragan_l@ukr.net

1 Інститут рибного господарства НААН, Вул. Обухівська 135, Київ, Україна, 03164
2 Київський національний університет ім. Тараса Шевченка, Вул. Володимирська, 60, Київ, Україна, 01033

Мета. Оцінити вплив низьких температур на ліпідний склад репродуктивних клітин самців стерляді (Acipenser ruthenus L.) за присутності кріозахисного середовища.

Методи. Визначення якості сперми (колір, консистенція, концентрацію та рухливість сперматозоїдів) оцінювали загальноприйнятими біохімічними методами та методом світлової мікроскопії з застосуванням стандартного обладнання. За допомогою тонкошарової хроматографії в спермі 10 досліджуваних рівень хроматографії в спермі 10 досліджуваних риб з трьох різних річкових регіонів – Дунаю, Дніпра та Волги, було виявлено п’ять фракцій нейтральних ліпі-дів. Всі фракції ліпідів (фосфоліпіди, холестерол, вільні жирні кислоти, триацилгіlycerоли та сфінголіпіди) продемонстрували низькі середні відсоткові показники (70, 12, 10, 5 та 3% відповідно) щодо трьох популяцій стерляді. Також було зафіксовано значне погіршення рухливості (в середньому на 50%). Результати. Отримані показники активності сперми стерляді до і після її кріоконсервації, свідчать про високу якість
нитивной спермы и её суттєве погіршення після заморожування/розморожування. **Висновки.** Вплив низьких температур на ліпідний склад репродуктивних клітин самців стерляді (Acipenser ruthenus L.) в присутності криозахисного середовища призводить до порушення fosfolіпідного бішару їх мембрани. Встановлено, що при відтауні замороженних спермів, які зберегли життєздатність після криоконсервації, відновляються в деякій мірі їх рухливість і здатність до запліднення яйцеклітини, при цьому відновлюється рухомість спермів.

**Ключові слова:** ліпіди, криозахисне середовище, кріо-банк.

**Вплив криоконсервації на ліпідний состав репродуктивних клеток самцов стерляді (Acipenser ruthenus L.) в присутстві криозахисної среды. Методы.** Определение качества спермы (цвет, консистенцию, концентрацию и подвижность сперматозоидов) оценивали общепринятые биохимическими методами и методом световой микроскопии с применением стандартного оборудования. С помощью тонкослойной хроматографии в течении 10 исследуемых рыб из трех разных речных регионов – Дунай, Днепр и Волги – было обнаружено пять фракций нейтральных липидов. Все фракции липидов (фосфолипиды, холестерол, свободные жирные кислоты, триацилглицеролы и эфиры холестерола) продемонстрировали более низкие средние процентные показатели (70, 12, 10, 5 и 3 % соответственно) в трех популяциях стерляді. Также было зафиксировано значительное ухудшение активности (в среднем на 50 %). **Результаты.** Полученные показатели активности спермы стерляди и после ее криоконсервации, свидетельствуют о высоком качестве нитивной спермы и ее существенное ухудшение после замораживания/размораживания.

**Выводы.** Влияние низких температур на липидный состав репродуктивных клеток самцов стерляді (Acipenser ruthenus L.) в присутствии криозахисной среды приводит к нарушению фосфолипидного бишара их мембран. Установлено, что при оттаивании замороженных сперматозоидов, которые сохранили жизнеспособность после криоконсервации, восстанавливает-ся в некоторой степени их подвижность и способность к оплодотворению яйцеклетки, при этом замедляется подвижность сперматозоидов.

**References**

13. Kuznetsov VI, Morrison VV, Lisko OB, Tsareva TD, Sreienskaya DA, Gavrilova IB, Hiebozarova OA. Lipids


ПРАВИЛА ДЛЯ АВТОРІВ

У журналах «Agricultural Science and Practice» публікуються результати фундаментальних і прикладних досліджень з питань ґрунтознавства, землеробства, рослинництва, ветеринарії, тваринництва, кормовиробництва, генетики, селекції та біотехнології, механізації, агроекології, радиології, меліорації, переробки та зберігання сільськогосподарської продукції, економіки, інноваційної діяльності.

Друкуються статті, які раніше не видавалися, огляди літератури, короткі повідомлення. Статті обов’язково реєструються на конфіденційній основі.


Комплект документів, необхідних для реєстрації статті

1. На нанері подаються (надсилаються):
   • один примірник рукопису українською або російською мовою (разом з таблицями і рисунками), пронумерований з першої до останньої сторінки і підписаний на останній сторінці тексту всіма авторами;
   • договір про передачу авторських прав, оформленний окремо кожним із співавторів, наприклад, 4 автори – 4 договори;
   • Звертаємо Вашу увагу на те, що договір про передачу авторських прав набуває чинності після прийняття статті до публікації. У разі відхилення Вашої статті редколегією журналу договір автоматично втрачає силу. Підписання договору автором (авторами) означає, що він (вони) ознайомлені та згодні з умовами договору;
   • лист – направлення від організації.

2. В електронному вигляді (електронною поштою або на CD/DVD дисках) представляються:
   • рукопис, ідентичний паперовій версії (прохання називати файл прізвищем першого автора статті англійською мовою, наприклад orlyk.doc);
   • всі ілюстрації у кольоровому і чорно-білому варіантах в одному зі стандартних графічних форматів – «ppt», «xls» або «psd» (ris1_orlyk.ppt, ris2_orlyk.xls);
   • інформація про авторів (auth_orlyk.doc): прізвища, імена, по батькові всіх авторів трьома мовами; при цьому необхідно використати одного з них для листування та зазначити його e-mail і номер телефону (з кодом), а також назви і поштові адresи установ з індексами, де виконано роботу, англійською, українською і російською мовами.
   Статті обов’язково супроводжуються англійською(російсько)-англійським словником специфічних термінів (не менше 30), використаних у статті (voc_orlyk.doc).

Оформлення рукопису

Матеріали для публікації необхідно подавати у форматі, підтримуваному Microsoft Word, розмір наперу A4, книжкова орієнтація, шрифт Times New Roman – розмір 14, міжрядковий інтерwał – 1,5.

Повний обсяг (включаючи текст, таблиці, рисунки та підписи до них, резюме трьома мовами, ключові слова і перелік літератури) експериментальної статті не повинен перевищувати 29 000 знаків з пробілами (~ 15 сторінок), оглядової статті - 52 000 знаків (26 сторінки), короткого повідомлення – 14 000 знаків (8 сторінок).

Рукопис має містити:
індекс УДК;
назву статті українською, російською і англійською мовами;
прізвища та ініціали усіх авторів трьома мовами;
назву і поштову адресу(и) установи(в), де працює(ють) автор(и), трьома мовами;
електронну пошту автора для листування.
Таблиці повинні мати заголовок і порядковий номер. Примітки до таблиць розміщують безпосередньо під ними.
Кількість ілюстрацій не може перевищувати 4 в оглядах, 6 – в експериментальних статтях і 2 – у короткому повідомленні. Всі громіздкі написи на рисунку слід замінити цифровими або літерними позначеннями, а їхнє пояснення перенести в підпис.
У пункті «Підтримка» при посиланнях на гранти необхідно вказувати фонд, назву гранту та/або номер.
Перелік літератури складається винятково англійською мовою (назви статей з періодичних видань повинні відповідати таким з англомовних резюме, розміщених у зазначених виданнях; заголовки монографій або статей з них також мають бути перекладені англійською мовою, транслітерація допускається лише у разі назв українсько-російсько-мовних періодичних видань (Agrarna nauka i osvita, російською мовою, місць видання та видавництв (Kharkiv, NNC «IGA im. О. Н. Sokolovsky»)) у порядку цитування, оформлення джерел слід здійснювати за прийнятим в журналі стандартом (див. приклади).
Посилання в переліку нумерують у порядку їхнього цитування в тексті, де їх позначають цифрою у квадратних дужках. Неприпустимо залишати гіперпосилання і посилатися на сайти в інтернеті. Джерела повинні бути загальномістю доступними, не можна посилатися на автореферати дисертацій.
Приклади оформлення списку літератури:
посилання на книгу –

на статтю з журналу –

на статтю з книги –


Увага!
Статті, оформлені не за правилами, повертаються авторам без реєстрації та розгляду.
Рецензування статей виконують незалежні експерти, призначені редакційною колегією, після чого автору надсилається примірник рукопису статті із зауваженнями рецензентів. Виправлені авторами варіант статті, погоджений з рецензентами, вважається остаточним і має бути підписаний рецензентами та авторами «До друку», після чого неприпустимими стають заміни тексту, рисунків або таблиць. При публікації статей редакція керується датою надходження останнього варіанту.
У разі відхилення рецензентами статті редакція надсилає автору письмове (e-mail) повідомлення.