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Heat pre-treatment as an initial step in vermicomposting significantly influences worm population and cocoon production

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Abstract

Heat treatment of vermicompost and cow manure is mandated before their use as fertilizers to address the risk of pathogenicity in vermicompost derived from cow manure. While vermicomposting under mesophilic conditions does not significantly reduce microorganisms or degrade enzymes and proteins, organic matter passing through the earthworm's digestive system is effectively composted and sterilized. This study focused on cow manure and subjected it to three heat treatments: 25 °C (HT1), 70 °C for 1 hour (HT2), and 121 °C at 1.5 atm for 15 minutes (HT3). We assessed these treatments over five incubation periods (0, 7, 15, 30, and 45 days) on earthworm (*Eisenia fetida* L.) biomass, juvenile counts, and cocoon production. The results showed that all earthworms in HT3 died at the second incubation, while the HT1 and HT2 groups survived. Cocoon counts in HT1 and HT2 increased with each period (P<0.01). Remarkably, the number of cocoons in the control group exceeded that of the HT2 group throughout the study (P<0.01), highlighting the significant impact of heat treatment on vermicompost quality and earthworm productivity. Earthworm productivity was significantly affected by the carbon-to-nitrogen ratio (C/N), organic carbon (%), total nitrogen (%), NH_4^+-N, and NO_3^--N. Elevated NH_4^+-N from heat treatments had a negative effect on earthworm activity.

Introduction

Vermicompost is a product derived from composting crop residues and animal waste with the help of earthworms under aerobic and mesophilic conditions. This method offers an alternative to traditional composting techniques for recycling organic material. During vermicomposting, organic materials are rapidly humified, detoxified, and disinfected, aided by beneficial microorganisms and earthworms (Kızılkaya and Hepşen, 2014; Rakıcıoğlu and Kızılkaya, 2021). The epigeic earthworm species, *Eisenia foetida* L., is preferred for vermicomposting due to its outstanding traits, such as a high reproductive rate, adaptability, and salt tolerance (Dominguez et al., 2001; Edwards et al., 2011; Garg et al., 2005; Namlı et al., 2014). Vermicompost has a higher concentration of beneficial microbial populations, readily available plant nutrients, and plant growth regulators compared to conventional compost. It also effectively suppresses plant diseases without issues related to odor or proliferation.

Vermicompost is a finely divided, peat-like substance with a low C: N ratio, superior structure, porosity, aeration, drainage, and water-holding capacity (Demir, 2019; 2020; Yılmaz and Uğur, 2022). It provides a balanced mineral content, enhances the availability of plant nutrients, and can act as a complex nutrient granule (Tomati and Galli, 1995; Tomati et al., 1987; 1988; Türkay and Öztürk, 2023). These qualities endow vermicompost with significant potential economic value as a soil conditioner or plant nutrient source. However, there is some risk of pathogenicity in vermicomposting since it employs animal waste (typically cow manure) and operates under mesophilic conditions, unlike thermophilic composting. Consequently, vermicompost is categorized as an animal by-product. As such, a heat treatment at 70 °C for 1 hour is mandated for vermicompost, as outlined by Regulation (EC) No. 1774/2002 (Animal By-Products Regulation) in the European Union and the Regulation on Animal By-Products not Intended for Human Consumption (dated 24/12/2011 with reference number 28152) in Turkey. The efficiency of vermicomposting depends on the biomass of earthworms and the proportion of adults and juveniles (population structure) in the population (Rajapaksha et al., 2013). The main element of population structure is biological reproduction and biomass is related to growth rate, both of which depend on environmental conditions such as light, temperature, and humidity (Auerswald et al., 1996; Sarwar et al., 2006).

Studies have shown that vermicomposting inherently serves as a sanitizing process. As organic materials traverse the intestinal system of earthworms, pathogenic microorganisms are suppressed by the intestinal flora due to antagonistic mechanisms (Eastman et al., 2001). Consequently, the final product does not necessitate additional heat treatment. Such treatments can also eradicate beneficial microorganisms, which are among the most valuable features of vermicompost (Boran et al., 2017; Brown and Mitchell, 1981). One suggested approach involves heat-treating the primary material, which is animal organic waste, instead of the end product, vermicompost. This strategy remains under discussion. An alternative method that avoids heat treatment, while adhering to legal stipulations, might be worth investigating. The necessity of heat-treating primary material for earthworm consumption is debated, as it can be unnecessary and detrimental to vermicompost quality.

This study aimed to develop a different heat treatment technique in the production of vermicompost. Given that heat treatment of the pre-product already reduces the risk of pathogenicity of vermicompost, and considering the sanitization of the precursor as it traverses the worm's digestive system, such treatment seems both redundant and harmful. Moreover, the heat treatment of both the vermicompost and the pre-product imposes additional energy costs for the producers. In this study, we investigated the effects of different heat treatments on the primary product (cow manure) and recorded the number of worm cocoons in each heat treatment. The chemical properties of organic wastes determine their suitability for vermicomposting. The evaluation of organic wastes is usually based on their initial physicochemical properties before the addition of earthworms, so the results should be evaluated by comparing them with the differences in the initial physicochemical properties of the organic wastes. (Zhou et. al., 2021).

The objective of this study was to evaluate the influence of the pre-treatments on the reproductive abilities of earthworms during the vermicomposting process across the four incubation periods. In particular, we assessed the impacts in terms of cocoon count, juvenile count, and overall earthworm biomass.

Materials and Methods

We examined the effects of three different heat treatments (HT1, control 25 °C; HT2, 70 °C 1 h; HT3, 121 °C, 1.5 atm, 15 min) over four incubation periods (7, 15, 30, 45 days) on cocoon count, juvenile count, and the overall biomass of earthworms. To prepare the feed for the earthworms, the cow manure (the primary product) was sieved and pre-composted for a duration of 45 days. The cow manure used in the experiment was rinsed with water using a separator device. The study was structured as a randomized block design, executed with three replicates under laboratory conditions. We conducted an analysis of the cow manure to determine various chemical properties, including pH, EC, total N%, organic C%, organic matter content, mineral N, NH_4^+-N, NO_3^--N, C/N ratio, and plant nutrient contents. At the beginning of the experiment, different heat treatments were administered to 1000 grams of pre-composted cow manure. Subsequently, 80 adult *Eisenia fetida* L. earthworms were introduced into each pot. Table 1 lists some biological characteristics of *Eisenia fetida*.

Throughout the vermicomposting period, the moisture content of the cow manure was maintained at approximately 60% of its maximum water-holding capacity through daily weighing of the pots. We counted the number of earthworms, juveniles, and cocoons during each incubation period, and collected vermicompost samples. At the experiment's conclusion, we analyzed the chemical properties of three distinct vermicomposts, each subjected to a unique pre-heat treatment. These properties included pH, EC, total N%, organic C%, organic matter content,

Table 1. Some characteristics of worms belonging to the species *Eisenia fetida* L.

Attributes of *Eisenia fetida*	Specific Details
Color	Brown-red
Life Expectancy	45-51 days
Temperature Limits of Media	0-35 ˚C
Optimum Temperature Requirement	25 ˚C
Humidity Limits of Media	70-90%
Optimum Humidity Requirement	80-85%
Time Until the Adolescent Phase	21-28 day
Number of Cocoons Produced Per Day	0.35-0.50
Cocoon Sizes	4.8 mm × 2.82 mm
Survival Rate of Fry Emerging from Cocoons	20%

mineral N, NH_4^+-N, NO_3^--N, C/N ratio, and plant nutrient contents.

This study was conducted as an incubation experiment in the laboratories of Kırşehir Ahi Evran University's Faculty of Agriculture, following a randomized block experimental design. For the laboratory experiment, we used *E. fetida* worms, which are known for their high adaptability and resistance to conditions such as salinity and drought. They are among the species with the highest reproductive capacity. Notably, *E. fetida* is the only species used for vermicompost production in our country and is considered the most widely used earthworm species worldwide. The experiment consisted of four distinct incubation periods (0, 7, 15, 30, 45 days), with sampling and counting scheduled for each period, culminating at 45 days. The duration of the experiment was determined based on the reproductive cycles of *E. fetida*, the number of earthworms, the volume of the pots, and the quantity of vermicompost. We selected pots with a height of 30 cm to allow the worms to replicate their natural vertical movements in the burrows. Each pot was populated with a total of 80 adult *E. fetida* worms, weighing between 22-25 g collectively, and was provided with 1000 g of earthworm food (sourced from cow manure) based on its oven-dry weight for the 45-day period. Before the introduction to the pots, the worm food underwent various heat treatment processes in line with the study's objectives. The HT2 heat treatment was carried out in an oven, while the HT3 sterilization occurred in an autoclave, typically used for sterilizing materials like soil. Table 2 gives an overview of the chemical composition of the cow manure utilized in the experiment.

The experiment was conducted in a randomized block design and involved 36 pots. This accounted for 4 different incubation periods, 3 distinct heat treatments, and 3 replications for each combination. After setting up the pots, the ambient moisture content was adjusted to 80% of the worm food's maximum water holding capacity using distilled water. Throughout the experiment, this moisture level was sustained by adding earthworms and offsetting the daily water deficit with pure water. Furthermore, the ambient temperature was kept constant under laboratory conditions.

At the end of each incubation period, each worm, juvenile, and cocoon in the pots was carefully counted and documented using laboratory tweezers. We also collected samples from the medium to assess their chemical properties. By the end of the experiment, we had examined the variations in earthworm biomass, juvenile count, cocoon count, and the chemical composition of the medium at different temperatures. To determine the chemical properties of the three types of cow manure (each of which was subjected to a unique heat treatment and utilized as primary materials for the experiment), we performed analyses for of pH, EC, total nitrogen, and organic matter

Table 2. Methods used to determine the nutrient content and chemical properties of cow manure (Kacar, 1972, 1995; Bayraklı, 1986; Ryan et al., 2001).

Analysis	Methods
Organic matter	Dry burning (adding 1 mL of 5% H2SO4 dissolved in ethyl alcohol to each 1 g of material and burning at 550 °C in porcelain crucibles)
Total Nitrogen	Kjeldahl method
pH	1:10 (w/v), pH-meter in soil: organic waste mixture
EC	1:10 (w/v), EC-meter in soil: organic waste mixture
Total Phosphorus	The extract obtained by dry digestion was analyzed spectrophotometrically
Total Potassium	Flame photometry of the extract obtained by dry combustion
Total Ca, Mg, Zn, Cu, Fe, Mn	The extract obtained by dry digestion was analyzed by Atomic Absorption Spectrophotometer

content, following the methods described by Kacar (1995).

Data analysis

In this study, we utilized Pearson correlation coefficients to ascertain the relationships between heat treatments, physicochemical properties, and the measured biological parameters. For each physicochemical property, the mean values of the three heat treatments (HT1, HT2, and HT3) were correlated with the mean values of the biological parameters (such as biomass increase %, cocoon count, and juvenile count) for each specific time interval (7th, 15th, 30th, and 45th day). The significance of these correlations was assessed at two levels: $P<0.05$ and $P<0.01$. Statistical analyses were performed using SPSS version 15.

Results and Discussion

In this study, cow manure was used as food for the worms. To reduce the presence of pathogens, the manure was subjected to heat treatment before being provided to the earthworms. To evaluate the effect of heat treatment on the worms' essential activities at different temperatures, we implemented two specific temperature treatments. These were compared to a control group that did not undergo any heat treatment.

Table 3. Physicochemical properties of cow manure and heat-treated cow manure used in this trial (Kacar, 1972, 1995; Ryan et al., 2001).

Physicochemical parameters	HT1 (Control, 25 °C)	HT2 (70 °C, 1 h)	HT3 (121 °C, 1.5 atm, 15 min)
pH	7.78	7.36	6.92
EC, dS/m	3.95	4.25	4.1
Organic matter, %	39.25	38.98	40.04
Organic Carbon (C), %	22.76	22.61	23.22
Total Nitrogen (N), %	1.685	1.619	1.712
Mineral N, %	0.247	0.250	0.256
NH_4^+-N, mg kg^{-1}	188.320	580.36	1141.29
NO_3^--N, mg kg^{-1}	2287.82	1595.64	1364.73
Organic N, %	1.686	1.624	1.618
C/N	13.507	13.965	13.567
Total Phosphorus (P), %	2.035	2.036	2.021
Total Potassium (K), %	3.432	3.487	3.447
Total Calcium (Ca), %	1.726	1.794	1.755
Total Magnesium (Mg), %	6.212	6.317	6.294
Fe, mg kg^{-1}	41634.11	41867.96	41992.84
Cu, mg kg^{-1}	322.69	323.17	323.11
Zn, mg kg^{-1}	17721.58	17801.32	17814.57
Mn, mg kg^{-1}	1723.80	1743.28	1749.46
Cr, mg kg^{-1}	701,11	701,96	702,13

After applying the three heat treatments to the cow manure prepared for vermicomposting, we analyzed certain chemical properties of the manures, which are listed in Table 3.

During the experiment, the changes in the worm biota, which initially consisted of adult individuals, were determined according to the differences in heat treatment. In the pots subjected to the HT3 treatment, all earthworms died by the second incubation period (7th day) across all replicates. In contrast, in both the control and HT2 treatments, the earthworms survived throughout all incubation periods. Although the number of earthworm cocoons increased in each incubation period in both control and HT2-treated pots, the control group consistently recorded higher cocoon counts than the HT2-treated group across all periods.

When the data on cocoon numbers were evaluated, as depicted in Figure 1, the cocoon numbers in the HT1, HT2, and HT3 heat treatments decreased, respectively, compared to the HT1-control group without heat treatment. Notably, there was no cocoon production in the HT3-sterilisation treatment. As the

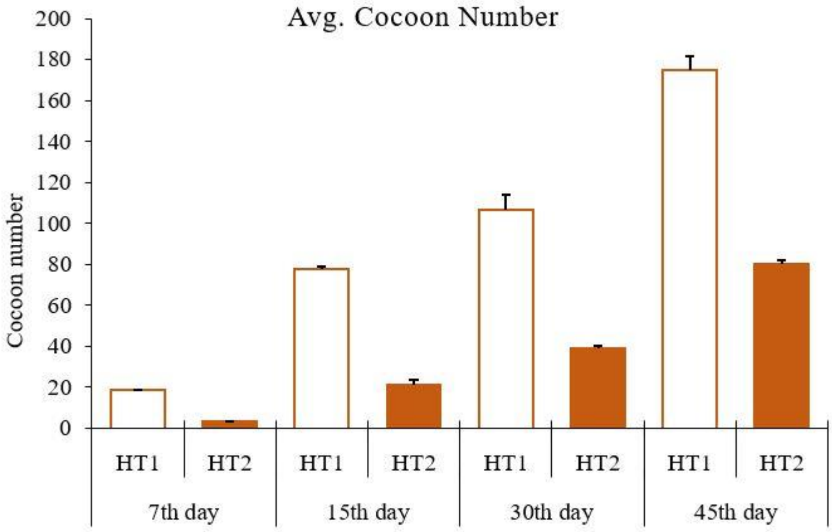

Figure 1. Variations in the average number of cocoons in pots during the incubation period. HT1: Control, 25 °C; HT2: 70 °C 1h treatment. No cocoons were found in pots for HT3 (121 °C for 15 min at 1.5 atm), and thus they are not shown in the diagram.

Upon analyzing the data on earthworm biomass from the experiment, as depicted in Figure 2, it was observed that the earthworm biomass decreased in the HT1, HT2, and HT3-sterilisation treatments compared to the HT1-control group without heat treatment. In the HT3-sterilisation treatment, all worms perished, leaving no biomass behind. With respect to the increasing incubation periods, there was no significant growth in biomass during the first three incubation periods. However, a significant increase in earthworm biomass was observed during the last incubation period. During the 4th incubation period, the juveniles emerging from the cocoons were also accounted for in the biomass. While the number of adults remained unchanged compared to the beginning of the experiment, the weight of the adults increased noticeably.

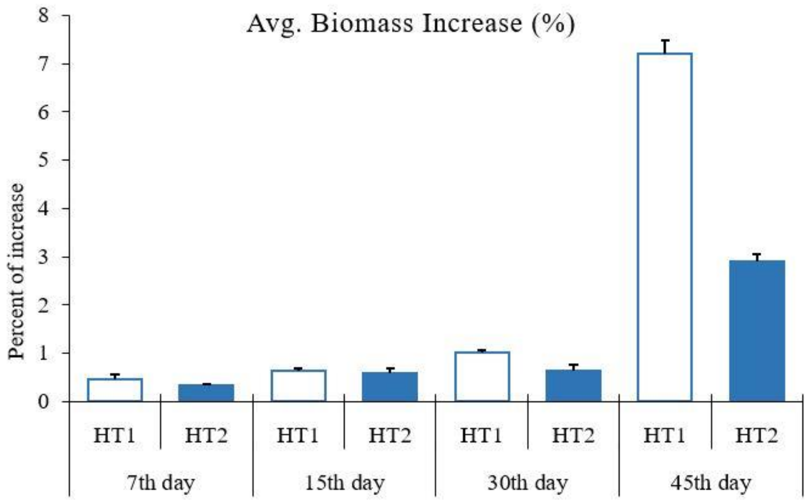

Figure 2. Variations in the average biomass increase in pots during the incubation period. HT1: Control, 25 °C; HT2: 70 °C 1h treatment. No biomass increase was observed in pots for HT3 (121 °C for 15 min at 1.5 atm), and thus they are not shown in the diagram.

After analyzing the data on the number of juveniles from the experiment, as depicted in Figure 3, it was observed that the number of juveniles in the HT1, HT2, and HT3 heat treatments decreased, respectively, compared to the HT1-control group without heat treatment. There were no juveniles in the HT3-sterilisation due to the death of all worms. As the incubation period progressed, there was no significant increase in juvenile numbers in the first three periods. However, in the last incubation period, a significant increase was observed. During the 4th incubation period, the surge in juvenile numbers corresponded to the growth of earthworm biomass. During the initial incubation period, there were no juveniles because the cocoons had not yet completed their maturity cycle.

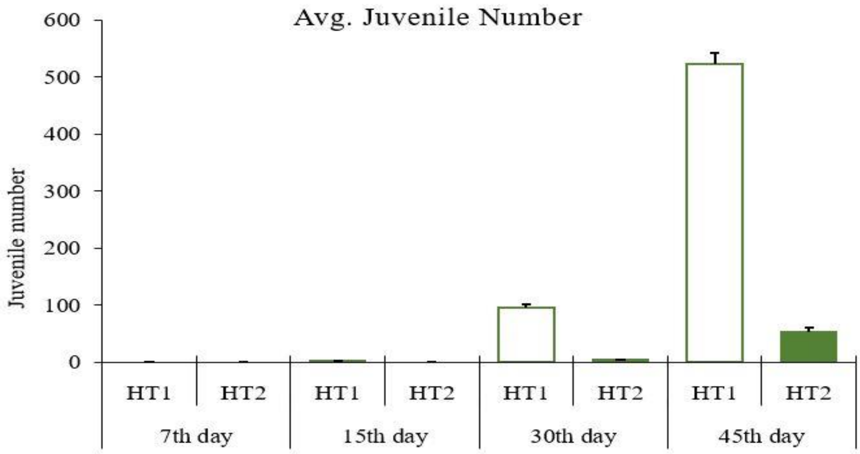

Figure 3. Variations in the average number of juveniles in pots during the incubation period. HT1: Control, 25 °C; HT2: 70 °C 1h treatment. No juveniles were observed in pots for HT3 (121 °C for 15 min at 1.5 atm), and thus they are not shown in the diagram.

incubation periods increased, and cocoon numbers in all treatments rose compared to the initial incubation period.

Both the HT2 and HT3 heat treatments resulted in a decrease in earthworm biomass and cocoon numbers compared to the control group. Especially in the HT3 treatment, the earthworms did not survive (Figure 1). An analysis of the chemical content of the treated materials showed variations in pH, EC, and NH_4^+-N levels, as listed in Table 1. In treatments with higher temperatures than the control, these crucial limiting factors increased to potentially hazardous levels. The HT2 treatment was carried out in an oven with dry air conditions, whereas the HT3 treatment was performed in an autoclave under sterilization conditions.

The mortality of the worms in our study was associated with the ammonia released during the heat treatment of the preproduct under the sterilization condition (HT3). Earthworms are very sensitive to ammonia and have difficulty surviving in organic waste with a high content of this cation, such as fresh poultry litter. They also cannot endure in wastes rich in inorganic salts. Both ammonia and inorganic salts have specific thresholds that delineate toxic from non-toxic levels (<1 mg g^{-1} for ammonia and >0.5% for salts), as highlighted by Edwards (1988). The optimal breeding conditions for *E. foetida* and *E. andrei* closely resemble those favorable for other species (Edwards, 1988). In addition to environmental factors, population density also plays a role in influencing the growth and reproduction rate of earthworms. Even if the physical and chemical conditions are ideal for vermicomposting, overcrowding of worms can still present problems. The findings of Reinecke and Viljoen (1990) and Domínguez (1997) indicate that when *E. foetida* was fed cow manure and *E. andrei* was given pig manure, earthworms in densely populated containers exhibited slower growth and reached a reduced final body weight. Nonetheless, the overall weight of earthworm biomass produced per unit of waste was greater when both species were cultivated at different population densities. The maturation rate, as indicated by the development of the clitellum, also fluctuated based on population density, resulting in earthworms of the same age reaching maturity at different intervals.

Statistical analysis indicated that earthworm biomass was primarily influenced by the C/N ratio, Organic C, Total N, NH_4^+-N, and NO_3^--N of cow manure subjected to various heat treatments. The total biomass of earthworms, cocoon numbers, and juvenile counts were correlated with the C/N ratio, OC, and TN (Table 4). The biological parameters of earthworms were strongly influenced by the C/N ratio, OC, TN, NH_4^+-N, and NO_3^--N. Increases in NH_4^+-N concentrations resulting from heat treatments were negatively associated with earthworm biological activity (Table 4).

According to a study by Zhou et al. (2021),

Table 4. Correlation coefficients between physicochemical properties and biological parameters following heat treatments. Values without asterisks are not statistically significant. '*' indicates *P*<0.05, and '**' indicates *P*<0.01.

Physicochemical Parameters	Biomass increase	Cocoon number	Juvenile number
pH	-0.874	-0.837	-0.895
EC, dS/m	0.512*	0.487	0.503*
Organic matter, %	0.634**	0.621**	0.642**
Organic Carbon (OC), %	0.598**	0.587**	0.605**
Total Nitrogen (TN), %	0.512*	0.503*	0.517*
Mineral N, %	-0.312	-0.298	-0.307
$NH4^+$-N, mg kg^{-1}	-0.874**	-0.865**	-0.881**
$NO3^-$-N, mg kg^{-1}	0.742**	0.731**	0.748**
Organic N, %	0.513*	0.504*	0.518*
C/N	-0.612**	-0.601**	-0.618**
Total Phosphorus (P), %	0.287	0.276	0.293
Total Potassium (K), %	0.315	0.304	0.322
Total Calcium (Ca), %	-0.287	-0.276	-0.293
Total Magnesium (Mg), %	0.298	0.289	0.305
Fe, mg kg^{-1}	0.276	0.268	0.282
Cu, mg kg^{-1}	-0.265	-0.257	-0.270
Zn, mg kg^{-1}	0.257	0.249	0.262
Mn, mg kg^{-1}	-0.248	-0.240	-0.253
Cr, mg kg^{-1}	0.240	0.233	0.245

increases in NH_4^+-N and NO_3^--N concentrations, coupled with decreases in total organic carbon content, adversely impacted earthworm growth and reproduction. The chemical composition of organic residues, particularly the C/N ratio, plays a pivotal role in determining earthworm abundance and population dynamics (Lavelle et al., 2006). Organic residues that possess a C/N ratio ranging between 20 and 30 are deemed optimal for earthworm development (Fusilero et al., 2013). This is attributed to the fact that the activity and community structure of decomposer microbes are influenced by the C/N ratio of the organic residues (Sarathchandra et al., 2006; Pang et al., 2009). In addition to the C/N ratio, earthworms are also sensitive to the pH and salinity of organic residues, parameters that can be determined by measuring the electrical conductivity.

Alterations in the physicochemical properties of organic residues during the vermicomposting process influence both earthworm biomass and population structure. Nitrogen concentration has an impact on earthworm reproduction, with the C/N ratio, NH_4^+-N, and NO_3^--N emerging as predominant factors negatively affecting the adult population. Research indicates that the number of offspring depends largely on total organic C, whereas the number of cocoons is influenced by total N, the C/N ratio, and NH_4^+-N (Zhou et al., 2021). Consequently, lower N concentrations combined with a favorable C/N ratio are viewed as conducive for earthworm growth and reproduction. In contrast, elevated N concentrations (TN, NH_4^+-N, and NO_3^--N) lead to a decline in earthworm abundance and biomass.

It was noted that the heat treatment of the initial product could be an alternative to treating the final product. However, this preproduct heat treatment had a detrimental effect on the reproductive and survival capacities of the worms compared to the control group. The application of heat treatment to products that are inherently at a low lisk of pathogenicity and therefore do not require such treatment has also been found to be counterproductive. While heat-treating the pre-product hampers worm reproduction and survival during vermicomposting, treating the end-product can undermine its beneficial attributes.

Conclusion

To mitigate the risk of pathogenic contamination in vermicompost, we proposed and implemented heat treatment of the initial product, using vermicompost prepared from cow manure, as an alternative to treating the end product. The results of the study showed that all heat treatment methods, applied to the initial material at different temperatures, had a significant negative impact on the cocoon counts of earthworms, thereby affecting their reproductive capacity and rate. Among the three heat treatments, including the control group, cocoon numbers diminished with increasing treatment temperatures. The control group registered the highest cocoon count, while the HT3 sterilization condition, being the most intense treatment, yielded the lowest. Notably, all worms that consumed the initial product treated under the HT3 sterilization conditions did not survive. The chemical analysis for the HT1 treatment showed a smaller increase in ammonia compared to HT3. The decline in biological parameters could be linked to the HT1 treatment carried out at 70 °C in an oven, which likely diminished the activity of microorganisms, crucial nutrients for the earthworms. Earthworms are sensitive to particular environmental conditions in waste, especially substances such as ammonia, salts, and other chemicals. However, these can be effectively removed or reduced through thermophilic pre-composting processes. While heat-treating the product has its drawbacks, applying this treatment to the initial product also presents several adverse effects. These include a reduction in beneficial microorganisms, degradation of proteins and enzymes, and hindrance to the biology and reproduction of earthworms. Nevertheless, the pathogen load in the initial product naturally decreases during the vermicomposting process. This makes vermicomposting a suitable method to counter the negative impacts of heat treatment arising from concerns about pathogenicity. Additionally, heat-treating the initial product affects the essential activities of earthworms by rapidly converting nitrogen to ammonia in the organic material. Higher temperatures can lead to an increased release of ammonia, which can lead to the death of earthworms. It is understood that pathogenic microorganisms in worm food, which probably originate from the digestive systems of warm-blooded animals, are neutralized by the microorganisms in the digestive tract of the worms. Therefore, based on the findings of this study, we conclude that heat-treating the initial product, which has a negative effect on earthworm viability and reproduction, is both unwarranted and impractical. Instead, we recommend regular sampling and pathogen load tests to keep track of any unexpected pathogen presence in the final vermicompost.

Conflict of Interest

The authors declare that there is no conflict of interest.

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