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Investigation of microbiological processes during long-term storage of grey forest soil samples

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Abstract. Investigation of a decrease in the viability of microorganism cells in the soil samples, stored for a long time in an air-dry state, has both theoretical and practical significance since in agrochemistry and the soil science it is a custom to store the soil samples for many years and decades, taking it as an axiom that the properties of these samples remain unchanged. To find out what are the patterns of survival of microorganisms of various ecological-trophic, functional and systematic groups, their viability was studied in samples of gray forest soil, stored for 32 months in an air-dry state. It has been shown that the number of microorganisms of most groups decreases by 42–94 times, the number of polysaccharides-synthesizing microorganisms decreases maximum-by 3,993–18,210 times, depending on the agricultural practices, used in a stationary experiment. the number of spores and cysts decreases. The microorganisms which have the least decrease in the number of colony-forming units of micromycetes and *Azotobacter* as groups that have forms of surviving unfavourable conditions during storage are spores and cysts. In addition, the physiological and biochemical activity of micromycetes decreases significantly, compared to their activity in the original (initial) fresh soil. During storage the number and share in the total number of melanin-synthesizing micromycetes sharply decreases from 65.8–94.6% to 2.48–5.17%. When storing soil in an air-dry state, the rate of decline in the number of microorganisms depends on the functional affiliation of the group and on agrotechnical techniques that were previously used in the stationary experiments: liming, application of mineral fertilizers, ploughing in the by-products of the predecessor crop in the crop rotation, and the biomass of the sideral crop. The organic matter, ploughed into the soil, promotes the survival of ammonifiers, mineral nitrogen immobilizers, *Azotobacter* and polysaccharide-synthesizing microorganisms. Ploughing in of crop by-products reduce the number and proportion of melanin-synthesizing micromycetes. Verification of the obtained data, using long-term stored soil samples, is not permissible since microbiological processes occur in the soil during which the soil microbiota consumes the macro- and microelements, present in it, organic and organomineral complexes, including humus.

Key words: storage, soil, ammonifiers, *Azotobacter*, micromycetes, number, physiological and biochemical activity.

INTRODUCTION

Interest in the problem of long-term preservation of the viability of microorganisms in various substrates and under various conditions has both theoretical and practical interest, associated with the need to store soil samples for as long time as possible. Long-term storage of soils is an inevitable approach when samples are used for the future, chronological or ecological investigations (Funa et al., 2006). Archival soils are extremely valuable and indispensable samples for microbiological environmental studies, allowing reanalysis of the published data and direct comparison of investigations, using the newest technologies.

There is reason to believe that microorganisms can maintain their viability for a long time, especially in frozen substrates, soils and ice. Reports have been published on the discovery of viable microorganisms that were in a state of anabiosis (suspended animation) for 13 thousand and even 1 million years (Kudriashova et al., 2013). However, remains little studied the question of maintaining the viability of microorganisms in the soil samples in an air-dry state. Although frozen storage (-80 °C) is widely accepted as the optimal method for preserving the chemical and biological components of soils, desiccation (i.e., air drying) is more often used for large–scale soil archives or multigenerational soil archives (Dolfing & Feng, 2015). Dried soils were often used for chemical composition analysis even after decades of storage. According to the authors (Wang et al., 2021), long-term (up to 8,192 hours) storage of soils in an air-dry state has a slight effect on the profile of the soil microbial community, laying the basis for the use of stored soils in the research of their microbial cenoses. Using the Illumina sequencing method, it was shown that both the prokaryotic and fungal communities did not change significantly during air drying and long-term storage. After 341 days it was still possible to determine the nature of the effect of fertilizers on the structure of the microbial community. Studies on archival fertilized soils, stored for 7 years, showed that they retained more than 90% of bacterial genera, which demonstrated different life strategies in relation to the desiccation stress. Nevertheless, long-term drying did not have a significant effect on bacterial diversity, community structure, and resistance levels (Hu et al., 2023). The impact of desiccation upon soil bacterial communities can be methodically mitigated by removing relict DNA.

However, authors (Dolfing & Feng, 2015) caution in the case of archived soils that air drying as a preservation method disrupts bacterial and eukaryotic diversity in the samples; so, caution is required when conducting quantitative studies that examine microbial abundance.

The multitude and composition of bacteria were studied in 24 soil samples, collected in the mainland of China in 1934–1939 and stored in the Soil Archive of the Institute of Soil Science of the Chinese Academy of Sciences, air-dried for more than 70 years (Zhao et al., 2021). It was found that the soils still contained measurable amounts of 16S rRNA gene, ranging from 10^3 to 10^8 gene copies g^{-1} of the dry soil, which is significantly lower than that observed in fresh soils, typically containing 10^7–10^9 of bacterial cells g^{-1} soil. Among all identified taxa, *Paenibacillus, Bacillales, Firmicutes, Alicyclobacillus, Brevibacillus, Actinobacteria* demonstrated the greatest growth

activity (increase in the number of genes by more than 1,000 times) after soil rewetting. The total number of bacteria in the soils was $1.9 \cdot 10^3$–$1.7\ 10^8$ copies of the dry soil gene g^{-1} in dried soils and reached $2.6\ 10^3$–$4.1\ 10^8$ copies of the dry soil gene g^{-1} after incubation with re–wetting. As a result of wetting the bacterial abundance increased significantly in 18 soils ($P < 0.05$) and remained unchanged in 5 soils.

Studies (De Nobili et al. 2006) obtained a concentration of soil microbial biomass and its activity in soils that had previously been stored in an air-dry state for different periods (from 2 to 103 years) in the Rothamsted sample archive. Storage of air-dry soils reduced the ability of microbial biomass to restore ATP concentrations. For example, the concentration of ATP in soil taken from an uprooted (i.e., tree seedlings, saplings and shrubs, often cut to the ground level) pasture during the Rothamsted continuous wheat experiment and then air-dried for 2 years was only about 14% of the concentration in the fresh soil. In soils, archived since 1852, the ATP concentration levels (after rewetting) were 52%–57% of those in the fresh soils. From long-term stored soils there was released more than twice as much CO_2–C than from the freshly sampled soils. Specific respiration of the microbial biomass did not change much after the first 12 years of storage.

Thus, information about the amount and activity of the microbial component of soils, stored for different times, is rather contradictory. In addition to it, most of the information was obtained after rewetting the soils. Therefore, the investigations of microbial communities in the soils that were stored in an air-dry state and were not re-wetted are relevant from both the practical and the theoretical points of view.

MATERIALS AND METHODS

The research was carried out on soil samples collected in a stationary experiment at the Department of Agro-Soil Science and Soil Microbiology, NSC Institute of Agriculture, National Academy of Sciences. The soil of the experimental plot is gray forest coarse-silty-light loamy one, characterized by the agrochemical indicators: humus content - 1.44%; pH_{sol} - 4.6; hydrolytic acidity - 3.6 mg eqv/100 g^{-1} of soil; exchangeable bases: calcium - 3.9 mg eqv/100 g^{-1}; magnesium - 0.58 mg eqv/100 g^{-1} of soil; the degree of base saturation - 56%; the content of alkali hydrolysable nitrogen compounds 7–9 mg; mobile phosphates - 13–25 mg, exchangeable potassium - 8–17 mg/100 g^{-1} of soil. Lime (limestone and dolomite flour) was applied in 1992, and again in 2005 in 1.0 and 1.5 doses, according to the value of hydrolytic acidity (the full dose of 1.0 Ng was 4.5–6.0 $CaCO_3$ t ha^{-1}), 1/7 dose-annually for each crop rotation culture and to neutralize the acidity of physiologically acidic mineral fertilizers. The sideral (green manure) crop-meadow clover. In 2013, in the studied variants, soybeans of the *Ustya* variety were grown, the predecessor being millet. The experiment was repeated 4 times, the area of the sowing plot was 60 m^2 (10×6), the registration plot was 24 m^2.

The soil samples were stored in a special sample room on wooden shelves, in the light, in dense transparent plastic bags, not hermetically sealed, in an air-dry state for 32 months.

The number of microorganisms of the main ecological and trophic groups was assessed by sowing a soil suspension on appropriate general, special and selective nutrient media: ammonifiers that consume nitrogen on meat-peptone agar, mineral nitrogen

immobilizers on starch-ammonia agar, pedotrophs on soil agar (based on a soil extract of the same type of soil from the control variant), oligonitrophils - on starvation agar, cellulolytic microorganisms - on Czapek-Dox medium, autochthonous microorganisms - on nitrine starvation agar according to Winogradsky, *Azotobacter* - on Ashby's ordinary medium, streptomycetes - on starch ammonia agar, micromycetes - on Chapek's medium, mineral phosphate mobilizers - on Muromtsev's medium (Paul, 2015; Nkongolo & Narendrula-Kotha, 2020).

The indicators of the intensity of mineralization processes and the physiological and biochemical activity of the microorganism cells, and the phytotoxic properties of the soil were determined in accordance with what was described previously (Malynovska et al., 2023). For a general assessment of the biological state of the soil indicators of the total biological activity (TBA) were calculated, using the method of relative values (Rusakova, 2013). The probability of colony formation reflects the physiological and biochemical activity of the microbial cells in the natural environment.

To determine the metabolic activity of microorganisms directly in the soil, the method of analyzing the dynamics of the appearance of colonies was used, which makes it possible to simultaneously determine the number and composition of the complex of chemoorganoheterotrophic bacteria in soils (Philippot et al., 2012; Blagodatskaya & Kuzyakov, 2013).

The study of the soil phytotoxicity took place as follows: the soil, which was stored for 32 months, was weighed by 60 g, placed in the Petri dishes, moistened to 60% of the full moisture capacity and 25 seeds of winter wheat were laid out on its surface, the Petri dishes were closed (3 for each variant of the experiment), they were placed in a thermostat for 3 days, where the seeds germinate, then the cups were opened and placed on a table, illuminated by a lamp for the plant growth. Every day, the seedlings were watered with the same amount of water since the surface of the Petri dish is large, and the depth of the soil layer is insignificant and drying occurs very quickly. After 7 days, the roots of the grown plants were thoroughly washed from the soil, excess moisture was removed with filter paper, and first the intact plants were weighed, then the roots were separated from the stems with a scalpel and weighed separately.

The statistical processing of the results was carried out with the use of the statistical programmes Microsoft Excel and Statistica 10.

RESULTS AND DISCUSSION

Data on the number of microbial cells in the original fresh soil samples were published in the article (Malynovska et al., 2014). Storing the samples of the grey forest soil for 32 months in different variants leads to a decrease in the number of ammonifers without fertilisers (reference) by 68.1 times, with the application of mineral fertilisers - by 71.5 times, with the application of mineral fertilisers against the background of ploughing the by-products of the previous crop in the crop rotation - by 50.5 times, with the application of mineral fertilisers against the background of ploughing the by-products of the previous crop in the crop rotation and liming - by 58.2 times (Table 1.). The data obtained indicate that in variants with ploughing of the by-products of a predecessor crop in the crop rotation, the rate of decline in the amount of ammonifiers is reduced, which indicates the protective effect of the exogenous organic matter.

Table 1. Influence of agricultural practices on the number of microorganisms in the gray forest soil after 32 months of storage in an air-dry state, 10^4 KUO*/ g^{-1} absolutely dry soil

Variant	Ammonifiers	Immobilizers of mineral nitrogen	Oligonitrophils	*Azotobacter*, % contamination of soil	Pedotrophs	Polysaccharide Synthesizing Bacteria	Streptomycetes	Micromycetes	Melanin–synthesizing micromycetes	Acid–forming	**Total number**
Without fertilizers (reference)	713.0 ± 50.1	259.1 ± 22.3	88.0 ± 9.11	4.98 ± 0.51	168.2 ± 18.2	0.048 ± 0.004	14.0 ± 1.01	10.1 ± 0.97	0.248 ± 0.020	40.2 ± 3.55	**1.339.1**
$N_{30}P_{30}K_{45}$	1.075.0 ± 77.2	242.6 ± 30.1	80.6 ± 9.11	2.17 ± 0.15	328.8 ± 30.1	0.038 ± 0.004	13.9 ± 0.98	13.8 ± 1.12	0.713 ± 0.092	31.0 ± 2.77	**1.864.8**
$N_{30}P_{30}K_{45}$ Ciderate + by-product	1.520.0 ± 78.3	435.4 ± 40.3	64.2 ± 6.99	6.32 ± 0.61	321.2 ± 30.3	0.150 ± 0.010	13.0 ± 2.07	12.0 ± 1.15	0.435 ± 0.088	21.6 ± 2.03	**2.615.9**
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0Hg) Ciderate + by-product	1.705.0 ± 112.2	277.0 ± 25.5	92.5 ± 10.5	11.9 ± 1.09	328.0 ± 32.8	0.298 ± 0.011	10.5 ± 0.98	20.6 ± 1.14	0.689 ± 0.084	20.5 ± 1.65	**2.568.6**

Note: CFU* – a colony forming unit.

Similar trends are revealed when analysing the decline in the number of mineral nitrogen immobilizers, which are followers of ammonifiers in the conversion cycle of the nitrogen compounds, and polysaccharide-synthesizing microorganisms. Consequently, the remains of the organic matter of the plants that were ploughed into the soil help maintain the number of bacteria of these three groups. Why the microorganisms of these groups are so closely related to the presence of exogenous organic matter in the soil, is not quite clear. If polysaccharide-forming microorganisms are more dependent on the C:N ratio in the soil, that is, excess carbon, then microorganisms of the first two groups-ammonifiers and immobilizers of mineral nitrogen-are closely related in their existence to vegetative plants and their root secretions. If we consider the role of the organic matter as a substrate, then the situation with polysaccharide–synthesizing microorganisms is completely understandable: their number in the options with ploughing of exogenous organic matter exceeds the number of these microorganisms in the options without exogenous organic matter (EOM) by 3–6 times. But then it must be assumed that in the air-dry soil the processes of mineralization of the organic matter continue just like other microbiological processes. This is confirmed by the high physiological and biochemical activity of the microbial cells in the soil samples, stored for 32 months (Table 2). Thus, the physiological and biochemical activity of ammonifiers increased during storage by an average of 8.90%, mineral nitrogen immobilizers-by 20.9%. An explanation for this phenomenon may be the fact that finely dispersed substrates, such as the soil samples, due to the large total surface of the soil particles, absorb the water vapor from the air and thereby maintain

soil moisture acceptable for the life of microorganisms. This is confirmed by the moisture content of the soil samples after 32 months of storage; it is about 1.19%. From the obtained fact of maintaining a certain level of vital activity of microorganisms in the air-dry soil it follows that it is impossible to preserve the soil samples in an unchanged state for analysis, which has been practiced by agrochemists and soil scientists for many years (a comparison with the original (initial) samples). In the stored soil microbiological mineralization and synthetic processes slowly but almost constantly occur during which the macro and microelements, organic matter and humus are consumed, the content of which is the subject of study by the soil scientists.

For microorganisms of the other groups different patterns are observed. Thus, the number of oligonitrophils in the soil of various variants decreases depending on the agrotechnical practices used-by 58.2–93.9 times, micromycetes-by 1.25–2.31 times (Table 1).

The group that decreased the number of CFU to the least extent during storage were micromycetes. The reduction in their numbers is 1.68 times without fertilizers (reference), 1.62 times with the application of mineral fertilizers, 2.31 times with the application of mineral fertilizers against the background of ploughing the by-products of the predecessor crop in the crop rotation, with the application of mineral fertilizers against the background ploughing the by-products of the predecessor crop in the crop rotation and liming - 1.25 times (Table 1).

It should be noted that there is a strong decrease in their physiological and biochemical activity. This is reflected in the fact that the fungal colonies appear on the agar medium only after 72 hours whereas, when sowing fresh soil samples, they appear after 28–30 hours. The probability of the formation of their colonies is reduced in comparison with the indicators of fresh soil in the following variants: without fertiliser (reference) - 25.0%, with the application of mineral fertiliser - 61.8, with the application of mineral fertiliser against the background of ploughing the by-products of the previous crop in the crop rotation - 65.2, with the application of mineral fertiliser against the background of ploughing the by-products of the previous crop in the crop rotation and liming - 14.4% (Table 2). A possible explanation for this could be the large size of fungal hyphae and their low resistance to the soil drying out. Most of the mycelium fragments most likely died during storage and colonies are formed only by spores, the germination time of which is 72 hours.

When analysing the abundance of the melanin–synthesizing forms of fungi, the same patterns are revealed as in fresh soil samples: the reference (control) variant is characterized by the lowest abundance, and the variant with the application of mineral fertilizers is the largest. Many years of research, conducted by us, have shown that the synthesis of the melanoid pigments is a nonspecific reaction of fungi to the soil contamination with heavy metals, petroleum products, and other pollutants (Malynovska, 2017). When studying the prevalence of the melanin-synthesizing micromycetes, one can consider not only their number but also the share in the total number of micromycetes in the soil of a particular experimental variant, since the total number of fungi is influenced by many factors: the presence of substrates, soil moisture and pH, the crop being grown, etc. The analysis showed that, as a result of long-term storage of the soil, the proportion of the melanin–synthesizing micromycetes in their total number sharply decreased. If in the initial soil the proportion of the melanin-synthesizing fungi was 88.8,

94.6, 78.0, and 65.8%, then after 32 months of storage the corresponding figures were 2.48, 5.17, 3.63, and 3.34%. The reason of this may be the lack of pollutants entering the soil, which leads to a decrease in the competitive advantages of the melanin-synthesizing forms. Noteworthy is the persistence of the influence of the exogenous organic matter on the number and proportion of the melanin-synthesizing micromycetes-they are reduced, compared to the soil variants without addition of exogenous organic matter: the number of the melanin-synthesizing micromycetes is reduced by 63.9%, their share-by 42.4%. The corresponding initial indicators for fresh soil were 4.34% and 27.5%. Thus, the organic substance of the plant origin has a high sorption capacity relative to various pollutants, and it protects the biota of the soil and plants from the negative effects of pollutants the indicator of which is the reduced content of the melanin-synthesizing fungi in the variants of the experiment with ploughing in by-products of the precursor in the crop rotation and the biomass of the cideral culture. The corresponding initial values for fresh soil were 4.34% and 27.5%. Consequently, the organic matter of the plant origin has a high sorbing capacity for a variety of pollutants and protects the soil and plant biota from the negative effects of pollutants, which is indicated by the reduced content of the melanin-synthesizing fungi in the experimental variants with ploughing in of the by-products of the predecessor in the crop rotation and the biomass of the green manure (siderate) crop.

Table 2. Probability of the formation of colonies of microorganisms (λ, year^{-1} 10^{-2}) in gray forest soil after 32 months of storage in an air-dry state

Variant		Ammonifiers	Immobilizers of mineral nitrogen	Oligonitrophils	*Azotobacter*	Pedotrophs	Streptomycetes	Micromycetes	Acid–forming
Without fertilizers (reference)		3.17	1.12	0.914	0.306	2.49	1.00	0.84	1.35
$N_{30}P_{30}K_{45}$		2.46	1.28	0.973	0.553	2.36	1.05	1.02	1.77
$N_{30}P_{30}K_{45}$	Siderate + by-product	2.17	1.36	1.25	0.492	4.19	1.58	1.58	1.06
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg)	Siderate + by-product	3.13	1.29	0.613	0.154	3.42	1.28	2.16	3.14
NSR_{05}		0.04	0.06	0.051	0.050	0.05	0.04	0.11	0.13

Also noteworthy is the fact of the protective impact of exogenous organic matter upon microorganisms of various ecological and trophic groups: the organic matter promotes greater survival of microorganisms. So, the number of ammonifiers decreases in variants without the addition of the exogenous organic matter by 68.1–71.5 times, and with the addition of EOM-by 50.5–58.2 times (Table 1); similar indicators for the mineral nitrogen immobilizers were 70.1–82.9 times (without EOM) and 29.9–34.5 times with the addition of EOM, *Azotobacter* - 2.40–2.45 times (without EOM) and 1.47–1.87 times with the addition of EOM.

Secondary metabolites such as exopolysaccharides are important for the survival of the soil bacteria under extreme conditions. They perform many important functions for the producer cells, one of which is increasing their viability under the drought

conditions since polysaccharides are hydrophilic and capable of retaining water in their matrix for a long time (Mulyukin et al., 2017). When cultivated on a dried sand matrix, bacteria produce more polysaccharides and fewer proteins than those growing with sufficient water.

It is quite natural to expect a slower rate of decline in the number of the polysaccharide-synthesizing bacteria during long-term storage. However, this does not apply to all the representatives of this group but only to *Azotobacter* whose numbers are decreasing at the slowest rate among the studied groups of bacteria and fungi. And, on the contrary, the number of the polysaccharide-synthesizing bacteria decreased to the greatest extent over 32 months of storage the following variants: without fertilizers (reference) - 12.520.8 times, with the application of mineral fertilizers - 1.8210.3 times, with the application of mineral fertilizers against the background of ploughing in of the crop by-products-predecessor in the crop rotation - 6.093.3, with the application of mineral fertilizers against the background of ploughing in the by-products of the predecessor crop in the crop rotation and liming - 3.993.3 times (Table 1). A possible reason for the observed pattern may be that during the first storage period-up to 12 months-the polysaccharides perform their protective function but later they are consumed by producer cells as a substrate under starvation conditions and the cells become more vulnerable and less competitive, compared to the cells which had not previously synthesized polysaccharides. It is also possible that the observed phenomenon is due to the ability of *Azotobacter* to form cysts that are 2 times more resistant to unfavourable conditions (including drying) than the vegetative cells (Funa et al., 2006). It should be noted that ploughing the by-products of the predecessor in the crop rotation greatly affects the number of the polysaccharide–synthesizing bacteria and the rate of decline in their numbers during storage of the soil samples: the organic matter increases the number of these micro It should be noted that ploughing the by-products of the predecessor in crop rotation greatly affects the number of polysaccharide-synthesizing bacteria and the rate of decline in their numbers during storage of soil samples: organic matter increases the number of these microorganisms (by 32.1%) and reduces the rate of their death by 3.95 times organisms (by 32.1%) and reduces the rate of their death by 3.95 times.

As mentioned above, the rate of decrease in the number of *Azotobacter* is one of the lowest among the studied groups of microorganisms in different variants: without fertilizers (reference) - 2.40 times, with the application of mineral fertilizers- 2.45 times, with the application of mineral fertilizers against the background of ploughing in the by-products of the previous crop in the crop rotation - 1.47 times, with the application of mineral fertilizers against the background of ploughing in the by-products of the previous crop in the crop rotation and liming - 1.87 times (Table 1). This fact does not agree with the idea of *Azotobacter* as a culture with increased xero-sensitivity, obtained in experiments on the contact-convective dehydration, the conditions of which are close to the drying out of the bacteria in nature in contact with dry soil and air (Funa et al., 2006). A possible reason for the observed contradictions may be a more gradual decrease in the soil moisture during storage and the possibility of formation of cysts by the *Azotobacter* cells during slow dehydration, while in the experiments (Funa et al., 2006), dehydration occurred at a faster pace and the cells did not have time to form cysts.

Groups the number of which decreases as a result of ploughing in of exogenous organic matter include streptomycetes and acid-forming microorganisms (Table 1). The rate of decline in the number of streptomycetes increases in variants with ploughing in of exogenous organic matter by 29.8%; for acid-forming bacteria this figure was 128.7%.

The decline rates in the total number of microorganisms coincides in the first two options (72.1–73.0 times) and decreases under the influence of ploughing in EOM by 29.2%.

Analysis of the survival of bacteria in the soil samples with different nitrogen concentrations (control (without fertilizers) and the variant with the application of mineral fertilizers) showed that the survival of the ammonifiers, mineral nitrogen immobilizers, oligonitrophils, pedotrophs, polysaccharide-synthesizing microorganisms and streptomycetes is reduced in the soil with the application of mineral fertilizers, compared with reference (control) soil on average by 15.6%.

Table 3. Indicators of the intensity of the mineralization processes and accumulation of phytotoxic substances in the gray forest soil after 32 months of storage in an air-dry state

Variant		Pedotrophicity index	Nitrogen mineralization coefficient	Oligotrophic coefficient	Total biological activity, %	Mass (Weight) of 100 plants of the test culture-winter wheat, g
Without fertilizers (reference)		0.236	0.363	0.123	100.0	12.5
$N_{30}P_{30}K_{45}$		0.306	0.226	0.075	106.4	12.7
$N_{30}P_{30}K_{45}$	Ciderate + by-product	0.211	0.286	0.042	129.9	13.2
$N_{30}P_{30}K_{45}$ + $CaCO_3$ (1.0 Hg)	Ciderate + by-product	0.192	0.279	0.054	135.2	13.5
HCP_{05}		0.011	0.005	0.010		0.10

Neutralization of the soil solution against the background of the addition of the exogenous organic matter affects the survival of cells of different groups in different ways: it increases the rate of death of the ammonifiers, mineral nitrogen immobilizers, pedotrophs, and streptomycetes. And, conversely, it reduces the rate of death of the oligonitrophils, *Azotobacter*, polysaccharide-synthesizing and acid-forming microorganisms, micromycetes. During storage the toxicity of the soil samples increased by an average of 28.1%; however, these are preliminary data since the toxicity test is performed using the winter wheat seeds, and the germination of the seeds and the growth of the seedlings of the test culture are influenced by quite a lot of factors: the season of the year, the temperature in the room, the light intensity, etc. (Table 3). The first toxicity research was carried out in June 2013, and the second in March 2016; that is, according to the above indicators, these periods are very different. To substantiate the thesis about the increase in toxicity, it is necessary to conduct research also in June or July. The correlation between the soil toxicity of the studied variants remained the same: the most toxic is the reference (control) soil, then the variant with the addition of $N_{30}P_{30}K_{45}$, the least toxic is the soil of the variant with the application of mineral fertilizers, ploughing of EOM and liming.

The value of the soil toxicity inversely significantly ($r = – 0.983$) correlates with the value of the total biological activity, calculated by the method of relative values (Table 3). The soil of the reference (control) variant is characterized by the lowest biological activity, with the maximum activity (35.2% higher than the SBA of the soil of the control variant) is the soil with the application of mineral fertilizers, ploughing of EOM and liming. The application of mineral fertilizers allows one to maintain a greater (6.4%) SBA than in the soil without fertilizers; ploughing in the by-products of the predecessor in the crop rotation also allows one to maintain a greater (29.9%) biological activity.

CONCLUSIONS

When storing the soil for 32 months, the number of *Azotobacter* decreases to a minimal extent in the different variants: without fertilizers (reference) - 2.40 times, with the application of mineral fertilizers - 2.45 times, with the application of mineral fertilizers against the background of ploughing in the by-products of the predecessor crop in the crop rotation - 1.47 times, with the application of mineral fertilizers against the background of ploughing in the by-products of the predecessor crop in the crop rotation and liming - 1.87 times. A possible explanation for this may be the ability of *Azotobacter* to form cysts to survive unfavourable conditions.

The number of polysaccharide-synthesising bacteria decreases the most during 32 months of storage in the following variants: without fertilisers (reference) - 12.520.8 times, with the application of mineral fertilisers - 18.210. 3 times, with the application of mineral fertilisers against the background of ploughing in the by-products of the previous crop in the crop rotation - 6.093.3 times, with the application of mineral fertilisers against the background of ploughing in the by-products of the previous crop in the crop rotation and liming - 3.993.3 times.

The rate of decrease in the number of micromycetes is one of the lowest among the studied groups of microorganisms in the different variants: in the reference without fertiliser - 1.68 times, with the application of mineral fertiliser - 1.62 times, with the application of mineral fertiliser against the background of ploughing in the by-products of the previous crop in the crop rotation - 2.31 times, with the application of mineral fertiliser against the background of ploughing in the by-products of the previous crop in the crop rotation and liming - 1.25 times. The small decrease in the number is probably due to the presence of the forms of these microorganisms that survive unfavourable conditions - spores and conidia, and accompanied by a significant decrease in the physiological and biochemical activity of the cells.

The organic matter of the plant origin, ploughed into the soil, has a protective effect against several studied groups of microorganisms and promotes the survival of ammonifiers, mineral nitrogen immobilizers, Azotobacter and the polysaccharide - synthesizing microorganisms during the long-term soil storage in an air-dry state.

It is not recommended to use long-term stored soil samples for analysis and comparison of the data with the original samples since the microbiological mineralization and synthetic processes are constantly taking place in the soil, during which macro and microelements, organic matter and humus are consumed, as a result of which the soil indicators change.

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