Scientific Papers. Series A. Agronomy, Vol. LX, 2017
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

BIOTA OF LEACHED CHERNOZEMS OF THE CENTRAL ZONE OF THE REPUBLIC OF MOLDOVA IN ASPECT OF ITS EVOLUTION AND RECOVERY

Irina SENICOVSCAIA

"Nicolae Dimo" Institute of Soil Science, Agrochemistry and Soil Protection,
100 Ialoveni Street, Chisinau, Republic of Moldova

Corresponding author email: irina_sen@mail.ru

Abstract

The influence of different land use management on the modification of the biological properties of leached chernozem located in the central zone of the Republic of Moldova has been investigated. Database of invertebrates, microorganisms and enzymatic activities has been formed. The current status of biota of the old-arable leached chernozem is characterized by the reduction in comparison with the level of the 1960s and with soil's standards that are in conditions of natural ecosystems. The values of most soil biological indices decrease in the sequence: old fallow land → arable land under organic system with farming manure and incorporation of crop residues → arable unfertilized land → arable land with mineral fertilization of $N_{90\text{-}300}$. The highest values of biota's abundance were registered in fallow soils in the woodland belt and on plots with manure application with plant residues additives. Biological indices in soil profiles decreased with the depth and depended of the form of farming system. The negative effects on biota and humus status have been observed as a result of the long-term land management practices without organic fertilizers. Annual losses due to mineralization processes constitute 0.019%. The growth of humus-mineralizing microorganisms has been noticed. Trends and regression equations describing the growth of humus-mineralizing microorganisms and the fall of humus content in old-arable leached chernozems have been calculated.

Key words: *biota, humus, leached chernozem, organic and mineral fertilizers, trends.*

INTRODUCTION

In the past decade, the idea of ecological agriculture has become significant in the Republic of Moldova (Boincean, 1999, 2013). Enhancing the carbon sink to agricultural land, the creation of stable soils with the high level of biodiversity and the enzymatic activity are the major advantages in ecological agriculture. There is a growing interest in developing valuable and sensitive indicators of soil quality, which will be able to reflect the effects of land management and ensure a long-term sustainability of soil fertility. In this context, soil biological indices can be used for the evaluation and comparison of mineral and organic farming systems. In addition, the study of the evolution of soils, which are under different land management, is also interesting from the point of view of the national soil quality standards.

One of the main problems in predicting soil biological status is that it is influenced by a multitude of interacting factors, including soil carbon quantity and quality, physical and chemical soil properties, nutrient status, vegetation type, climatic conditions and etc. Therefore, reliable differences can be found in the experimental data of several years in conditions of long-term stationary experiments. Long-term monitoring researches carrying out on site experiments made it possible to produce data bases of biota state of zonal soils, their statistical processing and the elaboration of evaluation system of degradation and recovery processes.

The purpose of the research was to determine the state and temporary variability of biota in the leached chernozem under different land management practices aiming to develop the methods of soil quality restoration of old arable soils.

MATERIALS AND METHODS

The experimental site is located in the central zone of the Republic of Moldova, in the wooded steppe of the central - Moldovan forest province, in the district No. 8 of gray forest soils and leached chernozems of the wooded steppe of hilly Kodru Forests (Figure 1), in the Ivancha village, Orhei district (lat. 47° 18′ N, long. 28° 54 ′ E, elevation 170-180 m). Leached chernozem occupies more than 395,000 ha of the coutry in the north and central regions (Andriesh et al., 2014).

Figure 1. Fragments of natural and agricultural landscapes located in the central zone of the Republic of Moldova

Biota's state in the leached chernozem in the condition of long-term arable has been investigated in comparison with fallow soils in the 60 years-old woodland belt.

Sampling for microbiological and enzymatic indicators was carried out in 2 profiles per soil horizons to a depth of 200 cm and from 0-30 cm layer separately during 1988-1998 and 2005-2016. Additionally, some microbiological and enzymatic indicators were compared with the level of the 1960s. Invertebrates sampling was carried out from 5-32 soil semi-profiles to a depth of 60 cm over the period 1991-2010.

Status of invertebrates was identified from test cuts by manual sampling of the soil layers to the depth of soil fauna occurrence (Gilyarov and Striganova, 1987).

Microbiological properties. The microbial biomass carbon was measured by rehydration method (Blagodatsky et al., 1987). Counts of culturable microorganisms (heterotrophic bacteria, humus-mineralizing microorganisms, actinomycetes, bacteria from the family of the *Azotobacter* and fungi) were obtained on agar plates (Zvyagintsev, 1991).

Enzymatic activity. The urease activity was measured by estimating the ammonium released on incubation of soil with buffered urea solution by colorimetric procedure (Haziev, 2005). The catalase activity was determined by the volumetric method by the rate of hydrogen peroxide's decomposition during its interaction with the soil and by the volume of released oxygen (Galstyan, 1978). The dehydrogenase activity was determined by the colorimetric technique on the basis of triphenylformazan (TPF) presence from TTC (2,3,5-triphenyltetrazolium chloride) added to soil (Haziev, 2005). The polyphenoloxidase and peroxidase activities were determined by the colorimetric technique using hydroquinone as a substrate (Karyagina and Mikhailovskaya, 1986).

Soil chemical properties. Organic carbon was determined by dichromate oxidation; the humus content was estimated using the coefficient 1.724 (Arinushkina, 1970).

Soil biological indices were evaluated by analysis of variance and correlation. Statistical parameters of soil invertebrates were calculated taking into account the depth of soil fauna occurrence, microorganisms and enzymes – for the layer of 0-30 cm.

RESULTS AND DISCUSSIONS

The state of biota in fallow and arable leached chernozem. The current state of biota in the leached chernozem is the result of a long-term influence of human activity, through agricultural land management. The average abundance and biomass of invertebrates and *Lumbricidae* family in the fallow and old arable chernozem were approximately the same (Table 1). The diversity of invertebrates was different depending on the soil management. The soil under 60-year-old fallow is characterized by a greater diversity of invertebrates. In addition to the *Lumbricidae* family, species of the *Formicidae, Glomeridae, Cerambycidae, Apidae, Araneae, Coccinellidae, Forficulidae* and *Carabidae* families were found. The abundant presence of the *Formicidae* family representatives is observed. In general, the soil under fallow contains 8-10 families of invertebrates, while the soil under arable only 3-5 families of edaphic fauna. According to the average statistical data, the weight of one individual of the earthworm in the soil under arable management practices is 0.16 g, which is 1.5 times lower than in the fallow land.

Conventional tillage practices are generally unfavorable to heterotrophic bacteria, various fungi and enzymatic activities (Table 1). Soil microbial biomass decreased on average from 492.5 to 314.7 μg C g^{-1} soil as a result of a long-term arable land management without application of organic fertilizers. The long-term use of the soil under arable affected the structure of soil microbial communities. The ratio between bacteria and fungi increased from 90 to 142.

There was a 3.6 - fold increase in the number of humus - mineralizing microorganisms.

A similar trend in decrease has been noticed in the number of the heterotrophic bacteria and fungi. Activities of soil enzymes reduced: urease - by 2.8, catalase - 2.1, dehydrogenase - by 1.6, polyphenoloxidase - by 1.4 times and peroxidase - by 20.3 %.

Biological indices of investigated soils are characterized by the medium and considerable variability. There is a tendency of increase in the variation coefficient from the old fallow to the arable chernozem on some indicators. Taking into consideration the decrease in the total level and increase in the amplitude of the biological parameters oscillations in degraded soils, this testifies the decline of soil's ecological resistance to anthropogenic impacts. The level and size of homeostasis zones and therefore the biota stability reached the maximum levels in the fallow soil.

The highest level of the microbial biomass has been determined in the A_1 horizon of the soils under natural vegetation and whereas the lowest – in the BC and C horizons of both profiles (Figure 1). Microorganisms in leached chernozems under fallow are concentrated in 0-60 cm layer, the biomass index decreases sharply in the soil profile to a depth of 70-100 cm. The concentration of microorganisms in the top layer reaches 1512.2 μ C g^{-1} soil.

Table 1. Statistical parameters of biota in the leached chernozem under different land management in the Central zone of the Republic of Moldova

Index	Fallow leached chernozem					Arable leached chernozem				
	min	max	mean value	V,%	confidence interval ($P \leq 0.05$)	min	max	mean value	V,%	confidence interval ($P \leq 0.05$)
Invertebrates (n=5-32)										
Number of invertebrates, ex m^{-2}	40.0	168.0	81.6	61	19.6-143.4	32.0	152.0	84.9	41	74.6-95.3
Biomass of invertebrates, g m^{-2}	4.5	26.4	11.5	75	0.7-22.2	4.9	18.7	9.8	47	8.1-11.5
Number of *Lumbricidae* fam., ex m^{-2}	16.0	56.0	28.8	54	9.4-48.2	21.0	128.0	61.3	51	52.0-70.5
Biomass of *Lumbricidae* fam., g m^{-2}	1.6	15.2	6.8	75	11.5-10.7	4.2	18.2	9.7	44	8.1-11.4
Microorganisms (n=3-33)										
Microbial biomass, μ g C g^{-1} soil	459.9	525.1	492.5	7	411.6-573.4	191.0	434.0	314.7	21	286.8-342.6
Heterotrophic bacteria, CFU g^{-1}soil*10^6	4.8	6.0	5.4	11	3.9-6.9	2.6	6.8	4.3	32	3.5-5.2
Humus-mineralizing microorganisms, CFU g^{-1} soil*10^6	2.5	2.9	2.7	7	2.2-3.2	4.6	15.5	9.6	32	7.8-11.4
Actinomycetes, CFU g^{-1} soil*10^6	1.4	1.8	1.6	13	1.1-2.1	1.4	4.1	2.8	30	2.3-3.2
Fungi, CFUg^{-1} soil*10^3	54.6	65.4	60.0	9	45.6-72.4	21.0	39.0	30.2	19	26.9-33.5
Azotobacter gen., CFUg^{-1} soil	10.0	12.4	11.2	11	8.2-14.2	13.0	253.0	91.6	56	64.8-138.4
Enzyme activity (n=3-34)										
Urease, mg NH_3 10 g^{-1}soil 24 h^{-1}	7.5	9.7	8.6	13	5.9-11.3	1.4	5.1	3.1	43	2.1-4.1
Catalase, cm^3 O_2 g^{-1} soil min^{-1}	5.6	5.8	5.7	2	5.3-5.9	1.4	5.2	2.7	41	2.1-3.3
Dehydrogenase, mg TPF 10g^{-1} soil 24h^{-1}	2.13	2.50	2.31	8	1.85-2.77	0.75	2.63	1.47	43	1.23-1.71
Polyphenoloxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	5.0	6.0	5.5	9	4.3-6.7	2.0	7.0	3.9	34	3.4-4.4
Peroxidase, mg 1,4-p-benzoquinone 10 g^{-1} soil 30 min^{-1}	35.0	37.0	36.0	33	33.5-38.5	15.0	37.0	28.7	19	26.8-30.6

In the arable chernozem the base mass of microbes is concentrated in 0-40 cm layer. Arable soils are characterized by the gradual decrease of biomass with the depth as compared to soils of natural ecosystems.

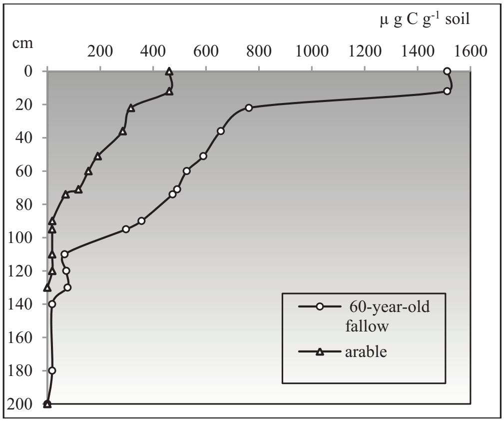

Figure 1. The profile distribution of microbial biomass in the leached chernozem in conditions of fallow and arable land

More intensive land-use involving of soil tillage stimulates the microbial decomposition of organic matter and tends to result in decrease in humus content in the arable chernozem. The soil layer of 0-10 cm is exposed by the highest mineralization. The humus content in 0-30 cm layer constitutes in average 4.05 % in the soil under fallow and 3.42 % in the arable soil (Table 2).

Table 2. Humus content* in the leached chernozem under different land management (%)

Depth, cm	Fallow land	Arable land
0-10	5.44	3.46
10-20	3.55	3.52
20-30	3.16	3.27
0-30	4.05	3.42

* mean values (n=10 for each layer)

Evolution of microorganisms and humus status in the arable leached chernozem. The current state of microbial community in arable leached chernozems is characterized by a decline of abundance compared to the level registered in 1960s (Figures 2, 3). The biological degradation of arable soils is interconnected with the dehumification processes, compaction and destruction of soil structure. A major reason for the deterioration of soil biological properties and for the decline of humus content under arable agriculture is annual tillage, which aerates soil, inevitably increases its oxygen content and breaks up aggregates where microbes are living. The selection process of species that can survive in conditions of a lower organic matter content and deterioration of physicochemical parameters of soil systems is taking place among the microorganisms.

A characteristic feature of long-term dynamics of arable leached chernozems is a significant decrease in the number of heterotrophic bacteria and fungi (Figures 2, 3). The time trend of the heterotrophic bacteria and fungi is described by polynomial function and reveals the moderate link ($R^2 = 0.67$ and 0.64).

The content of actinomycetes in soil, on the contrary, increases over time, which indirectly indicates the growing mineralization processes (Figure 4). The time trend is described by polynomial function and shows the moderate link ($R^2 = 0.48$).

During the 52 years of the utilization of arable leached chernozems the humus content decreased on average by 23.6% from its initial level. Annual losses due to mineralization processes account for 0.019%. Mineralization processes are dominated in soils, which are degraded as a result of long-term arable use. The growth of humus-mineralizing microorganisms has been noticed (Figure 5).

The database of humus-mineralizing microorganisms and the humus content was processed separately by the correlation and regression analysis during periods of observations. Regression equations with the highest correlation coefficients were chosen from all of the regression equations.

Humus-mineralizing microorganisms' trend is described by the power function. Trend has the moderate correlation coefficient: 0.59.

Trend of the humus content is described by the polynomial function. Correlation coefficient constitutes 0.88. These results indicate that the humus content was closely linked to the content of humus-mineralizing microorganisms in soil. The intensification of mineralization processes in arable leached chernozems leads to a steady decline in humus content and reserves.

The temporary long-term variability of the enzymatic complex of arable leached chernozems managed to trace to catalase activity (Figure 6). Soil has lost 68% of its catalase activity in comparison with initial level as a result of 52 years of arable use. Trend has the moderate correlation coefficient: 0.64. The time trend is described by logarithmic function.

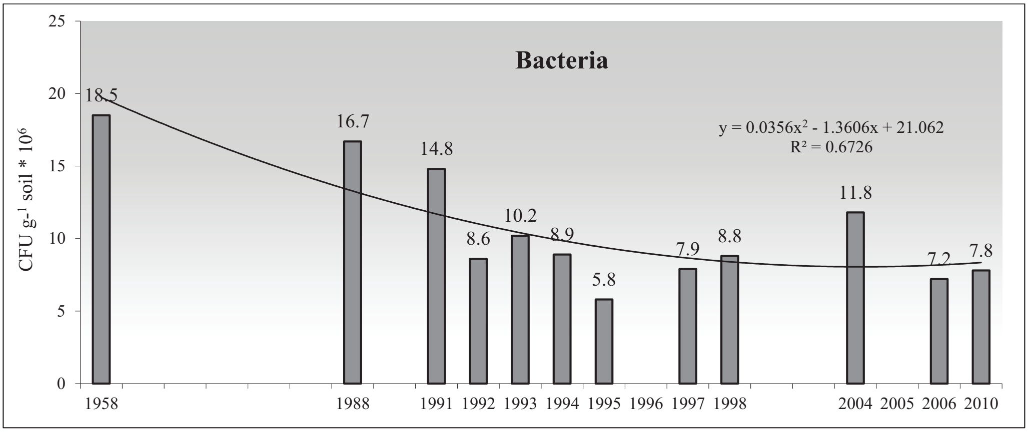

Figure 2. Dynamics of the bacteria's content in the arable leached chernozem

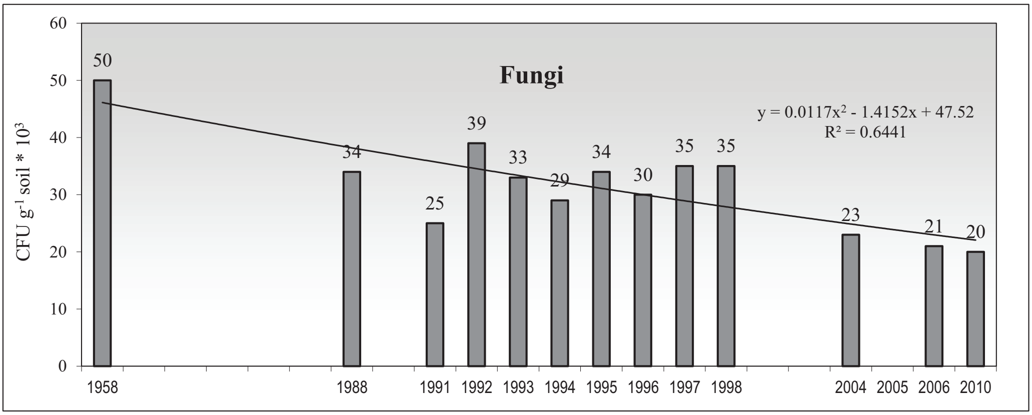

Figure 3. Dynamics of the fungi's content in the arable leached chernozem

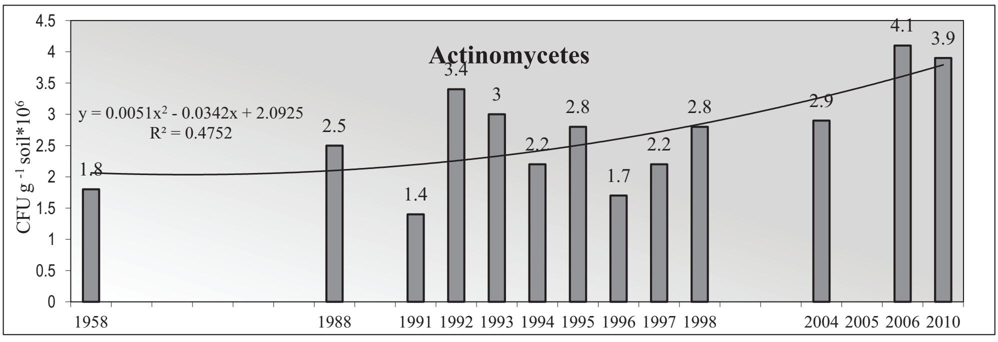

Figure 4. Dynamics of the actinomycetes content in the arable leached chernozem

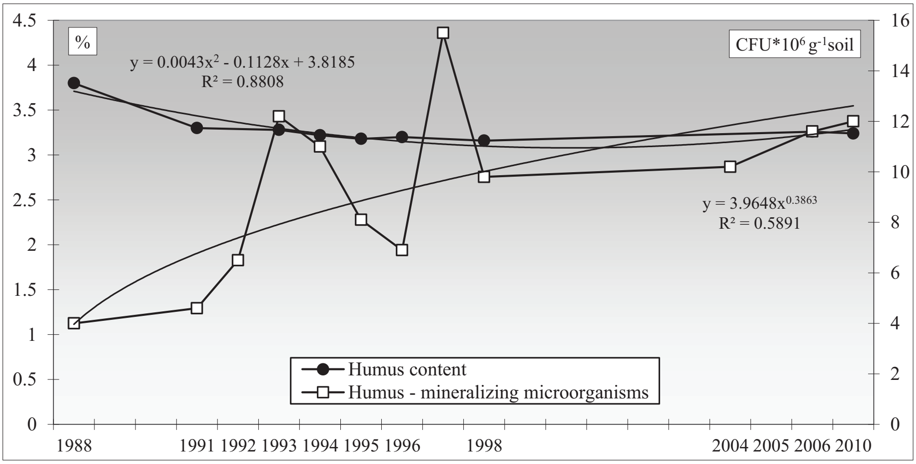

Figure 5. Dynamics of the humus and humus-mineralizing microorganisms' content in the arable leached chernozem

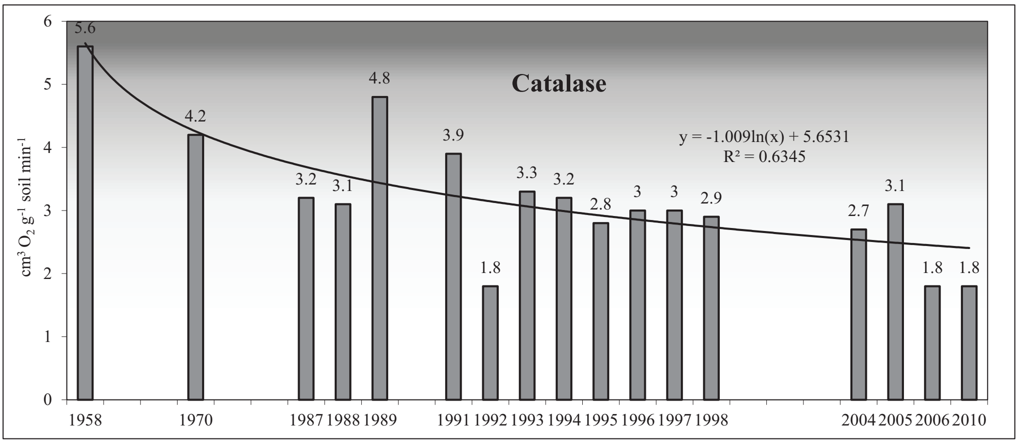

Figure 6. Dynamics of the catalase activity in the arable leached chernozem

Biota in conditions of mineral fertilizers application. Leached chernozem has changed its quality after prolonged cessation of inorganic fertilizers and that was reflected in all its properties. The soil acidification has been observed, as evidenced by decrease in pH from 6.7-6.8 in control plot to 6.3-6.5 in plot with $N_{90\text{-}300}P_{60}K_{60}$. Hydrolytic acidity raised significantly with the increase of doses of mineral fertilizers, in average from 2.7 me 100 g^{-1} soil in the control to 4.7 me 100 g^{-1} soil in the plot with $N_{90\text{-}300}P_{60}K_{60}$. Mineral fertilizers are one of the main factors that regulate the activity of soil biota. The abundance and activity of soil biota are determined by doses of fertilizers. Prolonged use of moderate doses of fertilizers ($N_{60}P_{60}K_{60}$) on the leached chernozem does not significantly impair ecological equilibrium and changes in majority of faunal and microbiological parameters are in the range of the soil system homeostasis and can be classified as reversible.

The long-term use of high doses of fertilizers ($N_{90\text{-}300}P_{60}K_{60}$) leads to the inhibition of invertebrates in the leached chernozem. The total number decreased by 1.9 times and biomass – by 2.1 times, mainly due to reduction in 4-5 times the amount of saprophagous (Table 3). The widespread occurrence phytophagous' larvae of *Melolontha melolontha* have been observed. The diversity of soil fauna, especially species of the *Lumbricidae* and *Enchytraeidae* families is significantly reduced. The largest part of soil fauna (75%) and all *Lumbricidae* fam. (100%) are concentrated in the 20-40 cm layer. The profile distribution of *Lumbricidae* fam. representatives exhibits a peak family activity in a layer of 30-40 cm in contrast to controls where their maximum number has been registered in slightly higher profile – in 20-30 cm layer. Thus, migration of earthworms to subsoil layers is typical for leached chernozems which were fertilized by $N_{90-300}P_{60}K_{60}$ for a long time.

Table 3. Influence of the long-term application of mineral fertilizers on invertebrates in the leached chernozem

Variant	Number of invertebrates, ex m^{-2}		Biomass of invertebrates, g m^{-2}	
	total	*Lumbricidae* fam.	total	*Lumbricidae* fam.
Control	168.0 ± 58.8	108.0 ± 22.0	13.2 ± 5.2	8.3 ± 3.5
$N_{60}P_{60}K_{60}$	152.0 ± 29.4	40.0 ± 29.4	16.6 ± 4.8	9.1 ± 6.8
$N_{90-300}P_{60}K_{60}$	88.0 ± 44.1	44.0 ± 7.4	6.4 ± 1.5	4.7 ± 0.9

The share of fungi in the microbial community increased, the number of species phytotoxic grew by 23-33%. The ratio between hydrolytic and redox enzymes has been broken. Polyphenoloxidase and dehydrogenase active-ties significantly inhibited (1.7-2.1 times) as a result of the prolonged application of mineral fertilizers in high doses (Figure 7).

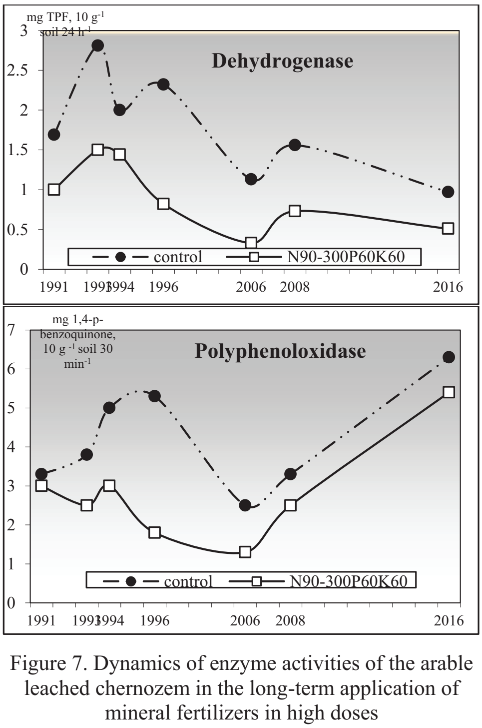

Figure 7. Dynamics of enzyme activities of the arable leached chernozem in the long-term application of mineral fertilizers in high doses

The inhibition becomes irreversible, as evidenced by long-term dynamics of the activity of these enzymes.

Enzyme activities were lower than the optimum level, providing the soil system stability. Negative shifts in the state of enzymes were accompanied by an increase in the soil acidity, disturbance in the humification–mineralization equilibrium and by soil degradation in the whole. Enzyme activities under mineral system with maximum doses were suppressed even in 10 years after the cessation of inorganic fertilizers use. The use of high doses of nitrogen fertilizers has a long after-effect and persists for some indicators on the organic fertilizers backgrounds.

Restoration of biota in leached chernozem by using organic amendments. The process of natural recovery of the soil biota composition and activity in soil with the mineral fertilizers after-effect has been determined as slow. The biomass of biota is restored quicker, its diversity and enzymatic activity – to a lesser extent (Senicovscaia, 2012).

The organic farming system with a long-term application of 60 t ha^{-1} of manure (once a crop rotation) and incorporation of crop residues returns the organic matter to the soil and creates conditions for carbon sink. This can be important in the light of the mitigation of carbon losses, the compensation of the CO_2 emissions by soils and in maintaining the soil microorganisms' nutrition.

According to statistical parameters the content of microbial biomass in leached chernozem increases by 1.5 times, dehydrogenase activity raises from 1.47 to 1.99 mg TPF 10 g^{-1} soil 24 h^{-1}. A similar trend was evident in catalase, polyphenoloxidase and peroxidase activities (Figure 8).

The humus content level was higher under application of organic fertilizers by 0.25%. The manure application with plant residues additives and $N_{60}P_{60}K_{60}$ restores the biota of old arable leached chernozem to the homeostasis zone. The decline of humus-mineralizing microorganisms and the activation of enzymes have been registered. However, the use of this technique on the soil, where were introduced higher doses of mineral fertilizers ($N_{90-300}P_{60}K_{60}$) had a softening effect only.

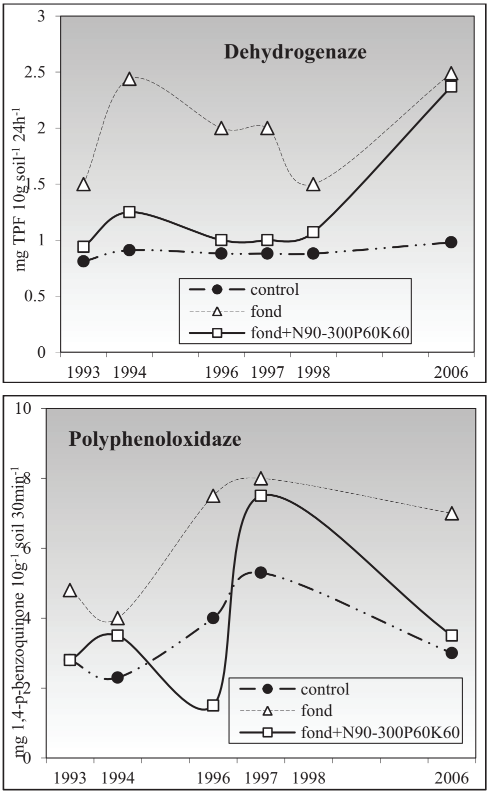

Figure 8. Dynamics of enzyme activities of the arable leached chernozem in the long-term application of organic and mineral fertilizers in high doses

Thus, the organic farming system greatly improves the enzymatic status and fertility of arable leached chernozem, but areas with high doses of inorganic fertilizers require the more radical recovery techniques of soil biota.

CONCLUSIONS

The evolution of biota in the old-arable leached chernozem is characterized by the significant reduction in its number, biomass, activity and diversity in comparison with the level of 1960s and with soil's standards that are in conditions of natural ecosystems. Time trends are described by the polynomial, power and logarithmic function with moderate and high correlation coefficients.

The values of most soil biological indices decrease in the sequence: 60 years-old fallow land → arable land under organic system with farming manure and incorporation of crop residues → arable unfertilized land → arable land with mineral fertilization of N_{90-300}.

Biological indices of investigated chernozems are characterized by the medium and considerable variability. The level and size of homeostasis zones and therefore the biota stability reached the maximum levels in the old fallow soil.

The negative effects on biota and humus status have been observed as a result of the long-term land management practices without organic fertilizers. Annual losses due to mineralization processes constitute 0.019%.

The long-term use of high doses of fertilizers ($N_{90-300}P_{60}K_{60}$) alters the habitat of soil biota. The soil changes its quality that leads to the inhibition of biota in leached chernozem.

Management of old-arable chernozems with the use of organic fertilizers and crop residues contributes to the restoration of biota. However, its parameters do not reach the level of the soil under old fallow.

REFERENCES

Andriesh S., Lungu V., Leah N., 2014. Long-term field experiments as a foundation for conserving and enhancing soil fertility. Soil as World Heritage (Ed. David Dent), Springer Science + Business Media Dordrecht, p. 201-207.

Arinushkina E.V., 1970. Guide for chemical analysis of soils. MGU, Moscow.

Blagodatsky S.A., Blagodatskaya E.V., Gorbenko A.J., Panikov N.S., 1987. Rehydration method for the determining of the microbial biomass in the soil. Pochvovedenie, Moscow, 4: p. 64-71.

Boincean B. Ecological restructuring of agriculture in the Republic of Moldova, 2013. In: "Chernozems of Moldova - evolution, protection and restoration of their fertility". Kishinev, p. 81-85.

Boincean B.P., 1999. Organic agriculture in the Republic of Moldova (crop rotation and soil organic matter). Stiinta, Chisinau.

Galstyan A. Sh., 1978. Determination of soil enzyme activity (methodical instructions). Yerevan: B.p.

Gilyarov M.S., Striganova B.R. (Ed.), 1987. Quantitative methods in soil zoology. Nauka, Moscow.

Haziev F.H., 2005. Methods of soil enzymology. Russian Academy of Sciences, Ufa.

Karyagina L.A., Mikhailovskaya N.A., 1986, Determination of polyphenoloxidase and peroxidase activities in the soil. Journal of the Academy of Sciences of BSSR, 2: p. 40- 41.

Senicovscaia Irina, 2012. Biota of degraded soils and methods for its restoration. AGROLIFE, scientific journal, volume 1, Bucharest, Romania, p. 78-83.

Zvyagintsev D.G. (Ed.), 1991. Methods of soil microbiology and biochemistry. MSU, Moscow.