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# Sustainable Management of the Organic Fraction of Municipal Solid Waste: Microbiological Quality Control During Composting and Its Application in Agriculture on a Pilot Scale

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**Abstract:** Within the Life-NADAPTA project (LIFE16 IPC/ES/000001), and in the framework of sustainable waste management, a study was carried out on the microbiological evolution during the composting process of the organic fraction of municipal solid waste (FORSU) using aerated static piles and their agricultural application on a pilot scale. This is necessary to ensure effective sanitization of the compost and that its application does not pose any risk. The microbiological parameters considered were as follows: *Salmonella* sp., *Escherichia coli*, total coliforms, and *Enterococcus* sp. The physicochemical parameters moisture, total solids, organic matter, nitrogen, phosphorus, and heavy metals were also evaluated. *Salmonella* sp. was not detected throughout the process, and the concentration of the three microbiological indicators decreased to the sanitary conditions recommended by legislation. As a result, the compost obtained complied with the requirements set out in the regulations on fertilizer products and was highly stabilized and mature for application on agricultural land. Tests were carried out on the soil before, during and after the vegetative cycle of the crop and on the irrigation water. The soil results showed that the addition of the organic amendment did not alter the populations of the tested micro-organisms at the end of the crop growing cycle. Thus, an adequate treatment of the residues allows them to be used in a sustainable way, but an adequate monitoring of the operational parameters is necessary to ensure this.

**Keywords:** organic waste valorization; waste sanitization; coliforms; *Escherichia coli*; *Enterococcus* sp.; *Salmonella* sp.; circular economy

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## 1. Introduction

The incorporation of a sustainable waste management approach plays a pivotal role in transitioning from a linear economy to a new circular model [1,2]. In this context, waste is acknowledged as a valuable resource that can be intelligently repurposed for various applications. This approach not only reduces the space required for waste storage but also mitigates soil, water, and air pollution stemming from the leaching of toxic substances or waste incineration, which releases harmful gases and particles into the atmosphere [3].

Moreover, it contributes to a decrease in greenhouse gas emissions and limits the proliferation of pests and rodents.

Furthermore, the recovery of materials from waste enables the recovery of valuable molecules, including nutrients that may be deficient in soil. This reduces the need for exploiting natural resources and the associated costs [3–5]. Consequently, the identification, sorting, and utilization of safe municipal solid waste are initiatives of broad societal interest.

Municipal solid waste comprises various components, with the organic fraction being the most predominant, constituting 44–49% of the waste by weight [6]. This fraction can be treated by aerobic or anaerobic biological processes with the aim of stabilizing organic matter, preserving essential nutrients and obtaining a sanitized product [7]. The resultant material, known as compost, serves as an agricultural amendment by enhancing soil biofertility, improving soil structure and water retention, and mitigating soil erosion [6].

According to the Ministry for Ecological Transition and the Demographic Challenge, Spain has witnessed a significant rise in composting as a percentage of total waste treatment over the past decade, accompanied by a considerable decrease in landfill usage [7]. Recent data indicates that out of the total of 22 million tons of waste generated in 2021, 22.4% underwent biological treatments leading to compost generation, marking an increase from previous years such as in 2018 when around 18 million tons of municipal waste were collected, with 17% being composted [6].

These activities in Spain have been governed by Law 22/2011 [8] since 2011, which was later replaced by Law 7/2022, enacted on 8 April 2022 [9]. Known as the law on waste and contaminated soils for a circular economy, its objectives include preventing and reducing waste production and its adverse effects on the environment and human health. Moreover, it aims to diminish the overall impact of resource consumption and enhance resource utilization efficiency, ultimately safeguarding the environment and public health. Furthermore, it aligns with the transition toward a circular economy to ensure the internal market's efficiency and Spain's sustained competitiveness in the future [9]. Consequently, Spain is in accordance with the Thematic Strategy on Sustainable Use of Natural Resources, the European Climate Change Programme, and the pursuit of the UN Sustainable Development Goals (SDGs), particularly SDG 12: responsible production and consumption.

In order to ensure the safe utilization of compost in agricultural soils from a health perspective, Royal Decree 506/2013, on fertilizer products [10], as amended by Royal Decree 999/2017 [11], provides guidelines regarding such products. Specifically, it stipulates that the maximum permissible concentrations of microorganisms (*Salmonella* and *Escherichia coli*) and heavy metals in the final material destined for use as organic fertilizer. This regulation aims to guarantee the microbiological safety of the material applied to the soil, as any material that is not at least harmless may disrupt the ecological balance of native microbial communities. Pathogen inactivation during composting refers to the elimination of microorganisms, which is expected to occur if the entire compost has temperatures above 55 °C for at least three days. However, good control of the operating parameters during composting is important for efficient sanitization. In fact, according to the existing literature, after proper removal of pathogenic bacteria there is a possibility of regrowth of some pathogenic bacteria. For this reason, continuous monitoring of the process may be necessary to ensure sanitization.

In the Autonomous Community of Navarra (Spain), where this study was conducted, the Waste Plan of Navarra 2017–2027 was devised, with objectives including the advancement towards high-quality selective collection to maximize waste recovery and recycling. This plan also aims to ensure the shared responsibility of waste generators (citizens,

businesses, etc.), while considering the principle of gender equality. This involves utilizing both traditional management channels and emerging processing channels, particularly for household bio-waste and industrial waste.

Recognizing the opportunity to contribute to the shift towards more sustainable practices, Navarra de Infraestructuras Locales S.A. (NILSA), which oversees sanitation and water treatment and manages the Waste Consortium of the Autonomous Community of Navarra, is collaborating with the Water and Environmental Health research group. This group is a recognized reference group endorsed by the Government of Aragon (Spain) and affiliated with the University Institute for Research in Environmental Sciences of Aragon at the University of Zaragoza. Together, they are spearheading a project aimed at promoting proper bio-waste management in this autonomous community, with an emphasis on encouraging recycling through composting processes, from both environmental and sanitary perspectives.

Their efforts are concentrated on executing composting processes for the treatment and recovery of organic waste, with the goal of promoting its use as agricultural fertilizer while minimizing associated environmental and health risks. This project seeks to establish action guidelines facilitating the adoption of good agricultural practices and the application of various fertilizers obtained through composting. Moreover, it aims to align the strategy for organic waste management from the public sector with the agricultural sector's needs in a sustainable way.

As part of this objective, a specific aim is focused on studying the microbiological quality (including *Salmonella* sp., *Escherichia coli*, total coliforms, and *Enterococcus* sp.) during the composting process of the organic fraction of municipal solid waste (OFMSW) using aerated static piles at a pilot scale. The regrowth and recontamination of pathogens in compost, common challenges in composting, have been widely documented. This study helps reinforce the importance of proper monitoring of the composting process, especially when conducted at pilot scale and under real-world conditions. Additionally, the study investigates microbiological quality during the application of compost in real agricultural soil throughout the vegetative cycle of a crop, specifically corn. This aims to enhance understanding of the processes involved in monitoring, controlling, and evaluating the composting process, including the tools and variables necessary to ensure its sanitization. Furthermore, it seeks to investigate the microbiological impact on the composting soil in order to check whether its application is really sustainable. Sharing experiences helps to highlight commonalities and differences between strategies employed in each case, thereby generating knowledge that can be applied in other composting processes and their field applications.

## 2. Materials and Methods

### *2.1. Composting Inputs: Municipal Solid Waste, Structural Material and Water*

The initial organic waste for composting, OFMSW, was sourced from a waste treatment plant situated in the Autonomous Community of Navarra (Spain) where a previous separation of all the OFMSW that arrived was carried out. Pruning waste served as the structural material (SM) for composting. OFMSW and SM were not shredded. Additionally, water utilized to regulate the moisture levels throughout the composting process was obtained from groundwater near the urban wastewater treatment plant in Tudela, Navarra, Spain.

### *2.2. Composting Facility*

The OFMSW composting process was conducted on a pilot scale using four aerated static piles, each with a capacity of 6–7 $m^3$ (approximate dimensions 1.5 m high × 1.6 m

wide × 2.7 m long). Each pile received air through three floor-installed pipes, each with 12 orifices through which air is distributed by a blower, while water was supplied through a drip system at various heights within the pile. The humidity level was maintained at 30–40% through controlled water supply. To minimize moisture loss, the piles were covered with tarpaulins. A PT100 sensor was used to automate the control of both pile and ambient temperatures. Aeration to cool the stacks was automated, so that when the probe detected a maximum temperature of 63–65 °C, aeration was switched on until the temperature dropped to 58–60°. Water was added by means of a drip irrigation system, placing pipes at three heights and activating it manually to adjust the humidity when it was less than 30%. Figure 1 illustrates the setup of the aerated static piles.



(**a**) (**b**) (**c**)

**Figure 1.** Aerated static piles of composting: (**a**) forced-air system: (**b**) drip watering system: (**c**) aerated compost pile.

The piles comprised a 1:1 (*v*/*v*) mixture of OFMSW and SM. Aeration and water addition were not conducted periodically; rather, the criteria for their implementation were based on the temperature and humidity of the pile. The composting process took place over four winter months, spanning from November to March.

### *2.3. Agricultural Land and Irrigation Water*

Figure 2 illustrates the agricultural land where the composted material was applied, covering an area of 2 hectares. The characterization showed that the texture was clayey, with clay content exceeding 30%. With particle sizes below 0.002 mm, it corresponds to a fine texture that retains water well and transfers heat efficiently, but offers low permeability and poor aeration, as noted by Weil and Brady [12].



**Figure 2.** Agricultural soil used for compost application.

The test plot was divided into experimental areas or fields where three different conditions are evaluated. One area served as a control where inorganic fertilizer was applied

(170 NFU). In the second area, no organic amendment was added, serving as a reference for the plot. Lastly, the third area utilized organic amendment, specifically compost obtained from OFMSW. The land was irrigated based on the crop's requirements, with water sourced from the nearest canal to the agricultural land. The origin of this water was a river in the Ebro basin. Sprinkler irrigation was employed as the method of irrigation.

### *2.4. Application of Compost on Agricultural Land*

After the thermophilic and mesophilic stages in the compost piles, undesirable materials were mechanically sieved out and stored until they were ready for use as an amendment. The screening consisted of several stages so that the final product obtained passed through a 1 cm mesh. What was not considered final product was considered inappropriate and was discarded as it was a pilot scale test and returned to the waste treatment plant. Prior to applying the compost to the soil, the agricultural land was tilled and aerated using a rototiller. The compost was then spread over 0.5 hectares at a rate of 18.9 tons per hectare, calculated based on soil and crop requirements (after analyzing the nitrogen in the soil, considering the needs of the crop for an average yield and taking into account the dose to be applied), by means of a surface application using an agricultural fertilizer spreader. After the compost was applied to the crop area, it was plowed to incorporate the materials from the surface layers into the deeper soil layers. The compost amendment was applied to the soil in April. Two days after addition and soil preparation, corn was sown using an agricultural seed drill. The corn crop was harvested the following January.

### *2.5. Sampling*

Sampling of OFMSW and SM, the initial materials for compost, and the matured material followed the Test Methods for the Examination of Composting and Compost [13]. In summary, this involved collecting various sample portions at random and combining them. The mixture was then thoroughly homogenized, and a 500 g representative sample was obtained using the quartering method for further analysis.

Sampling of agricultural soil, both before and after compost amendment, followed the standard method based on Carter [14]. The 0.5-hectare area was divided into 24 square plots of approximately 200 $m^2$ each. From each plot, a portion of soil was extracted at a depth of 15 cm. All soil samples were homogenized, and the quartering method was used to obtain a final representative sample weighing 500 g for subsequent analysis.

These methods have been widely used in similar studies, demonstrating their effectiveness in similar contexts.

Water samples were collected in accordance with ISO 5667-3:2018 standard procedures [15].

### *2.6. Analytical Methodology*

A pre-treatment of the solid samples (OFMSW, SM, compost, soil) was conducted to analyze both microbiological and physicochemical parameters, following the method described by Carter [14]. For microbiological analysis, 10 g of the solid sample were mixed with 90 mL of distilled water, ground for 5 min, and decanted to separate the liquid from the solid. For physicochemical analysis, 20 g of the solid sample were combined with 100 mL of distilled water, stirred for 2 h, centrifuged at 12,000 rpm for 20 min, and filtered using a 1.5 μm Whatman filter (Sigma-Aldrich) collected during the study were analyzed according to the standard methods described below.

2.6.1. Microbiological Parameters

The microbiological characterization of the samples involved the analysis of total coliforms, *Escherichia coli*, *Enterococcus* sp., and *Salmonella* sp. The choice was based on the fact that *Salmonella* sp. and *Escherichia coli* have established maximum concentration limits in compost according to RD 506/2013. Furthermore, total coliforms and *Enterococcus* sp. were included because of their Gram-negative and Gram-positive staining properties, respectively, and their common role as microbiological indicators of environmental contamination, given their potential fecal origin.

The culture media used and standard methods of analysis are shown in Table 1.

**Table 1.** Microbiological parameters, culture media, and standard methodology.

| Bacteria | Culture Media | Standard Methodology |
|---|---|---|
| Total coliforms | Chromogenic Coliform Agar (CCA) | ISO 9308-1 [16]<br>9215B-C-D [17] |
| *Escherichia coli* | Chromogenic Coliform Agar (CCA)<br>Glucuronic Agar tryptone and bile (TBX) | ISO 9308-1 [16]<br>9215B-C-D and 9222D [17] |
| *Enterococcus* sp. | Slanetx and Bartley Agar | ISO 7899-2 [18]<br>9215B-C-D [17] |
| *Salmonella* sp. | XLD Agar<br>Chromogenic Agar Salmonella Latex test | ISO 6579-1 [19] |

Seeding was carried out using either the surface or membrane filtration method, depending on the specific bacterium analyzed. The appropriate incubation period was applied to each sample for the respective bacterium, with specific times and temperatures tailored to optimize bacterial growth and enumeration: 24 h at 37 °C for total coliforms; 24 h at 44 °C for *Escherichia coli*; 48 h at 37 °C for *Enterococcus* sp.; 24 h at 37 °C for *Salmonella* sp. All samples were analyzed using the plate count method, and the bacterial concentration was expressed as colony-forming units (CFU) per gram of dry matter, measured as total solids. For water samples, the microbiological concentration was expressed as CFU per 100 mL. This methodology ensured consistent and standardized analysis of bacterial populations in both solid and liquid samples.

2.6.2. Physico-Chemical Parameters

The physico-chemical characterization involved analyzing several parameters including moisture, total solids, pH, organic matter, nitrogen, and phosphorus. Additionally, heavy metals were analyzed in the initial materials used for composting, specifically the organic fraction of municipal solid waste (OFMSW) and structural material (SM). To monitor the composting process, automatic temperature control was implemented once the process began, along with pH and humidity monitoring. Table 2 presents all the physicochemical parameters analyzed, the equipment utilized, and the standard methods applied in the analysis.

**Table 2.** Physico-chemical parameters, standard equipment, and methodology.

| Parameter | Equipment | Standard Methodology |
|---|---|---|
| pH | Multiparametric meter Orion Star A3295 | 4500H+-B [17] |
| Humidity (%) | Scale, cooker | ISO 11465:1993 [20] |

| Total solids (%) | Scale, cooker | ISO 11465:1993 [20] |
|---|---|---|
| Organic matter (% o.d.m. [1]) | Carbon analyzer | 5310B [17] |
| Nitrogen (% o.d.m. [1]) | System Kjeldahl | 4500-N [17] |
| Phosphorus (mg $P_2O_5$ $kg^{-1}$) | Inductively Coupled Plasma Mass Spectrometer (ICP-MS) | 4500-P [17] |
| Cadmium, cobalt, nickel, lead, zinc, mercury, chromium | Atomic Emission Spectroscope with Inductively Coupled Plasma (ICP-OES) | 3120B [17] |

[1] o.d.m.: on dry matter.

In addition, a study of the maturity and stability of the compost obtained was conducted using two techniques:

- Rottegrade Determination (UNE-EN 16087-2:2012 [21]): This method classifies compost maturity based on the highest temperature attained during a 10-day period. The test is performed under controlled conditions with a consistent ambient temperature of 20 °C and a sample humidity of 40%. The maximum temperature attained during this period provides insights into the degree of compost maturity.
- Solvita® Test: This test evaluates the stability of compost by qualitatively measuring $CO_2$ and ammoniacal nitrogen using a colorimetric technique. It assesses the decomposition rate and the release of ammonia, which are indicators of compost stability. This test provides results that help categorize compost according to its maturity, considering its resistance to decomposition and the lack of ammonia, organic acids, and phytotoxic elements.

## 3. Results and Discussion

### *3.1. Initial Properties of OFMSW, SM, and Water Used in Composting Process*

Table 3 presents the bacterial concentrations measured in the OFMSW and SM used for composting before mixing.

**Table 3.** Initial microbiological characteristics of OFMSW and SM.

| Bacteria | OFMSW (CFU $g^{-1}$) | SM (CFU $g^{-1}$) |
|---|---|---|
| Total coliforms | $9.70 \pm 5.30 \times 10^7$ | $1.24 \pm 1.23 \times 10^7$ |
| *Escherichia coli* | $7.75 \pm 5.25 \times 10^7$ | $2.04 \pm 1.10 \times 10^4$ |
| *Enterococcus* sp. | $3.70 \pm 2.60 \times 10^8$ | $3.20 \pm 0.70 \times 10^3$ |
| *Salmonella* sp. | Not detected | Not detected |

The data presented in Table 3 indicate that the organic fraction of municipal solid waste (OFMSW) exhibits higher bacterial concentrations for all the analyzed bacteria compared to the structural material (SM). Notably, OFMSW shows particularly elevated values for *Escherichia coli* and *Enterococcus* sp., which are taxonomic groups commonly used as indicators of fecal contamination. Specifically, the total coliforms are present at concentrations two logarithmic units higher than *Escherichia coli* in SM, suggesting the presence of free-living coliforms in addition to *Escherichia coli*. Furthermore, it is noteworthy that *Salmonella* sp. was not detected in either OFMSW or SM, indicating the absence of this pathogenic bacterium in the initial materials used for composting.

Regarding the remaining physico-chemical parameters analyzed in the initial materials (OFMSW, SM), they exhibit a pH close to neutrality (around 7.2–7.5), with approximately 75% organic matter content (% o.d.m.). The heavy metal content does not exceed the maximum concentrations established in RD 506/2013 at any point. The main

distinction between the two materials lies in their moisture content, with OFMSW having a higher moisture content (61%) compared to SM (32%).

Microbiological tests performed on the irrigation water, which supplied moisture during the composting process, showed significant variation in bacterial concentrations depending on the timing of the analysis throughout the process, with values $6.2 \times 10^2$–$3.1 \times 10^5$ CFU 100 mL$^{-1}$ of total coliforms; $5.0 \times 10^1$–$1.4 \times 10^5$ CFU 100 mL$^{-1}$ of *Escherichia coli*; and $5.0 \times 10^1$–$1.1 \times 10^4$ CFU 100 mL$^{-1}$ of *Enterococcus* sp. *Salmonella* sp. was not detected in these water samples either.

### 3.2. Evolution During the Composting Process

Figure 3 depicts the development of bacterial concentrations (total coliforms, *Escherichia coli*, and *Enterococcus* sp.) in the compost throughout the composting process using aerated static piles.

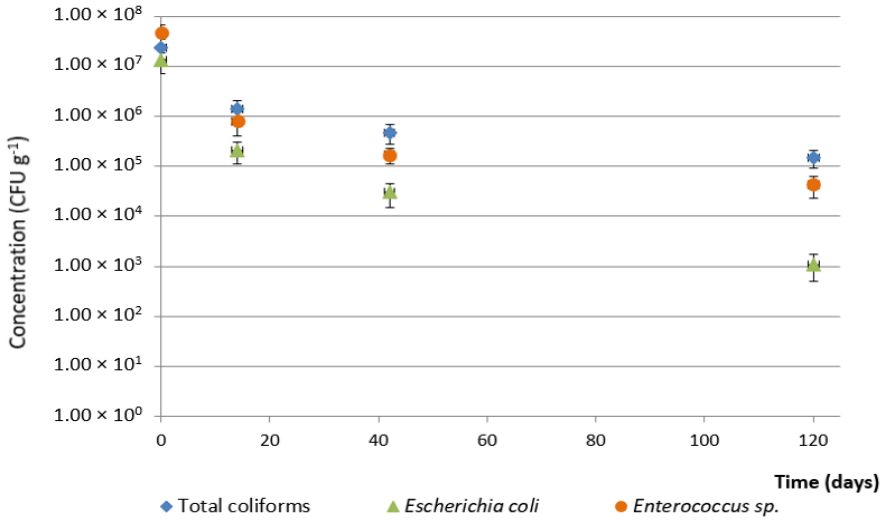



**Figure 3.** Evolution of bacterial concentrations during composting using aerated static pile.

The initial bacterial concentrations were approximately $10^7$ CFU g$^{-1}$, decrease between $10^2$ and $10^4$ CFU g$^{-1}$, with *Escherichia coli* being the bacterium that reduces its concentration the most (four logarithmic units), followed by the decrease in *Enterococcus* sp. (three logarithmic units) and finally total coliforms (two logarithmic units). The concentration of the three bacteria decreases progressively throughout the entire process, with the reduction being more pronounced during the initial days.

Figure 4 illustrates the evolution of temperature in the compost during the composting process using aerated static piles.

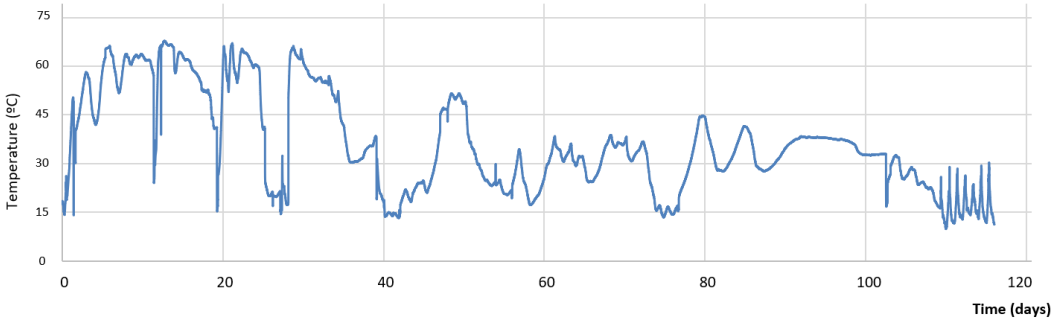



**Figure 4.** Evolution of temperature during composting using aerated static pile.

During the composting process, four cycles of thermophilic–mesophilic conditions were observed. The first two cycles occurred within the first eight weeks, with peak temperatures occurring in the second and fifth weeks, exceeding 67 °C. These were followed by periods with temperatures ranging between 30 and 55 °C. The subsequent two cycles reached maximum temperatures of 45 °C in weeks 11 and 12, respectively. Following these peaks, the temperatures decreased to ranges between 20 and 30 °C, corresponding to ambient temperatures typical of the summer season in the study area.

In aerobic composting, the inactivation of pathogens primarily stems from the elevated temperatures generated by microbial processes. However, as highlighted by Gurtler et al. [22], several conditions merit consideration to prevent the attainment of lethal temperatures during aerobic composting. These factors encompass the surface-to-volume ratio of the compost pile, the influence of ambient temperatures, the C/N ratio, and the presence of temperature gradients within the compost pile. It is important to closely monitor the process to ensure the sanitization of the composted material as well as the potential impact of application on agricultural land to ensure a sustainable application of these wastes.

It is imperative to acknowledge that the inactivation of pathogens during composting hinges primarily on two factors: the inherent heat resistance of microorganisms themselves and the operational and environmental conditions throughout the process. In terms of how heat impacts microorganisms, considerable strides have been made in discerning the mechanisms underlying cell inactivation. It is widely recognized that heat exerts a versatile influence on bacteria, causing damage to various components of the cell structure, including membranes, DNA, RNA, ribosomes, and specific enzymes [23]. However, the specific damage responsible for triggering inactivation remains undetermined, so it is linked with the potential involvement of an oxidative component [24]. Oxidative stress is defined by an imbalance between substances that encourage oxidation and those that serve as antioxidants, with a tendency toward the former [25]. Heightened levels of oxidative species within the cytoplasm of microorganisms, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), have the potential to do damage on various components of the cell, including proteins, membranes, and genetic material [26]. Bacteria have a certain ability to neutralize these reactive species and restore damaged structures; nevertheless, if this capability becomes overwhelmed, cell viability could be compromised. Nonetheless, the mechanisms of thermotolerance or resistance to temperature fluctuations vary among species. Some degree of thermotolerance above 50 °C has been documented for *Enterococcus* sp., whereas for *Escherichia coli*, resistance to such temperature thresholds appears to be less pronounced [27–29]. This is likely due to the fact that one of the components affected by heat is the outer membrane in Gram-negative cells. The degradation of this component makes the cells more susceptible to external agents [23,27,28]. This vulnerability of *Escherichia coli* aligns with the findings we present, as it is the bacterium that exhibits the most significant impact during the thermophilic stages observed throughout the composting process. The higher heat resistance of *Enterococcus* sp. is primarily attributed to its enhanced capacity to adapt to oxidative stress through the production of protective species, in contrast to other bacterial types [27,29].

When evaluating the operational conditions, alongside other factors, internal temperature gradients within the piles, pH levels, and the C/N ratio play pivotal roles in determining the efficacy of sanitizing the composted material [30–32]. Concerning temperature variations within the pile, the heterogeneity of the compost can lead to localized inactivation of bacteria, which may subsequently result in bacterial repopulation if adequate organic material for survival is present [33]. Regions with temperatures close to the optimal growth values of the tested microorganisms will not undergo inactivation; zones with temperatures above or below this optimal range will induce dormancy. Only those areas

experiencing temperatures exceeding this optimal range by more than 10 °C and sustained for a prolonged duration will effectively sanitize the composted material [31,32]. A robust temperature control system and regular turning of compost piles are crucial, as an unevenly distributed pile may allow pathogens to survive on its surface [34,35], given that environmental conditions can impact both inactivation and regrowth processes [36]. In this investigation, temperature was monitored at least at two points within the compost pile—an internal static point and periodic surface monitoring. These temperature measurements played a pivotal role in determining the frequency and duration of the aeration and irrigation cycles necessary to regulate the thermophilic–mesophilic transitions. Aeration, turning (if dynamic) and watering are regulated in frequency considering the temperature. Extreme values required more frequent aeration and turning (daily) because, as high temperatures, higher than 45 °C at the beginning of the process, decrease the humidity, the digestion of the compost is slowed down because it reduces the viability of microorganisms (mesophiles) and ammonia volatilization is favored, which reduces the nitrogen content in the final compost and generates bad smells. Likewise, in order to avoid low temperature values in the thermophilic stage, aeration and turning was restricted, as avoiding the thermophilic stage consequently hinders sanitization, causing incomplete degradation of complex organic matter such as cellulose or proteins, thus requiring longer maturation times and resulting in a lower quality compost. The observed rate of *E. coli* and coliform inactivation in the aerated piles under study aligns with findings from prior research [37,38].

Conversely, studies have indicated that pathogen inactivation is notably enhanced when employing initial C/N ratios falling within the range of 15 to 20 [33]. This effectiveness is attributed to the impact of these ratios on ammonia volatilization, as the conversion of proteins to ammonium during composting induces elevations in temperature, pH, and ammonia levels, conditions that are unfavorable for pathogens [39]. Indeed, studies have indicated that Gram-negative bacteria, such as *E. coli* $O_{157}$:$H_7$ and *Salmonella enterica*, are effectively eradicated when C/N ratios approach 20. Conversely, C/N ratios exceeding 40 are associated with increased likelihoods of bacterial survival [38,40,41]. In this study, both initial (compost pile) and final (in the composted material) values of organic carbon and nitrogen were assessed, with the ratio between these values targeted around a 40 C/N ratio. Hence, for further experiments, it would be beneficial to fine-tune the C/N ratio even more precisely. Adjusting the C/N ratio can have significant implications on the efficiency and stability of the process although it is complicated due to the heterogeneity of the materials used. A balanced C/N ratio, ideally between 25:1 and 30:1, favors optimal microbial activity, accelerating the decomposition of organic matter, improving the thermophilic phase and, consequently, the sanitization of the compost. In addition, a fine adjustment of the C/N ratio reduces ammonia emission, avoiding nitrogen losses and bad odors, and improves the final quality of the compost, making it more stable, mature and with better agronomic properties. It also facilitates greater control of the process, reducing composting times and increasing its predictability. To achieve this, the quantities of both compounds in the unmixed starting materials could be meticulously measured. Subsequently, these measurements could inform the creation of compost piles with precisely targeted ratios of starting materials. By doing so, researchers could assess whether such adjustments lead to enhanced sanitation of the compost pile under the revised operational conditions. It is worth noting that altering the ratio of starting materials not only impacts the C/N ratio but also influences all other control parameters within the pile.

Figure 5 illustrates the evolution of pH and humidity in the compost during the composting process using aerated static piles.

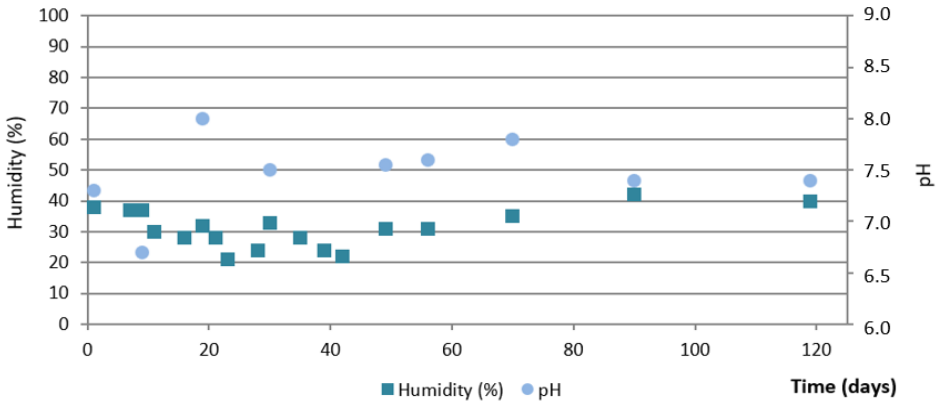



**Figure 5.** Evolution of pH and humidity during composting using aerated static pile.

During pH monitoring of the composting pile, values ranging from 6.5 to 8.0 were consistently observed from the initiation to the culmination of the process. This suggests the conversion of proteins to ammonia, which promotes the sanitization process of the pile, with the final compost pH recorded at 7.4. Regarding humidity levels throughout the process, they fluctuated between 20% and 45%, meticulously adjusted to facilitate microbiological activity essential for the decomposition of organic matter. However, it was also crucial to avoid prematurely sanitizing the compost pile. These humidity levels were maintained through four water irrigations.

Finally, concerning the degree of stability and maturity of the compost obtained at the final of the process, both the Rottegrade grade and the Solvita® test affirm that the compost is highly stabilized and adequately matured (grade V as per Rottegrade and a maturity index of 6 according to Solvita®). This classification indicates that the compost is ready for application in agricultural soil.

### *3.3. Evolution on Agricultural Land*

The changes in bacterial concentrations (total coliforms, *Escherichia coli*, and *Enterococcus* sp.) in the agricultural soil treated with compost during the crop's vegetative cycle, compared to untreated soil, are illustrated in Figure 6. Regarding the microbiological analysis of the irrigation water used in the agricultural soil, concentrations of total coliforms, *Escherichia coli*, and *Enterococcus* sp. were observed to be less than $1.0 \times 10^3$ CFU 100 $mL^{-1}$. *Salmonella* sp. was not detected in either the soil samples or the irrigation water.

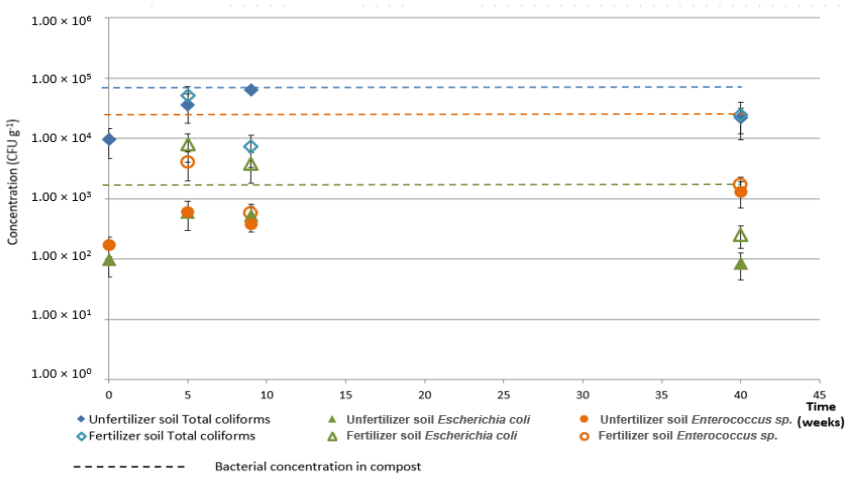



**Figure 6.** Evolution of bacterial concentrations in agricultural soil with and without compost amendment during the vegetative cycle of the crop.

As depicted, the concentrations of the three analyzed bacteria in the compost used as an amendment exceed those initially found in the agricultural soil. Initial values in the compost reveal bacterial concentrations ranging between $10^3$ and $10^5$ CFU $g^{-1}$ whereas in the agricultural soil, concentrations ranging between $10^2$ and $10^4$ CFU $g^{-1}$ are observed, with total coliforms predominating in both instances.

Throughout the vegetative cycle of the crop, minor fluctuations are noticed in the concentrations of the three bacteria, with none exceeding two logarithmic units. These variations could be attributed to the limited bacterial input from the compost amendment and irrigation water, as well as the prevailing soil and climatic conditions in the area [42].

At the end of the process, the observed bacterial concentrations closely resemble those initially present in the agricultural soil before compost amendment. Therefore, it can be stated that the application of compost does not lead to a decrease in the microbiological quality of the soil in this case. This is corroborated by the absence of notable disparities in the bacterial concentrations identified in the agricultural soil, either with or without the amendment, throughout the duration of the study. Before the application of compost, the agricultural soil exhibited low levels of organic matter (1.9% o.d.m.) and nitrogen (0.1% o.d.m.). However, following the addition of compost and at the time of crop harvest, these values increased significantly, reaching 33% o.d.m. for organic matter and 0.7% o.d.m. for nitrogen. These data reflect an improvement in soil quality, directly attributable to the application of compost. Organic matter acts as a key component in improving soil structure, increasing porosity, water holding capacity, and aeration, which promotes root development and beneficial microbial activity. In addition, increased nitrogen content indicates increased availability of essential plant nutrients, which can lead to more vigorous plant growth and higher crop yields. Compost application also contributes to soil pH stabilization, reduced erosion and improved cation exchange capacity, which improves the retention of other nutrients such as phosphorus, potassium, calcium, and magnesium.

More broadly, the use of compost promotes the sustainability of the agricultural system by reducing the reliance on chemical fertilizers, closing the nutrient cycle and valorizing organic residues. Taken together, these benefits reinforce the role of compost not only as an organic amendment but as an integral tool for improving soil fertility and long-term soil health.

## 4. Conclusions

Bacterial concentrations indicating microbiological contamination in the final compost were found to be lower than in the initial mixture of components used for its production, thus meeting the standards outlined in RD 506/2013 for fertilizer products. Additionally, throughout the composting process, it is evident that the aerated static pile method effectively monitored physicochemical parameters, producing compost that is stable and mature enough for agricultural use.

Initially, the compost obtained exhibits higher bacterial concentrations compared to the agricultural soil used for amendment. Following compost application, minor fluctuations in the concentration of analyzed bacteria are observed; nonetheless, there are no significant disparities in their progression between the soil amended with compost and the unamended soil. At the end of the vegetative cycle of the crop, bacterial concentrations similar to the initial levels are observed, affirming that the application of OFMSW-derived compost does not lead to a decrease in the microbiological quality of the soil in this study.

In conclusion, the work carried out at a pilot scale demonstrates that proper control of the process's operational parameters, along with effective monitoring of hygienization, makes it possible to obtain microbiologically safe compost. These results support the feasibility of using compost derived from organic waste as an agricultural amendment,

provided that good management practices and consistent monitoring are applied throughout the composting process.

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