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MICROBIOLOGICAL DIVERSITY AND CONTROL OF SOILS AND WATERS FROM FOREST AND AGROECOSYSTEMS UNDER DIFFERENT HYDROTHERMAL SOIL REGIMES AND AS FACTORS FOR HIGH-QUALITY AND SAFE COMPOSTING

Boyka MALCHEVA, Rozalina KOLEVA, Bilyana GRIGOROVA-PESHEVA, Pavel PAVLOV

University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: boika.malcheva@gmail.com

Abstract

Microbiological indicators of soils and waters from forest and agroecosystems have been studied. The main share in the composition of the soil microfl of microorganisms – actinomycetes and mold fungi, and an increase in anaerobes in over moistened soils have been established. In general, the total quantity of microorganisms is higher in agrogenic soils, which is also due to the fact that they are arable and with a controlled humidity regime. In these soils, a clearly higher quantity of microorganisms is established in spring compared to summer. The total microflora depends significantly on humidity, and the mineralization activity moderately on soil temperature. Pathogenic microorganisms have been established in some of the soils and waters, and for soils their quantity depends on the presence of pathogens in the waters that moisten them, as well as on other factors. Part of the soils were used as a substrate for composting, and part of the waters were used to moisten the compostable mixtures, after microbiological control.

Key words: *soil microflora, aquatic microflora, microbiological control, forest and agroecosystems.*

INTRODUCTION

Microorganisms are sensitive biomarkers characterizing changes and ongoing processes in any environment, in soils, compost and waters, in forest and agroecosystems, as well as in the composting phases. Soil and compost microflora depend on a complex of factors - temperature, humidity and pH of the substrate, mechanical composition of the soil, composition of the compostable mixture and other factors. The activity of microorganisms also depends on their quantity, but not as an independent factor, as well as on the composition of the microbiota.

The quantity and activity of soil microorganisms depends on soil temperature and humidity, as well as on the sampling depth, and based on these indicators, there are statistical models for recognizing and predicting soil microbiological activity by indirect signs, and it is determined that the most informative for indirect assessment of the number of soil microflora is the set of two diagnostic signs: sampling depth and soil temperature (Konsulova et al., 2017a,b,c), although microbiological indicators are highly dynamic, which determines the conduct of analyses in seasonal and annual dynamics. The moisture content of the compostable mixture is a dominant factor that affects aerobic microbial activity, to a greater extent than temperature, and the dependence between the two factors shows that the improvement of composting caused by an increase in temperature can only be achieved by increasing the moisture content (Liang et al., 2003). Moisture content of the compostable mixture 50-60%, monitoring of temperature in the composting phases, appropriate C:N ratio, pH, nutrient content are important factors for obtaining quality compost (Schulze, 1962; Suler and Finstein, 1977; Bertoldi et al., 1985; McKinley et al., 1986; Jackson and Line, 1997; Tiquia et al., 1998; Liang et al., 2003; Yordanova, 2020). Overwetting leads to a decrease in the amount of oxygen in the compostable mass and a slowdown in the rate of decomposition of organic matter (Lin, 2006). A similar trend is also found in overwetted soils (Naskova et al., 2015). In addition to the moisture content of the soil and compost, the microflora in the irrigation water also affects the soil and compost microbiome, and microbiological control of the irrigation water should be carried out by testing for the presence of pathogenic microflora that could pass into the soil, compost and vegetation (Koleva et al., 2024). Similar microbiological control of water flowing to the soil, without a specific irrigation regime, is also carried out in forest areas. The problem is that the pathogenic microflora in soils is not standardized, while for water and compost there are regulatory documents with specified norms regarding pathogenic microorganisms such as *Escherichia coli*, *Salmonella* sp., *Enterococcus* sp., *Pseudomonas aeroginosa* and others. In agrogenic soils under different hydrothermal regimes and the influence of anthropogenic and environmental factors (irrigation, fertilization, meteorological conditions), as well as in the composting process, in general, the percentage of bacteria (non-spore-forming bacteria and bacilli) is higher than that of mycelial forms (actinomycetes and molds), and after irrigation the proportion of anaerobic bacteria increases, but their quantity remains lower than that of aerobes, and the quantity of molds, which are moisture-loving, also increases, as the additives used and their rate, and irrigation techniques affect the structure of the soil and compost microbial community (Malcheva, 2021; Malcheva et al., 2018a; 2018b; 2019a; 2019b; 2020; Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Frene et al., 2022; Guo et al., 2022; Koleva et al., 2024; Grigorova-Pesheva et al., 2024a,b). A similar percentage distribution of the main groups of microorganisms is also found in forest soils - the strongest development of non-spore-forming bacteria, followed by bacilli, and the least represented are actinomycetes and micromycetes (molds) (Bogdanov et al., 2015; Malcheva, 2020a; Malcheva et al., 2024a; Grigorova-Pesheva et al., 2024c). In soils after flooding, some authors found a higher amount of anaerobes compared to aerobes (Naskova et al., 2015). Some authors point to *Bacillus megaterium* and *Bacillus cereus* as the main representatives of the bacillary microflora (Malcheva, 2020b; 2020c; Malcheva et al., 2019a) in agrogenic and forest soils, other authors found an increase in the development of bacteria from the genera *Asticacaulis*, *Aquicella* and *Acromobacter* and an altered development of mold fungi - *Aspergillus* and *Alternaria* were reduced, while *Stagonospora* and *Metarhizium* increased in irrigated soils for 5 years in agriculture (Frene et al., 2022).

The aim of the study was to establish microbial diversity and conduct microbiological control in soils and waters from forests and agroecosystems, under different hydrothermal regimes and in the creation of high-quality and safe compost.

MATERIALS AND METHODS

Waters and soils from 4 regions of the Sofia Municipality were studied: village of Zheleznitsa, village of Pasarel, Vrazhdebna district, Vitosha Nature Park.

22 sites were analyzed, in seasonal dynamics (spring and summer) by soil indicators - at two depths (0-10 cm and 10-20 cm) - 44 soil samples per season, with and without influence from analyzed water bodies.

13 water bodies were analyzed - 1 mineral well, 1 natural spring, 2 artificial wells, 1 irrigation basin, 7 local water sources (water from fountains), 1 surface water source (river), where soils were also studied, with and without influence from the water source (Table 1).

Table 1. Soil and water variants

Variants (soils)
S1-Zheleznitsa - mineral drilling
S2-Zheleznitsa - to mineral drilling
S3-Zheleznitsa - natural spring
S4-Zheleznitsa - to natural spring
S5-Passarel, roadside fountain
S6-Passarel, to roadside fountain
S7-Passarel, to "Colorful fountain"
S8-Vrazhdebna, soil watered from a pool
S9-Vrazhdebna, soil watered from Probe 1
S10-Vrazhdebna, soil watered from Probe 2
S11-Vrazhdebna, soil watered from a fountain
S12-Vrazhdebna, unwatered soil
S13-Vitosha tulip, fountain
S14-Vitosha tulip, to fountain
S15-Vitosha, fountain
S16-Vitosha, to fountain
S17-Vitosha, "Stone fountain"
S18-Vitosha, next to "Stone fountain"
S19-Vitosha, Pavlova fountain
S20-Vitosha, next to Pavlova fountain
S21-Golden Bridges, Vladayska River
S22-Golden Bridges, next to Vladayska River
Variants (waters)
W1-Zheleznitsa - mineral drilling

W2-Zheleznitsa - natural spring
W3-Passarel, roadside fountain
W4-Passarel, "Colorful fountain"
W5-Vrazhdebna, watering pool
W6-Vrazhdebna, Probe 1
W7-Vrazhdebna, Probe 2
W8-Vrazhdebna, water from an outdoor fountain
W9-Vitosha tulip, fountain
W10-Vitosha, fountain
W11-Vitosha, "Stone fountain"
W12-Vitosha, "Pavlova fountain"
W13-Golden Bridges, Vladayska River

To prepare the studied compost, a compostable mixture of starter (soil and last year's compost), zucchini, dry and green weeds, whole plants of lavender, oregano, basil and thyme was used. Composting was done indoors, in a composter.

Microbiological soil analyses were carried out using the limiting dilution method and inoculations on solid nutrient media: meatpeptone agar (non-spore-forming bacteria and bacilli), Čapek-Dox agar (mold fungi), Actinomycete isolation agar (actinomycetes and bacteria that absorb mineral nitrogen) (Mishustin & Emtsev, 1989; Gushterov et al., 1977). The total microflora and the mineralization coefficient were determined by computational methods after enumerating the individual groups of microorganisms (Mishustin & Runov, 1957; Malcheva & Naskova, 2018).

Pathogenic microflora (coliforms and *Escherichia coli*, *Enterococcus* sp., *Salmonella* sp., *Pseudomonas aeroginosa*, *Clostridium perfringens*, molds and yeasts, total number of mesophilic aerobic microorganisms) in water was determined by applying membrane filtration and placing the filter on specific nutrient media for each pathogenic species. Cultivation was in a thermostat under aerobic conditions, with the exception of *Clostridium perfringens* - cultivation in a jar (anaerobic environment). The nutrient media and confirmatory tests are specified in the relevant regulatory documents: BDS EN ISO 9308-1:2014/A1:2017, BDS EN ISO 7899-2:2003, BDS EN ISO 19250:2013, BDS EN 26461-2:2004, BDS EN ISO 16266:2008, BDS EN ISO 6222:2002, BDS EN ISO 6222:2002.

The temperature of the samples was determined in the field using a temperature probe, and the pH was determined potentiometrically using a pH meter. The moisture content of the soil and compost samples was determined using a moisture balance, model DBS.

The statistical processing of the results included determining the average value of three repetitions and the coefficient of variation for the soil and compost samples. The correlation analysis was performed using the Excel 2010 program.

RESULTS AND DISCUSSIONS

The biogenicity of the studied soils in seasonal dynamics is presented in Tables 2 and 3.

Table 2. Quantity and composition of soil microflora (x 10^3 CFU/g; spring)

Variants	Depth, cm	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes	Micromycetes	Bacteria Assimilating mineral nitrogen
S1	0-10	1540	980	400	20	140	3040
	10-20	1460	960	360	20	120	3000
S2	0-10	2320	1700	480	20	120	3060
	10-20	296	224	58	2	12	342
S3	0-10	4700	3000	1600	20	80	4510
	10-20	536	360	148	20	8	475
S4	0-10	4300	2960	1260	20	60	4320
	10-20	510	350	132	20	8	466
S5	0-10	1340	860	400	0	80	2500
	10-20	192	136	50	0	6	272
S6	0-10	1360	900	380	20	60	2400
	10-20	186	132	48	2	4	264
S7	0-10	1360	880	400	20	60	2220
	10-20	148	100	40	2	6	236
S8	0-10	6320	5360	660	140	160	4850
	10-20	738	642	66	14	16	475
S9	0-10	6000	5200	620	120	60	4000
	10-20	618	532	66	12	8	420
S10	0-10	3880	3320	460	60	40	3850
	10-20	446	376	58	6	6	395
S11	0-10	4340	3880	400	40	20	3210
	10-20	448	400	40	6	2	334
S12	0-10	5560	5000	520	20	20	3020
	10-20	560	500	56	2	2	320
S13	0-10	3460	2800	540	20	100	4280
	10-20	2880	2400	400	20	60	3940
S14	0-10	3400	2760	540	20	80	4250
	10-20	516	390	100	10	16	484
S15	0-10	3080	2720	360	0	0	4830
	10-20	2620	2380	240	0	0	4080
S16	0-10	3080	2680	360	20	20	4750
	10-20	2700	2400	260	20	20	4500
S17	0-10	2980	2760	220	0	0	3900
	10-20	2520	2380	140	0	0	3420
S18	0-10	3020	2740	240	20	20	3860
	10-20	322	290	28	2	2	390
S19	0-10	400	280	120	0	0	620
	10-20	280	220	60	0	0	400
S20	0-10	1320	1000	280	20	20	1320
	10-20	150	112	34	2	2	148
S21	0-10	400	280	100	0	20	600
	10-20	360	240	100	0	20	460
S22	0-10	1880	1500	300	40	40	1120
	10-20	246	200	38	4	4	112

Table 3. Quantity and composition of soil microflora (x 10^3 CFU/g; summer)

Variants	Depth, cm	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actinomycetes flora is occupied by non-spore-forming bacteria, followed by bacilli. Trends for weaker development of mycelial groups test	Micromycetes	Bacteria assimi-lating mineral nitrogen
S1	0-10	4520	4160	280	40	40	4150
	10-20	3200	3000	160	20	20	4150
S2	0-10	2240	1680	460	20	80	3000
	10-20	286	220	56	2	8	334
S3	0-10	5280	3000	2180	20	80	4510
	10-20	3010	2000	1000	2	8	3510
S4	0-10	4060	2800	1200	20	40	3830
	10-20	476	340	128	2	6	446
S5	0-10	1580	1100	400	0	80	2520
	10-20	164	112	40	0	12	260
S6	0-10	1600	1140	380	20	60	2460
	10-20	158	110	38	2	8	256
S7	0-10	1480	1000	400	20	60	2700
	10-20	158	104	46	2	6	278
S8	0-10	5900	5100	560	120	120	4300
	10-20	604	518	60	12	14	452
S9	0-10	5760	5060	580	100	20	3760
	10-20	600	520	66	12	2	389
S10	0-10	3680	3200	400	40	40	3550
	10-20	404	340	56	4	4	365
S11	0-10	4220	3800	360	40	20	3210
	10-20	450	390	54	4	2	332
S12	0-10	5540	5000	500	20	20	3020
	10-20	576	512	60	2	2	325
S13	0-10	3720	3000	600	20	100	5850
	10-20	3120	2600	440	20	60	4850
S14	0-10	3760	3040	600	20	100	5850
	10-20	398	316	68	2	12	608
S15	0-10	3120	2760	360	0	0	5550
	10-20	2200	2000	200	0	0	3760
S16	0-10	3260	2800	400	20	40	5780
	10-20	346	294	46	2	4	601
S17	0-10	3040	2800	240	0	0	4000
	10-20	2780	2600	180	0	0	3680
S18	0-10	3280	2880	320	40	40	4100
	10-20	362	308	42	4	8	418
S19	0-10	420	300	120	0	0	820
	10-20	400	280	120	0	0	700
S20	0-10	940	540	320	40	40	860
	10-20	136	74	52	4	6	92
S21	0-10	440	300	120	0	20	800
	10-20	400	280	100	0	20	720
S22	0-10	1300	1080	160	20	40	1000
	10-20	148	120	20	2	6	110

The main share in the composition of the soil microflora is occupied by non-spore-forming bacteria, followed by bacilli. Similar trends for agrogenic and forest soils are also established in other studies (Malcheva, 2021; Malcheva et al., 2018a; 2019a; Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Frene et al., 2022; Guo et al., 2022; Koleva et al., 2024; Bogdanov et al., 2015; Malcheva, 2020a). Trends for weaker and absent development of mycelial groups of microorganisms – actinomycetes and mold fungi in overmoistened soils are established due to the anaerobic conditions created. The mycelial structure of these groups of microorganisms and the fact that they are aerobic limits their development in an anaerobic environment. In most sites, the remaining groups of microorganisms also decrease in overmoistened soils, but less so compared to actinomycetes and micromycetes. Molds are acid-resistant and moisture-loving, but their development is limited in systematically overmoistened soils. In general, the total amount of microorganisms is higher in agrogenic soils from the Vrazhdebna district, which is also due to the fact that they are arable and with a controlled humidity regime. In these soils, a clearly higher total amount of microorganisms is established in spring compared to summer – compared to uncultivated soils. The rate of mineralization does not depend solely on the amount of microorganisms (Malcheva, 2021; Malcheva et al., 2018a; 2019a; Naskova et al., 2015; 2016; Plamenov et al., 2016; Yankova et al., 2016; Koleva et al., 2024; Bogdanov et al., 2015; Malcheva, 2020a) (Figures 1 and 2).

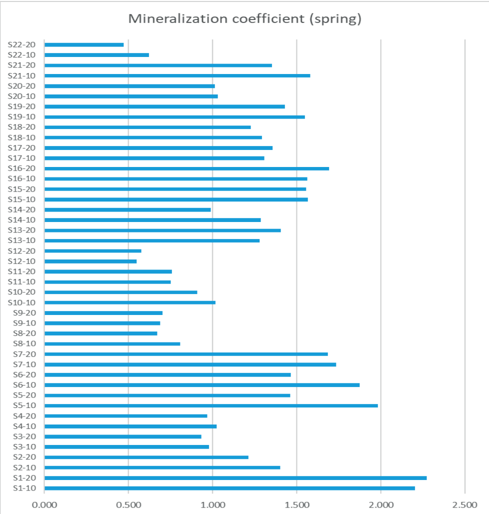

Figure 1. Mineralization coefficient values (spring)

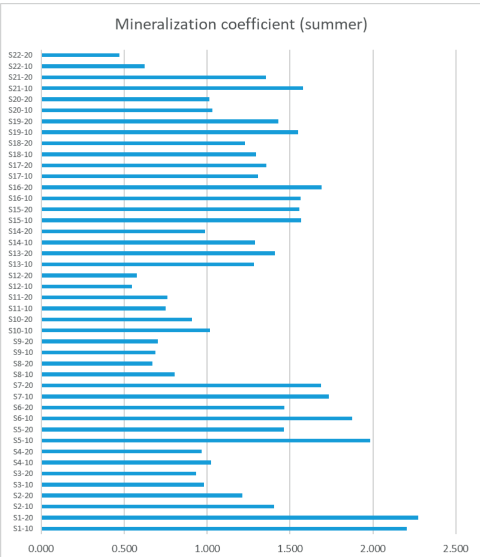

Figure 2. Mineralization coefficient values (summer)

The highest mineralization rate was found at S1 (Zheleznitsa, mineral drilling), and the lowest at S22 (Golden Bridges, next to the next to Vladayska River), which does not correlate with the highest and lowest total amount of microorganisms compared to all studied soils. A complex of factors has an influence: amount of microorganisms, temperature and humidity of the soils, mechanical composition of the soil, nutrients in the soils and other factors.

Soil humidity and temperature are interdependent factors affecting the soil microflora. When the soils are overmoistened, conditions are created for the development of anaerobic microorganisms and putrefactive processes. In soils after flooding, some authors found a higher amount of anaerobes compared to aerobes (Naskova et al., 2015). The soil humidity is higher at the sites of Vitosha, Pasarel and Zheleznitsa, where there is uncontrolled water inflow. At most of these sites, the amount of microorganisms generally decreases, especially for the mycelial groups of microorganisms, which are aerobic (actinomycetes, mold fungi). In spring, the temperatures in the arable soils from Vrazhdebna, as well as from the villages of Zheleznitsa and Pasarel, are slightly higher than in the soils from Vitosha. In the depth of the soil, the temperature decreases. The studied microorganisms are mainly mesophiles - in spring they develop at a temperature of the studied soils in the range of 9-15°C, and in summer - 28-32°C. In summer, the soil temperature increases, and the soil humidity decreases, which has the strongest impact on the development of the microflora in the arable soils. In the soils from Vitosha, Zheleznitsa and Pasarel, the soils have an acidic reaction, while the soils from Vrazhdebna have a neutral reaction. Microorganisms can also change the pH within limited limits. The more acidic environment creates conditions for more active development of mold fungi, but they are limited by the anaerobic conditions created in the overmoistened soils. The ratio of aerobes to anaerobes at different ranges of soil moisture is presented in Figures 3 and 4.

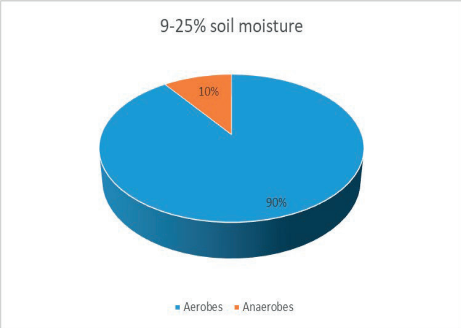

Figure 3. Aerobic: anaerobic ratio at soil moisture in the range of 9-25%

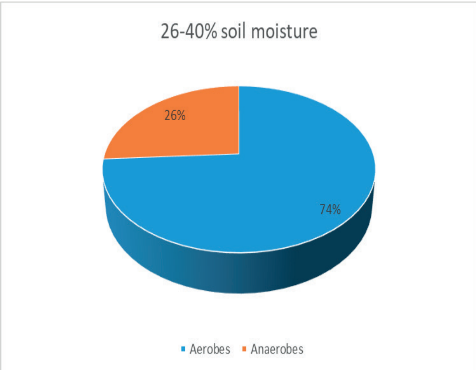

Figure 4. Aerobic: anaerobic ratio at soil moisture in the range of 26-40%

The correlations between the total microflora (TM), mineralization coefficient (MC), temperature (T), humidity (W) and pH of the soils are presented in Table 4.

Table 4. Correlation coefficients

Spring	TM	MC	T	W	pH
TM	1				
MC	-0.182	1			
T	-0.106	0.395	1		
W	0.569	0.109	-0.072	1	
pH	0.142	-0.465	-0.491	0.073	1
Summer	**TM**	**MC**	**T**	**W**	**pH**
TM	1				
MC	-0.276	1			
T	-0.015	0.394	1		
W	0.630	-0.218	-0.165	1	
pH	0.131	-0.624	-0.451	0.236	1

The total microflora depends significantly on the humidity and slightly on the pH of the soils, and the mineralization activity of microorganisms depends moderately on the temperature of the soils.

Pathogenic microorganisms have been identified in some of the soils, which mainly correlates with the presence of pathogens in the waters that moisten them, but in some sites other factors also have an influence - subsoil pollution, fecal pollution from animals, anthropogenic activity and other factors. In some of the waters, the presence of pathogenic microorganisms has been identified, mainly coliforms, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas aeruginosa*. In the soils moistened by these waters, the same pathogens are found, without *Pseudomonas aeruginosa*. The microbiological control of different types of water is also carried out by the health inspections under a monitoring program. For the purposes of this report and in connection with a national composting project being developed, compost, with starter arable soil from sq. Hostile with the highest total microflora, as indicated, and watering in three variants to the thermophilic phase: with borehole water, pool water and tap water. No pathogenic microorganisms were found in the water from an external tap and from a borehole, while the presence of coliforms, *Escherichia coli* and *Enterococcus* sp. was found in the water from the pool. At the same time, the water from the pool contained the highest amount of total microflora of bacteria, actinomycetes and mold fungi, which are actively involved in the decomposition of organic matter in soil and compost. After composting, no pathogenic microorganisms were found in all three variants, and the total amount of microorganisms was higher in the variant watered with pool water, followed by the variant watered with borehole water and the total was lower in the variant watered with water from an external tap (Figure 5). The amount of non-spore-forming bacteria and bacilli was higher than that of actinomycetes and mold fungi in the composition of the total microflora in the studied compost. Similar participation of major groups of microorganisms in compost variants has been presented in other scientific studies (Malcheva et al., 2018b; 2019b; 2020; Koleva et al., 2024; Grigorova-Pesheva et al., 2024a; 2024b).

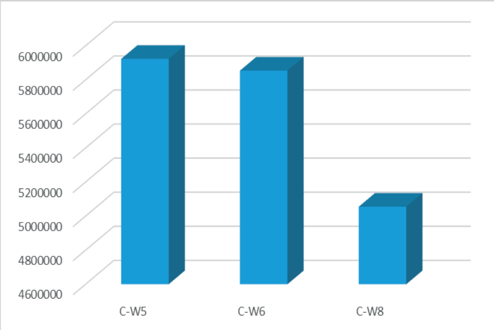

Figure 5. Total microflora in compost irrigated with water from a different source

On the one hand, the passage of the compost through a thermophilic phase disinfects it, but on the other hand, when the temperature increases, mesophilic microflora dies. The decomposition processes continue with thermophilic microorganisms. Temperature, humidity and pH have a major impact on the development of microorganisms in soils and compost. The amount and composition of the microflora in the starter, the type of waste used as a composition of the compostable mass and the irrigation water also have an impact on the production of high-quality and safe compost. The essential oil crops used (*Lavandula angustifolia* Mill., *Origanum vulgare* L., *Ocimum basilicum* L., *Thymus vulgaris* L.) have an antibacterial effect against *Escherichia coli*, *Enterococcus faecalis* and other pathogenic microorganisms (Man et al., 2019; Malcheva et al., 2024b; 2024c).

CONCLUSIONS

The total microflora is higher in agrogenic soils compared to forest soils, which is also due to the fact that they are arable and with a controlled humidity regime, with clearer trends for a higher amount of microorganisms in spring compared to summer. The rate of mineralization does not depend solely on the amount of microorganisms. The total microflora depends significantly on humidity and weakly on the pH of the soils, and the mineralization activity of microorganisms depends moderately on the temperature of the soils.

Bacteria predominate over the mycelial groups of microorganisms, with the amount of mold fungi increasing in soils with higher humidity, but not in overmoistened soils, in which the proportion of anaerobes increases.

In some of the waters, the presence of pathogenic microorganisms was detected, mainly coliforms, *Escherichia coli*, *Enterococcus* sp., *Pseudomonas aeruginosa*, which are also found in the soils moistened by these waters (with the exception of *Pseudomonas aeruginosa*). Watering the compost variant with water containing pathogenic microflora does not lead to the content of pathogenic microorganisms in the finished compost, they die during the thermophilic phase, but enriches the compost with bacteria, actinomycetes and mold fungi, which actively participate in the decomposition of organic matter. The essential oil crops used with antibacterial effect also have an effect on the destruction of pathogenic microflora.

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