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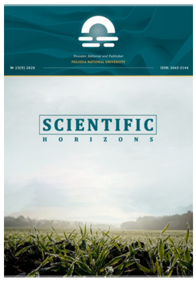

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Number of melanin-synthesising microorganisms under mineral fertilisation and liming of Albebeluvisoil

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Abstract. The study of the main patterns of distribution of microorganisms that synthesise melanins, which are precursors and components of humus molecules in agrocenosis soils is an urgent scientific task. The purpose of this study was to determine the influence of agrochemical factors on the number of melanin-synthesising microbial

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species and bacteria in grey forest soil. Microbiological, laboratory and analytical, and statistical methods were employed in the study. It was found for the first time that the number of melanin-synthesising micromycetes is minimal in the soil of the variant without fertilisers, liming with one dose according to hydrolytic acidity contributes to an increase in their number by 86.8%, application of mineral fertilisers in a dose of $N_{30}P_{30}K_{45}$ – 2.0 times, compatible use of lime and mineral fertilisers – 2.94 times. A 1.5-fold increase in the dose of mineral fertilisers leads to a 2.54-fold increase in the number of colony-forming units (CFU) of melanin-synthesising microbial species compared to a single dose of fertilisers, and a 2.0-2.62-fold increase in the dose of fertilisers, which coincides with the results of the analysis of the humus content in the soil of these variants – it exceeds the humus content in the variant with a single dose of fertilisers by 26.5% and 16.3%, respectively (correlation coefficient is 0.811). The number of melanin-synthesising bacteria in grey forest soil is 2-3 orders of magnitude higher than the number of melanin-synthesising micromycetes. However, the patterns of influence of anthropogenic factors on their number coincide with those established for micromycetes: liming with one dose of hydrolytic acidity leads to an increase in the number of melanin-synthesizing bacteria by 26.1%, application of mineral fertilizers in a dose of $N_{30}R_{30}K_{45}$ – 2.03 times, combined use of lime and mineral fertilisers – 2.48 times. A 1.5-fold increase in the dose of mineral fertilisers leads to a 5.8-fold increase in the number of melanin-synthesising bacteria compared to a single dose of fertiliser, while a 2.0-fold increase in the dose of fertiliser leads to a 13.3-fold increase, respectively. The correlation coefficient between the number of melanin-synthesising bacteria and the humus content in the soil is 0.417. The findings of the presented research can be used to develop recommendations for measures to increase the humus content of agricultural soils

Keywords: melanin-synthesising microorganisms; micromycetes; bacteria; azotobacter; liming; mineral fertiliser; humus

INTRODUCTION

Humus plays a fundamental role in the existence of biogeocenoses and agrocenoses. Its quantity and qualitative properties affect the soil's accumulation capacity, ensuring the level of effective fertility, and it accumulates carbon dioxide for a long time, reducing the negative impact of agriculture on climate change, etc. Therefore, the study of all processes that affect the accumulation of humus, and specifically, the spread of melanin-synthesising microorganisms, is relevant and practically significant. The importance of melanins for the synthesis of humus is underlined by the fact that a separate class of substances, haematomelanic acids, is now being identified as part of humus.

Melanins are the oldest biological pigments synthesised by representatives of all kingdoms of life (Zhdanova & Vasilevskaya, 1988). They are known for their ability to absorb broad-spectrum radiation, serve as an antioxidant, a photoprotector that effectively absorbs and dissipates solar radiation in the form of heat, a sorbent that chelates metals and binds organic compounds, and an organic semiconductor (Barreto *et al.*, 2023; El-Zawawy *et al.*, 2024). Specifically, A.S. Abd-El-Aziz *et al.* (2024), for melanin from the black fungus *Curvularia soli* AS21 ON076460, found antimicrobial, antiviral, and antitumour activity. The zones of growth inhibition of *Klebsiella pneumoniae ATCC 13883* and *Pseudomonas digitatum* were 37.51 ± 0.012 and 44.25 ± 0.214 mm, respectively. *Curvularia soli AS21* melanin demonstrated significant antiviral efficacy (77% inhibition) of herpes simplex virus (HSV1), as well as cytotoxic effects against human breast cancer and skin cancer cell lines (Mcf7 and A431), while causing a low percentage of cell death in normal human skin cell lines (Hfb4).

Fungi, especially ascomycetes, synthesise melanin-molecules in their cell walls to protect against fungal pathogens and harsh abiotic conditions (Baskaran *et al.*, 2019). A range of soil microorganisms of the genera *Bacillus*, *Aeromonas*, *Mycobacterium*, *Azotobacter*, *Streptomyces*, *Stachybotris*, *Aspergillus*, etc. synthesise melanins, which are analogous in their characteristics to humus molecules (Gerke, 2018). Model experiments have shown that these pigments are incorporated into humus molecules. The absorption spectra of melanin pigments of streptomycetes are comparable to those of natural humic acids. Their acidic properties are also analogous. In terms of optical characteristics and molecular weight, melanoproteins of streptomycetes are comparable to young humic acids. Humic compounds are rich in phenoxyl radicals, which can bind various organic and inorganic molecules, including amino acids, peptides, sugars, and melanins (Kulikova & Perminova, 2021).

In Ukraine, studies are currently underway to investigate the antioxidant or prooxidant properties of haematomelanic acids in radical chain oxidation processes, depending on the nature of the oxidative substrate (Efimova *et al.*, 2022). Specifically, it was shown that the addition of haematomelanic acids to the coumol and ethylbenzene oxidation system causes a slowdown in oxidation, with the observed effect increasing with increasing concentration of haematomelanic acids. It was also shown that the opposite effect is observed in the oxidation of ascorbic acid – acceleration of oxidation with the addition of haematomelanic acids, and the effect of acid concentration dependence was also established.

However, the ecology of melanin-synthesising microorganisms – what anthropogenic and natural factors and how they affect the number of melanin-synthesising microbes and bacteria (including azotobacter) – is still unexplored. This is especially important for agrocenosis soils, as the process of melaninogenesis directly affects the accumulation of humus and the level of potential soil fertility.

MATERIALS AND METHODS

The study was conducted in the stationary experiment of the Department of Agrosoil Science and Soil Microbiology of the National Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine" "Development and Improvement of Intensive Crop Growing Technologies Based on Extended Reproduction of Soil Fertility". The soil of the experimental plots is grey forest coarse-dusty light loam. The initial parameters of the humus state of the studied soil in the layer 0-20 cm were as follows: total humus content – 1.44%, type of humus formation – humate-fulvate – Cha – 31.9, Cfa – 40.4, Cnhr – 27.7%; Cha:Cfa = 0.79. The composition of the humus was dominated by the aggressive group of fulvic acids of fractions 1a and 1, which resulted in acidic, easily mobile soil humus, as fractions associated with mobile half-acids predominated. According to the classification of soils by granulometric composition, the soil of the experimental site belongs to the coarse dust-light loamy type: the coarse dust fraction prevails in the arable layer – 52.4%, the content of physical clay is 20.5%, and silt – 12.9%. The high content of the dust fraction and low content of the silt fraction determines a range of physicochemical and agrochemical properties of the soil under study, among which the key are low absorption capacity and low humus content, which causes low natural fertility of grey forest soil.

The object of research was the soil of the variants of the stationary experiment: 1 – without fertilisers (control); 2 – liming according to the indicator of hydrolytic acidity with a full dose of 1.0 Hg; 3 – introduction of mineral fertilisers in a dose of $N_{30}P_{30}K_{45}$; 4 – $N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg); fertilisation variants against the background of ploughing by-products of crop production (winter wheat) and biomass of green manure crops (fodder radish): 6 – $N_{30}P_{30}K_{45}$; 7 – $N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg); 8 – $N_{45}R_{45}K_{68;}$ 9 – $N_{60}R_{60}K_{90}$. Lime (limestone and dolomite powder) was applied in 1992 and again in 2005 in 1.0 and 1.5 doses according to hydrolytic acidity, 1/7 dose annually for each crop of the crop rotation and to neutralise the acidity of physiologically acidic mineral fertilisers (according to the value of hydrolytic acidity, the full dose of 1.0 Hg was 4.5-6.0 t/ha $CaCO_3$).

In 2019, white lupine of the ultra-early variety "Serpnevyi" was grown in the experimental variants, with winter wheat as the predecessor. The repetition of the experiment was fourfold, the area of the sowing plot was 60 m^2 (10×6), the accounting plot was 24 m^2 (6×4). Soil samples were taken from the root zone of plants on the day of sowing the soil suspension on the nutrient media. The number of microorganisms of the main ecological, trophic, functional, and systematic groups was assessed by sowing a water-soil suspension on the appropriate general, selective, and special nutrient media in three replicates (Alef & Nannipieri, 1995; Paul, 2015): ammonifiers consuming nitrogen from organic compounds – on meat-peptone agar, mineral nitrogen immobilisers – on starch-ammonia agar, pedotrophs – on soil agar (based on soil extract), oligotrophic microorganisms – on starvation agar, autochthonous microorganisms – on Vinogradskyi nitrite starvation agar, and azotobacter – on Ashby's medium. The number of colonies was counted for 21 days, depending on the growth rate and physiological characteristics of microorganisms of a particular ecological and trophic group. Indicators of the intensity of the processes of mineralisation of nitrogen compounds, soil organic matter, humus, probability of bacterial colony formation (BCF), and total biological activity (TBA) were determined using the method of relative values as described previously (Malynovska *et al.*, 2023). Generalisation of materials, calculation of research results, reliability of differences between samples were assessed using analysis of variance with subsequent assessment of least significant differences (LSD_{05}) using Statistica 5 software.

RESULTS

The results of the authors' research on the distribution of melanin-synthesising microorganisms under the influence of agrochemical factors in grey forest soil are presented in Table 1 and Figure 1. At the current stage of scientific development, the synthesis of melanoid pigments is considered to be a protective reaction of microorganisms to anthropogenic pollution of ecotopes, specifically, by radionuclides and heavy metals (Malynovska, 2017). It was shown that with an increase in the level of soil contamination with heavy metals from 5 to 100 MPC, the number of melanin-synthesising microbial species increases, while nitrogen-bacteria decreases: at a dose of heavy metals of 5 MPC – by 90.8%, at 10 MPC – 83.3%, at 100 MPC – 114.9%. The number and proportion of melanin-synthesising micromycetes in the total number of fungi can be used as a diagnostic indicator for grey forest soil contamination only if the contamination period does not exceed 24 months. It was shown that the nature of heavy metals' impact on the number of microorganisms depends on the presence of phytocoenosis in the monitoring system, the dose of heavy metals, the period of contamination, and the type of previous soil use.

These results confirm the following thesis: in the control variant of the experiment, the number of melanin-synthesising microbial species is minimal, since the soil of the control variant is the least contaminated with pollutants (Table 1). Liming leads to an increase in the number of melanin-synthesizing micromycetes by 86.8%, application of mineral fertilisers ($N_{30}P_{30}K_{45}$) – by 2.0 times, combined application of lime and mineral fertilisers – by 2.94 times. As a result of the application of agricultural practices, not only the number of melanin-synthesising micro-mycetes increases, but also their share in the total number of fungi. Thus, with liming in one dose, the proportion of melanin-synthesising microbial species increases by 73.7% in terms of hydrolytic acidity, with mineral fertilisation – by 1.9 times, and with the combined use of agricultural practices – by 3.99 times. A 1.5-fold increase in the dose of mineral fertilisers leads to a 2.54-fold increase in the number of melanin-synthesising microbial communities, and a 2.0-fold increase leads to a 2.62-fold increase. There is an increase not only in the number of melanin-synthesising microbial cells, but also in their proportion: at a one-and-a-half dose of fertiliser, it is 2.22 times higher, and at a double dose, it is 2.38 times higher. This confirms the assumption that the synthesis of melanoid pigments is a protective reaction not only to soil contamination by radionuclides, but also to general contamination of agrophytocenoses, which includes various pesticides, impurities in mineral fertilisers, residues of ameliorants, heavy metals, etc.

***Table 1**. Influence of agrochemical measures on the number of microorganisms in grey forest soil, mln CFU*/g of dehydrated soil*

Variant		Azotobacter, % fouling soil clod	Pedotrophs	Autochthonous	Micromycetes	Melanin-synthesising micromycetes	The share of melanin-synthetic micromycetes in the total amount, %	Melanin-synthesising bacteria	Proportion of melanin-synthesising bacteria in the total number, %	Total number of microorganisms
Without fertilisers (control)		69.3	48.6	17.1	0.448	0.0068	1.52	1.15	0.110	1,047.5
$CaCO_3$ (1.0 Hg)		92.6	129.8	14.8	0.483	0.0127	2.64	1.45	0.120	1,206.5
$N_{30}P_{30}K_{45}$		12.0	85.9	26.0	0.470	0.0136	2.89	2.33	0.182	1,283.3
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg)		98.7	51.0	15.8	0.337	0.0204	6.05	2.85	0.249	1,144.1
$N_{30}P_{30}K_{45}$	Green manure + by-products	15.3	22.5	22.5	0.340	0.0034	1.00	2.05	0.177	1,154.9
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg)	Green manure + by-products	94.0	15.9	15.9	0.432	0.0103	2.38	2.12	0.205	1,031.9
$N_{45}P_{45}K_{68}$ + $CaCO_3$ (1.0 Hg)	Green manure + by-products	73.3	22.7	22.7	0.496	0.0262	5.28	12.3	0.820	1,491.6
$N_{60}P_{60}K_{90}$ + $CaCO_3$ (1.0 Hg)	Green manure + by-products	80.7	52.7	52.7	0.476	0.0270	5.67	28.2	1.63	1,733.7
$LSD_{0.5}$		2.84	6.85	0.85	0.85	0.002	0.11	1.04	0.02	10.2

***Source:** compiled by the authors of this study*

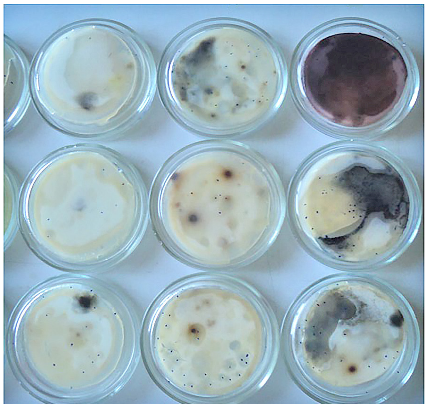


***Figure 1**. Synthesis of melanoid pigments by microorganisms of grey forest soil under ploughing of by-products of predecessor in crop rotation and green manure biomass*

***Note:** 1 – $N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg); 2 – $N_{45}P_{45}K_{68}$ + $CaCO_3$ (1.0 Hg); 3 – $N_{60}P_{60}K_{90}$ + $CaCO_3$ (1.0 Hg)*

***Source:** compiled by the authors of this study*

The patterns of distribution of bacteria capable of synthesising melanins and melanin-like pigments were also investigated. As Table 1 shows, the number of melanin-synthesising bacteria is 2-3 orders of magnitude higher than the number of melanin-synthesising fungi. However, the patterns observed for microorganisms of this group almost completely coincide with those obtained for melanin-synthesising micromycetes, specifically, it was shown that liming leads to an increase in the number of melanin-synthesising bacteria by 26.1%, the introduction of mineral fertilisers ($N_{30}P_{30}K_{45}$) – by 2.03 times, the combined use of lime and mineral fertilisers – by 2.48 times. As a result of the use of agricultural practices, not only the number of melanin-synthesising bacteria increases, but also their share in the total number of microorganisms. Thus, with liming with a single dose of hydrolytic acidity, the proportion of melanin-synthesising bacteria increases by 9.09% compared to the control, with mineral fertilisation with a dose of $N_{30}P_{30}K_{45}$ – by 65.5%, with the combined use of agromeasures – by 2.26 times.

A 1.5-fold increase in the dose of mineral fertilisers leads to a 5.8-fold increase in the number of melanin-synthesising bacteria compared to a single dose of fertiliser, and a 4.0-fold increase in their share (Table 1, Fig. 1). A 2.0-fold increase in fertiliser dose contributes to a 13.3-fold increase in the number of melanin-synthesising bacteria and a 7.95-fold increase in their share in the total number of microorganisms.

In lupine cultivation, liming enhances the mineralisation of soil organic matter without mineral fertilisation by 95.3%, against the background of ploughing exogenous organic matter (EOM) – by 13.9%, and with mineral fertilisation – does not weaken it (Fig. 2). These results are substantially different from those obtained in previous years, when liming weakened the mineralisation of soil organic matter and hindered the preservation of the pool of organic and organic-mineral substances. This may have been due to a decrease in mineralisation in the soil of the no-fertiliser variant, against which the results of the other variants are compared. Thus, in 2012, the pedotrophicity index in the control was 0.523 (winter wheat), in 2013 – 0.124 (soybean), in 2014 – 0.325 (spring wheat), in 2015 – 0.296 (buckwheat), which indicates that the content of organic matter in the soil of the control variant is gradually decreasing and, as a result, the intensity of organic matter mineralisation is decreasing. The index of oligotrophicity as a result of liming without mineral fertilisation increases by 80.4%, and against the background of mineral fertilisation and ploughing of green manure biomass and by-products of the predecessor crop in the crop rotation – decreases by 69.7% and 229.4%, respectively. A different trend was revealed when studying the mineralisation-immobilisation of nitrogen compounds: when liming without fertilisers, the mineralisation intensity increases by 71.4%, when using mineral fertilisers – by 14.3%, when ploughing green manure and by-products of the predecessor – by 66.9%.

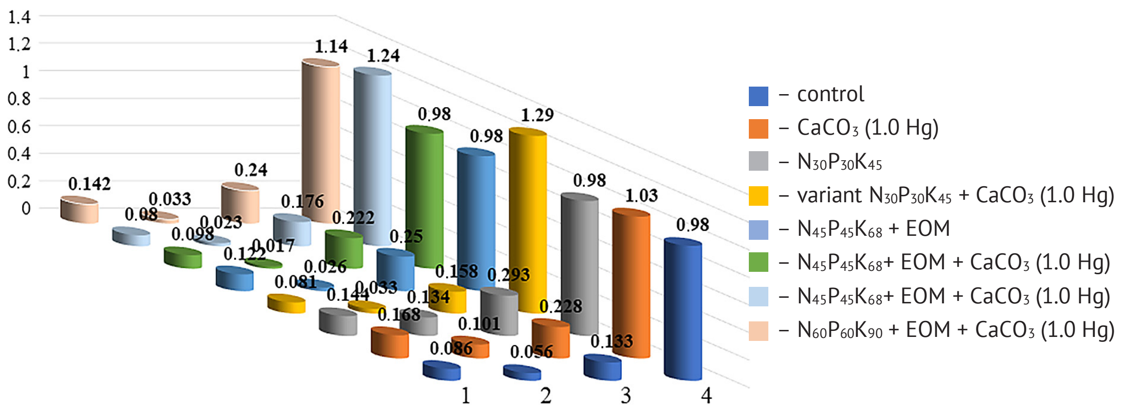


Figure 2*. Indicators of the intensity of mineralisation processes and humus content in grey forest soil under different agrochemical measures*

Note: *1 – pedotrophic index; 2 – oligotrophic coefficient 3 –nitrogen compounds mineralisation coefficient, 4 – humus content %*

Source: *developed by the authors of this study*

The regularities obtained in previous years regarding the effect of liming on the activity of humus mineralisation were also confirmed: it decreases as a result of liming the soil with a dose of 1 Hg without mineral fertiliser by 9.32%, with mineral fertiliser – by 13.5%, and against the background of ploughing green manure biomass and by-products of the predecessor – by 15.8% (Fig. 3). The application of mineral fertilisers in a single dose also slows down the mineralisation of humus compared to the control by 16.2%, together with liming – by 13.5%, against the background of ploughing by-products – by 19.3%; for the application of 1.5 doses of fertilisers against the background of ploughing by-products – by 9.66%; for the application of a double dose of fertilisers against the background of ploughing by-products, the mineralisation of humus is activated by 4.9% (Fig. 3).

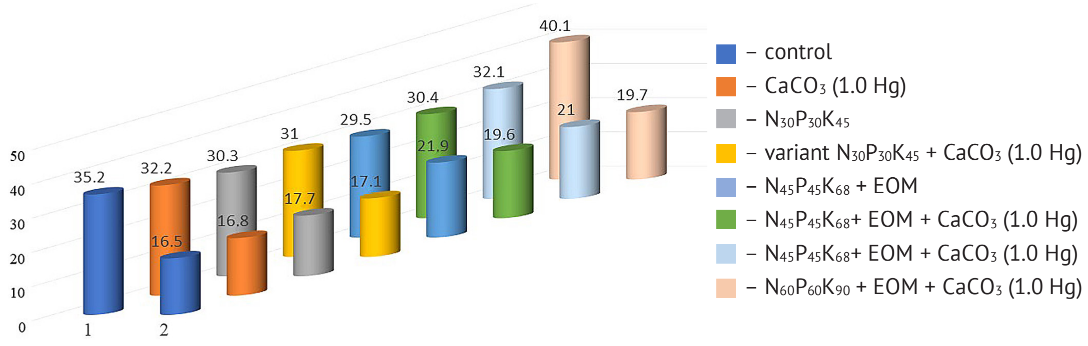


***Figure 3**. Indicators of humus mineralisation activity and phytotoxic properties of grey forest soil under different agrochemical measures*
***Note:** 1 – activity of humus mineralisation, %; 2 – weight of 100 plants of the test crop – winter wheat, g*
***Source:** developed by the authors of this study*

Neutralisation of excessive acidity of the soil solution allows increasing the total biological activity of the soil of the variant without mineral fertilisation by 31.3%, with mineral fertilisation – by 7.41, and against the background of ploughing exogenous organic matter (EOM) – by 6.79% (Fig. 4). Liming helps to reduce the level of soil phytotoxicity in all variants of application: without mineral fertiliser, with mineral fertiliser and with mineral fertiliser in the background of EOM application. The positive effect of liming is enhanced in variants with the introduction of organic matter (by-products of the predecessor and green manure) into the soil.

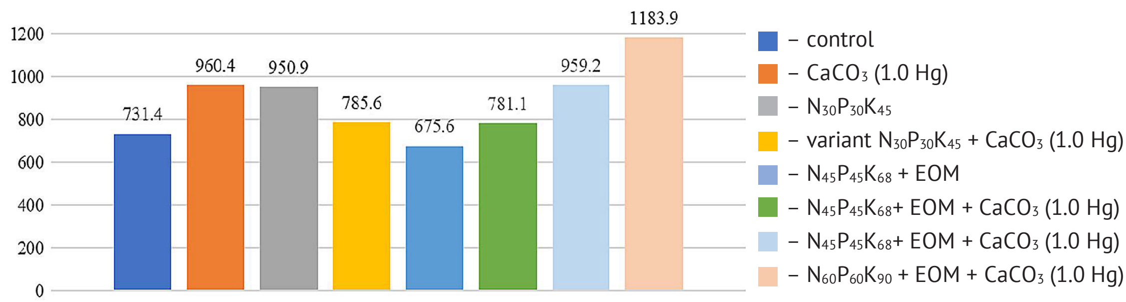
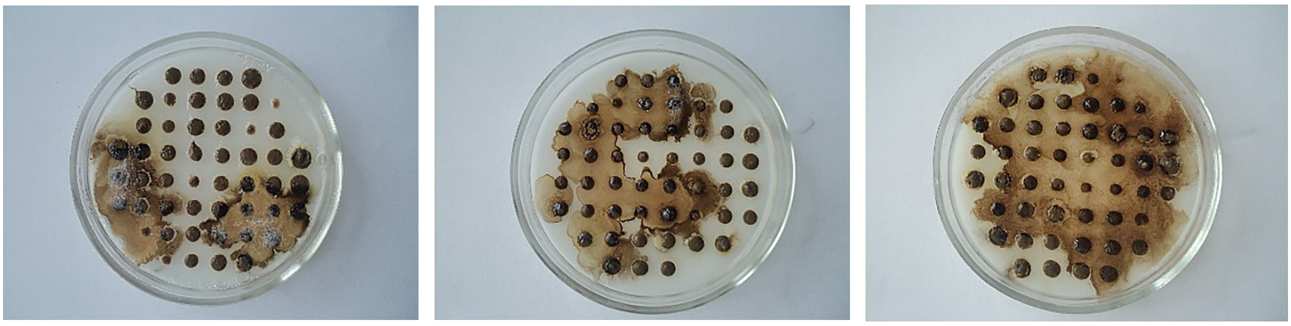


***Figure 4**. Indicators of total biological activity of grey forest soil under different agrochemical measures*
***Source:** developed by the authors of this study*

The total biological activity also increases under the influence of mineral fertilisation. The application of mineral fertilisers in a single dose leads to a 30.0% increase in TBA; in combination with liming – by 7.41%; against the background of ploughing by-products in combination with liming – by 6.80%; with the application of 1.5 dose of fertilisers against the background of ploughing by-products – by 31.1%; with the application of a double dose of fertilisers against the background of ploughing by-products – by 61.9% (Fig. 4). This highlights the overall impact of mineral fertilisers as a source of nutrients on the functioning of the phytos and microbiome.

Microorganisms of the genus *Azotobacter* also can synthesise melanin (Fig. 5). As Table 1 shows, the number of nitrogen-bacteria is closely related to the application of lime, its number reaches a maximum in the soil of the variants where the pH of the soil solution was neutralised. Liming without mineral fertilisers exceeds the control figures by 33.6%, while liming in combination with mineral fertiliser exceeds the control figures by 42.4%. The application of mineral fertilisers in a single dose without liming leads to a 5.78-fold reduction in the number of nitrogen-bacteria, while ploughing by-products reduces the number of nitrogen-bacteria by 4.53 times. An increase in the dose of fertiliser, even with simultaneous liming, helps to reduce the number of nitrogen-bacteria: a dose and a half of fertiliser inhibits the viability of this microorganism by 28.2%, and a double dose – by 16.5%.



a *b* *c*

***Figure 5**. Synthesis of melanins by bacteria of the genus Azotobacter under ploughing of by-products of the predecessor in crop rotation and green manure biomass*
***Note:** a – $N_{30}P_{30}K_{45}$; b – $N_{45}P_{45}K_{68}$ + $CaCO_3$ (1.0 Hg); c – $N_{60}P_{60}K_{90}$ + $CaCO_3$ (1.0 Hg)*
***Source:** developed by the authors of this study*

To establish the relationship between the indicators under study, a correlation analysis was conducted, which showed that the humus content in grey forest soil under white lupine cultivation is closely related to the number of melanin-synthesising micromycetes (r = 0.811) and their share in the total number of fungi (r = 0.915) (Table 2). Melanin-synthesising bacteria, despite being much more numerous than micromycetes, have a medium level of significance with humus content (r = 0.417), as does their share in the total number of microorganisms (r = 0.450). Therewith, the number of melanin-synthesising bacteria has a prominent level of correlation with the number of melanin-synthesising microbial species (r = 0.761), which indicates, in our opinion, that the distribution of melanin-synthesising fungi and bacteria is controlled by the same environmental factors. Notably, the number of melanin-synthesising bacteria is closely related to the activity of humus mineralisation (r = 0.805) and the number of autochthonous bacteria (r = 0.701), which are responsible for humus mineralisation. In general, all indicators of melanin synthesis (the number of producers, their share in the total number of microorganisms in this kingdom) are closely related.

Despite the high activity of melanin synthesis (Figure 5), the correlation between the number of nitrogen-bacteria and humus content has an average level of significance (r = 0.482) (Table 2). The determination of the physiological and biochemical activity of producer cells, in this case, the colony formation probability (CFP) of producers on agarified medium, is important for understanding the patterns of synthesis of any product or the intensity of the process in the soil. To determine the metabolic activity of microorganisms directly in the soil, the method of analysing the dynamics of colony formation was used, which makes it possible to simultaneously determine the number and composition of the complex of chemo-organoheterotrophic bacteria in the soil. Colony formation probability (CFP) reflects the physiological and biochemical activity of microbial cells in the natural environment. As Table 2 shows, only nitrogen-bacteria and autochthonous microorganisms have a medium level of significance with humus content. The correlation between the CFP of microbial species and humus content is insignificantly negative, possibly because the physiological and biochemical activity of all microbial species, not just melanin-synthesising ones, was determined. This is also clear from the fact that the physiological and biochemical activity of microbial cells depends on a range of factors: the moisture content of the soil in which they exist, the availability of substrates and energy sources, the phase of the population development cycle, etc. Therefore, the probability of colony formation can vary substantially during the growing season and generally does not correlate with the humus content of the soil.

***Table 2**. Correlation coefficients between indicators of microbial cenosis and humus content in grey forest soil for growing white lupine*

Indicator	Azotobacter, % fouling soil clod	Autochthonous	Micromycetes	Melanin-synthesising micromycetes	Share of melanin-synthetic micromycetes in the total amount, %	Melanin-synthesising bacteria	Proportion of melanin-synthesising bacteria in the total number, %	Total number of microorganisms	Humus mineralisation activity, %	PCF of micromycetes	PCF of azotobacter	CFP of autochthonous microorganisms	Humus content, %
Azotobacter, % fouling soil clod	1.000												
Autochthonous	0.097	1.000											
Micromycetes	0.132	0.489	1.000										
Melanin-synthesising micromycetes	0.418	0.431	0.390	1.000									
Share of melanin-synthetic micromycetes in total number, %	0.482	0.274	0.116	0.948	1.000								
Melanin-synthesising bacteria	0.183	0.701	0.378	0.761	0.628	1.000							
Share of melanin-synthesising bacteria in the total number, %	0.195	0.669	0.372	0.783	0.652	0.998	1.000						
Total number of microorganisms	0.007	0.743	0.483	0.806	0.643	0.931	0.932	1.000					
Humus mineralisation activity, %	0.322	0.673	0.434	0.492	0.377	0.805	0.782	0.658	1.000				
PCF of micromycetes	0.044	0.141	0.138	-0.158	-0.123	-0.496	-0.512	-0.262	-0.336	1.000			
PCF of azotobacter	0.525	-0.364	-0.469	0.164	0.393	-0.104	-0.085	-0.285	-0.193	-0.185	1.000		
PFC of autochthonous microorganisms	0.586	-0.291	-0.248	0.134	0.360	-0.241	-0.239	-0.361	-0.018	0.200	0.785	1.000	
Humus content, %	0.482	0.034	-0.102	0.811	0.915	0.417	0.450	0.445	0.176	-0.069	0.360	0.334	1.000

Source: *compiled by the authors of this study*

Thus, melanin-synthesising microorganisms of grey forest soil, synthesising melanins, have a substantial impact on humus accumulation. The spread of melanin-synthesising microorganisms depends on the agronomic practices used in a given agrocenosis, soil type, and the level of soil pollution by pollutants.

DISCUSSION

Experimental data showing that increasing the dose of mineral fertilisers by 1.5 and 2.0 times leads to an increase in the number of melanin-synthesising bacteria and fungi confirms the assumption that with the deterioration of environmental conditions: pollution by agrochemical residues and an increase in the dose of mineral fertilisers, the synthesis of melanoid pigments is enhanced by both the bacterial and fungal components of the soil microbiota (Malynovska, 2017). The increase in the synthesis of melanoid pigments, especially with the introduction of one and a half and double doses of mineral fertilisers, coincides with the results of the analysis of the humus content in the soil of these variants – it exceeds the humus content in the soil of the variant with the introduction of a single dose of mineral fertilisers by 26.5% and 16.3%, respectively (Fig. 1, 2). However, it is possible that the increase in melanin synthesis in this case is associated not only with the deterioration of environmental conditions, but also with the optimisation of mineral nutrition of the phytocoenosis of this ecotope and, as a result, an increase in the number of soil microorganisms in the root zone, including melanin-synthesising ones (Jacoby *et al.*, 2017; Dang *et al.*, 2022). Addressing this issue is a task for the development of a methodological framework and further research in this area.

The fact that the number of azotobacter decreased with increasing fertiliser doses, even with simultaneous liming, indicates the inhibitory effect of nitrogen fertilisers on the number of CFU of freely existing nitrogen-fixing organisms, which include azotobacter. This is consistent with the well-known fact that high doses of nitrogen-containing substances inhibit the development of nitrogen-fixing microorganisms (Noar & Bruno-Bárcena, 2018; Knutson *et al.*, 2018). Thus, the patterns of distribution of azotobacter as a melanin producer largely contradict those observed for melanin-synthesising bacteria and micromycetes, whose numbers increase with increased doses of mineral fertilisers. The authors of this study believe that azotobacter is one of the microorganisms-indicators of ecological cleanliness of soil by pollutants of anthropogenic origin, its number decreases with increasing pollution levels (Malynovska, 2017) and, accordingly, melanin synthesis is more active in ecologically safe ecotopes (variants of agrocenosis). Thus, the environmental and agrochemical properties of the soil affect the synthesis of melanins by nitrogen bacteria and melanin-synthesising bacteria and micromycetes in opposite ways, which makes it difficult to assess the contribution of individual components of the soil microbiota to humus accumulation.

The high activity of melanin synthesis by azotobacter cells (Fig. 5) is not accompanied by a close correlation between the number of nitrogenobacteria and humus content (r = 0.482). This may be due to the fact that a large number of nitrogenobacteria with high melanin synthesis activity are observed in the soil under lupine cultivation, while other crop rotation crops have the opposite effect on the number and activity of nitrogenobacteria cells, and the overall contribution of nitrogenobacteria to the accumulation of melanins, which are precursors of humus substances, should be investigated and evaluated during the crop rotation under all crops.

Organic matter of plant origin is known to have a high sorption capacity for various pollutants (Duarte *et al.*, 2017; Tran-Ly *et al.*, 2020a; 2020b) and protects soil biota and plants from the negative effects of pollutants, which is reflected in the reduction in the number of melanin-synthesising microbes and bacteria in the experimental variants with the introduction of exogenous organic matter. The thesis about reducing the content of toxic substances in the soil as a result of ploughing exogenous organic matter is confirmed by the results of the study of soil phytotoxicity: it decreases in variants with the introduction of EOM by an average of 20.9% (Fig. 3). Liming has a multifaceted effect on humus accumulation. Firstly, due to a decrease in the number of autochthonous bacteria (by 15.5%), their physiological and biochemical activity (by 10.4%), a decrease in the activity of humus mineralisation (by 9.32%), and an increase in the number of melanin-synthesising microorganisms: azotobacter, micromycetes, and bacteria.

Earlier, for typical chernozem, it was found that the relationship between humus content and the number of melanin-synthesising bacteria (r = 0.528) and their share in the total number of microorganisms (r = 0.470) is of medium significance, which is much higher than the level of the relationship between humus content and the number of melanin-synthesising microbial communities (r = -0.052) (Malynovska *et al.*, 2023). This suggests that in black soil, typical for sunflower cultivation, humus is formed mainly by melanins of bacterial origin. For grey forest soil, the opposite trends are observed: it is with the involvement of micromycetes melanins that humus molecules are synthesised in this type of soil when growing white lupine (r = 0.811). In typical chernozem, a low inverse correlation coefficient (r = -0.099) was found between the humus content and the number of azotobacter, and for grey forest soil the corresponding indicator was 0.482 (with the PFC of Azotobacter r = 0.360), which suggests that melanins of bacteria of the genus *Azotobacter* are insignificantly involved in the synthesis of humus molecules when growing sunflower on typical black soil, but this trend is reversed when growing white lupine on grey forest soil (Feszterová & Hudec, 2022).

For grey forest soil, a correlation of medium significance was found between the number of polysaccharide-synthesising bacteria and humus content (r =0.417). For the typical chernozem, the existence of a direct average relationship between the physiological and biochemical activity of polysaccharide-synthesising bacteriacells and humus content (r = 0.532) was confirmed, which reflects the increased resistance of humus molecules to mineralisation by autochthonous microorganisms in soils of both types.

Thus, the agricultural practices used in modern agriculture – neutralising excessive acidity of the soil solution, applying mineral fertilisers, especially in high doses – contribute to the growth of microorganisms that synthesise the constituent components of humus molecules, such as melanoid pigments. Ploughing up crop by-products and green manure biomass leads to de-intensification of melanin synthesis due to a decrease in the distribution of their producers.

CONCLUSIONS

The investigated agrochemical measures affect the process of melaninogenesis in the soils of agrocenoses by representatives of the main kingdoms of life – prokaryotes and fungi (micromycetes). The nature and direction of the impact depend on the type of agricultural measure, the crop being grown, and the systematic, as well as functional, affiliation of the group of microorganisms producing melanins. Liming with one dose according to hydrolytic acidity leads to an increase in the number of melanin-synthesising micromycetes and bacteria by 86.8% and 26.1%, respectively; application of mineral fertilisers in a dose of $N_{30}P_{30}K_{45}$ – 2.0 and 2.04 times, combined application of lime and mineral fertilisers – 2.94 and 2.48 times, respectively. A 1.5-fold increase in the dose of mineral fertilisers leads to a 2.54-fold increase in the number of CFU of melanin-synthesising micromycetes compared to a single dose of fertilisers, and a 5.8-fold increase in bacteria; a 2.62-fold and 13.3-fold increase in the dose of fertilisers doubles the number of melanin producers, respectively.

It was shown that when growing white lupine on grey forest soil, the humus content is closely related to the number of melanin-synthesising microbial species (r = 0.811) and their share in the total number of fungi (r = 0.915), the correlation coefficient with the number of melanin-synthesising bacteria and nitrogen-bacteria is 0.417 and 0.482, respectively. The distribution of melanin-synthesising fungi and bacteria is controlled by the same environmental or agronomic factors, as evidenced by the existence of a high level of correlation between the number of melanin-synthesising bacteria and the number of CFU of melanin-synthesising micromycetes (r = 0.761).

The study of the influence of agrochemical factors on the processes of melanin synthesis by microorganisms of grey forest soil has the prospect of continuing with the involvement of other types of soils and soils with different levels of anthropogenic pollution affecting the synthesis of melanoid pigments.

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CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

REFERENCES

[1] Abd-El-Aziz, A.S., Abed, N.N., Mahfouz, A.Y., & Fathy, R.M. (2024). Production and haracterization of melanin pigment from black fungus *Curvularia soli* AS21 ON076460 assisted gamma rays for promising medical uses. *Microbial Cell Factories*, 23, article number 68. doi: 10.1186/s12934-024-02335-y.

[2] Alef, K., & Nannipieri, P. (1995). *Methods in applied soil microbiology and biochemistry.* Elsevier: Academy Press. doi: 10.1016/B978-0-12-513840-6.X5014-9.

[3] Barreto, J.V.O., Casanova, L.M., Junior, A.N., Reis-Mansur, M.C.P.P., & Vermelho, A.B. (2023). Microbial pigments: Major groups and industrial applications. *Microorganisms,* 11(12), article number 2920. doi: 10.3390/microorganisms11122920.

[4] Baskaran, P., Ekblad, A., Soucémarianadin, L., Hyvönen, R., Schleucher, J., & Lindah, B. (2019). Nitrogen dynamics of decomposing Scots pine needle litter depends on colonizing fungal species. *FEMS Microbiology Ecology*, 95(6), article number fiz059. doi: 10.1093/femsec/fiz059.

[5] Dang, P., Li, C., Lu, Ch., Zhang, M., Huang, T., Wan, Ch., Wang, H., Chen, Y., Qin, X., Liao, Yu., & Siddique, K.H.M. (2022). Effect of fertilizer management on the soil bacterial community in agroecosystems across the Globe. *Agriculture, Ecosystems & Environment*, 326, article number 107795. doi: 10.1016/j.agee.2021.107795.

[6] Duarte, A., Cachada, A., & Rocha-Santos, T. (2017). *Soil pollution: From monitoring to remediation* (1st Ed.). London: Academic Press.

[7] Efimova, I.V., Smirnova, O.V., & Bessarabov, V.I. (2022). Oxidation of organic substrates of different natures in the presence of humic and hematomelanic acids. *Technologies and Engineering*, 6, 82-88. doi: 10.30857/2786-5371.2022.6.8.

[8] El-Zawawy, N.A., Kenawy, E.R., Ahmed, S., & El-Sapagh, S. (2024). Bioproduction and optimization of newly characterized melanin pigment from *Streptomyces djakartensis* NSS-3 with its anticancer, antimicrobial, and radioprotective properties. *Microbial Cell Factories,* 23(1), article number 23. doi: 10.1186/s12934-023-02276-y.

[9] Feszterová, M., & Hudec, M. (2022). The distribution of metallic elements among humus substances in soil from volcanic rocks. *Sustainability*, 14(24), article number 16427. doi: 10.3390/su142416427.
[10] Gerke, J. (2018). Concepts and misconceptions of humic substances as the stable part of soil organic matter: A review. *Agronomy*, 8(5), article number 76. doi: 10.3390/agronomy8050076.
[11] Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., & Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition – current knowledge and future directions. *Frontiers in Plant Science*, 8, article number 1617. doi: 10.3389/fpls.2017.01617.
[12] Knutson, C.M., Plunkett, M.H., Liming, R.A., & Barney, B.M. (2018). Efforts toward optimization of aerobic biohydrogen reveal details of secondary regulation of biological nitrogen fixation by nitrogenous compounds in *Azotobacter vinelandii*. *Applied Microbiology and Biotechnology,* 102, 10315-10325. doi: 10.1007/s00253-018-9363-0.
[13] Kulikova, N.A., & Perminova, I.V. (2021). Interactions between humic substances and microorganisms and their implications for nature-like bioremediation technologies. *Molecules*, 26(9), article number 2706. doi: 10.3390/molecules26092706.
[14] Malynovska, I. (2017). Influence of heavy metal pollution on the number, physiological and biochemical activiti of Azotobacter and melanin-synthesizing micromicetes. *Biotechnologia Acta*, 10(3), 65-71. doi: 10.15407/biotech10.03.065.
[15] Malynovska, I., Kaminskyi, V., Zadubynna, Ye., Kurhak, V., & Karpenko, V. (2023). The influence of agrotechnical measures on the number of melanin-synthesizing microorganisms. *Scientific Horizons*, 26(8), 52-61. doi: 10.48077/scihor8.2023.52.
[16] Noar, J.D., & Bruno-Bárcena, J.M. (2018). *Azotobacter vinelandii*: The source of 100 years of discoveries and many more to come. *Microbiology*, 164(4), 421-436. doi: 10.1099/mic.0.000643.
[17] Paul, E.A. (2015). *Soil microbiology, ecology and biochemistry* (4th Ed.). Fort Collins: Elsevier Science. doi: 10.1016/C2011-0-05497-2.
[18] Tran-Ly, A.N., Reyes, C., Schwarze, F.W.M.R., & Ribera, J. (2020a). Microbial production of melanin and its various applications. *World Journal of Microbiology and Biotechnology,* 36, article number 170. doi: 10.1007/s11274-020-02941-z.
[19] Tran-Ly, A.N., Ribera, J., Schwarze, F.W., Brunelli, M., & Fortunato, G. (2020b). Fungal melanin-based electrospun membranes for heavy metal detoxification of water. *Sustainable Materials and Technologies,* 23, article number e00146. doi: 10.1016/j.susmat.2019.e00146.
[20] Zhdanova, N.N., & Vasilevskaya, A.N. (1988). *Melanin-containing mushrooms in extreme conditions*. Kyiv: Naukova Dumka.

Чисельність меланінсинтезувальних мікроорганізмів за мінерального удобрення і вапнування сірого лісового ґрунту

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Анотація. Дослідження основних закономірностей розповсюдження мікроорганізмів, що синтезують меланіни, які є попередниками і складовими гумусових молекул, в ґрунтах агроценозів є актуальним завданням науки. Метою представленої роботи було встановлення впливу агрохімічних чинників на чисельність меланінсинтезувальних мікроміцетів і бактерій у сірому лісовому ґрунті. В роботі було використано мікробіологічний, лабораторно-аналітичний, статистичний методи. Вперше встановлено, що чисельність меланінсинтезувальних мікроміцетів є мінімальною у ґрунті варіанту без добрив, вапнування однією дозою за гідролітичною кислотністю сприяє збільшенню їхньої чисельності на 86,8 %, внесення мінеральних добрив у дозі $N_{30}P_{30}K_{45}$ – в 2,0 рази, сумісне застосування вапна і мінеральних добрив – у 2,94 рази. Збільшення дози мінеральних добрив в 1,5 рази призводить до зростання кількості колонієутворювальних одиниць (КУО) меланінсинтезувальних мікроміцетів порівняно із одинарною дозою добрив у 2,54 рази, збільшення дози добрив у 2,0-2,62 рази, що співпадає із результатами аналізу вмісту гумусу у ґрунті цих варіантів – він перевищує вміст гумусу у варіанті із одинарною дозою добрив на 26,5 та 16,3 % відповідно (коефіцієнт кореляції становить 0,811). Чисельність меланінсинтезувальних бактерій в сірому лісовому ґрунті на 2-3 порядки вища за чисельність меланінсинтезувальних мікроміцетів, однак, закономірності впливу антропогенних чинників на їхню чисельність співпадають із встановленими для мікроміцетів: вапнування однією дозою за гідролітичною кислотністю призводить до збільшення чисельності меланінсинтезувальних бактерій на 26,1 %, внесення мінеральних добрив у дозі $N_{30}P_{30}K_{45}$ – в 2,03 рази, сумісне застосування вапна і мінеральних добрив – у 2,48 рази. Збільшення дози мінеральних добрив у 1,5 рази призводить до зростання чисельності меланінсинтезувальних бактерій порівняно із одинарною дозою добрив у 5,8 рази, збільшення дози добрив у 2,0 рази – відповідно у 13,3 рази. Коефіцієнт кореляції між чисельністю меланінсинтезувальних бактерій і вмістом гумусу у ґрунті складає 0,417. Результати представлених досліджень можуть бути використані при розробленні рекомендацій щодо заходів підвищення вмісту гумусу в ґрунтах сільськогосподарського використання

Ключові слова: меланінсинтезувальні мікроорганізми; мікроміцети; бактерії; азотобактер; вапнування; мінеральне удобрення; гумус