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On-site composting of waste hop biomass: the impact of covering piles on leachate quantity and compost quality

Barbara Čeh[1], Julija Polanšek[2], Žan Trošt[1], Ana Karničnik Klančnik[1]*

[1]Slovenian Institute of Hop Research and Brewing, Žalec, Slovenia
[2]Agricultural Institute of Slovenia, Ljubljana, Slovenia
**Corresponding author: barbara.ceh@ihsps.si*

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Abstract: Covering hop waste composting piles with semipermeable membrane after the thermophilic phase until spring, when the compost was ready to use, reduced the volume of leachate and leached nutrients amounts significantly; there was a negligible amount of leachate and low amount of leached nutrients during winter and spring at all treatments. At treatments with additives (biochar, preparation effective microorganisms) and larger percent of particles of 2–5 cm, it was indicated that composting pile should probably also be covered in the first months of composting and be opened only when turning/mixing it; on the other hand, the amount of leachate was much lower in a pile without additives and particles of 2–10 cm by the time of covering (2.2- and 2.5-fold less respectively) and the amount of leached nutrients as well. All treatments produced compost without a bad smell, had a total nitrogen content greater than 2%, could be considered as mature (the C:N ratio was below 20) and stable, and the biomass hygienisation threshold was reached. However, there were some significant differences among them. The compost of the pile with added biochar and starting hop waste biomass particles of 2–5 cm was considered phytotoxic (germination index of radish was 31%); also, its other results were less promising in comparison to other treatments. The pile with no additive and the starting particles of waste hop biomass 2 to 10 cm, mixed properly related to regular temperature measurements in the thermophilic phase, reached the best results; the leachate amount and leaked nutrients amount were significantly the lowest, while the final compost contained significantly higher amount of nutrients and had the highest germination index.

Keywords: *Humulus lupulus* L.; on-farm composting; environment protection; leachate; circular economy on farms; organic matter; waste management; recycling; soil fertility; germination test; respiratory test; bacteria and fungi

The process of composting involves the deliberate aerobic biological breakdown of organic matter, transforming it into a stable, humus-like substance known as compost. This method closely mirrors natural decomposition but is intensified and expedited by blending organic wastes with other components to maximise microbial development (USDA 1999). This waste management approach converts discarded materials into a valuable resource, yielding a recycled product composed of stabilised organic matter. It is rich in carbon and largely devoid of pathogens, and weed seeds can germinate (Pan et al. 2023). The enrichment of soil fertility, improving the activity and function of the soil microbiome and support for plant growth with compost arise from the infusion of organic matter and crucial nutrients into the soil (Jahangir et al. 2021).

On-farm composting is a cost-effective, efficient and also environmentally safe biological process for the recycling of residual agricultural biomass if done properly (Maniadakis et al. 2004). However, the process could also produce large amounts of

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leachate, which occurs with the percolation of water through the compost, which presents an environmental problem if it is not lead properly (Roy et al. 2018). On the other side, leaching leads to low nutrient content in the compost. Therefore, we strive to carry out the composting process on the farm as professionally as possible.

During harvest, hop (*Humulus lupulus* L.) plants are cut down. The whole aboveground biomass is removed from the field and driven to the harvest hall, where hop cones are picked, dried, and packed for the brewing industry, while large amounts of waste hop biomass (stems and leaves, which accounts for approximately two-thirds of the total harvested hop biomass, that is in average 15 tonnes (4.5 t/ha dry matter) from each harvested hectare) are left next to the harvest hall (Čeh et al. 2019). Correctly composting this biomass should help close the cycle of nutrients on the farm and produce the farm's own organic fertiliser. Whole waste hop biomass (leaves and stems) must be used in composting so it proceeds appropriately (Čeh et al. 2022b).

Implementing proper composting protocols on hop farms is pivotal for completing the nutrient cycle and generating organic fertiliser from hop plant residues. This practice not only sustains but also enhances soil fertility, all while minimising environmental impact. In response to the growing need for environmentally sustainable on-site composting solutions for waste hop biomass, our study aimed to bridge the knowledge gap regarding this promising technology on hop farms. In previous studies by Čeh et al. (2022b) and Luskar et al. (2022), a composting protocol for waste hop biomass was developed, resulting in good-quality composts. However, the quantity of leachate and the leached nutrients were identified as areas needing improvement. In this paper, we present an investigation into the volume and composition of leachate throughout the on-farm composting process, as well as the final properties of the composts, having composting piles covered with a semi-permeable membrane during the cooling and maturation phases.

MATERIAL AND METHODS

Experiment setting and performing. The experiment was conducted in Lower Savinja Valley, Slovenia, the biggest hop-growing region in Slovenia. After the hop harvest in September 2021, hop waste biomass (stems and leaves) was collected to prepare five composting piles. It comprised approximately 18% organic matter, 0.8% nitrogen, 0.3% potassium, and 0.1% phosphorus, with a carbon-to-nitrogen ratio of 13:1. Each pile was formed from hop waste biomass (leaves and stems) from a 2 ha hop field (approx. 30 t). The biomass also contained BioTHOP supporting twine (made from polylactic acid – PLA) that served as support for climbing hop plants during the growth on the field (approximately 95 kg/ha of hop field). This twine is degraded at proper on-site composting of waste hop biomass to water, CO_2 and organic matter in 7 months, having an OK compost certificate (Čeh et al. 2023).

The protocol among piles differed in start particle size (the hop harvest machine cuts the hop stems to a certain length depending on different reasons; in piles C and F, more particles were a bit longer than 5 cm, up to 10 cm, although also in other piles certain amount of such long particles were detected) and additives added at the pile preparation, according to the manufacturer's recommendations (Table 1). These additives, including biochar obtained from softwood in one pile (pile A) and effective microorganisms EM® containing water, sugar cane molasses, lactic acid bacteria, photosynthetic bacteria, yeasts, and sea salt in another pile (pile B), were added to the start biomass. Through their symbiotic relationships, effective microorganism mixtures can enhance the microbial ecosystems in compost piles; they are recognised for their ability to accelerate the composting process (Key 2012). In piles C, D and E, there was no additive. Piles A, B and C were mixed precisely according to average temperature measurements; when it reached above 60 °C, the pile was turned. Pile D was usually mixed when average temperatures reached 60 °C, but sometimes, even before that, it was mixed 9 times. The pile E was mixed 5 times, and not regularly; sometimes, the temperature reached more than 60 °C, but the pile was mixed a week or two later.

A customised TFA® thermometer probe was regularly used to measure the temperature at 50 and 100 cm depths in all four cardinal directions (north, south, east, and west) of each compost pile. Piles were turned/mixed after the average temperature (the average of temperatures at both depths and all four cardinal directions) of the compost piles exceeded 65 °C for two consecutive days. Trapezoidal-shaped compost piles, each with a height of 2 m, were reformed after each turning. Due to distinct temperature patterns in the piles, turning schedules varied (Table 1). At the end of the thermophilic phase (when

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Table 1. Treatments in the experiment

Treatment	Start particle size (cm)	Cover placed	Turnings/mixing the pile	Additive, mixed at pile construction (September 2021)
A – biochar	2–5	at the start of December	8 times, precisely according to temperature measurements	biochar (3.3 kg/t)
B – EM	2–5	at the end of November	5 times, precisely according to temperature measurements	effective microorganisms EM® (2 L/t)
C – control	2–10	at the end of November	5 times, precisely according to temperature measurements	–
D	2–5	at the end of November	9-times; more or less according to temperature measurements but sometimes before the rise above 60 °C	–
E	2–10	at the end of November	5 times; not related to temperature measurements	–

temperatures dropped below 45 °C (at the end of November or at the beginning of December 2021), turnings were ended, and piles were covered with a semipermeable membrane (TenCate Toptex, a gas-permeable fabric made of continuous 100% polypropylene fibres, chemically stable in the presence of acids and alkalis (pH 2–13), biologically stable against microbial decomposition and leachate (VDA and VOC), resistant to freezing and thawing, and UV resistant due to special UV stabilisation; density: 200 g/m^2 (Tencate 2024)) and tightened on the ground all around the piles.

The leachate collecting system was installed on the ground (soil) in September 2021 in piles A, B and C before placing the biomass on it, and it remained in place until the end of the experiment in April 2022. The system was constructed from three metal drip trays, dug in the ground under each pile to prevent its disturbance during the mixing of the composting pile. Each tray measured 1 m^2 and was 10 cm high. To collect leachate from trays, containers were added to the collecting system; each tray was connected to one 30 L container with a plastic tube. Containers were buried in the ground in 50 cm deep dug-up holes approximately 1 m from the pile. The holes at the top of the containers were hermetically sealed and opened only when it was time to collect the leachate. The inclination of the trays was crucial to

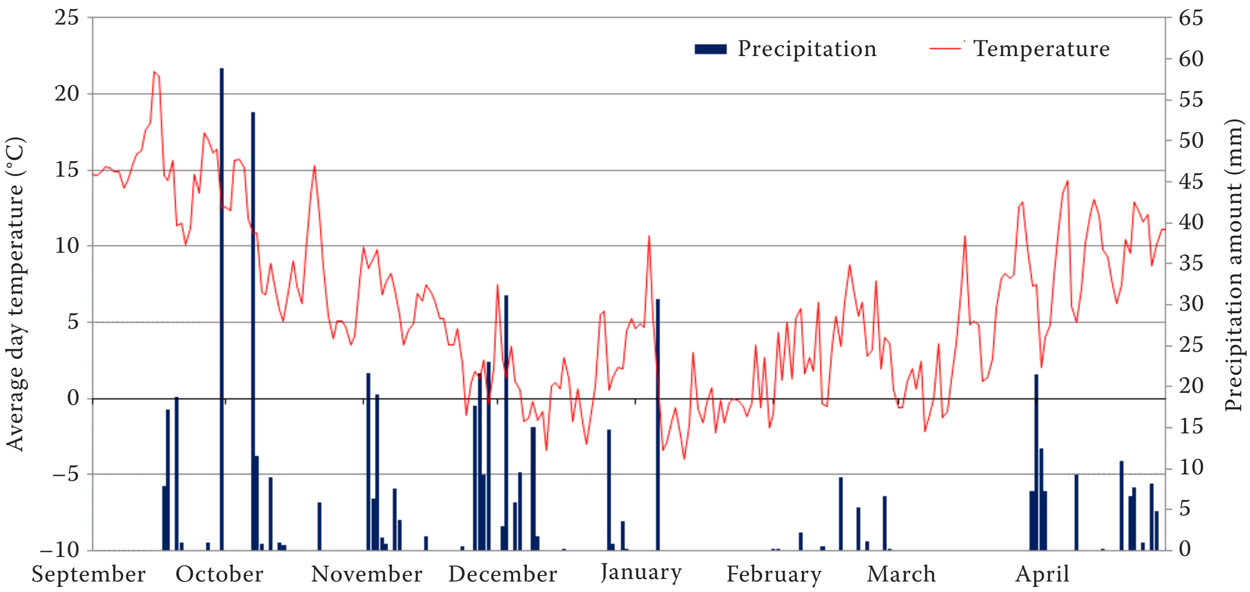

Figure 1. Precipitation quantity and average temperatures in the time of composting from the beginning of September 2021 to the end of April 2022 (data obtained at Agromet 2024)

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ensure that the leachate drained into containers and did not stagnate under the compost piles.

Weather conditions during the experiment. Daily precipitation quantity and average temperatures during the time of composting experiment from September 2021 to April 2022 are shown in Figure 1. The measurements are from the automatic meteorological station Celje-Medlog (Agromet 2024). At the end of November, there was a relatively big amount of precipitation (72 mm in the last 10 days of November and 8 cm of snow). The piles were covered afterwards with a semipermeable membrane up to April.

Leachate sampling and analysing. Regular leachate sampling occurred at the end of each calendar month, starting September 2021. The containers were opened during sampling, and the leachate was poured out for analysis. After sampling, the containers were closed and placed back in the dug holes. Leachate was thoroughly mixed; its volume was precisely measured, and a representative sample of 1 L was obtained from each container and frozen until chemical analysis.

The chemical composition of leachate was determined using the following methods: total nitrogen (method SIST ISO 11261: 1996), ammonium nitrogen (the method by Jackson (1958), nitrate nitrogen (method DIN EN 12014-7), total phosphorus and total potassium (the method by Hodnik (1988). The leachate pH was measured using the Reflectoquant® test. The quantity of nutrients in the leachate was calculated based on leachate concentration and volume, expressed as the mass of leached nutrients from the compost pile per m^2.

Compost sampling and analysing. In September 2021, sampling of the start hop waste biomass was done. After 7 months of composting, in April 2022, sampling of resulting compost was done. Composts had a temperature similar to ambient, were dark brown and had a pleasant smell. The tops of each pile were removed, and the core material was taken for various analyses. The sampling methods and the types of the performed analyses are shown in Table 2.

Chemistry. Fresh samples were analysed for pH and ammoniacal nitrogen (SIST ISO 14255: 1999, chapter 7, modified), whereas dry samples were analysed for organic C and humus content (method SIST ISO 14235: 1999), nitrate nitrogen (ISIST ISO 14255: 1999), total N (SIST ISO 11261: 1996), phosphorus (SIST ISO 6491: 1999, modified) and potassium (SIST EN ISO 6869: 2001, modified) content. The water content was determined after drying at 60 °C for 24 h until constant mass was obtained.

Germination test. Zucconi employed the method used in our experiment (1981). The method combines seed germination index and root elongation of cress seeds and garden radishes (*Lepidium sativum* L. and *Raphanus sativus* L.). Each sample of fresh compost was placed in distilled water at a ratio of 1:5 (*w*/*v*). The suspensions were shaken for 1 h at 120 rpm and left overnight to settle. The supernatant was filtered (black laboratory filter). Each compost sample was collected in triplicates. 5 mL of extract was placed in a Petri dish (90 mm) with one sheet of filter paper (MN 640), whereas controls received 5 mL of distilled water. 10 seeds were placed in a Petri dish in 3 replicates per extract, and the test was conducted in the dark at 22 °C for 48 h. The number of germinated seeds was then counted, and the overall length of seedlings (root) was evaluated. The GI (germination index, %) was calculated using Zucconi's formula (1981).

$$GI = \frac{\text{mean radicle length (sample)} \times \text{number of germinated seeds (sample)}}{\text{mean radicle length (control)} \times \text{number of germinated seeds (control)}} \times 100\%$$

Growth test. Composts were mixed in a 1:3 (*v*/*v*) ratio with commercial plant growth substrate (S25-Biotray (Villeurbanne, France) + Eco-mix 70L/45EP-Gramoflor (Vechta, Germany)) for planting in

Table 2. The type of analysis and sampling method per compost pile

Type of analysis	Sampling method
Chemistry	3 samples/pile, each from 12 different spots
Growth test	1 sample/pile from 12 different spots
Respiratory test	3 samples/pile, each from 4 different spots
Germination test	3 samples/pile, each from 4 different spots
Microbiology, bacteria and fungi count	1 sample/pile, each from 4 different spots

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4 replicates, while only substrate was used for control (marked as F in Table 5). 10 seeds of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*) were sown in each pot (12 cm diameter pots (volume 1 L)) and grown in the controlled environment chamber at 24 °C (day)/17 °C (night) with a 13-h photoperiod (Warman 1999). After 21 days, the fresh above-ground biomass was weighed.

Respiratory test. The Oxitop® system was used to measure microbial respiration using a modified method by Kaurin et al. (2020). Compost samples were collected in triplicates from four different points. Fresh compost (20 g of dry matter (DM) eq.) was placed in a jar with a beaker glass of 10 mL 25% NaOH and incubated for 5 days at 22 °C. Pressure drop was measured every 24 min and converted into O_2 consumption with the ideal gas law equation.

Several bacteria and fungi. Each compost sample was collected from 4 different points. All samples were analysed in duplicates. 50 g of sample was mixed with 200 mL of sterile water. Ten-fold serial dilutions were prepared and applied to PDA plates to enumerate fungi and TSA plates for total bacteria (Milinković et al. 2019). Plates were counted after five days of incubation at room temperature.

Data processing. The dataset was meticulously analysed using computational tools, specifically Microsoft Excel (Redmond, USA) and Statgraphics Centurion XVI (Plains, USA). A two-way analysis of variance (ANOVA) was employed to evaluate the statistically significant effects of the factors under consideration and their interactions on the observed parameters, adhering to a 95% confidence interval. Duncan's multiple range test was executed to elucidate distinctions among the mean values, facilitating the identification of statistically significant disparities.

RESULTS AND DISCUSSION

The impact of treatment and time on the leachate amount and leaked nutrients

Leachate amount. The amount of leachate was significantly the highest in the first composting month, followed by the fourth month (December), which coincided with high precipitation right before covering the piles at the end of November/start of December (Table 3). In the other two autumn months (October and November), the amount was significantly lower, while from January until April (the piles were covered at that time), the amount of leachate was significantly the lowest.

The sum of the leachate from the piles A and B was significantly higher compared to the C – control treatment (70 L/m^2 and 75 L/m^2 compared to 30 L/m^2; that means 140, 150 and 60 L/m^3 of the composting biomass – the pile was 2 m high). When the pile was covered from January to April, there was a low amount of leachate (around 1 L/m^2 per month and less) (Figure 2). Interaction treatment × month for

Table 3. Precipitation amount and quantitative analysis results of leachate parameters: monthly sampling from September (1) to April (8) and month average related to treatments

Month	Precipitation	Leachate volume	pH	NH_4-N	NO_3-N	TN	TP	TK
	(L/m^2)			(g/m^2)				
1 – Sept.	105	25.3^d	7.6^a	10.0^d	0.0^a	24.7^d	0.045^b	53.9^c
2 – Oct.	82	7.9^b	8.2^b	46.0^c	0.0^a	2.2^{ab}	0.003^a	17.4^{ab}
3 – Nov.	134	9.0^b	8.2^b	2.2^b	0.9^a	4.4^b	0.006^a	42.5^{bc}
4 – Dec.	86	13.7^c	8.2^b	1.4^{ab}	2.9^b	9.2^c	0.008^a	114.6^d
5 – Jan.	31	1.1^a	8.1^b	0.3^a	0.6^a	1.2^{ab}	0.000^a	15.7^{ab}
6 – Feb.	25	0.7^a	8.8^c	0.3^a	0.3^a	1.0^{ab}	0.000^a	11.1^a
7 – Mar.	7	0.7^a	8.8^c	0.1^a	0.0^a	0.5^{ab}	0.001^a	2.8^a
8 – Apr.	51	0.0^a	8.1^b	0.0^a	0.0^a	0.0^a	0.000^a	0.1^a
Treatment								
A – biochar		8.6^b	8.1^a	2.7^b	1.1^b	6.2^b	0.005^a	45.1^b
B – EM		9.4^b	7.9^a	3.6^c	0.3^a	7.6^b	0.016^a	37.3^b
C – control		3.7^a	8.7^b	0.8^a	0.4^a	2.4^a	0.003^a	14.3^a

The same letter in the column within one factor (month; treatment) means that there is no significant difference between the values. TN – total nitrogen; TP – total phosphorus; TK – total potassium; EM – effective microorganism

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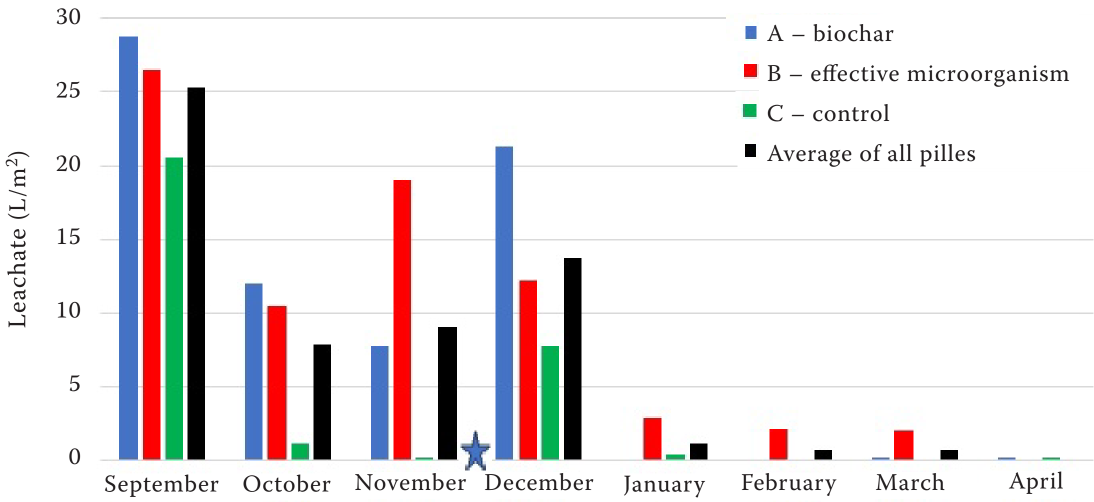
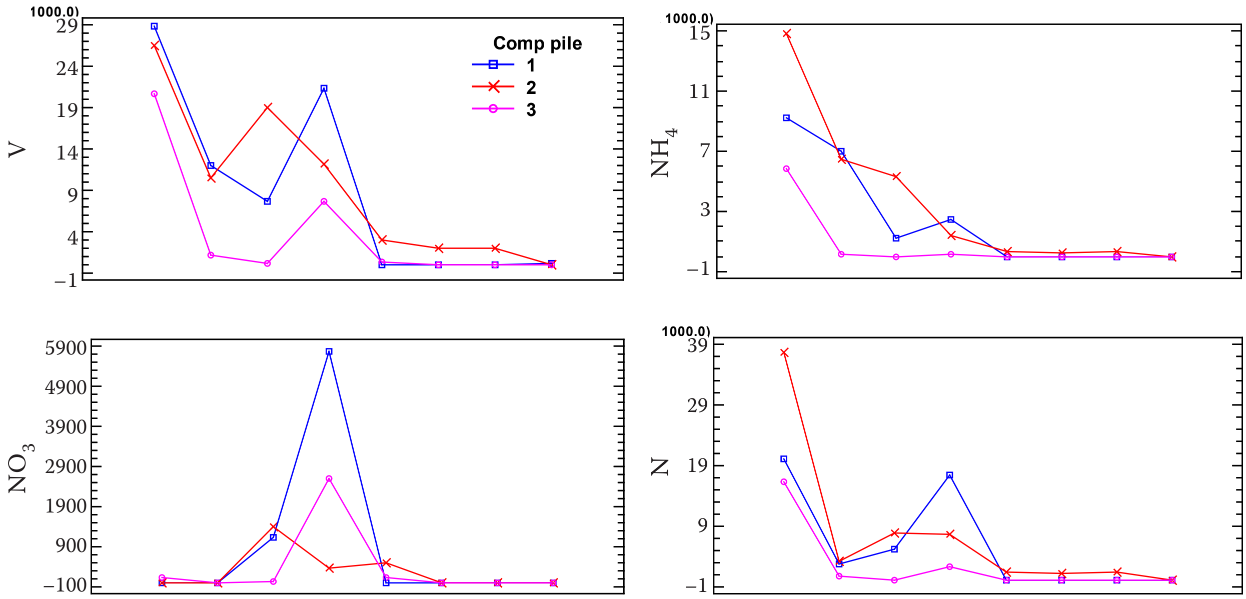


Figure 2. Leachate from the composting pile's amount (L/m^2) related to calendar month (September 2021 to April 2022) and treatment (A – biochar; B – EM; C – control); ✳ – time when piles were covered

the volume of the leachate was significant (Figure 3). The difference was that at pile B – EM, the amount of leachate increased significantly in November and then was lower in December, while at the other two piles, the leachate amount increased significantly in December.

In the waste hop biomass composting experiment by Čeh et al. (2022b), where the pile with added bio-



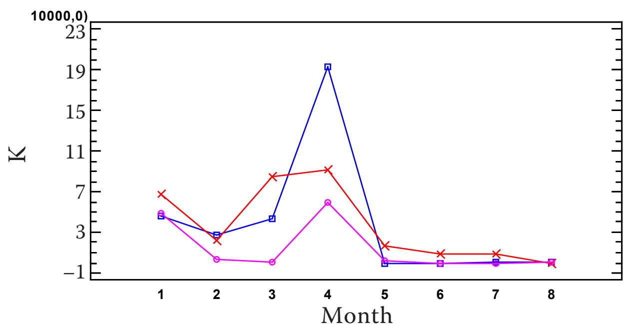


Figure 3. Interaction treatment (A – biochar; B – EM (effective microorganism); C – control) × month (1 – September to 8 – April) for volume of leachate (left up; L/m^2), for NH_4-N leaked (right up; g/m^2), for NO_3-N leaked (middle left; g/m^2), for total nitrogen (TN) leaked (middle right; g/m^2), and for total potassium (TK) leaked (bottom left; g/m^2)

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char and the one without additives were not covered throughout the composting, in the first two months, the highest leachate quantity was in a pile with added biochar (11 kg/t), with 17 L/m^2 in the first and 34 L/m^2 in the second month of composting, while at the pile without additives (control), the peak leachate quantity occurred during the maturation phase (more than 50 L/m^2 in period from November to April). Comparing the research results, we can conclude that covering the pile during maturation significantly improved the on-site (on-farm) composting protocol. When adding biochar (treatment A), the pile should be covered urgently in the thermophilic phase (opened only for mixing/turning the pile). In this treatment, the leachate amount in the first 3 months was 49 L/m^2, in treatment B 56 L/m^2, while it was 22 L/m^2 at treatment C.

Leachate pH. According to Van der Wurff et al. (2016), at the beginning of the composting process, the pH value is lower due to the presence of organic acids. The alkaline pH of leachate is due to the release of NH_4^+ (Wang et al. 2021). According to Amatya et al. (2011), the optimum pH range for nitrification is between 7.8 and 9.0. Nitrification is reduced outside this range. In our experiment, the pH of the pile significantly increased during the composting process and decreased again in April, a pattern similar to results by Čeh et al. (2022b) with not-covered waste hop biomass composting piles. The leachate from the C – control compost pile (without additives) exhibited a significantly higher pH (8.7) than A – biochar and B – EM in our study. There was no significant difference between composting piles with additives (biochar and EM®). In the study by Čeh et al. (2022b), the lowest pH values were observed in the leachate of the pile with added biochar (8.0), and the control compost pile without additives had a pH of 8.6. That indicates that lower pH in the initial stages of composting in composting pile B – EM could slow down nitrification in this pile.

While there was no leachate or the quantity was minimal from January to April, there were also no/low amounts of leached nutrients.

Leached nitrogen related to composting protocol and time. Total nitrogen and ammonia are one of the most important pollutants in compost leachate (Pirsaheb et al. 2021). Nitrate can also be a very important pollutant, especially in the phase of compost maturation phase (Čeh et al. 2022b).

The sum of NH_4-N leached out was significantly the highest at treatment B – EM (30 g/m^2), followed by treatment A – biochar (20 g/m^2), and significantly the lowest at treatment C – control (6 g/m^2). So, the composting approach B – EM had the least perspective considering the NH_4-N leached amount, which was significantly the highest in this pile. Every month, a higher amount of NH_4-N was leached from pile B than the other piles, except in October, probably due to EM activity in the pile connected to small particle size (1–5 cm). At all piles, this parameter decreased over the composting time.

In the research by Čeh et al. (2022b), where the waste hop biomass composting piles were not covered, NO_3 content in leachate increased significantly during the maturation phase (56.6 g/m^2). Their results of nitrogen (NH_4, NO_3, total N) content in leachate led to the conclusion that the composting pile should probably be covered at least after the thermophilic phase and keep it covered up to spring, which was done in this experiment.

The sum of NO_3 amount leaked from the pile was significantly higher in the treatment A – biochar compared to the other two piles but still relatively low compared to the NH_4-N amount (7 g/m^2 at A – biochar, 2 g/m^2 at B – EM and 3 g/m^2 at C – control). The observed effects of biochar on nitrate leaching could be due to its impact on microbial communities, adsorption properties, or its effect on the physical structure of the compost. Biochar can adsorb ammonia, potentially reducing the availability of ammonia for nitrifying bacteria and thus affecting the nitrification process.

On the other hand, the leachate at the pile A – biochar was also the highest among the investigated treatments in the thermophilic phase. In the compost pile B – EM, temperatures fell under 65 °C at the start of October (temperatures over 55 °C lasted for around 50 days, while in the trial by Čeh et al. (2022b), they lasted about 1 month). The average temperature in the pile C – control never exceeded 65 °C, and the temperatures over 55 °C lasted around 20 days. The guidelines set by the U.S. Environmental Protection Agency (EPA 2024) for composting processes that ensure the reduction of pathogens to safe levels require that the composting pile must maintain a temperature of 55 °C or higher for several consecutive days, during which the pile must be turned occasionally to ensure even exposure of all material to these conditions.

NH_4-N in leachate was decreasing significantly with the time of composting (from 10.0 g/m^2 in September to 0.3 mg/m^2 in April), while NO_3-N increased signifi-

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cantly in December, the fourth composting month; at that time, obviously, the nitrification process started (decreasing NH_4 and increasing NO_3). The composting piles started to cool down in November (Figure 4), which affected nitrification. Nitrifying bacteria are effective and active at temperatures lower than 40 °C in composting piles (Confesor et al. 2009). Nitrification probably continued within the piles then, but there was no longer much leachate due to the cover, so NO_3-N stayed inside the pile.

Čeh et al. (2022b) reported that the EM® treated composting pile had the least NH_4 loss, likely due to being covered with black foil after one month of composting and up to the end of the maturation phase (the other two treatments were not covered throughout the season at all). The control pile without additives had a slightly lower NH_4 loss, while the pile with added biochar showed the highest losses, mainly in the second month of composting. Examining weather data revealed increased rainfall during this period. Therefore, it is advisable to cover the pile before the expected heavy rain during the thermophilic phase to prevent NH_4 loss for effective leachate management when adding biochar.

The same results are for TN; the highest amount was leached in the first month, followed by the fourth; in all the other months, the amount was lower, with the lowest and decreasing values from January to April. The concentration of leached TN decreased with the compost's age, as in the green waste biomass experiment conducted by Al-Bataina et al. (2016).

Related to the results of NH_4-N and NO_3-N, the amount of TN leached was significantly the highest in the first, followed by the fourth composting month, compared to the other composting months, while at the same time, the amount in spring was very low. The sum of TN leached was significantly lower in the treatment C – control (20 g/m^2) compared to A – biochar (46 g/m^2) and B – EM (61 g/m^2). If we

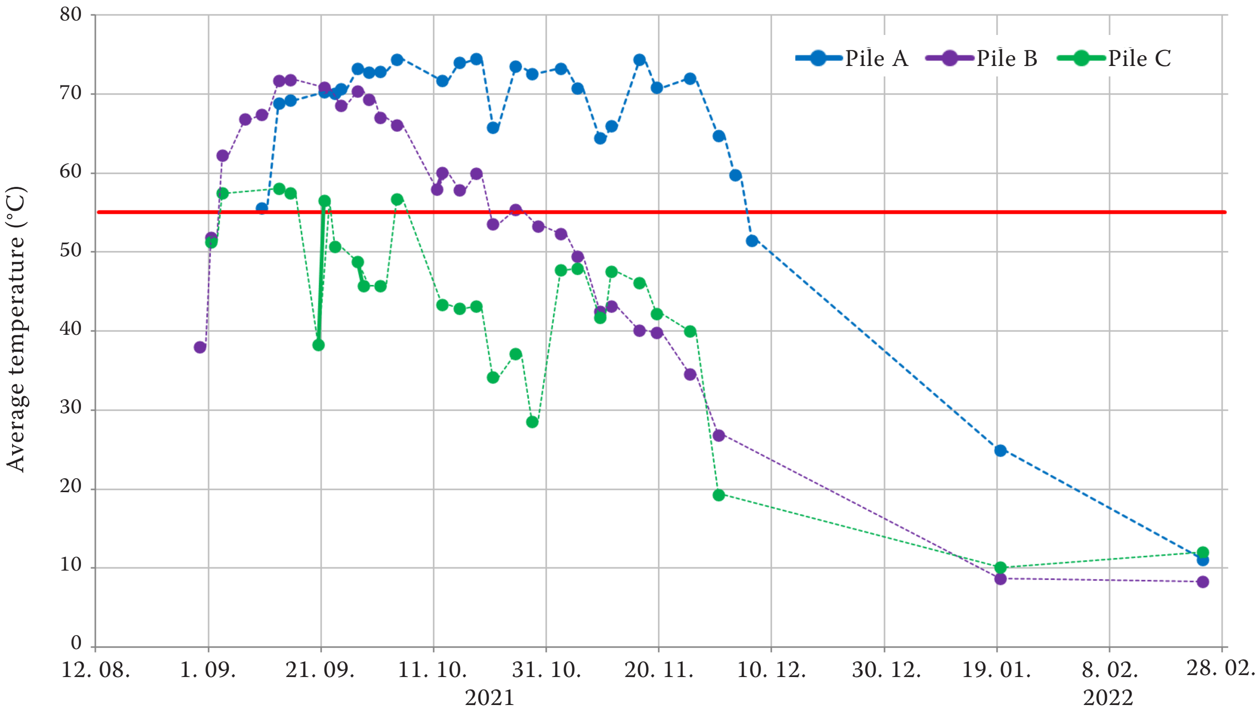

Figure 4. Temporal temperature variation in composting piles with different treatments (A – biochar; B – effective microorganisms; C – control) from September 2021 to February 2022. The red line indicates the biomass hygienisation threshold (EPA 2024) and the start of biodegradable BioTHOP twine degradation at 55 °C (Karamanlioglu and Robson 2013). We are considering the temperature of 55 °C because this is the biomass hygienisation threshold, and the biodegradable BioTHOP (made of polylactic acid – PLA) twine degradation starts at 55 °C (Karamanlioglu and Robson 2013). PLA is a low-weight, *in vivo* biodegradable and compostable semi-crystalline bio-based polymer synthesised from natural sources like corn starch, sugarcane or cassava roots (Grigoras 2021). PLA is a biodegradable polyester; it degrades under proper composting processes, including a rich oxygen environment with higher temperatures, high humidity (> 60% moisture) as well as the presence of micro-organisms (thermophilic bacteria) (Ainali et al. 2022)

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subtract the mineral N (NH_4-N + NO_3-N) from the total N and calculate the organic N (Regulatory 2024) in the leachate, it amounts to 11 g/m^2 in the treatment C – control, 19 g/m^2 in treatment A – biochar and 30 g/m^2 in the treatment B – EM.

The interactions treatment × month for NH_4-N leached of the piles, NO_3-N leached of the piles, TN leached, and TK leached were significant (Figure 3). The suggestion that the piles of treatments A and B would be covered from the start of the composting process also comes from the results that in the first three composting months, there were 28, 49 and 17 g/m^2 TN leached in piles A, B and C, respectively.

Leached potassium related to time and composting protocol. Leachate also contains other elements, such as potassium and phosphorus, as well as high levels of salts (Romero et al. 2013). In our experiment, the leached TK quantity was the lowest among examined parameters, from 115 g/m^2 at pile C – control, where it was significantly lower compared to piles A – biochar and B – EM with a sum of 309 g/m^2 and 299 g/m^2, respectively. TK leached had a similar pattern to leached NO_3-N; significantly, the highest amount of TK was leached in December (115 g/m^2), followed by September (54 g/m^2) and November (43 g/m^2). From January to April, the amount was low (from 16 g/m^2 in January to 0.09 g/m^2 in April). Comparing the piles, while the leached TK amount was comparable among other months, it was significantly the highest in the first and fourth months in the piles B – EM and C – control, while in A – biochar, it was far the highest in December (the fourth month; 193 g/m^2).

Leached phosphorus related to time and composting protocol. The amount of leached total phosphorus was much lower than TK and TN. Also, other authors report that phosphorus retention in the biomass during composting is considerably better (Wang et al. 2021). A significantly higher amount of leached TP was in the first composting month compared to all the other months (45.0 mg/m^2 and less than 8.0 mg/m^2, respectively).

From the experiment by Čeh et al. (2022b), we can also see that leached TP was significantly lower (3.27 mg/m^2) in the cooling and maturation phase (from November to April) than in the previous two months (13.7 mg/m^2 in September and 15.6 mg/m^2 in October). Compared to covered piles in this experiment, we can see that covering the piles did not influence the leaching of the TP; it was relatively low in both cases. Interaction treatment × month for total phosphorus was not significant (Figure 3). The amount of leached TP was relatively low and comparable among treatments.

To summarise the chapter on the impact of treatment and time on the leachate amount and leaked nutrients, we can see that the amount of leached TK was the highest among the examined parameters, especially in December, before covering the piles (115 g/m^2), followed by September (54 g/m^2) – the first composting month. From January to April, the amount was low (from 16 g/m^2 in January to 0.09 g/m^2 in April). The amount of leached total nitrogen was highest at the start month of composting, in September (25 g/m^2), followed by December (9 g/m^2). We have to mention here heavy precipitation at the end of November/start of December. From January to April, it was much lower, similar to TK. The amount of NH_4-N was expected to be high in October (46 g/m^2), followed by September (10 g/m^2), while it was very low in the later stages of composting. The amount of leached NO_3-N was relatively low compared to other nutrients, down to 0 g/m^2 in almost all months except 2.9 g/m^2 in December. The amount of leached total phosphorus was much lower than TK and TN (not detectable in all months) due to better retaining of phosphorus in the biomass during composting.

The maturation phase is the period when the compost continues to decompose at a slower rate, further stabilising and developing the properties that make it beneficial as soil amendment, such as improved structure, moisture-holding capacity, and microbial activity. This phase is crucial for the development of a mature compost that is safe and beneficial for plant growth. Covering the waste hop biomass composting piles after the thermophilic phase with the semipermeable membrane (which prevents rain from percolating into the pile) and let them be covered during the whole maturation phase, significantly lowered the volume of leachate in the winter/spring, compared to previous researches with piles that were not cowered throughout the process; this is a very promising way of preventing groundwater pollution and retaining nutrients in the pile. There was a negligible amount of leachate during winter and spring at all treatments in our research, namely, and also a higher amount of TN, TC and TK in the final composts, higher pH values, indicating a more alkaline condition, higher levels of NO_3-N, alongside decreased concentrations of NH_4-N, likely resulting from the more comprehensive nitrification process.

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Analysis of mature composts

Chemistry. Upon comparison with the chemical parameters of composts reported by Čeh et al. (2022b), in which composting piles were not covered throughout the season, it is evident that the average concentrations of TN, TP, TK and TC in piles were higher in the final composts in our research (in average 2.7 g and 3.2 g of TN, 0.38 g and 0.52 g of TP, 1.08 g and 1.98 g of TK in 100 g of dry matter, respectively); it means 8.1 and 9.6 kg of TN, 1.1 and 1.6 kg of TP and 3.2 and 5.9 kg of TK in 1 t of the compost with 70% moisture, respectively. Piles covered averagely contained more TN than cattle manure, as reported by Babnik et al. (2006), and more TK and TP. pH values were higher as well, indicating a more alkaline condition, and higher were the levels of NO_3-N, alongside lower concentrations of NH_4-N, likely resulting from the more comprehensive nitrification process facilitated by having the piles covered throughout the maturation phase. Compost and manure with a total nitrogen content greater than 2% can be used as a fertiliser (Bary et al. 2016), so all composts in both researches covered this requirement.

The pile C, which produced the least amount of leachate and had the lowest levels of nutrients leaked among A, B and C piles, retained the highest quantity of nutrients within the biomass (Table 4). Additionally, this pile exhibited the highest pH values among all piles; the compost's pH was notably high at 9.2, with the leachate presenting a slightly lower pH (pH = 8.7).

Conversely, pile E demonstrated the lowest TK, TN, C_{org}, and humus content levels. This pile underwent fewer mixings compared to pile C, but it was not mixed consistently and not according to temperature measurements. Evidently, approach E is not good; we can assume that the nutrient loss was higher compared to the other piles.

According to Zucconi and de Bertoldi (1987), the NH_4-N concentration in mature compost should be below 0.4 g/kg; all the composts fulfilled this requirement.

Healthy, high-producing hop plants were found to have leaf zinc concentrations above 15 mg/kg at the bloom stage (Boawn 1965). Zinc content in our composts was significantly the highest in composts A and D and lowest in pile B. However, none of the composts exceeded legally permitted values for composts, which is up to 0.4 g/kg for first-class compost and up to 1.8 g/kg for second-class compost. Significantly, the lowest zinc content was in the compost B – EM.

Compost requires a moisture level of between 45% and 60% – a figure recommended both by Oshins et al. (2022) and Cornell Waste Management Institute (1996). According to McFarland (2001), compost should have 30–50% DM content. However, for the composting waste hop biomass, the ideal moisture is 60–70%, according to Čeh et al. (2022a). The composts in the research reached this requirement. The moisture content was around 3% lower than the one in the not covered composting piles in the research by Čeh et al. (2022b) (in average of the piles 65.2% and 68.8%, respectively).

Compost can be characterised as mature when the C:N ratio is below 20 (10 to 20), and nitrogen content is above 3% (Chowdhury et al. 2013).

Germination test. While there was no significant difference in the mean root length of garden cress, the mean root length of radish was significantly the lowest at treatment A and significantly the highest at the control (F). Except compost of the pile A, where the GI of radish was 31%, all the other composts in our

Table 4. Basic chemical characteristics of composts after seven months of composting in April 2022

	Moisture	Humus	TP[1]	TK[1]	C_{org}[1]	TN[1]	NO_3-N[2]	NH_4-N[2]	Zinc[1]	pH	C:N
	(%)		(g/kg)		(%)		(g/kg)				
A	60^{a}	49.6^{bc}	4.6^{a}	21.0^{b}	28.8^{bc}	3.4^{bc}	1.6^{c}	0.07^{b}	0.10^{d}	8.6^{b}	9:1
B	71^{c}	53.7^{c}	5.0^{a}	15.0^{a}	31.1^{c}	3.6^{c}	0.5^{a}	0.03^{a}	0.07^{a}	8.2^{a}	9:1
C	68^{bc}	64.6^{d}	5.6^{b}	26.5^{c}	37.5^{d}	3.6^{c}	1.1^{b}	0.05^{ab}	0.08^{b}	9.2^{c}	10:1
D	66^{b}	42.1^{ab}	4.9^{a}	22.1^{b}	24.4^{ab}	3.1^{b}	1.2^{b}	0.06^{b}	0.10^{d}	8.5^{b}	8:1
E	61^{a}	39.0^{a}	5.8^{b}	14.2^{a}	22.6^{a}	2.3^{a}	0.7^{a}	0.02^{a}	0.09^{c}	8.3^{a}	10:1

[1]Measured in dry matter; [2]Measured in fresh matter; TP – total phosphorus; TK – total potassium; TN – total nitrogen; TC – total carbon; NO_3-N – nitrogen in nitrate form; NH_4-N – nitrogen in ammoniacal form. The same letter in the column indicates there is no significant difference between the values (Duncan´s test, $P < 0.05$)

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research we can consider as not phytotoxic (GI < 65) according to the Zucconi (1981) criteria. The reason for the bad result of pile A may be the temperature, which was very high for a long time at the pile, which may have killed some beneficial microorganisms; the high temperature in this pile (over 65 °C) lasted till the end of November (Figure 4). In the experiment by Čeh et al. (2022b), the temperatures in the hop biomass composting pile with biochar were the highest among treatments as well, stayed above 55 °C the longest (around 3 months), which is similar to this trial (85 days), including temperatures above 70 °C (around 30 days in trial by Čeh et al. (2022a), comparing to 50 days in this trial). Biochar may be insulated compost piles, potentially maintaining higher temperatures for longer periods. The other reason for the lower GI of the pile A compost may be an imbalance in the microbial community, with a dominance of harmful microorganisms over beneficial ones, which could inhibit seed germination and root development. Also, if the pH of the pile is too high, it could have an adverse effect on seed germination and root development (pH of pile A was 8.6). There may also be inadequate oxygen levels during composting, which can result in anaerobic conditions, producing toxic compounds that harm plant growth (based on a respiratory test, there was 3.0 mg O_2/g DM).

Growth test. The results of the growth test are presented in Table 5. Due to nutrients, which may have a greater impact on plant growth than on germination, this test has proved to be more sensitive than the seed germination test (Warman 1999). The more mature the compost is, the better the growth of plants is (Van der Wurff et al. 2016). There were no significant differences in the number of emerged plants; however, the number was relatively the lowest at treatment A and the highest at treatment F (only substrate in the pots). It is mentioned because the results were also reflected in the parameter above ground plant weight (measured after 21 days from the sowing); not-so-good results were also indicated at this parameter at treatment A. Significantly, the lowest was the plant weight at treatments A and E. Significantly, the highest 1 plant weight as well as mean green mass per pot, so the best results, were got at treatment B. Treatments C and D were comparable to treatment F with only substrate in the pot for 1 plant weight, but significantly lower when comparing the mean green mass per pot. In the experiment by Čeh et al. (2022b), the results were similar; compost

Table 5. Germination tests with cress and radish seeds were compared to the average root length (mm), number of germinated seeds, and germination index (GI, %) based on extracts of 5 different composts compared to the control (only substrate). Results of Chinese cabbage growth test after 21 days of sowing; germination performance and mass of one shoot related to tested compost. Results of the respiratory test and several colony-forming units of bacteria and fungi related to treatment

Compost/ treatment	Germination test						Growth test			Respiratory test	Bacteria	Fungi
	garden cress (*Lepidium sativum*)			radish (*Raphanus sativus*)			Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*)					
	mean root length	number of germinated seeds	GI	mean length	number of germinated seeds	GI	germination (%)	mean green mass per pot (g)	mass of one shoot (g)	(mg O_2/ g DM)	(CFU 10^6/ g compost)	
A	13^{b}	10^{a}	161^{b}	4.1^{a}	7.0^{a}	31^{a}	7.3^{a}	1.5^{a}	0.22^{a}	3.0^{a*}	423^{c}	71^{d}
B	15^{b}	10^{a}	189^{b}	10.0^{b}	10.0^{b}	79^{b}	8.7^{a}	5.5^{d}	0.65^{c}	10.7^{d}	50^{a}	2^{a}
C	15^{b}	10^{a}	180^{b}	10.2^{bc}	10.0^{b}	85^{bc}	8.5^{a}	3.6^{b}	0.41^{b}	6.2^{cc}	291^{b}	21^{b}
D	16^{b}	10^{a}	193^{b}	12.2^{cd}	10.0^{b}	103^{d}	8.8^{a}	3.5^{b}	0.40^{b}	3.0^{a}	248^{b}	39^{c}
E	17^{b}	10^{a}	196^{b}	11.3^{bc}	10.0^{b}	96^{cd}	8.0^{a}	2.2^{a}	0.27^{a}	4.6^{b}	138^{a}	3^{a}
F	8^{a}	10^{a}	100^{a}	13.4^{d}	10.7^{b}	100^{cd}	9.5^{a}	4.4^{c}	0.47^{b}	–		

The same letter in the column marks no statistical differences between values (Duncan´s test, $P < 0.05$); Control (F) – only substrate, without any compost for the growth test and distilled water for the germination test; CFU – colony-forming units

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from the pile with added biochar was significantly less quality than the control in terms of green mass and mass of one shoot. Similarly, compost with added EM was the best at these parameters.

Respiratory test. Compost stability is reflected in microbial respiration activity; it refers to the extent to which the organic material in compost has been decomposed. Stable compost will not undergo significant further decomposition and will not heat up significantly if moistened and aerated. Stability is important to ensure that the compost will not harm plant roots or seedlings by drawing nitrogen from the soil for further decomposition. The presence of phytotoxic effects on plant growth is predominantly associated with compost that has not yet reached full maturity, underscoring the critical importance of this attribute. Conversely, low microbial respiration rates serve as a hallmark of compost stability, signifying the composting process's completion and the compost's readiness for agricultural use (Tamás et al. 2019). The upper limit value of oxygen in one gram of dry weight of first-class biologically stable compost, according to Slovenia's regulation (Regulation 2013), is 15 mg after four days, while the European Union recommends it to be below 10 mg O_2/g DM after four days (European 2001).

The highest value of the respiratory test is observed in treatment B (10.7 mg O_2/g DM), followed by treatment C (6.2 mg O_2/g DM), indicating a high level of biological activity and organic matter degradation in the compost. The lowest values of the respiratory test are observed in treatments A and D (both 3.0 mg O_2/g DM), suggesting a lower level of biological activity in these treatments compared to others. A lower respiratory test value means that the microorganisms in the compost use less oxygen for biological processes, which may indicate that most of the organic matter has already been decomposed and that the compost has already reached a stable phase and is mature. According to Slovenian regulations, all composts are stable and mature (Luskar et al. 2022).

Bacteria and fungi in compost. The proportion of bacteria in composts was much higher than the proportion of fungi. The same results were reported by Luskar et al. (2022). For vegetables and other annual plants, compost with a higher bacterial content might be more beneficial, as bacteria work faster to release readily available nutrients to fast-growing plants. For perennials, shrubs, and trees, a compost with a more balanced or even fungi-dominant ratio might be preferable, as fungi are excellent at breaking down complex organic materials over time, providing a slow release of nutrients.

Significantly, the highest number of bacteria was observed in treatment A (423 × 106/g compost), followed by treatment C (291 × 106/g compost) and treatment D (248 × 106/g compost), while significantly, the lowest number was observed in treatment B (50 × 10^6/g compost). This may indicate that the compost of treatment A was favourable for the growth and proliferation of bacteria, while the compost of treatment B showed a lower presence of bacteria.

The highest number of fungi was observed in the compost of treatment A (71 × 10^4/g compost), while the lowest number was observed in the compost of treatment E (3 × 10^4/g compost). Treatment A appears to be most favourable for the growth and proliferation of fungi, while treatment E is less favourable for the presence of fungi in the compost. On the other side, in the investigation by Luskar et al. (2022), the compost from the pile with added biochar and small starting particles (smaller than 5 cm) had the least bacteria and fungi, reported that was probably due to very high temperatures in the thermophilic phase of this pile.

Considering the ratio between bacteria and fungi (Table 5), waste hop biomass composts would be better for fertilising vegetables and other annual plants than perennials. However, a high amount of bacteria and fungi is not always a guarantee of compost quality. The presence of diverse microbial life can indicate even better compost suitable for use in agriculture, horticulture or other purposes.

To summarise the chapter Analysis of mature composts, we can see that all treatments produced compost without a bad smell; all of them had a total nitrogen content more significant than 2% so that they could be used as fertiliser; they could all be considered as mature (the C:N ratio was below 20 and nitrogen content was above 3%), all were stable; however, there were some significant differences among them. The normal range for total nitrogen in finished composts is 0.5 to 2.5 on a dry basis (Regulatory 2024).

The most effective composting approach, at the conditions of the 2021/22 season experiment, considering the leachate amount, the leached amounts of nutrients and the final compost quality, was observed in pile C – control with no additive and the starting particle size of 2–10 cm. Total nitrogen, potassium, NH_4-N and NO_3-N leached out – all were significantly lower in pile C – control than the other two piles. The final compost of this pile contained

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a significantly higher amount of TK, humus, C_{org}, TN and had the highest GI.

The incorporation of biochar as an additive at the commencement of the composting process (treatment A), with starting hop waste biomass particles of 2–5 cm, yielded less promising results than other treatments. The leaching of nitrate (NO_3-N) was the highest among the studied treatments. Additionally, this treatment experienced rapid and prolonged increases in temperature, even above 70 °C, necessitating frequent turning of the compost pile by the farmer. This represents a significant inconvenience for practical agricultural applications. Except for the compost of pile A, where the GI of radish was 31%, the other composts in our research were not phytotoxic (GI < 65) criteria based on germination index percentage.

Treatment B stood out for its high biological activity and reached the best results in the growth test – it had significantly the highest 1 plant weight as well as mean green mass per pot, but it has fewer bacteria and fungi compared to other treatments, significantly lower TK, NO_3-N and NH_4-N content.

The composting pile should be mixed according to temperature measurements; otherwise, we lose the quality of the final compost; the pile that was mixed improperly (pile E) had significantly the lowest content of TK, TN, NO_3-N, NH_4-N and C_{org}, significantly the lowest CFU of bacteria and fungi and also significantly the lowest parameters of the growth test.

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