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[1]Natalia KOVALZHY[1], Serhii RIEZNIK[1], Andrii BUTENKO[2*], Dmytro HAVVA[1], Vasyl DEGTYARJOV[1], Anna HOTVIANSKA[3], Oksana BONDARENKO[3], Nataliia NOZDRINA[3]

ACTIVITY OF CELLULOSE–DEGRADING MICROORGANISMS IN TYPICAL CHERNOZEM UNDER DIFFERENT FERTILIZATION SYSTEMS OF STRAWBERRIES (*FRAGARIA*)

SUMMARY

To determine the effect of fertilization systems on indicators of cellulose–degrading activity of microorganisms of a typical deep heavy loamy chernozem under the bed technology of growing garden strawberries using drip irrigation. Methods. Comparative profile, microbiological, mathematical and statistical. The cellulose–destroying activity of soils was carried out by the method of deep sowing of soil suspension on a dense agarized medium (Hutchinson–Clayton agar). The glucose formed after the decomposition of carboxymethyl cellulose (CMC) was determined by titration with sodium hyposulfite. Also, the activity of cellulose–destroying microorganisms was additionally evaluated by the rate of decomposition of cotton fabric (size 10*10 cm) during six months. The degree of decomposition was calculated based on the difference in weight. Results. Statistical analysis of the results of our research confirmed the significant influence on indicators of the number of cellulose–degrading microorganisms and cellulase activity of such factors as sampling depth and fertilization system, in particular, sampling depth has a more significant effect than fertilization system and vice versa, the fertilization system has a more significant effect on decomposition fabrics The highest activity of cellulase is characteristic of chernozem of the fallow area, and among the strawberry variants, the highest indicators were recorded under the condition of using organic fertilizers. An increase in cellulase activity at a depth of 20–30 cm in arable land as a result of

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[1]Natalia Kovalzhy, Serhii Rieznik, Dmytro Havva, Vasyl Degtyarjov, State Biotechnological University, Alchevsky St., 44, UA61000, Kharkiv, UKRAINE
[2]Andrii Butenko*, (Corresponding author: andb201727@ukr.net), Sumy National Agrarian University, 160 H. Kondratieva St., UA40021, Sumy, UKRAINE;
[3]Anna Hotvianska, Oksana Bondarenko, Nataliia Nozdrina, Dnipro State Agrarian and Economic University, 25 Sergey Efremov St., UA49600, Dnipro, UKRAINE.
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plowing plant residues was noted. The highest cellulase activity was recorded in chernozem from the fallow area (6.4–11.2 μg of glucose per 1 g of soil in 7 days) and somewhat lower in strawberries, and comparing fertilization systems, we are talking about the positive effect of organic fertilizers (5.2–6.7 μg of glucose per 1 g of soil for 7 days). Conclusion. The largest number of cellulose–destroying microorganisms was recorded in summer, but enzymatic activity is greater in spring and autumn. Perennial strawberry plantations have higher biological activity compared to arable land, and a comparison of different fertilization systems shows the advantage of organic fertilization. Cultivated soils are characterized by a decrease in the activity of soil enzymes compared to fallow. In the strawberry area, under the organo–mineral system of fertilization, the decomposition of cotton fabric is more intense, even compared to fallow.

Keywords: typical chernozem, fertilization systems, soil microorganisms, enzymatic activity, cellulase.

INTRODUCTION

At the present stage of soil genesis, the most influential factor there is human agricultural activity (Tikhonenko, 2011; Bulyhin *et al.* 2018). Agrogenic soil formation is fundamentally different from natural, especially the rate of transformation of organic and mineral parts of the soil (Demydenko, 2021; Rieznik *et al.* 2023). Activity cellulose–degrading microorganisms in the soil is one of the most important indicators of its overall biological activity (Soares *et al.* 2012; Rieznik *et al.* 2021). The decomposition of cellulose is brought about by complex communities of interacting microorganisms (Mahanta *et al.* 2014; Dimova *et al.* 2023). Products of cellulose hydrolysis are available as carbon and energy sources for other microbes that inhabit environments in soil (Fomenko *et al.* 2021; Long *et al.* 2024; Karbivska *et al.* 2020). Human agricultural activity often leads to the suppression of soil biosphere functions and has a negative impact on the ecosystem (Kanivets, 2015; Volkohon *et al.* 2019; Kolisnyk *et al.* 2024). Therefore, the study of soil biological activity under different farming practices is especially relevant.

MATERIAL AND METHODS

Typical deep, medium–humus, heavy loam chernozems on the loess (molik, mollisol) on the territory of the State Biotechnological University, Kharkiv, Ukraine (Forest–Steppe zone of Ukraine), were chosen as the objects of research. The options for the study were selected plots under a natural phytocenosis (fallow land since 1946), a plot under a traditional system of cultivation (corn, the predecessor of which was sunflower with yield 2.7 t ha^{-1}, arable land for more than 100 years) and options for growing garden strawberries with drip irrigation under different fertilization systems. In the variant of growing corn, 40 t ha^{-1} of cattle manure was applied as the main fertilizer in the fall, followed by plowing to a depth of 22–24 cm. In March, harrowing was carried out, and in May the Leka hybrid corn (a simple interline corn hybrid of

Ukrainians selection) was sown with a sowing rate of 70,000 seeds ha^{-1}. Pre–sowing cultivation and sowing were carried out to a depth of 4–6 cm. During sowing, 160 kg ha^{-1} of nitroammophoska ($N_{16}P_{16}K_{16}$) were applied. Top dressing was carried out in the phase of 5–7 leaves with ammonium nitrate (N – 34.4%) in a dose of 80 kg ha^{-1}. Fallow and arable land in our studies served as a kind of control in the study of microbiological indicators of the soil. An experiment with strawberries of the Roxana variety was planted in the fall of 2018 on an area of 0.3 ha, the predecessor of which was pure steam, and the average yield is 15–17 t ha^{-1}. The plot is divided into 4 options (each option has 4 rows): 1. Without fertilizer. 2. Mineral fertilizer system ($N_{64}P_{64}K_{64}$). 3. Organo–mineral fertilization system ($N_{64}P_{64}K_{64}$ + 50 t ha^{-1} of humus). 4. Organic fertilization system (50 t ha^{-1} of humus). Nitroamophoska ($N_{16}P_{16}K_{16}$) was used as a fertilizer at a dose of 400 kg ha^{-1} and cattle manure – 50 t ha^{-1}. Strawberries were planted using a tape method of 25*130 cm with row shift, the distance between plants 25 cm. The formed ridges were covered with a black plastic wrap after laying the drip tape. Watering was carried out as needed to ensure constant soil moisture within 75% of the full soil moisture content.

The growing technology involves mulching the rows with straw and treatment with herbicides based on clopyralid 300 g l^{-1} + fluazifop–p–butyl 150 g l^{-1} at the end of May. Plant feeding is carried out by fertigation with a solution of urea–ammonium nitrate (UAN–32) at the rate of 120 kg ha^{-1} at the end of March. Protection of plants from pests and diseases was carried out in several stages with the following tank mixtures: at the beginning of May, an acaricide based on two active substances, hexityazox 204 g l^{-1} + abamectin 36 g l^{-1}, is applied; after two weeks, the insecticide pyridaben, 150 g l^{-1} + pirimifos–methyl, 200 g l^{-1} + acetamiprid, 50 g l^{-1} was applied with the addition of liquid complex fertilizers with trace elements in chelated form; two weeks later, the fungicide cyprodinil 375 g l^{-1} + fludioxonil 250 g l^{-1} was added, also with the addition of liquid complex fertilizers with trace elements in chelated form. All drugs are introduced in accordance with the manufacturer's recommendations.

The selection of soil samples was carried out three times, layer by layer, every 10 cm to a depth of 40 cm, and in strawberry areas – and from the ridge. The research was conducted in 2021, and individual soil samples were taken by season. To count the number of cellulose–destroying microorganisms, the method of deep seeding of a soil–water suspension on a dense agar medium (Hutchinson–Clayton agar) containing carboxymethylcellulose (CMC) was used. Cellulase activity was determined based on the ability of the enzyme to decompose biopolymers (CMC) to glucose, the amount of which is determined iodometrically by back titration with sodium hyposulfite. The activity of cellulose–destroying microorganisms was additionally evaluated by the rate of decomposition of cotton fabric. Pieces of cotton fabric measuring 10*10 cm were buried in the ground for six months, and the degree of decomposition was calculated based on the difference in weight. All specified methods of studying the biological activity of the soil are described by Volkohon *et al.* (2010) and

Shchukovs′kyy *et al.* (2002). Analyzes were performed in triplicate. Analyzes were performed in triplicate.

Mathematical analysis of the data was performed with Microsoft Excel 2010 and Statgraphics 18.1 trial. Multifactor ANOVA and Correlation analysis were used.

RESULTS AND DISCUSSION

Our previous studies indicate a significant increase in biological activity and acceleration of the processes of mineralization of organic substances under the organo–mineral fertilization system (Kovalzhy, 2021; Karbivska *et al.* 2022). It was also confirmed that the improvement of physical and chemical indicators under the organic and organo–mineral fertilization system (Dehtiar'ov *et al.* 2021). In this article, we decided to highlight in more detail the annual dynamics of the activity of cellulose–destroying microorganisms (Table 1).

First of all, it should be noted that the number of cellulose–decomposing microorganisms is greatest in the upper part of the soil profile at a depth of up to 10 centimeters. The exception is the option of arable land, where the soil is turned over annually during plowing. Therefore, the number of bacteria and their activity will increase in the spring and autumn at the depth of wrapping plant residues and fertilizers (10–20 cm). In other versions, the number of cellulose–destroying microorganisms is regulated by hydrothermal indicators and the amount of plant residues that enter the soil. Therefore, in variants with strawberry, an unnatural situation is also created with the formation of a ridge mulched with a non–transparent plastic wrap. Under the plastic wrap, optimal conditions are created for the rapid decomposition of cellulose, and the supply of fresh plant residues is limited. Therefore, the largest number of cellulose–destroying microorganisms in the soil was recorded in the fallow variant (45.3 CFU*106 per 1 g dry soil), and in the experiment with strawberry – under the condition of applying organic fertilizers (6.0 CFU*106 per 1 g dry soil). This is consistent with the results of the study obtained by a team of Indian scientists Sangma *et al.* (2018).

Enzymatic activity of chernozem gradually decreases with depth and has similar dynamics with the number of cellulose–destroying microorganisms. In strawberry variants, a significant decrease into enzymatic activity into the ridge was recorded due to rapid mineralization of plant residues and organic fertilizers (Table 2). We also note the increase in cellulase activity at a depth of 20–30 cm in arable land as a result of plowing plant residues. The seasonal dynamics of enzymatic activity is slightly different from the dynamics of the number of microorganisms, and the correlation analysis showed a weak relationship between these indicators r=23. An increase in enzymatic activity is observed in spring and autumn, which indicates the additional influence of other factors such as: humidity, the presence of a substrate and the content of humus in the soil. In general, it was found that the highest activity of cellulase is characteristic of chernozem of the fallow area, and among strawberry variants, the highest indicators were recorded under the condition of using organic fertilizers. At that time, it is worth noting that the enzymatic activity of the typical chernozem we studied is significantly lower than in the chernozems of the forest–steppe zone of Ukraine, but the seasonal dynamics and general trends are similar (Rieznik *et al.* 2021; Radchenko *et al.* 2022; Radchenko *et al.* 2024). In particular, the highest activity of cellulase was recorded in chernozem in the fallow area (6.4–11.2 µg of glucose per 1 g of soil for 7 days) and somewhat lower in strawberries, and comparing fertilization systems we are talking about the positive effect of organic fertilizers (5.2–6.7 µg of glucose per 1 g of soil for 7 days).

Table 1. Dynamics of the number of cellulose–destroying microorganisms

Variants	Depth, cm	Hutchinson–Clayton agar, CFU*10^6 per 1 g dry soil		
		spring	summer	autumn
Fallow	0–10	3.98	45.32	1.32
	10–20	3.07	31.61	0.67
	20–30	0.61	4.55	0.28
	30–40	0.38	3.13	0.12
Corn for grain	0–10	3.14	9.37	0.63
	10–20	4.34	3.55	1.56
	20–30	0.73	1.10	0.43
	30–40	0.50	0.58	0.28
Strawberries (without fertilizers)	ridge	3.12	2.17	0.82
	0–10	4.27	8.82	1.10
	10–20	3.06	4.02	0.65
	20–30	0.47	1.92	0.23
	30–40	0.32	0.21	0.14
Strawberries (organic fertilization system)	ridge	3.12	5.97	0.70
	0–10	4.91	28.31	2.66
	10–20	3.10	6.77	1.48
	20–30	0.56	0.96	0.28
	30–40	0.33	0.81	0.20
Strawberries (organo–mineral fertilization system)	ridge	3.80	6.01	2.55
	0–10	4.83	18.09	1.57
	10–20	2.76	8.28	0.77
	20–30	0.48	0.89	0.20
	30–40	0.31	0.74	0.15
Strawberries (mineral fertilization system)	ridge	3.49	4.40	1.00
	0–10	3.87	15.33	1.35
	10–20	2.41	6.66	0.74
	20–30	0.48	2.07	0.19
	30–40	0.28	1.38	0.15
Fertilizer system	S.E.		1.05	
	$LSD_{0.5}$		2.73	
Soil layer	S.E.		0.83	
	$LSD_{0.5}$		2,32	

We observed a similar trend in the decomposition of cotton fabric, but the lack of correlation with other indicators indicates that the fabric and plant remains are decomposed by enzyme systems of a complex of microorganisms (Dimova *et al.* 2021). And according to data Datsko & Zakharchenko (2023) the intensity of tissue decomposition is significantly influenced by hydrothermal parameters, tillage, fertilization system and other parameters. In particular, according to Zinaida Dehtiarovas (2022) data, favorable conditions for increasing cellulolytic activity at a depth of 20–30 cm are often found in fields. Similar data on the decomposition of cotton fabric were obtained in our research also.

Table 2. Dynamics of the activity of cellulose–destroying microorganisms

Variants	Depth, cm	Cellulase, µg of glucose per 1 g of soil for 7 days			The degree of decomposition of cotton fabric, %
		spring	summer	autumn	
Fallow	0–10	11.2	6.4	9.9	28.9
	10–20	8.9	7.7	6.4	23.8
	20–30	6.7	5.4	5.2	23.6
	30–40	7.0	1.5	3.6	18.1
Corn for grain	0–10	3.6	4.9	7.7	16.9
	10–20	4.6	4.6	5.4	10.5
	20–30	5.2	2.4	4.0	13.2
	30–40	3.1	1.7	3.1	9.5
Strawberries (without fertilizers)	ridge	2.0	2.8	3.3	23.2
	0–10	8.6	3.6	5.2	34.2
	10–20	4.6	3.3	3.0	29.3
	20–30	2.7	2.7	2.6	26.2
	30–40	2.4	2.4	2.1	19.5
Strawberries (organic fertilization system)	ridge	2.7	4.2	2.6	19.0
	0–10	6.7	5.2	6.7	25.9
	10–20	4.8	4.6	5.8	24.5
	20–30	4.2	3.3	3.0	25.2
	30–40	2.0	2.0	2.6	19.8
Strawberries (organo–mineral fertilization system)	ridge	3.0	3.0	2.7	32.6
	0–10	6.4	4.8	6.8	35.7
	10–20	5.4	2.5	5.6	33.8
	20–30	4.2	2.1	3.7	30.1
	30–40	4.0	2.0	1.1	25.9
Strawberries (mineral fertilization system)	ridge	2.0	1.6	2.7	24.1
	0–10	6.7	3.6	5.8	28.6
	10–20	5.8	3.0	4.0	27.7
	20–30	3.3	2.4	3.3	26.9
	30–40	2.7	1.0	2.4	17.7
Fertilizer system	S.E.		0.22		0.84
	$LSD_{0.5}$		0.53		2.21
Soil layer	S.E.		0.18		0.67
	$LSD_{0.5}$		0.49		1.88

The factor analysis confirmed the significant influence on indicators of soil biological activity of such factors as sampling depth and fertilization system (Table 3). But the statistical analysis of the results of our research indicates a more significant influence of the sampling depth than the fertilization system on the indicators of the number of cellulose–degrading microorganisms and cellulase activity. At the same time, let's note that on the contrary, the fertilizer system has a more significant effect on the decomposition of the fabric.

Table 3. A two–way ANOVA for the effects of fertilizer system and soil layers on the number of cellulose–destroying microorganisms and their activity

Influence factor		Cellulose–destroying microorganisms	Cellulase	The degree of decomposition of cotton fabric
Fertilizer system	F–Ratio	4.60	28.74	65.25
	P–Value	0,0005	0,0000	0,0000
Soil layer	F–Ratio	16.21	69.84	30.11
	P–Value	0,0000	0,0000	0,0000

CONCLUSIONS

Mathematical analysis data indicates significant changes in biological processes under the influence of human agricultural activity and allows to distinguish "agrochernozem" from natural analogues.

Agrogenic soils are characterized by a decrease in the number of cellulose–destroying microorganisms. Cultivated soils are characterized by decrease in activity of soil enzymes in comparison with a fallow. Perennial strawberry plantations have higher biological activity compared to arable land, and a comparison of different fertilization systems shows the advantage of organic fertilization. The highest number of cellulose–destroying microorganisms was recorded in summer, but the enzymatic activity is greater in spring and autumn.

On the strawberry site, under the organo–mineral fertilization system, cotton fabric decomposition is more intense, even compared to fallow.

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