



SUSTAINABLE MANAGEMENT OF SEWAGE SLUDGE VIA VERMICOMPOSTING: SYNERGISTIC EFFECTS OF HAZELNUT HUSK AND COW MANURE ON MICROBIAL DYNAMICS

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Abstract: This study was conducted to investigate the stabilization and transformational potential of anaerobically digested sewage sludge (SS) through vermicomposting, using hazelnut husk (HH) and cow manure (CM) as bulking agents and carbon sources. The research focused on the performance of the epigeic earthworm *Eisenia fetida* in managing these organic wastes and monitored the resulting changes in microbial dynamics. An incubation experiment was established with eleven different feed mixtures, ranging from 0% to 100% SS proportions by weight. The mixtures were incubated under controlled laboratory conditions at 20 °C in total darkness for a period of 90 days, with moisture levels maintained at 80% of the water-holding capacity. Microbial activity indicators, specifically basal soil respiration (BSR) and microbial biomass carbon (Cmic), were systematically analyzed at 15-day intervals (days 15, 30, 45, 60, 75, and 90). The experimental results revealed that *Eisenia fetida* could not maintain viability in mixtures containing 60% or higher doses of sewage sludge, identifying a critical toxicity threshold for the vermicomposting process. Statistical analysis showed significant differences (P<0.001) in microbiological properties across all feed rations. Both BSR and Cmic levels reached their maximum values on the 30th day of incubation, followed by a gradual decline, indicating the onset of organic matter stabilization. The study concluded that mixing sewage sludge with HH and CM at proportions below 50% SS provides an ideal environment for earthworm activity and microbial proliferation. These findings suggest that the produced vermicompost serves as a sustainable and efficient soil conditioner for land restoration and agricultural practices.

Keywords: Basal respiration, *Eisenia fetida*, Hazelnut husk, Microbial biomass C, Sewage sludge, Easte management

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1. Introduction

The rapidly growing global population and the consequent need for food security pose the risk of expanding agricultural lands at the expense of forests; this situation triggers environmental problems such as erosion and global warming (Lal, 2024). The intensive use of chemical fertilizers and pesticides to increase yield per unit area has led to the deterioration of soil structure and the destabilization of ecosystem balance. To overcome these adverse effects, the recovery of organic wastes through biotechnological methods stands out as one of the most effective techniques within sustainable agricultural practices (Bellitürk and Sundari, 2024; Gong et al., 2023). In addition to waste stabilization, contemporary research emphasizes the integration of biotechnological pipelines and green chemistry principles to establish sustainable production systems (Türkay, 2025).

Vermicomposting, employed for the stabilization of organic wastes, is a process of biochemical

decomposition through the synergistic action of epigeic earthworm species, such as *E. fetida*, and microorganisms (Edwards et al., 2010). It has been reported in the literature that organic waste applications enhance soil enzyme activities, and the presence of earthworms stimulates microbial activity and CO₂ production (Srivastava et al., 2020). However, preserving the biological properties of the soil ecosystem is of critical importance when using 'objectionable' wastes, such as municipal sewage sludge, which carry the risk of heavy metal contamination. It is well documented that heavy metals suppress soil microbial community structures and enzyme activities (Rusănescu et al., 2022).

During the vermicomposting process, the C/N ratio of the feed mixtures is a decisive factor for the stability and quality of the final product. Previous studies have determined that vermicomposting results in a decrease in the C/N ratio, while total N and P contents increase (Kızilkaya et al., 2012). In terms of microbial dynamics, it has been reported that the biological activity, which is high at the beginning of the process, tends to decline in



the later stages of incubation as the substrate becomes stabilized (Gómez-Brandón et al., 2022). This situation proves how strategic microbial biomass carbon (Cmic) and basal soil respiration (BSR) parameters are in determining the maturation level of organic wastes.

This study aimed to ensure the sustainable management of municipal sewage sludge (SS), categorized as an objectionable organic waste, by vermicomposting it with hazelnut husk (HH)—a strategic waste for the Black Sea region in need of agricultural evaluation—and cow manure (CM). Within this scope, the research intended to determine the most ideal vermicompost ration ratio and incubation period for the earthworm species *E. fetida*. Furthermore, the study aimed to reveal the changes occurring in critical biological properties, such as microbial biomass carbon and basal soil respiration, throughout the 90-day process.

2. Materials and Methods

2.1. Research Materials

In this study, anaerobically stabilized sewage sludge (SS), hazelnut husk (HH), cow manure (CM), and earthworms of the species *E. fetida* were used as the primary materials. The sewage sludge was obtained from the Ankara Central Wastewater Treatment Plant (AMAAT); the hazelnut husk was procured from hazelnut orchards in the Ordu province; and the cow manure was sourced from cattle farms in the Samsun province. The *E. fetida* earthworms used in the study were collected from cow manure piles in the Samsun province and prepared for the experiment under laboratory conditions.

2.2. Mixture Ratios and Experimental Design

The organic wastes used in the incubation experiment were completely dried, ground, and passed through a 0.5 mm sieve before being mixed on a weight basis. Within the scope of the experiment, 11 different mixture ratios ranging from 0% to 100% were established. The specific mixing ratios created for the study are presented in Table 1.

2.3. Establishment of the Incubation Experiment

To establish the incubation experiment, 500 g of the

prepared mixtures were placed into 1-liter light-proof cylindrical plastic pots (8 cm in diameter, 25 cm in height). Three *E. fetida* earthworms of equal weight were introduced into each pot. The tops of the pots were covered with mesh to allow aeration while preventing escape, and water was added to provide optimum conditions for the earthworms (Reinecke et al., 1992; Ma et al., 2024). Water addition was adjusted to maintain a moisture content of 80% for each mixture. The pots were incubated in darkness at 20 °C for three months. Throughout the incubation, the pots were weighed daily, and any moisture loss was replenished by adding water. Since *E. fetida* produces vermicompost up to a maximum of 75% of its body weight per day (Ndegwa et al., 2000). The total incubation period was limited to 90 days. Vermicompost samples were collected on days 15, 30, 45, 60, 75, and 90 to perform the relevant indicator analyses (Table 2).

The incubation experiment was initially designed with a total of 198 pots [6 (sampling periods) x 3 (replications) x 11 (mixtures) = 198], utilizing 594 earthworms. However, because the earthworms could not survive in mixtures containing sewage sludge doses higher than 50%, the pots containing 60%, 70%, 80%, 90%, and 100% sewage sludge were excluded from the experiment following the initial setup. At the end of the incubation period, the earthworms were separated from the vermicompost and kept in their original bedding until the completion of the study.

2.4. Laboratory Analyses

The methods used to determine the properties of the organic wastes and the samples collected on days 15, 30, 45, 60, 75, and 90 of the incubation experiment are presented in Table 2 (Kacar, 1994; Ryan et al., 2001).

2.5. Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) using the SPSS statistical software package. The significance of the differences between parameters was determined by the Least Significant Difference (LSD) test at confidence levels of $P < 0.05$ and $P < 0.001$.

Table 1. Mixture ratios of organic wastes used in the vermicomposting process

Mixtures	Organic waste mixture ratios
Mixture 1	0% SS + 100% HH and CM (50% HH + 50% CM)
Mixture 2	10% SS + 90% HH and CM (45% HH + 45% CM)
Mixture 3	20% SS + 80% HH and CM (40% HH + 40% CM)
Mixture 4	30% SS + 70% HH and CM (35% HH + 35% CM)
Mixture 5	40% SS + 60% HH and CM (30% HH + 30% CM)
Mixture 6	50% SS + 50% HH and CM (25% HH + 25% CM)
Mixture 7	60% SS + 40% HH and CM (20% HH + 20% CM)
Mixture 8	70% SS + 30% HH and CM (15% HH + 15% CM)
Mixture 9	80% SS + 20% HH and CM (10% HH + 10% CM)
Mixture 10	90% SS + 10% HH and CM (5% HH + 5% CM)
Mixture 11	100% SS + 0% HH and CM (0% HH + 0% CM)

Table 2. Analytical methods used for the characterization of organic wastes and vermicompost samples during the incubation period

Analysis	Methods
Organic matter	The dry ashing method (incineration in porcelain crucibles at 550 °C following the addition of 5% H ₂ SO ₄ dissolved in ethyl alcohol at a ratio of 1 g:1 ml).
Total Nitrogen	Kjeldahl Method
pH	1:10 (w/v), organic matter: distilled water, pH-meter
EC	1:10 (w/v), organic matter: distilled water EC-meter
Total Phosphorous	Spectrophotometrically in the filtrate obtained through dry ashing
Total Potassium	Flame photometry in the filtrate obtained through dry ashing
Basal Soil Respiration (BSR)	Anderson (1982)
Microbial biomass C	Anderson and Domsch (1978)

Table 3. Protocols for biological analyses of vermicompost samples

Analysis	Protocol
Basal Respiration (CO ₂ -C)	50 g of soil is moistened with distilled water until it reaches 55% of its maximum water holding capacity and placed into 1 L Isermeyer jars. 25 mL of 0.05 M NaOH is added to the alkaline tube of the jar, and the jars are incubated at 25 °C for 3 days. The CO ₂ released by microbial respiration is trapped by the alkali, and the remaining OH ⁻ is titrated with standardized HCl in the presence of phenolphthalein indicator. The result is expressed as µg CO ₂ -C g ⁻¹ dry soil.
Microbial Biomass C (MBC-C)	50 g of soil is moistened with distilled water until it reaches 55% of its maximum water holding capacity, then 200 mg of glucose is added and placed into 1 L Isermeyer jars. The amount of CO ₂ released from the soil is determined hourly. The maximum respiration at the end of 4 h is calculated using the equation of 40.04 µg CO ₂ g ⁻¹ + 3.75, and the result is expressed as µg CO ₂ -C g ⁻¹ dry soil.

Table 4. Properties of organic wastes used in the incubation experiment

Analysis	Sewage Sludge	Hazelnut Husk	Cow Manure
Organic matter, %	39.490	92.880	35.746
Organic carbon (C), %	22.907	53.875	20.734
Total nitrogen (N), %	2.544	1.136	1.695
C/N	9.00	47.42	12.23
Mineral N ((NH ₄ ⁺ + NO ₃ ⁻) - N), %	0.406	0.287	0.248
NH ₄ ⁺ - N, mg kg ⁻¹	2184.00	378.560	189.280
NO ₃ ⁻ - N, mg kg ⁻¹	1873.39	2489.76	2293.93
Organic - N, %	2.326	1.098	1.676
Total Phosphorus (P), %	2.426	0.343	2.662
Total Potassium (K), %	1.136	2.193	3.941
Total Calcium (Ca), %	4.276	1.389	1.989
Total Magnesium (Mg), %	2.050	0.183	6.165

3. Results and Discussion

The vermicomposting process, involving the biochemical decomposition and stabilization of organic wastes through the synergistic action of epigeic earthworms like *E. fetida* and microorganisms, leads to significant changes in the properties of starting materials. In this study, basal soil respiration (BSR) and microbial biomass carbon (Cmic), key indicators of microbial activity and organic matter stability, were monitored over a 90-day period across different sewage sludge (SS), hazelnut husk (HH), and cow manure (CM) mixtures.

3.1. Characterization of Initial Organic Wastes

Some chemical and physical properties of the organic wastes used in the research are presented in Table 4. Among these materials, sewage sludge (SS) exhibited the highest total N content and the lowest C/N ratio (9.0). Additionally, SS was characterized by high amounts of mineral N. In contrast, hazelnut husk (HH) had a significantly larger particle size and displayed a physiological acid reaction. Compared to sewage sludge and cow manure, hazelnut husk contained relatively lower levels of P, Ca, and Mg.

3.2. Changes in the Biological Properties During Vermicomposting

3.2.1. General statistical evaluation

The effects of incubation time, sewage sludge doses, and their interactions on basal soil respiration (BSR) and microbial biomass C (Cmic) were evaluated using variance analysis. The summary of F-values and significance levels obtained from the analysis is presented in Table 5. According to the ANOVA results, independent variables (time and mixture) and their interactions (Time × Mixture) were found to be statistically significant ($P < 0.001$) for both biological parameters investigated.

3.2.2. Changes in basal soil respiration (BSR)

Statistically significant differences were determined in basal soil respiration (BSR) values depending on the incubation period and mixture ratios during the vermicomposting process of sewage sludge (SS), hazelnut husk, and cow manure mixtures ($P < 0.001$).

When time-dependent changes were examined, a distinct increase (peak) in microbial activity was generally observed in all mixtures on the 30th day of incubation (Figure 1). The highest respiration value was measured as $2.896 \mu\text{g CO}_2\text{-C g}^{-1}$ in mixture 4 (30% SS + 35% Hazelnut Husk + 35% Cow Manure) on the 30th day. After this period, with the decomposition of organic matter and the progression of the stabilization process, a gradual decrease occurred in respiration values towards the 90th day. In particular, it was observed that respiration values fell below the initial levels (15th day) at the end of the incubation (90th day), indicating that the maturation process of the vermicompost was completed. Furthermore, Akanbi et al. (2026) recently suggested that monitoring these biological indicators is crucial for determining compost maturity in sewage sludge vermicomposting, supporting our findings on the stabilization of the organic matrix by the end of the 90-day period.

When the effect of mixture ratios on general means was evaluated, the highest mean BSR value was obtained in mixture 4 (30% SS) with $1.508 \mu\text{g CO}_2\text{-C g}^{-1}$, which was statistically placed in group "a" (Figure 2). This was followed by mixture 3 (20% SS) and mixture 5 (40% SS), respectively. The control group, mixture 1 (0% SS), yielded the lowest mean respiration value with $0.811 \mu\text{g CO}_2\text{-C g}^{-1}$ and was placed in group "f". This finding indicates that the addition of sewage sludge at certain rates (30-40%) promotes microbial activity and the biological degradation process in the medium.

3.2.3. Evaluation of microbial respiration dynamics

Significant differences were observed among the sampling periods of the 90-day incubation ($P < 0.001$). The microbial respiration content of the medium increased until the 30th day of incubation and subsequently decreased depending on the incubation stage. Statistically significant differences were detected between the mixtures in each incubation period;

specifically, a decrease in microbial respiration was observed when the sewage sludge (SS) level in the mixture exceeded 30%. Across all organic waste mixtures and incubation periods, the maximum microbial respiration was achieved on the 30th day in Mixture 4 (30% SS + 35% HH + 35% CM).

These findings are consistent with previous literature. The decline in microbial parameters after the 30th day signifies the transition from the active decomposition phase to the maturation phase, confirming the stabilization of the organic matrix (Vuković et al., 2021). Similarly, Chaoui et al. (2003) reported in an incubation study investigating the effects of vermicompost, compost, and mineral fertilizer applications that the highest microbial respiration and microbial biomass contents occurred in the vermicompost application on the 35th day of incubation. Furthermore, Tejada et al. (2009) determined that the application of vermicompost derived from various plant sources to soils significantly increased the microbial respiration content of the soil.

3.2.4. Changes in microbial biomass C (Cmic)

The microbial biomass C (Cmic) contents of mixtures containing different doses of sewage sludge showed statistically significant differences in terms of both incubation period and mixture ratios ($P < 0.001$). Throughout the incubation period, Cmic values followed a trend similar to the BSR results, generally reaching their highest levels on the 30th and 45th days (Figure 3). Mixture 4 reached the highest biomass value with $51.165 \mu\text{g C g}^{-1}$ on the 30th day, while the biomass values of mixture 6 containing a high dose of sewage sludge (50% SS) remained quite low throughout the incubation.

When the general performances of the mixtures were compared, mixture 4 (30% SS) was found to be statistically superior to all other treatments with a mean value of $43.093 \mu\text{g C g}^{-1}$, forming group "a" (Figure 4). In contrast, in mixture 6 where the sewage sludge ratio increased to 50%, the mean biomass value dropped to $17.554 \mu\text{g C g}^{-1}$ and was placed in the lowest statistical group (group "e"). This situation coincides with the finding that sewage sludge doses of 50% and above create a potential toxic or inhibitory effect on *E. fetida* and microorganisms, suppressing biological activity.

3.2.5. Evaluation of microbial biomass carbon dynamics

Throughout the 90-day incubation experiment, significant differences ($P < 0.001$) were determined in microbial biomass C (Cmic) levels among the sampling periods, and significant differences ($P < 0.001$) were also detected between the mixtures in each 15-day sampling interval.

Table 5. Summary of ANOVA results (F-values) for basal soil respiration (BSR) and microbial biomass C (Cmic) during the vermicomposting process

Source of Variation	df	BSR F-values	Cmic F-values
Incubation Time (T)	5	2802.27***	96.94***
Mixture Dose (M)	5	591.99***	192.05***
Interaction (T x M)	25	100.98***	8.47***

Significance levels: *** P<0.001, ** P<0.01, * P<0.05, ns= not significant, df= degrees of freedom.

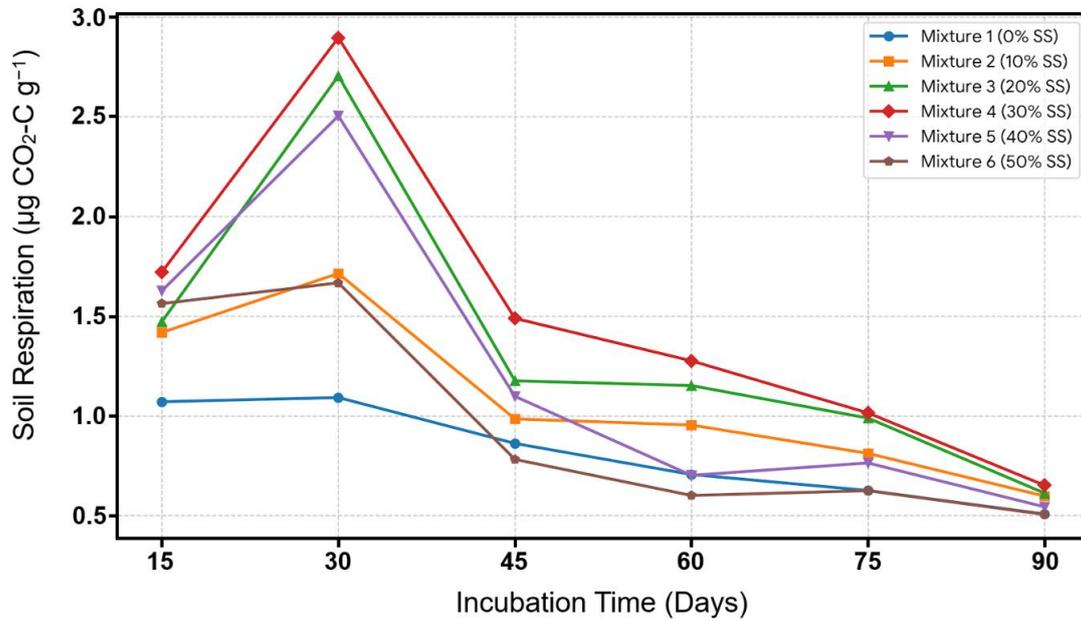


Figure 1. Changes in basal soil respiration (BSR) levels ($\mu\text{g CO}_2\text{-C g}^{-1}$ dry soil) in different feed mixtures during the 90-day vermicomposting period.

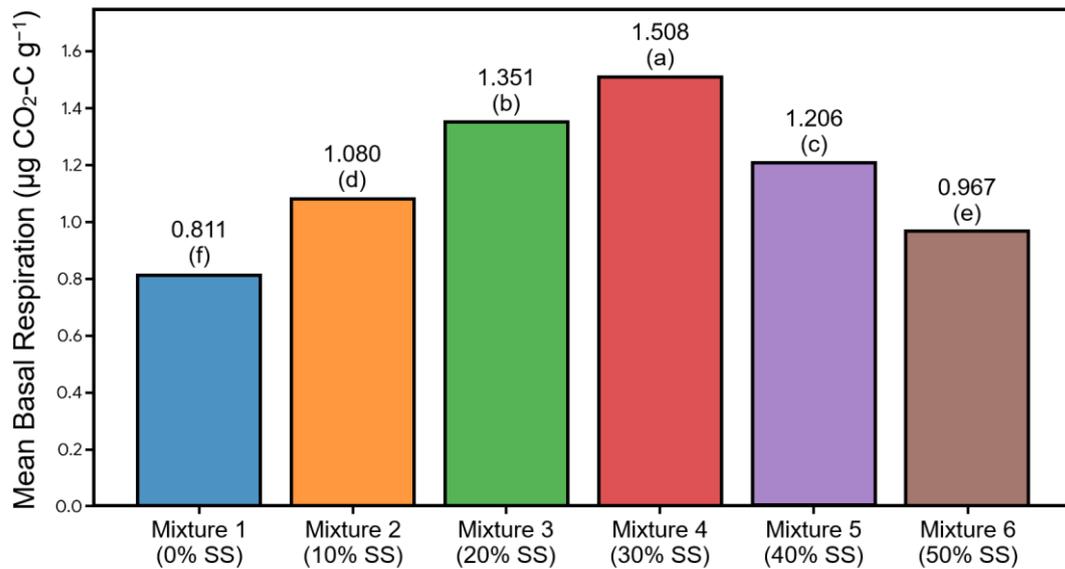


Figure 2. The effect of different sewage sludge doses on the mean basal soil respiration (BSR) values throughout the vermicomposting process. Different letters indicate statistically significant differences at P<0.05 according to the LSD test.

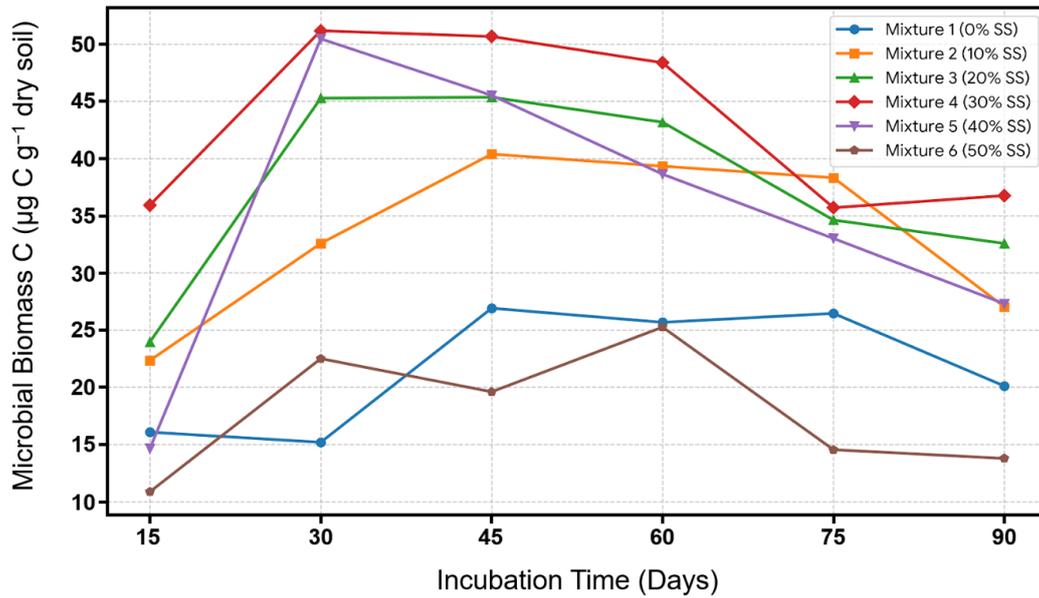


Figure 3. Changes in microbial biomass C (Cmic) contents (µg C g⁻¹ dry soil) in different feed mixtures during the 90-day vermicomposting period.

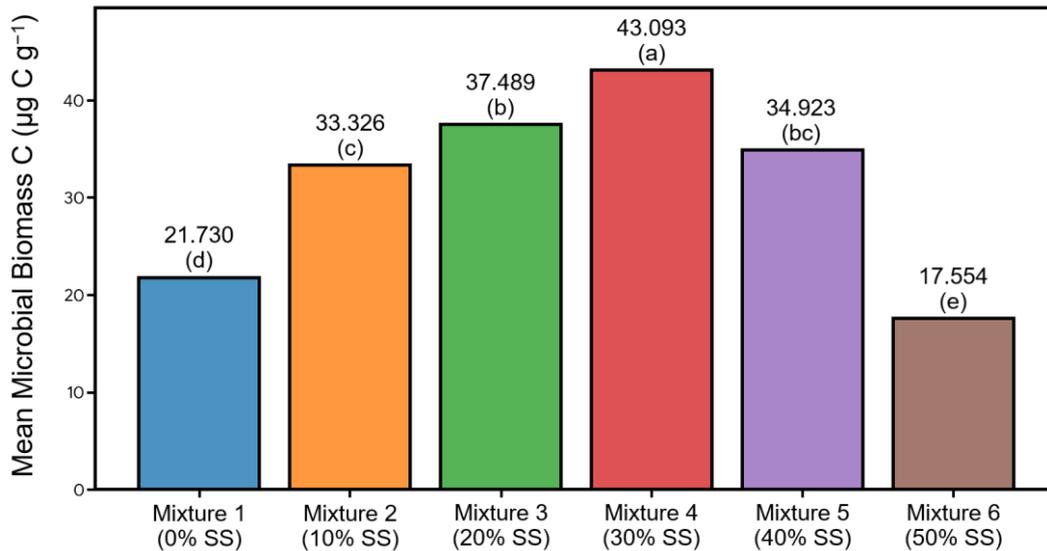


Figure 4. The effect of different sewage sludge doses on the mean microbial biomass C (Cmic) values throughout the vermicomposting process. Different letters indicate statistically significant differences at P < 0.05 according to the LSD test.

Cmic levels generally showed an increase after the initial period followed by a gradual decrease throughout the remainder of the incubation. Mixture 4 (30% SS + 35% HH + 35% CM) consistently yielded the highest Cmic values across all periods.

These results are supported by various studies in the literature. The reduction in microbial biomass towards the end of the incubation also reflects the transition of the vermicompost into a more stable and mature state. As noted by Vuković et al. (2021) the decrease in microbial parameters over time is often associated with the exhaustion of easily degradable organic fractions and the successful stabilization of the waste matrix during the vermicomposting process. In a 100-day incubation study

by Tiunov and Scheu (2000) evaluating the microbial biomass C of *L. terrestris* casts of different ages, it was found that biological activities were higher in fresh casts, but significant decreases in Cmic occurred as the incubation progressed. Yakushev et al. (2009) reported that peat-based vermicomposts with high organic matter content exhibited higher Cmic levels than vermicomposts derived from other wastes, while non-vermicomposted mixtures showed 2.7 times lower values. In their study, vermicomposts using farm manure showed similar Cmic levels at the beginning and the end of the process. Furthermore, Chaoui et al. (2003) observed in a 28-day laboratory study that Cmic levels in vermicomposts were highest within the first 15 days, followed by a significant

decrease compared to farm manure by the end of the study. The positive effect of sewage sludge on microbial populations was also noted by Kunito et al. (2001), who found that the application of sewage sludge to sandy loam soils increased the Cmic content and enzyme activities. Conversely, Zhang et al. (2000) investigated the biological properties of soil samples incubated with different earthworm species (*Metaphire guillelmi* and *E. fetida*) and reported that earthworm inoculation actually decreased microbial biomass, suggesting that the impact of earthworms can vary depending on the species and environmental conditions. The initial increase in BSR and Cmic levels during the first 30 days of incubation can be explained by the synergistic interaction between *E. fetida* and the cow manure added to the mixtures. As recently emphasized by Gao et al. (2023), the presence of earthworms and manure modifications significantly enhances the microbial community structure and stimulates biological decomposition processes through increased nutrient availability.

3.2.6. Relationship between basal soil respiration and microbial biomass C

The results of the correlation analysis performed to determine the relationship between BSR and Cmic data obtained during the vermicomposting process are presented in Figure 5. As a result of the analysis, a positive and statistically significant relationship ($r=0.410$, $P<0.05$) was determined between basal soil

respiration and microbial biomass C. This positive correlation confirms that as the microbial mass in the medium increases, respiration, which is an indicator of metabolic activity, also increases as expected.

4. Conclusion

This study demonstrates the sustainable management potential of vermicomposting anaerobically digested municipal sewage sludge (SS) by using hazelnut husk (HH) and cow manure (CM). The 90-day incubation data successfully identified the ideal ration and biological transformation processes for *E. fetida*. A critical finding is the toxicity threshold; SS proportions of 60% and above exerted a lethal effect on earthworms, likely due to high ammonia or heavy metal loading. Therefore, maintaining SS below 50% is essential for a successful process. The mixture of 30% SS + 35% HH + 35% CM emerged as the most ideal biological environment, yielding the highest basal soil respiration and microbial biomass carbon. The peak in microbial activity on day 30, followed by a decline, confirms the stabilization and maturation of the organic wastes. Utilizing regional hazelnut husk with sewage sludge provides a dual solution for environmental protection and sustainable soil conditioner production. Future research should focus on the long-term effects of these ideal mixtures on different crop patterns and heavy metal mobility under field conditions.

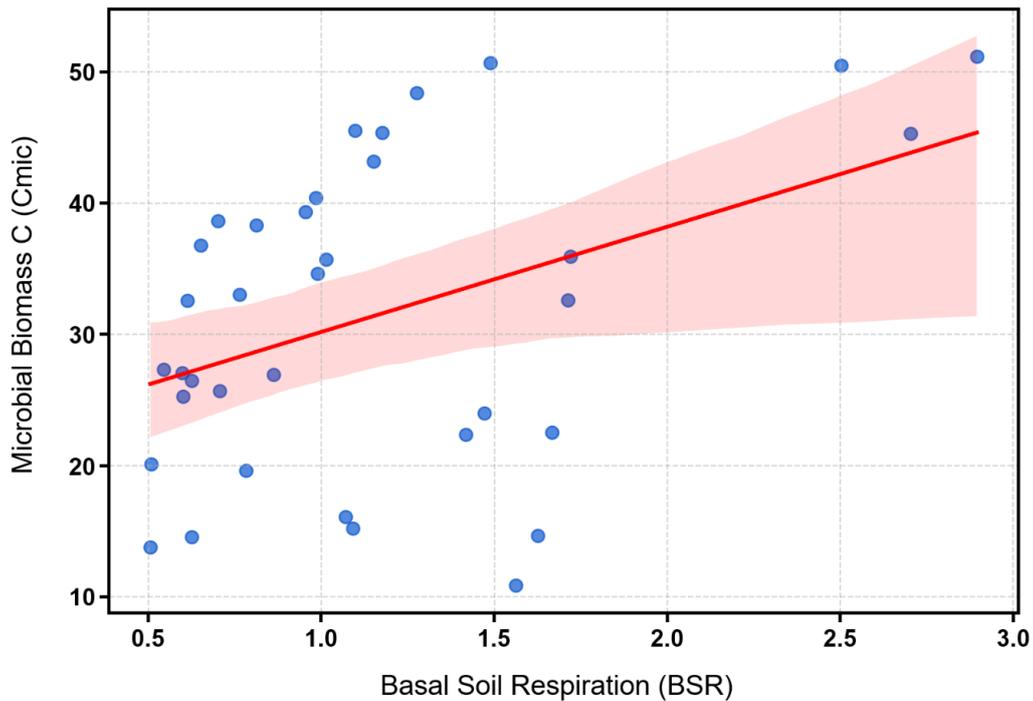


Figure 5. The relationship between basal soil respiration (BSR) and microbial biomass C (Cmic) during the vermicomposting of sewage sludge mixed with hazelnut husk and cow manure (Pearson correlation analysis, n=36).

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	F.Ş.H.T.	R.K.
C	60	40
D	50	50
S		100
DCP	100	
DAI	70	30
L	70	30
W	70	30
CR	50	50
SR	100	
PM		100

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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