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Influence of *Bacillus subtilis* on soil microbiocenosis

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Abstract. The peculiarities of the formation and functioning of the microbial coenosis of podzolized chernozem soil and the intensity of soil-biological processes when using probiotic preparations in different concentrations containing *Bacillus subtilis* were studied.

Probiotic preparations were applied to the soil in different concentrations and doses in separate areas, and the viability of the soil microbial coenosis of agricultural land was assessed in the spring and autumn periods on the 15th and 30th days after the application of the mixtures. The soil without any substances was considered a control option.

The analysis of the coefficients of mineralization – immobilization, oligotrophicity and pedotrophicity determined that the use of probiotics helps to increase the content of nutrients in the soil for various ecological and trophic groups of microorganisms, showed that the best result for the functioning of the microbial coenosis of podzolic chernozem soil is observed when using a probiotic in a dilution of 1:10 in a dose of 100 l ha-1. Thus, the use of probiotics in a dilution of 1:10 at a dose of 100 l ha-1 can be used as an environmentally friendly fertilizer in organic farming, which will improve the biological parameters of the soil.

Keywords: soil, probiotic preparations, vital activity of soil microorganisms, soil and biological processes.

1. Introduction

In the complex of natural and anthropogenic factors influencing the formation of soil fertility, biochemical activity of microorganisms plays the leading role. This activity predetermines the transformation of organic matter and humus synthesis. (Aranda & Comino, 2014; climate conditions and vegetation type on soil organic matter (SOM Bogomazov et al., 2016)climate conditions and vegetation type on soil organic matter (SOM

As pointed out (Aktar et al., 2009; Margesin & Niklinska, 2019; Möhring et al., 2020), pesticide application in the agrosphere negatively affects the volume and structural and functional characteristics of microbial groupings and soil biodynamic processes. It was established (Huliaieva et al., 2019; Volkogon et al., 2021), that beneficial soil microorganisms introduced into agrocenoses, inhabiting the root-containing spheres, permanently block plant infection with pathogenic bacteria and micromycetes and synthesize complex organic compounds, including biologically active substances that ensure active development of plants. In the soil-microorganisms-plant system, soil bacteria are an indispensable and integral component. This is why a plant provided with an appropriate complex of microorganisms receives adequate nutrition and, as a consequence, realises its yield potential (Zhang et al., 2008; Patyka et al., 2016).

Therefore, there is a need to apply agronomic techniques aimed at increasing the number of agronomically valuable

microorganisms, or artificial provision of agrocenoses with the necessary bacteria. Almost all modern agrocenoses need this, because soils, as already mentioned, are biologically degraded (Vlasiuk, 2020; Pysarenko et al., 2021). That is why this area is of interest for scientific research, because there is a need to select strains and doses of microorganisms to create the most favorable conditions for the development of soil microflora, which under favorable conditions is the basis for the reproduction of soil fertility.

Scientific research (Vandenberghe et al., 2017) showed that the probiotic based on *Bacillus subtilis* is an alternative fertilizer in agriculture and improves soil and plant nutrition and does not cause adverse effects. The use of probiotics in crop production is promising, but this issue requires further investigation. In particular, a number of scientists (Li et al., 2014; Kravchenko & Perederii, 2017; Vandenberghe et al., 2017; Pysarenko et al., 2021) point out the positive influence of probiotic preparations, in particular the main bacteria of the genus *Bacillus*, on improving the activity and phytosanitary condition of agrocenoses. Therefore, the issue of using probiotic preparations, in particular based on bacteria of the genus *Bacillus*, to improve soil microbiota activity is relevant and poorly investigated today. It is reasonable to determine optimal doses of probiotic preparations to justify environmentally safe system of new fertilizers use.

The aim of the work was to study the features of formation and functioning of microbial cenosis and intensity of soil and biological processes when probiotic preparations of different concentration and dose are applied.

2. Materials and methods

Field experiments were carried out on the experimental farm of PSAU (Poltava State Agrarian University) over the period of 2016-2021. Different doses and concentrations of probiotic preparations were applied to the soil on the selected plots and the viability of soil microbial cenoses of farmland soil (crops grown in the studied agrocenoses) was assessed. The experiment was conducted throughout 5 years: every spring (April-May) and every autumn in October. In this research we used probiotic preparation based on *Bacillus subtilis* microorganisms.

The experiment envisaged investigating the influence of the probiotic *Bacillus subtilis* of different concentration (dilution 1:10, 1:100, 1:1000) in different doses (50 l ha^{-1}, 100 l ha^{-1}; 150 l ha^{-1}) on the number of major groups of microorganisms in the soil (number of cells in 1 g absolutely dry soil of agrocenosis).

The following experimental soil plots were established taking into account two factors – probiotic concentration and dose:

1 – control;

2a – dosing with probiotic at a dilution of 1:10 at a rate of 50 l ha-1;

2b – dosing with probiotic at a dilution of 1:10 at a rate of 100 l ha^{-1};

2c – dosing with probiotic at a dilution of 1:10 at a rate of 150 l ha^{-1};

3a – dosing with probiotic at a dilution of 1:100 at a rate of 50 l ha^{-1};

3b – dosing with probiotic at a dilution of 1:100 at a rate of 100 l ha^{-1};

3c – dosing with probiotic at a dilution of 1:100 at a rate of 150 l ha^{-1};

4a – dosing with probiotic at a dilution of 1:1000 at a rate 50 l ha^{-1};

4b – dosing with probiotic at a dilution of 1:1000 at a rate 100 l ha^{-1};

4c – dosing with probiotic at a dilution of 1:1000 at a rate 150 l ha^{-1}.

Soil samples were taken from the experimental field of PSAU (Poltava region, Brechkovka) in the spring and summer period. Soil samples were taken in size 30×30×30 cm and established in a fourfold replication. The effect of probiotic preparations on soil microorganisms compared to the control was evaluated in the spring and autumn periods on the 15^{th} and 30^{th} days after application.

Weather conditions in the research years were typical for the zone of unstable moisture in the central forest-steppe of Ukraine. Soil of the experimental field is typical black soil deep low humus medium loamy soil type – Haplic Luvisol (according to WRB, 2014): organic matter – 3.17%, nitrogen (N) – 81 mg kg^{-1} dry soil, phosphorus (P) – 139 mg kg^{-1} dry soil, potassium (K) – 118 mg kg^{-1} dry soil, acidity (pH) – 6.8.

For microbiological analyses, 10 g of soil was taken from each variant of the experiment, and the experiments were carried out in three repetitions. The samples were placed in sterile mortars and the microorganisms were dispersed by D. Zvyagintsev method (Zvyagintsev, 1991). A tenfold dilution of the original soil suspension was used for seeding on selective media.

The ecological and trophic groups of soil microorganisms were defined by seeding certain dilutions of soil suspensions on appropriate nutrient media (Iutynska, 2017; Øvreås, 2021). Microorganisms numbers were determined by seeding the soil suspension on standard nutrient media: ammonifying bacteria on meat-and-peptone agar (MPA), streptomycetes and bacteria using mineral nitrogen (amylolytic bacteria) on starch ammonia agar (SAA), pedotrophic – on soil agar (PA), number of microscopic fungi on Chapek agarized medium with lactic acid, oligotrophic microorganisms on starvation agar (SA) (media manufacturing company Titan Biotech LTD, India). After seeding the nutrient media, they were

incubated at a temperature of 28°C for 5-14 days (depending on the growth rate of certain groups of microorganisms) (Titova & Kozlov, 2012). The number of microorganisms was recorded in colony-forming units (CFU) per 1 g of absolutely dry soil. For this purpose, the moisture content of the soil sample taken for the experiment was determined by the thermostatic weight method, and the obtained number of colonies was recalculated taking into account the moisture coefficient and dilution of the soil suspension. The experiments were performed in three repetitions. The direction of microbiological processes in the soil was assessed by the mineralization-immobilization, oligotrophic and pedotrophic coefficients (Romero-Olivares et al., 2017). Statistical analysis was performed by the analysis of variance in Excel and Statistica 6.0.

The next step was to investigate the parameters of microbiological coefficients of the intensity of soil and biological processes in soils at different doses of CSW (concominant stratum water) application. The following parameters were calculated:

- index of mineralization-immobilization (IMI) is the ratio of amylolytic microorganisms that use ammonia (mineral) nitrogen to ammonifying microorganisms that assimilate organic nitrogen (soil proteins). IMI > 1 indicates an increased rate of humus decomposition or unfavourable conditions for microorganisms development (Mary & Recous, 1994);
- index of pedotrophy (IP) is the ratio of pedotrophic microorganisms involved in the conversion of the water-soluble fraction of soil nutrients to ammonifying microorganisms that assimilate organic nitrogen. IP>1 indicates humus recovery and approach to virgin land (>6) (Bongiorno et al., 2020);
- index of oligotrophy (IO) is the ratio of oligotrophic microorganisms that complete the mineralization of soil organic compounds to ammonifying microorganisms that assimilate organic nitrogen. IO>1 indicates unfavourable degradation processes in the soil (Primpas & Karydis, 2011).

3. Results and discussion

Microbiological indication of the studied soil was carried out on the 15th and 30th days (Zvyagintsev, 1991) after the establishment of the experiment, which showed that the probiotic preparations created a certain level of biological activity in the upper soil layer, which led to specific conditions of organic matter transformation and agrobiocoenosis productivity.

The studies of the main ecological and trophic groups of microorganisms showed that in the spring period the soil was more enriched with microorganisms compared to the autumn period, which is explained by the active recovery of the microbiota in autumn (Table 1).

It was found that the influence of probiotic *Bacillus subtilis* on soil microbial cenosis depends on the dose and concentration of application, as well as on the period of aftereffect. The most active effect is shown on the 30th day after application, on the 15th day the activation of microbiological processes is observed. The best variant of the experiment for improving the viability of soil microbial cenoses in both spring and autumn periods was found to be the variant with *Bacillus subtilis* diluted 1:10 and in a dose of 100 l ha^{-1}. In particular, the total number of all groups of bacteria in the soil increases with the use of probiotics at a dilution of 1:10 by 6-33% compared to the control and is maximal when using probiotics in a dose of 100 l ha^{-1} and dilution of 1:10 (increases by 33% in spring and 25% in autumn compared to the control).

The same correlation is observed for other groups of soil microflora (Table 1). The number of pedotrophic microorganisms increased at a dilution of 1:10 by 47-78% on day 15 of application and by 50-173% on day 30, respectively, compared to the control. At this concentration, the best result was recorded at a probiotic dose of 100 l ha^{-1}, with the number of paedotrophic microorganisms increasing by 78% in spring and 173% in autumn compared with the control. The dilution of probiotic 1:100 increased the number of pedotrophic microorganisms in the spring period to 15.5 million CFU/g soil in a dose of 100 l ha^{-1} after 30 days of application, which is lower compared with the dilution of probiotic 1:10 and application of this dose (20.5 million CFU/g soil). In autumn, the dilution of probiotic 1:100 resulted in an increase of pedotrophic microorganisms up to 10.1 million CFU/g soil in a dose of 100 l ha^{-1} on day 30, which is also lower compared with probiotic dilution 1:10 and application of this dose (15.5 million CFU/g soil). At a dilution of 1:1000 the increase of pedotrophic microorganisms does not exceed 20% compared with control. Thus, the highest activity for pedotrophic microorganisms was found when probiotic was applied in a dose of 100 l ha^{-1} and probiotic diluted 1:10 on the 30th day after application (15.5 million CFU/g soil in spring sampling and 10.1 million CFU/g soil in autumn sampling, respectively).

It was found that the content of oligotrophic microorganisms decreased with probiotic in 1:10 and 1:100 concentrations by 1-9% on day 15 compared to the control, but on day 30, it slightly increased and was at a control level. Thus, no significant aftereffect of the probiotic was observed for this group of microorganisms.

Ammonificators and amylolytic microorganisms play an important role in the biological cycle of nutrients, particularly nitrogen. It was established that the number of ammonifying bacteria increases by 3-17% in the spring

Table 1. The number of the main groups of microorganisms in soil, number of cells in 1 gramme of absolutely dry soil (spring sampling, average for 2016-2021, mln CFU/g soil)

Variant of the experiment		Total number of bacteria, mln	Pedotrophic microorganisms (PA), mln.	Oligotrophic microorganisms (SA), mln	Ammonifiers (MPA), mln	Amylolytic microorganisms (SAA), mln	Actinomycetes, mln	Microscopic fungi, thousand
Probiotic concentration, *Bacillus subtilis* dilution	Dose of probiotic, *Bacillus subtilis* l ha^{-1}							
Day 15								
Control		2.2± 0.01	4.6± 0.11	9.0± 0.12	6.8± 0.10	6.9± 0.10	0.056± 0.002	15.5± 0.30
1:10 (10%)	50	2.5± 0.04	5.5± 0.15	9.1± 0.15	7.9± 0.25	6.3± 0.25	0.060± 0.002	17.7± 0.37
	100	4.1± 0.11	9.5± 0.22	8.3± 0.23	9.4± 0.39	6.5± 0.08	0.069± 0.001	22.5± 0.50
	150	3.5± 0.14	7.1± 0.30	8.9± 0.36	7.7± 0.40	7.0± 0.36	0.048± 0.002	16.9± 0.78
1:100 (1%)	50	2.4± 0.08	5.1± 0.24	8.7± 0.32	7.9± 0.32	6.5± 0.25	0.055± 0.001	16.3± 0.52
	100	3.2± 0.09	8.4± 0.36	8.5± 0.10	9.1± 0.45	6.9± 0.13	0.062± 0.002	19.2± 0.72
	150	3.0± 0.10	4.1± 0.45	8.9± 0.15	8.0± 0.11	6.1± 0.10	0.060± 0.003	17.2± 0.29
1:10 (10%)	50	3.0± 0.14	4.9± 0.12	9.1± 0.25	6.9± 0.08	7.0± 0.25	0.059± 0.002	15.9± 0.46
	100	3.1± 0.15	5.9± 0.20	9.0± 0.08	7.4± 0.10	7.1± 0.13	0.060± 0.003	17.3± 0.12
	150	2.8± 0.07	2.7± 0.10	8.5± 0.42	6.7± 0.12	6.5± 0.17	0.057± 0.000	16.2± 0.38
Day 30								
Control		3.5± 0.10	5.9± 0.27	10.2± 0.30	8.5± 0.36	9.9± 0.30	0.099± 0.004	20.4± 0.97
1:10 (10%)	50	4.1± 0.11	8.2± 0.32	10.3± 0.24	9.1± 0.12	9.8± 0.05	0.100± 0.003	21.5± 1.01
	100	6.5± 0.22	15.5± 0.41	8.4± 0.13	11.5± 0.25	9.5± 0.45	0.112± 0.003	29.6± 0.91
	150	5.8± 0.13	10.1± 0.13	8.5± 0.07	10.2± 0.12	10.2± 0.12	0.102± 0.002	25.2± 0.30
1:100 (1%)	50	5.5± 0.21	6.8± 0.07	9.8± 0.45	9.4± 0.41	10.2± 0.10	0.095± 0.005	22.1± 0.46
	100	5.8± 0.11	10.1± 0.10	10.1± 0.34	10.2± 0.30	10.0± 0.13	0.108± 0.002	25.1± 0.71
	150	5.3± 0.08	7.1± 0.12	10.0± 0.11	8.7± 0.14	9.9± 0.05	0.087± 0.004	20.3± 0.03
1:10 (10%)	50	3.6± 0.10	6.8± 0.09	10.1± 0.05	8.8± 0.30	10.1± 0.03	0.093± 0.001	19.8± 0.10
	100	4.4± 0.01	7.2± 0.11	9.8± 0.32	9.8± 0.11	9.7± 0.30	0.100± 0.001	22.5± 0.36
	150	3.8± 0.22	7.0± 0.07	10.5± 0.41	9.1± 0.42	10.0± 0.24	0.098± 0.004	20.7± 0.20

Table 2. The number of the main groups of microorganisms in soil, number of cells in 1 gramme of absolutely dry soil (autumn sampling, average for 2016-2021, mln CFU/g soil)

Variant of the experiment		Total number of bacteria, mln	Pedotrophic micro-organisms (PA), mln	Oligotrophic micro-organisms (SA), mln	Ammonifiers (MPA), mln	Amylolytic micro-organisms (SAA), mln	Actino-mycetes, mln	Microscopic fungi, thousand
Probiotic concen-tration, dilution	Dose of pro-biotic l ha^{-1}							
Day 15								
Control		2.2± 0.01	4.6± 0.11	9.0± 0.12	6.8± 0.10	6.9± 0.10	0.056± 0.002	15.5± 0.30
1:10 (10%)	50	2.5± 0.04	5.5± 0.15	9.1± 0.15	7.9± 0.25	6.3± 0.25	0.060± 0.002	17.7± 0.37
	100	4.1± 0.11	9.5± 0.22	8.3± 0.23	9.4± 0.39	6.5± 0.08	0.069± 0.001	22.5± 0.50
	150	3.5± 0.14	7.1± 0.30	8.9± 0.36	7.7± 0.40	7.0± 0.36	0.048± 0.002	16.9± 0.78
1:100 (1%)	50	2.4± 0.08	5.1± 0.24	8.7± 0.32	7.9± 0.32	6.5± 0.25	0.055± 0.001	16.3± 0.52
	100	3.2± 0.09	8.4± 0.36	8.5± 0.10	9.1± 0.45	6.9± 0.13	0.062± 0.002	19.2± 0.72
	150	3.0± 0.10	4.1± 0.45	8.9± 0.15	8.0± 0.11	6.1± 0.10	0.060± 0.003	17.2± 0.29
1:10 (10%)	50	3.0± 0.14	4.9± 0.12	9.1± 0.25	6.9± 0.08	7.0± 0.25	0.059± 0.002	15.9± 0.46
	100	3.1± 0.15	5.9± 0.20	9.0± 0.08	7.4± 0.10	7.1± 0.13	0.060± 0.003	17.3± 0.12
	150	2.8± 0.07	2.7± 0.10	8.5± 0.42	6.7± 0.12	6.5± 0.17	0.057± 0.000	16.2± 0.38
Day 30								
Control		3.5± 0.10	5.9± 0.27	10.2± 0.30	8.5± 0.36	9.9± 0.30	0.099± 0.004	20.4± 0.97
1:10 (10%)	50	4.1± 0.11	8.2± 0.32	10.3± 0.24	9.1± 0.12	9.8± 0.05	0.100± 0.003	21.5± 1.01
	100	6.5± 0.22	15.5± 0.41	8.4± 0.13	11.5± 0.25	9.5± 0.45	0.112± 0.003	29.6± 0.91
	150	5.8± 0.13	10.1± 0.13	8.5± 0.07	10.2± 0.12	10.2± 0.12	0.102± 0.002	25.2± 0.30
1:100 (1%)	50	5.5± 0.21	6.8± 0.07	9.8± 0.45	9.4± 0.41	10.2± 0.10	0.095± 0.005	22.1± 0.46
	100	5.8± 0.11	10.1± 0.10	10.1± 0.34	10.2± 0.30	10.0± 0.13	0.108± 0.002	25.1± 0.71
	150	5.3± 0.08	7.1± 0.12	10.0± 0.11	8.7± 0.14	9.9± 0.05	0.087± 0.004	20.3± 0.03
1:10 (10%)	50	3.6± 0.10	6.8± 0.09	10.1± 0.05	8.8± 0.30	10.1± 0.03	0.093± 0.001	19.8± 0.10
	100	4.4± 0.01	7.2± 0.11	9.8± 0.32	9.8± 0.11	9.7± 0.30	0.100± 0.001	22.5± 0.36
	150	3.8± 0.22	7.0± 0.07	10.5± 0.41	9.1± 0.42	10.0± 0.24	0.098± 0.004	20.7± 0.20

period and by 7-38% in autumn compared with control when the probiotics were used in 1:10 dilution. At dilutions of 1:100 and 1:1000, a significant increase in the number of ammonifying bacteria is observed only in a dose of 100 l ha^{-1} (by 3-9% in the spring period and 8-15% in the autumn period compared with the control). It was studied that the number of amylolytic microorganisms in the spring period when probiotics are used at a 1:10 dilution decreases by 3-4% on day 15 compared to the control and approaches the control on day 30. In the autumn period, both on day 15 and on day 30 at a given concentration there is a slight reduction in the number of amylolytic microorganisms in doses of 50 l ha^{-1} and 100 l ha^{-1}. This is because the application of probiotics at a dilution concentration of 1:10 intensifies the development of microorganisms that absorb organic nitrogen, while the number of microorganisms using ammonia (mineral) nitrogen is slightly reduced, but not significantly compared to the control.

The number of actinomycetes increased when the probiotic was applied at a dilution of 1:10 in a dose of 50 and 100 l ha^{-1} both in the spring and autumn periods. The highest number of microorganisms in this group was recorded when probiotic diluted 1:10 and a dose of 100 l ha^{-1} (0.452 million CFU/g soil on day 15, 0.590 million CFU/g soil on day 30 in spring, and 0.069 million CFU/g soil on day 15, 0.112 million CFU/g soil on day 30 in autumn, which means an increase of 1.2-1.5 times compared to control).

The analysis of the total number of microscopic fungi showed that on the variant with probiotic dilution of 1:10, the number of this ecological and trophic group was significantly higher compared to the control (10-55% higher compared to the control on day 15 and 5-31% higher on day 30 in the spring period, 9-45% higher on day 15 and 5-23% higher on day 30 in the autumn period). It was also found that the greatest growth of microscopic fungi under the influence of probiotic microorganisms occurred on the 15th day.

The parameters of microbiological coefficients of intensity of soil biological processes in the soil – mineralization-immobilization of nitrogen (IMI=SAA/MPA); pedotrophy (IP=PA/MPA), oligotrophy (IO=SA/MPA), at different concentrations and doses of probiotic application were studied (Table 3).

It was found that in the control samples in both spring and autumn periods IMI> 1, that indicates the predominance of organic matter destruction processes over synthesis. When the probiotic was applied at 1:10 dilution (100 l ha^{-1}), the minimum value of IMI was observed in the dose, which proves the decrease of humus decomposition rate and creation of favorable conditions for the development of soil microorganisms. In the spring period, the decrease in the IMI index was 12-14%, in autumn – 28-31% compared to the control.

Positive effects on soil microorganisms at dilutions of probiotic 1:100 and 1:1000 are recorded only in a dose of

Table 3. Microbiological coefficients of intensity of soil and biological processes in the soil (spring and autumn sampling, average for 2016-2021)

Microbiological coefficients	Variants of the experiment									
	Control	Probiotic dilution 10%			Probiotic dilution 1%			Probiotic dilution 0.1%		
	Dose of probiotic, l ha^{-1}									
		50	100	150	50	100	150	50	100	150
1. Spring sample										
Day 15										
IMI	1.02	0.96	0.87	0.91	1.01	0.89	1.01	1.00	0.92	0.97
IP	0.76	1.08	1.21	1.06	0.90	1.03	0.86	0.95	0.83	0.79
IO	1.09	1.00	0.92	0.95	1.06	0.97	0.99	1.07	0.97	1.02
Day 30										
IMI	1.05	0.98	0.92	0.93	1.01	0.99	1.02	1.06	0.97	1.05
IP	0.56	1.11	1.30	0.70	0.76	1.11	0.68	0.67	0.70	0.57
IO	1.10	0.97	0.79	0.87	1.01	0.96	1.04	1.09	1.05	1.10
2. Autumn sample										
Day 15										
IMI	1.01	0.80	0.69	0.91	0.82	0.76	0.76	1.01	0.96	0.97
IP	0.68	0.70	1.01	0.92	0.65	0.92	0.51	0.71	0.80	0.40
IO	1.32	1.15	0.88	1.16	1.10	0.93	1.11	1.32	1.22	1.27
Day 30										
IMI	1.16	1.08	0.83	1.00	1.09	0.98	1.14	1.15	0.99	1.10
IP	0.69	0.90	1.35	0.99	0.72	0.99	0.82	0.77	0.73	0.77
IO	1.20	1.13	0.73	0.83	1.04	0.99	1.15	1.15	1.00	1.15

100 l ha^{-1} (reduction of IMI index in the spring period was 6-13% at dilution of probiotic 1:100 and 3-7% at dilution 1:1000 compared to control, in autumn 16-25% and 5-15% respectively).

The growth of the pedotrophy coefficient indicates an increase in the intensity of soil organic matter decomposition. On the control sample, both in the spring and autumn periods, in most cases, IP<1, which indicates a low level of humus recovery. With probitic, the best effect was obtained at a dilution of 1:10 and a dose of 100 l ha^{-1}, this index was greater than 1 in all variants, with a 59% increase in IP on day 15 and a 132% increase on day 30 in spring compared to control, and a 48% increase in IP on day 15 and a 95% increase on day 30 in autumn compared to control. Thus, the maximum values of pedotrophy coefficient in the experiment were obtained when using the probiotic in a dose of 100 l ha^{-1} and dilution of 1:10 on the 30th day after application, which corresponds to an increase in the intensity of decomposition of soil organic matter to meet the needs of plants in nutrients.

The highest values of oligotrophy coefficient (IO) were found in the control variant, and in all variants of the experiment this indicator was higher than 1, that indicates the unfavourable degradation processes in the soil. In all variants of the use of dilution probiotic 1:10 and 1:100 on day 15 and 30 this indicator was better in comparison with control. Probiotic dose 1:10 dilution of 100 l ha^{-1} had the best effect (IO decreased by 15-28% in spring and by 33-39% in autumn compared with control), indicating an increase in available nutrients for microorganisms and high provision of nutrients. When the probiotic was diluted 1:1000, the values of oligotrophicity coefficient in all samples were close to the control.

So, it is possible to point out that the use of probiotic in concentration 1:10 increases the content of available nutrients for microorganisms, but the best option was a dose of 100 l ha^{-1}. Although the best result was achieved on day 30, the improvement of soil nutrients was also observed on day 15, which is associated with the active action of probiotics (increase of nutrients for different ecological and trophic groups of microorganisms).

4. Conclusion

The analysis of literature sources established that the use of *Bacillus subtilis* in crop production is promising, but these assumptions require further research. In particular, a number of scientists (Li et al., 2014; Kravchenko & Perederii, 2017; Vandenberghe et al., 2017; Pysarenko et al., 2021) note the positive effect of probiotic preparations, in particular on the main bacteria of the genus *Bacillu*s, on improving soil microbiota activity and phytosanitary effects on agrocenoses. However, it is necessary to study the specifics of the formation and functioning of microbial cenosis and the intensity of soil biological processes under the conditions of application of *Bacillus subtilis* of different concentrations and doses

The prospects for future research are field experiments of *Bacillus subtilis* depending on the crop type.

Based on the analysis of mineralisation-immobilisation, oligotrophic and pedotrophic coefficients, it was found that the use of *Bacillus subtilis* increases the nutrient content in the soil for different ecological and trophic groups of microorganisms, reduces the humus degradation rate and creates favourable conditions for the development of soil microorganisms. It was found that the best experiment variant both in spring and in autumn for improvement of soil microbial cenosis activity was the variant with *Bacillus subtilis* dilution concentration 1:10 and application rate of working solution 100 l/ha. In this variant, the total number of all groups of soil bacteria increased by 33% in the spring and by 25% in the autumn period compared with the control. The number of pedotrophic microorganisms increased with the application of *Bacillus subtilis* at a dilution of 1:10 by 47-78% on the 15th day of application and by 50-173% on the 30^{th} day compared to the control. The number of ammonifying bacteria increased by 3-17% in the spring period and by 7-38% in the autumn period when *Bacillus subtilis* was applied at a dilution of 1:10 as compared with the control, whereas at dilutions of 1:100 and 1:1000 a significant increase in the number of ammonifying bacteria was observed only in a dose of 100 l/ha.

Thus, the use of a probiotic at a dilution of 1:10 in a dose of 100 l/ha can be used as an environmentally friendly fertiliser in organic farming, which will improve soil and biological parameters.

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