

Enhanced Wheat Straw Bioconversion via *Bacillus* spp. Inoculation: Advancing Sustainable, High-Value Compost Production

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Research Article

Keywords: composting, wheat straw, *Bacillus* spp., biofertilizer, mineral composition, phytotoxicity

Posted Date: November 5th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7759193/v1>

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Abstract

Wheat straw, as a lignocellulosic residue, can be effectively converted into compost, representing a sustainable approach to agro-industrial waste management. This study investigates the effect of inoculating wheat straw with *Bacillus* strains on composting dynamics and organic matter degradation efficiency, with implications for soil quality improvement and environmental sustainability. Compost maturity and quality were evaluated through chemical and biological parameters over a 40-day period. Results showed that *Bacillus*-inoculated compost exhibited traits of a stable and mature product: the $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$ ratio was 0.625, the C/N ratio was 25.88, and the pH was 7.26. The mineral composition of the inoculated compost was significantly increased compared to the control compost, namely: Ca (36%), Mg (58%), Cu (62%), and Zn (75%), with the greatest increase observed in Mn concentration (threefold). Phosphorus content in the treated compost increased eightfold compared to the initial value and exceeded that of the control. The germination index of white mustard seeds (*Sinapis alba* L.) was 30% higher in the inoculated sample. *Bacillus* strains accelerated the degradation of lignocellulosic material derived from wheat straw, yielding a mineral-rich, phytotoxin-free compost suitable for agricultural application and with enhanced biofertilization potential.

1 Introduction

Wheat straw, as a representative form of lignocellulosic biomass, is among the most abundant agro-industrial residues and constitutes a renewable and economically attractive feedstock for the sustainable production of high-value biotechnological derivatives, including compost, biofuels, industrial enzymes, and other bio-based materials. The bioconversion of wheat straw through composting presents an effective strategy for mitigating its excessive accumulation in agricultural systems, which can otherwise lead to long-term degradation of soil properties, as well as reductions in crop yield and quality [1]. It also contributes to mitigating environmental pollution by reducing greenhouse gas emissions associated with waste incineration, a method frequently used in conventional waste management [2, 3]. The advancement of composting technologies is therefore of significant relevance for environmental sustainability, as it aligns with the principles of the circular economy by promoting the transformation of waste into a valuable resource, thereby addressing both ecological and economic dimensions of waste management [4–6]. Through the bioconversion of agro-industrial waste into organic fertilizer-compost it is possible to significantly reduce the reliance on synthetic fertilizers in agricultural production. This strategy encourages the adoption of high-quality, certified compost produced under controlled conditions using environmentally sustainable inputs, thereby supporting both soil health and ecological integrity [7–9]. The agronomic effectiveness of compost is primarily attributed to its physicochemical and biological properties, including optimal pH and electrical conductivity levels, balanced nutrient composition, elevated concentrations of humic substances, and the presence of a diverse and functionally active microbial community. Due to its high biofertilization potential, compost derived from the processing residues of medicinal plants represents a viable alternative to conventional mineral fertilizers and has demonstrated positive effects on both the yield-related parameters and

quality attributes of *Calendula officinalis* (pot marigold) flowers under cultivation [10, 11]. During the composting process, microbial communities facilitate the biodegradation of organic matter, resulting in the formation of biomass, the release of thermal energy, and the emission of carbon dioxide and ammonia as metabolic by-products [12–14]. One of the fundamental mechanisms through which microorganisms interact with organic substrates involves the synthesis of intracellular and extracellular enzymes that catalyze the breakdown of complex organic compounds, particularly those constituting lignocellulosic structures. These microbial enzymes play a pivotal role in the biotransformation of organic matter during the composting process [15]. The bioconversion of lignocellulosic materials represents a complex and multi-stage process that often necessitates the application of pretreatment strategies, including mechanical, chemical, or biological methods, to enhance substrate accessibility. Agricultural lignocellulosic biomass primarily consists of cellulose (35–50%), hemicellulose (20–30%), and lignin (10–20%), which form a highly recalcitrant matrix. Numerous studies have demonstrated that the inoculation of compost with specific microbial consortia, particularly bacterial strains, can enhance the degradation of organic matter including lignocellulosic fractions and thereby improve the overall efficiency and kinetics of the composting process [16].

According to numerous studies, bacterial strains from the genus *Bacillus* are extensively employed in the composting of agro-industrial residues due to their pronounced lignocellulolytic activity. In particular, co-inoculation with *Bacillus subtilis* has been shown to shorten the composting cycle, enhance the degradation rate of lignocellulosic components in agricultural waste, and extend the duration of the thermophilic phase, thereby improving composting efficiency and process stability [1, 17].

Bacillus species have also been reported to promote an elevation in composting temperature, which subsequently enhanced the degradation efficiency of lignocellulosic substrates by approximately 18% to 42% during a 45-day composting process involving wheat straw and livestock manure [18]. These microorganisms are capable of producing a diverse array of hydrolytic and oxidative enzymes essential for the breakdown of organic waste, including celluloses, amylases, laccases, β -glucosidases, peroxidases, and proteases [19–21].

Su et al. (2024) [22] examined the impact of *Bacillus licheniformis* inoculation on the decomposition of organic constituents during the composting of food waste. Their findings demonstrated that the introduction of *B. licheniformis* enhanced the degradation of carbohydrates by 31.2%. Cao et al. (2025) [23] additionally employed the thermophilic cellulolytic bacterium *Bacillus amyloliquefaciens* in the composting of cabbage residues combined with maize straw. Complementary studies have shown that the application of lignocellulose-degrading microbial consortia comprising fungi such as *Trichoderma harzianum*, *T. viride*, *T. koningii*, and *Aspergillus niger*, alongside bacterial strains including *Bacillus subtilis*, *B. amyloliquefaciens*, and *B. megaterium* significantly reduced the composting duration and enhanced the breakdown of lignocellulosic components in corn straw waste mixed with poultry manure and spent mushroom substrate [1, 24].

In our previous study, we demonstrated that a consortium of plant growth-promoting bacteria comprising *Streptomyces* sp., *Paenibacillus* sp., *Bacillus* sp., and *Hymenobacter* sp. contributed to a reduction in the composting duration of medicinal plant residues supplemented with spent coffee grounds, while simultaneously enhancing the biofertilizer potential of the resulting compost [25]. The strains *Bacillus amyloliquefaciens* ssp. *plantarum* PPM3 and *Bacillus altitudinis* PPT1, classified as mesophilic microorganisms, exhibit optimal growth within a temperature range of 25°C to 40°C and are capable of adapting to a broad spectrum of pH values under aerobic conditions.

The aim of this study was to evaluate the effect of a consortium comprising six *Bacillus* strains on the reduction of the composting duration of wheat straw, as well as on the quality and biofertilization potential of the resulting mature compost. Additionally, this study aimed to employ artificial neural networks (ANNs) as an effective mathematical tool for modeling systems characterized by high variability and nonlinear parameters [26]. This modeling approach is particularly valuable in industrial contexts, such as food production, where conventional assays are often time- and resource-intensive [27]. ANN models do not require predefined physical parameters but can effectively extract meaningful insights from experimental data, manage complex nonlinear systems, and capture intricate interactions among variables [28]. The potential for predicting enhanced wheat straw bioconversion through *Bacillus* spp. inoculation, aimed at advancing sustainable and high-value compost production, was investigated. Mathematical modeling was conducted using an artificial neural network (ANN).

2 Materials and Methods

2.1 Substrate and chemicals

The lignocellulosic waste from wheat straw used for composting was obtained from local agricultural producers.

A microbiological preparation containing *Bacillus* strains at a concentration of 2% (v/w) was used as an inoculum (cca 10⁸ CFU/mL). The preparation was obtained from Agridaeus (Italy) and consists of a mixture of six *Bacillus* strains: *B. subtilis* AIBS-001, *B. licheniformis* AIBL-002, *B. simplex* DMSZ 1515, *B. amyloliquefaciens* SD-2, *B. pumilus* 30004, and *B. megatherium* MEG20011.

A preparation containing liquid organic nitrogen and potassium (composition: K₂O 12%, organic N 3%, amino acids of plant origin 7%), used as an additive to the waste sample, was obtained from Itapollina S.p.A. (Italy).

White mustard seeds (*Sinapis alba* L.) were obtained from an agricultural store.

2.2 Composting Process

For the purpose of the research, wooden containers were constructed and connected with threaded rods and nails, resulting in a semi-enclosed structure with a volume of 1 m³ per container (dimensions: 1 m × 1 m × 1 m), inside which plant waste was stored. Two bales of wheat straw, with a total mass of about

20 kg, were placed in each compost container. The plant material was previously processed mechanically by chopping and grinding to a particle size of 1–4 cm using crusher machines designed for grinding plant material. The samples for the composting process were prepared according to the instructions presented in Table 1.

Table 1
Samples for the composting process

Sample	Composition
RS	20 kg wheat straw + <i>Bacillus</i> sp. 2 % (v/w) inoculum (cca 10 ⁸ /mL) + organic N 3% + K ₂ O 12%
C (control)	
C-0 (control in day 0)	20 kg wheat straw
	20 kg wheat straw

Wheat straw, in an amount of 20 kg, was treated with prepared aqueous solutions of the *Bacillus* preparation using a sprayer, with constant mixing and turning of the pile (RS sample). Before inoculation, the waste samples were moistened with water to a moisture content of 60%. The compost was mixed twice a week by turning the pile to allow aeration. Moisture of the composting material was maintained at 60% to 65% by adding tap water once a week. The decomposition of plant waste and the composting process were monitored for 40 days.

2.3 Sampling and Analysis

Sampling was performed at different composting stages on days 0, 10, 20, and 40 of the process. Each individual sample, taken from each container, was obtained by combining six subsamples collected from representative locations, three from the top and three from the bottom of the composting material. The results were expressed as mean values ± standard deviation of three independent replicates and were statistically analyzed. Before chemical analysis, the samples were dried in an air oven at 80°C to constant weight, then homogenized by grinding to a particle size of 0.5-1.0 mm. After grinding, the samples were dried again at 80°C to constant weight. The results of the chemical analyses are expressed per gram of dry mass.

2.3.1 pH Measurement

Samples were extracted using a solid-to-liquid ratio of 1:10 (w/v) with distilled water to determine pH. After 4 hours of extraction, the suspensions were centrifuged at 4500 rpm for 10 minutes. The pH of the aqueous extracts was measured using a digital pH meter [29].

2.3.2 Electrical Conductivity (EC)

Electrical conductivity was measured in distilled water extracts using a WTW 315i conduct meter. Extracts were prepared by mixing compost samples with distilled water at a 1:5 (w/v) ratio and agitating on a rotary shaker for 60 minutes at room temperature.

2.3.3 Organic Matter Content

Organic matter (OM) content was determined gravimetrically by calculating the difference between the dry weight and ash content of the samples, and expressed as a percentage of total dry mass [30].

2.3.4 Total Phosphorus and Potassium

Available P_2O_5 was determined spectrophotometrically using a UV/VIS spectrophotometer (Labomed Inc., model 1166), based on the formation of 12-molybdophosphoric acid through the reaction of phosphate with molybdate in an acidic medium, and followed by its reduction to a phosphomolybdenum blue complex [31].

Available K_2O was measured using a Jenway PFP7 flame photometer, following ammonium lactate extraction as described by [32].

2.3.5 Total Carbon, Nitrogen, NH_4^+-N and $NO_3^- -N$

The concentrations of available NH_4^+-N and $NO_3^- -N$ were determined via steam distillation following a 2-hour extraction with 2 M KCl (solid-to-liquid ratio 1:10, w/v), with nitrate reduction performed using Devarda's alloy [33]. Moisture content of the raw materials and composting mixtures was automatically measured using a moisture analyzer (MLS 65-3A, KERN & SOHN, Balingen, Germany). Total nitrogen (TN) was quantified after sample digestion with concentrated sulfuric acid (H_2SO_4) in the presence of a catalyst mixture (100:1:1000 $CuSO_4 \times 5H_2O/Se/K_2SO_4$) [34]. This procedure converts organic nitrogen into ammonium (NH_4^+-N), which serves as the basis for subsequent quantification of nitrogen (N) and phosphorus (P). Ammonium from the Kjeldahl digests was determined titrimetrically following alkaline steam distillation [33]. Organic carbon content was estimated through dry ashing in a muffle furnace at 500–550°C until constant weight. The percentage of ash was subtracted from the initial dry mass to calculate total organic carbon.

2.3.6 Evaluation of Phytotoxicity

The seed germination test is a widely recognized method for evaluating compost phytotoxicity and maturity [35]. In this study, water extracts of compost samples were prepared by diluting fresh material at a 1:10 (w/v) ratio in distilled water, followed by agitation for 1 hour at 150 rpm on an orbital shaker. Sterile Whatman No. 1 filter paper was placed in glass Petri dishes ($\varnothing$ 8.5 cm), and 5 mL of each extract was added as the test solution; distilled water (5 mL) served as the control. Germination of *Sinapis alba* L. seeds was assessed using 25 seeds per replicate, with three replicates per treatment. Seeds were incubated in the dark at 25°C for 72 hours, after which the number of germinated seeds and root lengths were recorded. The germination index (GI) was then calculated according to the following formula:

$$GI (\%) = 100 \times G/G_c \times L/L_c (1)$$

where G and L stand for the germination and root length of the samples, respectively, and G_c and L_c stand for the corresponding values for the control (distilled water).

2.3.7 Mineral composition of compost (Ca, Mg, Fe, Mn, Cu and Zn)

Available calcium (Ca) and magnesium (Mg) contents were quantified by atomic absorption spectrophotometry (AAS; SpectrAA220FS, Varian) after a 30-minute extraction with 1 M NH₄OAc (pH 7.0) at a solid-to-liquid ratio of 1:50 (w/v) [34]. Available iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) were similarly determined by AAS following extraction with 0.005 M DTPA [36]

2.4 ANN modelling

In this study, an artificial neural network was established using a multi-layer perceptron (MLP) architecture consisting of three interconnected layers: input, hidden, and output. The MLP is widely applied for the approximation of nonlinear functions [37]. Prior to computation, the input data were normalized to improve model performance. During model training, the dataset was iteratively presented to the network [37]. The Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm was employed as an iterative optimization method for solving unconstrained nonlinear problems within the ANN framework. The dataset was randomly divided into training (70%) and testing (30%) subsets. Model training was considered successful when the learning and cross-validation error curves converged to minimal values. The weights and biases of the hidden and output layers are denoted as W_1 and B_1 , and W_2 and B_2 , respectively, while the transfer functions of the hidden and output layers are represented by f_1 and f_2 . The input variables are expressed as the vector X [38].

$$Y = f_1 (W_2 \cdot f_2 (W_1 \cdot X + B_1) + B_2)$$

2

Throughout the learning cycle, the weight coefficients (matrices W_1 and B_1 , and W_2 and B_2) were iteratively updated using the Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm, with the objective of minimizing the estimation error of the artificial neural network (ANN) [39]. This optimization procedure, based on the sum of squares (SOS) criterion, enabled faster and more stable convergence [40]. The predictive performance of the developed ANN model was evaluated using the coefficient of determination.

To explore the effect of inoculating wheat straw with *Bacillus* strains on the composting rate and the efficiency of organic matter decomposition, ANN modeling was applied. The network architecture, including the biases and weight coefficients, was determined by the initial configuration of the input matrix parameters, which critically influenced the model's development and its fitting to experimental data.

2.5 Statistical analysis

Multivariate analysis of variance (MANOVA) was performed to identify statistically significant differences among the sample means. In addition, Principal Component Analysis (PCA) was applied as a pattern recognition technique to explore descriptor variability and to visualize the distribution and separation of the investigated samples. All statistical analyses were conducted using STATISTICA 10.0 software (StatSoft Inc., Tulsa, OK, USA) (STATISTICA, 2010).

The numerical validation of the ANN model's accuracy was evaluated using several statistical metrics, including the coefficient of determination (R^2), reduced chi-square (χ^2), mean bias error (MBE), root mean square error (RMSE) and mean percentage error (MPE) [41]:

$$\chi^2 = \frac{\sum_{i=1}^N (x_{\text{exp},i} - x_{\text{pre},i})^2}{N - n}$$

3
,

$$RMSE = \left[\frac{1}{N} \cdot \sum_{i=1}^N (x_{\text{exp},i} - x_{\text{pre},i})^2 \right]^{1/2}$$

4
,

$$MBE = \frac{1}{N} \cdot \sum_{i=1}^N (x_{\text{exp},i} - x_{\text{pre},i})$$

5
,

$$MPE = \frac{100}{N} \cdot \sum_{i=1}^N \left(\frac{|x_{\text{exp},i} - x_{\text{pre},i}|}{x_{\text{exp},i}} \right)$$

6
,

$$SSE = \sum_{i=1}^N (x_{\text{pre},i} - x_{\text{exp},i})^2$$

7

$$AARD = \frac{1}{N} \cdot \sum_{i=1}^N \left| \frac{x_{exp,i} - x_{pre,i}}{x_{exp,i}} \right|$$

8

where $x_{exp,i}$ is the experimental value and $x_{pre,i}$ is the ANN calculated value, N and n are the number of observations and the number of constants, respectively.

3. Results and Discussion

3.1 Monitoring of the Composting Process

This study monitored the biodegradation of wheat straw during a 40-day composting process, with samples collected at four time points (days 0, 10, 20, and 40). To evaluate the impact of *Bacillus* strain inoculation on compost quality and maturity, key chemical and biological parameters were assessed, including temperature, pH, organic matter loss, electrical conductivity (EC), total carbon, nitrogen, phosphorus, potassium, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, C/N ratio, mineral composition, and phytotoxicity.

3.1.1 Temperature change during the composting period

Temperature is a key parameter for monitoring composting dynamics and evaluating compost maturity and stability. It is primarily driven by microbial metabolic activity and influenced by factors such as substrate type, composting method, ambient conditions, and moisture content [42]. In this study, the initial temperature of the composting mixture was $17.3 \pm 1.17^\circ\text{C}$. During the thermophilic phase, a peak temperature of $49.1 \pm 2.55^\circ\text{C}$ was recorded on day 25 in the *Bacillus*-inoculated treatment (RS). By the end of the composting period, the temperature in the RS pile had decreased to $24.4 \pm 0.56^\circ\text{C}$, while the control (C) maintained a slightly higher final temperature of $28.7 \pm 0.89^\circ\text{C}$ (Fig. 1). These results indicate that mesophilic conditions dominated both the initial and final phases of composting, whereas thermophilic conditions prevailed during the active decomposition stage.

The moisture content in the samples remained stable and controlled at 60% to 65% at the beginning and during the composting process, but dropped below 30% by the end

3.1.2 Change in chemical parameters of the compost during the composting period

As shown in Table 2, all samples exhibited a statistically significant increase in pH to values above 8 within the first 10 days of composting, relative to the initial pH of 6.38 ± 0.14 . This shift is attributed to the release of ammonia and other alkaline metabolites produced during the mineralization of proteins, amino acids, and peptides. By the end of the composting process, the pH stabilized at 7.26 ± 0.09 in the *Bacillus*-inoculated sample (RS), and at 7.80 ± 0.30 in the control (C), consistent with findings reported in previous studies [25, 43].

Table 2
Change in chemical parameters of the wheat straw during the composting period

Composting days	Sample	pH	EC	NH ₄ ⁺ -N	NO ₃ ⁻ -N
			(mS/cm)	mg/kg	mg/kg
0	C-0	6.38 ± 0.14 ^d	0.004 ± 0.0005 ^e	28.0 ± 2.78 ^a	25.9 ± 1.61 ^a
10	RS	8.20 ± 0.15 ^a	0.55 ± 0.05 ^a	9.10 ± 0.44 ^c	2.80 ± 0.16 ^d
	C	8.13 ± 0.12 ^a	0.57 ± 0.01 ^a	2.10 ± 0.18 ^e	0.70 ± 0.09 ^e
20	RS	7.62 ± 0.30 ^b	0.28 ± 0.04 ^b	8.40 ± 0.20 ^b	4.90 ± 0.36 ^c
	C	7.37 ± 0.17 ^b	0.23 ± 0.01 ^b	8.40 ± 0.35 ^b	4.20 ± 0.44 ^c
40	RS	7.26 ± 0.09 ^c	0.10 ± 0.02 ^d	3.50 ± 0.35 ^{d, e}	5.60 ± 0.46 ^b
	C	7.80 ± 0.30 ^c	0.22 ± 0.03 ^c	5.60 ± 0.30 ^d	4.90 ± 0.09 ^b

*Statistic was performed within the columns.

Electrical conductivity (EC) is an important indicator of compost safety, as it reflects the concentration of soluble salts that may affect plant growth [44]. In this study, EC increased during the initial phase of composting, reaching 0.55 ± 0.05 mS/cm on day 10 in the *Bacillus*-inoculated treatment (RS), followed by a gradual decline toward the end of the process. The initial rise in EC is likely linked to microbial activity induced by *Bacillus* inoculation, which promoted the release of low-molecular-weight compounds from organic matter. By the end of the composting period, EC values differed significantly between treatments, with the RS sample exhibiting notably lower values compared to the control (Table 2).

Organic matter (OM) content progressively decreased throughout the composting process. Starting from an initial value of 90 ± 2.0%, OM content declined to 78 ± 2.7% in the RS sample and 81 ± 1.0% in the control after 40 days (Fig. 2a), indicating enhanced degradation of organic substrates in the inoculated treatment. Nitrogen in compost exists in various forms, such as N₂, NH₄⁺-N, and NO₃⁻-N, which can be converted into each other by microorganisms. It was observed that the total nitrogen (TN) content increased significantly after the 20th day during the thermophilic phase, reaching 1.43 ± 0.08% in the inoculated sample (RS), while the value in the control was slightly lower but showed no statistically significant difference (Fig. 2b). This phenomenon is due to the increased activity of microorganisms, which led to the decomposition of nitrogen-containing organic compounds such as nucleic acids, amino acids, and proteins. This process was accompanied by a rise in temperature, which was higher in the bacteria-treated sample compared to the control, where the temperature remained lower (Fig. 1). The NH₄⁺-N value decreases mainly due to the emission of ammonia, which microorganisms partially convert into NO₃⁻-N and subsequently into N₂ [44] (Table 2). Inoculation with bacteria improves the transformation of NH₄⁺-N into NO₃⁻-N and in mature compost this NH₄⁺-N/NO₃⁻-N ratio should be less

than 1 [45, 46]. Our results align with these findings, showing that by day 40, the ratio in the bacteria-treated sample (RS) was 0.63, while in the control sample, it remained above 1 (Table 2). According to Wan et al. (2023) [20] this ratio was below 1 (ranging from 0.325–0.650) in compost produced from garden waste using the fungi *Sordaria tomentosalba* (MSDA1) and *Thermomyces lanuginosus* (HDGA2). The authors divided the composting process into two phases: the fermentation phase, lasting the first 40 days, and the maturation phase, occurring between days 40 and 60.

The bacteria damage the straw epidermis and the vascular bundles lose their very compact structure. The inoculated sample showed a greater degradation of cellulose and hemicellulose compared to the control sample. The degradation of cellulose and hemicellulose mainly occurs during the heating and thermophilic phases of the composting process [44]. Wheat straw is a typical lignocellulosic plant material with an initial C/N ratio of 57.02 (Fig. 2c). The *Bacillus* strains in the compost utilize cellulose and hemicellulose as carbon sources throughout the composting process. During the initial phase (day 10), the bacteria are most active and their population increase, as shown by the sharp drop in the C/N ratio to 47.90 in the treated sample (RS), likely due to bacterial carbon consumption. Unlike the treated sample, in the control the C/N ratio was unchanged and very close to the initial sample before composting. The carbon and nitrogen sources required by *Bacillus* strains were abundant at this stage, so the microorganisms could survive well in the compost. In the later stage of composting, most of the cellulose and hemicellulose were degraded, reducing the available carbon sources. This led to a decline in *Bacillus* activity and stabilization of the C/N ratio. By day 40, the C/N ratio was lowest in the sample inoculated with *Bacillus* strains (RS), with the final-to-initial C/N ratio reaching 0.45. The C/N ratio in the control sample aligned with our previous research [25, 44]. Compost is generally considered to be mature when the C/N ratio is between 15 and 25, and the final-to-initial C/N ratio ranges from 0.5 to 0.6 [47].

The potassium content decreases during the first days of composting but increases again by the end of the process, reaching values slightly below the initial levels (Fig. 3a). In contrast, phosphorus gradually increases throughout composting in all samples, with the highest increase observed in the treated sample (RS), reaching approximately eight times the initial content by the end (Fig. 3b). This finding aligns with our previous research on composting medicinal plant waste, where phosphorus increased fivefold by the end of the process [43]. The release of organic acids during the composting process helps dissolve insoluble phosphorus, and phosphate-solubilizing bacteria also contribute to this, which mineralize organic phosphorus. Similar results regarding phosphorus and potassium content during grape pomace composting were reported by Matiz et al. (2021) [48] and Moldes et al. (2007) [49]. Phosphorus is the second most limiting nutrient after nitrogen in most soils which is important for crop production, and many attempts have been made to produce compost with increased phosphorus content [47]. Phosphorus sources are essential for maintaining sustainable crop production, closing the phosphorus cycle, and promoting food security and environmental health [50].

3.1.3 Phytotoxicity test

Phytotoxicity was assessed using the germination index (GI) of white mustard (*Sinapis alba* L.), a widely used biological indicator for evaluating compost maturity and stability [51, 52]. The GI is considered one of the most reliable indicators of compost phytotoxicity, with values exceeding 80% generally indicating loss of toxicity and a mature compost product [53, 54]. Low GI values in the early stages of composting are often attributed to the presence of phytotoxic compounds such as volatile fatty acids or elevated levels of $\text{NH}_4^+\text{-N}$ [4].

According to proposed classification criteria, substrates with $\text{GI} < 25\%$ are considered highly phytotoxic; values between 26–65% indicate moderate phytotoxicity; while GI between 66–100% denotes non-phytotoxic material suitable for agricultural application. GI values above 101% suggest phytonutrient or phytostimulant properties [47].

In this study, compost samples showed phytotoxic effects during the first 30 days of composting (Fig. 4). However, GI values after day 30 indicated the absence of phytotoxicity. By day 40, the *Bacillus*-inoculated compost (RS) reached a $\text{GI of } 114.8 \pm 5.6\%$, representing a statistically significant ($\sim 30\%$) increase compared to the control, which reached $88.3 \pm 4.4\%$. This result classifies the inoculated compost not only as non-phytotoxic but also as a phytonutrient-phytostimulant, supporting its use as a value-added biofertilizer in agriculture [47].

In contrast, while the control compost was also non-phytotoxic ($\text{GI} > 80\%$), it did not exhibit biofertilization potential. These findings suggest that inoculation with *Bacillus* strains effectively enhances compost maturity, accelerates lignocellulose degradation, and improves the agronomic quality of the final product.

Research by Wang L. et al. (2023) [18] also shows the potential of *Bacillus* in improving lignocellulose degradation, compost fertility, and plant growth promoting effects with increased aboveground biomass and increased chlorophyll.

3.1.4 Mineral composition of compost during the composting period

The results showed that inoculation of wheat straw with *Bacillus* strains during the composting process lead to a significant increase in the content of Ca (36%), Mg (58%), Cu (62%), and Zn (75%), with the greatest effect observed in the increase of Mn concentration (3fold increase) in the mature compost produced compared to the compost control produced without treatment (Table 3). Our results are consistent with those reported by Matiz et al. (2021) [48].

Table 3
Mineral composition of the wheat straw during the composting period

Composting days	Sample	Ca mg/kg	Mg mg/kg	Fe mg/kg	Mn mg/kg	Cu mg/kg	Zn mg/kg
0	C-0	1462.3 ^{c,d,e}	646.7 ^c	0.40 ^d	6.14 ^b	0.19 ^{b,c}	0.70 ^{b,c}
10	RS	1431.2 ^d	341.3 ^d	0.15 ^d	0.45 ^d	0.04 ^d	0.15 ^d
	C	974.4 ^f	324.1 ^d	0.20 ^d	0.58 ^d	0.04 ^d	0.10 ^d
20	RS	1180.1 ^e	299.5 ^d	2.28 ^{c,b}	4.15 ^b	0.12 ^{c,d}	0.31 ^{c,d}
	C	1594.6 ^c	343.9 ^d	1.96 ^c	0.73 ^c	0.09 ^{c,d}	0.38 ^{c,d}
40	RS	2466.3 ^a	1288.8 ^a	3.58 ^b	11.21 ^a	0.39 ^a	1.97 ^a
	C	1807.3 ^b	813.5 ^b	12.75 ^a	3.80 ^b	0.24 ^b	1.12 ^b

*Statistic was performed within the columns.

These results demonstrate that the application of *Bacillus* strains enhances the mineral composition of compost derived from wheat straw. The resulting microelement-enriched compost has the potential to significantly improve soil quality, thereby supporting optimal plant growth and development. The availability of these essential micronutrients facilitates key physiological processes in plants, including chlorophyll synthesis and photosynthesis, enhances resistance to various diseases, and ultimately contributes to increased efficiency and sustainability of agricultural production.

3.2 Correlation analysis

The correlation analysis of physicochemical parameters revealed several strong and statistically significant relationships. Compost pH exhibited a strong positive correlation with electrical conductivity (EC; $r = 0.900$, $p = 0.006$), while being negatively correlated with both ammonium nitrogen ($\text{NH}_4^+\text{-N}$; $r = -0.785$, $p = 0.037$) and nitrate nitrogen ($\text{NO}_3^-\text{-N}$; $r = -0.893$, $p = 0.007$). These results indicate that higher pH values are associated with increased EC but reduced concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$. A strong negative, though not statistically significant, association was also observed between EC and $\text{NO}_3^-\text{-N}$ ($r = -0.706$, $p = 0.076$), while $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ showed a very strong positive correlation ($r = 0.951$, $p = 0.001$), suggesting that these nitrogen forms tend to accumulate simultaneously in the studied samples.

The second correlation matrix, focused on mineral nutrients, also demonstrated distinct patterns. Calcium (Ca) was strongly and positively correlated with magnesium (Mg; $r = 0.910$, $p = 0.004$), manganese (Mn; $r = 0.758$, $p = 0.049$), copper (Cu; $r = 0.887$, $p = 0.008$), and zinc (Zn; $r = 0.934$, $p = 0.002$), highlighting a coupled enrichment of these cations. Magnesium showed very strong correlations with Cu ($r = 0.969$, $p < 0.001$) and Zn ($r = 0.987$, $p < 0.001$), as well as a strong association with Mn ($r = 0.885$, $p =$

0.008), suggesting that these elements may share similar geochemical behavior or sources. Among the micronutrients, Mn was strongly correlated with both Zn ($r = 0.893$, $p = 0.007$) and Cu ($r = 0.935$, $p = 0.002$), while the strongest overall association was observed between Cu and Zn ($r = 0.987$, $p < 0.001$). In contrast, Fe displayed only weak and nonsignificant correlations with the other elements, indicating its independent variation. Collectively, these results indicate that cations such as Ca, Mg, Mn, Cu, and Zn tend to co-occur, while Fe behaves differently, possibly reflecting differences in mobility, solubility, or source contributions.

3.3 PCA analysis

The PCA analyses provided complementary insights into the relationships among physicochemical and mineral parameters. In the first PCA, based on pH, EC, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$, two principal components explained 98.38% of the total variance, with PC1 accounting for 84.95%. pH (27.76) and EC (21.29) contributed strongly and positively to PC1, whereas $\text{NH}_4^+\text{-N}$ (−23.47) and $\text{NO}_3^-\text{-N}$ (−27.47) contributed negatively, clearly separating compost reaction and salinity from mineral nitrogen availability. This indicates that higher pH and EC values are associated with lower concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$, reflecting a strong antagonistic relationship. PC2 explained only 13.43% of the variance, with moderate negative contributions from EC and $\text{NH}_4^+\text{-N}$, but the variance structure was predominantly defined by the contrasting behavior along PC1.

The second PCA, including mineral nutrients (Ca, Mg, Fe, Mn, Cu, and Zn), also revealed a well-structured system, with two components explaining 95.53% of the total variance. PC1, which captured 81.24% of the variance, was characterized by high negative loadings of Ca (−17.71), Mg (−19.84), Mn (−16.63), Cu (−20.20), and Zn (−20.42), indicating a strong co-variation and clustering of these elements. In contrast, Fe contributed only weakly to PC1 (−5.20) but loaded strongly and negatively on PC2 (−85.88), which explained an additional 14.29% of the variance. This pattern suggests that Fe behaves independently of the other cations, reflecting differences in mobility and solubility under the prevailing compost conditions.

When considered together, the two PCA results highlight that compost pH and EC are key environmental factors that control nitrogen availability, while simultaneously influencing the distribution of mineral nutrients. The close clustering of Ca, Mg, Mn, Cu, and Zn suggests common sources or synchronized geochemical behavior, likely favored under higher pH and EC conditions, whereas Fe emerges as a chemically distinct element with its own variability pattern. This integrated view indicates that shifts in compost reaction and salinity not only regulate nitrogen dynamics but also shape the balance between co-occurring cations and independently varying micronutrients.

3.4. ANN model

The number of neurons in the hidden layer significantly influences both ANN model's behavior. To minimize the impact of random correlations arising from the initial assumptions and weight initialization,

each network topology was trained 100,000 times. Based on this approach, the highest coefficient of determination (R^2) during the training phase was achieved using nine hidden neurons.

The model was trained for 100 epochs, and the performance metrics-training accuracy and error (loss). Training accuracy steadily improved with the number of epochs, plateauing around the 70th to 80th epoch. The highest training accuracy and lowest loss were observed within this range. Beyond 80 epochs, minor improvements in accuracy and further reduction in loss were noted, indicating the onset of overfitting. Therefore, limiting the training to approximately 70 epochs was deemed sufficient to achieve high model accuracy while avoiding overfitting.

The verification of both ANN models confirmed their strong predictive performance across all evaluated statistical criteria. For the first network (MLP 4-5-4), developed to estimate pH, EC, $\text{NH}_4^+\text{-N}$, and $\text{NO}_3^-\text{-N}$, the error indices were remarkably low ($\chi^2 = 0.0143$, RMSE = 0.0947, SSE = 0.0536), indicating that deviations between measured and predicted values were minimal. Relative error indices also remained within acceptable limits, with MPE = 1.82% and AARD = 2.11%, demonstrating that the model systematically reproduced the observed data with very little bias. The high determination coefficient ($R^2 = 0.986$) and correlation values above 0.97 for all variables highlight the model's robustness in capturing the nonlinear relationships between physicochemical parameters. The near-perfect match between experimental and predicted values, particularly for pH and EC, confirms that the ANN adequately learned the underlying data patterns without overfitting.

The second model (MLP 4-3-6), which targeted Ca, Mg, Fe, Mn, Cu, and Zn, also displayed excellent predictive capacity. Although its error values ($\chi^2 = 0.0219$, RMSE = 0.1285, SSE = 0.0812) were slightly higher than those of the first model, they still fall within an acceptable range for complex multivariate systems. The relative error indices (MPE = 2.64%, AARD = 2.95%) remain below 3%, confirming that systematic deviation from experimental values was negligible. The determination coefficient ($R^2 = 0.981$) and the strong correlation coefficients ($r > 0.98$ for all nutrients) further validate the reliability of the model. Some larger deviations were observed for Fe and Mn, reflecting their higher natural variability and complex geochemical interactions, but the model still succeeded in maintaining high predictive precision for these elements.

When comparing both networks, it is evident that the MLP 4-5-4 architecture provided a slightly better overall performance, particularly due to the lower residual errors and higher consistency across all predicted variables. Nevertheless, both models achieved prediction accuracies above 98%, error indices well below critical thresholds, and excellent generalization ability on test data, confirming that the BFGS training algorithm with nonlinear activation functions was well suited for this problem.

The verification results demonstrate that both ANN models provide statistically robust, unbiased, and highly reliable predictions. The combination of high R^2 values, very low error metrics, and stable correlation coefficients indicates that the developed ANN frameworks are suitable for practical application in modeling compost physicochemical properties and nutrient dynamics. These findings

confirm the effectiveness of ANN in capturing complex nonlinear interactions in environmental data, outperforming traditional regression approaches in predictive accuracy and flexibility.

3.5 Limitations of the ANN model prediction

Although the dataset used for model development comprised a relatively small number of samples, such limitations are common in agricultural investigations, where seasonal cycles, environmental conditions, and experimental costs restrict sample collection. Despite this, an artificial neural network model was successfully developed and trained to capture the nonlinear relationships among the studied parameters.

Model verification was performed using statistical indicators, and these metrics indicated good agreement between predicted and observed values, showing that the model is capable of accurately describing the experimental results. The findings confirm that ANN can be applied even when data availability is limited, offering an advantage over conventional methods by handling nonlinear and multivariate interactions typical of agricultural systems. The restricted sample size must be acknowledged as a limitation. Incorporating additional variables, such as environmental or management factors, could also enhance predictive performance. Future work should focus on validating the ANN against independent datasets and exploring hybrid approaches with other machine learning methods.

4 Conclusion

This study investigated the use of *Bacillus* strains as inoculants in the composting process of wheat straw. Due to its renewable and biodegradable nature, wheat straw biomass is increasingly recognized as a valuable agro-industrial by-product with diverse applications, including compost production. The composting process results in a final product that, owing to its technological, ecological, and economic advantages, represents a high-quality and sustainable alternative to synthetic fertilizers in organic agriculture. *Bacillus* strains promote lignocellulose decomposition, accelerate compost maturation, and significantly enhance both the mineral composition and the biofertilizing potential of the compost. Organic compost derived from improved wheat straw bioconversion exhibits the necessary technological properties for advanced sustainable plant production, while also contributing to the improvement of soil quality.

Declarations

Data Availability

Data will be made available on request.

Acknowledgements

This work was supported by joint funding from the Ministry of Science, Technological

Development and Innovation of the Republic of Serbia. Agreement number: grant nos. 451-03-136/2025-03/200053, 451-03-65/2025-03/200135, 451-03-136/2025-03/200051, 451-03-136/2025-03/200054, 451-03-137/2025-03/200116 and 451-03-136/2025-03/200032

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Competing Interests

The authors declare no conflicts of interest

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Figures

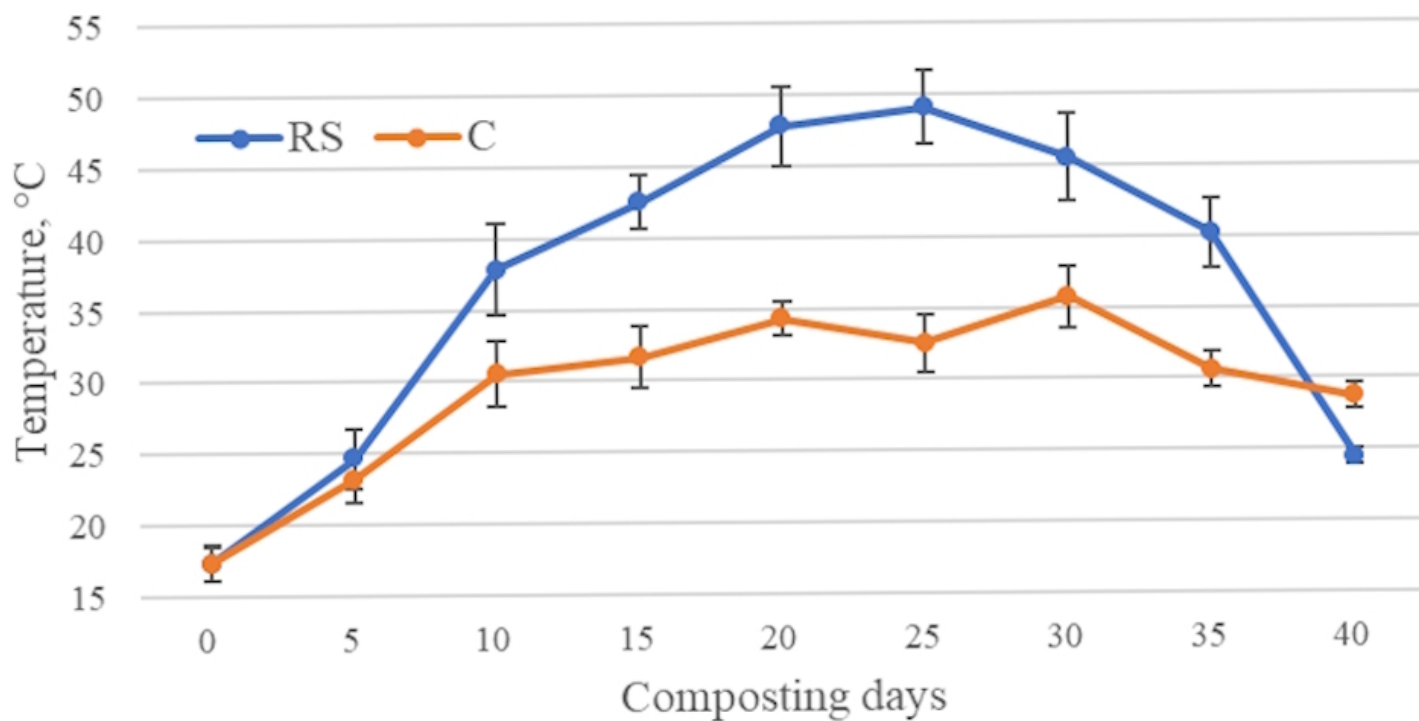


Figure 1

Temperature change during the wheat straw composting period

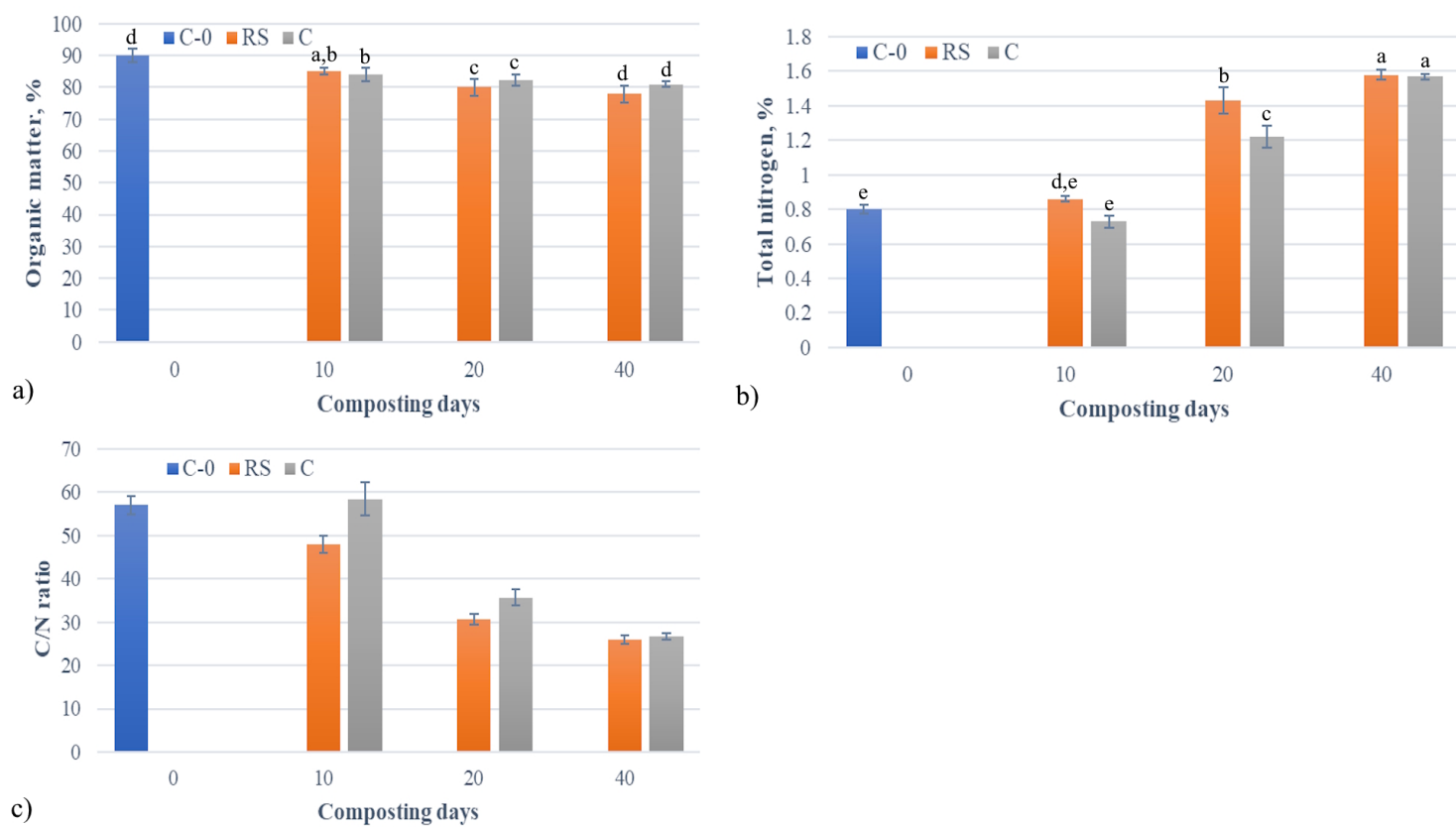


Figure 2

Change in organic matter (a), total nitrogen (b), and C/N ratio (c) of the wheat straw during the composting period

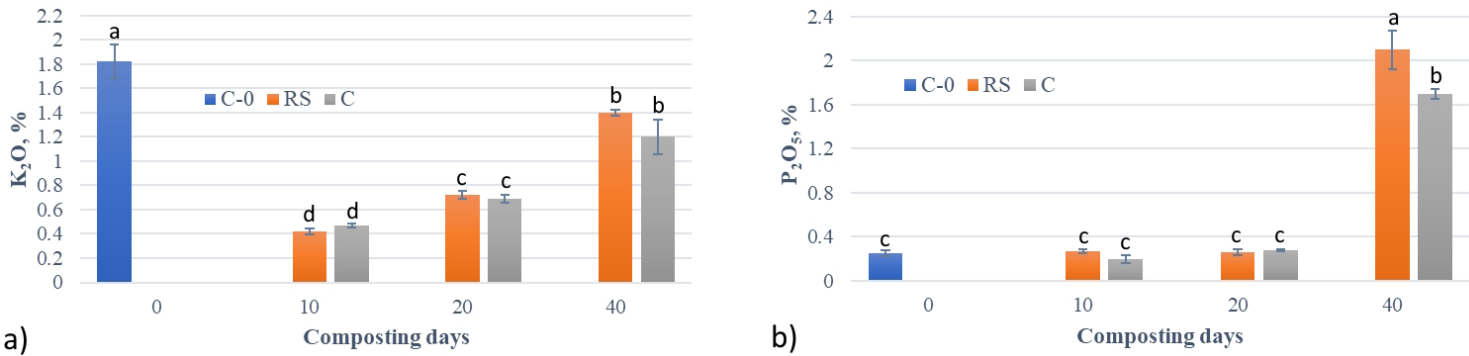


Figure 3

Change in potassium (a) and phosphorus (b) content of the wheat straw during the composting period

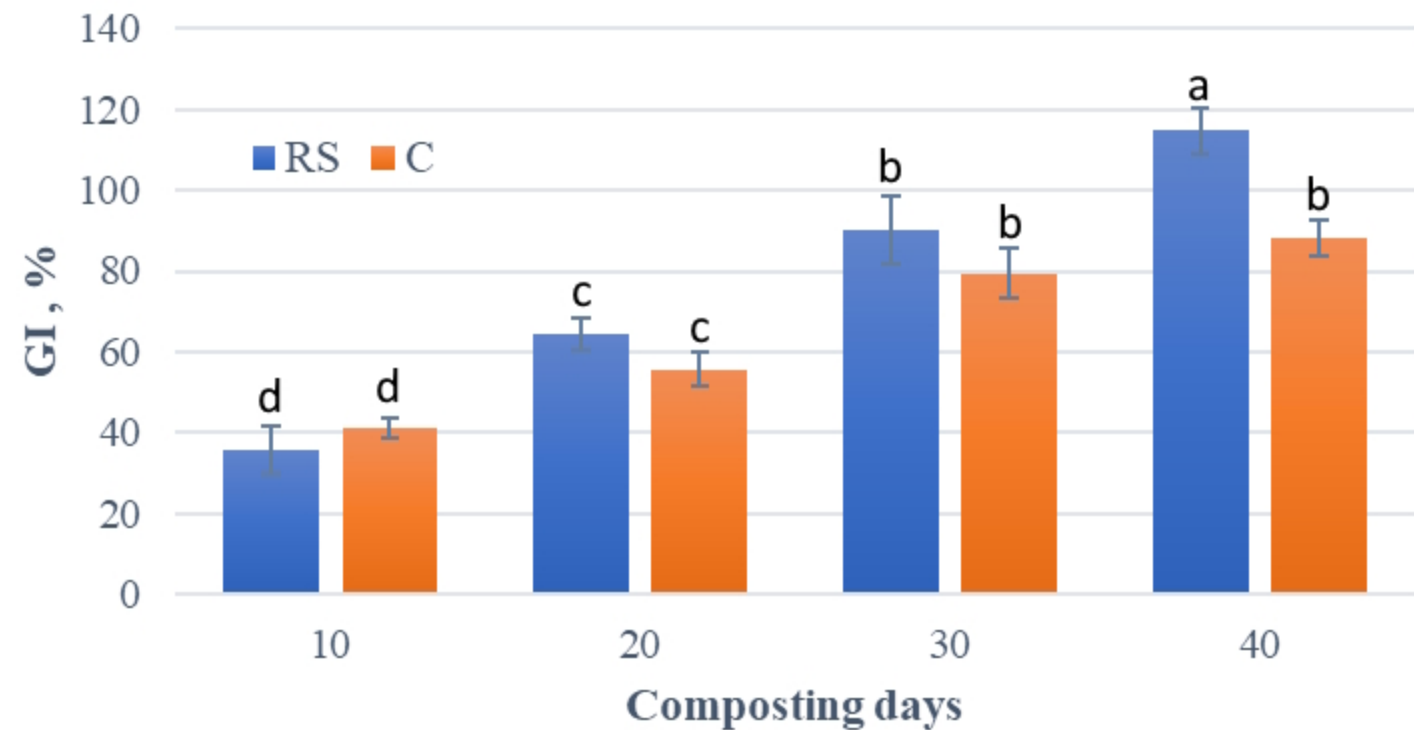


Figure 4

The phytotoxicity test of the wheat straw compost using the germination index of white mustard seeds (*Sinapis alba* L.)

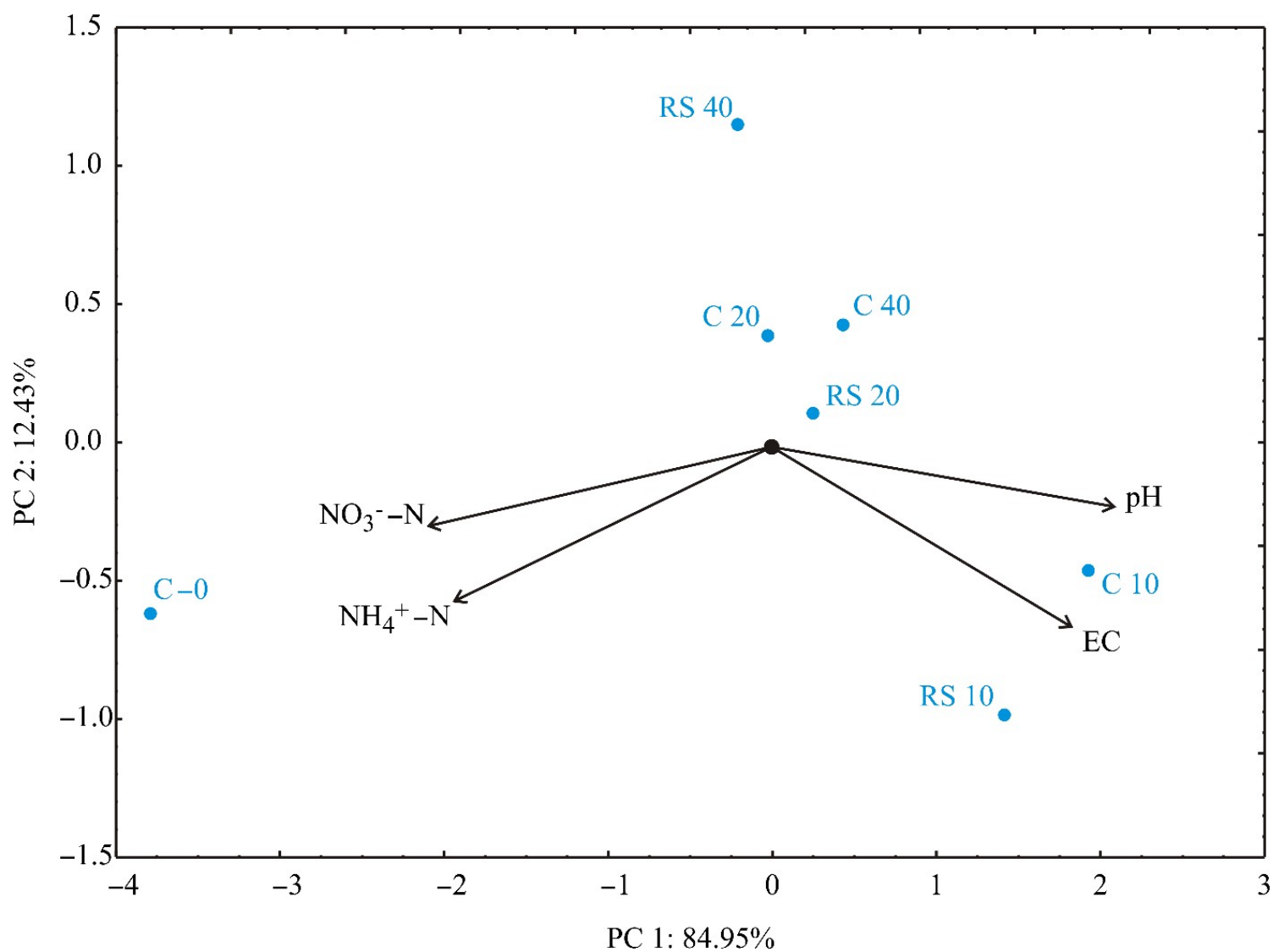


Figure 5

PCA analysis of the chemical parameters of the wheat straw during the composting period

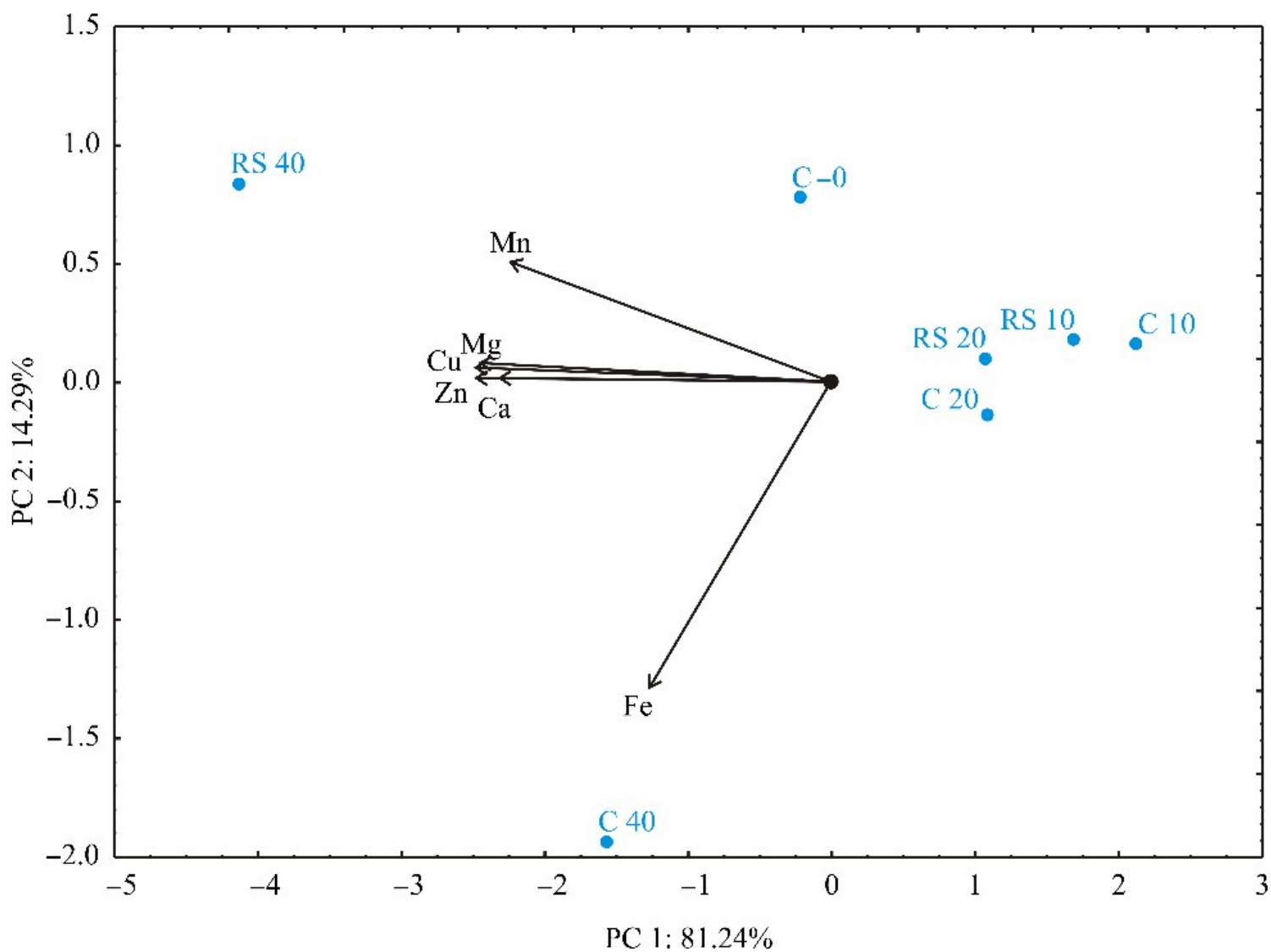


Figure 6

PCA analysis of the mineral parameters

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