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INFLUENCE OF MICROFLORA IN IRRIGATION WATER ON THE SOIL AND SOIL-COMPOST MICROBIOME

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Abstract

Sanitary-microbiological control of water for irrigation of soils and composts with agricultural crops was carried out. The analyzed water meets the regulatory requirements for indicators of pathogenic microorganisms. The main groups of microorganisms before and after irrigation were studied: non-spore-forming bacteria, bacilli, actinomycetes, micromycetes, bacteria assimilating mineral nitrogen, as well as their mineralization and enzyme activities (catalase, cellulase). The quantity and activity of the studied soil aerobic microorganisms decreased immediately after irrigation, increased up to 12 hours after irrigation, and then decreased again. This tendency is not expressed for the molds. A major share in the composition of the total microflora is occupied by bacteria. Immediately after irrigation, the number of anaerobes increases but remains lower than that of aerobes. The quantity, composition, and activity of the soil microflora depend on the humidity of the soils and soil-compost mixtures, as well as on the microflora introduced with the irrigation water and vary with different agricultural crops. The activity of microorganisms highly depends on their quantity, but it is not an independent factor for their activity.

Key words: *water microflora, soil-compost microflora, irrigation, catalase, cellulase.*

INTRODUCTION

Humidity and temperature are the main factors influencing the development and activity of soil and soil-compost microflora. Irrigation is essential to improve soil fertility and crop yields. The good humidity regime of the soils and soil-compost substrates determines a good development of aerobic groups of microorganisms, which is also facilitated by the aeration of the soils during agrotechnical processing. Waterlogging of soils and composts creates anaerobic conditions and, accordingly, leads to increased development of anaerobic groups of microorganisms (Lin, 2006; Naskova et al., 2015). Li et al. (2021) found that irrigation had an even greater impact on the abundance, diversity and composition of soil bacteria than nitrogen fertilization. According to these authors, irrigation, but not nitrogen fertilization, significantly affected the bacterial alpha diversity index, with species of Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Gemmatimonadetes and Firmicutes being the predominant phylum. Changes in soil moisture affect soil microbiomes more strongly in the surface layer than in deeper layers, while for rhizosphere communities the influence of irrigation is similar at different depths (Naylor et al., 2023). Frenk et al. (2018) found that irrigation water quality affects soil functionality and bacterial community stability in response to thermal disturbances. According to the study of these authors, bacterial communities under conditions of high availability of mineral and organic carbon (artificial wastewater) differ from the native bacterial community and show proteobacterial dominance. These bacterial communities (main abundance of bacilli) have a lower resistance to disturbance by heat treatment than soils under conditions of low resource availability (high-quality treated wastewater or freshwater) (Frenk et al., 2018). The composition and activity of the soil and soil-compost microbiome depend on a complex of anthropogenic and ecological factors, such as the applied levels of irrigation, fertilization, and cultivation technologies specifically affect the soil microbiocenosis (Malcheva, 2021; Malcheva et al., 2018a; 2018b; 2019a; 2019b; 2020; Naskova et al., 2015; 2016; Plamenov et al., 2016; Styła & Sawicka, 2010; Tasseva et al., 2006; Taseva and Zdravkova, 2016; Yankova et al., 2016; Zydlik et al., 2006). Proteobacteria, Chloroflexi, Acidobacteria, and

Gemmatimonadetes in soil irrigated with recycled water were more abundant than that irrigated with clean water as irrigation techniques and methods also affected the structure of soil bacterial communities (Guo et al., 2022). Irrigation water quality is a major force in shaping the root-associated microbiome, leading to altered microbial community structure at the plant-soil interface, and rhizosphere microflora can mediate this interaction (Zolti et al., 2019). In cultivated irrigated soils over 5 years, Frene et al. (2022) found increased bacterial development (*Asticacaulis*, *Aquicella* and *Acromobacter*), altered fungal development - *Aspergillus* and *Alternari*a were reduced, while *Stagonospora* and *Metarhizium* were increased in irrigated agriculture (Frene et al., 2022). Konsulova et al. (2017) developed a statistical model for recognizing and predicting soil microbiological activity by indirect signs, including in the model some of the main factors affecting the composition and activity of microorganisms: soil temperature and humidity, and sampling depth.

The research aimed to follow the influence of water, from different sources, with different microbiological indicators, in complex with the use of compost, on the soil and soil-compost microbiome during the germination of different agricultural crops.

MATERIALS AND METHODS

A germination test was conducted with spinach and radishes in two variants - sowing in pots with soil and with a soil-compost mixture, as well as control variants without vegetation. The experiment was conducted in greenhouse conditions, prepared in two versions according to the type of water for irrigation - fresh and borehole water. The options are watered daily. Both types of water are tested for the presence of *Escherichia coli* and coliforms, as well as intestinal bacteria (*Enterococcus*) according to the requirements of Ordinance 18/2009 for irrigation water, by inoculating specific nutrient media (Endo agar for *Escherichia coli* and coliforms, and *Enterococcus* agar for *Enterococcus* sp.) and subsequent cultivation in a thermostat at 37°C. Water, soil and soil-compost mixture were examined for main groups of aerobic microorganisms occurring in soils: bacteria, actinomycetes, micromycetes - for water by direct dense inoculations (cfu/ml), and for soils and soil-compost mixtures by inoculation after dilution by the method of limiting dilutions and subsequent sowings (cfu/g dry substrate) (Mishustin & Emtsev, 1989; Gushterov et al., 1977). The following nutrient media were used: meat-peptone agar for non-spore-forming bacteria and bacilli, Actinomycetes isolation agar for actinomycetes and mineral nitrogen-assimilating bacteria, and Chapek-Dox agar for mold fungi. Cultures for these groups of microorganisms were cultivated in a thermostat at 25°C. Anaerobes are isolated by plating on Anaerobe agar and culturing in a jar with reagents to create an anaerobic environment. The total microflora and mineralization coefficient (MC) were determined for the soil and soil-compost samples (Mishustin & Runov, 1957; Malcheva & Naskova, 2018).

The variants were as follows:

V1 - Control (soil, without vegetation);
V2 - Control (compost, without vegetation);
V3 - Spinach (*Spinacia oleraceae* L.) (soil);
V4 - Spinach (*Spinacia oleraceae* L.) (compost);
V5 - Radishes (*Raphanus sativus* L.) (soil);
V6 - Radishes (*Raphanus sativus* L.) (compost).

The catalase activity of microorganisms was determined by a titration manganimetry method (Khaziev, 1976).

Cellulase activity of microorganisms was determined by reading the percentage of degraded area of cellulosic material (Khaziev, 1976).

The humidity of the soil and soil-compost variants was determined on a moisture balance, model DBS.

Statistical processing of the results included determination of the mean value of three replicates for the microbiological indicators and the coefficient of variation. Correlation analysis performed using the Excel 2010 program was applied.

RESULTS AND DISCUSSIONS

The tested waters used for irrigation meet the regulatory requirements for such waters - no pathogenic types of microorganisms (*Escherichia coli* and coliforms, intestinal bacteria - *Enterococcus*) were found, both in the fresh and the borehole water. In the freshwater were found 50 cfu/ml non-pathogenic non-spore-forming bacteria, and in the borehole water - 200 cfu/ml spore-forming bacteria and 400 cfu/ml non-spore-forming bacteria. The quantity, composition, and activity of microflora in soil and soil-compost mixture before irrigation are presented in Table 1.

Table 1. Quantity, composition and activity of the microflora in the studied substrates before irrigation (x 10^3 cfu/g dry substrate)

Variants	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
Before irrigation							
V 1	4900	3300	960	600	40	1320	0.31
V 2	5920	3780	1120	960	60	1640	0.34
V 3	5160	3460	1000	640	60	1460	0.33
V 4	6140	3900	1160	1000	80	1740	0.34
V 5	5380	3540	1060	700	80	1660	0.36
V 6	6380	4020	1200	1060	100	2000	0.38

Before irrigation, the total microflora was highest in radishes, followed by spinach and the samples without vegetation. Compost increases soil biogenicity. Non-spore-forming bacteria and bacilli occupy the main share in the composition of the total microflora, followed by actinomycetes, and the least represented are micromycetes (mold fungi). The mineralization activity is higher in the vegetation and compost variants and follows the total microflora. The rate of decomposition of organic matter in radishes is slightly higher than in spinach, which correlates with the total amount of microorganisms in the individual variants. Immediately after irrigation, the quantity of aerobic groups of microorganisms decreases, while the number of anaerobes, represented by anaerobic non-spore-forming and spore-forming bacteria, increases (Table 2).

Table 2. Quantity, composition and activity of the microflora in the studied substrates immediately after irrigation (x 10^3 cfu/g dry substrate)

Variants	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
Immediately after irrigation							
Fresh water							
V 1	4420	3120	840	400	60	1160	0.29
V 2	4700	3200	920	500	80	1320	0.32
V 3	4660	3200	920	460	80	1280	0.31
V 4	4800	3260	940	500	100	1360	0.32
V 5	4860	3280	960	500	120	1380	0.33
V 6	4960	3320	1000	520	120	1480	0.34
Borehole water							
V 1	4860	3260	940	580	80	1300	0.31
V 2	5820	3720	1080	920	100	1600	0.33
V 3	5060	3400	960	600	100	1440	0.33
V 4	6020	3840	1120	940	120	1720	0.35
V 5	5280	3540	1000	620	120	1680	0.37
V 6	6220	3940	1140	1000	140	1920	0.38

Immediately after irrigation in the tested variants, the total aerobic microflora decreases, and only the amount of mold fungi, which develop better in a moist environment, slightly increases. In the composition of the total microflora, however, the amount of mold fungi is again the lowest, and the highest percentage share is occupied by non-spore-forming bacteria, followed by bacilli and actinomycetes. The total amount of microorganisms in the borehole irrigated variants was higher than that in the fresh water irrigated variants, mainly due to a higher amount of non-sporulating bacteria and bacilli. This trend is partly determined by the higher number of bacteria found in the borehole water. The values of the mineralization coefficient in the variants irrigated with borehole water are close to those before irrigation, while in the variants irrigated with fresh water, the rate of mineralization immediately after irrigation slightly decreases, which correlates with a decrease in the total amount of microorganisms. Irrigation water quality affects soil functionality and bacterial community stability in response to thermal disturbances (Frenk et al., 2018). Li et al. (2021) found that irrigation had an even greater impact on the abundance, diversity and composition of soil bacteria than nitrogen fertilization.

After 12 hours after irrigation, the number of aerobic groups of microorganisms increased and the total microflora had higher values than those before irrigation (Table 3).

Table 3. Quantity, composition and activity of the microflora in the studied substrates 12 hours after irrigation (x 10^3 cfu/g dry substrate)

	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
12 hour after irrigation							
Freshwater							
V1	5860	3780	1100	920	60	1680	0.34
V2	6080	3820	1180	980	100	1780	0.36
V3	6120	3900	1160	980	80	1920	0.38
V4	6320	4000	1200	1020	100	2020	0.39
V5	6300	4000	1200	1000	100	2120	0.41
V6	6560	4120	1260	1060	120	2260	0.42
Borehole water							
V1	6080	3900	1140	960	80	1820	0.36
V2	6320	4000	1200	1000	120	1920	0.37
V3	6220	3940	1180	1000	100	2000	0.39
V4	6380	4020	1220	1020	120	2180	0.42
V5	6320	3980	1200	1040	100	2240	0.43
V6	6500	4060	1260	1060	120	2400	0.45

The quantity of microorganisms was again higher in the variants irrigated with borehole water, with compost and with vegetation, and the biogenicity was higher in radish compared to spinach. The trend regarding the distribution of the investigated groups of microorganisms in the composition of the general microflora remains similar to the previous research periods. The amount of mold fungi is close to the results immediately after irrigation, with a tendency to decrease with drying. The mineralization activity in this period is higher than that before irrigation and immediately after irrigation, to a higher degree in the variants irrigated with borehole water. As humidity decreases (24 hour after irrigation), the number of microorganisms and their mineralization activity also decreases (Table 4).

Table 4. Quantity, composition and activity of the microflora in the studied substrates 24 hours after irrigation (x 10^3 cfu/g dry substrate)

Variants	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
24 hour after irrigation							
Fresh water							
V 1	4980	3320	1000	620	40	1360	0.32
V 2	5960	3800	1140	960	60	1660	0.34
V 3	5240	3500	1020	660	60	1500	0.33
V 4	6200	3920	1180	1020	80	1760	0.35
V 5	5460	3580	1080	720	80	1700	0.37
V 6	6440	4060	1220	1060	100	2040	0.39
Borehole water							
V 1	5080	3360	1060	620	40	1380	0.31
V 2	6040	3840	1180	960	60	1700	0.34
V 3	5320	3540	1040	680	60	1540	0.35
V 4	6260	3960	1200	1020	80	1820	0.35
V 5	5520	3620	1100	720	80	1740	0.37
V 6	6540	4120	1260	1060	100	2060	0.38

For one day after irrigation, the quantity and activity of microorganisms is close to that before irrigation, which confirms the importance of proper irrigation for soil fertility. The trends regarding the number of microorganisms in the individual variants and their distribution in the microbiocenosis remain similar. It reduces the amount of mold fungi the most. The preserved higher quantity of microorganisms in the soil-compost variants is due both to the nutrients in the compost and to its other properties: it improves the physico-chemical and biological properties of the soil, it improves the infiltration of air and water in the root zone, it improves the structure of the soil, increases the soil's water holding capacity and reduces surface runoff (Karadag et al., 2013; Yordanova, 2020).

As we mentioned, immediately after irrigation, the number of aerobic microorganisms decreases. The results show that in this period, however, the quantity of anaerobic bacteria increases, remaining lower than that of aerobes. Regarding the total microflora, the percentage ratio between aerobes: anaerobes varies by period: immediately after irrigation the ratio of aerobes: anaerobes is 60:40, 12 hours after

watering the ratio of aerobes: anaerobes is 90:10, 24 hours after watering the ratio of aerobes to anaerobes is 96:4. Some authors found a higher quantity of anaerobes than aerobes in soils after flooding (Naskova et al., 2015). Excess moisture in compost reduces air flow and thus the rate of organic matter degradation (Lin, 2006).
Enzyme activity (catalase and cellulase) of soil and soil-compost microflora correlated with trends in total microbial abundance and mineralization activity can be observed within Figure 1 and Figure 2.

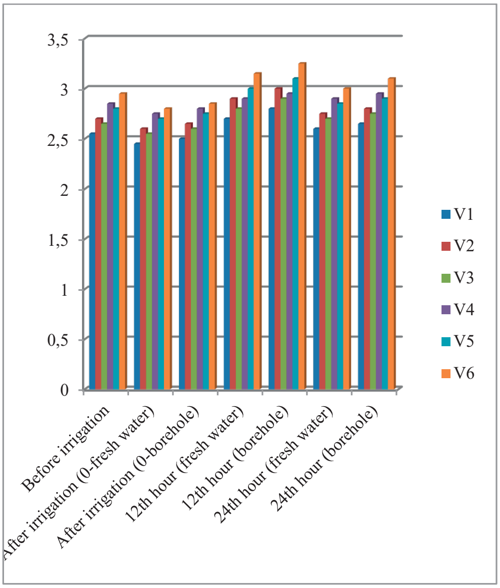

Figure 1. Catalase activity (ml O_2/30 min)

The analysed enzyme activities were highest 12 hours after irrigation, in the variants irrigated with borehole water, in the soil-compost mixture and in the variants with radishes. The values of the enzymes in the individual research periods depend on the quantity and composition of the microflora in the variants, the irrigation regime, the type of water used, the presence and type of vegetation.

Changes in the activity of cellulase and catalase enzymes in soils can be used as biochemical indicators of processes after flooding and fertilization (Malcheva et al., 2015; 2016; 2018a; 2019a; Malcheva, 2021; Naskova et al., 2016).

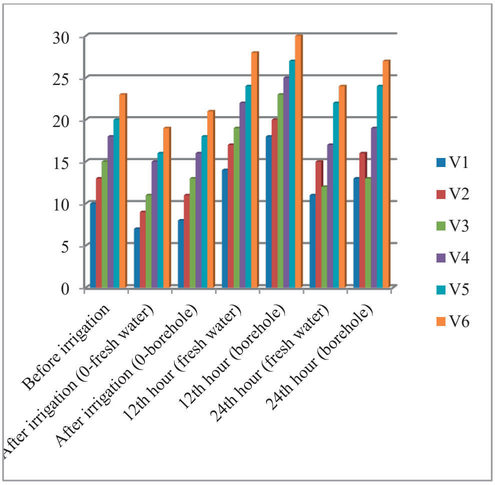

Figure 2. Cellulase activity (% degraded area)

The composition and activity of the microflora in soils and compost depend on a complex of anthropogenic and natural factors, such as the applied levels of irrigation, fertilization, and cultivation technologies specifically affect the soil microbiocenosis (Malcheva, 2021; Malcheva et al., 2018a; 2018b; 2019a; 2019b; 2020; Naskova et al., 2015; 2016; Plamenov et al., 2016; Styła and Sawicka, 2010; Tasseva et al., 2006; Taseva & Zdravkova, 2016; Yankova et al., 2016; Zydlik et al., 2006).
The higher biogenicity of the soil and soil-compost variants when irrigated with borehole water is not the only factor determining the activity of microorganisms. As we mentioned, these sensitive indicators, and accordingly their activity, depend on a complex of factors. Correlation analysis showed a high positive correlation between the total microflora, the amount of non-spore-forming bacteria, bacilli, actinomycetes, and mineral nitrogen-assimilating bacteria and their activity. There is a moderate positive relationship between the quantity and activity of mold fungi. The correlation coefficients determining the indicated dependencies are slightly higher in the variants irrigated with freshwater compared to those irrigated with well water, which once again confirms that the quantity of microorganisms is not the only prerequisite for their activity (Table 5).

Table 5. Correlation dependencies

Indicator	Total micro-flora	Non-spore-bac-teria	Baci-lli	Acti-nomy-cetes	Micro-myce-tes	Bac-teria-N	MC	Cata-lase	Cellulase
Fresh water									
Total micro-flora	1								
Non-spore-bac-teria	1	1							
Bacilli	0.99	0.98	1						
Actino-myce-tes	0.99	0.99	0.977	1					
Micro-myce-tes	0.24	0.24	0.283	0.138	1				
Bacte-ria-N	0.96	0.96	0.958	0.917	0.398	1			
MC	0.87	0.88	0.884	0.809	0.506	0.97	1		
Cata-lase	0.86	0.85	0.892	0.796	0.603	0.92	0.9	1	
Cellu-lase	0.78	0.78	0.819	0.702	0.647	0.9	0.9	0.95	1
Borehole water									
Total micro-flora	1								
Non-spore-bac-teria	1	1							
Bacilli	0.95	0.95	1						
Actino-myce-tes	0.99	0.97	0.927	1					
Micro-myce-tes	0.48	0.49	0.272	0.429	1				
Bacte-ria-N	0.88	0.89	0.869	0.829	0.547	1			
MC	0.73	0.74	0.717	0.659	0.568	0.96	1		
Cata-lase	0.84	0.85	0.892	0.769	0.387	0.93	0.9	1	
Cellu-lase	0.76	0.78	0.806	0.672	0.446	0.93	0.9	0.94	1

CONCLUSIONS

The tested waters do not contain pathogenic microorganisms, which is a prerequisite for their use for irrigation. Borehole water contains a higher quantity of bacteria than freshwater, which are found in soils and are involved in the decomposition of organic matter. In this regard, the results show that the use of borehole water increases the biogenicity of the soil and soil-compost variants after irrigation to a higher degree, compared to the use of fresh water. The activity of microorganisms highly depends on their quantity, but it is not an independent factor for their activity.

The total aerobic microflora decreases immediately after irrigation, at the expense of increasing the number of anaerobes, but the aerobic microflora remains predominant. Only the quantity of mold fungi does not decrease since they more closely depend on the humidity of the substrates in which they develop. Twelve hours after irrigation, the total number of microorganisms increased to the highest degree and reached values higher than those before irrigation. After 24 hours of irrigation, the number of microorganisms decreases and reaches values close to those before irrigation. These trends confirm the need for daily irrigation during the germination of agricultural crops to maintain good fertility of the studied substrates.

Biogenicity is influenced by the irrigation regime, the period of study after irrigation, the composition of the microflora, the type of irrigation water, the use of compost, the presence of vegetation, and the type of vegetation. It is higher in the options irrigated with borehole water compared to fresh, in the soil-compost options compared to those with only soil, and in the radish options compared to those with spinach. Non-spore-forming bacteria and bacilli occupy a major share in the composition of the general microflora, followed by actinomycetes, and mold fungi are the least represented.

The catalase and cellulase activity of microorganisms, as well as their mineralization activity, depend on the quantity of microorganisms, the humidity of the samples, the type of irrigation water and the option – soil, soil-compost mixture, the period of study after irrigation, the presence of vegetation and the type of vegetation. The activity of the microorganisms is highest at 12 hours after irrigation, in the soil-compost mixture, in the variants with radishes.

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