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# ENZYMATIC ACTIVITY OF TYPICAL CHERNOZEMS UNDER THE CONDITIONS OF THE ORGANIC FARMING SYSTEMS

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***Abstract***

*Soil enzymes play a major environmental function by participating in biochemical processes related to the conversion of substances and energy into soil. The purpose of the research is to study the biological processes occurring in the soil under the influence of different farming systems. The activity of the following soil enzymes was studied: catalase, urease, dehydrogenase, protease and cellulase. The largest changes in the activity of soil enzymes were observed in the layer of 0-10 cm. Organic farming enhances the enzymatic activity at the level at which is found in uncultivated soil (fallow). The use of green manure increases the activity of most soil enzymes. The intensive farming system is characterized by a much lower enzyme activity in the 0-10 cm layer, as well as an increase enzyme activity in in the 20-30 cm layer, which is related to the soil tillage features (layer turnover). Hence the enzymatic activity directly depends on the farming system.*

***Key words****: enzyme, chernozem, farming system, fallow.*

## INTRODUCTION

It is known that the enzymatic activity of the soil is associated with the activity of fungi, microorganisms, algae, protozoa and root secretions of plants. The highest potential activity of enzymes is in the upper part of the humus-accumulative horizon, because it is here that the largest reserves of organic matter and microbial biomass are concentrated. Thus, enzymatic activity reflects those changes in soil evolution that are caused by plowing, so it can characterize the biological activity of the soil to the greatest extent (Shi, 2011; Bautista-Cruz, 2015; Zhang, 2020).

One of the most stable indicators of soil biological activity is its enzymatic activity. Soil enzymes perform the most important ecological function, participating in biogeochemical processes related to the conversion of minerals, organic matter and energy.

Many years of studies have proven the effectiveness of diagnosing soil cover by biochemical methods. Low experimental error and high sensitivity to external factors contribute to the use of soil enzymatic activity as a diagnostic indicator of soil fertility and ecological status (Sinegani, 2013; Coleman, 2018; Zimmerman, 2011).

The aim of the study was to determine the impact of different farming systems (organic and intensive) on the change in the enzymatic activity of chernozem soils.

## MATERIALS AND METHODS

Typical deep medium loamy chernozems on the loess (molik, mollisol) located on the territory of Zinkiv district of Poltava region were selected for research. (forest-steppe zone, Ukraine). The soils are located on the plateau of the watershed between the rivers Psel and Vorskla. The area is wide undulating plain densely permeated with ravine-beam systems.

The selection of individual soil samples took place during 2018-2020 in the fields of farms operating under two radically different systems of agriculture. Organic technology farms abandoned plowing in 1975, herbicides and other agrochemicals in 1978, and mineral fertilizers a few years later. In the fields of the enterprise, working on traditional technology, a system of different tillage is used: deep loosening, plowing, disking and cultivation. The technology of growing crops involves the use of seeds, fertilizers and plant protection products only from the best domestic and foreign producers. New agricultural machinery is used in the fields of the farm and elements of precision agriculture are introduced into production: GPS-monitoring systems, autopiloting, remote sensing methods, yield monitoring, variable sowing rates and differentiated fertilizer application.

The first soil profile is located on field with an area of 143 hectares, where in the crop rotation link a vetch yara (*Vicia sativa* L.) is grown for green manure – is a variant of the organic farming system (green manure). The crop rotation is shown in Table 1, and technological operations in Table 2.

Table 1. Crop rotation and fertilizer system during the research period

| Variant/year | 2018 | 2019 | 2020 |
|---|---|---|---|
| Organic farming system (green manure) | vetch yara on green manure | winter wheat (green manure, 15 t/ha of green mass) | wintering peas - moved corn to silage |
| Fallow | weeds | | |
| Organic farming system (compost) | corn for grain (20 t/ha of compost from cattle manure) | oat | soybeans |
| Intensive farming system | corn for grain (N130P30K30) | sunflower (N35P15K30) | corn for grain (N130P30K30) |

Table 2. Technological operations for the period 2018-2020, variant organic farming system (green manure)

| 2018 | 2019 | 2020 |
|---|---|---|
| - harvest of the predecessor in 2017<br>- earnings of crop residues by a disk cultivator to a depth of 6-8 cm<br>- disking (12-14 cm)<br>- early spring cultivation (4 cm)<br>- pre-sowing cultivation (4 cm)<br>- sowing of vetch yara (continuous sowing)<br>- disking of green manure to a depth (6-8 cm) in two tracks<br>- pre-sowing cultivation (5 cm)<br>- sowing of winter wheat | - early spring harrowing<br>- harvesting by direct combining<br>- harvesting of straw<br>- earnings of crop residues by a disk cultivator (10-12 cm)<br>- cultivation (6-8 cm)<br>- pre-sowing cultivation (5 cm)<br>- sowing of winter peas | - cultivation (6-8 cm)<br>- earnings of crop residues by a disk cultivator to a depth of 12-14 cm<br>- pre-sowing cultivation (6 cm)<br>- sowing corn for silage because the peas are gone<br>- two inter-row cultivation<br>- collection of green mass on a silo<br>- cultivation (12-14 cm) |

The second soil profile is located on a plot that has not been cultivated for over 30 years – it is variant fallow. Grow in the field association of legumes, grasses and cereals. The variant of fallow is control.

The third soil profile is located on field with an area of 94 hectares, where the compost is applied at a dose of 20 t/ha – is a variant of the organic farming system. The crop rotation is shown in Table 1, and technological operations in Table 3.

Table 3. Technological operations for the period 2018-2020, variant organic farming system (compost)

| 2018 | 2019 | 2020 |
|---|---|---|
| - harvest of the predecessor in 2017<br>- earnings of crop residues by a disk cultivator (6-8 cm)<br>- disking (12-14 cm)<br>- rolling by heavy ring-spur rollers (spring)<br>- export and application of humus (compost)<br>- cultivation to a depth of 6-8 cm<br>- pre-sowing cultivation (6 cm)<br>- sowing of corn<br>- harrowing of ladders<br>- three inter-row cultivation, and the last with hilling<br>- harvesting<br>- earnings of crop residues by a disk cultivator (6-8 cm)<br>- cultivation to a depth of 12-14 cm | - spring provocative cultivation (3-4 cm)<br>- pre-sowing cultivation (4 cm)<br>- sowing of oats<br>- post-emergence harrowing<br>- harvesting by separate method<br>- harvesting of straw<br>- earnings of crop residues by a disk cultivator (10-12 cm) | - early spring harrowing<br>- pre-sowing cultivation (4 cm)<br>- soybean sowing<br>- pre-sowing cultivation (4 cm)<br>- new soybean sowing<br>- post-emergence harrowing<br>- three inter-row cultivation<br>- harvesting by direct combining<br>- earnings of crop residues by a disk cultivator (12-14 cm)<br>- deep loosening to a depth of 26 cm |

The fourth soil profile is located on field with an area of 125 hectares, where they use the full range of plant protection products and fertilizers. The crop rotation is shown in Table 1, and technological operations in Table 4.

The activity of the following soil enzymes was studied: catalase, urease, dehydrogenase, protease and cellulase.

The activity of the enzyme catalase was determined by the gasometric method by Galstyan (1974). The essence of the method is to determine the amount of oxygen released during the decomposition of hydrogen peroxide. The activity of the enzyme invertase was determined by a modified colorimetric method of Khaziev (1957). The essence of the method is to determine the optical density of the solution after the reduction of copper by glucose and fructose, released during the hydrolysis of sucrose. Urease activity was determined by the colorimetric method of Scherbakova (1983), by determining the amount of ammonium released using Nessler's reagent. Dehydrogenase activity was determined by Galstyan (1962), by photocolorimetric determination of the amount of formed triphenylformazan (TFF). Protease activity was determined by the method of Galstyan-Harutyunyan (1976), a method based on the ability of proteases to decompose the protein substrate into amino acids, followed by photocolorimetric determination of their amount using ninhydrin. Cellulase activity was determined based on the ability of the enzyme to decompose biopolymers to glucose, the amount of which is determined iodometrically by back titration with sodium hyposulfite (Khaziev, 2005; Titova, 2012).

Mathematical analysis of the data were performed with Microsoft Excel 2010 and Statgraphics 18.1 trial.

Table 4. Technological operations for the period 2018-2020, variant intensive farming system

| 2018 | 2019 | 2020 |
|---|---|---|
| - collection of the predecessor in 2017<br>- disking (12-15 cm)<br>- deep loosening 35-37 cm (autumn)<br>- application of urea 250 kg / ha (spring)<br>- cultivation (12-15 cm)<br>- discusing (8-10 cm)<br>- sowing of corn together with introduction of a diamophos of 120 kg/hectare 10:26:26<br>- introduction of soil herbicide<br>- care 1-2: application of insurance herbicide + foliar fertilization<br>- care 3: application of insecticide (on the panicle)<br>- harvesting by direct combining<br>- disking (12-15 cm)<br>- plowing (25-28 cm) | - application of Ammonium Sulfate 100 kg/ha<br>- cultivation (12-15 cm)<br>- harrowing<br>- sowing of sunflower with the introduction of complex fertilizers 115 kg/ha 8:24:24<br>- introduction of soil herbicide<br>- care 1: herbicide around the perimeter of the field and inter-row tillage<br>- care 2: application of graminicide, fungicide, growth regulator and feeding on the leaves (4-5 pairs of true leaves)<br>- care 3: application of insecticide, fungicide and foliar fertilization (asterisk)<br>- harvesting by direct combining<br>- disking (12-15 cm)<br>- deep loosening (35-37cm)<br>- application of urea 250 kg/ha<br>- cultivation (12-15 cm) | - harrowing<br>- sowing of corn together with introduction of a diamophos of 125 kg/hectare 9:25:25<br>- introduction of soil herbicide<br>- care 1: application of insurance herbicide + foliar fertilization (3-5 leaves)<br>- care 2: foliar fertilization (7-8 leaves)<br>- care 3: application of insecticide (on the panicle)<br>- harvesting by direct combining<br>- disking (12-15 cm)<br>- deep loosening (35-37cm)<br>- application of urea 250 kg/ha<br>- cultivation (12-15 cm) |

## RESULTS AND DISCUSSIONS

Analyzing the obtained data (Table 5) should pay attention to a significant decrease in invertase activity in the conditions of intensive farming system compared to the rest of the studied options, especially in layers 0-10 and 10-20 cm. The most significant difference was recorded in 0-10 cm layer. The highest values of invertase activity are characterized by variants of fallow and organic farming system with the use of green manure. Similar data were reported by the researcher Mao-hua, 2012. We compare the obtained activity data of all enzymes with the comparative scale (Table 7) proposed by Zvyagintsev (1978). According to Table 7, all studied soils have a medium degree of invertase activity.

Soils involved in organic farming have the highest rates of urease enzymatic activity, which is confirmed by Meysner, 2013.The reason for this is the periodic application of organic fertilizers and the use of green manures. The lowest activity of this enzyme was in the variant of intensive farming system. High urease activity in the variant of intensive technology is a consequence of the introduction of high doses of urea in the cultivation of corn. This leads to an increase in urease activity at a depth of 10-20 cm.

The studied soils are characterized by an average degree of catalase activity. However, it is worth noting the significant difference between all the options studied, in particular, the highest rates were recorded in the variant of the organic farming system with the use of green manure, and the lowest in the variant of the intensive farming system. Different agricultural uses of soils lead to significant changes in the activity of catalase and many other enzymes (Kuscu, 2018; Petcu, 2014).

The highest protease activity is characterized by the variant of fallow, and the lowest - the variant of intensive farming system where the activity of this enzyme in the 0-10 cm layer is more than 4 times lower. A large percentage of cereals in crop rotation causes a decrease in protease activity. In general, it should be noted that the most significant difference between the studied variants is observed in the layers of 0-10 and 10-20 cm. Among the variants of the organic farming system, the variant with the use of vetch yara is characterized by higher prosthesis activity. The degree of protease activity is average in the variant of intensive farming system and very high in other variants. The thesis of increasing the activity of soil enzymes, especially proteases, under the conditions of the organic system of agriculture, has been reported in numerous other studies by other authors (Kwiatkowski, 2020; Melero, 2004; García-Orenes, 2016).

All variants have a high degree of dehydrogenase activity in the 0-10 cm layer. The exception is the variant of the intensive system of agriculture, which has a medium level. There was an increase in dehydrogenase activity in the layers of 20-30 and 30-40 cm and vice versa decrease in 0-10 and 10-20 cm in the variant of intensive farming system. Intensive farming system causes a decrease in dehydrogenase activity, and in organic farming, on the contrary - an increase, which also is confirmed by the data Kobierski, 2020 also.

There is information in the literature that cellulase activity is a fairly stable indicator that most likely responds to the amount of substrate entering the soil (Doyle, 2006; Cenciani, 2011). Analyzing the obtained data, we note the weak cellulose-destroying activity of all studied soils. The highest values are in fallow, and the lowest – in the organic system using of compost. Also, an interesting fact is the increase in cellulase activity in the layer of 10-20 cm within the variant of organic farming system (green manure) and a drop in the variant of intensive farming system. In our opinion, the small difference between the indicators of cellulase activity in the studied soils also indicates a greater influence of the quantity and quality of organic matter entering the soil than the system of agriculture.

The analysis of the obtained data (Table 5) shows that the soil samples form the organic system are characterized by a higher biological activity for almost all these indicators and in all layers. On the contrary, the soil from the variant of the intensive growth system is characterized by a decrease in biological activity in the layer of 0-10 cm and an increase in the indicators in the layer of 30-40 cm. According to Two-way ANOVA analysis of farming system and soil depth on soil enzyme activity (Table 6), both factors have a significant effect on the activity of soil enzymes, with the exception of cellulase. However, the value of F indicates that the effect of depths is much higher (except for catalase).

Table 5. Enzymatic activity of chernozems under different farming system, the average for the years 2018-2020

| Variant | Depth, cm | Catalase, $cm^3$ $O_2$ per 1 g of soil for 1 min | Dehydrogenase, mg of TFF per 10 g of soil for 24 hours | Invertase, mg of glucose per 1 g of soil for 24 hours | Urease, mg $NH_3$ per 10 g of soil for 24 hours | Protease, mg of glycine per 1 g of soil for 24 hours | Cellulase, µg of glucose per 10 g of soil for 48 hours |
|---|---|---|---|---|---|---|---|
| green manure | 0-10 | 7,54a | 11,68a | 29,89a | 25,61a | 17,10a | 5,93a |
| | | 0,25 | 0,43 | 0,70 | 1,51 | 1,40 | ‘0,44 |
| | 10-20 | 7,36 | 9,42ab | 20,94b | 18,54b | 5,41 | 5,84a |
| | | 0,28 | 0,42 | 0,35d | 1,63 | 0,36 | 0,30 |
| | 20-30 | 7,00 | 6,11c | 12,82c | 14,03c | 3,59 | 2,60b |
| | | 0,23 | 0,22 | 0,97 | 1,29 | 0,32 | 0,21 |
| | 30-40 | 5,79ab | 4,27d | 7,78d | 12,20d | 4,08a | 2,09b |
| | | 0,27 | 0,20 | 0,48 | 1,23 | 0,21a | 0,18 |
| compost | 0-10 | 6,35a | 11,83a | 22,47a | 16,07a | 11,65 | 6,2 |
| | | 0,20 | 0,49 | 0,70 | 1,28 | 0,72a | 0,49a |
| | 10-20 | 6,24ab | 9,37b | 18,83b | 16,15b | 5,91 | 5,22 |
| | | 0,24 | 0,46 | 1,06 | 1,46 | 0,39 | 0,36 |
| | 20-30 | 6,12ab | 7,32c | 11,45c | 13,28c | 3,55b | 2,60b |
| | | 0,22 | 0,43 | 0,41 | 1,08 | 0,24 | 0,18 |
| | 30-40 | 5,46ab | 5,89d | 8,44d | 11,93d | 2,44c | 1,76c |
| | | 0,21 | 0,35 | 0,41 | 0,14 | 0,17 | 0,14 |
| Fallow | 0-10 | 5,63a | 12,38a | 35,83a | 14,61a | 21,96a | 6,38a |
| | | 0,30 | 0,47 | 2,35 | 1,23 | 1,29 | 0,44 |
| | 10-20 | 4,92ab | 9,92b | 19,41b | 11,96b | 9,70b | 5,30b |
| | | 0,20 | 0,35 | 1,54 | 1,09 | 0,53 | 0,46 |
| | 20-30 | 5,01 | 7,82c | 15,56 | 12,05c | 3,81 | 3,08 |
| | | 0,21 | 0,27 | 1,26 | 1,21 | 0,33 | 0,25 |
| | 30-40 | 4,39ac | 4,72d | 9,40c | 10,47d | 2,04c | 1,86c |
| | | 0,17 | 0,15 | 0,71 | 1,12 | 0,16 | 0,12 |
| Intensive | 0-10 | 4,28a | 8,88a | 15,92a | 13,71 | 4,71 | 6,13a |
| | | 0,23 | 0,41 | 0,94 | 0,94 | 0,51 | 0,45 |
| | 10-20 | 4,03 | 9,11ab | 16,12 | 14,60 | 3,65a | 3,76 |
| | | 0,21 | 0,51 | 0,84 | 1,30 | 0,26 | 0,44 |
| | 20-30 | 3,97 | 8,68 | 15,78 | 12,34 | 3,23 | 3,31 |
| | | 0,17 | 0,36 | 1,02 | 1,16 | 0,24 | 0,22 |
| | 30-40 | 3,56ac | 6,85a | 10,65 | 12,20a | 1,70b | 2,34c |
| | | 0,17 | 0,38 | 0,65 | 1,27 | 0,14 | 0,15 |

Notes. The numerator is the average value; denominator – standard error. The letters in the columns of variants are significant differences between different layers of soil ($p < 0.05$).

Table 6. A two-way ANOVA for the effects of farming system and soil layers on soil enzyme activities

| Influence factor | | Catalase | Dehydrogenase | Invertase | Urease | Protease | Cellulase |
|---|---|---|---|---|---|---|---|
| Farm system | F | 130.62 | 21.47 | 13.61 | 3.76 | 62.41 | 0.81 |
| | P | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.4912 |
| Soil Layer | F | 18.79 | 181.62 | 16.78 | 168.38 | 310.59 | 135.20 |
| | P | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| Interaction | F | 1.20 | 17.46 | 3.75 | 10.29 | 35.57 | 2.77 |
| | P | 0.2925 | 0.0000 | 0.00001 | 0.0000 | 0.0000 | 0.0037 |

Table 7. Scale of comparative assessment of soil enzymatic activity (Zvyagintsev, 1978; Titova, 2012)

| Enzyme activity | Catalase, $cm^3$ $O_2$ per 1 g of soil for 1 min | Dehydrogenase, mg of TFF per 10 g of soil for 24 hours | Invertase, mg of glucose per 1 g of soil for 24 hours | Urease, mg $NH_3$ per 10 g of soil for 24 hours | Protease, mg of glycine per 1 g of soil for 24 hours | Cellulase, μg of glucose per 10 g of soil for 48 hours |
|---|---|---|---|---|---|---|
| Very low | <1 | <1 | <5 | <3 | <1 | <10 |
| Low | 1-3 | 1-3 | 5-15 | 3-10 | 1-3 | 10-20 |
| Medium | 3-10 | 3-10 | 15-50 | 10-30 | 3-5 | 20-50 |
| High | 10-30 | 10-30 | 50-150 | 30-100 | 5-8 | 50-100 |
| Very high | >30 | >30 | >150 | >100 | >8 | >100 |

## CONCLUSIONS

Agricultural using of soils leads to significant changes in their biological activity. The nature and extent of these changes depend on the system of agriculture, crop rotation and fertilization system. This once again confirms the significant sensitivity of soil enzymatic activity to any anthropogenic impact.

The data obtained within our studies indicate a decrease in the activity of such enzymes: invertase, protease, dehydrogenase and cellulase under conditions of agrogenic use of typical chernozems. But, at the same time, the activity of urease and catalase increases. The enzyme activity changes with soil depth in accordance with the content of organic matter and microbiological activity. So, the activity all studied enzymes usually decreases with soil depth. In the variant of intensive farming system, as a result of plowing, homogenization of a 0-30 centimeter layer of soil is observed, which leads to equalization of indicators at these depths. A feature of the variant of the intensive farming system is an increase in the activity of urease at a depth of 10-20 cm, which is a consequence of the introduction of urea. The application of organic fertilizers in the variants of the organic system of agriculture (especially the use of green manure) increases the activity of all studied enzymes in comparison with the variant of the intensive system of agriculture.

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