

CHANGES IN MICROBIAL ABUNDANCE OF COMPOST AFTER TREATMENT WITH SPECIFIC ADDITIVES

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Abstract

The present study aimed to follow the change in the microbial community of finished compost after application of different additives. Six variants and one control (C) were prepared: V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - mineral + microbial fertilizer. Initial sampling was performed, as well as sampling at days 5, 10, and 15. In V2 V5, V6 and V7, the microbial biota increases with time. On the fifth day, the amounts of microorganisms were highest in the samples with added organic aqueous extracts. On the tenth day, the V7 has the highest microbial abundance. On the fifteenth day, the microbial abundance of V5 is again the lowest. The highest number of microorganisms has V7. The data showed that the water-based organic extracts slightly increased the amount of the microbial community compared to the control. The pure microbial additive has a suppressive effect on the compost microflora, but combined with a mineral additive gives good results and can be used for improvement of compost parameters.

Key words: microorganisms, compost, composting process, additives.

INTRODUCTION

Composting represents an economically advantageous methodology for biological decomposition. The regulation of the composting process is predominantly governed by microorganisms. This intricate process is significantly influenced by the physicochemical parameters, including thermal conditions, oxygenation levels, moisture content, carbon to nitrogen ratio, and pH (Cogger et al., 2008; Ventorino et al., 2019).

FAO defines composting as a specific aerobic process in which aerobically composted organic matter is converted into a mass called "compost". This mass is used to improve soil structure and provide nutrients (FAO, 2015). During the composting process, microorganisms decompose organic material, resulting in the generation of carbon dioxide, water, heat, and humus - the comparatively stable organic resultant product. Under conditions deemed optimal, the composting sequence progresses through four distinct phases: 1) the mesophilic or moderate-temperature phase; 2) the thermophilic or elevated-temperature phase; 3) a cooling phase and 4) maturation phase, spanning several

months (Papale et al., 2021). Specific groups of microorganisms are involved in all phases of the composting process. The products obtained as a result of metabolic processes of one type of microorganisms serve as nutrients or a source of energy for another type of microorganisms (Mira et al., 2003). The main participants in the composting process are bacteria, microscopic fungi and actinomycetes. Bacteria play the main role in the individual stages of composting (Silva & Naik, 2007).

Composting as a process is a hot topic in agriculture. Composting achieves simultaneous treatment and disposal of the generated agricultural waste and subsequent production of a valuable product - compost (Wagas et al., 2023; Papale et al., 2021).

Across Europe, agricultural soils are degraded and in poor condition both in terms of their physical performance and nutrient content (Ferreira et al., 2022; Lawrence & Melgar, 2023). Composting is an economically beneficial process that can help both small farmers and medium and large farmers to help restore the soil. (Karthika, et al., 2022). When applying compost to the soil, a number of improvements in its characteristics are observed - the structure of the soil is improved,

the water-air regime is improved, the pH is balanced and slow-release nutrients are introduced (Lucchetta et al., 2023; Ahmed et al., 2023). Compost is an increasingly preferred product over mineral fertilizers, because when it is applied, the nutrient elements are absorbed slowly, which minimizes the possibility of competition between soil microorganisms and the cultivated crop (Maucieri et al., 2019). In addition, when compost is applied, it is not washed away in depth, as is the case with inorganic fertilizers. Composting is a promising environmentally friendly process for dealing with organic waste generated by agriculture (Sayara et al., 2020). The introduction of specific additives to the finished compost can improve its qualities, both from a sanitary point of view and from a microbiological point of view (Barthod et al., 2018).

The **main** objective of the present study is to follow the change in the microbial community of compost after the application of different additives in it.

MATERIALS AND METHODS

For the purpose of the present study, a compost obtained by mixing sprouted potatoes: wood chips: lettuce: soil in a ratio of 20:2.5:10:7.5 was used. The ratio of carbon to nitrogen in the starting mixture is 31:1.

Six variants and one control (C) were prepared. The extract variants were prepared according to the Decoction method: The extracts were prepared from whole plants of the essential oil crops used (lavender, thyme, basil etc.) by boiling the crushed plant material from the respective plant in solvent water and subsequent hot filtration. The analyzed variants are as follows:

V1 - lavender extract, V2 - thyme extract, V3 - basil extract, V4 - mix of lavender, thyme and basil extract, V5 - microbial fertilizer, V6 - mineral fertilizer, V7 - mineral + microbial fertilizer. Basic microbiological indicators were investigated immediately after preparation of the mixes and the control, as well as after 5, 10 and 15 days.

Basic microbiological indicators include the determination of non-spore-forming bacteria, spore-forming bacteria (such as *Bacillus* sp., etc.) *Lactobacillus* spp., actinomycetes (*Saccharomonospora* spp., *Streptomyces* spp.) and microscopic fungi (*Aspergillus* sp, *Penicillium* sp. etc.). The analysis for the number of microorganisms was performed by microbiological groups by inoculation of (semi)selective agar media. No separate identification of species from the groups of non-spore-forming bacteria, spore-forming bacteria, actinomycetes and microscopic fungi was carried out. Based on the obtained data, the total microbial count of each of the samples was calculated. For microbiological analyses, the standard Koch method was used, including serial dilutions and using plate counts in CFU on (semi)selective agar media. Water extracts of lavender (V1), thyme (V2), basil (V3), a mixture of the three (V4), microbial fertilizer (V5), mineral fertilizer (V6) and a mixture of microbial and mineral fertilizer (V7) were used to prepare the different variants.

RESULTS AND DISCUSSIONS

Table 1. presents the results of the performed microbiological analyses. The results show an increase in microbial abundance compared to the initial phase in all variants, including the control.

In the starting initial compost, non-spore-forming bacteria represented 65% of the microbial community, followed by spore-forming bacteria at 16%. With almost equal amounts in the microbial community are micromycetes and actinomycetes, respectively 9% and 8%. The participation of lactobacilli is the least (2%).

Total microbial results vary by variant and time of reporting (Figure 1.)

The results show that the introduction of additives affects the total microbial count of the finished compost. All variants showed an increase in the total amount of microorganisms on the fifth day compared to the initial compost (Figure 1).

Table 1. Main microbiological parameters (CFU*10⁶/g compost)

	TMN	Non-spore-forming bacteria	Spore-forming bacteria	<i>Lactobacillus</i> spp.	Actinomycetes	Micromycetes
0	1.18	2.15	0.54	0.07	0.27	0.30
5th day						
K	5.04 ± 0.80	3.18 ± 0.89	0.94 ± 0.07	0.07 ± 0.10	0.81 ± 0.30	0.05 ± 0.06
1	5.36 ± 0.80	3.28 ± 0.89	1.04 ± 0.07	0.07 ± 0.10	0.87 ± 0.30	0.10 ± 0.06
2	5.36 ± 0.80	3.19 ± 0.89	0.96 ± 0.07	0.34 ± 0.10	0.77 ± 0.30	0.10 ± 0.06
3	5.59 ± 0.80	3.19 ± 0.89	1.01 ± 0.07	0.08 ± 0.10	1.18 ± 0.30	0.13 ± 0.06
4	5.22 ± 0.80	3.18 ± 0.89	0.89 ± 0.07	0.02 ± 0.10	1.01 ± 0.30	0.13 ± 0.06
5	4.45 ± 0.80	1.44 ± 0.89	1.06 ± 0.07	0.03 ± 0.10	1.68 ± 0.30	0.24 ± 0.06
6	3.36 ± 0.80	1.38 ± 0.89	0.87 ± 0.07	0.13 ± 0.10	0.87 ± 0.30	0.10 ± 0.06
7	3.90 ± 0.80	1.66 ± 0.89	1.01 ± 0.07	0.08 ± 0.10	1.06 ± 0.30	0.08 ± 0.06
10th day						
K	4.84 ± 0.46	2.52 ± 0.43	0.97 ± 0.06	0.10 ± 0.13	1.18 ± 0.25	0.07 ± 0.02
1	5.34 ± 0.46	3.29 ± 0.43	1.02 ± 0.06	0.08 ± 0.13	0.87 ± 0.25	0.07 ± 0.02
2	5.71 ± 0.46	3.33 ± 0.43	0.97 ± 0.06	0.45 ± 0.13	0.84 ± 0.25	0.12 ± 0.02
3	4.99 ± 0.46	3.28 ± 0.43	0.96 ± 0.06	0.10 ± 0.13	0.59 ± 0.25	0.07 ± 0.02
4	5.48 ± 0.46	3.24 ± 0.43	0.91 ± 0.06	0.02 ± 0.13	1.21 ± 0.25	0.10 ± 0.02
5	4.54 ± 0.46	2.18 ± 0.43	1.04 ± 0.06	0.05 ± 0.13	1.18 ± 0.25	0.08 ± 0.02
6	5.06 ± 0.46	3.02 ± 0.43	0.89 ± 0.06	0.17 ± 0.13	0.87 ± 0.25	0.10 ± 0.02
7	5.93 ± 0.46	3.31 ± 0.43	1.04 ± 0.06	0.10 ± 0.13	1.34 ± 0.25	0.13 ± 0.02
15th day						
K	4.91 ± 0.50	2.55 ± 0.43	0.99 ± 0.06	0.10 ± 0.13	1.18 ± 0.23	0.08 ± 0.03
1	5.66 ± 0.50	3.38 ± 0.43	1.08 ± 0.06	0.10 ± 0.13	1.01 ± 0.23	0.10 ± 0.03
2	5.93 ± 0.50	3.36 ± 0.43	1.01 ± 0.06	0.45 ± 0.13	0.97 ± 0.23	0.13 ± 0.03
3	5.56 ± 0.50	3.36 ± 0.43	1.02 ± 0.06	0.13 ± 0.13	0.94 ± 0.23	0.10 ± 0.03
4	5.06 ± 0.50	3.29 ± 0.43	0.94 ± 0.06	0.03 ± 0.13	0.67 ± 0.23	0.12 ± 0.03
5	4.79 ± 0.50	2.27 ± 0.43	1.08 ± 0.06	0.08 ± 0.13	1.24 ± 0.23	0.12 ± 0.03
6	5.38 ± 0.50	3.16 ± 0.43	0.92 ± 0.06	0.20 ± 0.13	0.97 ± 0.23	0.12 ± 0.03
7	6.20 ± 0.50	3.39 ± 0.43	1.08 ± 0.06	0.13 ± 0.13	1.43 ± 0.23	0.17 ± 0.03

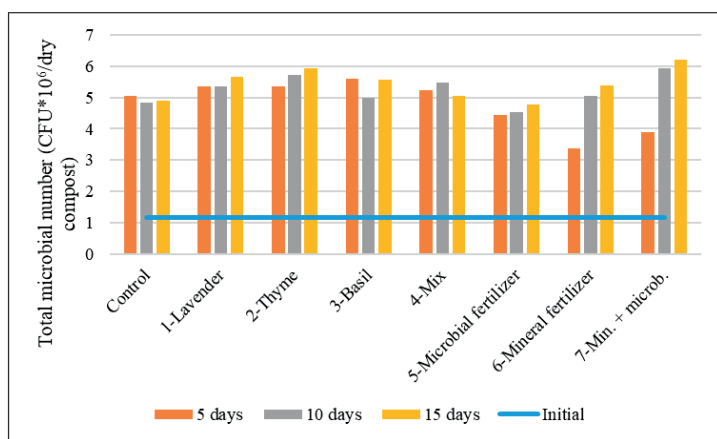


Figure 1. Total microbial number (CFU*10⁶/g compost)

All variants with added plant extracts showed higher biogenicity than the control. With the lowest microbial abundance on the fifth day are the variants with added microbial supplement, mineral supplement and a mix of both: V5=4.45*10⁶ CFU/g compost mass, V6=3.36*10⁶ CFU/g compost mass, V7=3.90*10⁶ CFU/g compost mass.

The highest biogenicity on the fifth day was reported for sample three - with added water extract from basil.

The results on the tenth day showed an increase in the microbial biota in sample 4 and 5. An increase in microbial abundance was also observed in the thyme variants (Sample 3). However, the greatest increase in microbial biota was observed in sample 6 and sample 7 (Figure 1). These are the samples with delayed microbial community development on the fifth day compared to the initial compost.

On day ten, variants 6 and 7 reached an approximate value as the others, but on day 15 variant seven had the highest total microbial count. These data show that a combination of microbial fertilizer and mineral fertilizer at the beginning of enrichment on day 5 does not stimulate the microbial biota, but after that the data show a positive effect on the amount of microorganisms.

On the fifteenth day, we have an increase in the amount of microorganisms in all the examined samples compared to the tenth day, with the exception of the sample with an added mix of plant water extracts. Of the tested mixtures, only the mixture of water extracts showed a decrease in the microflora on day 15. The control data is the most passive. After the rise on day 5 we have a slight decline on day 10 and a slight rise again on day 15.

The information generated by the present study shows that the application of the mineral fertilizer and the complex of mineral fertilizer and microbiological additive to the finished compost increases the microbial potential of the compost, due to the heights microbial number of V7 at day 15.

Lavender in principle shows antimicrobial activity against pathogenic and non-pathogenic microorganisms (Kwiatkowski et al, 2020),

therefore we assume that it has a restraining effect on the microbial biota, in the initial days of the experiment, as there is almost no increase in total microbial numbers.

Of interest is Variant 5, which is only with added microbial fertilizer. There, a retention of microbial abundance was observed, with levels lower than those of the control during all three samplings. Such data show the presence of competition between the autochthonous microbiota of the compost and the imported allochthonous microbiota with the microbial fertilizer. In other soil-related studies when allochthonous microorganisms were introduced, the autochthonous did not struggle and outcompeted it (Podmirseg et al., 2019).

These results show the higher sensitivity of the local microorganisms in the compost compared to introduced external microorganisms. The present study found a suppressive effect of the imported microbial supplement in all three consecutive samplings. In all the analyzes performed, the sample with only the microbial additive introduced showed lower biogenicity compared to the control.

Figure 2 presents results for the percentage participation of individual microbiological groups.

In the initial compost, the group of non-spore-forming bacteria was the most involved, followed by bacilli. Actinomycetes and micromycetes have an almost equal share. Lactobacilli have the smallest share of less than 3%. Through all three subsequent sampling and analysis of the compost variants, the data were almost identical.

The participation of actinomycetes increased, at the expense of non-sporulating bacteria and micromycetes. This trend persists until day 15. In almost all variants except the variant with microbial fertilizer, the group of non-spore-forming bacteria dominates. An exception is the sample with added microbial fertilizer.

The predominance of non-spore-forming bacteria is typical because these microorganisms are involved in the cooling phase of the compost as well as during the maturation phase (Varma et al., 2018).

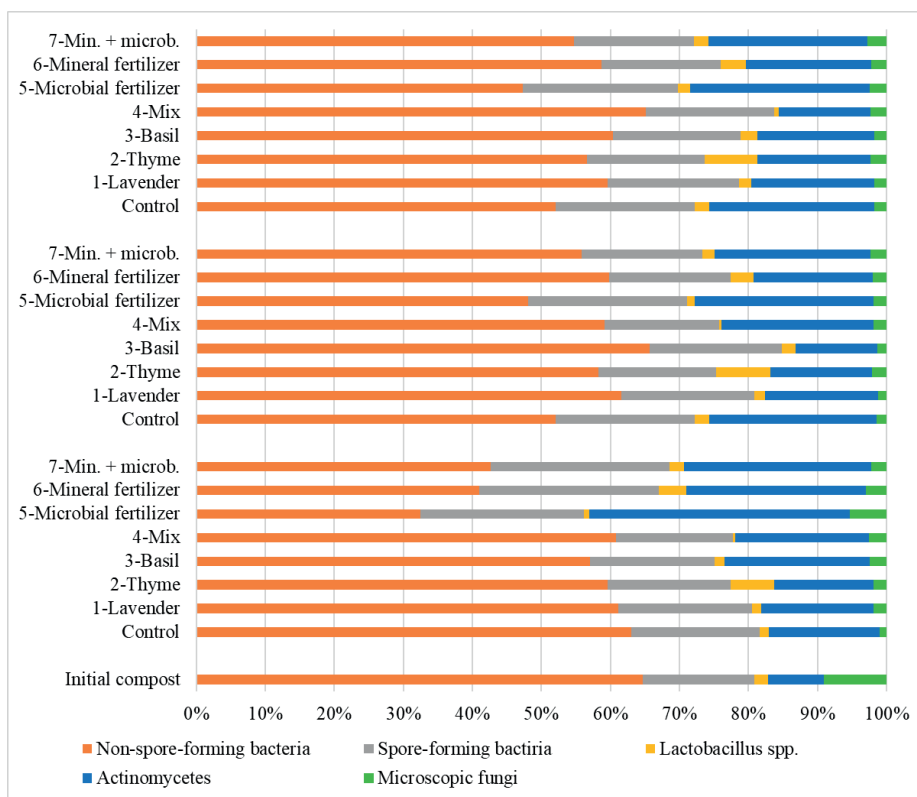


Figure 2. Percentage participation of individual microbial groups

The large amount of bacteria in general relative to the total biogenicity of compost is related to the ability of these microorganisms to adapt to different environmental conditions much faster than other microbial groups (Shi et al., 2022; Aguilar-Paredes et al., 2023). Bacteria are the main microorganisms carrying out the composting process, as they are adapted to the consumption and decomposition of various organic substances (Golueke, 1992; Epstein, 1997).

With the smallest percentage share are *Lactobacillus* spp., given their increased participation in the early stages of the composting process (Kurola et al., 2011; Tran et al., 2019)

When considering the percentage participation of the individual functional groups of microorganisms in relation to the totality of the microbial community, certain trends are clearly visible. From the obtained results it is evident that the microbial additive suppresses the development of the more dominant in the

compost, a group of non-compost-forming bacteria.

Of all the investigated variants, the sample with the added microbial supplement showed the greatest reduction in the percentage of non-spore-forming bacteria. On the fifth day after the introduction of microbiological fertilizer, the percentage of non-spore-forming bacteria drops to approx. 30% due to the increased participation of actinomycetes with nearly 40% participation.

A decrease in the percentage of lactobacilli was observed in the variant with a mix of plant extracts. Based on these results, we do not recommend the use of a mixture of the investigated extracts to improve the compost qualities, since lactobacilli, even with a small percentage, play an extremely important role. *Lactobacillus* spp. are associated with stopping unpleasant odors during composting. Lactobacilli use ammonia as a food source, which is released during decomposition and

can lead to the release of an unpleasant odor (Varma et al., 2018).

Given the results generated, when unpleasant odors are present in the compost, the addition of thyme extracts will increase the percentage of Lactobacilli and regulate the depletion of generated ammonia.

We considered that the imported additives, with the exception of the microbial extract, as well as the variant with aqueous thyme extract, do not have a significant impact on the distribution of the percentage participation of the microbial groups.

CONCLUSIONS

Seven variants of compost mixes and one control were studied. The results show that the applying of additives affects the total microbial number of the finished compost. The present study found a suppressive effect of the added microbial supplement in all three consecutive samplings. In all the analyzes performed, the sample with an imported microbial supplement showed lower biogenicity compared to the control.

However, when using a combination of microbial supplement and mineral fertilizer, the highest levels of microbial abundance were generated relative to the control on day 15.

In all variants with added aqueous extract, an increase in the total microbial number was observed compared to the control.

The obtained results for the percentage distribution of the microbial groups, again show that the used microbial additive is not recommended as an improver of the qualities of the finished compost.

In this sample, the amount of non-spore-forming bacteria decreases sharply, at the expense of actinomycetes. Also, the microbial fertilizer reduces the percentage of lactobacilli. The present study found that of the plant extracts, the addition of thyme extract was the most suitable option for improving the qualities of the finished compost. When adding an aqueous extract of thyme, a general increase in the number of microorganisms was observed, as well as an increased percentage of Lactobacilli.

We also consider the combination of microbial fertilizer and mineral fertilizer as a suitable

supplement, but after standing the mixture for fifteen days.

The present study can serve as a basis for future studies related to the influence of specific additives introduced into finished compost.

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