



Enhancement of Microbial Activity during the Composting Process of Agricultural and Farm Waste Using Zeolite Solid and Suspended Nanoparticles

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Abstract

The use of nanomaterials in agricultural waste composting has emerged as a promising strategy to enhance the process and the agronomic quality of the product. Among the different materials, zeolite is characterised by its potential to reduce nutrient loss and favour microbial activity and organic matter degradation. This study compares the effect of the addition of solid and suspended zeolite nanoparticles on the composting process of agricultural waste and product quality. The study was conducted in reactors with 40 kg of substrate mixture under laboratory conditions. Zeolite in both application forms led to better process conditions, such as higher temperature peaks (65.3 °C and 63.8 °C for TZS and TZL, respectively; 60.5 °C for the control), higher abundance of bacteria in the thermophilic phase (9.97×10^{11} copies/g DM y 2.87×10^{11} copies/g DM for TZS and TZL; 2.42×10^{11} copies/g DM for the control), increased degradation of organic matter (a reduction in volatile solids of 28% and 23% for TZS and TZL, respectively; and 21% for the control) and a relatively higher nitrogen content in the product (2.4% and 2.1% for TZS and TZL, respectively; 2.2% for the control). In addition, zeolite treatments showed the highest abundance of the *amoA* gene during the process, suggesting that this amendment promotes nitrification in composting. This study demonstrates that the application of zeolite nanoparticles favours microbial activity. Its application in solid form shows the best results in the process conditions and the nitrogen content in the product.

Statement of Novelty

This manuscript presents a study of the addition of large zeolite nanoparticles in composting agricultural residues in two forms of application: in solid state and in aqueous solution. Although other studies have evaluated the application of zeolite in composting, to the authors' knowledge, the effect of zeolite nanoparticles in solid state and suspension on both process variables and product quality has not been compared. In addition, the analysis of three functional genes of the nitrogen cycle (*nifH*, *amoA* for bacteria and archaea and *nirS*) and the abundance of bacteria and archaea during the four phases of composting are included. Therefore, this information is novel for the scientific community interested in this topic and allows consolidating strategies to optimize the use of zeolite in composting.

Keywords Biological activity · Composting · Nitrification · Nitrogen cycle genes · Organic matter degradation · Zeolite

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Introduction

Population growth has brought with it the need to increase food production. By 2022, the production of primary crops reached 9.6 billion tonnes, an increase of 56% compared to 2000. In addition, 360 million tonnes of meat were produced, with chicken being the main meat produced [1]. The inadequate management of agricultural waste has contributed to the contamination of soil and water sources and the generation of greenhouse gases [2–4]. Therefore, it is necessary to implement agricultural waste management strategies that consider environmental, social and economic aspects [5]. Composting has emerged as a suitable method to treat organic solid waste and reincorporate organic matter and nutrients in the circular economy framework to support fertilisation and reduce synthetic fertilizer use [6].

Various materials have been used to improve composting of agricultural waste; however, the use of nanomaterials in composting, although still very emerging, has gained interest for their enhancement of microbial activity [7] by promoting the selection of beneficial microorganisms, suppressing pathogens and improving product quality [8]. It is commonly accepted that nanomaterials have particle sizes between 1 and 100 nm; however, ISO 80004-1:2023 suggests that, in some particular cases, such as zeolite, materials with particle sizes larger than 100 nm could be considered large nanomaterials if they retain nanometric properties such as a high surface-to-volume ratio. In addition, different organisations around the world also accept upper limits above 300 (Chathan House and Friends of Earth), 500 (Swiss public health office) and 1000 nm (House of Lords Science Committee) to refer to a nanoscale material [9]. Unlike smaller nanoparticles (< 10 nm), which can penetrate cells and generate cytotoxic effects on microorganisms, large nanoparticles (100–500 nm) offer a safer and more predictable interaction with tissues [10], without affecting microorganisms that could limit their development and impact biological processes such as composting.

Tian et al. [3] reported that the use of iron-based nanomaterials reduces carbon dioxide, methane, and nitrous oxide emissions by 20.5%, 39.7% and 55.4%, respectively in chicken manure composting. Also, Liu et al. [11] used iron-based nanoscale particle sizes and found that they enhance humification and promote microplastic degradation during sewage sludge composting. Biochar nanoparticles were also shown to improve the maturity of municipal waste compost and reduce phytotoxicity, with total nitrogen content increased by 63.4% compared to using biochar in a traditional presentation [7].

Zeolite (in a wide range of configurations) is among the materials that can be used in composting. Unlike other nanomaterials, such as iron nanomaterials, zeolite has been used

in agricultural activities because of its lower potential for soil and compost contamination. It is a mineral composed of aluminosilicates, whose structure is based on three-dimensional tetrahedron of AlO_4 and SiO_4 , which are infinitely linked to form highly crystalline and porous networks. Thanks to these structures, zeolite allows the retention of ions such as ammonium, helping to delay the nitrification process [12, 13]. Zeolite is useful in the composting process as it modifies the physicochemical properties of the product [14], as it has the ability to increase water retention and aeration due to its structure [15]. In general, zeolite helps to minimize nitrogen loss [16], immobilises heavy metals [17], reduces antibiotic resistance genes [18, 19] and promotes organic matter degradation during processing [4, 14, 20].

Generally, zeolite application is done in solid form, with particle sizes above 0.1 mm and dosages ranging from 5 to 30% (w/w ratio) [14]. The addition of 5% (w/w) natural zeolite in chicken manure composting can reduce methane emission by 56% [20], which could be related to the ability of zeolite to improve the porosity of the compost mixture and to retain nutrients [16]. In other studies, the application of 10% (w/w) zeolite enhanced nitrogen retention by reducing ammonia emissions by 28% and nitrous oxide emissions by 55% in chicken manure composting. Zeolite also decreased the abundance of denitrification genes associated with nitrogen losses [4].

To the authors' knowledge, the study of large zeolite nanoparticles and its effect on composting has been scarcely treated in literature, both in solid particles and as particles in aqueous suspension. Fine zeolite particle sizes (< 500 μm) show better ammonium adsorption than larger sizes [21], so it is possible to use zeolite doses lower than 5% (w/w) in composting experiments and obtain favourable results when the zeolite is in powder form [22]. For example, in sewage sludge composting, a 1% dose of zeolite powder (particle size 200 μm) increased nitrogen retention by 48% compared to treatments without zeolite addition. Also, antibiotic resistance genes were reduced by implementing 1% (w/w) zeolite in sludge composting [23].

On the other hand, the use of zeolite in suspended particles is being positioned in agricultural applications, due to its nutrient and water retention capacity [24]. For example, a 16 g/L dose of zeolite was used to evaluate its ability to retain ammonium and prevent its release in a wheat crop and it was found that not all of the nitrogen retained by zeolite is available to the plant in the first crop cycle [24]. In the case of the study by Mahmoud et al. [25], zeolite nanoparticles were added through the irrigation systems of a potato crop to measure their effects on soil salinity and observed that zeolite significantly increased plant growth compared to the control treatment.

In parallel to these findings, microorganisms involved in the nitrogen cycle can influence compost quality and participate in greenhouse gas emissions during composting [26]. Current research focuses on the microbial community and its effect on process variables and product quality [27]; however, the study of the functional genes of the nitrogen cycle has remained in the background with a focus on gene abundance, without investigating the effect of additives such as zeolite, especially in substrates with high total nitrogen content such as chicken manure and, in consequence, susceptible to high ammonia emissions.

This study's initial hypothesis is that applying zeolite in the form of suspended particles in the composting process could reduce the required amount of material and, consequently, the acquisition costs. Since no studies comparing the use of zeolite nanoparticles in their two forms (solid and aqueous suspension) have been identified, this research evaluates the effects of both alternatives on process variables, product quality and the abundance of three functional genes of the nitrogen cycle (*nifH*, *amoA* and *nirS*) in the co-composting of agricultural waste (chicken manure and green onion residues), using phosphate rock and sawdust as amendment materials and bulking agent, respectively.

Materials and Methods

Materials Used for Composting

Chicken manure (CM) and green onion residues (*Allium fistulosum*) (OR) were used for the study. Both materials are characteristic of agricultural activities in the study area. The CM was obtained from a farm in Girón (Colombia), while OR residues were collected from the Centro de Abastos de Bucaramanga (Colombia). Additionally, sawdust (Sd) was used as a bulking agent and phosphate rock (PR) as an

amendment material. The PR used was commercial, with the following composition: 38% CaO, 28% P₂O₅, 1% TOC, 0.5% Al₂O₃, 0.4% Fe₂O, and 0.1% K₂O. Table 1 presents the physicochemical characteristics of the materials used.

Zeolite nanoparticles were obtained from a commercial mixture (Antracitas de Cundinamarca, Colombia) extracted from mines in the region, using a RETSCH PM1000 planetary mill in a 500 mL volume oxide vial until a particle size of 300 nm (large nanoparticles) was reached. Grinding was dry with a filler volume fraction of 40% and a ball distribution 40/30/20 with diameters between 1, 3 and 5 mm respectively. The grinding time was 3 h, the rotational speed was 300 rpm and a ball to powder ratio (BPR) of 10:1 was maintained for all samples. The particles were characterised by X-ray diffraction (XRD), scanning electron microscopy (SEM) and dynamic light scattering (DLS) to identify the crystalline phases and changes in size and morphology of the particles to be used in the experiment. The XRD (Bruker D8 Advance diffractometer in Bragg-Brentano $\theta/2\theta$ mode in a range of 5°–70°, with a speed of 2°/min), SEM (Anton Paar/LiteSizer500) and DLS (Jeol JSM 6490 LV electron microscope in SE mode) results for the obtained zeolite are presented in Supplementary material (Figure S1). After the extraction process, the zeolite particles were kept in an oven at 105 °C to avoid wetting.

Regarding the results of the zeolite milling process, it can be concluded that it did not significantly alter the crystallinity or the phases present in the commercial starting material, which is mainly composed of Mordenite (63%), Quartz (34%) and to a lesser extent Muscovite and Calcite (3%), as well as distribution size (Supplementary material, Figures S2 and S3).

Composting Experiments

The experiment was carried out at the Universidad Industrial de Santander (UIS), located in Bucaramanga (Colombia) (average temperature 22.6 °C). The experimental units corresponded to laboratory-scale reactors with a capacity of 250 L with co-composting mixtures of 40 kg. The reactors are made of high-density polyethylene and have a passive oxygen supply, through perimeter holes of 2 cm in diameter and located equidistantly at 15 cm and a perforated tube located in the centre of each reactor [28]; in addition, the vessels were covered with a Thermolon thermal insulation material (thickness 12 mm) to avoid heat losses.

Three treatments were evaluated using the same substrate mixture: chicken manure 62%, green onion waste 23%, sawdust 14% and phosphate rock 1% (wet weight), and adding zeolite with and without suspension, and a control experiment: TC: no zeolite, TZS: 2% zeolite in solid form (w/w over total mass) and TZL: 2% zeolite in aqueous form

Table 1 Characterization of materials used for composting

Parameter	CM	OR	Sd
pH	9.0±0.03	5.1±0.04	4.7±0.03
EC (μS/cm)	4230.0±116.8	180.6±4.4	152.5±11.6
Moisture (%)	22.0±2.1	90.0±4.5	9.0±1.5
VS (%)	74.3±3.9	81.3±1.9	9.8±1.4
TOC (%)	30.5	33.6	48.7
N total (%)	3.7	1.7	0.2
C/N (%)	8.2	19.8	243.5
P total (%)	3.3	0.4	ND
K total (%)	5.1	2.1	0.1
Hemicellulose (%)	18.1	6.4	12.9
Cellulose (%)	2.2	0.7	7
Lignin (%)	24.1	34.9	69.2

CM: chicken manure, OR: green onion residues, sd: sawdust; EC: electrical conductivity; VS: volatile solids and TOC: total organic carbon. All the parameters are presented in dry matter basis

(w/v). The mixtures were defined considering an initial C/N ratio between 25:1 and 30:1 [29].

For TZS, the application was carried out by vigorously mixing the solid particles to the substrate mixture at the beginning of the process. In the case of TZL, a solution of distilled water and 2% zeolite (20 g/L) was prepared, stirred and added by irrigation of 2 L of solution to the substrate mixture at the beginning of the process. After addition zeolite in solid and suspended form, the entire solid mixture was turned to ensure a good zeolite distribution.

Monitoring of Composting Parameters and Sampling

The process was periodically monitored and experimental data were taken on temperature, pH, electrical conductivity (EC), moisture, volatile solids (VS), germination index (GI), total nitrogen (TN), total organic carbon (TOC), total phosphorus (TP), hemicellulose, cellulose and lignin. Temperature was measured twice a day for the first two weeks and then measured daily at five points in each reactor with a K-type thermocouple thermometer (HANNA Instruments) with a 70 cm long metal probe. Ambient temperature was taken from the records of the Institute of Hydrology, Meteorology and Environmental Studies (IDEAM) [30].

pH and EC were measured daily for the first two weeks and then three times a week, while moisture and VS were measured every three days. GI was measured four times: at the beginning of the process, in the thermophilic phase, in the cooling phase and the maturation phase. The other parameters (TN, TOC, TP, hemicellulose, cellulose and lignin) were measured three times during the process: at the beginning, in the thermophilic phase and in the cooling phase.

To determine these parameters, samples were taken from four equidistant points of each experimental unit with approximately 300 g of material and mixed to obtain a representative sample from each reactor.

Analytical Procedures

The analyzed parameters were selected according to NTC 5167 norm [31]: moisture (gravimetry after drying the sample at 105 °C), VS (sample calcination method at 505 °C), pH and EC (potentiometric method after water extraction, ratio 1:10, mass/volume), TOC (Walkley-Black method, which estimates TOC through chemical oxidation with potassium dichromate in an acidic medium), TN (Kjeldahl total nitrogen, which measures total nitrogen by converting it to ammonium through acid digestion, then distilling it and titrating it), and TP (spectrophotometric method, which measures the concentration of a substance in a sample based

on light absorption at a specific wavelength). Lignocellulose was determined by the colorimetric method, and cellulose and hemicellulose through the Van Soest method [32]. The organic matter loss in terms of VS was calculated, according to Eq. 1 [33]:

$$VS (\%) = 100 - 100 \cdot \frac{X_1(100 - X_2)}{X_2(100 - X_1)} \quad (1)$$

where X_1 and X_2 correspond to the initial and final ash content, being SV the volatile solids content.

GI was determined using *Raphanus sativus* seeds. 1 g of sample was taken and diluted in 10 mL of distilled water in a 1:10 (m/v) ratio for each treatment. Ten seeds were used for each sowing and the process was carried out in triplicate [34]. 10 mL of distilled water was used for the control. GI was calculated based on the methodology described by Issarakraisila et al. [35] (Eq. 2). GI values < 50% indicate the presence of phytotoxic substances, GI between 50% and 80% shows moderate phytotoxicity, GI between 80% and 120% shows no phytotoxicity, and GI > 120% shows biostimulant effects.

$$GI = \frac{\% \text{Germinated seeds (treatment)}}{\% \text{Germinated seeds (control)}} \cdot \frac{\text{Root length (treatment)}}{\text{Root length (control)}} \cdot 100 \quad (2)$$

Once the reactors reached room temperature after the composting process, self-heating tests were carried out using the RM 82 equipment (Umwelt Elektronik, Germany) to determine the degree of stabilisation of the organic matter.

Molecular Biology Analysis

Samples were taken from the first three stages of the process (i.e., day 0, day 14, day 40) and the final unscreened product for total genomic DNA extraction and quantification by quantitative PCR - qPCR (SYBR-green) method (ExcelTaq™ 2X Q-PCR Master Mix extraction kit (SYBR, not ROX), 500 RXN). Bacterial (rpoB) and archaeal (16 S rRNA) abundance and functional genes of the nitrogen cycle were monitored during fixation (*nifH*); nitrification, (*amoA*) for both bacteria and archaea; and denitrification (*nirS*).

Compost Quality

Once the process was finished, the product was sieved with a No. 35 sieve according to NTC 5167 [31]. Subsequently, samples were taken to measure the physicochemical and microbiological parameters: moisture, ash, cation exchange capacity (CEC), TOC, water retention capacity (WRC),

Fig. 1 Evolution of the temperature in the composting process of several mixtures: TC: control, TZS: solid zeolite, TZL: suspended zeolite

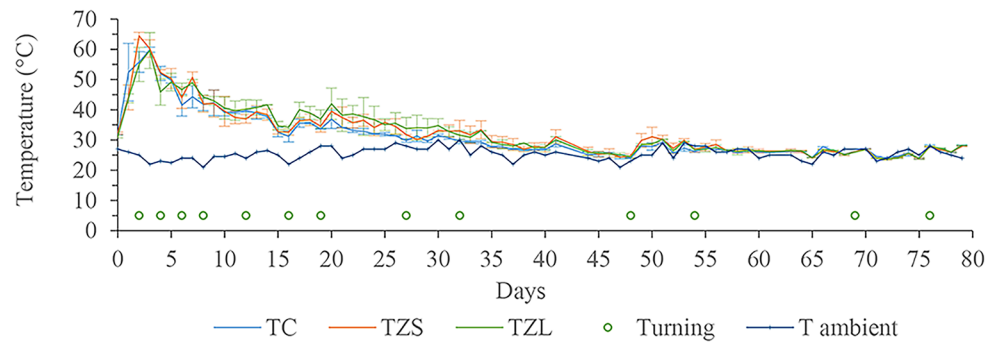


Table 2 Temperature main facts

Treatment	Temp. max. (°C)	Thermophilic stage (starting day)	Thermophilic stage duration (days)	Cooling stage duration (days)	Time to reach ambient temperature ± 2 °C (days)
TC	60.5	1	8	19	27
TZS	65.3	1	7	31	38
TZL	63.8	1	8	24	32

bulk density, pH, EC, TN, TP, TK, hemicellulose, cellulose, lignin, GI, total and faecal coliforms, enterobacteria and *Salmonella* sp. The techniques used followed the standard NTC 5167 [31].

Statistical Analysis

One-way analysis of variance (ANOVA) with a significance level $\alpha=0.05$ was implemented to determine the effect of the studied factors on the response parameters. Differences between treatments were calculated using Tukey's test. The statistical analysis was carried out in the R software through its RStudio interface (version 4.4.0).

Results and Discussion

Characteristics of Composting Substrates

In general, it can be seen from Table 1 that the physico-chemical characteristics of the substrates allow for a mixture suitable for the composting process. CM has an alkaline pH (> 8) and a high EC (> 2 mS/cm), unlike OR and Sd, which have an acid pH (< 6) and a lower EC. The high total nitrogen content of CM ($> 3\%$) is in line with that reported in other studies (3–5%) [36–38]. The high TOC of Sd contributes to balance the C/N ratio of the substrate mixture defined for composting. The phosphorus content of CM is higher than 3%, which coincides with that reported by Wei et al. [38], and contrasts with the 1% obtained by Oviedo-Ocaña et al. [37] and Wang et al. [4]. This high phosphorus content could be due to the diet of chickens and their rapid metabolism that causes the phosphorus from the supplements to go

into the excreta instead of being utilised by the organism. In the case of potassium, 5.1% was found, which is higher than that reported in other studies (1%, 3.2%) [4, 38], which may also be related to the different diets fed to the chickens [39].

Performance of the Composting Process

Temperature

Figure 1 shows the temperature evolution of the composting process. The process had a duration of 79 days, longer than that reported by Latifah et al. [17] in composting CM with 5% (w/w) zeolite (54 days) and by Geng et al. [27] in composting food waste amended with 5% (w/w) zeolite (60 days).

Although there were no differences between treatments in reaching the thermophilic phase (i.e., from day 1 of the process), there were higher peak temperatures in the zeolite treatments (Table 2). This could be related to higher biological activity associated with improved mixing conditions, such as increased porosity and moisture retention. Other studies using zeolite in CM composting [4] or swine manure composting [22] found similar results with doses of 1 and 2% (w/w) zeolite.

Likewise, there were no significant effects on the duration of the thermophilic stage (7 days for TZS and 8 days for TC and TZL, respectively), but a more intense biological activity reflected higher temperature values. In all cases, temperatures allowed the elimination of pathogenic microorganisms in all three treatments (i.e., total coliforms < 3 NMP/g) because they exceeded 60 °C [40] and were above 50 °C for more than three days [41].

In Table 2, it can also be observed that there were differences in the duration of the cooling phase, which was longer in the zeolite treatments. This may be associated with better process conditions for the presence of microorganisms with a specific role in the degradation of these organic substances, which was manifested in a lower cellulose and lignin content at the end of the process in both zeolite treatments, as explained later (e.g. lignin content of 43.3% in TZS, 47.2% in TZL and 48.4% in TC, respectively).

In summary, although there were no significant differences among the temperature of the three treatments ($p > 0.05$), it is observed that at the beginning of the process, TZS was the one that increased its temperature more rapidly, with higher peak values, probably because the application of solid zeolite favoured the microbial activity, the degradation of organic matter and consequently, released more energy in the form of heat [42], which is consistent with the higher increase of bacteria from the mesophilic to the thermophilic phase in this treatment, as explained in the next point.

Relative Abundance of Prokaryotes (Archaea and Bacteria)

Regarding the relative abundance of bacteria and archaea, it was observed that the abundance of bacteria was higher than that of archaea during the first three phases of the composting process (Fig. 2), which could be related to the oligotrophic characteristics of archaea and the longer generation periods of bacteria [43]. The abundance of bacteria showed a decreasing trend for the three treatments (Fig. 2a), except the first phase in TZS, which showed an increase in growth and biological activity, which may be related to the better conditions for bacterial growth with the addition of solid zeolite. From the thermophilic phase to the cooling phase, the archaeal content was reduced in the zeolite treatments. At the same time, it increased in the control, probably because

during the cooling phase, there were still easily degradable compounds that were not degraded in the previous phases.

pH

Despite the acidic characteristics of the green onion and sawdust residues (Table 1), at the beginning of the process all three treatments had alkaline pH values (> 8) that remained throughout the entire period (Fig. 3a). This is probably due to the rapid biodegradation of the most labile organic matter in the substrate mixture which are released in the form of volatile ammonia, carbon dioxide and mineralised organic nitrogen [44]. At the end of the process, a slight decrease in pH value is observed, except for TZL, which could be related to the affinity of zeolite for ammonium ion [22], indicating that suspended zeolite may have a higher retention of ammonium ion than solid zeolite.

Electrical Conductivity

Figure 3b shows the influence of zeolite addition on the EC in the co-substrate mixture. The zeolite treatments started with EC higher than 5 mS/cm while the control started with a value less than 3 mS/cm. Similar results were reported by Cui et al. [19], using zeolite powder in organic waste composting. The high EC in the three treatments is related to the nature of the CM [45], which presents values between 4.7 and 9.6 mS/cm [46–48], similar to those found in this study (Table 1).

In the cooling stage, the EC of the three treatments gradually increased, possibly due to the release of soluble ions after organic matter degradation [49, 50]. This behaviour could also be related to a higher concentration of mineral cations that did not leach or bind to more stable organic compounds as organic matter degraded and the mass of the mixture was reduced [51].

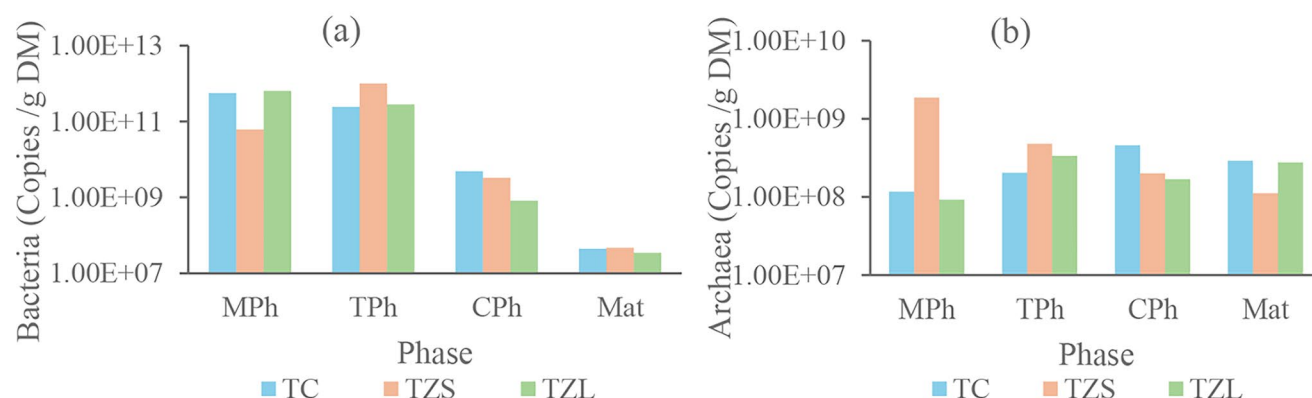


Fig. 2 Relative abundance prokaryotes for the composting treatments (TC: control, TZS: solid zeolite, TZL: suspended zeolite): **a)** bacteria, **b)** archaea, during the four phases of composting: mesophilic (MPh), thermophilic (TPh), cooling (CPh) and maturation (Mat)

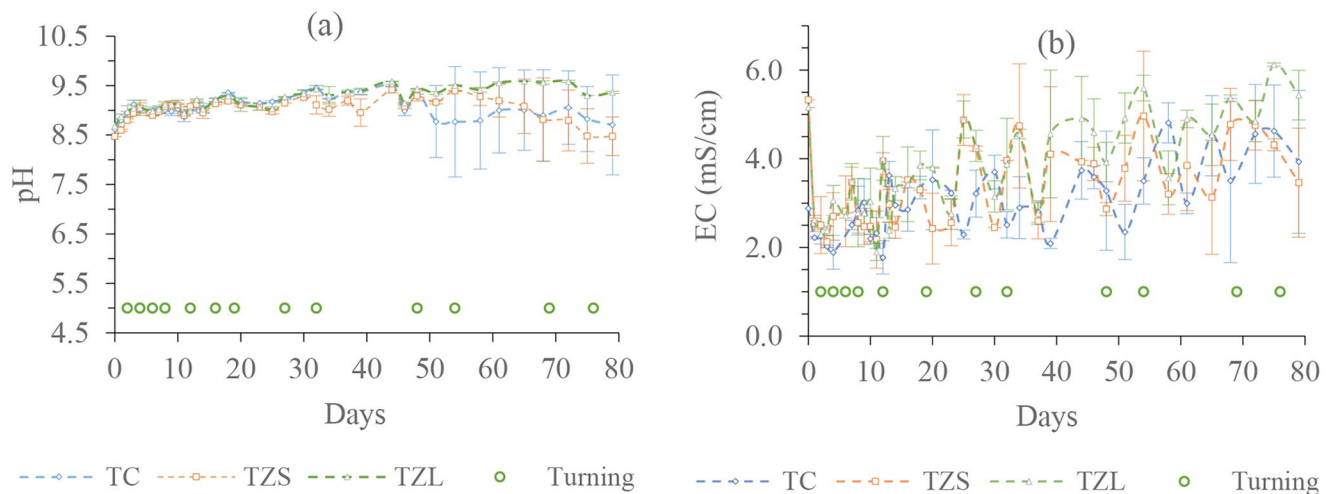


Fig. 3 Evolution of pH (a) and EC (b) for the composting treatments (TC: control, TZS: solid zeolite, TZL: suspended zeolite)

About the different treatments, towards the end of the process, TZS presented the lowest EC, so it is possible that the crystalline structure of the solid zeolite and its high cation exchange capacity (120–160/100 g), allows the exchange and adsorption of ions on its surface, helping to reduce the EC [50]. This behaviour was also reported by Chan et al. [50] with doses of 5% and 10% (w/w) zeolite in food waste composting and Doni et al. [52] with doses of 10% zeolite (w/w) in vineyard waste composting.

Biodegradation of Organic Matter and Fibres

In general, for both VS and TOC (Fig. 4a and b), a decreasing trend in the values of both parameters is observed. In none of the cases there were statistically significant differences between treatments. The values at the beginning of the process are influenced by the presence of lignocellulosic compounds associated with sawdust as a bulking agent. The observed reduction in the two parameters is related to the degradation processes of the organic matter in the material mixtures (e.g. more pronounced decrease in VS at the beginning of the process due to the decomposition of easily degradable compounds).

The reduction in VS was higher in the zeolite treatments, reaching values of 21%, 28% and 23% for TC, TZS and TZL, respectively. These reductions are in agreement with those reported in other studies, where 26% VS reduction was achieved by adding 5% (w/w) zeolite in agricultural waste composting versus 23% in the control [42] and 31% SV reduction using 10% (w/w) zeolite versus 22% in the control [53] in food waste composting. In this case, zeolite could facilitate the degradation processes, due to its porous structure, that promotes the retention of water and nutrients and favouring microbial activity [54] as previously mentioned.

Regarding fibres, it is observed that the hemicellulose content decreased towards the cooling stage in the three treatments, being lower in TZS (10.3%) (Fig. 4c). Zeolite in solid state application had a greater effect on hemicellulose degradation during the cooling stage, as it helps maintain favorable pH, moisture, and nutrient conditions, thereby promoting residual enzymatic activity [14]. Cellulose presented an increasing trend during the first three phases, with a lower concentration in TZS during the first two phases of the process (Fig. 4d). This behavior could be associated with water loss, which reduces the enzymatic degradation of this compound, and with the persistence of lignin that encases the cellulose fibers [55]. In relation to lignin, it is the compound found in higher proportion compared to cellulose and hemicellulose, both in the co-substrates (Table 1) and in the composting mixtures, which is explained by the presence of the bulking agent used in this study. The treatment with the lowest lignin concentration in the cooling phase was TZS (Fig. 4e). For the three parameters there were no statistically significant differences between treatments ($p > 0.05$).

Nitrogen and Involved Genes in its Cycle

In general, the TN content was higher in the zeolite treatments in the first three stages of the process and decreased as the process progressed (Fig. 5a). Wang et al. [4] reported that the addition of zeolite reduced nitrogen loss in the form of ammonia in the first two stages of the process, given the ability of zeolite to retain nitrogen [56, 57].

Concerning the functional genes involved in the nitrogen cycle, the *nif* genes encode proteins required for nitrogen fixation. The *nifH* gene is essential for the conversion of atmospheric nitrogen to ammonia [58]. The *amoA* gene catalyzes the conversion of ammonium to hydroxylamine (NH_2OH), the first step in nitrification. The *nirS* gene is

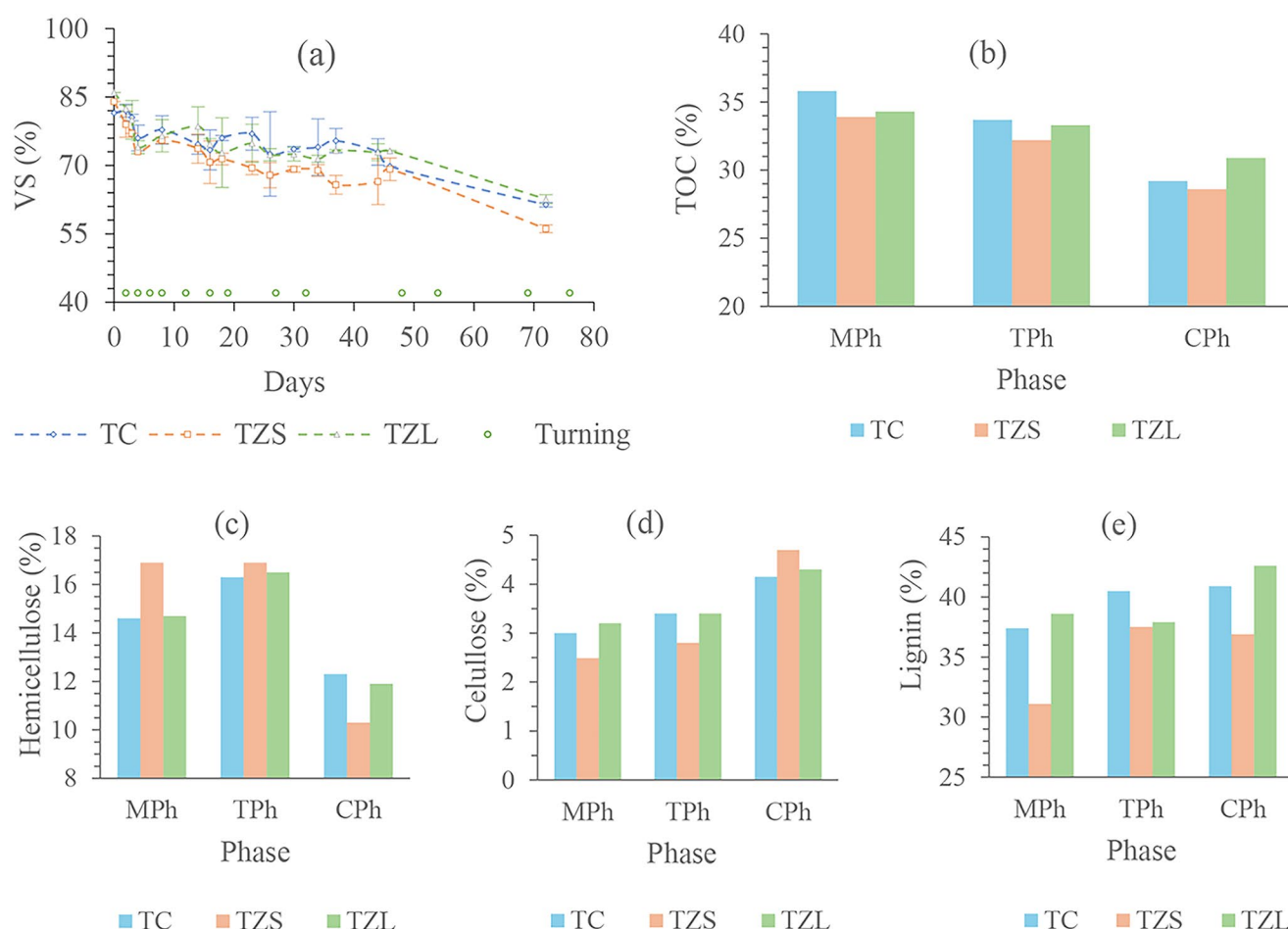


Fig. 4 Evolution of VS (a), TOC (b), hemicellulose (c), cellulose (d) and lignin (e) for the composting treatments (TC: control, TZS: solid zeolite, TZL: suspended zeolite) during the phases of composting: mesophilic (MPh), thermophilic (TPh) and cooling (CPh)

present during denitrification. In this study, as shown in Fig. 5b, nitrogen fixation occurred mainly in the first two phases of the process; in TZS, an increasing trend in the *nifH* gene is observed between the mesophilic and thermophilic phases, which could indicate biological activity associated with nitrogen fixation processes in this treatment. Qian et al. [59] and Zhong et al. [60] also found that the *nifH* gene is more abundant in the early stages of composting.

During the first three phases, it was observed that the abundance of *amoA* in bacteria was higher in the zeolite treatments than in the control. This behaviour could suggest that zeolite promotes nitrification in the early stages of composting, according to Geng et al. [27]. In the thermophilic stage, high temperatures and high oxygen requirements may affect the growth and activity of ammonia-oxidising bacteria [61], which could explain the reduction of *amoA* abundance from the thermophilic to the cooling stage and then the increase in maturation [56]. The relative abundance of the *amoA* gene of archaea was lower than that of bacteria (Fig. 5c and d), with the control having a higher relative

abundance, so it is likely that zeolite influences the abundance of this gene in archaea.

Nitrogen loss occurs mainly via ammonia volatilization in the mesophilic and thermophilic stages [62]. Denitrification, related to nitrogen losses, occurs more in these first two stages [63]. This result is congruent with the higher abundance of the *nirS* gene in all three treatments during these phases (Fig. 5e). This is corroborated by the reduced TN of the treatments (Fig. 5a).

The TZL treatment presented the lowest *nirS* abundance during the process, which is related to lower nitrogen loss [27]. In contrast, TZS had the highest abundance of the *nirS* gene throughout the composting process, contrary to what was found in other studies [4, 27, 56]; however, it did not seem to have much influence on nitrogen loss in this treatment (Fig. 5a). This could be explained by the findings of Wu et al. [42], who observed that, with increasing temperature in the thermophilic stage, zeolite inhibited nitrification and denitrification was stimulated, causing nitrate concentration to decrease in the first few days. In TC, there was

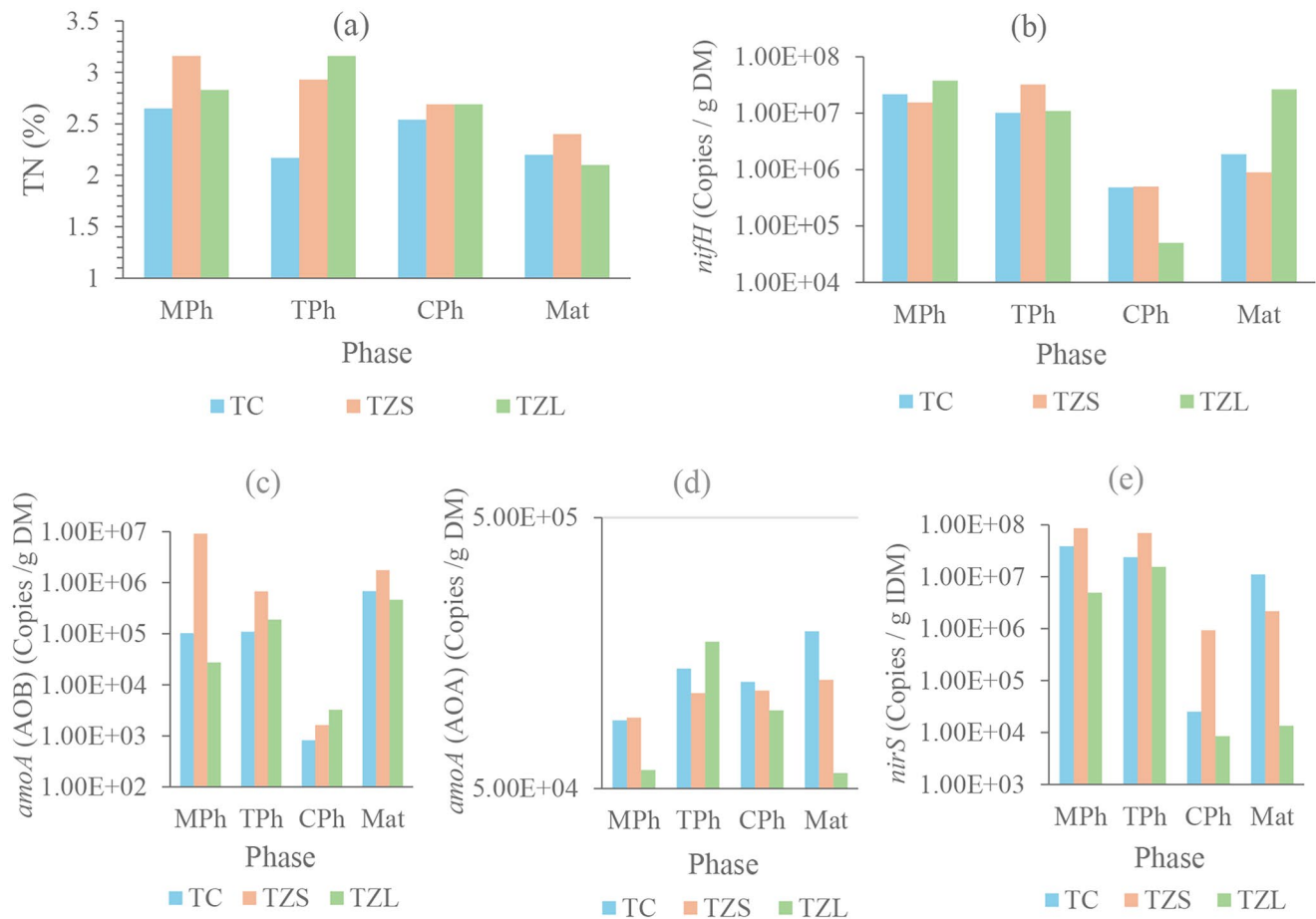


Fig. 5 Evolution of total nitrogen (a) and abundance of functional genes in the nitrogen cycle: *nifH* (b), *amoA* (AOB) (c), *amoA* (AOA) (d) and *nirS* (e) for the composting treatments (TC: control, TZS: solid

zeolite, TZL: suspended zeolite) during the four phases of composting: mesophilic (MPh), thermophilic (TPh), cooling (CPh) and maturation (Mat)

an increase in the abundance of *nirS* during the maturation phase, which may have favoured nitrogen loss in this phase.

At the end of the process, the TN content was 2.2%, 2.4% and 2.1% for TC, TZS and TZL, respectively. Although there were no significant differences between treatments ($p > 0.05$), the ability of solid particulate zeolite to retain nitrogen [14, 22] in the thermophilic stage is highlighted. Furthermore, it may be related to the fact that zeolite increases the adsorption of ammonium ions, reducing ammonia loss, as reported by Chan et al. [50].

Although the suspended zeolite presented a lower nitrogen loss in the thermophilic phase, probably due to the lower temperatures, it had an adverse effect at the end of the process with a lower nitrogen concentration than the control treatment. Gamze and Nuri [64] suggested that higher doses of zeolite improve nitrogen retention; however, Venglovsky et al. [22] used zeolite powder at low doses between 1% and 2% (w/w) and observed a significant reduction of nitrogen losses in pig manure composting. Therefore, it is possible that zeolite in suspension loses its ammonium adsorption

effect after the thermophilic stage, coupled with the high pH of this treatment, so reapplication could be considered when starting the cooling stage or increasing the concentration of the initially applied solution. Future studies could analyse the effect of different types of zeolites, considering that, depending on the specific characteristics of the zeolite, different results could be obtained when applied in an aqueous solution [65].

Total Phosphorous

Regarding total phosphorus, zeolite had no significant effect on this parameter ($p > 0.05$). In all three treatments, the total phosphorus content started below 3%. During the maturation phase, it ranged between 3% and 4% in all three treatments, which is attributed to a concentration effect following the degradation of organic matter [66].

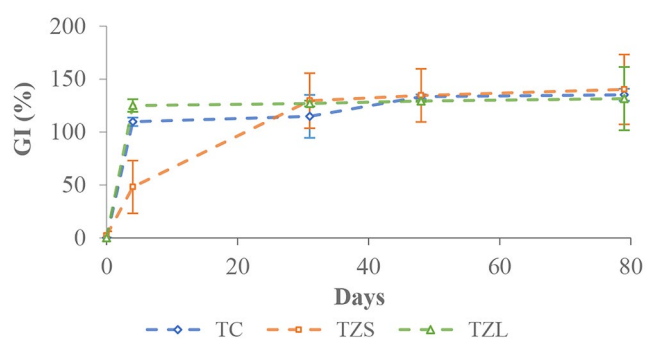


Fig. 6 Evolution of germination index for the composting treatments (TC: control, TZS: solid zeolite, TZL: suspended zeolite)

Germination Index

GI is an indicator of maturity, related to the presence of phytotoxic substances in the compost mixture. In this study, on day 0, all mixtures showed a high level of phytotoxicity (Fig. 6). This is typically due to volatile fatty acids and free ammonia [45, 67].

Afterwards, TC and TZS overcome phytotoxicity from the thermophilic phase by achieving GI above 80%. In the cooling and maturation phases, TZS reached a higher GI than the other two treatments and remained above them towards the end of the process. However, no significant differences were observed between the three treatments ($p > 0.05$). The above results show that the application of zeolite particles has no adverse effect on the phytotoxicity of the material and, on the contrary, may help reduce the presence of substances that inhibit germination, due to their ability to adsorb toxic compounds such as ammonia, organic acids, and heavy metals.

Compost Quality

A complete characterization of the end products obtained for each treatment is presented in Table 3.

Conclusions

The application of 2% zeolite nanoparticles in solid state increased the relative abundance of bacteria and the peak temperature in the first two phases of the process. It also favoured organic matter degradation and nitrogen retention compared to the control and suspended zeolite treatment. Moreover, increased the abundance of the *amoA* gene, suggesting that this amendment could promote nitrification in composting. Zeolite application did not show statistically significant negative effects on product quality compared to the control treatment. Future studies should evaluate higher concentrations of zeolite suspension and higher doses of

Table 3 Characterisation of the three end products obtained in this study (TC: control, TZS: solid zeolite, TZL: suspended zeolite)

Parameter	TC	TZS	TZL	NTC 5167
<i>Physicochemical</i>				
Moisture (%)	46.0 ± 10.0 ^a	43.0 ± 6.4 ^a	35.5 ± 7.8 ^a	<35
Ash (%)	27.3 ± 0.0 ^a	30.7 ± 1.4 ^a	28.9 ± 4.0 ^a	<60
CEC (meq/100 g)	90.2 ± 1.8 ^a	87.2 ± 14.4 ^a	89.1 ± 6.4 ^a	>30
TOC (%)	32.1 ± 0.4 ^a	32.5 ± 3.5 ^a	34.5 ± 1.8 ^a	>15
WRC (%)	236 ± 31.5 ^a	192.0 ± 3.8 ^a	246.8 ± 11.0 ^a	>100
Bulk density (g/cm ³)	0.4 ± 0.01 ^a	0.5 ± 0.03 ^a	0.4 ± 0.01 ^a	0.6
pH	8.7 ± 1.0 ^a	8.5 ± 0.4 ^a	9.4 ± 0.02 ^a	>4-<9
EC (mS/cm)	3.9 ± 2.0 ^a	3.5 ± 1 ^a	5.4 ± 0.6 ^a	-
TN (%)	2.2 ± 0.2 ^a	2.4 ± 0.1 ^a	2.1 ± 0.2 ^a	>1
C/N	14.4 ± 1.2 ^a	13.3 ± 1.0 ^a	16.5 ± 2.3 ^a	-
TP (%)	4.3 ± 0.2 ^a	3.9 ± 0.6 ^a	3.8 ± 0.7 ^a	>1
TK (%)	6.6 ± 1.2 ^a	6.6 ± 1.1 ^a	6.5 ± 0.4 ^a	>1
Hemicellulose (%)	8.3 ± 3.5 ^a	10.5 ± 2.7 ^a	10.2 ± 0.5 ^a	-
Cellulose (%)	9.0 ± 0.3 ^a	5.6 ± 0.5 ^b	4.6 ± 0.5 ^b	-
Lignin (%)	48.4 ± 0.4 ^a	43.3 ± 0.2 ^a	47.2 ± 6.1 ^a	-
GI (%)	135 ± 23.2 ^a	140.3 ± 33 ^a	131.6 ± 29.9 ^a	-
<i>Microbiology</i>				
Total coliforms (MPN/g)	<3	<3	<3	<1000
Faecal coliforms (MPN/g)	<3	<3	<3	<1000
Enterobacter (CFU/g)	0	0	0	<1000
Salmonella /25 g	Absent	Absent	Absent	Absent

Values of the standard NTC 5167 are also presented for comparison purposes

Abbreviations: CEC: cation exchange capacity, TOC: total organic carbon, WRC: water retention capacity, EC: electrical conductivity, GI: germination index, MPN: most probable number, CFU: colony forming units. Different letters indicate significant statistical differences

solid zeolite to assess whether there is a greater influence on product quality parameters. Additionally, the different forms of nitrogen must be measured to relate functional genes with nitrate, nitrite, ammonium and ammonia content.

The products of the three treatments do not show statistically significant differences ($p < 0.05$), except cellulose (i.e., the zeolite treatments had lower values than the control). In general, compliance with the standards required by the Colombian technical norm (NTC 5167) for organic

amendments to be used in agricultural activities is accomplished. The products are characterised by high cation exchange capacity, water retention capacity, and a nutrient content higher than 1% (i.e., TN, TP and TK), which implies an agronomic potential of the compost obtained. Similarly, a minimal presence of pathogenic microorganisms in the products (e.g. total coliforms, faecal coliforms, enterobacter and *Salmonella*) will be highlighted. Finally, it is important to mention that the product with the best conditions is TZS, characterised by higher nitrogen content, lower pH and electrical conductivity, lower lignin and cellulose concentration and higher germination index than the other two treatments.

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Data Availability Data will be available on request.

Declarations

Competing Interests The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work presented in this article.

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