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Variability of Soil Microorganism Numbers in Response to Exogenous Organic Matter and Water-Absorbing Substrate

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ABSTRACT

The aim of the research was to determine the variability of the total numbers of bacteria, actinomycetes and fungi under stress associated with the presence of exogenous organic matter in the soil. Additonaly, the ratio of the total number of bacteria and actinomycetes to the number of fungi was calculated. Another goal of the experiment was to observe the effect of substrates absorbing water and minimizing drought stress on the number of soil microorganisms. Organic waste materials applied to the soil did not significantly affect the number of microorganisms, i.e. bacteria, actinomycetes or the ratio of bacteria and actinomycetes on the one hand to fungi on the other. The results of the research prove the possibility of utilizing organic matter produced by sewage treatment plants, waste treatment plants or mushroom farms as substances enriching the soil of urban green areas.

Keywords: actinomycetes, bacterias, microscopic fungi, soil, compost.

INTRODUCTION

Microbes living in soil pores account for up to 50% of total soil volume (Wu et al. 1990). The presence of microorganisms in the soil affects its fertility and health. Soil beneficial microorganisms, for example, reduce the number of pathogenic ones. By mineralizing organic matter, microorganisms provide plants with nutrients and improve soil structure (Blagodatskaya and Kuzyakov 2008). Organic matter is a factor determining the number of soil microorganisms affecting humus transformation in many ways. However, soil organic substances are also affected by hydrothermal conditions and soil physicochemical properties and its contamination (Kallenbach et al. 2016). The most numerous groups of soil microorganisms include bacteria proper, actinomycetes and fungi (Mendes et al. 2013).

Involved in biogeochemical cycles, bacteria play a key role in soil biological processes (Ranjard and Richaume 2001). They act as an auxiliary factor in the bioremediation of organic and metallic pollutants (Glick 2010). They also stimulate plant growth by fixing atmospheric nitrogen and producing phytohormones, i.e. auxins, cytokinins and gibberellins, but they also synthesize siderophores, thus facilitating iron uptake by plants, and they dissolve minerals so that they become more easily available to plants (Glick et al. 2007). Actinomycetes are gram-positive bacteria, but they are unique because of their ability to form spores and build mycelial structures (Agadagba 2014; Solecka et al. 2013). It is a group of microorganisms widely distributed in the natural environment, with about 90% of them living in the soil. As the second after bacteria, they transform complex carbon and nitrogen compounds in the soil (Gałązka and Kocoń 2015). They are isolated from the soil environment for pharmaceutical or agricultural industry purposes (Bawazir and Shantaram 2018). Interestingly, actinomycetes are used to produce natural antibiotics. Studies by Sapkota et al. (2020) indicate that about 46% of actinomycete isolates are efficient producers of antimicrobials. In addition, secondary metabolites of soil-isolated actinomycetes are potent inhibitors of many plant pathogens (Jeffrey 2008).

Oksay et al. (2004) point out that soil actinomycetes are able to inhibit Erwinia amylovora and Agrobacterium tumefaciens.

Together with bacteria and actinomycetes, fungi participate in the process of soil nutrient cycling. They have physiological abilities to accumulate water, and they take part in soil-forming and plant nutrition processes. Saprophytic fungi are involved in intensive mineralization of organic matter, which positively affects soil fertility (Ritz and Young 2004; Gałązka and Kocoń 2015). In active and inactive forms, fungi perform many different functions in the soil, and their soil propagules are present in the state of dormancy (Bridge and Spooner 2001).

In recent years, new applications for composted organic matter originating from discharges obtained from waste treatment plants, sewage treatment plants or mushroom farms have been intensively searched for. This kind of waste is often a good quality material with high content of organic matter. However, due to its varied amounts of heavy metals, it is not allowed to be used in agriculture, but it could be used in urban green areas. It contains a wide variety of organic compounds, and it can be an excellent base for setting up permanent urban lawns or flowerbeds. One-off introduction of exogenous organic matter improves soil physical and chemical structure and facilitates the germination of plant seeds. Additionally, soil porosity improves and its ability to store water increases. Soil microorganisms break down organic matter, making numerous macro and microelements available to emerging plants (Allen-King et al. 2002).

The aim of the research was to determine the variability of the total numbers of bacteria, actinomycetes and fungi under stress associated with the presence of exogenous organic matter in the soil. Another goal of the experiment was to observe the effect of substrates absorbing water and minimizing drought stress on the number of soil microorganisms.

Table 1. Soil composition

Component	Content
N_{total}	0.259 g·kg^{-1} DM
Organic matter	5.64 g·kg^{-1} DM
C_{org}	3.27 g·kg^{-1} DM
pH	6.92
P_2O_5	106 mg 100 g^{-1} of soil
K_2O	15.5 mg 100 g^{-1} of soil
Mg	8.7 mg 100 g^{-1} of soil

MATERIALS AND METHODS

Characteristics of the experimental place

The lawn experiment was established at the experimental facility of the University of Natural Sciences and Humanities in Siedlce (N: 52°10’11.63” E: 22°17’14.99”) in the autumn of 2017 and continued throughout the growing seasons of 2018 and 2019. Plots with an area of 4m^2 were arranged with a split-plot design. The loamy sand soil was of the Technosol type according to the FAO classification (Schad et al. 2014). Its composition is presented in Table 1.

Lawn establishment and experimental factors

To establish the lawns, perennial ryegrass (*Lolium perenne L.*) of the Boxer variety was sown in autumn 2017, according to the seeding standard of 3 kg 100 m^{-2}, with seeds provided by the Grunwald Plant Breeding Ltd. Boxer is an early lawn grass variety, a diploid of great durability and compactness. In addition, it is not susceptible to diseases of leaves and shoots.

The maintenance of lawns was limited; they were not irrigated in order to investigate the effect of weather conditions on the number of microorganisms. The lack of irrigation also made verification of the sorption capacity of water-absorbing substrates easier. After winter, lawn care consisted in scarification and aeration. All plots were treated with the same dose of nitrogen and the same dose of potassium. After the first mowing, 40% of nitrogen and potassium were applied, another two doses, each of 30%, were used in May/June and September/October. Fertilizer doses were as follows: 1.5 N kg 100m^{-2} and 0.8 K kg 100m^{-2}.

Due to its high content in the soil, phosphorus was not applied.

Experimental factors:

- Treatments with exogenous organic matter constituted the first factor:
  - Control;
  - WTP – compost from a waste treatment plant at a dose of 100 kg 100 m^{-2},
  - STP – granules from a sewage treatment plant at a dose of 50 kg 100 m^{-2},
  - MS – compost from mushroom substrate at a dose of 75 kg 100 m^{-2}.

The dose of the materials was dependent on their content of organic matter. Those materials were placed at a depth of about 5 cm below the soil surface. Table 2 presents the composition of exogenously applied organic matter of different origin.

Water absorbing substrates introduced into the soil constituted the second factor:
- Control;
- Coconut fibre substrate – 200 $dm^3 \cdot 100\ m^{-2}$;
- Hydrogel substrate –5000 $g \cdot 100\ m^{-2}$;
- Coconut fibre substrate – 100 $dm^3 \cdot 100\ m^{-2}$ + Hydrogel substrate – 2500 $g \cdot 100\ m^{-2}$.

Both absorbents were placed in the soil at a depth of 10 cm below its surface.

Dependent variables:
- The total number of aerobic bacteria was determined by serial dilution using Bount-Rovira medium (Bount and Rovira 1955) with the addition of soil extract. The samples were seeded deep and cultured at 28°C for 72 hours.
- The Actinomycete number was determined by serial dilution using Gauze medium (1983) with the addition of starch. The samples were seeded deep and cultured at 28°C for 5 days.
- The total number of microscopic fungi was determined by serial dilution using peptone-glucose agar with Rose Bengal according to Martin (1950) and the addition of streptomycin. The samples were seeded deep and cultured at 28°C for 72 hours. At the end of the incubation period, the number of microorganisms was expressed in colony-forming units (CFU) per 1 gram of soil.
- The ratio of the sum of the number of bacteria and actinomycetes to the number of fungi.

Soil samples were collected from each plot once a year in the second half of September. Three samples were taken from each object and subjected to the following analyses. The soil was sampled using a cylindrical device with a capacity of 1500 cm^3. Then, for microbiological analysis from a large sample of soils, five smaller ones were collected at different points and then mixed thoroughly.

Hydrothermal conditions

The average air temperature during the growing seasons of 2018–2019 was 2°C higher than the multiannual average. Particularly exceptional was April, where the average daily temperature was about 13°C (4.5°C more than the multiannual average). The year 2018 was more favourable in terms of the amount of precipitation than the following year. In 2019, the beginning and end of the growing period were with very low rainfall.

Statistics

The results of the research were processed using the Statistica 13 program (TIBCO Software Inc., PaloAlto, CA, USA). The data were analyzed at the significance level of $p<0.05$. The differences between means were assessed using ANOVA and Tukey's test. Differences between means are indicated by letters in the Tables.

Table 2. Chemical properties of the substrates

Chemical properties	Type of exogenous organic matter		
	WTP	STP	MS
pH	7.4	6.8	7.1
Organic matter content	16.73%	69.01%	51.09%
Available components [mg kg^{-1}]			
Mn	156.0	563.0	313.0
Cu	18.3	125.0	22.5
Zn	125.0	938.0	219.0
Fe	1325.0	15000.0	1360
Available components [mg $100g^{-1}$]			
P_2O_4	396.0	1129.0	1103
K_2O	73.2	59.2	1653.0
Mg	216.0	535.0	409.0
Heavy metal content [mg kg^{-1}DM]			
Pb	43.4	19.1	28.3
Cd	0.36	0.89	0.44

Table 3. Average monthly air temperature (°C) and monthly total precipitation (mm)

Year	Month							
	April	May	June	July	Aug.	Sept.	Oct.	Means
Temperature (°C)								
2018	12.9	16.5	18.1	19.1	19.8	15.6	9.3	15.9
2019	13.1	17.0	18.3	20.4	20.6	15.9	9.6	16.4
Means	13.0	16.8	18.2	19.8	20.2	15.8	9.5	16.2
Multiannual average	8.5	14.0	17.4	19.8	18.9	13.2	7.9	14.2
Precipitation (mm)								
2018	42	26	75	98	27	42	44	51
2019	6	60	36	30	49	17	10	30
Means	24	43	56	64	38	30	27	40
Multiannual average	33	52	52	65	56	48	28	48

RESULTS

For water-absorbing substrates average bacterial counts (Table 4) were the highest on the plot without water absorbing substrate (103.54 10^5CFU g^{-1}FM of soil). The use of coconut fibre reduced the number of bacteria by 15%, and hydrogel did it by 30%. In turn, both substrates used together contributed to a 17% decrease in colony numbers. Yet the above differences were not statistically significant.

The use of water-absorbing substrates did not significantly affect the count of actinomycetes in the soil either.

The smallest number of microscopic fungi was found on the plot where coconut fibre was used together with hydrogel, with the value similar to that recorded on the control plot. Slightly more of these microorganisms, by about 12%, were in soil with hydrogel substrate and by about 17% more on the plot with coconut fibre. These differences were not statistically significant.

Comparing the average effects of water-absorbing substrates on ratio values, no statistically significant differences were noted. However, the ratios slightly differed from each other on the control plot and on the one on which coconut fibre and hydrogel were used together. The values on the plot with coconut fibre and hydrogel, each used on its own, were narrower by 23 and 28% than the control ratio, respectively.

On average, the most numerous bacterial colonies (103.13 10^5 CFU g^{-1}FM of soil) were found on plots without exogenous organic matter (Table 5). On plots with exogenous organic matter the number of bacteria decreased relative to the control plot by 7% for WTP, by 32% for STP and by 19% for MS. However, differences in bacterial numbers between the control plot and other plots were not statistically significant.

The number of actinomycetes did not vary significantly across types of exogenous organic matter and ranged from 70 to 80 $\cdot 10^4$ CFU g^{-1}FM of soil. The total numbers of microscopic fungi ranged from 28 to $38 \cdot 10^3$ CFU g^{-1}FM of soil. Differences between average counts on plots with different exogenous matter were statistically insignificant. Yet, the smallest numbers of microscopic fungi were recorded on the control plot and on that with STP granules. On the plot with

Table 4. Average number of microorganisms depending on the soil absorbent used

Type of microorganism	No water-absorbing substrate	Coconut fibre	Hydrogel	Coconut fibre + Hydrogel
Total bacterial numbers (10^5 CFU g^{-1}FM of soil)	103.54^{a} ±57.09	87.58^{a} ±23.87	75.67^{a} ±17.63	85.54^{a} ±24.09
Actinomycete numbers (10^4 CFU g^{-1}FM of soil)	77.58^{a} ±13.56	75.50^{a} ±3.47	70.63^{a} ±5.96	70.08^{a} ±11.73
Total numbers of microscopic fungi (10^3CFU g^{-1}FM of soil)	31.29^{a} ±7.26	36.08^{a} ±15.89	34.46^{a} ±7.19	29.50^{a} ±4.30
The ratio of the total number of bacteria and actinomycetes to the number of fungi	376.19^{a} ±252.82	289.25^{a} ±87.64	270.37^{a} ±96.51	323.79^{a} ±104.85

Note: The values with different superscript letters in a row are significantly different ($p < 0.05$).

Table 5. Average number of microorganisms depending on the exogenous matter used

Type of microorganism	Control	WTP	STP	MS
Total bacterial numbers (10^5 CFU g^{-1}FM of soil)	103.13^{a} ±17.00	95.79^{a} ±56.35	69.96^{a} ±14.89	83.46^{a} ±26.51
Actinomycete numbers (10^4 CFU g^{-1}FM of soil)	79.92^{a} ±7.38	71.00^{a} ±14.27	70.29^{a} ±8.25	72.58^{a} ±4.34
Total numbers of microscopic fungi (10^3CFU g^{-1}FM of soil)	30.04^{a} ±8.52	34.29^{a} ±9.39	28.58^{a} ±4.71	38.42^{a} ±12.21
The ratio of the total number of bacteria and actinomycetes to the number of fungi.	382.52^{a} ±75.14	347.67^{ab} ±272.67	288.15^{ab} ±65.54	241.26^{b} ±23.21

Note: The values with different superscript letters in a row are significantly different ($p < 0.05$).

WTP the colony number was by 15% larger and on the one with MS by about 24%.

The predominance of bacteria and actinomycetes over microscopic fungi is favourable for the soil. According to the results, the average, most favourable ratio between individual microorganisms was on the control plot. A slightly narrower ratio, but with the difference statistically insignificant, was recorded on the WTP plot (by 9%) and on the STP plot (by 25%). The least favourable ratio of the sum of bacteria and actinomycetes to microscopic fungi (37% lower in relation to control) was on the plot where MS compost was used.

Double infection with total bacteria using exogenous organic matter and absorbent at the same time showed no significant differences (Figure 1). It can be assumed that the use of a soil absorbent neutralizes the effect of organic matter. This is particularly visible in the case of WTP matter, where in the object without absorbents the number of bacteria was more than twice as high as in the objects where this matter was used with absorbents.

The highest number of actinomycetes was recorded in the facility where only exogenous matter was used without absorbent, in the control facility and after WTP application (Figure 2). Large differences in the number of actinomycetes resulted from the use of exogenous organic matter on objects with coconut fiber + hydrogel

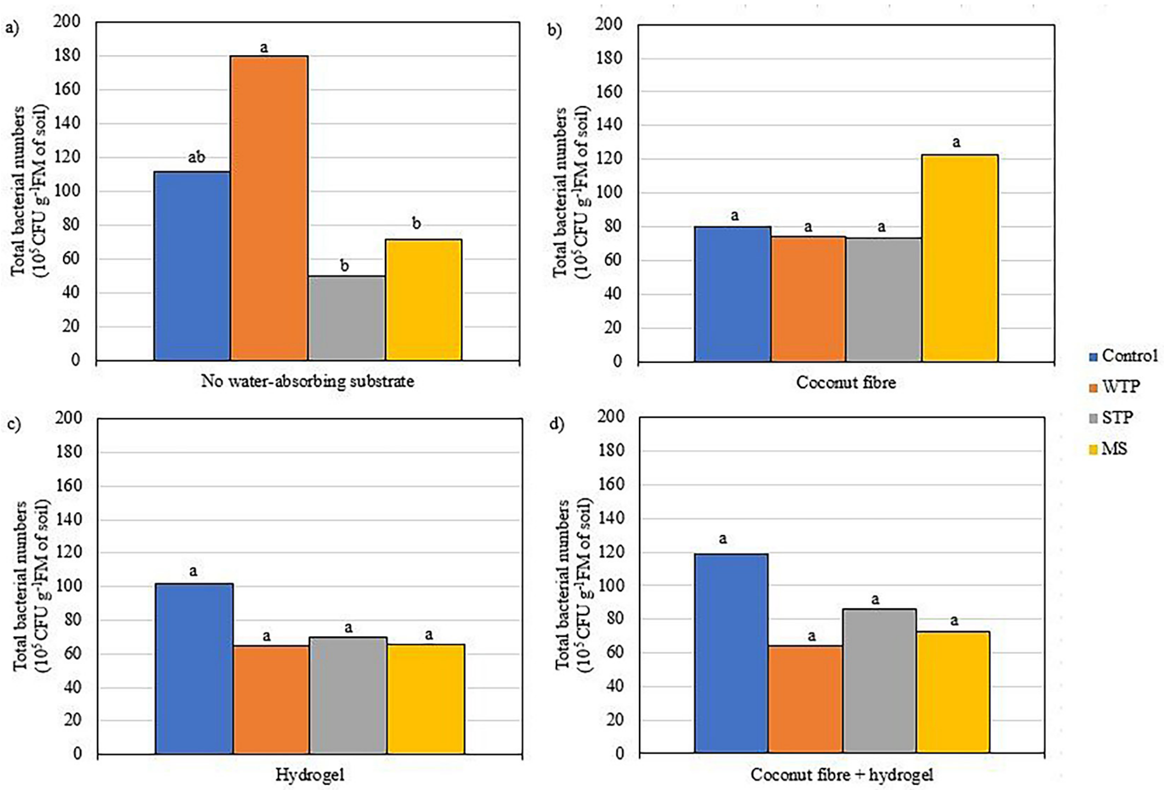

Figure 1. The number of bacteria depending on the absorbent used and exogenous organic matter

Note: The values with different superscript letters are significantly different ($p < 0.05$)

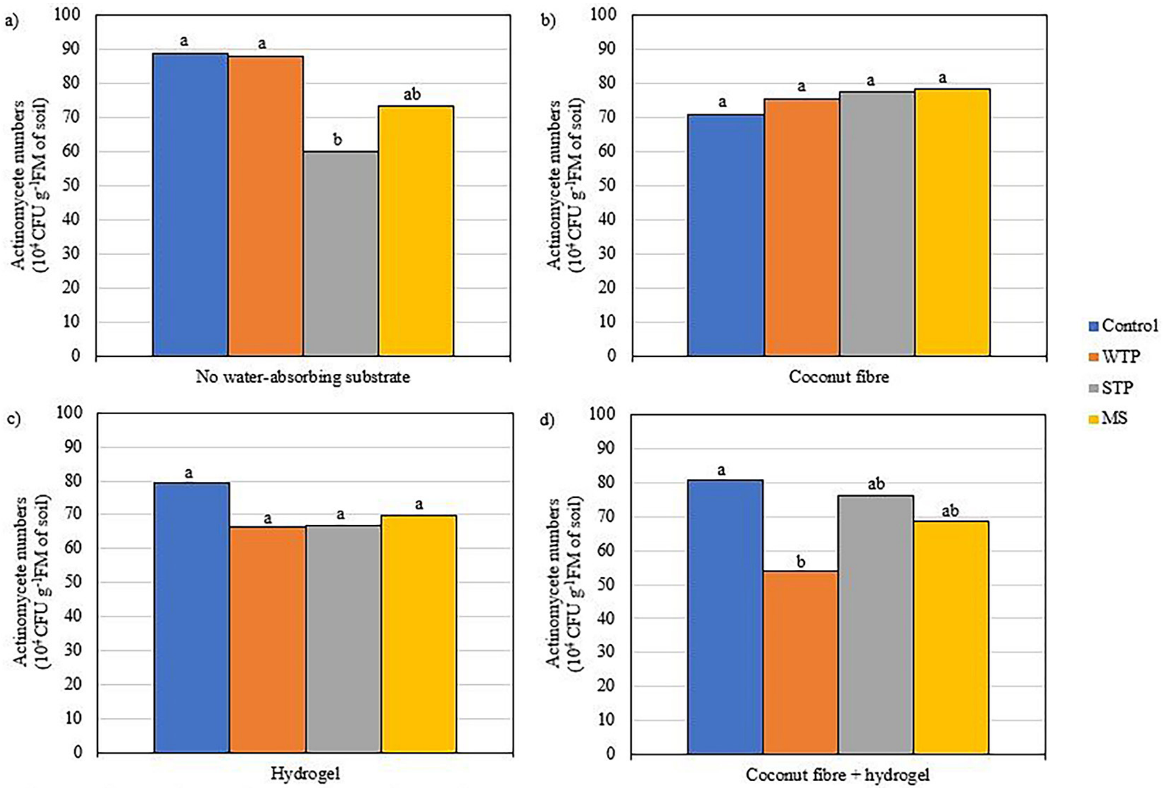

Figure 2. The number of actinomycetes depending on the absorbent used and exogenous organic matter

Note: The values with different superscript letters in a row are significantly different ($p < 0.05$)

absorbent. An undesirable effect was noted due to the application of WTP to these objects, as the number of actinomycetes decreased significantly compared to the control object.

In most cases, neither the applied absorbents nor exogenous materials influenced the fungi content in the soil (Figure 3). The exception are objects made of coconut fiber. After the combined use of coconut fiber and MS, the fungi content was twice as high compared to the control and STP treatment.

There were no significant differences in the ratio of the sum of the number of bacteria and actinomycetes to the number of fungi on coconut fiber and hydrogel objects. In the case of objects without soil absorbents, the object with WTP had the most favorable relationship of microorganisms (Figure 4).

In the second year of the study (2019), significantly higher numbers of bacterial colonies were recorded than in the first year of the study (2018), in particular in the control objects (increase by 42%) and in the objects supplied with WTP (increase by 55%) and WS (increase by 50%). The number of actinomycetes was distributed similarly over the two years of research, although the increases in numbers were smaller – 19% for WTP and 17% for WS. The smallest discrepancies in the number of microorganisms over the years were recorded for fungi. A significant difference was recorded only in 2019 for MS – where this value was the highest. In the case of microorganisms, all objects show a continuing trend of lower values in the first year and slightly higher values in the following year. Both years of research differed significantly in hydrothermal conditions. Despite differences in climatic conditions, the relationship between the numbers of individual microorganisms was similar in both years of the study.

Considering the average number of bacteria across years, significantly more colonies were found in 2019, a year with less rainfall, than in 2018, when the rainfall was much higher (Figure 6). However, differences in the number of these microorganisms were notable between both years of research. Actinomycetes, like bacteria, were found in greater numbers in 2019, when they were on average 14% more numerous than in the previous year. Like bacteria and actinomycetes, microscopic fungi also appeared in greater numbers in the second year of research. Their increase was 39% compared to the first year, and it was a statistically significant difference.

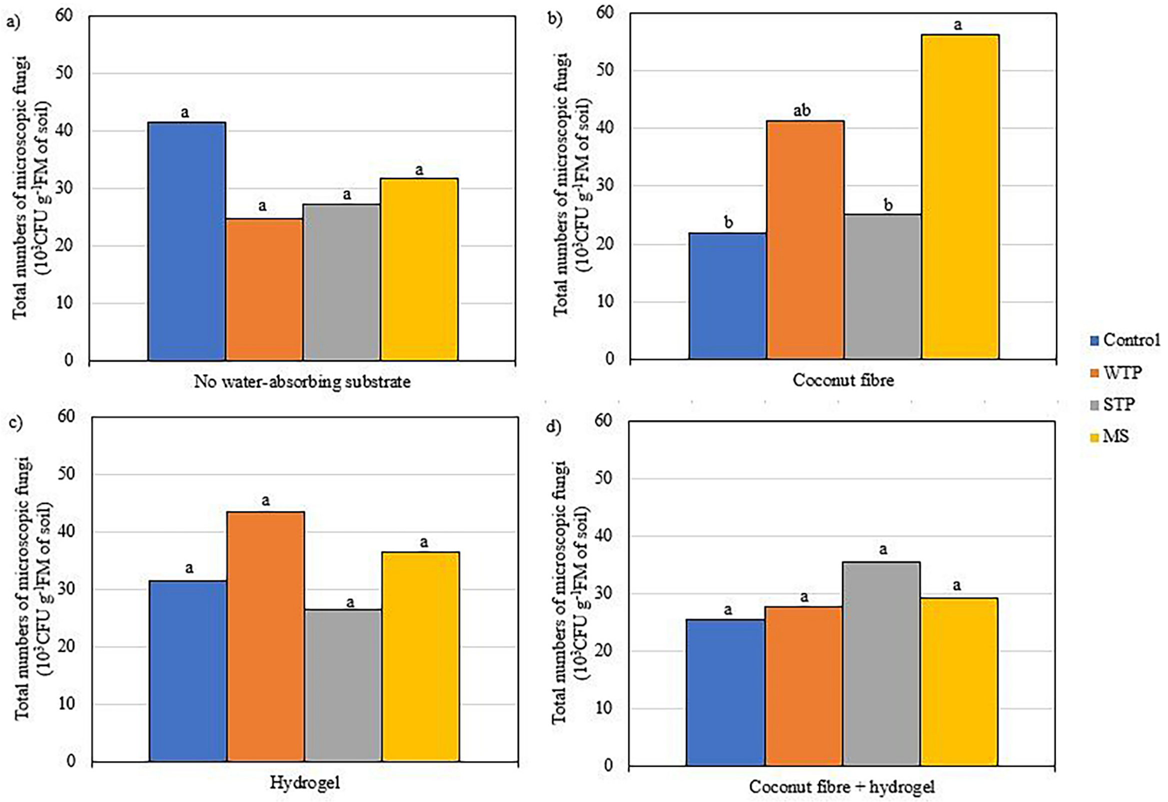

Figure 3. The number of fungi depending on the absorbent used and exogenous organic matter

Note: The values with different superscript letters in a row are significantly different ($p < 0.05$)

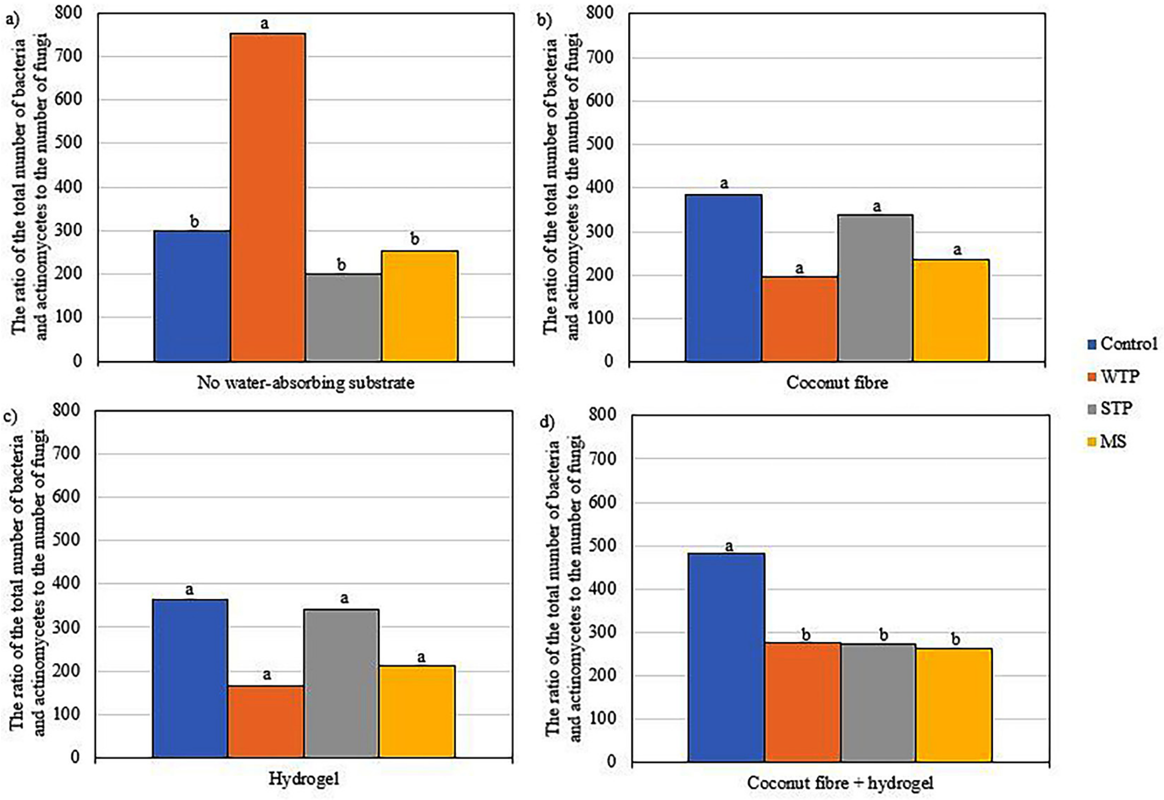

Figure 4. The ratio of the sum of the number of bacteria and actinomycetes to the number of fungi depending on the absorbent used and exogenous organic matter

Note: The values with different superscript letters in a row are significantly different ($p < 0.05$)

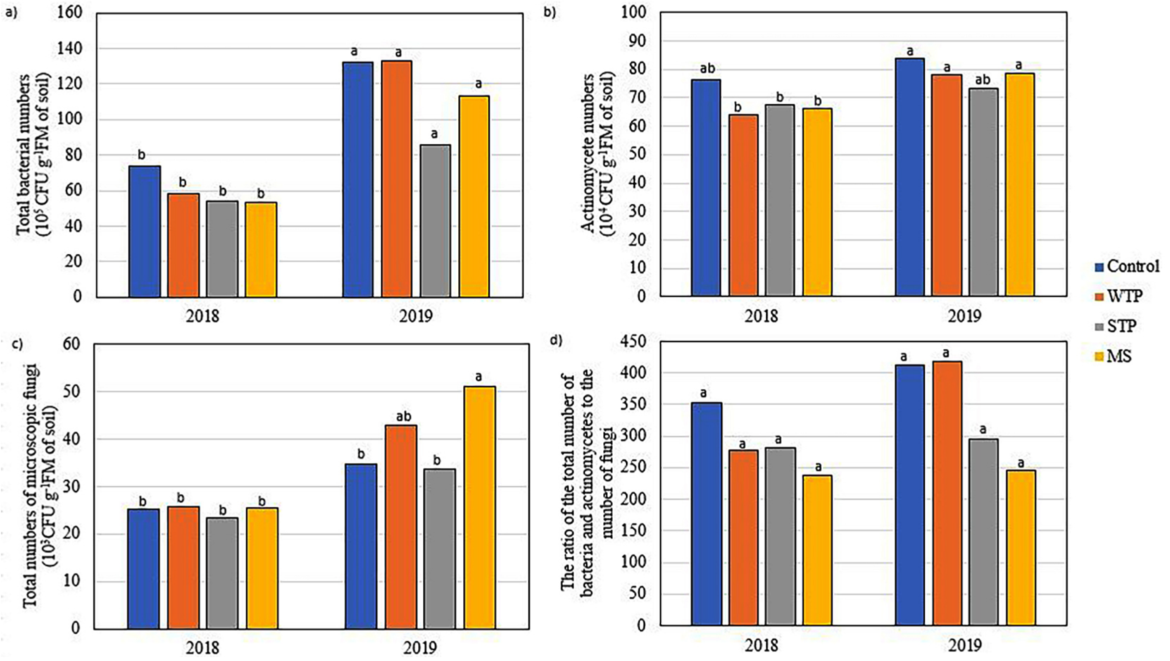

Figure 5. The number of bacteria, actinomycetes and fungi depending on the exogenous organic matter used over the years of research

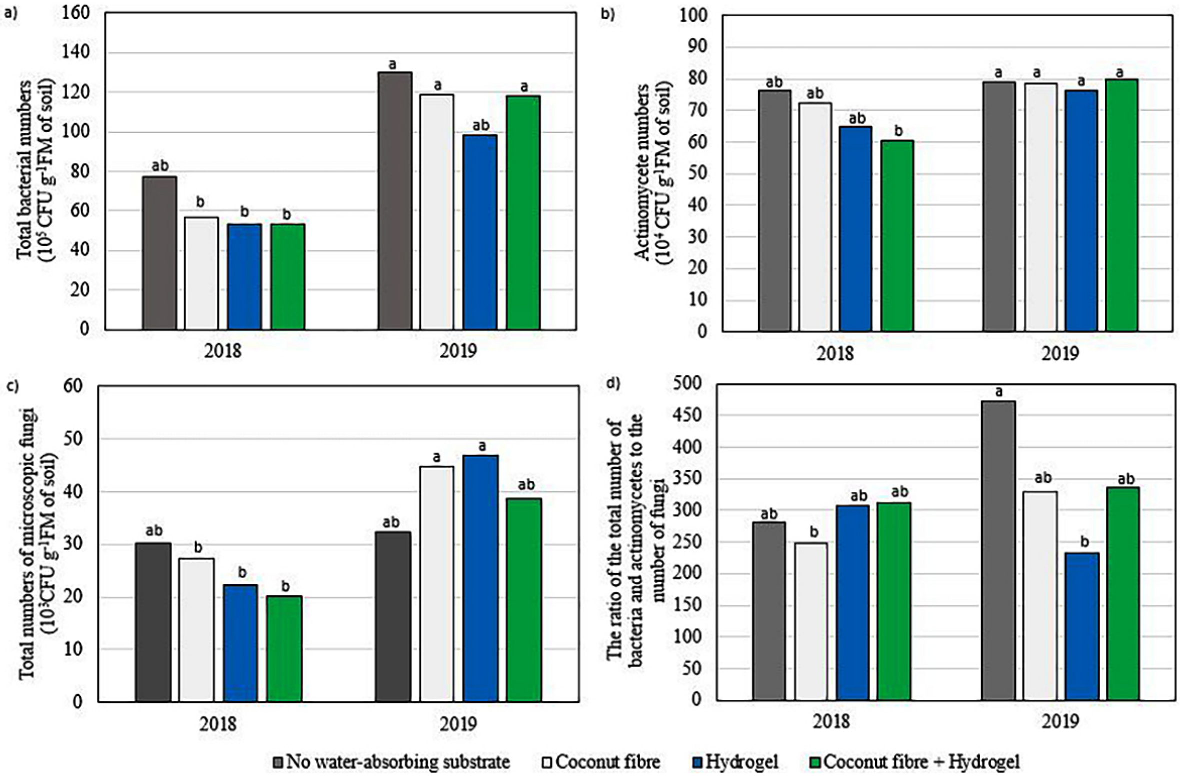

Figure 6. The number of bacteria, actinomycetes and fungi depending on the soil absorbent used over the years of research

DISCUSSION

Bacterial colonies in urban soil can be affected by metal contamination (Dell'Amico et al. 2005). In the present experiment, STP sediments contained the most cadmium, which could have affected the number of bacteria, actinomycetes and fungi. Thus, on plots with STP granules the smallest amounts of microorganisms were recorded, but the differences in the number of microorganisms between plots with different exogenous organic matter were not statistically significant. Kabata-Pendias and Pendias (1993) state that cadmium is a very mobile element, and its activation can be induced after the introduction of waste with its substantial content into the soil. In turn, Gondek (2003) reports that the addition of sediments, composts and vermicomposts from tanning sludge to the soil has little effect on changes in the content of available and total amounts of cadmium and lead. According to Wuertz and Mergeay (1997), soil contamination with cadmium negatively affects microbial diversity and inhibits many microbial processes. Wyszkowska et al. (2007) have found that this element is highly toxic to bacteria of the *Azotobacter* genus and significantly reduces the number of other soil microorganisms. Toxic amounts of cadmium inhibit the uptake of nutrients by plants. However, there are reports that certain groups of bacteria counteract cadmium inhibition of nutrient uptake by stimulating plant root growth and increasing the activity of nutrient uptake processes (Safronova et al. 2006).

Exogenous organic matter from WTP contained the most lead among other kinds of waste examined in the present experiment. However, there was no significant decrease in the number of microorganisms on plots where the substrate with the highest amount of lead was used. Lead is a highly toxic metal, but, at the same times it is present in all soils (Silvetti et al. 2014). According to Zhanget al. (2017), it can be immobilized into a form that does not threaten soil microorganisms and plants. This is due to bacteria that release phosphorus by breaking down phosphates into forms that block lead bioavailability.

The overall bacterial numbers in response to different forms of organic matter and water-absorbing substrate did not vary significantly. On the other hand, the number of bacteria was greater in the year with less rainfall (2019). Lower precipitation affects ecosystem processes and, consequently, the microbial community. However, the response of the soil microbial community and its interaction with ecosystem processes still remain unclear (Ren et al. 2018). According to Zhang et al. (2017), the interactions between bacterial numbers on the one hand and precipitation and nutrients supplied to them on the other are not significant. In turn, Borowik and Wyszkowska (2016a) reported that optimal soil moisture content was 20% for the development of organotrophic bacteria and 40% for *Azotobacter* spp.

The present results indicated that technosol soil contained a similar number of actinomycetes across all kinds of exogenous organic matter and water-absorbing substrate, with smaller differences than in the case of bacteria. However, differences in the number of actinomycetes were found across the years so hydrothermal conditions during a given growing period were a factor affecting that value. A greater amount of precipitation and lower temperatures in the first year of research (2018) apparently did not promote their development. According to the literature (Borowik and Wyszkowska 2016a), moisture determined the microbiological and biochemical activity of the soil to a much lesser extent than the soil type, with the highest number of actinomycetes recorded at the moisture content of 40%. Similarly, Arifuzzaman et al. (2010) argues that the number and diversity of actinomycetes depends on the specificity of soils, cultivation methods and organic matter content.

The number of fungi in the present experiment was greater in 2019, a year with lower precipitation and higher air temperature. Contrary to that, Zhang et al. (2017) reported that the number of fungi was significantly reduced in response to decreased rainfall. A higher temperature of 25-30°C promotes the development of soil microorganisms, i.e. bacteria and fungi. If the temperature drops, their development is slowed down, yet without causing the extinction of colonies (Rousk and Baath 2011). According to Borowik and Wyszkowska (2016a, b), fungi thrive best at 60% of soil moisture, but soil temperature only slightly changes the ecophysiology of diverse microbial groups. In turn, Unger et al. (2009) report that stagnant water reduces their numbers.

In the present experiment, the highest number of each microorganism group was recorded in the soil with coconut fibre substrate, but no significant differences were found between the effects of the two different substrates on soil microorganisms. Unfortunately, there is a lack of literature reports on this matter to discuss the results.

CONCLUSIONS

Organic waste materials applied to the soil did not significantly affect the number of microorganisms, i.e. bacteria, actinomycetes or the ratio of bacteria and actinomycetes on the one hand to fungi on the other. Therefore, these substances can be considered safe for soil microorganisms. The results of the research prove the possibility of utilizing organic matter produced by sewage treatment plants, waste treatment plants or mushroom farms as substances enriching the soil of urban green areas. Because the results significantly varied mainly over the years, it can be assumed that the number of microorganisms was affected by the amount of precipitation and air temperature.

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