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Evolution of Microbial Composition and Enzymatic Activities during the Composting of Textile Waste

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Abstract: The production of stable and mature compost often depends on the performance of microbes and their enzymatic activity. Environmental and nutritional conditions influence the characteristics of microbial communities and, therefore, the dynamics of major metabolic activities. Using three waste mixtures (textile waste mixed with either green, paper, or cardboard waste), the maturity of the compost produced was assessed by following the physico-chemical parameters and enzymatic activities provided by the microorganisms that were identified using next-generation sequencing (NGS). Among the three mixtures used, it was found that the two best mixtures showed C/N ratios of 16.30 and 16.96, total nitrogen of 1.37 and 1.39%, cellulase activities of 50.62 and 52.67 Ug^{-1}, acid phosphatase activities of 38.81 and 68.77 Ug^{-1}, and alkaline phosphatase activities of 51.12 and 56.86 Ug^{-1}. In addition, several lignocellulosic species, together with those that are able to solubilize phosphate, were identified. Among those known for cellulase and acid/alkaline phosphatase activities, bacteria belonging to the Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes phyla were shown. The presence of species belonging to the Ascomycota and Basidiomycota phyla of Fungi, which are known for their ability to produce cellulase and acid/alkaline phosphatases, was demonstrated. These findings provide a basis for the production of stable and mature compost based on textile waste.

Keywords: composting; organic waste; alkaline phosphatase; acid phosphatase; cellulase; next-generation sequencing

1. Introduction

The evolution of waste quantity closely follows the development of society. Also, waste that in the past was essentially organic (effluents from animal husbandry, food waste, plant residues, etc.) today contains a very heterogeneous range of products (green waste, food waste, livestock and industrial waste, paper, plastic and metal packaging, etc.). This increased diversity of waste encourages researchers to find a solution to treat and recover it [1].

Over the last decades, the increased affluence of the production industry has resulted in greater quantities of waste produced yearly. One of the most challenging environmental tasks for scientists is definitely the correct management and discarding of industrial solid waste. Presently, the textile

industry is considered to be the one of the industries generating a large amount of solid waste, which is disposed directly into the environment without any treatment [2]. Furthermore, the abundant organic fraction in textile waste can be bio-converted into a useful end-product called bio-fertilizer, which could be utilized as a supplement to the soil on farm lands [3].

Composting is a biotransformation process in which a solid organic fraction turns into mature and stabilized material by means of microbial action in aerobic conditions [4]. Compost is a noteworthy soil amendment, with its application as an organic fertilizer improving the level of soil organic matter, long-term soil fertility, and productivity [5]. Several studies have been devoted to the characterization of the composting process in order to follow its progress. Among the most useful parameters for assessing compost maturity are the percentage of total organic carbon (TOC), total nitrogen level, C/N ratio, heavy metals, and enzymatic activities [6].

As the composting process is a biological process, microorganisms play a key role. Furthermore, most of the transformations that organic matter undergoes during the bio-oxidative stage are ascribable to the action of microbes and their enzymatic activities [4]. Several investigations concerning the characterization of the composting microbiot a have been published previously [7]. Furthermore, diversity studies regarding composting substrate are limited, since most reports are focused only on the valorization of solid wastes [4]. In this context, there is a paucity of reports depicting complete biochemical characterization of the microbiota involved in the composting. Many authors have revealed specific microbial communities [7], others elucidated only the enzymatic evolution [8]. In fact, the metabolic abilities of microorganisms in relation to organic waste transformation, along with the nature of the residual material, affect the enzymatic features in biodegradation reactions. An assessment of the dynamics of microbes during composting provides a better understanding of the evolution of the process from a microbiological perspective and an overview of the connections between biotic and abiotic factors.

In this context, to identify the microbiota involved in the composting, a metagenomic approach has been widely used [9]. Direct Deoxyribonucleic acid (DNA) sequencing techniques have been emerging recently, increasing the capacity and rate of discovery of previously unknown genes and functional sequences of previously unknown microorganisms [10], allowing the detection of more complete biodiversity in a given environment. They also allow studies of the metabolic pathways of microbiota. High-throughput sequencing (next-generation sequencing (NGS)) is one of the most advanced, rapid, efficient, and accurate technologies used in metagenomics studies [11].

Therefore, the main purpose of this work was to study the dynamics of the microbiota according to its metabolic ability profile during the composting of textile waste. A detailed analysis of the metabolic potential of microorganisms throughout the biotransformation process could contribute to the understanding of microbes role in composting and the influence of process conditions on the composition of the microbes.

2. Materialsand Methods

2.1. Composting Process

The composting processes were carried out on the following organic residues: Textile waste collected from the MULTIWACH textile plant located in the Sidi Ibrahim industrial district in Fez (Morocco) [12]. This kind of waste is known for its high lignocellulosic fiber content in biodegradable organic matter [2]. To this kind of waste, green, paper, and cardboard waste from the Faculty of Sciences, Dhar El Mahraz, Fez, which are also rich in cellulose, were added [1].

As a composting technique, aerobic composting in a silo was used, and the follow-up lasted 11 months; each silo received 40 kg of total waste according to the proportions indicated below:

Mixture A: (ratio of 40%: 30%: 30% of textile waste, green waste and paper and cardboard waste).

Mixture B: (ratio of 60%: 20%: 20% of textile waste, green waste and paper and cardboard waste).

Mixture C: (ratio of 80%: 10%: 10% of textile waste, green waste and paper and cardboard waste).

The initial moisture content of the mixture was 60%, and aeration was provided by manual shaking each week. Regarding the temperature monitoring, daily measurements were recorded in order to assess the temperature evolution during composting. Table 1 summarizes the main physico-chemical characteristics of the feedstock.

Table 1. Physico-chemical characterization of feedstock (textile waste, green waste, and paper and cardboard waste).

Physico-Chemical Parameter	Textile Waste	Green Waste	Paper and Cardboard Waste
Moisture %	51.28 ± 1.03	61.49 ± 1.41	11.28 ± 1.07
pH	7.47 ± 0.15	6.61 ± 0.53	7.23 ± 0.35
Total OrganicCarbon (TOC %)	31.63 ± 1.48	45.67 ± 1.37	59.35 ± 0.90
Total Nitrogen (TN %)	0.57 ± 0.04	1.23 ± 0.04	1.05 ± 0.05
C/N ratio	55.16	37.23	56.71

The choice of these proportions was aimed to determine the mixture that would provide the best results in terms of physico–chemical and microbiological parameters, but also to determine whether it is possible to obtain a good-quality compost even with a high concentration of textile waste. The evolution of organic matter was followed throughout 11 months. Samples were taken from the various mixtures at different stages (1, 6, 9, 18, 24, 28, 36, 40, and 44 weeks) and stored at 4 °C until further analysis.

2.2. Physico-Chemical Analysis

Measurements of the temperature, moisture, pH, and electrical conductivity were carried out according to the protocol described by French Association for Standardization AFNOR [13]. The total organic carbon and nitrogen levels were determined by preparing an extract from the compost samples. Water extracts were prepared using a 10:50 (*w/v*) suspension after shaking for 4h in a shaker (Rs12 Rotoshake, Gerhardt) according to a method described previously [14]. The samples were filtered, and the amounts of total organic carbon and nitrogen were determined using a TOC analyzer (Shimadzu-V CSN) [15]. The measurement of heavy metal concentrations was carried out using inductively coupled plasma spectrometry (ICP-AES) according to Alsac [16]. For this, 0.5g of a sample previously dried at 40 °C/16 h was mineralized with 6mL of hydrochloric acid and 2mL of nitric acid at 95 °C/75 min. The mineralized solution was then adjusted to 50 mL with distilled water. Appropriate dilution was then carried out as required before the analysis.

2.3. Infrared Spectroscopy Analysis

Infrared spectroscopy analysis of the three feedstocks used (textile waste, green waste, and paper and cardboard waste) was carried out using a BRUKER VERTEX 70 spectrophotometer according to Acharya et al. [17]. For this, 2 mg of each waste was oven dried, finely crushed, and homogenized with 400 mg KBr under vacuum to limit moisture interference. The spectra were plotted from 400 to 4000 cm^{-1} with a resolution of 2 cm^{-1}.

2.4. Germination Testing

Germination tests were carried out in the dark and at room temperature (25 °C) for 72 h. For this purpose, 20 corn seeds were placed on filter paper in petri dishes soaked with 5 mL of water-soluble extracts of composts of different mixtures at different stages (1, 28, and 44 weeks) (10g/100 mL of distilled water) in triplicate [18].

2.5. Microbiological Analysis

The amounts of culturable bacteria, fungi, and actinomycetes were assessed using a standard serial dilution procedure. The compost suspension was produced using 5g of compost in 45 mL

of sterile phosphate sodium buffer (0.1 M, pH 7) with Tween 80 (0.05%) and shaken for 30 min at 30 °C. Serial dilutions were made in 0.85% NaCl aqueous solution. For each microbial species, a specific medium was used: Pochon and Tardieux medium (glycerol 2.5 g L^{-1}, L-asparagine 0.25 g L^{-1}, $K_2HPO_4$1g L^{-1}, agar 15 g L^{-1}) for actinomycetes; yeast peptone glucose agar (yeast extract 5 g L^{-1}, peptone 5 g L^{-1}, glucose 7.5 g L^{-1}, agar 15 g L^{-1}) supplied with 1 mL L^{-1}amphotericin B (100 mg L^{-1}) for total microflora; and potato dextrose agar (PDA) (39g per L) for fungi [19]. All microorganisms were incubated at 30 °C, and the viable count measurements were performed in triplicate.

2.6. Enzymatic Activity Assays

Different enzymatic activities were estimated in triplicate as described below.

2.6.1. Cellulase Activity

Cellulase activity was measured after preparation of the enzymatic extract by mixing 3 g of compost with 15 mL of acetate buffer (50mM, pH 5) and stirring for one hour on an agitator table (120oscillations/min), followed by centrifugation. A sterile filter with a cellulose acetate membrane was used to sterilize and filter the supernatant [20]. Cellulase activity was measured by mixing 500 µL of enzyme extract with 500µL of 1% carboxymethyl cellulose (CMC) aqueous solution. After incubation for 1h at 50 °C, the released glucose was assayed as described in [21]. The results were expressed as units of enzymatic activity per gram of dry matter (U/g), a unit corresponding to the number of micromoles of glucose released per minute.

2.6.2. Phosphatase Activity

Enzymatic extracts for the measurement of phosphatase activities were prepared by mixing 1g of compost with 5mL of a buffer solution which was prepared from a mixture of 10mM p-nitrophenylphosphate with either an acetate buffer (0.1 M, pH 5) in the case of acid phosphatase activity or a glycine buffer (0.1M, pH 9) in the case of alkaline phosphatase activity, at 50 °C, stirred for 1h [22]. One milliliter of 0.5M $CaCl_2$ and 4 mL of 0.5MNaOH were added and the flask vortexed for a few seconds to stop the reaction [23], then samples were centrifuged. The intensity of yellow color (release of p-nitrophenol, $\varepsilon M = 1.9 \times 10^4 M^{-1}\ cm^{-1}$) was measured at 412 nm in a spectrophotometer (Thermo Scientific GENESYS 10S Series UV–Visible spectrometer).

2.6.3. Identification of Microorganisms Using NGS

The genomic DNA of the community samples was extracted directly from compost and purified using the PureLink Microbiome DNA Purification Kit, according to the instructions of the manufacturer. NGS sequencing of the DNA from sample B was performed by Macrogen (Republic of Korea). The 16S rRNA gene targeting the V3-V4 region was amplified from 16S rRNA primers specific to bacteria:

(Forward 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and Reverse:5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC). For the target amplicon of fungi, the internal transcribed spacer ITS2 region was amplified using the following primers: forward primer ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) and reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′).

Bioinformatics analysis and annotation of the data were carried out using Mothur to analyze the 16S rRNA gene fragments for bacteria and DADA2 to analyze the ITS region for fungi. Mothur is an open source bioinformatics tool for performing the analysis of microbial DNA from raw sequencing data generated on Illumina or other sequencing platforms [24]. DADA2 is an open source pipeline that uses different algorithms for sequence clustering, which is considered to be more accurate and faster than Operational Taxonomic Unit (OTU)clustering [25].

2.6.4. Statistical Analyses

The relationships between biotic and abiotic factors were analyzed by determining the Pearson correlation coefficient. The level of significance was set to 99% ($p < 0.01$). All statistical analyses were performed using the statistical package for the social sciences (SPSS) v.21 program.

3. Results

3.1. Infrared Spectroscopy Analysis

The infrared spectra of the feedstock samples are reported in Figure 1, and their assignments are summarized in Table 2.

Table 2. Assignments of the main vibrations of the infrared spectra.

Wavenumber (cm^{-1})	Band Assignments
3700–3000	H-OH groups of alcohols, phenols, and organicacids, as well as N–H groups of amides and amines
3000–2850	C–H stretching in aliphatic structures
1650–1630	C=C stretching in aromatics, C=O stretching in amides (I), C=Ostretching in ketone and quinone groups
1400	C–H stretching in aliphatic structures
1121	Amide III, aliphaticalcohols, or acids, C–O and O–H deformation of carboxylic groups of carbohydrates, C–O stretch of arylethers, esters, and organo-sulphur compounds
1060	C–H vibration, OH deformation in carboxyls, C–O of ethers on an aromatic ring
	N–H amides II
<1000	Vibrations of aromaticethers, polysaccharides (C–H vibration)

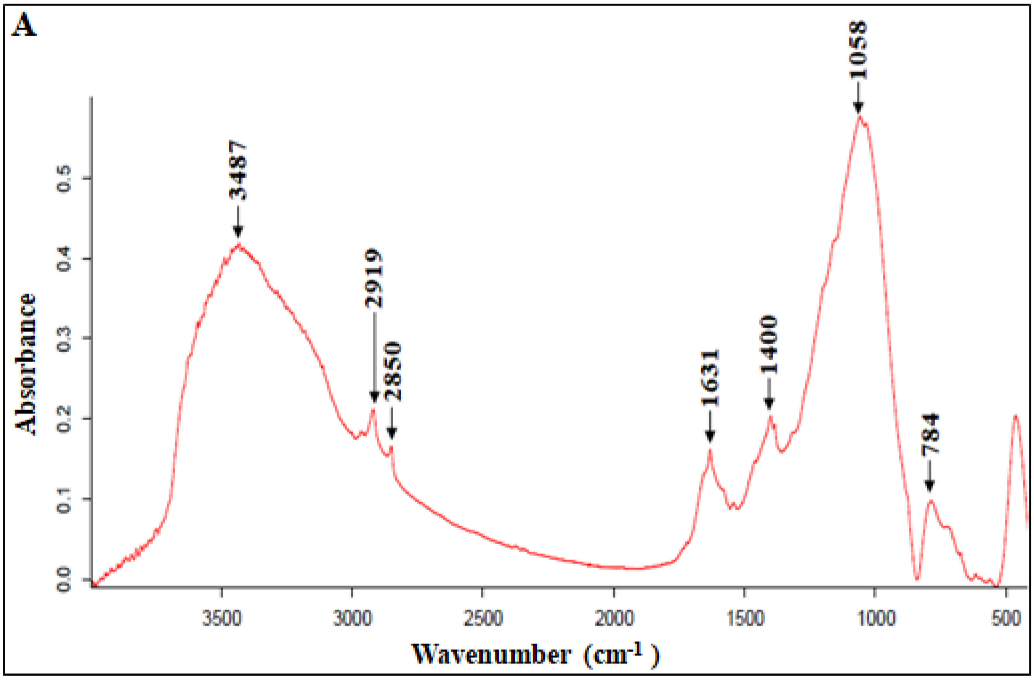

Figure 1. *Cont.*

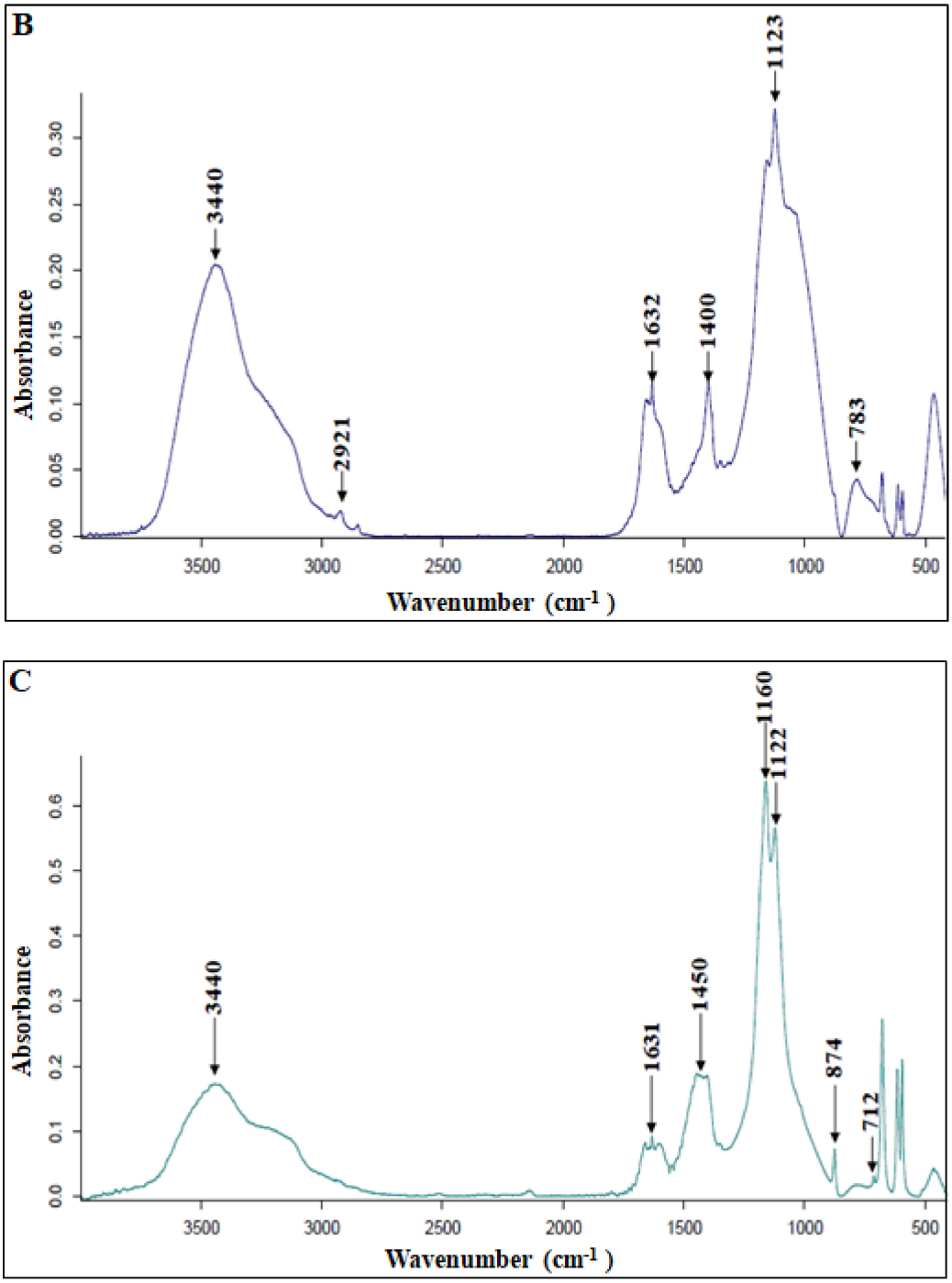

Figure 1. IR spectra of feedstock used (**A**): Textile waste, (**B**): Green waste, and (**C**): Paper and cardboard waste).

Infrared spectroscopy was used in order to identify the functional groups present in the feedstock used. The common predominant peaks observed at 3400 cm^{-1} indicate the hydrogen vibrations of alcoholic O–H, marking the presence of phenols, aromatic, polysaccharides, and saturated aliphatic species in all feedstock used [26]. Aliphatic methylene groups (C-H) were also detected between 2941 and 2892 cm^{-1} in textile waste and paper and cardboard waste. The shoulder at 1631 cm^{-1} in textile waste and in paper and cardboard waste indicates the formation of ketones, aldehydes, and carboxylic acid derivatives owing to the C=O stretching vibrations. Aromatic components in all feedstocks used were further detected via the peaks at 1400 cm^{-1} caused by C=C stretching vibrations. The peaks at

1120 and 1111 cm^{-1}, respectively, for green waste and for paper and cardboard waste are attributed to the stretching of polysaccharide [27]. The peak at 1060 cm^{-1} in textile waste characterizes primary alcohols, polysaccharides, aromatic ethers, and esters. C–H vibration was detected via the peak at 783 cm^{-1}, ascribed to aromatic ethers and polysaccharides [28].

3.2. Changes in Total Organic Carbon and Total Nitrogen Concentrations and the C/N Ratio during the Composting Process

The initial value of total organic carbon (TOC) ranged between 32.64 ± 1.50% and 43.96 ± 0.90% (Table 3). During the composting, the amount of total organic carbon decreased to 22.23 ± 0.92%, 23.57 ± 1.39%, and 23.53 ± 2.39% for mixes A, B, and C, respectively (Table 3). At the same time, the amount of total nitrogen increased to reach values at the end of treatment of 1.37 ± 0.07%, 1.39 ± 0.06%, and 1.10 ± 0.14% for the mixtures A, B, and C, respectively (Table 3). Due to the nitrogen concentration increase and the decrease in organic carbon during composting, a decrease in the C/N ratio was observed. From the first weeks onward, a sharp decrease in the C/N ratio was observed: from 32.56 to 20.87, from 39.15 to 25.04, and from 58.29 to 30.42, respectively, for mixes A, B, and C (Table 3), reflecting sustained decomposition of organic matter. Consequently, it was found at the end of the treatment that the C/N ratios were 16.30, 16.96, and 21.39 for mixes A, B, and C, respectively, which proved that the compost was reaching the stage of maturity.

Table 3. Changes in total organic carbon, total nitrogen, and C/N ratio during the composting of different wastes.

Time (Weeks)	Total Organic Carbon (%)			Total Nitrogen (%)			C/N Ratio		
	Mix A	Mix B	Mix C	Mix A	Mix B	Mix C	Mix A	Mix B	Mix C
1	43.96 ± 0.90	40.72 ± 0.62	32.64 ± 1.50	1.35 ± 0.09	1.04 ± 0.06	0.56 ± 0.10	32.56	39.15	58.29
6	42.6 ± 0.88	36.61 ± 0.59	31.32 ± 2.21	1.63 ± 0.06	1.19 ± 0.20	0.66 ± 0.21	26.13	30.76	47.45
9	36.94 ± 0.67	35.05 ± 0.84	29.81 ± 1.70	1.77 ± 0.09	1.40 ± 0.08	0.98 ± 0.18	20.87	25.04	30.42
18	28.3 ± 0.57	29.42 ± 2.59	28.26 ± 2.11	0.75 ± 0.12	0.74 ± 0.19	0.76 ± 0.12	37.73	39.76	37.18
24	27.7 ± 0.56	27.15 ± 2.53	26.17 ± 2.55	1.20 ± 0.16	1.15 ± 0.13	1.11 ± 0.13	23.08	23.61	23.58
28	26.6 ± 0.55	26.52 ± 2.05	24.92 ± 2.26	1.29 ± 0.08	1.23 ± 0.15	1.14 ± 0.14	20.67	21.56	21.86
36	24.63 ± 1.13	25.63 ± 1.75	23.91 ± 2.57	1.35 ± 0.12	1.29 ± 0.07	1.17 ± 0.07	18.24	19.87	20.44
40	22.35 ± 1.03	23.89 ± 1.55	23.62 ± 2.01	1.42 ± 0.13	1.32 ± 0.09	1.19 ± 0.06	15.74	18.10	19.85
44	22.23 ± 0.92	23.57 ± 1,39	23.53 ± 2.39	1.37 ± 0.07	1.39 ± 0.06	1.10 ± 0.14	16.30	16.96	21.39

3.3. Changes in Temperature, Moisture, pH, Electrical Conductivity, Heavy Metal Contents, and Microbial Concentration during the Composting Process

The initial temperatures of the compost were 15 °C, 14.3 °C, and 10.5 °C, respectively, for mixes A, B, and C, which were close to the ambient temperature of 10 °C. As composting proceeded, these temperatures increased rapidly; the highest temperatures were observed after 28 weeks of treatment, with 53 ± 3 °C, 46.5 ± 1.2 °C, and 42 ± 1 °C, respectively, for mixes A, B, and C (Figure 2A), which far exceeded the ambient temperature of 36±1 °C. Afterwards, the temperatures decreased until the end of the treatment, to 39 ± 1 °C, 31 ± 1.3 °C, and 23 ± 2.7 °C, respectively, for mixes A, B, and C. Regarding the moisture content of the compost, initially it was 71.22 ± 1.22%, 78.22 ± 0.23%, and 69.97 ± 0.01%, respectively, for mixes A, B, and C. Throughout the duration of the treatment, decreases in humidity until the end of the treatment, to 10.82 ± 1.02%, 30.84 ± 0.40%, and 37.24 ± 2.30% (Figure 2B), were noted.

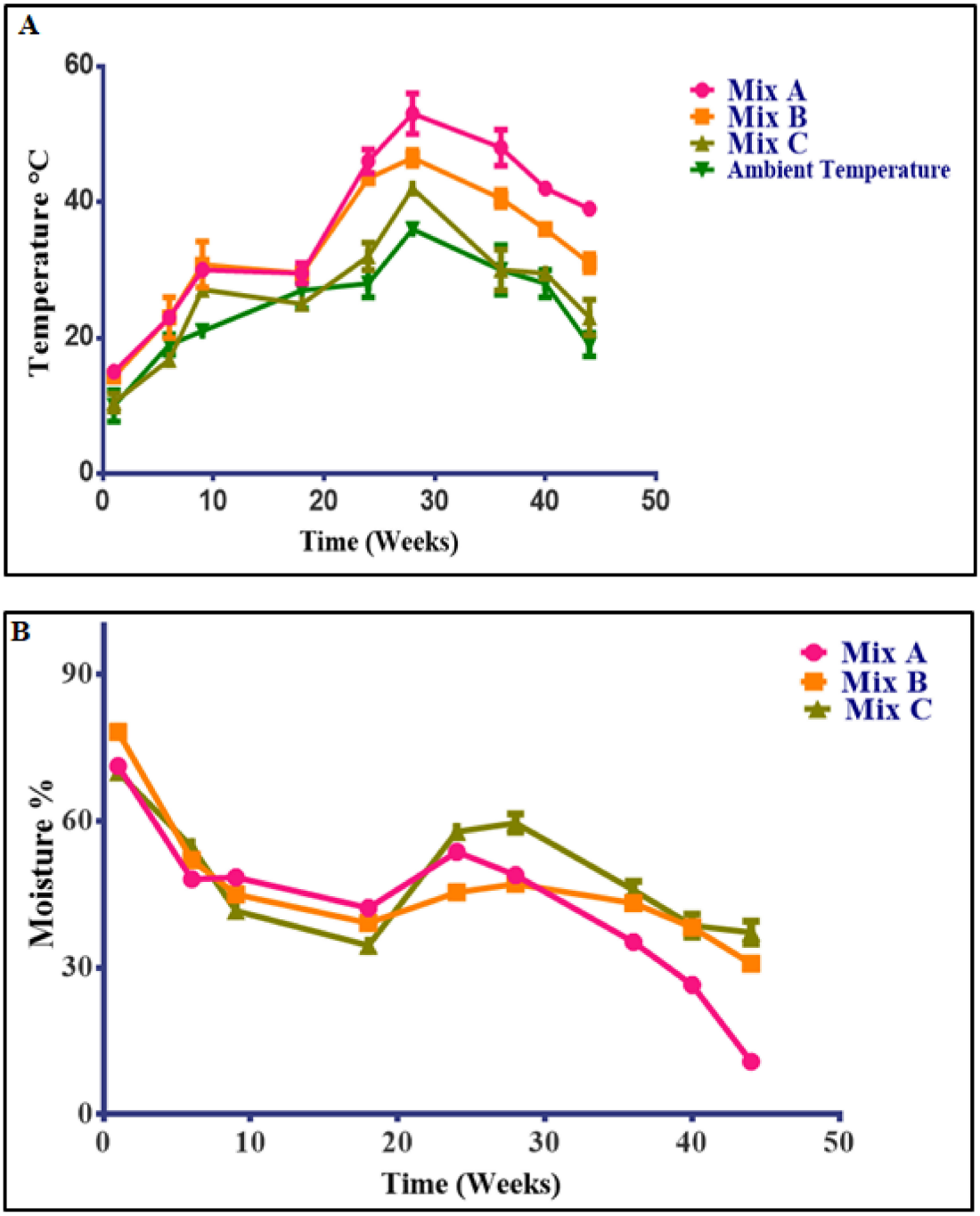

Figure 2. Changes in temperature (**A**) and moisture (**B**) during the composting of different mixtures.

Several studies have indicated that pH is one of the most important indicators of the final quality of compost and the progress of composting [29,30]. The evolution of the pH during composting is illustrated in Figure 3A. The initial pH values of the compost were 7.62 ± 0.08, 7.9 ± 0.23, and 8.37 ± 0.34, respectively, for mixes A, B, and C. At the middle of the composting period, an increase in pH values to 8.07 ± 0.08, 7.76 ± 0.20, and 8.59 ± 0.34 was recorded, followed by a decrease until the end of the treatment, to 6.41 ± 0.34, 6.12 ± 0.27, and 6.02 ± 0.18, respectively, for mixes A, B, and C (Figure 3A). Regarding the electrical conductivity, throughout the duration of the treatment, a noteworthy increase in electrical conductivity was observed up to the end of the treatment, to values of 1.79 ± 0.04, 1.74 ± 0.07, and 1.24 ± 0.09 (Figure 3B).

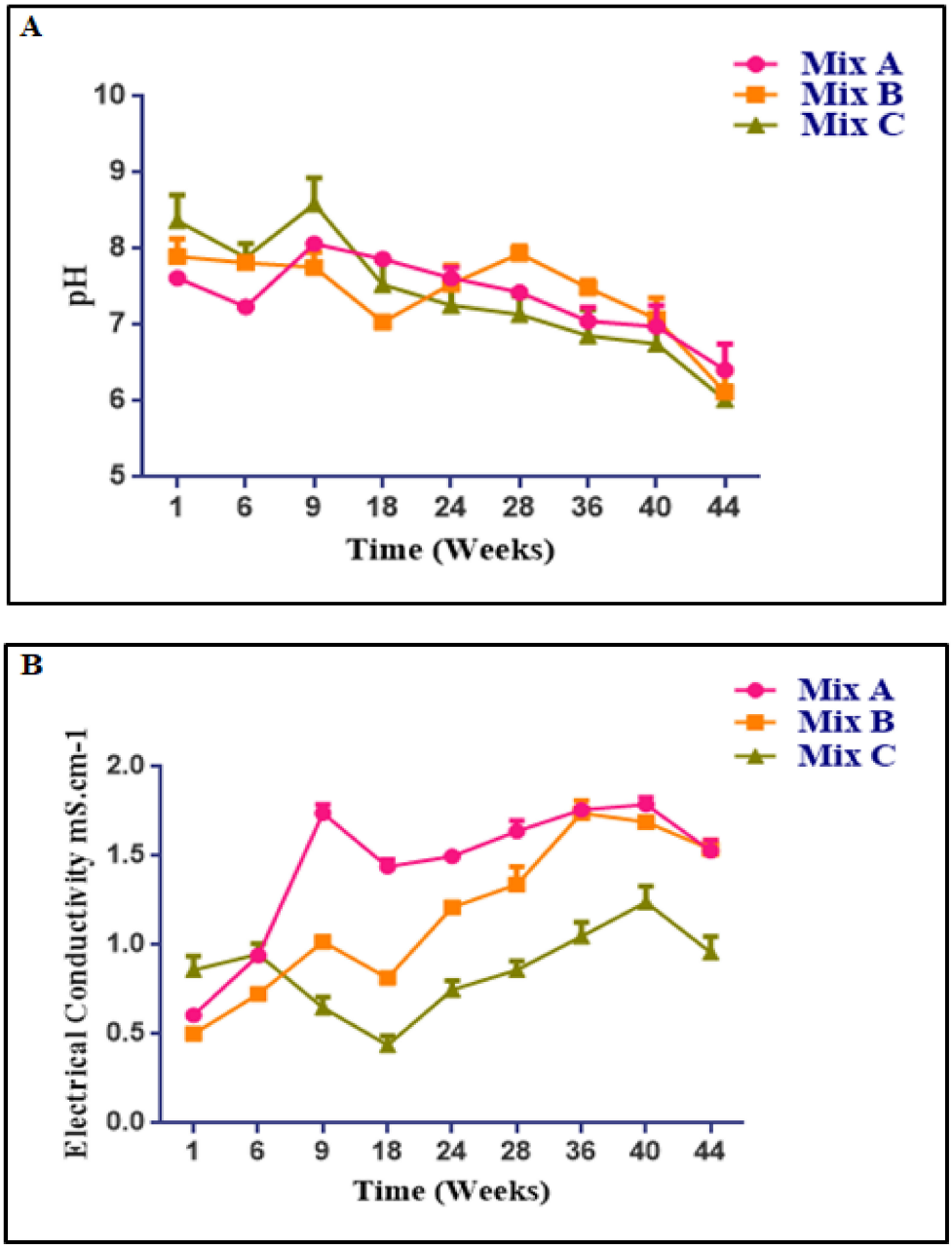

Figure 3. Changes in pH (**A**) and electrical conductivity (**B**) during the composting of different mixtures.

In order to assess the contents of heavy metals, four elements—chromium (Cr), copper (Cu), zinc (Zn), and nickel (Ni)—were studied at different stages of textile waste composting. A significant decrease in the concentration of all the heavy metals studied was recorded towards the end of the process for the mixes A, B, and C, reaching respective values of 68 ± 5.65 mg/kg, 65 ± 7.41 mg/kg, and 78 ± 4.24 mg/kg for Cr; 120 ± 1.24 mg/kg, 160 ± 6.36 mg/kg, and 100 ± 5.26 mg/kg for Cu; 320 ± 4.24 mg/kg, 210 ± 6.53 mg/kg, and 398 ± 2.24 mg/kg for Zn; and 60 ± 2.12mg/kg, 51 ± 6.53 mg/kg, and 59 ± 1.40 mg/kg for Ni (Figure 4a–c).

Regarding the phytotoxicity of the final composts and their ability to germinate corn seeds, an analysis of the germination index was carried out. Figure 5 shows that the germination index values for the final composts were 139.55%, 158.33%, and 116.42%, respectively, for mixes A, B, and C.

The evolution of microbial growth is illustrated in Figure 6. During the whole treatment, the concentrations of total microflora and fungi changed in the same direction, but with different intensity.

The initial concentration of total microflora ranged between $8.60*10^6 \pm 1.42$ CFU and $10^7 \pm 0.87$ CFU per 1 g of compost (Figure 6A).From the first weeks onward, an increase in the total microflora concentration was recorded, reaching maxima at week 28 with $2.3*10^6 \pm 2.85 CFU*g^{-1}$, $4.35*10^6 \pm 1.48$ CFU* g^{-1}, and $2.7*10^6 \pm 1.73 CFU * g^{-1}$, respectively, for mixes A, B, and C; this was followed by a decrease to $0.74*10^6 \pm 1.18 CFU*g^{-1}$, $0.98*10^6 \pm 2.76 CFU*g^{-1}$, and $0.48*10^6 \pm 1.56 CFU*g^{-1}$, respectively, for mixes A, B, and C at the end of experiment. The initial concentrations of fungi were $0.1*10^5 \pm 0.02 CFU*g^{-1}$, $1.60*10^5 \pm 0.6$ CFU*g^{-1}, and $1.90*10^5 \pm 0.37 CFU*g^{-1}$; these concentrations increased to maxima of $7.1*10^5 \pm 1.28$ CFU*g^{-1}, $8.80*10^5 \pm 1.10$ CFU*g^{-1}, and $5.00 * 10^5 \pm 1.01$ CFU*g^{-1}, and then decreased to $1.3 * 10^5 \pm 0.76$ CFU*g^{-1}, $3.00 * 10^5 \pm 1.25 CFU*g^{-1}$, and $1.90 * 10^5 \pm 0.26$ CFU*g^{-1} at the end of experiment (Figure 6B). Regarding actinomycetes, the curve reflecting their growth dynamics in the compost had the same shape as did those for the total microflora and fungi, but with a lag: at the beginning, the concentrations increased from $2.20*10^5 \pm 1.45$ CFU* g^{-1}, $0.5*10^5 \pm 0.21$ CFU* g^{-1}, and $2.10*10^5 \pm 0.91$ CFU*g^1 to $17*10^5 \pm 2$ CFU* g^{-1}, $15.60*10^5 \pm 2.04$ CFU* g^{-1}, and $13.50*10^5 \pm 2.25$ CFU* g^{-1}, respectively, for mixes A, B, and C (Figure 6C). The concentration decreased by the end of the treatment to $8.5*10^5 \pm 1.04 CFU*g^{-1}$, $3.50*10^5 \pm 1.24 CFU*g^{-1}$, and $2.50*10^5 \pm 0.64 CFU*g^{-1}$, respectively, for mixes A, B, and C.

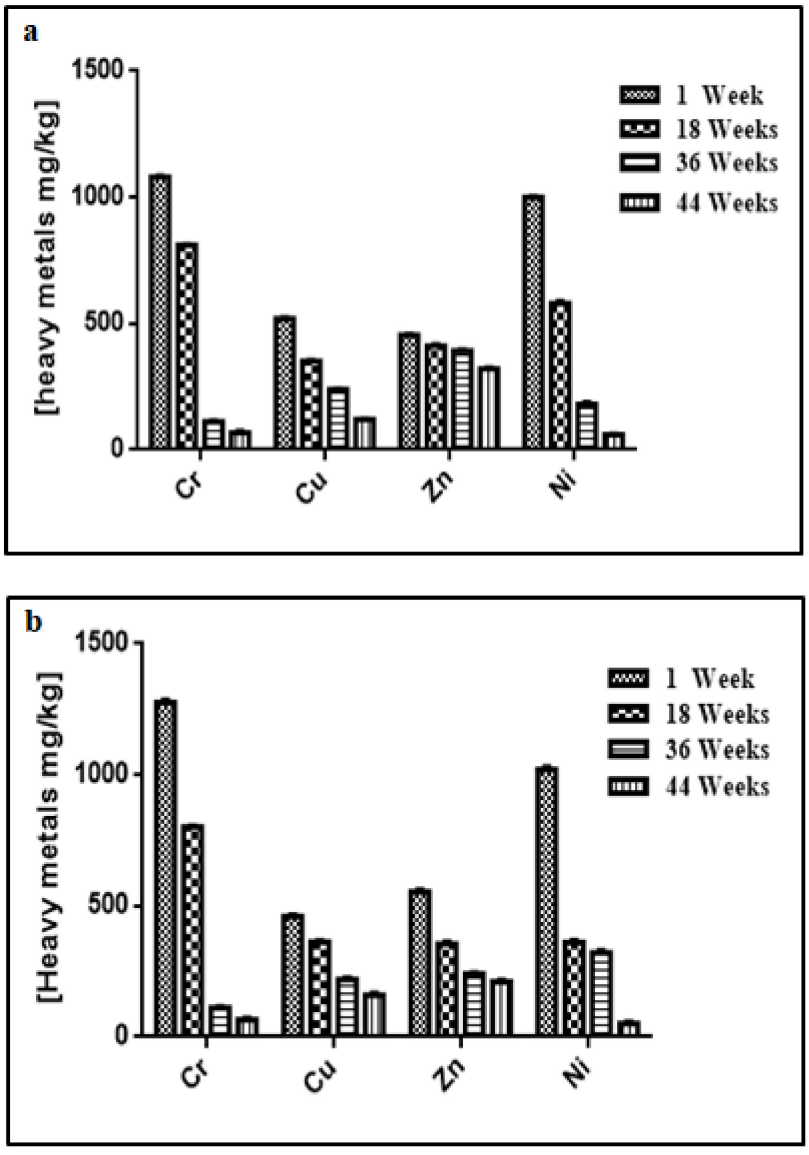

Figure 4. *Cont.*

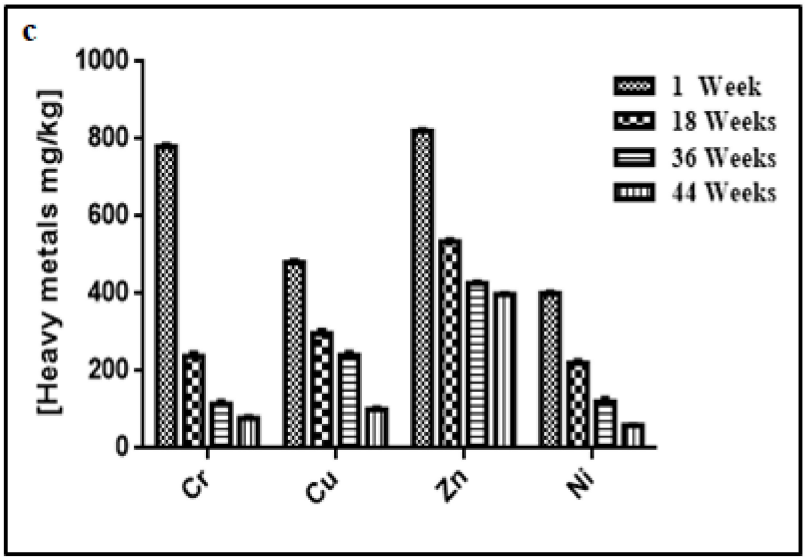

Figure 4. Contents of heavy metals (Cr, Cu, Zn, and Ni) during the co-composting process of mixes **A** (**a**), **B** (**b**), and **C** (**c**).

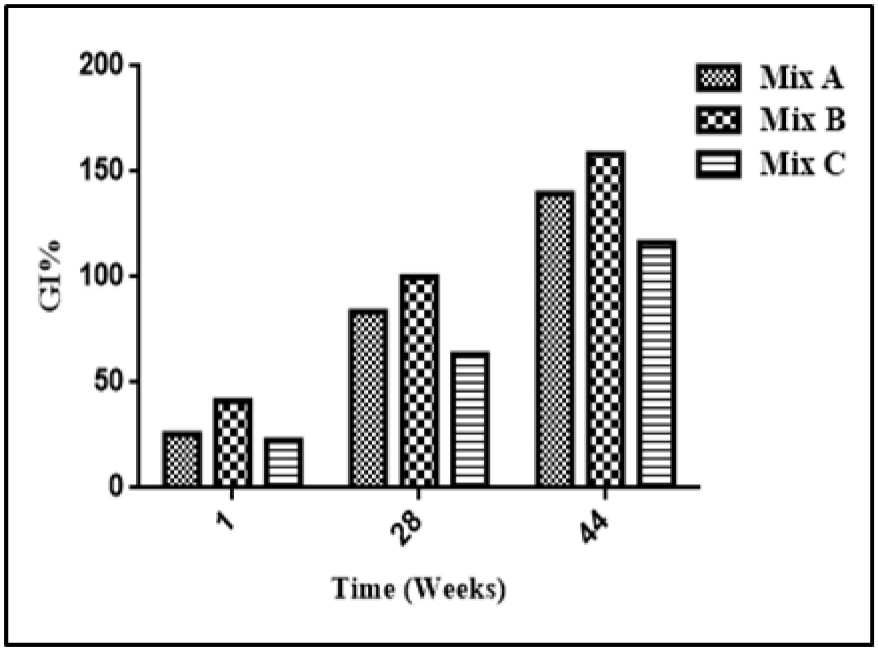

Figure 5. Evolution of phytotoxicity during the composting of mixes **A**, **B**, and **C**.

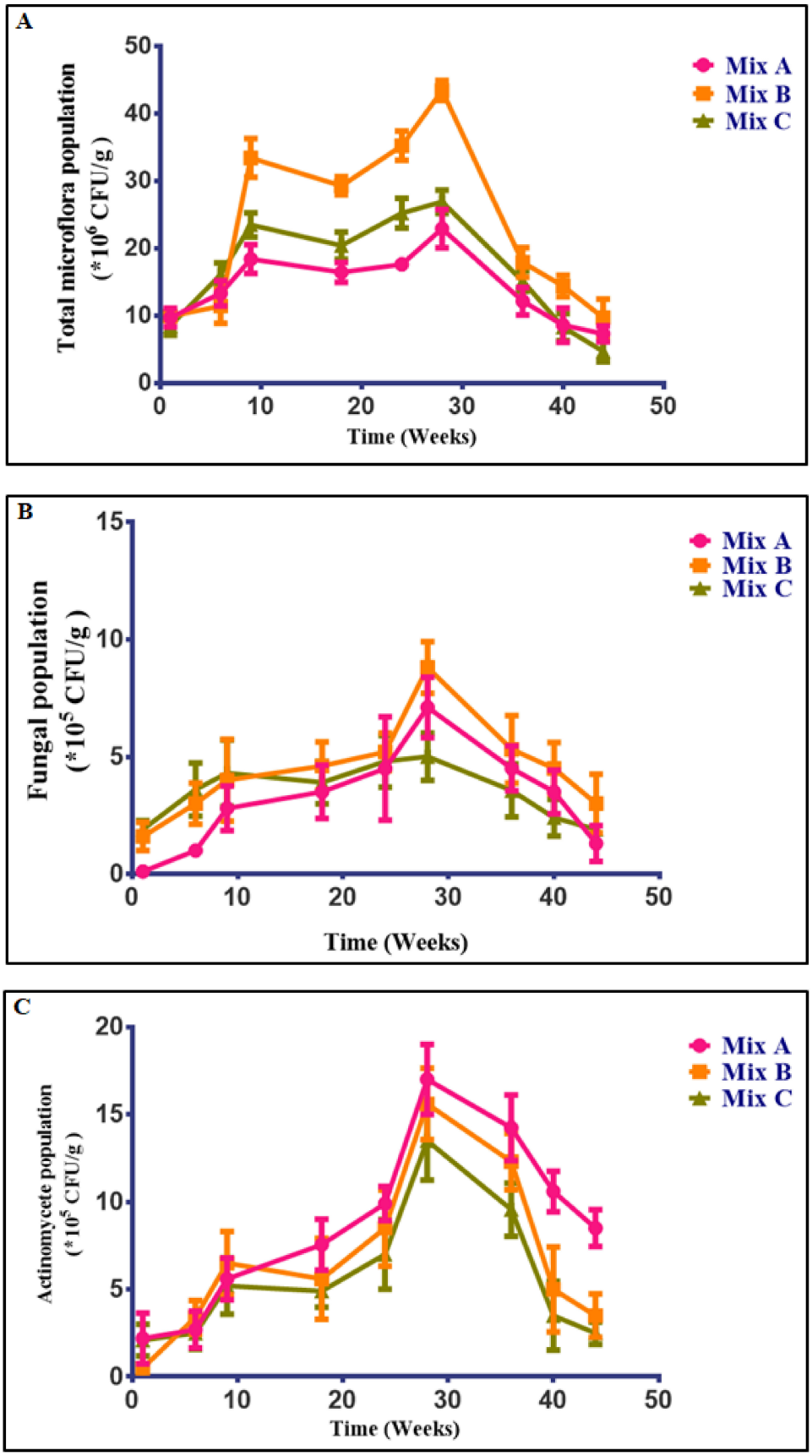

Figure 6. Changes in the total microflora (**A**), fungal (**B**), and actinomycete (**C**) populations during the composting of different mixtures.

3.4. Enzymatic Activity Changes during Composting

The assessment of different enzymatic activities provides information on the stability and the maturity of composts according to several authors [6,31], and is described below.

3.4.1. Changes in Cellulase Activity

The dynamics of cellulase activity were measured during the 44 weeks of compost maturation (Figure 7). Throughout the first 28 weeks, an increase in cellulase activity, reaching 50.62 ± 2.07 U* g^{-1}, 52.67 ± 2.53 U* g^{-1}, and 42.59 ± 2.24 U* g^{-1} for mixes A, B, and C, was observed. This is associated with an increase in the concentrations of total microflora, fungi, and actinomycetes (Figure 6), which degraded the organic matter using secreted enzymes. At the end of the treatment, cellulase activity had decreased to 9.88 ± 0.81 U* g^{-1}, 7 ± 3.38 U* g^{-1}, and 5.84 ± 1.43 U* g^{-1}. This decrease in activity was consequent to a reduction in microbial activity (Figure 6) and a decrease in temperature during the composting process (Figure 2A) [31].

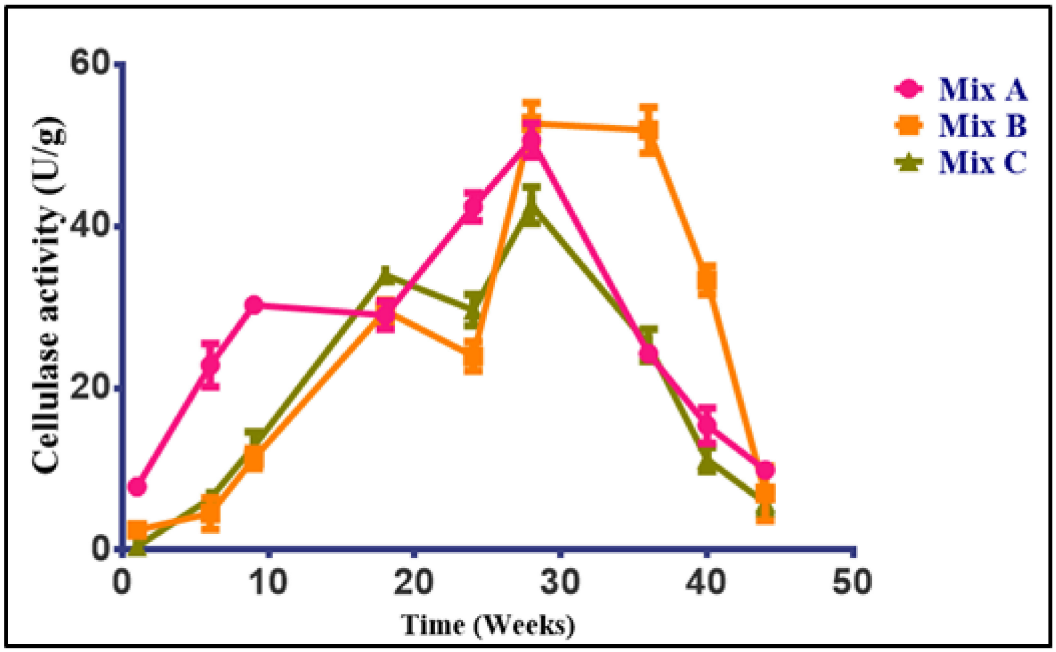

Figure 7. Changes in cellulose activity during the composting of different mixtures.

3.4.2. Changes in Phosphatase Activity

The acid and alkaline phosphatase activity levels were also measured for 44 weeks. These exhibited elevated enzyme activity throughout the duration of the treatment, with maximum values of 57.33 ± 1.29 U*g^{-1}, 68.77 ± 1.15 U*g^{-1}, and 47.58 ± 0.82 U*g^{-1} for acid phosphatase in mixes A, B, and C, respectively, and 51.12 ± 0.79 U*g^{-1}, 56.86 ± 0.87 U*g^{-1}, and 32.84 ± 2.14 U*g^{-1} for alkaline phosphatase in mixes A, B, and C, respectively (Figure 8). Afterwards, the enzymatic activities of both kind of phosphatases declined until the end of the process (Figure 8), which is in agreement with data obtained by several authors [8,22].

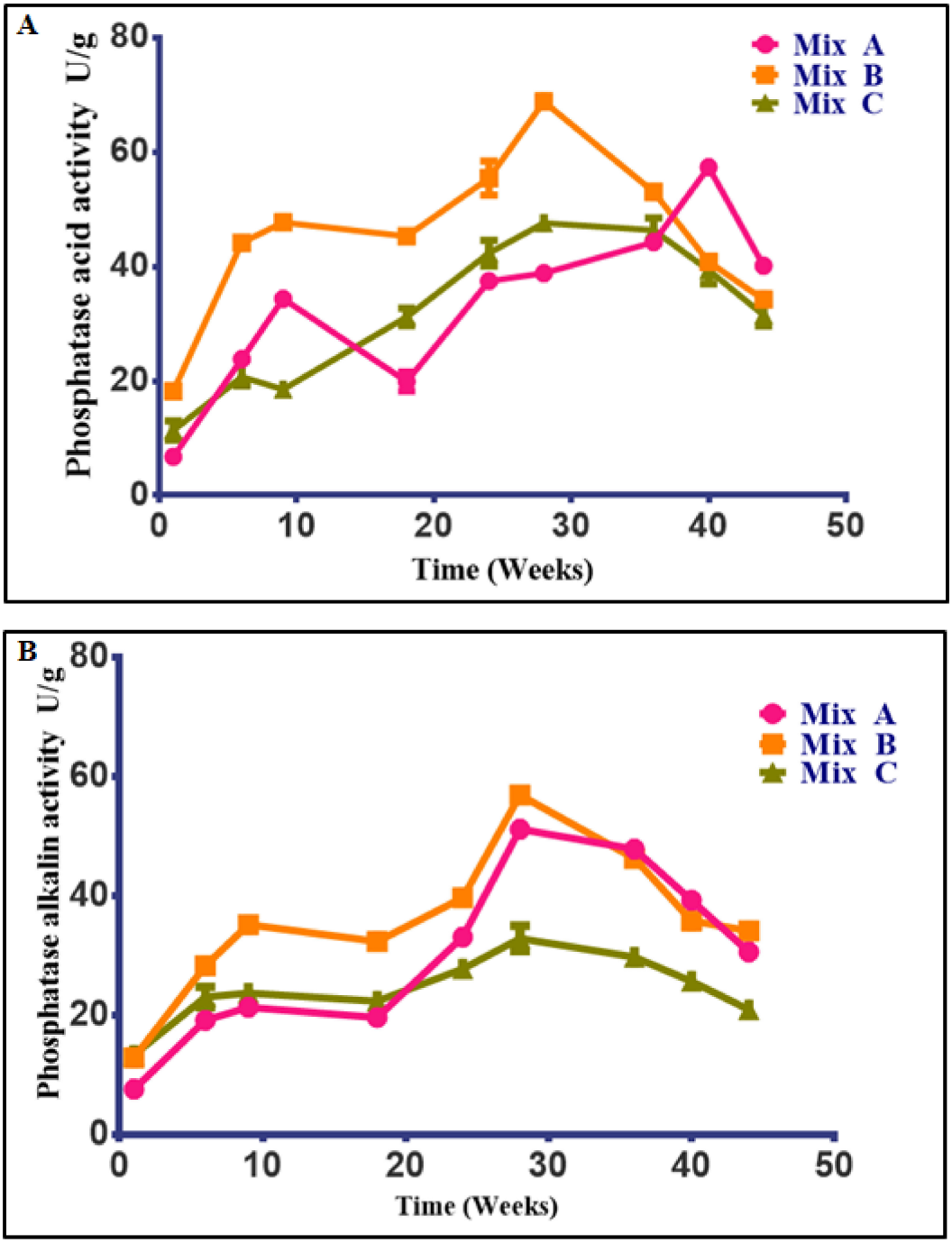

Figure 8. Changes in phosphatase (acid (**A**), alkaline (**B**)) activity during the composting of different mixtures.

3.5. Identification of Microbial Diversity by Next-Generation Sequencing

Figure 9 depicts the bacterial community in the compost. The bacterial community consisted of five majority phyla, with maximum representation by Proteobacteria(29%). This was followed by Bacteroidetes (21%), Actinobacteria (12%), Firmicutes (7%), and Chloroflexia (6%) (Figure 9). This representation was in agreement with the results found by Tkachuk et al. (2014) [10] showing that during composting, the most abundant phyla are those belonging to Actinobacteria, Firmicutes, and Proteobacteria.

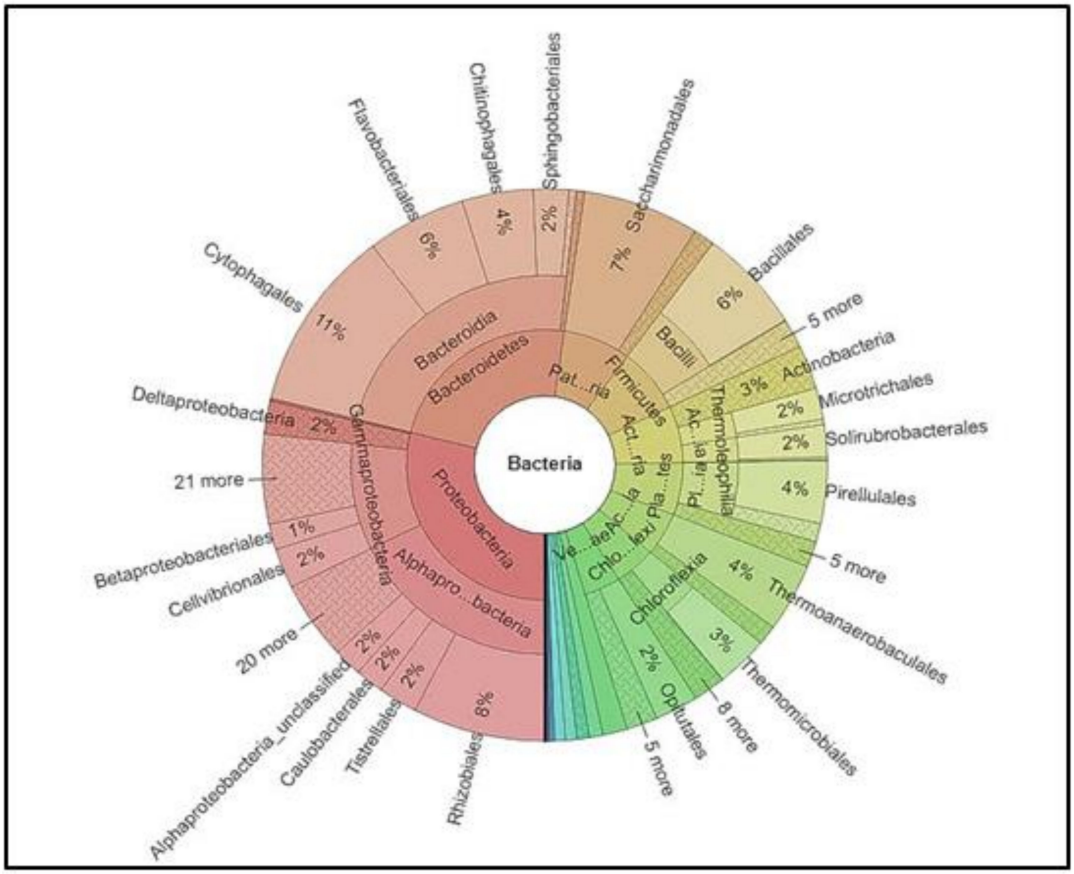

Figure 9. A Krona chart representing the distribution of the whole bacterial community in compost sample B.

At the class level, the taxonomic distribution was as follows: Alphaproteobacteria (61%), Bacilli (57%), Actinobacteria (58%), Chloroflexia (42%), and Bacteriodia (99%) present in majority. However, several classes like Gammaproteobacteria (27%), Clostridia (42%), and Thermoleophillia (21%) were present in minority (Figure 10).

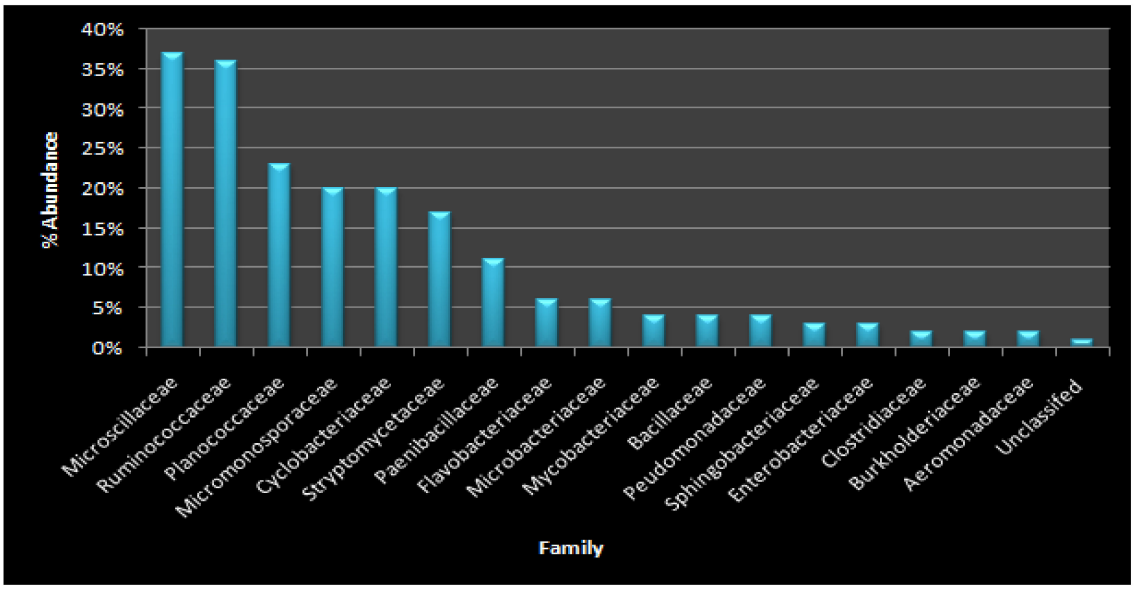

Figure 10. Family-level distribution of the bacteria groups present in compost sample B.

Molecular identification by the NGS technique allowed us to identify a wide range of the bacterial genera present in the composted waste. Among those which are known by their abilities to degrade lignocellulosic compounds and solubilize phosphate compounds, those that belong to the genera *Bacillus* (27%), *Paenibacillus* (28%), *Pseudomonas* (38%), *Clostridium* (31%), *Enterobacter* (10%), *Burkholderia* (88%), *Flavobacterium* (33%), *Streptomyces* (13%), *Microbacterium* (9%), and *Micromonospora* (96%) were found in abundance.

At the same time, the fungal community consisted of five major phyla, with maximum representation by species from Ascomycota (28%), followed by Mucoromycota (22%), Basidiomycota (21%), Rozellomycota (14%), and Mortierellomycota (6%) (Figure 11).

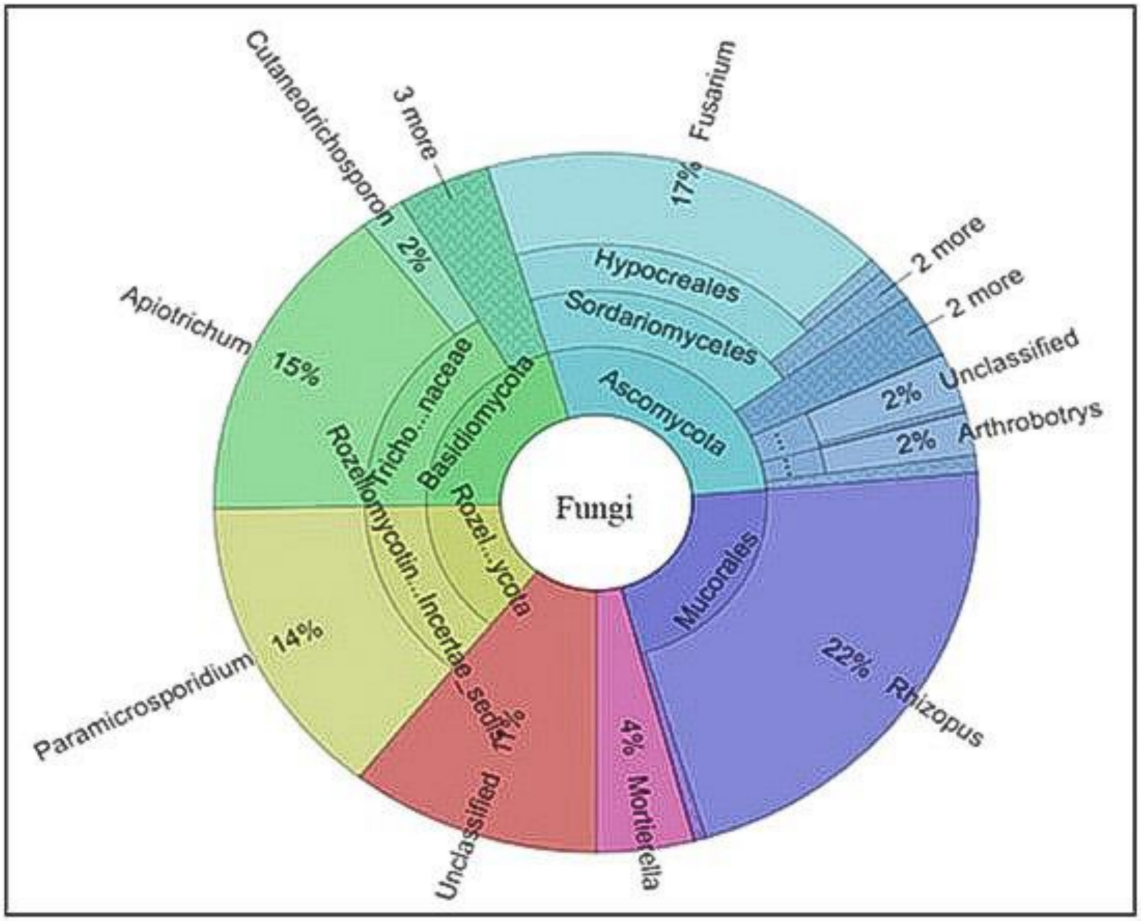

Figure 11. A Krona chart representing the whole fungalcommunity distribution in compost sample B.

In the class-level taxonomic distribution, members Trichosporonoceae (81%), Sordariomycetes (70%), Saccharomycetes (10%), Agaricomycetes (7%), and Eurotiomycetes (5%) were present in majority. The whole structure of the fungal community in the compost is presented through a Krona graph plotted using the Krona tool (Figure 11).

4. Discussion

Based on the physico-chemical and spectroscopic characterization of the feedstock, the waste used in this study has a remarkable concentration of organic matter, consisting mainly of aromatic compounds and phenolic, aliphatic, and polysaccharide structures. These results suggest that the waste might be rich in lignocellulosic material, among other unidentified molecules, which confirms the assumption regarding the abundance of the organic fraction in the mixtures studied; it provides an environment that is favorable to the proliferation of microorganisms during composting.

Throughout the composting procedure, a decrease in the total organic carbon was recorded. Such a decrease is characteristic of organic matter degradation and was observed by several authors [29,32]. In fact, similar decreases during composting have often been reported and attributed to the mineralization of organic matter by microorganisms [33]. Furthermore, together with the decrease of total organic carbon, an increase in total nitrogen was recorded. This effect is owing to the nitrogen that is produced during the degradation of carbon compounds by microorganisms [34]. A decrease in the nitrogen content during the middle stages of the composting process, mainly due to the volatilization of ammonia, was also observed, as described by Goyal et al. [35]. At the end of the composting, and owing to the assimilation of TOC, the C/N ratio decreased, as revealed by several previous studies [36,37]; such a decrease in the C/N ratio is often related to the degree of maturity. Previously, it was reported that changes in the C/N ratio reflect the decomposition and stabilization of organic matter [37]. Several studies have reported that a C/N ratio of 15–20 is indicative of acceptable maturity [32,38]. This is in agreement with the results obtained, showing that at the end of composting, the C/N values were between 15 and 20, even for mixture C with a high concentration (80%) of textile waste. Indeed, these results are in agreement with other studies that used other kinds of waste, such as sludge from water treatment plants and palm waste, and obtained similar C/N ratios at the end of composting [20,39]. In addition, this result allows us to conclude that the three mixes of composts reached a very high degree of maturity, and therefore allows to answer the main question about the feasibility of using composting for the treatment of textile waste.

Although composting is a biological treatment that relies primarily on microbial activity, the evaluation of such activity and the mechanism of action of these microorganisms on organic matter can only be achieved by monitoring changes in the environmental conditions, such as temperature, moisture, pH, and electrical conductivity. Several authors have explained that there is an interdependent relationship between environmental conditions (temperature, humidity, pH, and electrical conductivity), nutritional properties, and microbial activity [4]. In this context, the increase in activity of microbes and their metabolic reactions during composting causes a release of energy, and, therefore, an increase in temperature, which is followed by evaporation of the water [32]; this explains the increase in temperature and decrease in humidity during composting, and these results are equally linked to the increase in electrical conductivity, owing to the release of salt during the degradation of organic matter. The increase in electrical conductivity could explain the low germination index GI obtained with compost C compared to the other composts; the high concentration of salts in compost C could have a detrimental effect on plant growth. Indeed, the noteworthy decrease in pH could be linked to the presence of organic acids and/or the degradation of certain molecules such as carbohydrates by microorganisms [40]. Furthermore, according to several authors, a neutral pH could also be an indicator of compost maturity [32,34]. These results are also strengthened by the microbial diversity revealed by NGS analysis.

Taking into account the heavy metal contents, the higher concentration of heavy metals recorded at the beginning of study could be contributed by the addition of chemicals in the form of metal salts used during the manufacturing process of fabrics in the textile industry; this is the case for chromium, nickel, and copper [2]. Several authors have revealed reduction of heavy metals to the biosorption and/or the adsorption of these elements by microorganisms, due to changes in certain physico-chemical parameters such as pH and electrical conductivity, or even due to the immobilization of these heavy metals by organic matter. It may also be due to the formation of humus, which has a chelating effect on heavy metals [39,41,42]. Also, it can be attributed, according to several authors, to the synthesis of biogenic nanomaterials by several bacterial species which have the ability to bioreduce and recover heavy metal ions. Among these, we identified by NGS species belonging to the genera *Bacillus*, *Pseudomonas*, *Klebsiella*, *Escherichia*, *Staphylococcus*, *Morganella*, and *Serratia*. In fact, bacterial cells, as efficient biofactories, have an important capacity to bioreduce metal ions through metal oxidation, intracellular and extracellular sequestration of metals, metal–organic complexation, and the generation of chelators for metals, such as bio-surfactants and metallothionein, in the form of

nanocrystals of variable morphologies and sizes. This could explain this decrease in heavy metals [43,44]. Furthermore, a slight decreasing trend in the total Zn content was recorded for mix A, consisting of a high percentage of green waste (30% of green waste), which could be explained by the fact that the Zn was slowly released during the composting of green waste, making it less susceptible to loss by leaching [3]. The values obtained at the end are far below the French standards [45]. In addition, according to the compostability standard/ Quebec Bureau of Standardization CAN/BNQ 0413-200 (2012), which classifies compost into three categories (AA, A, and B), compost from textile waste belongs to category A, authorizing its use as an amendment without any risk. Ultimately, all of these obtained results explain the high germination index value at the end of composting, which confirms that the final composts had no phytotoxic effects. Indeed, according to the recommendations of the Food and Agriculture Organization (FAO) [46], with a germination index exceeding 50%, we can qualify the compost as mature, confirming that the obtained composts reach an advanced degree of maturity. These results are also in agreement with other studies that obtained similar GI% values using other kinds of waste [34,38].

The decrease in the amount of microorganisms at the end of the treatment can be explained by the decrease in carbon and nitrogen sources, mainly owing to the assimilation of organic matter by microorganisms [35]. These changes are particularly marked in the metabolic profiles, distinguishing less mature composts from those more advanced in the maturation process [19]. Therefore, to reach a better understanding of the relationship between organic matter degradation and the dynamics of microorganism metabolic activities, an enzymatic profile study focusing on cellulase and phosphatase (acid/alkaline) activities was conducted.

Cellulase activity is dependent on the types of cellulolytic microorganisms which develop on organic waste [36]. In fact, several authors have explained low initial cellulase activity by the low growth of cellulolytic fungus at the beginning of composting (Figure 6), which grows further during composting with a consequent increase in cellulose activity [35,47,48]; this was confirmed by statistical analyses indicating that the production of cellulase and the concentration of fungi correlated positively (p-value = 0.975) (Table 4). Several studies have explained that many fungal species, especially those belonging to the Ascomycetes and Basidiomycetes phyla, are known for their ability to degrade lignin, cellulose, or hemicellulose [4,48].These findings are in agreement with the NGS analysis recorded. Also, it can be suggested that the decreasing C/N ratio during composting allowed greater availability of nutrients and favored the growth of microbial biomass [49]; this assumption was confirmed by statistical analysis showing that the C/N ratio and cellulase activities correlated negatively (p-value = −0.366) (Table 4).

Table 4. Significant Pearson correlation coefficients between enzymatic activities and biotic and abiotic factors.

	Cellulase	Acid Phosphatase	Alkaline Phosphatase
C/N ratio	−0.366 **	−0.833 **	−0.786 **
Temperature	0.843	0.969	0.963
Moisture	−0.095	−0.502	−0.396
pH	−0.53	−0.040	−0.345
Electricalconductivity	0.417	0.851 **	0.792 *
Bacteria	0.788 *	0.459	0.495
Actinomycetes	0.925 **	0.853 **	0.941 **
Fungi	0.975 **	0.821 **	0.864 **

*: The correlation is significant at the 0.05 level (bilateral); **: The correlation is significant at the 0.01 level (bilateral).

During the examination of the distribution of microbial species among the taxonomic groups, it is important to consider the phylogenetics-based microbial taxonomy, rather than a set of arbitrary morphological or biochemical characteristics as used in classical taxonomy [50]. In this investigation, the whole genomic DNA of compost samples was analyzed through NGS. NGS analysis indicated that

compost B harbored a wealth of different microbial species, which means that the compost was an ideal habitat for various microbial life forms [10]. This richness could be owing to the wide range of organic matter present in the feedstock. It is known that the fungal kingdom contains a wide range of fungal species that can degrade cellulose [51], especially those belonging to phyla like Basidiomycota and Ascomycota.

In fact, among the cellulolytic fungi, representatives of the *Aspergillus* genus (*A. aculeatus)* which contain genes for the production of cellulase and xylanase have been found [52]. In addition, other species belonging to *Trichoderma (T. longibrachiatum)* and *Humicola insolens* have the ability to produce at least two exoglucanases (CBHI and CBHII), five endoglucanases (EGI, EGII, EGIII, EGIV, and EGV), and two glucosidases (BGLI and BGLII) [52]. Moreover, several authors have demonstrated that there are many other species that produce different types of cellulases to degrade cellulose; among them are *Oxysporus sp*, *Rhizopus oryzae*, *Candida pelliculosa* var. *acetaetherius*, *Kluyveromyces lactis, Pichia pastoris, Hansenula polymorpha, Yarrowia lipolytica, Leurotus ostreatus, Phanerochaete* (*P.chrysosporium*), *Penicillium (P.janthinellum* and *P.chrysosporium)*, *Lentinus edodes, Trametes versicolor, Schizophyllum*, *Saccharomyces cerevisiae*, and *Fusarium oxysporum* [7,53–56].

Additionally, among the marginally represented bacterial community members in the compost samples associated with the ability to produce enzymes capable of degrading cellulose, colonize substrates under conditions of low nutrient availability, and solubilize lignin were those belonging to the family of phyla including Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes (Figure 10) [57].

Moreover, several studies have shown that many bacterial species of Bacillus (B. pumilus, B. licheniformis, keratinolytic Bacillus cereus), Sporocytophaga (S. myxococcoides), Cytophaga (C. hutchinsonii), Pseudomonas (P. fluorescens var.cellulosa), Clostridium (C. cellulolyticus), and Pandorae (P. norimbergensis) can also use cellulose as a source of carbon and produce cellulase for its degradation [5,7,51,58]. Indeed, many species of Flavobacterium may be involved in the degradation of recalcitrant molecules (like lignocellulosic compounds) of organic matter [57].

In addition, actinomycetes are essential agents in the degradation of lignocellulose; their ability to degrade cellulose and lignin is not as great as that of fungi, but they are involved in the decomposition of cellulosic compounds present in organic matter [35]. Indeed, NGS demonstrated that in the compost samples, there were many species belonging to the Actinobacteria phylum capable of degrading cellulose compounds. Among them, many species of *Micromonospora* (*M. melanosporea)* and thermophilic filamentous bacteria *Thermobifida* (*T. fusca* and *T. cellulolytica)* which possess cellulose and lignin degrading capabilities were recorded. They equally use cellulose for growth [7,59]. Various strains of *Cellulomonas* (*C. flavigena* and *C. uda*) were reported to produce high yields of cellulase on cellulosic substrates: at least six endoglucanases and one exoglucanase. In addition, several species of *Streptomyces*, such as *S. reticuli* and *S. thermocarboxydus,* are able to degrade cellulose and hemicelluloses by oxidizing and solubilizing lignin components [60]. The study of microbial communities by NGS makes it possible to highlight the role they play in the transformations of cellulase activity which occur throughout the process, and has made it possible to explain the evolution of this activity in relation to the microbial concentration obtained. Indeed, the evolution of the microbial activity of compost C revealed a relative abundance of microflora compared to fungi, which confirms the NGS results; therefore, the production of cellulase could be owing not only to fungi but also to bacteria and actinomycetes.

This global identification by NGS of the microbial community allows us to understand the progress of organic matter degradation. This technique makes it possible to identify several kinds of microorganisms that are involved in cellulose degradation by the production of cellulases, as well as acid/alkaline phosphatases involved in phosphate solubilization.

Phosphate is an essential nutrient for plant growth. In the environment, it is found in two major forms: inorganic phosphate (Pi) that is mainly complexed with other minerals such as iron (Fe) and aluminum (Al), calcium (Ca), and magnesium (Mg), and organic phosphate (Po), which is largely immobilized in organic matter and decomposition residues. However, not all phosphate

is in a bioavailable form for the roots [8]; indeed, plants are only able to assimilate $H_2PO_4^-$ [61]. Composting is considered to be a treatment based on the activities of the two main classes of microorganisms, bacteria and fungi. They have an ability to solubilize phosphate via their enzymatic system (acid and alkaline phosphatases).

Moreover, several studies in this context have shown that phosphatase activities are clearly related to the respiratory patterns of microorganisms [8,62]. In fact, it was shown that an increase in the concentration of phosphates is related to organic matter degradation, which is mainly owing to the activity of microorganisms throughout the duration of the treatment [8,63]; this could explain the important phosphatase activities at the beginning and then their decrease, which is in agreement with statistical analysis showing that the phosphatase activity is positively correlated with the microbiota activity (Table 4). It was suggested that the decrease in phosphatase activities during composting could be attributed to the formation of complexes between enzymes and humic substances, thus disrupting enzyme–substrate interactions [64]. It can be equally explained by the global decrease in biological activity and abiotic parameters during the cooling phase of composting, which was confirmed by statistical analysis (Table 4). Alkaline phosphatase is the main phosphatase active during the composting process. Since plant roots are devoid of alkaline phosphatase, it can be inferred that the alkaline phosphatase should be attributed to bacteria and fungi [47]. However, it was suggested that, unlike microorganisms, plants produce only acid phosphomonoesterases [62]. Consequently, it is suggested that the phosphatase in the compost samples originated from microorganisms and not from green waste.

NGS analysis demonstrated the microbial diversity of species solubilizing the phosphate. In this context, several types of bacteria transform the non-bioavailable phosphate present in compost into forms usable by plants. Among the bacteria that are able to solubilize phosphate by producing alkaline and acid phosphatases are species belonging to *Rhizobium*, *Enterobacter*, *Serratia*, *Citrobacter*, *Proteus*, *Klebsiella*, *Pseudomonas (P.cepacia)*, *Flavobacterium*, *Achromobacter, Agrobacterium, Erwinia (E. herbicola), Escherichia, Mycobacterium*, and *Bacillus* [61]. The high concentration of phosphatase activity recorded with mixture B could be attributed to the bacterial diversity identified by NGS, which is in agreement with the results of the microflora microbial concentration obtained—higher in compost B compared to the other two compost mixes. Indeed, there are also bacteria belonging to the class of Gammaproteobacteria, such as *Burkholderia cepacia* and *Morganella morganii*, which contain *bceD* and *napA* genes, respectively, that encode phosphatases [65,66]. For fungal species, several authors showed that many species of *Aspergillus* (like *A. terreus*), *Saccharomyces cerevisiae*, *Neurospora crassa*, *Rhizopus delemar, Cryptococcus neoformans, Fosencaea pedrosoi*, *Humicola lutea, Sporothrix shenckii*, and *Fusarium moniliforme* possess the ability to produce huge amounts of acid phosphatase [62,64,67–69]. Other species have the ability to produce both types of phosphatases at the same time, such as *Aspergillus* (*A.niger, A. awamori*, and *A. fumigatus*), *Emmericella (E.nidulans* and *E.rugulosa), Penicillium (P.simplicissimum* and *P.rubrum)*, *Acrophialophora*, *Alternaria, Mucorrouxii*, *Coriolus versicolor*, and *Schizophyllum* [70,71]. In addition, alkaline phosphatase activity was reported for *Rhizopus stolonifer* and *A. rugulosus* [72].

5. Conclusions

In this study, the use of NGS allowed us to better understand the dynamics of microbes and their metabolic ability profile during the composting of textile waste. Through this investigation, the capacity of composting to treat and recover textile waste was discussed. The assessment of physico-chemical parameters and enzymatic activity (cellulase and phosphatase) allowed us to study the final quality of the three composts. Additionally, several species involved in cellulose degradation and phosphate solubilization belonging to bacterialphyla such as Proteobacteria, Bacteroidetes, Actinobacteria, and Firmicutes, as well as fungi such as Basidiomycota and Ascomycota, were identified by NGS.

Author Contributions: Conceptualization, S.B., M.M. and J.U.; Methodology, S.B., D.V., J.U., E.M. and S.V.; Software, S.B., T.D.; Validation, M.M. and J.U.; Formal analysis, S.B.; Investigation, S.B.; Resources, J.U., S.V.,

and S.B.; Data curation, S.B.; Writing—Original draft, S.B.; Writing—Review & editing, S.B., M.M., J.U. and S.V., Visualization, S.B., J.U.; Supervision, M.M., J.U., S.V. and M.B.; Project administration, M.M.; Funding acquisition, J.U. All authors have read and agree to the published version of the manuscript.

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