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# Moisture-Induced Effects on Lignocellulosic and Humification Fractions in Aerobically Composted Straw and Manure

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**Abstract:** Humic substances affect compost stability and maturation. However, the intricate structure of lignocellulosic materials hinders the biodegradation of cellulose, hemicellulose, and lignin, often promoting the use of synthetic additives which results in microbial inactivation and death. Therefore, this study examined the effects of optimal moisture levels (MC1 = 45%, MC2 = 55%, and MC3 = 65%) on lignocellulosic and humification fractions in aerobically composted straw and manure. The study showed that 65% moisture content was more efficient in decomposing cellulose, hemicellulose, and lignin, with hemicellulose (115.3% $w/w \approx$ 47.1%) degrading more than cellulose (76.0% $w/w \approx$ 39.5%) and lignin (39.9% $w/w \approx$ 25.9%). However, in compost heaps with 45% moisture, the humic acid concentration increased significantly by 12.4% (3.1% $w/w$) and 17.3% (4.3% $w/w$) compared with 55% and 65% moisture, respectively. All moisture levels increased the mineralization of humic substances, but the index measured was highest at 65% MC (23.8% $w/w$) and lowest at 45% MC (18% $w/w$). In addition, the humification rate showed the trend: 0.083% $w/w$ > 0.087% $w/w$ > 0.100% $w/w$ for MC1, MC2, and MC3, respectively. Overall, the results indicate that an initial moisture content of 65% is aerobically efficient for the conversion of corn straw and cow manure into stable and mature compost.

**Keywords:** moisture content; aerobic composting; corn straw; cow manure; lignocellulosic fractions; humification indices



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## 1. Introduction

Agricultural byproducts, mainly livestock manure (3.8 billion tons) and crop straw (0.8 billion tons), are largely produced in China due to intensive livestock farming and crop production [1,2]. The exacerbated development of these wastes put pressure on croplands, causing various pollutions in most farming communities [3]. Meanwhile, agricultural by-products are biodegradable and can be converted into organic amendments to improve soil quality for sustainable crop production [4]. Although most organic materials have intricate structures, aerobic composting has proven to be a viable technique for transformation and nutrient build-up and a standard approach for ensuring end-product stability and maturation [5]. In addition, humic substances are important end-products of composting and play a key role in compost quality [6]. As a macromolecular organic material, humic substances have oxidized functional groups, including a complex composition of humic acids (HA), fulvic acids (FA), and humins (HU). These compositions (HA, FA, and HU) together with the ratio of humic acid (HA) and fulvic acid (FA) play a significant role in determining the degree of humification, maturation, and stabilization of compost [7,8], meaning that decomposition is not efficiently achieved under conventional composting process [9,10].

To overcome the recalcitrance of lignocellulosic materials, various treatments such as alkaline additives (synthetic, microbial, and mineral), hydrogen peroxide, steam explosion, and heat treatments are commonly used [11,12]. However, the indispensable use of these treatments inhibits microbial richness, diversity, and robustness, and under critical conditions, reduces their population through the die-off effect, resulting in incomplete fermentation and reduced compost quality [5,13,14]. Although China ranks first in the world for crop straw production (17.29%) [13], nearly 50% of the resource (corn, rice, wheat, and potato straw) is either burned or incorporated directly into the soil without proper treatment [1,14]. Burning remains a widely used method of biomass disposal and a traditional land preparation practice in rural and suburban areas in northwest China [15]. However, apart from wasting valuable biological resources in the region, burning has often propelled seasonal air pollution, the loss of soil organic matter, and the occurrence of cloudy weather. According to Zhang et al. [16], this phenomenon releases significant amount of pollutants such as particulate matter (PM2.5), sulfur dioxide ($SO_2$), carbon monoxide (CO), ammonia ($NH_3$), volatile organic compounds (VOC), and nitrogen oxides ($NO_X$), which adversely affect human health. In addition, the direct incorporation of straw into the soil has been shown to cause seed dormancy due to excessive heat development, while allowing termites to proliferate on cropland [17], which can result in partial or complete crop loss. However, our previous study shows that crop straw is rich in organic nutrients, highlighting its potential value for compost production when exposed to optimal moisture conditions.

Water plays a crucial role in organic-residue decomposition and humification, but different moisture levels have been reported, leading to inconsistencies and uncertainties in their application [18,19]. The relationship between water and organic materials is unique and depends on the physical, chemical, and biological properties of the resource, but these indicators have often not been considered [5]. Therefore, recommendations for moisture in composting should be resource-specific rather than a general rule to avoid disparities. Given the uncertainties surrounding the effects of moisture in composting, the study aims to achieve the following objectives: (i) Elucidate the mechanistic effect of water on lignocellulosic residues; (ii) Evaluate the transformative effects of water on lignocellulosic and humification fractions; (iii) Identify the optimal moisture level for compost maturation and stability; (iv) Propose potential approaches for measuring pile moisture levels during composting. The results of the study could improve composting practices in resource-limited settings.

## 2. Materials and Methods

### *2.1. Study Area and Test Materials*

The research was conducted at Gansu Agricultural University in China, situated at an elevation of 2400 m above sea level (36°02 N, 104°25 E), during the winter months. Nine bioreactors of equal size were used in the study, with relative humidity and temperature maintained at 60% and 16.2 ± 0.23 °C, respectively. The organic materials, mainly cow manure and corn straw, were sourced from the fecal heap-site of Lanzhou Yili Dairy Company Limited and corn fields in Yuzhong County, China. The choice to use raw materials from Gansu province was to emphasize the significance of organic resources to local farmers in the region. Notably, the province remains one of the grain-basket areas in northwest China, accounting for about 19.3% of the country's production, and is home to about 40% of China's rural poor population [20]. However, due to large stockpiles and limited storage space [20], most farmers burn crop straw (maize, rice, wheat, and potatoes) after harvest. The main components of the compost pile, based on the dry weight of the raw materials, are summarized in Table 1.

**Table 1.** Initial physicochemical properties of corn straw, cow manure, and a mixture of cow manure and corn straw used to evaluate the performance of moisture on lignocellulosic and humification fractions.

| Parameter | Corn Straw | Cow Manure | Mixture |
|---|---|---|---|
| Moisture content (%) | 6.33 ± 0.22 | 50.90 ± 0.81 | 28.62 ± 0.52 |
| pH | 7.23 ± 0.91 | 7.71 ± 0.32 | 7.79 ± 0.09 |
| Electrical conductivity (mS/cm) | 3.02 ± 0.10 | 2.90 ± 0.04 | 3.04 ± 0.13 |
| Particle size (mm) | 120.0 ± 6.21 | 20.0 ± 2.21 | 98.0 ± 9.71 |
| Cellulose content (mg/g) | 270 ± 15.12 | 125 ± 8.4 | 178 ± 8.25 |
| Hemicellulose content (mg/g) | 350 ± 11.42 | 170 ± 6.91 | 248 ± 15.12 |
| Lignin content (mg/g) | 125 ± 6.12 | 80 ± 8.18 | 95 ± 10.35 |
| Cow manure/corn straw ratio (kg) | ms 3.8 | mm 15.0 | ≈15:4 |

mm = mass of cow manure (not a ratio). ms = mass of corn straw (not a ratio). These parameters were measured according to the Chinese National Standard for Testing Organic Resources (NY525-2002) [21,22].

## 2.2. Composting Vessels and Processes

The composting process was performed in nine cylindrical bioreactors, each with a volume of 60 L. The bioreactors were insulated with double-layer cotton wool enclosed in a stainless steel casing. An inlet valve, located at the base of the reactor allowed oxygenation into the composting materials, while its top had a tight-fitting lid with two outlet valves for holding the thermometer and a collection point for gases. There was also a 5 cm thick sealing film above the inlet valve which ensured the uniform distribution of air within the reactor system (Figure 1). The operational activities (agitation control, data monitoring and logging, temperature control, pH regulation, nutrient and gas control, etc.) on the bioreactors were controlled by the programmable control unit (PCU).

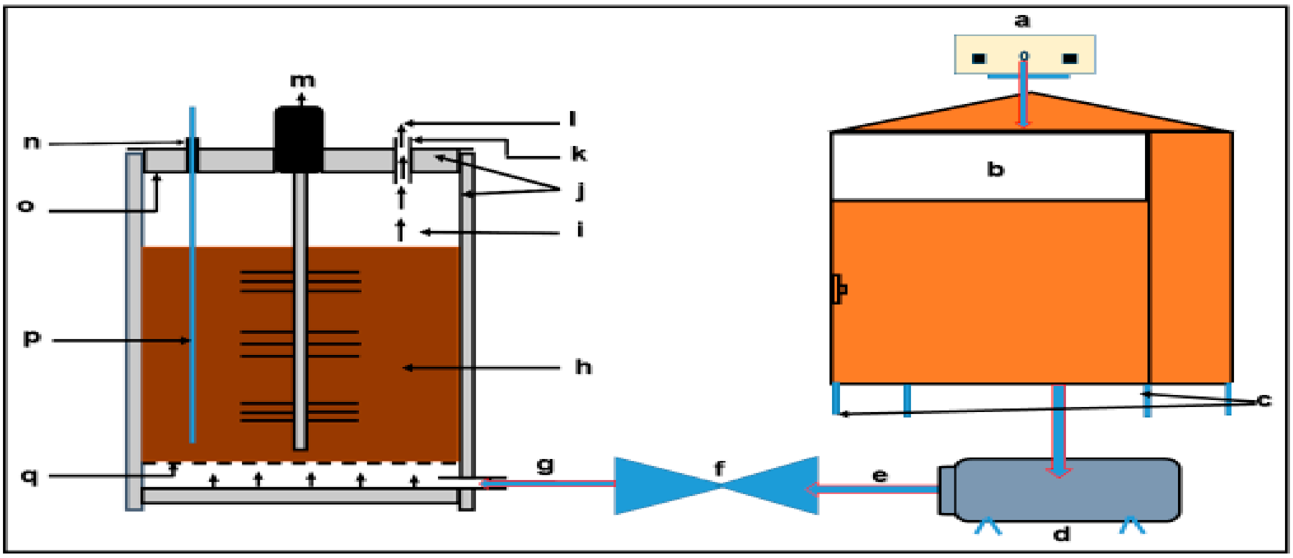


**Figure 1.** Schematic diagram of the bioreactor and its components: a = switch; b = programmable control unit (CPU); c = stand, d = gas cylinder; e = gas supply line; f = automatic point of oxygen regulation, g = inlet valve; h = manure and corn straw mixture; i = reactor headspace; j = insulator; k = outlet valve; l = point of gas collection; m = rotator; n = point of thermometer fixation; o = reactor lid; p = thermometer; and q = seal liner.

Prior to the decomposition process, the organic materials (corn straw and cow manure) were exposed to natural air and monitored until a constant mass was reached. The materials (≈15 kg cow manure: 4 kg corn straw) were then loaded into a commercial mixing machine and supplied with distilled water while on rotation to adjust the initial moisture content of the feedstock to 45% (MC1), 55% (MC2), and 65% (MC3), thereby ensuring uniform mixing.

The formulated piles were then fed into the bioreactors to monitor the decomposition process of each treatment, while maintaining an airflow rate of 0.4 L $min^{-1}$ (10 min on and 10 min off) for good oxygenation. The composting process was considered physically complete when the temperature values of the decomposing piles were almost identical to the ambient temperature value (16.2 ± 0.23 °C).

*2.3. Experimental Design and Sampling Protocol*

The study consisted of three optimal moisture levels (45%, 55%, and 65%) laid out in a completely randomized design (CRD) with three replicates. Moisture levels were designated MC1, MC2, and MC3 for 45%, 55%, and 65%, respectively. Sampling was conducted randomly and in triplicate from the top, middle, and bottom of the reactors at intervals of 2 days, except for moisture determination which was conducted at 5-day intervals. In addition, temperature and pH were measured daily. On average, 100 g of fresh solid samples were collected from different locations in each reactor and grouped in accordance with the treatments. The homogenized samples were divided into two: one for air drying and the other kept in the refrigerator at −4 °C. The air-dried samples were crushed with a pulverizer (Retsch ZM200, Germany) and sieved through a 0.25 mm mesh before measuring the lignocellulosic and humification fractions. In particular, the pH, electrical conductivity (EC), lignocellulosic contents (cellulose, hemicellulose, and lignin), humic substances (humic acid and fulvic acid), and humification indices (humification index, humification rate, and degree of polymerization) were determined from the air-dried samples, while the temperature, moisture content, and germination index were determined from the fresh solid samples.

The temperatures of the composting material and the composting environment (ambient) were recorded hourly on the programmable control unit (PCU) that controlled the operations on the reactor, averaging the temperature values on daily basis. The procedure described by Ren et al. [23] was used to determine the pH and electrical conductivity (EC), where representative samples (dry in nature) were mixed with deionized water at a ratio of 1:10 (*w*/*v*) and shaken for 30 min. The mixture was then allowed to settle for 30 min, prior to analysis with an MP521 pH/EC electrode (Shanghai, China) [23]. Humic substance (HS) determination depicted the methodology of Cao et al. [6]. Briefly, the pulverized samples obtained after grinding and sieving the compost dry samples (10 grams) were mixed with 100 milliliters of 0.1 M sodium hydroxide (NaOH) solution and sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) in a mass ratio of 1:20 and shaken at 20 °C for 24 h before centrifuging (11,000 rpm for 10 min) to obtain a supernatant containing the humic substance. The procedure was repeated twice more before pooling the extracts and then freeze-drying the HS for further analysis. The FA content was obtained by precipitating HA and extracting it with sodium hydroxide and sodium pyrophosphate at a pH of 1 after keeping the solution overnight (12 h at 4 °C). The HA content was calculated by subtracting the FA content from the HS content, while the concentrations of HS, HA, and FA were analyzed using a TOC analyzer. The humification indices were calculated using the following formulae:

$$\text{Humification index (HI)} = \frac{\text{Humic acid (HA)content}}{\text{Total organic carbon (TOC) content}} \times 100 \quad (1)$$

$$\text{Humification ratio (HR)} = \frac{\text{Humic acid (HA)content}}{\text{Fulvic acid (FA)content}} \times 100 \quad (2)$$

$$\text{Percentage humic acid (PHA)} = \frac{\text{Humic acid (HA)content}}{\text{Fulvic acid (FA)content}} \times 100 \quad (3)$$

$$\text{Degree of polymerization (DP)} = \frac{\text{Humic acid (HA)content}}{\text{Fulvic acid (FA)content}} \quad (4)$$

Additionally, the method of Van Soest et al. [24] was used to determine the lignocellulosic constituents (cellulose, hemicellulose, and lignin) via the incorporation of a fiber analyzer (Foss, Hillerød, Denmark). Beforehand, 1 g of compost was measured and added to a beaker containing 50 milliliters of a 72% sulfuric acid ($H_2SO_4$) solution to break down the cellulose and hemicellulose contents in the compost. The beaker was heated at 121 °C for 1 h to hydrolyze the lignocellulosic material and convert it into soluble sugars before cooling to a room temperature and adding 200 milliliters of deionized water to dissolve all soluble sugars for 2 h. By assessing the germination index (GI) of the compost, aqueous suspensions of the fresh compost samples (1:10, *w*:*v*) were derived and tested on Chinese cabbage (*Brassica rapa subsp. chinesis*).

$$\text{GI (\%)} = \frac{\textit{Seed germination in compost extract} \times \textit{Root length in compost extract}}{\textit{Seed germination in control extract} \times \textit{Root length in control extract}} \times 100 \quad (5)$$

### 2.4. Statistical Analyses

All measurements were performed in triplicate to ensure data reliability before being subjected to an analysis of variance (ANOVA) to test the level of significance between the treatments. Microsoft Excel (version 2016) was used to process the data, while within- and between-treatment means were analyzed using the IBM Statistical Package for Social Sciences (version 22) at a 5% probability level. To visualize the data, graphs were created using GraphPad Prism (version 8.0) and data were presented as mean ± standard error ($n$ = 3).

## 3. Results

### 3.1. Variation in Temperature, Matrix pH, and EC

Compost piles differed significantly in temperature ($p < 0.01$), pH ($p < 0.01$), and EC ($p < 0.02$) between the moisture levels (Figure 2a–c). There was a gradual increase in temperature in the first eight days, followed by a decrease over time. With the exception of pile MC1, all treatments showed an active thermophilic phase (>50 °C) on day 3, with varying peaks on days 7, 9, and 11 for piles MC2 (57.8 °C), MC1 (51.5 °C), and MC3 (56.7 °C), respectively. Heap formulations with 55% moisture content exhibited higher temperatures during the initial mesophilic and thermophilic phases than those induced with 45% and 65% moisture. Additionally, the 55% moisture content maintained temperatures above 50 °C for 7 days. The temperatures for all treatments were 60% higher at their peaks than at the initial mesophilic phase. Overall, pile MC3 exhibited the most significant temperature increase during the composting period.

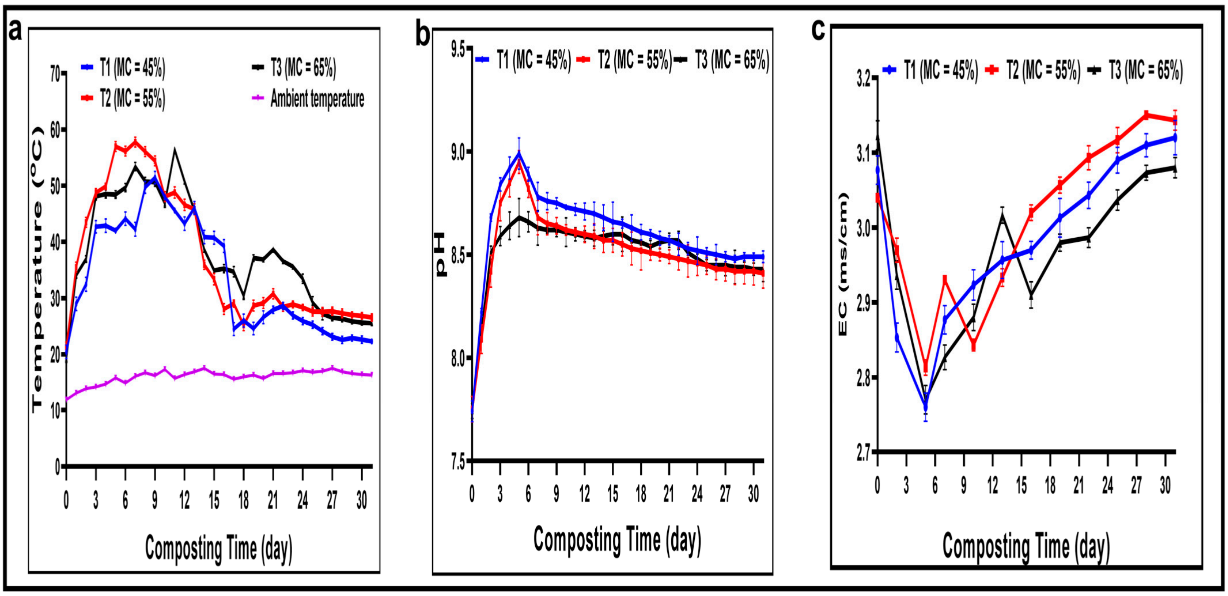


**Figure 2.** Effect of moisture conditions on the dynamics of temperature (**a**), pH (**b**), and EC (**c**) in aerobically composted straw and manure mixture. Error bars represent ± standard error of the mean values in three replicates.

As shown in Figure 2b, the pH of all treatments increased during the first 5 days (7.7–9.0 for MC1, 7.8–8.9 for MC2, and 7.7–8.7 for MC3) but slowly declined until the end of the composting process (9.0–8.5 for MC1, 8.9–8.4 for MC2, and 8.7–8.4 for MC3). The pH varied by 0.5 between pile MC1 and MC3, and by 0.2 between pile MC1 and MC3, but all treatments had alkaline indices at the end of the process.

Similarly to pH, all treatments showed a decrease in EC from the beginning of the composting process (3.05 mS/cm) to the 5th day (2.78 mS/cm), but steadily increased through to the end of the composting process. The difference in EC values between day 5 and day 31 was 0.36 mS/cm, 0.33 mS/cm, and 0.28 mS/cm for pile MC1, MC2, and MC3, respectively.

### *3.2. Changes in Humic Substance, Humic Acid, and Percentage Humic Acid*

The impact of moisture regimes on humic substance (HS), humic acid (HA), and percentage humic acid (PHA) are shown in Figure 3a–f. The results show that an increase in moisture content led to an increase in HS (Figure 3a,b) and HA (Figure 3c,d). The amount of HS peaked and stabilized during the cooling phase, with piles induced with 65% water showing 11% and 19.1% increases in HS compared to piles induced with 55% and 45% water, respectively. Moisture differences significantly ($p < 0.02$) affected the production of HS, with larger differences impacted by MC3. Although the amount of HS was generally high throughout the composting period in pile MC3, the amount detected on day 13 was statistically highest in pile MC2. HS increased by 18% *w*/*w* for pile MC1, 20.2% *w*/*w* for pile MC2, and 23.8% *w*/*w* for pile MC3 at the end of composting.

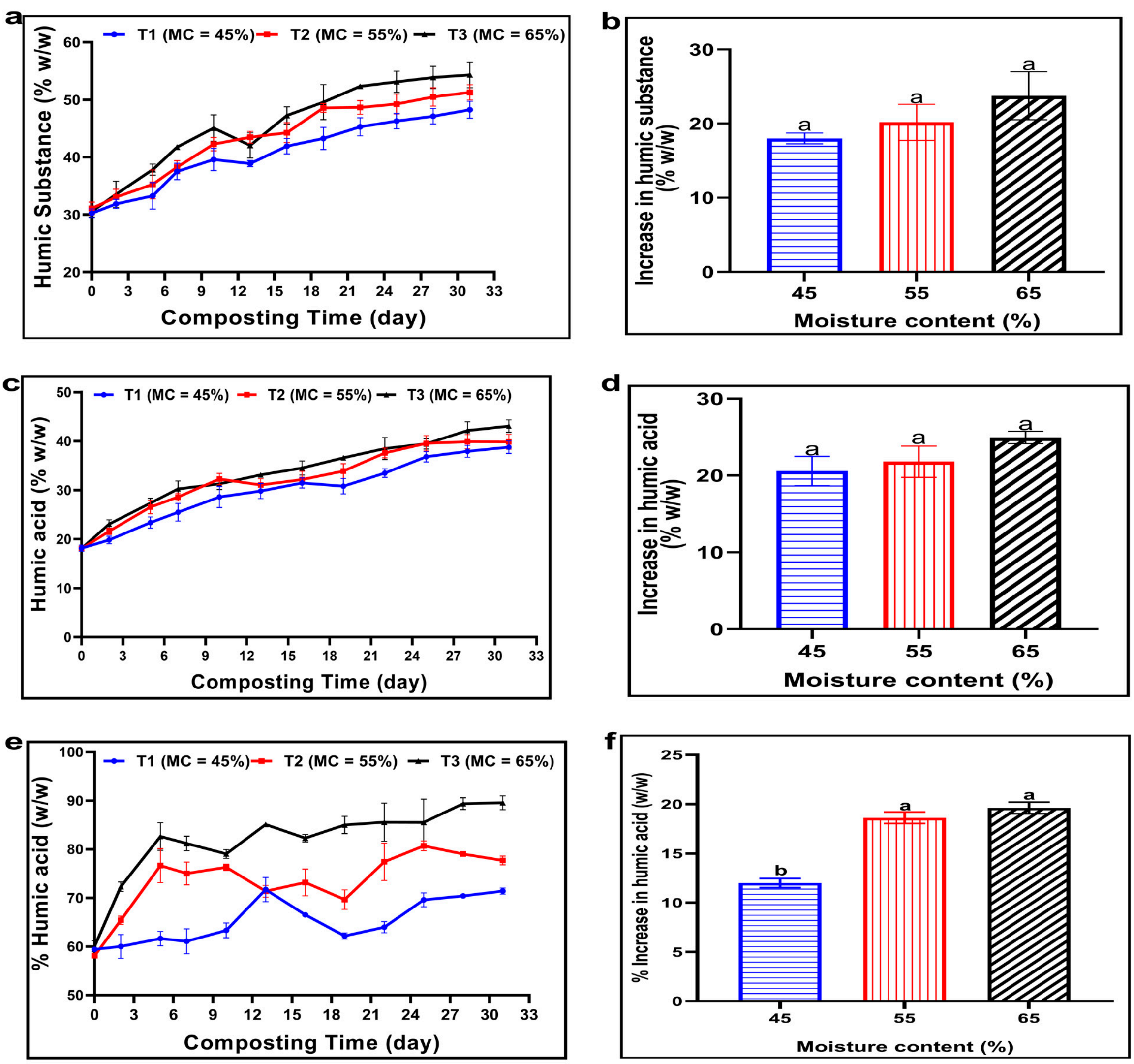


**Figure 3.** Effect of moisture conditions on the dynamics of humic substance (**a**,**b**), humic acid (**c**,**d**), and percentage humic acid (**e**,**f**) in aerobically composted straw and manure mixture. Error bars represent ± standard error of the mean values in three replicates. Different letters show different significant differences.

The amount of HA was highest in the thermophilic phase (43.0% *w*/*w* for MC1, 39.9% *w*/*w* for MC2, and 38.8% *w*/*w* for MC3) and lowest in the initial composting phase (18.2% *w*/*w* for MC1, 18.1% *w*/*w* for MC2, and 18.1% *w*/*w* for MC3), suggesting a two fold difference between the two phases. The concentration of HA showed a significant difference ($p < 0.03$) between MC1 and MC3, but the combined effect of the three moisture levels had no effect. The piles with 45% water content showed an increase in HA by 3.1% *w*/*w* (12.4%) and 4.3% *w*/*w* (17.3%) compared to piles with 55% and 65% water content, respectively. On average, the concentration of HA followed the trend: 29.52% *w*/*w* (MC3) > 31.71% *w*/*w* (MC2) > 33.12% *w*/*w* (MC1).

Percentage humic acid (PHA) showed significant variation ($p < 0.01$) between composting periods and moisture treatments. As the composting period advanced, the level of PHA (Figure 3e,f) increased. However, a reduction in moisture content led to a corresponding increase in PHA. The compost from MC1 had higher PHA content than MC2 (8.1% *w*/*w* ≈ 10.59%) and MC3 (16.4% *w*/*w* ≈ 25.2%). In contrast to the concentrations of HS and HA, which followed a consistent pattern of increase across the periods, PHA showed the reverse.

### 3.3. Variation in Fulvic Acid and Degree of Polymerization

Fulvic acid (FA) determination showed a significant difference ($p < 0.02$) between the moisture treatments (Figure 4a,b). The concentration of FA decreased from 24.9% *w*/*w* at the beginning of composting to 9.27% *w*/*w* at the end, a decrease of 62.7% (15.5% *w*/*w*). The largest decrease in FA (16.8% *w*/*w*) occurred in piles with 45% moisture, while it was least in piles with 55% (15.5% *w*/*w*) and 65% (14.3% *w*/*w*) moisture, respectively. The amount of FA showed an inverse relationship with humic acid (HA). In addition, the degree of polymerization (DP) increased from 0.83% *w*/*w* to 3.9% *w*/*w* during composting. For all moisture conditions, DP increased with composting time, but a greater difference was observed between pile MC3 and MC1 (11.5%) and least between pile MC3 and MC2 (2.3%). At 65% moisture, substrate polymerization improved by 9.8% (0.4% *w*/*w*) and 19.5% (0.8% *w*/*w*) compared to 55% and 45% moisture, respectively.

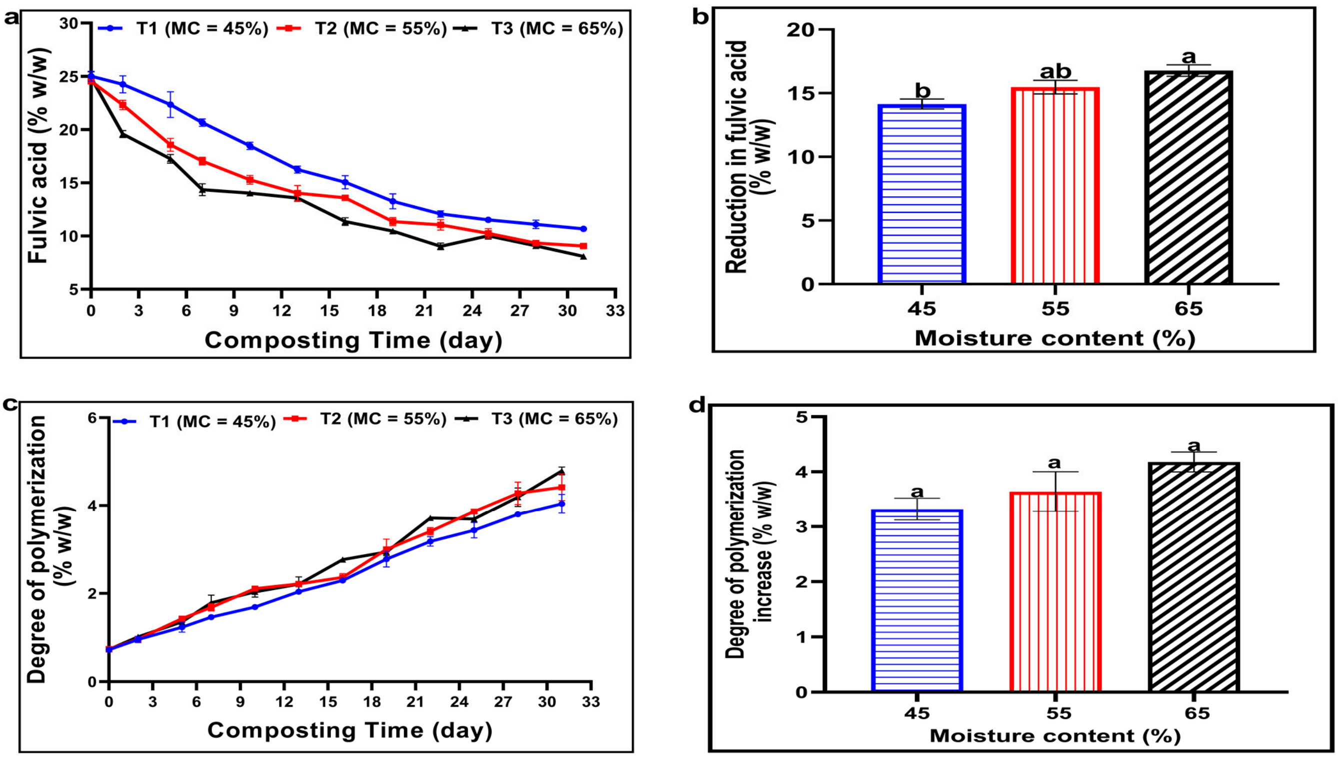


**Figure 4.** Effect of moisture conditions on the dynamics of fulvic acid (**a**,**b**) and the degree of polymerization (**c**,**d**) in aerobically composted straw and manure mixture. Error bars represent ± standard error of the mean values in three replicates. Different letters show different significant differences.

### 3.4. Variations in Humification Index and Humification Rate

The dynamics of substrate humification rate (HR) is shown in Figure 5a,b. As indicated by the figure, all moisture regimes showed a consistent increase from a minimum of 0.082% *w*/*w* to a maximum of 0.172% *w*/*w*. The levels of HR were higher during the cooling phase and lower during the initial mesophilic phase, resulting in a significant difference ($p < 0.01$) between the phases of composting (Figure 5a). Piles with 65% moisture showed the highest increase in HR, while those with 45% moisture showed the least effect. On average, the difference between the initial mesophilic phase and the cooling phase was 0.083% *w*/*w*, 0.087% *w*/*w*, and 0.100% *w*/*w* for MC1, MC2, and MC3, respectively. Although all moisture regimes resulted in an increase in HR, MC3 moisture showed an increase of 13% and 17% over the moisture contents of MC2 and MC1, respectively. Moreover, the humification index (HI) of the composted materials increased with time and ranged from 0.048% *w*/*w* (minimum) to 0.136% *w*/*w* (maximum). The HI (Figure 5c) was three times higher at the end of composting compared to the beginning, resulting in a significant ($p < 0.01$) difference between composting periods. In contrast to the 65% moisture regime, which showed an increase in HI, the 45% moisture regime had the least effect on HI, showing a trend of 0.934% *w*/*w* > 0.099% *w*/*w* > 0.103% *w*/*w* for MC1 > MC2 > MC3 moisture regimes.

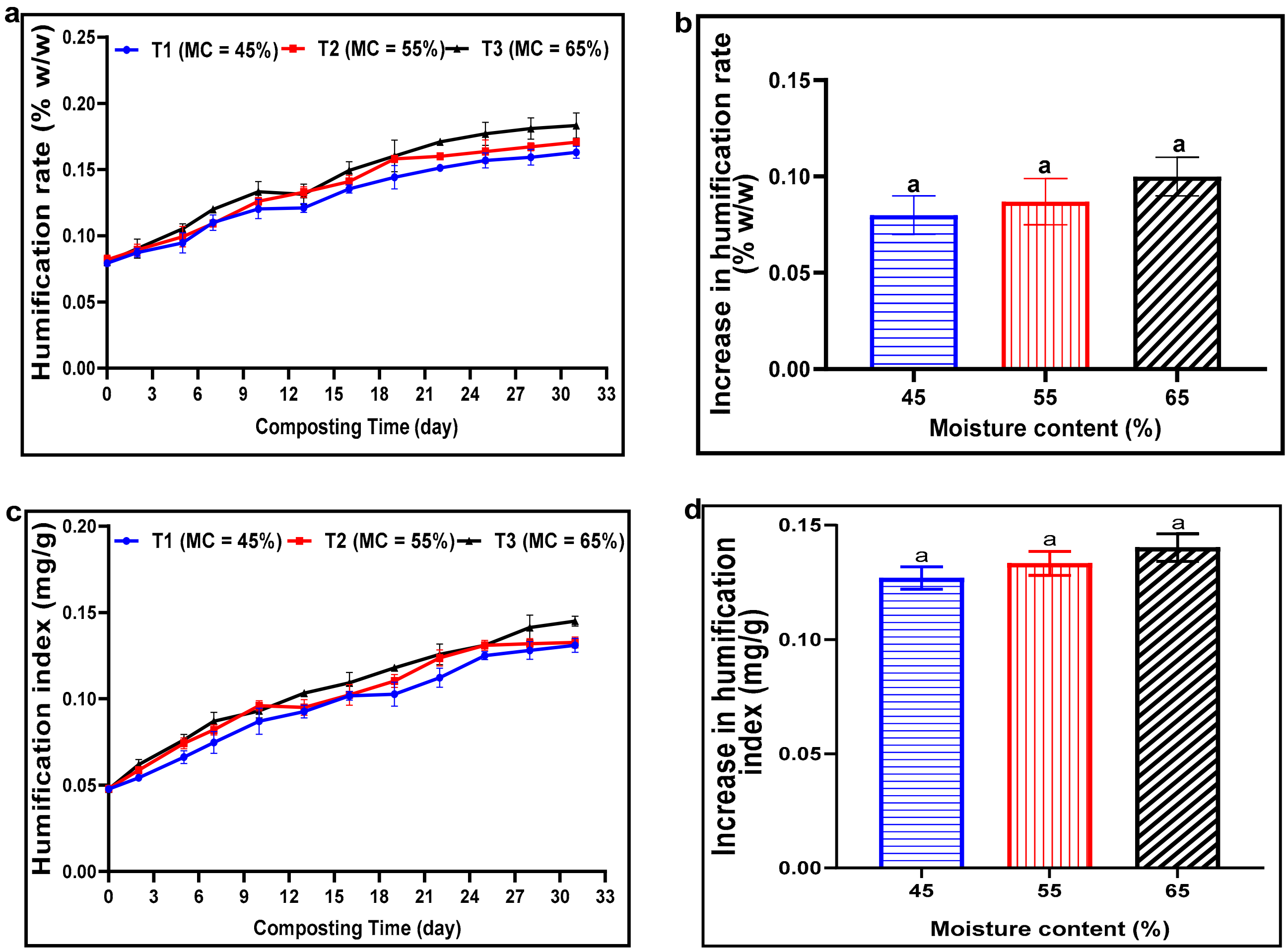


**Figure 5.** Effect of moisture conditions on the dynamics of the humification rate (**a**,**b**) and humification index (**c**,**d**) in aerobically composted straw and manure mixture. Error bars represent ± standard error of the mean values in three replicates. Different letters show different significant differences.

### 3.5. Changes in Cellulose, Hemicellulose, and Lignin Content

The dynamics of cellulose content are shown in Figure 6a,b. Cellulose degradation was initially slow but increased as the composting period progressed. Moisture and the duration of composting significantly affected ($p < 0.01$) the degradation of cellulose in the piles (Figure 6a). As expected, cellulose degradation increased with increasing moisture content, i.e., 30.0% for MC1, 42.8% for MC2, and 52.5% for MC3. Piles induced with 65% moisture increased cellulose degradation by

18.3% *w*/*w* compared to the average-induced treatment (55% MC) and by 41.8% *w*/*w* compared to the least-induced treatment (45% MC). Additionally, the amount of cellulose degraded was low compared to hemicellulose degradation.

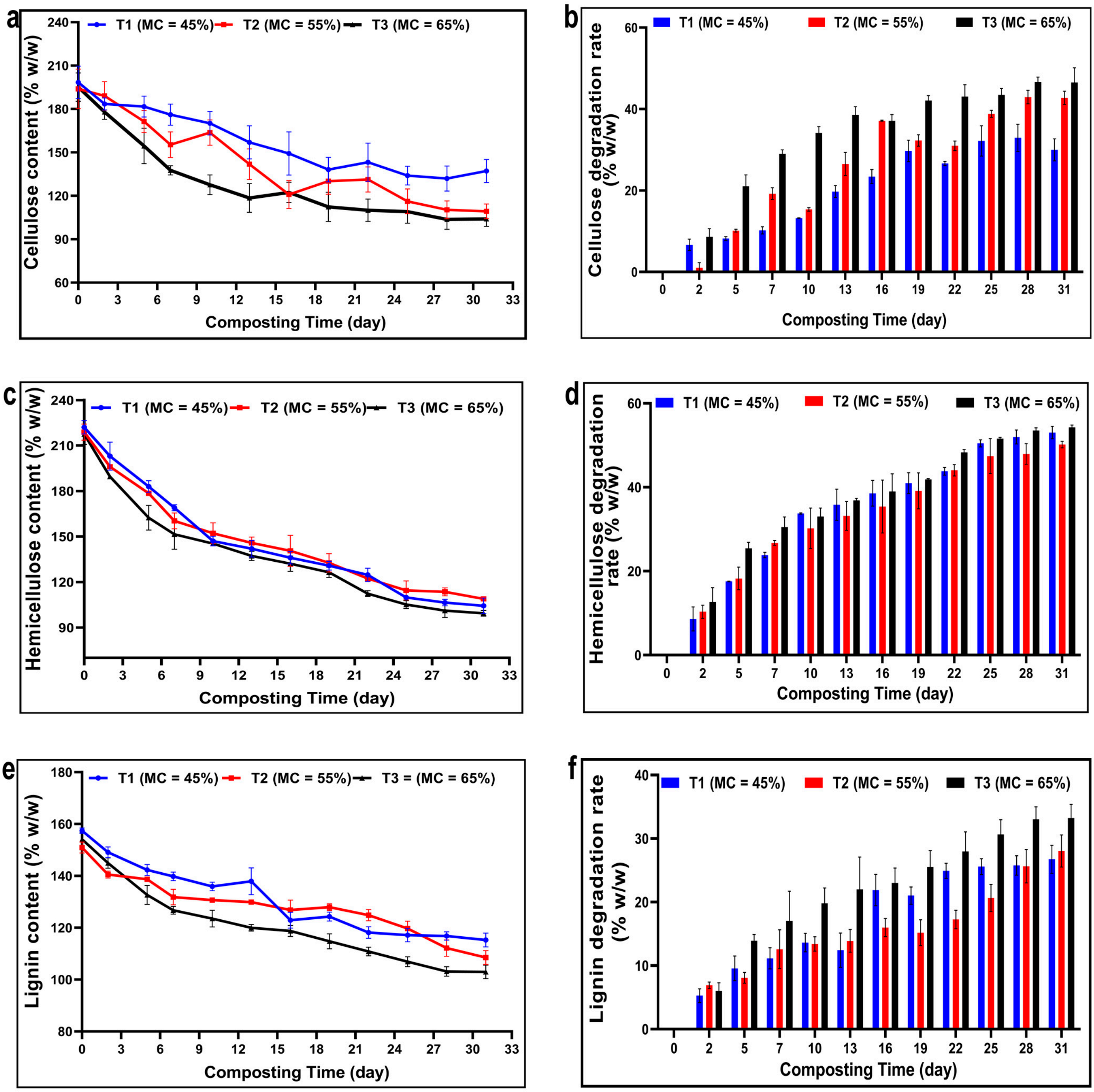


**Figure 6.** Effect of moisture conditions on the dynamics of cellulose (**a**,**b**), hemicellulose (**c**,**d**), and lignin (**e**,**f**) in aerobically composted straw and manure mixture. Error bars represent ± standard error of the mean values in three replicates.

Changes in hemicellulose (Figure 6c,d) and lignin (Figure 6e,f) degradation followed a similar pattern as that of cellulose (Figure 6a,b), but differed significantly ($p < 0.01$) between composting periods and moisture treatments. Hemicellulose degradation was 33.2% for MC1, 31.9% for MC2, and 37.2% for MC3. Hemicellulose degradation was also higher than that of cellulose (Figure 6a,b) and lignin (Figure 6e,f). Although degradation of lignin was low among the three components, its content was degraded from 154.1% *w*/*w* to 114.2% *w*/*w*, showing a similar pattern to cellulose and hemicellulose. The amount of lignin retained after composting was 121.7% *w*/*w* for MC1, 129.4%

*w*/*w* for MC2, and 139.3% *w*/*w* for MC3, indicating a degradation rate of 11.5% for MC1, 14.1% for MC2, and 21.0% for MC3. Nonetheless, the degradation effect of MC3 was almost twice that of MC1.

### *3.6. Variations in Moisture Content*

The moisture content in the compost piles varied greatly between treatments ($p < 0.01$) but decreased as the period of composting increased (Figure 7). All treatments showed steady decreases, except for MC3 which showed a slight increase on day 6. Piles supplied with 45% water lost 44% moisture at the end of composting, while piles supplied with 55% and 65% water lost 30.4% and 23.4%, respectively. Pile MC3 exhibited a more stable water loss at the cooling phase compared to MC1 and MC2.

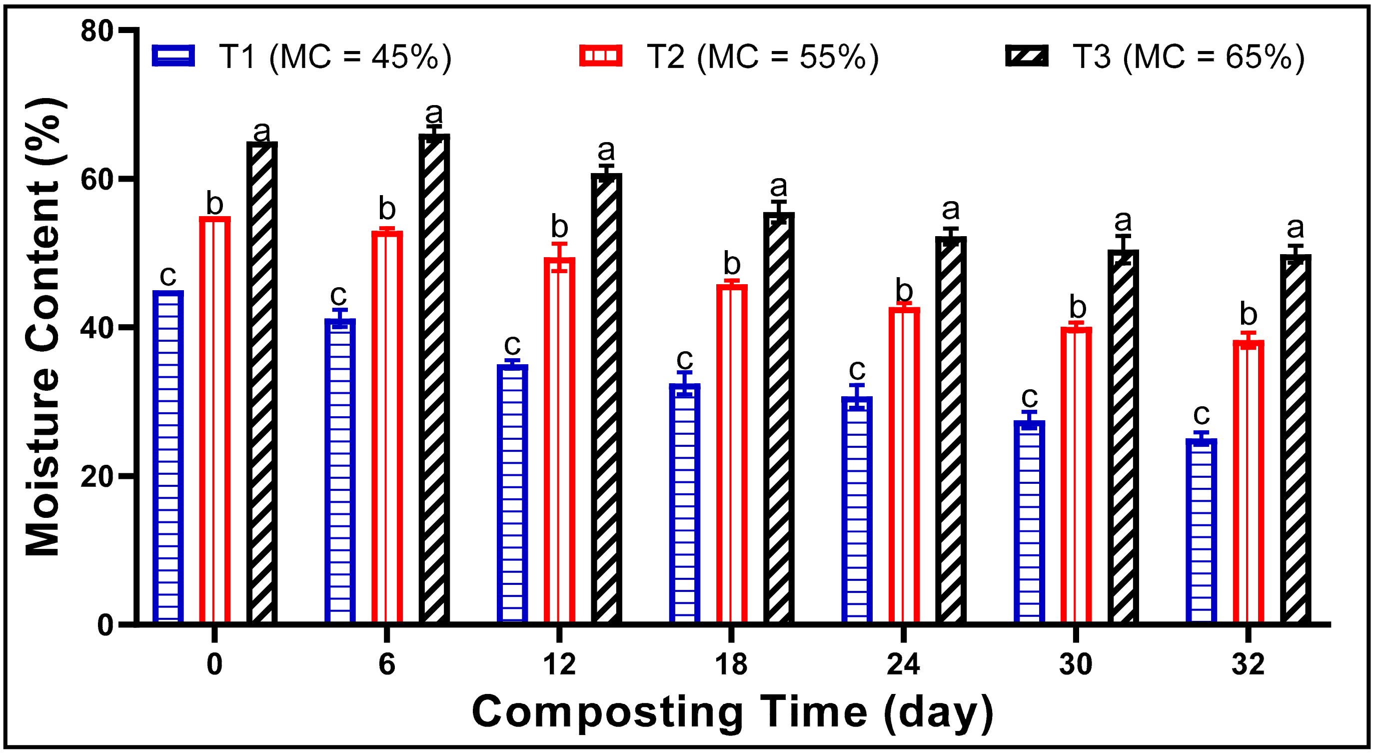


**Figure 7.** Changes in moisture content of aerobically composted straw and manure mixture. Error bars represent ± standard error of the mean values in three replicates. Different letters show different significant differences.

### *3.7. Redundancy Analysis (RDA) of Compost Physicochemical Indices as Affected by Moisture*

Redundancy analysis (RDA) was conducted to investigate the relationship between moisture and compost physicochemical properties (Figure 8, Table 2). The results showed that moisture significantly ($p < 0.05$) affected pH, fulvic acid (FA), percentage humic acid (PHA), and the degradation of cellulose, hemicellulose, and lignin, indicating a higher percentage variation of 43.0 to 74.2. This finding highlights a strong relationship between moisture and the aforementioned indices. Conversely, moisture had a weaker influence on humic acid (HA), humification index (HI), humic substance (HS), humification rate (HR), and the degree of polymerization (DP), explaining a lower variation of 0.9% to 42.8% between the indices.

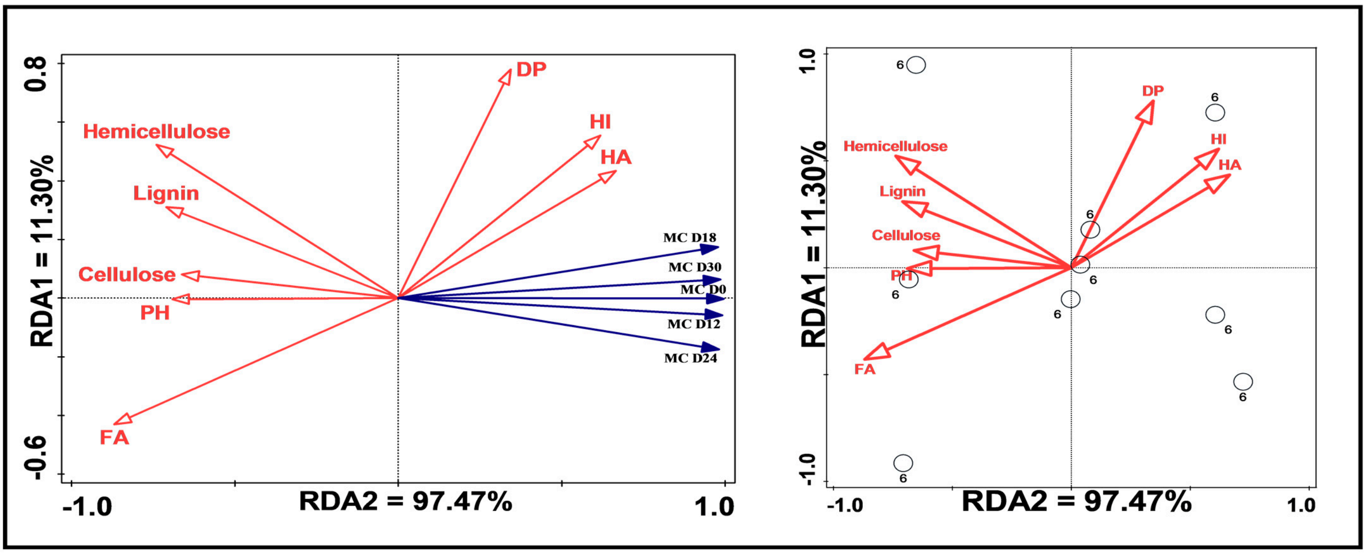


**Figure 8.** Redundancy analysis (RDA) on the dynamics of physicochemical indices of aerobically composted straw and manure mixture as affected by moisture.

**Table 2.** Permutation results between moisture and physicochemical indices of compost.

| Traits | % Explained | Pseudo-F | *p*-Value |
|---|---|---|---|
| pH | 46.5 | 6.1 | 0.036 * |
| FA | 74.2 | 20.1 | 0.002 ** |
| Percentage humic acid (PHA) | 48.3 | 6.5 | 0.044 * |
| Cellulose | 42.8 | 5.2 | 0.030 * |
| Hemicellulose | 53.8 | 8.1 | 0.014 * |
| Lignin | 49.4 | 6.8 | 0.008 ** |
| Humic acid (HA) | 43.0 | 5.3 | 0.056 NS |
| Humification index (HI) | 37.4 | 4.2 | 0.096 NS |
| Humic substance (HS) | 31.5 | 3.2 | 0.148 NS |
| Humification rate (HR) | 20.0 | 1.7 | 0.240 NS |
| Degree of polymerization (DP) | 12.3 | 1.0 | 0.412 NS |

NS Not significant at $p > 0.05$. * Significant correlations at $p < 0.05$. ** Significant correlations at $p < 0.01$.

## 4. Discussion

### *4.1. Temperature, Matrix pH, and EC Variations*

The biological activities that take place during composting are impacted by temperature. Therefore, the gradual heat development at the beginning of composting indicates a slow biodegradation process attributable to the week-long turning of piles. Although adequate turning of piles activates microbial communities and promotes biodegradation of organic materials [25], excessive turning disorganizes microorganisms, requiring more time for them to form new communities and ensure heat buildup [26]. Microorganisms have different oxygen requirements, and they occupy different niches within the pile. However, excessive turning may have depleted the oxygen and moisture level within the piles, thereby disrupting the delicate balance of microorganisms within the pile. As expected, heat production was barely stimulated by the 45% MC, a circumstance that could have promoted unstable biological processes. As Yang et al. [27] noted, dehydration occurs easily in piles that are insufficiently moisturized, thereby affecting biological processes. This implies that microorganisms have a high preference for compost piles that are adequately moistened, as such conditions promotes high temperatures for an extended duration [19].

Gases are inevitably produced during composting. Therefore, $NH_3$ losses and the decomposition of organic matter could possibly have increased the acidity of the compost [5]. $NH_3$ reacts with water vapor in the air to form ammonium hydroxide ($NH_4OH$), which increases the acidity (lowers the pH) of the compost [28]. The decomposition of organic matter during composting could have enhanced increases in pH as basic compounds such as ammonium ($NH_4^+$) and calcium ($Ca^{2+}$) ions are released. During the decomposition of organic matter by microorganisms, they produce compounds that have the ability to interact with hydrogen ions ($H^+$) present in the compost. As a result, the pH of the compost pile increases. This can be explained in part that, during the decomposition of nitrogen-rich organic matter such as animal manure or food waste, microorganisms convert nitrogen into ammonium ions through a process known as ammonification. Ammonium ions are alkaline in nature, and can react with and neutralize hydrogen ions in the compost, which can cause an increase in pH. In addition, the reason for the increase in EC is linked to the decomposability of organic matter which releases mineral salts in the form of ammonium and phosphate ions [5]. During composting, microorganisms break down organic matter in the presence of oxygen, releasing charged mineral ions such as ammonium ($NH_4^+$) and phosphate ($PO_4^-$) into the compost [29]. These ions have electrical charges that can conduct electricity, increasing the EC of the compost. Conversely, EC decreased with increasing moisture content could be due to an increase in moisture content which might have influenced greater neutralization and leaching of soluble salts [30]. As water moves through the compost, it inevitably carries soluble salts with it, reducing the overall concentration of salts in the compost and lowering its EC. Additionally, the high moisture level may have enhanced the growth of microorganisms that consume organic matter and produce organic acids. These organic acids also contribute to the leaching of salts out of the compost.

### *4.2. Variations in Humification Parameters*

The study showed that different moisture levels affected the concentrations of humic substance (HS), humic acid (HA), and percentage humic acid (PHA). Humification is the process by which organic matter is converted into stable and highly-polymerized organic compounds that contribute to the quality and stability of compost [31]. Therefore, changes in HS and its components (HA and FA) significantly affect the maturation of the compost [32]. The results showed that there was a steady increase in HS in all the treatments, indicating increased biodegradation and transformation of stable organic compounds [33]. The degradation of stable organic compounds such cellulose, hemicellulose, lignin, organic carbon, and organic matter may have acted as precursors for the formation of HS [34,35]. Compost piles with 65% MC (baseline) may have possibly provided the optimal moisture balance and aerobic conditions for microorganisms in the compost pile to convert lignin and cellulose into phenols and quinone compounds. Studies have shown that phenolic and quinone compounds are the precursors of humus and help to accelerate the production of humic substances (HS) [36]. In contrast, piles with 45% moisture (baseline) had relatively a low production of HS, possibly because the thermophilic phase was delayed. The biodegradation of organic matter may have enabled the production of polysaccharides and amino acids, as they necessitate the formation of HA [7]. The late onset of the thermophilic phase in the 45% moisture treatment may have restricted active microbial metabolism, reducing the production of polysaccharides and amino acids, which are important precursors in the formation of HA [37].

Humic and fulvic acids are essential organic compounds found in humic substances. However, fulvic acid exhibited a lower and inverse relation to humic acid [7,37,38]. In mature compost, FA often has a lower concentration than HA due to the degradation of lignocellulosic compounds [39,40]. In addition, its low molecular weight makes it more readily accessible and susceptible to microbial attacks and decomposition [41]. Microorganisms use FA during the decomposition of OM to synthesize HA [37], which implies that FA is more readily absorbed by plants through soil and water than humic acid. The dominance of HA over FA in this study suggests that HA is the major component in the formation of HS [7]. Likewise, the increase in the concentration of HA during the cooling phase of composting was due to the conversion of lignin to HA during the period. Previous studies have shown that humic substances are formed by the polymerization and condensation of lignin, proteins, amino acids, and nucleic acids, further solidifying the relationship between lignin and HA in the formation of humic substances [7,38].

The effect of moisture on the degree of polymerization showed an increase (>3.0% $w/w$), proving the effectiveness of each treatment in humification. As optimal moisture regimes, these treatments could have provided optimal balance and aerobic conditions that might have controlled the rate of organic matter decomposition. Optimal moisture content is necessary for microorganisms to efficiently break down organic matter into simpler compounds. The by-products of this decomposition process, including humic substances, have a higher degree of polymerization compared to the original substrate. Compost with a degree of polymerization greater than 1.9 is considered a stable and mature product [38]. The increase in the degree of polymerization of the heap indicates its conversion from fulvic acid to humic acid, resulting in humic substances with complex structures [6]. Increase in humification rate (HR = HS/TOC) at the cooling phase can be attributed to the increase in total organic carbon content, suggesting a strong humification and decomposition of organic matter [42]. The humification index also increased significantly, indicating intense humification of organic carbon [7].

*4.3. Changes in Lignocellulose Fractions*

The high cellulose content in the initial compost pile could be due to the cellulose-rich organic materials and the low temperature during the initial fermentation process. Cellulose degradation is a microbial process that is highly influenced by heat-tolerant microorganisms. However, these organisms have difficulty adapting to low temperatures, which affects degradation [43]. Piles supplied with 65% moisture had a high cellulose degradation rate due to the long duration of the thermophilic phase [33]. Optimal moisture content promotes temperature rise, allowing heat-tolerant microorganisms to invade and degrade organic materials [22], hence the reason for the increased rate of cellulose degradation. In contrast, cellulose degradation was lowest among piles with 45% MC. This can be attributed to insufficient moisture, which could have restricted microbial activities within the compost pile. This phenomenon may have created a lignin–hemicellulose bond between the cellulose to impede enzymatic action, leading to an accumulation of cellulose rather than its breakdown [35,44]. The rapidity in the decomposition of hemicellulose can in part be attributed to its low molecular weight. This attribution may have enabled microorganisms to use the polysaccharide as a carbon source for growth [44]. Similarly, the decrease in temperature after the thermophilic phase may have contributed to the the decrease in hemicellulose content [7], as this phenomenon tends to suppress the activities of most microorganisms. Microorganisms are sensitive to temperature fluctuations and their metabolic activity decreases with decreasing temperature.

Although lignin is a complex organic substance (three-dimensional organic substrate), there could be a synergistic relationship between microbial communities and biodegradable organic matter that led to the partial degradation [45]. Additionally, the inevitable production of ammonia during composting could have influenced the partial degradation of lignin. As opined, ammonia is an intermediate product of degradation that creates the necessary conditions for microorganisms to effectively degrade the lignin components in organic residues [46]. The lignin content of the compost pile with 45% moisture showed lower degradation efficiency due to low moisture supply. Lignin is a cross-linked polymer made up of phenylpropanoid monomers, which makes it resistant to penetration and hydrolysis when the moisture content is insufficient [47].

*4.4. Moisture Content*

The moisture levels played an active role in the composting process. The rapid decrease in moisture content during the first 15 days of composting was due to the release of gases [5], which was more significant at the beginning of composting. The increase in moisture loss by pile MC1 (45%) could be due to the fact that the treatment did not provide optimal balance and aerobic conditions for microorganisms to effectively degrade the substrate [19]. Insufficient moisture content leads to dehydration of the pile, hindering biological processes [27]. However, improving the matrix structure of the organic material for microbial invasion depends on optimal and balanced moisturization of the pile [48].

*4.5. Mechanistic Action of Water on Substrate Decomposition*

The mechanistic action of water on substrate decomposition refers to the process by which water breaks down complex organic compounds into simpler ones, leading to the release of nutrients, energy, and other compounds. The action of water on a substrate is dependent on temperature, pH, oxygen diffusion, water potential and activity, microbial growth rates, and particle size (Figure 9). Feedstock, particularly animal-based manure, contains microorganisms such as fungi, bacteria, actinomycetes and other microorganisms, which are responsible for breaking down biological materials [5]. Microorganisms move through ultra-thin water films and interact with organic matter to produce heat. The heat generated causes the pile to warm up, leading to four distinct temperature phases, including the mesophilic or warming phase (25–40 °C), the thermophilic phase (40–65 °C), the curing or cooling phase (25–40 °C), and the maturation phase (20–30 °C) [49]. The substrate decomposition is a complex process that involves several steps, and the presence of water plays a crucial role in this process:

1. Hydrolysis: Hydrolysis is the process by which water breaks down complex organic compounds into simpler compounds through the addition of water molecules. The process of hydrolysis is critical in the release of energy and nutrients from complex organic molecules.

2. Leaching: Leaching is the process by which water dissolves and transports minerals and other nutrients from the substrate into the surrounding environment. This process is important for plant growth due to the release of nutrients into the soil.
3. Microbial action: Microorganisms such as bacteria, fungi, actinomycetes, and protozoa play an important role in the decomposition of organic matter in water. These microorganisms feed on the organic matter and break it down into simpler compounds through metabolic processes. This process is essential for the release of energy and nutrients from the substrate.
4. Oxidation–reduction reactions: Water also act as a solvent for various redox reactions, and are critical for the breakdown of complex organic compounds. As a chemical reaction (redox reaction) occurs, electrons are transported from one molecule to the other, facilitating the release of energy and nutrients from the substrate.

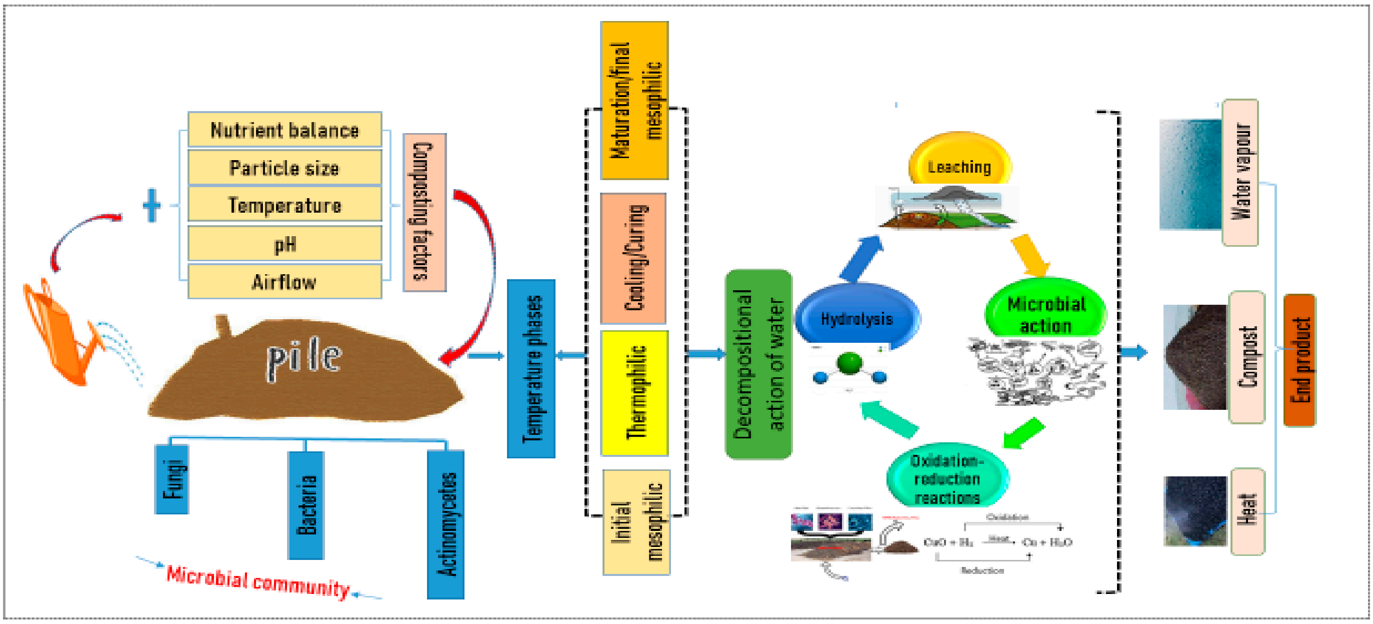


**Figure 9.** Schematic diagram on the mechanistic action of water during composting.

## 5. Conclusions

The study showed that increasing the initial moisture content (MC1 = 45%, MC2 = 55%, and MC3 = 65%) and composting time significantly improved the depolymerization and conversion of corn straw and cow manure into compost. The highest degradation of the substrates occurred during the thermophilic phase, while the maturation phase showed the lowest degradation effect. Increasing the initial moisture content of the pile to 65% resulted in higher degradation, with hemicellulose (115.3% $w/w \approx$ 47.1%) being more susceptible than cellulose (76.0% $w/w \approx$ 39.5%) and lignin (39.9% $w/w \approx$ 25.9%). However, increasing the initial moisture content of the pile led to a decrease in fulvic acid concentration (FA) and humification index, but an increase in humic substance (HS), humic acid (HA), humification rate (HR), and the degree of polymerization (DP). Overall, the study suggests that initial moisture content at 65% is optimal for the decomposition and conversion of corn straw and cow manure into stable and mature compost. Future research can target the use of magnetic resonance imaging (MRI) to evaluate the effects of moisture on substrate decomposition and transformation. These findings are relevant for improving composting processes in medium and commercial industries.

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