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Keratinolytic Bacteria (*Bacillus pumilus*) Enhance Decomposition of Poultry Feather Waste

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Abstract: A pressing demand exists for economically viable and environmentally sound strategies to valorize poultry feather waste, with keratinolytic bacteria demonstrating notable potential in meeting this imperative. The keratinolytic strain Bacillus pumilus NM03, isolated from live poultry, exhibits robust feather degradation capabilities. This study investigates the impact of temperature variations on compost quality, employing wheat and coriander straw, with and without thermophilic microbial inoculation. Temperature @ 37°C + inoculum significantly reduced total nitrogen loss (56.20 and 69.80%) and total organic carbon degradation (47.06 and 62.41%) in coriander and wheat straw composting, respectively, when compared to temperature @37°C. For different regression models, the higher and lower R^2 values were estimated for the linear-linear and log logistic models for coriander with feather composting, and the linear-linear and linear models for wheat with feather composting, respectively. The principal component loading matrix obtained from correlation matrix reveals that the first three components whose eigen values are greater than 1, together account for about 91.70% and 87.69 % of the total explained variance in coriander and wheat with feather composting, respectively. Furthermore, our results showed that temperature @ 37°C + inoculum were most suited for feather composting with wheat and coriander straw for total organic carbon and total nitrogen breakdown was expedited for increased bacterial richness and diversity, as well as *Bacillus pumilus* overrepresentation. The study highlights the effectiveness of *Bacillus pumilus* in converting poultry feathers into valuable resources, underscoring its potential for sustainable waste management and resource utilization.

Keywords: Poultry feather waste; Keratinolytic bacteria; Composting; Thermophilic microbial inoculation; Total nitrogen & carbon loss

Introduction

Over 847 and 135.7 thousand metric tonnes of coriander seeds were produced annually in India and Rajasthan in 2022–2023, respectively (https://dasd.gov.in). In coriander straw, the seed yield was around 1.5 times more. Therefore, coriander straw cannot be used as animal feed. As a result, underutilised coriander straw that is composted is an excellent option for producing wealth resources. In contrast, wheat straw is fed to animals (Mhaskey et al. 2021). In the current rabi (winter) season of the 2022–

23 crop year, wheat has been planted on 54,000 hectares, up 59% from 34,000 hectares at the same time previous year (https://economictimes.indiatimes.com). The necessity to develop a more sustainable and secure food supply chain is becoming more and more critical in today's society in order to reduce straw waste. In this context, composting is a natural and environmentally responsible procedure whereby organic waste is converted via biological processes into organic fertiliser and soil conditioners (Gautam et al. 2010; Alexander, 1999; Zahir et al. 2014).

Poultry is one of the fastest growing segments of the agricultural sector in India with around 8% growth rate per annum (Chatterjee and Rajkumar 2015). Poultry farming in Rajasthan has witnessed steady growth, driven by rising consumer demand for poultry products. However, this expansion has brought attention to the environmental challenges associated with poultry feather waste. Globally, 8.5 billion tons of feathers are generated annually, with approximately 350 million tons generated in India alone. The large number of feather waste causes environmental problems and contaminates the air, water, and soil. The research on the efficacy of keratinolytic bacteria, in decomposing poultry feathers represents a pioneering effort with significant potential impact on waste management and resource utilization. Poultry feather production has emerged as a concern due to its non-degradability and potential to cause environmental pollution. The accumulation of poultry feathers, which are rich in keratin, poses challenges in terms of disposal and management. Feather waste has the potential to release pollutants into soil and water bodies, leading to soil contamination and disrupting local ecosystems. Our research focuses on addressing this critical issue by exploring the efficacy of keratinolytic bacteria, particularly *Bacillus pumilus*, in decomposing poultry feathers, thereby offering a sustainable solution to mitigate the environmental impact of poultry feather waste in Rajasthan and India. To produce an end product with the best quality for success after field application, the composting processes must be appropriately handled and the gradual changes with time of the physical-chemical features of composts must be properly controlled (Ballardo et al. 2020; Hashim et al. 2022). Due to its impact on the activity and variety of microorganisms, compost temperature is a crucial component in the efficiency of composting (Ajmal et al. 2012; Sokač et al. 2022; Sayara et al. 2020).

The release of byproducts into the environment is linked to the intense growth of human economic activity, including agriculture, animal production, and the leather processing industries (Bazrafshan et al. 2016; Tesfaye et al. 2017). Even while the production of other animal products produces less waste animal tissues from the poultry sector, managing the waste of hardly biodegradable keratin, particularly feathers, presents considerable challenges (Krishnaiah et al. 2020; Farhad et al. 2021). Particularly, the use of microorganisms that secrete the keratinase enzyme for the removal and use of waste keratin has showed great promise (Duan et al. 2020). Rajput and Gupta (2013) analysed the previous study on the thermostable keratinase from *Bacillus pumilus* KS12. In this study, we looked into the degrading potential of straw made of wheat and coriander that had bristles shaped like those found on chickens. Then, we looked into *Bacillus pumilus* NM03's microbial activities and capacity to make clean compost products by composting.

The escalating accumulation of poultry feather waste presents a pressing environmental and economic challenge, necessitating innovative solutions for its management and valorization. Traditional methods for disposing of poultry feathers often prove inadequate, leading to environmental pollution and resource wastage. In this context, the utilization of keratinolytic bacteria, such as *Bacillus pumilus*, emerges as a promising strategy for converting poultry feathers into valuable resources. The necessity of this research lies in addressing the urgent need for economical and environmentally responsible approaches to manage poultry feather waste. By harnessing the keratinolytic properties of *Bacillus pumilus*, this study aims to explore novel avenues for transforming poultry feather waste into wealth, thereby mitigating environmental pollution and promoting sustainable resource utilization.

Overall, this study endeavors to bridge the gap between waste management and resource utilization by introducing a novel and innovative approach for converting poultry feather waste into valuable assets. Through its exploration of the efficacy of keratinolytic bacteria, this research aims to contribute to the development of sustainable solutions for waste management and resource recovery in the poultry industry.

The experiment was designed in a randomized block design with three replications, encompassing various temperature combinations involving *Bacillus pumilus* NM03. This comprehensive approach, comprising eight distinct treatments with different straw types, provides novel insights aimed at optimizing commercial composting practices. The present study investigates the microbiological degradation process of keratin by bacteria in wheat and coriander straw, supplemented with poultry feathers, under varying temperature conditions. The primary objective is to assess the abundance of keratin-degrading bacteria in response to compost ingredients, while also establishing bio-resources and cost-effective methods for keratin recycling. Specifically, the research aims to evaluate the efficacy of *Bacillus pumilus* NM03 in decomposing feather waste when applied alone or in conjunction with agricultural waste, and to analyze the nutritional quality of the resulting compost.

Material and methods

Preparation of compost using poultry feathers waste by B. pumilus NM03

Microbiological Inoculum

The microbiological inoculum was obtained from a ICAR-NRCSS, Ajmer's laboratory, enriched from poultry feathers. The two types of microorganism's inoculum contained, Keratinolytic bacteria (*Bacillus pumilus*) NM03 and *Bacillus licheniformis* NM18. *B. pumilus* NM03, as its ability to efficiently degrade poultry feathers within 48 hours of incubation with higher specific activity (1150.48 U/mg DNA) against *B. licheniformis* NM18 (789.68 U/mg DNA), was used to prepare compost using feather as a raw substrate. *Bacillus pumilus* is a versatile species, and several general-purpose media can support its growth. Nutrient agar or tryptic soy agar are commonly used, providing a balanced mix of nutrients. *Bacillus pumilus* typically grows well at temperatures around 30-37°C and a neutral to slightly alkaline pH.

Compost preparation

To obtain the appropriate C: N ratio, a carefully balanced mixture of organic materials possibly including coriander straw, poultry feathers, and other agricultural residues were used to prepare the substrate. Plastics baskets (500 gm) of size 30 cm*24 cm*10 cm were used with a final compost weight of 480 gm (Approximately 500 grams of baskets were utilized for compost formation, resulting in the production of approximately 480 grams of compost after a 60-day period). The ratio of the additives applied to the compost was maintained at a 5:2:1 ratio (300 gm feathers, 108/96 gm wheat straw/coriander straw, and 63 gm manure). The ratio was calculated as the initial C: N values in feather, wheat/coriander straw and manure. A crucial stage was microbial inoculation, in which *Bacillus pumilus* was probably added to promote keratin degradation and increase the overall effectiveness of composting. The precise management of variables including temperature (30–37°C), aeration, and moisture content (60–70%) to produce the perfect habitat for microbial activity was crucial. Composting bins or reactors were a part of the experimental setup, which allowed for controlled decomposition. Regular monitoring and changes were made to guarantee ideal conditions were maintained during the composting time. All compost trials at different temperature level have maintained moisture levels above their controls.

Treatment details

The total treatments for composting were of eight types summarized in Table 1.

Table 1 - The treatment types.

Treatment	Details
T1 (25°C)	Temperature @ 25°C
T2 (37°C)	Temperature @ 37°C
T3 (60°C)	Temperature @ 60°C
T4 (I+25°C)	Temperature @ 25°C + inoculum (*B. pumilus)*
T5 (I+37°C)	Temperature @ 37°C + inoculum (*B. pumilus)*
T6 (I+60°C)	Temperature @ 60°C + inoculum (*B. pumilus)*
T7 (N+C)	Natural condition + Control
T8 (N+I)	Natural condition + inoculum (*B. pumilus)*

The experiment conducted in the randomized block design with three replications. Bacterial inoculum was introduced at day 2 and the moisture content was set to the recommended level of 60% before the inoculation event. Compost was turned on alternate days and moisture level was maintained simultaneously by spraying water.

Measurement of the Physicochemical Characteristics of the compost

Compost temperature changes were recorded daily. Temperature levels at the surface, middle, and bottom of the layers were measured three times using digital thermometers. The average temperature of each sample was used for the analysis. All analytical tests *viz.* estimation of Carbon, Hydrogen, Nitrogen, Sulphur (CHNS analyser, Flash, 2000, *Thermoscientific*), Phosphorus (Piper, 1950), Potassium (Flame photometer) and analysis of micronutrients Cu, Zn, Mn, Fe (Atomic absorption spectrophotometer AAS4136A) were performed when complete degradation of feather and straw was achieved for treatments as well as controls. Composite samples were collected randomly from the baskets in triplicate and grind & oven dried prior to analysis. pH and electric conductivity (EC) was also measure using pH meter and Conductimeter 304 (Systronics).

Statistical Analyses

Differences in physicochemical parameters and microbiological inoculum were subjected to analysis of variance. The difference between any two observed data was compared using Duncan’s tests with R 3.3.2 at a statistical significance of $P < 0.05$. Pearson correlation and principal component analysis (PCA) were employed to evaluate relationships between physicochemical variables, using FactoMineR, ade4, stats, ca, MASS and ExPosition packages of R 3.3.2. Regression models of C: N ratio were evaluated by r-squared values using Agroreg packages of R 3.3.2.

Results and discussion

End-product characterizations

A final physical–chemical characterization of the raw materials and the compost (Coriander and wheat) samples is shown in table 2. Data analysis was done independently for each temperature in order to have a better understanding of the chemical changes brought on by heating and to assess how the compost's behaviour is affected by its early maturation. The higher C: N ratio (Figure 1) was observed with coriander straw at 37°C + inoculum *i.e.* 7.3, followed by compost prepared using wheat straw at 37°C + inoculum (7.0). However, compost prepare in natural conditions + inoculum have highest C: N ratio i.e. 7.8 using coriander straw as compared to wheat straw i.e. 7.7. The results in the present study showed that increase in C/N ratio was clearly observed in the treatments (compost with inoculum) level exceptionally at 25°C with both straw (Figure 1) due to release of nitrogen in the terms of ammonia from keratinous substrate (feather keratin) excepting the treatment level at 25°C with coriander straw. The linear decrease in the C: N ratio observed in all treatments over time would likely lead to an increased emission of both NH_3 and N_2O (Amlinger et al. 2008). According to Raut et al. (2008), this reliance could result from a drop in C/N. Microbial growth was promoted by the increase in nitrogen availability and reduction in organic carbon availability that occurred during the composting of lignocellulosic waste (Nahm 2003; Miyatake and Iwabuchi 2006; Waqas et al. 2018).

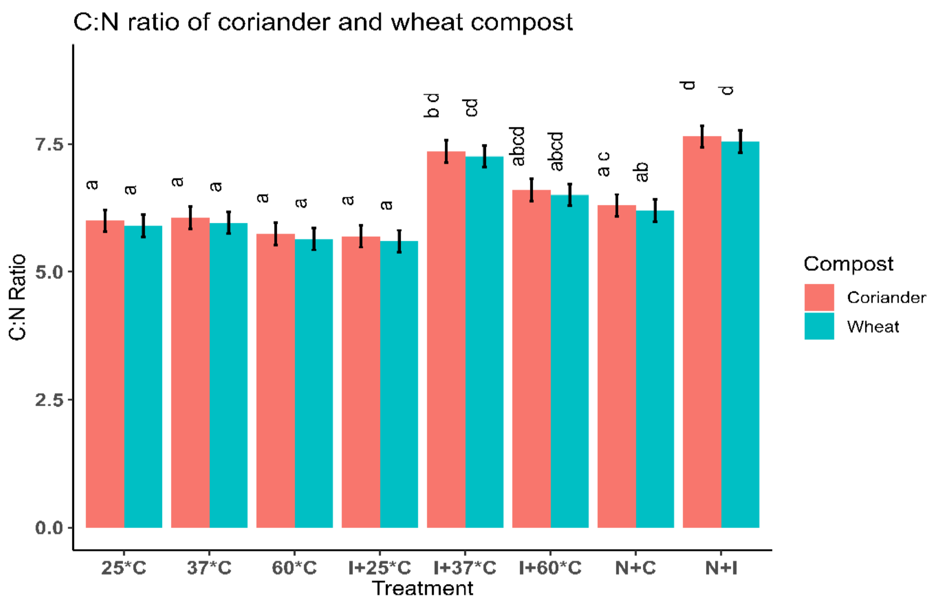

Figure 1 - Comparative analysis of C: N Ratio of coriander and wheat straw compost with feather

The N: P: K: S: H of the compost was found to lowest at Temperature @ 37°C + inoculum as compared to remaining treatments (Table 2). Mineral composition *i.e.* Cu^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+} were more or less similar for coriander and wheat straw at all the temperature. Temperature @ 37°C + inoculum significantly reduced total nitrogen loss (56.20 and 69.80%) and total organic carbon degradation (47.06 and 62.41%) in coriander and wheat straw composting, respectively, when compared to temperature @37°C. Feather degradation rate was found to be in decreasing order of temperature 60

>37 >25°C. However, no fungal growth was observed at 60°C whereas several colonies of fungal genera were seen in compost trial at 25 and 37°C. The compost without inoculum and supplement (control) has shown very little activity over the 60 days' period at the above mentioned temperatures. The waste contained large amounts of keratinolytic materials, so keratin degrading microorganisms were thought to be essential for biodegradation of the waste. The smell of ammonia, noted during composting, was most probably due to the accumulation of proteinacious keratin material at 37°C. No such smell was observed at 60°C and very little at 25°C during the 10 days' period. Comparing the time required to finish the composting process, the quality of end-product obtained (based on extent of degradation of waste, final C/N ratio and texture and structure of the final compost) coriander compost with feathers was found to be the best supplement for composting of such highly keratinoluytic waste. Similar investigations were also conducted by Kianirad et al. (2010) and Lalremruati and Devi (2021).

Throughout the composting process, it is anticipated that the C:N ratio will decrease, with its ultimate value serving as a key indicator of the relative maturity of the resulting product. The observation that all values of the C/N ratios fall below 15, which represents the maximum recommended threshold for agronomic application, suggests that the compost samples exhibit a maturity level suitable for such utilization, aligning with established agronomic guidelines.

The change of pH from slightly acidic (depending on the nature of waste and supplements used) to alkaline range can be attributed to the accumulation of alkaline metabolic products of microorganisms generated during the process of composting. At the final stage, the bacterial and keratinolytic bacterial populations showed marked increase while the fungal population showed a drastic decreased. The probable reason for bacterial dominance during the composting process is the increase in temperature, making conditions unfavourable for most of the fungal growth but allowing bacteria to dominate over fungi. The data were also confirmed with change in pH values where acidic pH range was observed for controls (alone temperature) and alkaline for all treatments (temperature+ inoculum) (Figure 2). The highest pH 7.63 was observed at 37°C for wheat composting. Also observed complete decomposition of both straws within 60 days which was started at 30 days. Aeration tends to decrease CO_2 level in the compost, which in turn will tend to increase pH (Singh et al.2012).

Electrical conductivity was found to be maximum at all control levels compared to treatments (compost with inoculum) (Figure 3). Highest EC was observed for coriander control trial at 25°C *i.e.* 3.17 dSm^{-1}. Maximum decrease in EC value was observed at Temperature @ 60°C + inoculum in coriander compost, which was 1.16 $dSm^{-1.}$ This is showing the more ions for conductivity and acidic nature of compost at control level whereas presence of inoculum in treatments level reduce the amount of ions thus decrease in conductivity was observed. Composting results in levels in the range of 6.5-7.5, as expected for this kind of material, as well as a progressive decrease in both moisture content and electrical conductivity and an increase in pH values. If electrical conductivity was higher, the composts would be considered immature (Figure 3). While the decline in EC values during the composting process is directly related to increased concentrations of nutrients like nitrates and nitrites (Pathak et al. 2012; Comino et al. 2017; Fuentes et al. 2020). The elevated electrical conductivity (EC) values observed suggest that additional maturation is necessary for all composting trials to achieve a level of safety suitable for plant utilization.

Table 2 - Analysis of compost prepare by B. pumilus NM03 at different temperature viz. 25, 37, 60°C using poultry feathers waste as a raw substrate supplemented with coriander and wheat straw

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Treatment	Coriander compost chemical properties									
	N	C	H	S	K	P	Cu	Mn	Zn	Fe
25°C	3.29^{c}	21.04^{b}	3.06^{bc}	0.56^{b}	8.60^{c}	5.40^{b}	0.24^{abc}	1.23^{bc}	0.92^{a}	12.24^{d}
37°C	4.43^{a}	25.73^{a}	3.38^{b}	0.61^{b}	9.51^{a}	5.60^{b}	0.31^{ab}	1.37^{abc}	1.01a	12.91^{d}
60°C	4.20^{b}	24.81^{a}	4.01^{a}	0.70^{a}	9.04^{b}	4.83^{c}	0.21^{cd}	1.26^{bc}	0.76^{b}	14.29^{c}
I+25°C	3.13^{c}	15.83^{d}	2.71^{d}	0.02^{d}	5.92^{f}	4.34^{d}	0.28^{abc}	1.30^{bc}	0.72^{b}	13.70^{c}
I+37°C	1.94^{f}	13.62^{e}	2.17^{e}	0.02^{d}	4.66^{h}	2.48^{g}	0.24^{bc}	1.35^{bc}	0.38^{e}	16.31^{a}
I+60°C	2.67^{d}	18.67^{c}	2.93^{c}	0.03^{d}	6.34^{e}	3.33^{e}	0.34^{a}	1.45^{ab}	0.56^{cd}	15.20^{b}
N+C	4.40^{ab}	26.18^{a}	4.25^{a}	0.32^{c}	7.79^{d}	5.84^{a}	0.13^{d}	1.18^{c}	0.64^{bc}	10.91^{e}
N+I	2.40^{e}	19.78^{bc}	3.15^{bc}	0.07^{d}	5.13^{g}	2.81^{f}	0.27^{abc}	1.59^{a}	0.52^{d}	15.45^{b}
SEm	0.07	0.45	0.11	0.03	0.11	0.07	0.03	0.08	0.03	0.24
LSD	0.21	1.37	0.33	0.08	0.34	0.21	0.09	0.23	0.11	0.72
Treatment	Wheat compost chemical properties									
	N	C	H	S	K	P	Cu	Mn	Zn	Fe
25°C	3.22^{c}	17.80^{d}	2.87^{cd}	0.28^{c}	8.24^{a}	5.04^{a}	0.30^{a}	1.30^{b}	0.89^{a}	11.79^{cd}
37°C	5.53^{a}	34.16^{a}	5.15^{a}	0.47^{a}	6.88^{b}	4.24^{b}	0.17^{bc}	1.37^{ab}	0.72^{b}	12.34^{bc}
60°C	3.19^{cd}	17.76^{d}	2.77^{d}	0.17^{d}	6.99^{b}	4.01^{b}	0.16^{c}	1.36^{ab}	0.70^{b}	11.05^{d}
I+25°C	2.77^{de}	17.14^{d}	2.80^{cd}	0.07^{ef}	7.42^{b}	4.31^{b}	0.23^{abc}	1.32^{b}	0.69^{bc}	13.24^{b}
I+37°C	1.67^{f}	12.84^{e}	1.73^{e}	0.02^{f}	5.59^{c}	2.55^{c}	0.19^{bc}	1.55^{ab}	0.40^{d}	15.84^{a}
I+60°C	1.74^{f}	10.47^{f}	1.69^{e}	0.05^{ef}	5.70^{c}	2.46^{c}	0.27^{ab}	1.66^{a}	0.53^{cd}	15.20^{a}
N+C	3.90^{b}	25.27^{b}	4.38^{b}	0.38^{b}	8.28^{a}	3.89^{b}	0.16^{c}	1.41^{ab}	0.98^{a}	8.58^{e}
N+I	2.74^{e}	19.29^{c}	3.17^{c}	0.12^{de}	5.90^{c}	2.75c	0.29^{a}	1.65^{a}	0.58^{bc}	9.09^{e}
SEm	0.14	0.37	0.12	0.02	0.24	0.15	0.035	0.102	0.05	0.32
LSD	0.43	1.12	0.37	0.085	0.73	0.46	0.104	0.307	0.16	0.98

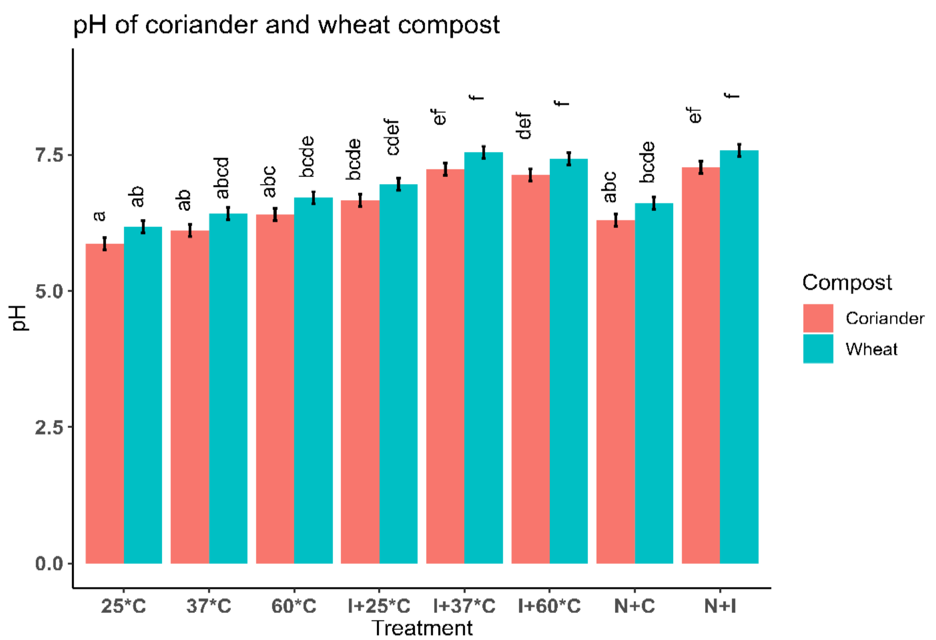

Figure 2 - Comparative analysis of pH of coriander and wheat straw compost

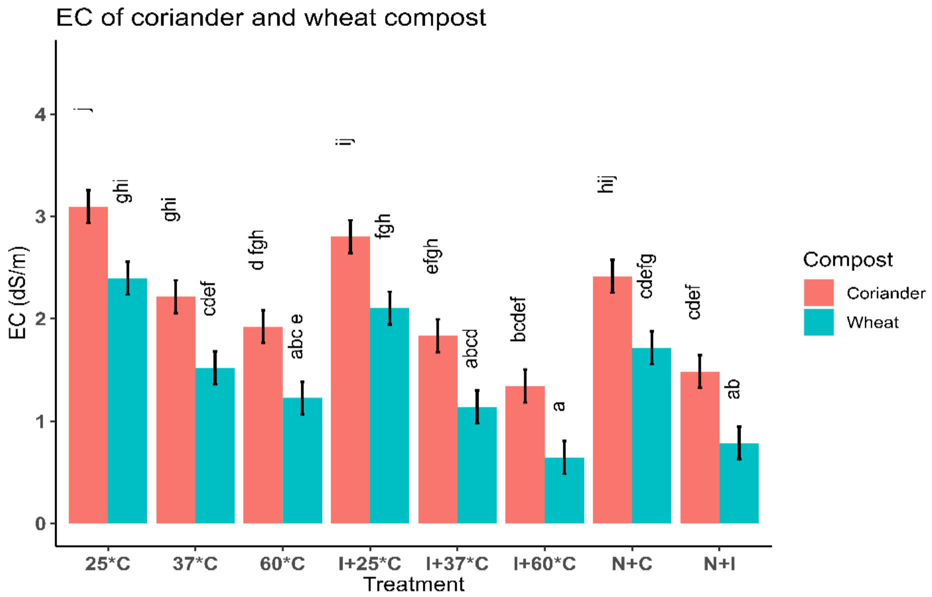

Figure 3 - Comparative analysis of EC of coriander and wheat straw compost

Regression models for coriander and wheat composting

The Regression parameters of the tested regression models concerning C: N Ratio (independent variable) and treatment (dependent variable) in coriander and wheat straw compost (Figure 4 and 5). For the examined linear and nonlinear models, significant R^2 values were confirmed. These values ranged from 0.17 to 0.54 with regard to C: N of coriander compost (Table 2) and 0.34 to 0.42 with regard to C: N of wheat compost (Table 3). The higher and the lower R^2 values were estimated for the linear-linear and log logistic models, respectively, for coriander compost and linear-linear and linear models, respectively, for wheat compost. The RMSE ranged from 0.615 to 0.824 and 0.543 to 0.581, with regard to the model inputs coriander and wheat compost, respectively. The lower and the higher values of RMSE were confirmed in the cases of linear-linear and log-logistic models for coriander and linear-linear and linear models for wheat, respectively, irrespective of the aforementioned variables. The following regression models are carried out for coriander compost:

Y = 5.41 +0.223x (Linear model) ………………………………………………Eq (1)

$Y = \frac{16.67}{1+e^{-0.15(\log(x)-4.39)}}$ (Logistic model) …………………..……………………Eq (2)

Y = 6.53-0248x (x<3.89) + 0.769x (x>3.89) (Linear. Linear model) ………… Eq (3)

$Y = 6.38 – 0.217\ x + 0.0063\ x^3$ (Cubic model) ……………………………… Eq (4)

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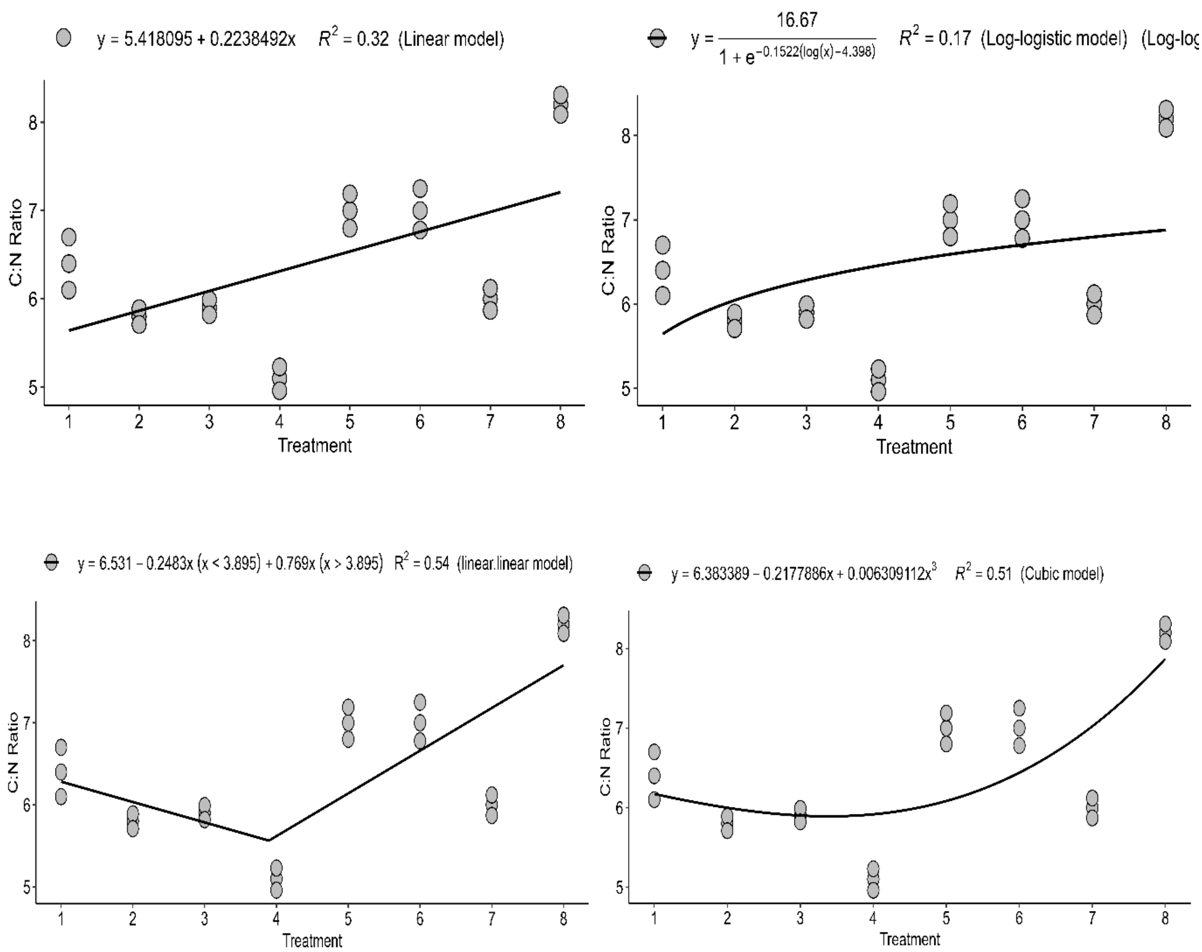


Figure 4 - Comparative study of regression models for dependent variable (C: N Ratio) and independent variable (treatment) in coriander composting. Regression models represent: (a) Linear; (b) log-logistic; (c) linear-linear; (d) cubic model.

Table 3 - Regression parameters of the tested regression models concerning C: N Ratio (independent variable) and treatment (dependent variable) in coriander.

Parameters	Linear	Log-logistic	Linear. Linear	Cubic
Mean Bias Error	0	-0.001	0	0
Relative Mean Bias Error	0	-0.011	0	0
Mean Absolute Error	0.616	0.697	0.5	0.524
Relative Mean Absolute Error	9.592	10.846	7.787	8.15
Squared error	13.469	16.301	9.078	9.741
Mean squared error	0.561	0.679	0.378	0.406
Root Mean Square Error	0.749	0.824	0.615	0.637
Relative Root Mean Square Error	11.659	12.826	9.572	9.915
Modelling Efficiency	0.319	0.176	0.541	0.508
Standard deviation of differences	0.765	0.842	0.628	0.651
Coefficient of Residual Mass	0	0	0	0

Agreement Coefficient	-0.646	-2.115	0.338	0.244
Unsystematic Agreement Coefficient	-0.188	-0.89	0.439	0.371
Systematic Agreement Coefficient	0.542	-0.225	0.899	0.873

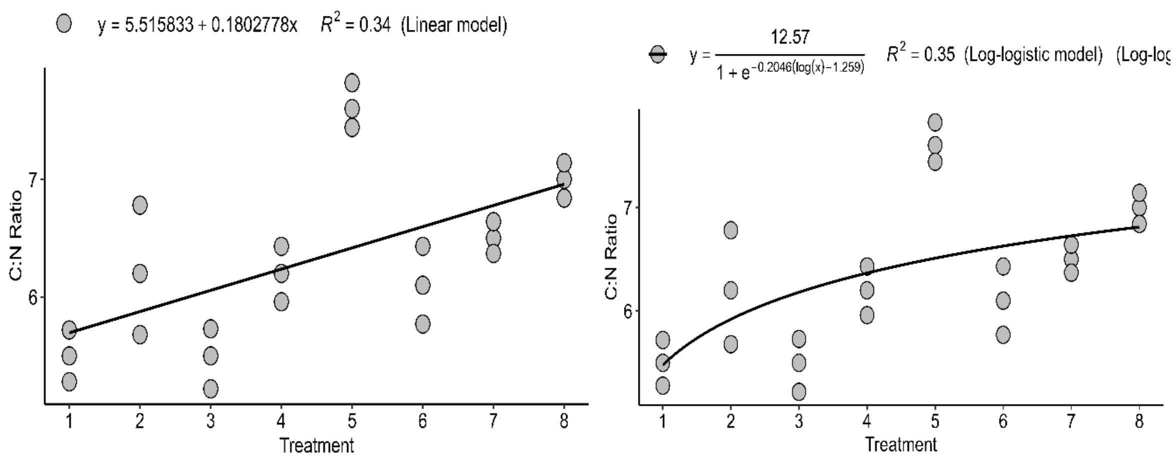
The goodness of fit of the tested regression models, from a purely statistical point of view, taking into account that the best and the worst models were characterized by the combinations of maximum R^2 with minimum RMSE and minimum R^2 with maximum RMSE, respectively, followed the descending orders linear-linear>cubic>linear>log-logistic of C: N for coriander compost as input variable (Table 3) and linear-linear>cubic≈log-logistic> linear of C: N for wheat input (Table 4). When comparing the regression models of the same type to investigate the effectiveness of the independent variables (C: N ratio) for predicting, coriander crop models was superior to wheat crop in all cases, due to its coincidence with higher R^2 and lower RMSE. The following regression models are carried out for wheat compost:

$$Y = 5.51 + 0.180x \text{ (Linear model)} \qquad \text{Eq (5)}$$

$$Y = \frac{12.57}{1+e^{-0.20(\log(x)-1.25)}} \text{ (Logistic model)} \qquad \text{Eq (6)}$$

$$Y = 5.13 - 0.325x\ (x<5) + 0.359x\ (x>5) \text{ (Linear. Linear model)} \qquad \text{Eq (7)}$$

$$Y = 5.27 - 0.289x + 0.0015x^3 \text{ (Cubic model)} \qquad \text{Eq (8)}$$



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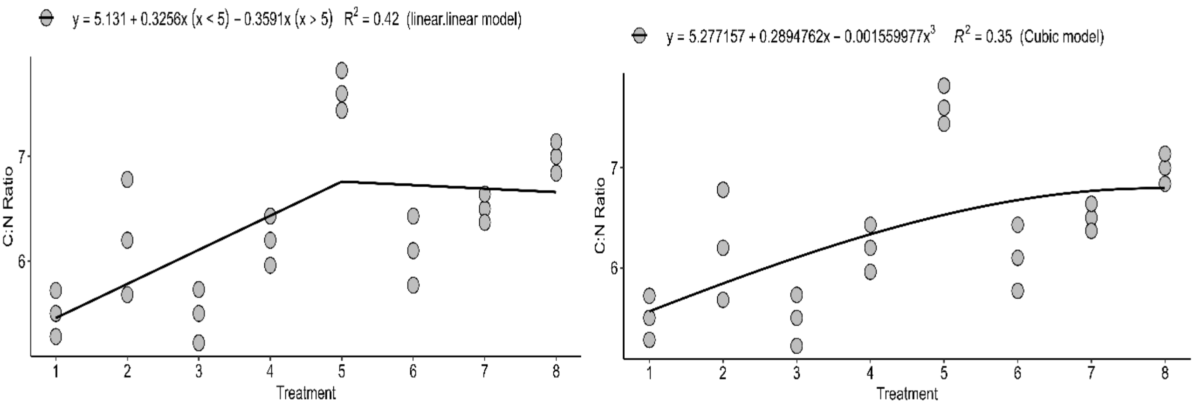

Figure 5 -Comparative study of regression models for dependent variable (C: N Ratio) and independent variable (treatment) in coriander composting. Regression models represent: (a) Linear; (b) log-logistic; (c) linear-linear; (d) cubic model.

Table 4 - Regression parameters of the tested regression models concerning C: N Ratio (independent variable) and treatment (dependent variable) in wheat composting.

Parameters	Linear	Log-logistic	Linear. Linear	Cubic
Mean Bias Error	0	0	0	0
Relative Mean Bias Error	0	0.005	0	0
Mean Absolute Error	0.44	0.446	0.442	0.451
Relative Mean Absolute Error	6.949	7.043	6.991	7.127
Squared error	8.088	7.911	7.075	7.86
Mean squared error	0.337	0.33	0.295	0.328
Root Mean Square Error	0.581	0.574	0.543	0.572
Relative Root Mean Square Error	9.175	9.074	8.581	9.045
Modelling Efficiency	0.336	0.351	0.419	0.355
Standard deviation of differences	0.593	0.586	0.555	0.585
Coefficient of Residual Mass	0	0	0	0
Agreement Coefficient	-0.635	-0.536	-0.117	-0.478
Unsystematic Agreement Coefficient	-0.2	-0.142	0.122	-0.104
Systematic Agreement Coefficient	0.565	0.606	0.761	0.625

Association of different temperature with physicochemical properties during the coriander composting

Two PCs accounted for 79.89% of the total variance, according to the results of the principal component analysis (PCA) done on the final study, which also included the mass loss during the thermal treatment as a variable. As shown in Figure 6's biplot of scores and loadings. The PC1 axis has more final compost sample locations. This is mostly caused by variations in C, N, and H content, which represent the samples' heterogeneity. The samples exhibit higher C and H content (with negative values for the loadings of PC1), whereas completed compost shows higher N contents (with positive values for the loadings of PC1), which results in a higher C/N ratio. As can be observed from its positive value for the PC2-loading, the percentage of mass loss increases with rising temperatures and is especially pronounced in samples of younger compost. This is caused by the fact that at higher temperatures, the H concentration decreases and the C content substantially rises.

The applicability of the PCA to the data sets used in this study was verified through the application of Bartlett's sphericity test. In the latter phases of composting, the PCA showed a significant impact of temperature on the physicochemical parameters of the compost ($R^2 = 0.94$, $P = 0.001$, Figure 6A). The first two principal components explained 65.6 % (PC1) and 14.3% (PC2) of the total variation of the compost properties (Figure 1A). The PC was mainly explained by variation in P (~11.5%), K (~10.5%), N (~10%), pH (~10%), S (~8.5%), Fe (~8.5%) and Zn (~8.3%), while EC (~27%), H (~24%), C (~16%), C: N (~13%) and Cu (~8.5%) explained of the variation on the second PC (Figure 6B).

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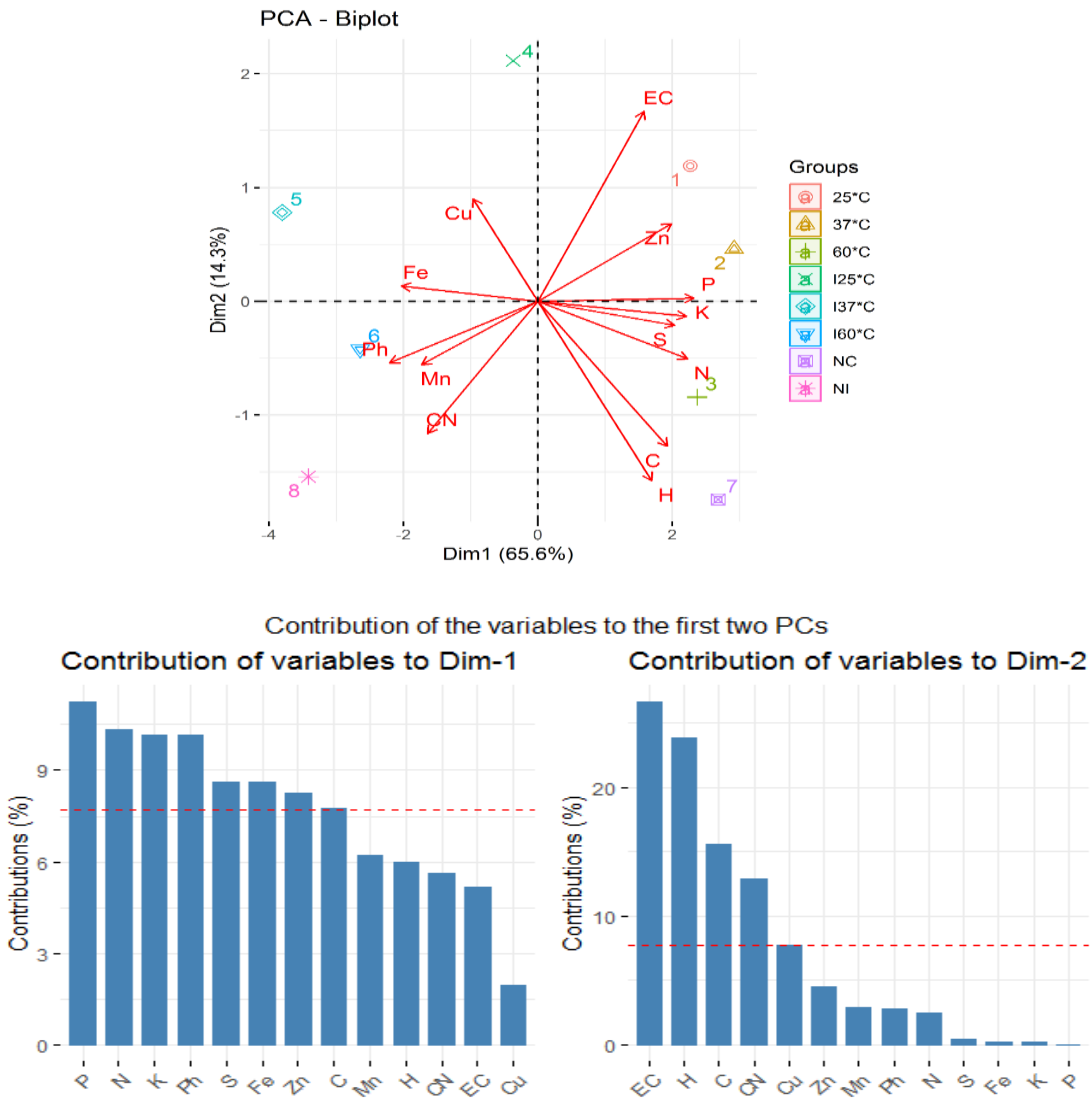

Figure 6 - Principal component analysis of the physicochemical compost properties at final (filled symbols) phases of composting. In panel (A), colours correspond to different types of tratment used for composting. Panel (B) shows the contribution of each physiochemical property on the first two principal components in panel A that explained 79.89% of the total variation (65.6 and 14.3% for PC1 and PC2, respectively). Red dashed lines in panel (B) indicate the theoretical contribution of physicochemical properties.

The results of PCA are summarized in 5. Since the eigenvalues of the first three Principal Components (PCs) are greater than 1, Kaiser's approach was applied to these PCs while excluding the other PCs. The Eigen value for that axis divided by the entire variance, or the sum of the diagonal of the cross-products matrix, yields the variance proportion. The component's varying loads are shown by the bolded number (loadings > 0.3). According to Table 4, the first PC displayed substantial loadings of N, K, and P with a positive impact and a negative impact on pH. The second PC had negative effects for C, C: N, and H and positive effects for EC. Cu, Mn, and Zn were negatively correlated with the third PC.

Table 5 - Summarization of Principal Component Analysis for coriander composting.

	PC1	PC2	PC3
Eigenvalue	8.53E+00	1.86E+00	1.53E+00
Variance (%)	6.56E+01	1.43E+01	1.18E+01
Cumulative variance (%)	65.6	79.89	91.7
Standard deviations	2.92E+00	1.36E+00	1.24E+00
Variables	Factor loadings		
N	0.321	-0.156	-0.032
C	-0.278	-0.394	-0.169
C:N	-0.236	-0.359	-0.211
H	-0.244	-0.487	0.049
S	0.293	-0.065	-0.228
K	0.318	-0.040	-0.242
P	0.335	0.009	0.035
Cu	-0.139	0.278	-0.620
Mn	-0.249	-0.171	-0.473
Zn	0.287	0.211	-0.353
Fe	-0.293	0.041	-0.189
pH	-0.318	-0.167	0.172
EC	0.227	0.515	0.105

Association of different temperature with physicochemical properties during the wheat composting

The principal component analysis is a good tool for screening out the insignificant parameters from the analysis. The PCA revealed a strong effect of different temperature effect on the physicochemical compost properties at final phases of composting (R^2 = 0.92, P = 0.001, Figure 1A). The first two principal components explained 58.6 % (PC1) and 18.4% (PC2) of the total variation of the compost properties (Figure 7A). According to the factor loadings, the PC was mainly explained by variation in pH (~10.5%), Zn (~10.2%), K (~10.1%), P (~9.8%), S (~9.7%), N (~9.2%), Mn (~8.7%) and H (~8.6%), while C (~17%), H (~13%), EC (~12.4%), Cu (~10.4%), C: N (~9.8%) and N (~9.7%) explained of the variation on the second PC (Figure 7B).

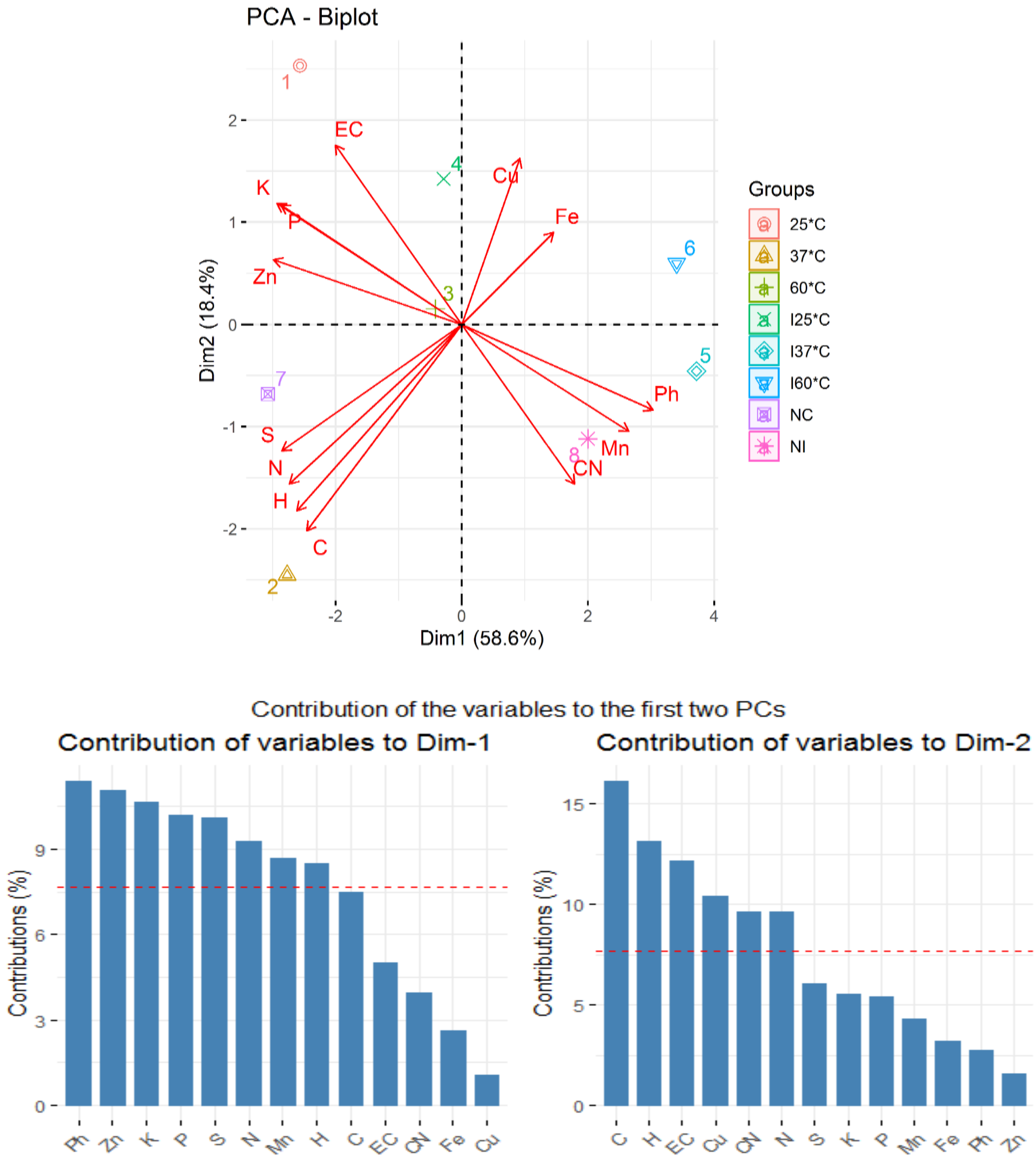

Figure 7 - Principal component analysis of the physicochemical compost properties at the initial (open symbols) and final (filled symbols) phases of composting. In panel (A), colours correspond to different types of manure used for composting. Panel (B) shows the contribution of each physiochemical property on the first two principal components in panel A that explained 77.0% of the total variation (58.6 and 18.4% for PC1 and PC2, respectively). Red dashed lines in panel (B) indicate the theoretical contribution of physicochemical properties.

The principal component loading matrix obtained from correlation matrix (Table 5) reveals that the first three components whose eigen values are greater than 1, together account for about 87.69 % of the total explained variance. Table 6 indicates that the first PC showed high loadings of N and pH with positive effect and K, S, Zn and P with negative effect. The second PC was associated with N, Cu and EC with positive effects and C, C: N and H with negative effect. The third PC was associated with Fe with positive effects and Cu, Mn and EC with negative effect. In conclusion, the PCA helped to reveal some relationships between some chemical properties of composting. It has proved to be useful approaches to characterization of soils based on their properties.

This microbe could degrade poultry feather within 60 days or before at 30-60°C temperature in combination with coriander and wheat straw resembles the general composting duration of other farm waste miraculously. Otherwise these feather remains undecomposed seasons together. Fast decomposition and quality produce (compost) solves the environmental problem and provides quality compost for farm input. Hence it makes useless waste of feather and coriander for useful purpose.

Table 6. Summarization of Principal Component Analysis for wheat composting

	PC1	PC2	PC3
Eigenvalue	7.62E+00	2.39E+00	1.38E+00
Variance (%)	5.86E+01	1.84E+01	1.06E+01
Cumulative variance (%)	58.64067	77.0412	87.69103
Standard deviations	2.76E+00	1.55E+00	1.18E+00
Variables		Factor loadings	
N	0.305	0.310	0.105
C	-0.274	-0.402	0.015
C:N	0.199	-0.310	-0.261
H	-0.292	-0.363	-0.094
S	-0.318	-0.246	-0.010
K	-0.326	0.236	-0.097
P	-0.319	0.233	0.160
Cu	0.102	0.323	-0.435
Mn	0.295	-0.208	-0.322
Zn	-0.332	0.126	-0.217
Fe	0.162	0.179	0.631
pH	0.337	-0.166	-0.110
EC	-0.223	0.349	-0.355

Practical applications of research

The research on the efficacy of Bacillus pumilus for decomposing poultry feathers, combined with coriander and wheat straw, presents scientifically grounded practical applications. The decomposed material, enriched with keratinolytic bacteria-derived nutrients, demonstrates significant potential for organic fertilizer production. This nutrient-rich amendment offers an environmentally sustainable alternative to synthetic fertilizers, contributing to soil fertility enhancement. The circular economy principles embedded in the study showcase a waste-to-wealth approach, where seemingly challenging poultry feathers are transformed into valuable agricultural resources. The application extends to improved soil structure and water retention, addressing concerns related to water scarcity in agriculture. Furthermore, the research offers insights into sustainable poultry farming practices by integrating on-farm feather decomposition, potentially reducing external waste disposal needs and fostering self-sufficiency. The potential for bio-based product development and contributions to mitigating greenhouse gas emissions further underscore the scientific and practical significance of the study in promoting sustainable agricultural and waste management strategies.

Conclusions

In conclusion, the utilization of chicken feather fiber in composite materials presents a novel avenue for the development of recyclable, cost-efficient, and environmentally advantageous materials. This investigation underscores the significant impact of temperature, both independently and in combination with microbial inoculation, on various physicochemical attributes of poultry composting involving coriander and wheat straw. Through enzymatic activity, the keratinolytic bacterium *Bacillus pumilus* NM03 plays a pivotal role in the stabilization and utilization of compost resources, thereby ensuring the production and maintenance of compost quality. Notably, the application of a temperature of 37°C in conjunction with inoculation significantly mitigated total nitrogen loss (by 56.20% and 69.80%) and total organic carbon degradation (by 47.06% and 62.41%) during coriander and wheat straw composting, respectively, compared to composting at 37°C alone. This microorganism, when introduced alongside coriander and wheat straw, accelerates the decomposition of chicken feathers within a timeframe of 60 days or less, under temperature conditions ranging from 30 to 60 ℃, resembling typical composting durations for other agricultural residues. Consequently, this approach not only resolves environmental concerns associated with waste accumulation but also yields high-quality compost rapidly, rendering it a valuable agricultural input. Furthermore, the observation that all C/N ratio values fall below 15, the recommended threshold for agronomic application, indicates that the compost samples have reached a maturity level suitable for agricultural utilization, thus aligning with established agronomic standards. As a result, the valorization of coriander and feather waste emerges as a sustainable practice with significant benefits for agricultural productivity and waste management.

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