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Research Article

Role of Microorganisms and Supplement Additives during Sugarcane Straw Composting Process

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Abstract

Microorganisms and supplement additives such as Poultry Manure (PM) - Cow Manure (CM) - Ammonium nitrate (NH_3) also have an essential role in enriching and accelerating the sugar cane straw (ScS) residues biodegradation to produce organic fertilizer (compost). Supplement additives may affect physical and chemical changes inside ScS heaps, such as (pH - internal temperature - humidity ratio). These changes also affect the activity of microorganisms in the decomposition of ScS residues. So the main objectives of this investigation are to find out convenient solutions to abate the environmental impact by focused on ScS and their microbiological studies, for production of compost. Microorganisms effect of the ScS decomposition, periods of decomposition and decomposition efficiency. The self-heating temperature increased after two weeks (66.5°C to 72.7°C) was attained. After one month, Self-heating temperature decreased gradually up to the finish of the experiment. PH values recorded after two weeks were ranged of 7.2 to 8.3 throughout the composting operation. Counts of all microorganisms increased during composting process as compared with their initial counts. The total counts of thermophilic bacteria and actinomycetes were present throughout the composting process 85.5 x 10^5 and 56.8 x 10^4 CFU/g, respectively. C/N ratio decreased by composting reaching about 8.9:1, acute microbiological activities likely be due to reduce C/N ratio and Organic Matter (OM) mineralization. The supplement additives from alternatives substances enhanced the biodegradation of composting mixtures. Generally, ScS compost can be used as a substitute for other organic manures for amending soils.

Keywords: Sugarcane straw; Composting; Spore-forming bacteria; Microbiome; Biodegradation

Introduction

In these last years, sugarcane straw (*Saccharum officeinarum* L.) has been viewed as great residues of wealth that is burned [1]. Burning sugar cane straw residues leads to a loss of useful soil biomass as well as harmful Environmental Impacts due to releasing of enormous amounts of fumes from greenhouse gasses (CO and CO_2). Increasing of CO_2 content in atmosphere might increase the mean surface temperature. In adding the burning of trash produces an increase in the concentration of fine particles (ash) in the atmosphere which cause changes in the heat balance of the earth because they reflect and adsorb radiation from the sun and the earth. Environmental pollution is one of main challenging issues nowadays that researchers have been trying to heading. Sugarcane straw is agricultural residues in Egypt, it is a residues of the sugarcane harvesting consisting of dry leaves and green tops, can be preserve on fields to get better the soil quality. It is of high nutritive values and produced in layer quantities. So it can be seen that the use of sugarcane straw conform necessity of utilization to produce compost and to prod act environment from pollution. Therefore, increasing reclamation and land poverty in organic matter is a major motivator for degrading organic residues fertilizers that benefit the soil. This is also done by biologically degrading the residues by the endogenous organisms in the residues and supplement additives that activate the demolition processes and turn them into organic fertilizers (compost). Composting is a biological aerobic process by major active groups of microorganisms under controlled optimum conditions of moisture content, aeration and temperature that transform heterogeneous organic residues to humus [2-5]. This product (compost) is used as a soil conditioner or as an organic fertilizer. Composting contains major active groups of bacteria and actinomycetes. Bacteria are the largest number group in compost organisms, it secretes many exogenous enzymes to chemically break down a variety of organic matters. Supplement additives can be a useful for decreasing the composting time and enhancing the properties of compost [6]. Tallou et al. [7], reported that the high surface/Volume ratio of microorganisms permits a fast exchange of solvent substrates into the cell, their ability to produce spores permitting surviving in unpositive natural condition is an advantage over other microorganisms.

Franke-Whittle et al. [8], stated that the environmental and nutritional conditions are not the only factors that can influence microbial growth; the presence of other microorganisms can impact the activity of the tried microorganism, either positively or negatively. Hassen et al., reported that biological treatment agriculture residues, it can produce good organic matter (compost).

In this part of study, Sugarcane straw was used to prepare different composts as organic fertilizers. These experiments aimed to conduct microbiological studies on the utilization of sugar cane straw and

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converting it into organic fertilizer in order to reduce environmental pollution and reduce the use of chemical fertilizers, Under Egyptian conditions.

Materials and Methods

Raw materials and composting preparation

The sugar cane straw (ScS) utilized in this study was obtained of a private farm in ELmaseed Village, Qena Governorate, fragmented into small pieces (2-5 cm length) and air dried. Then, divided to three treatments: I) ScS+ PM, II) ScS + CM and III) ScS + NH_3. Inorganic supplement NH3 (33.5%) was added to the compost, organic supplements, Poultry Manure (PM) and Caw Manure (CM), were obtained from Faculty of Agriculture South Valley University, Qena Governorate. Additives are materials other than water added at the compost making process to increase microbial populations [9]. The heaps were prepared from one ton (2m x 3m/ton and about 1m high) with or without nitrogen according to Abu-El-Fadl [10] and Acharya et al., [11]. The sugarcane straw was completely wetted and heap in symmetric layers. The heaps were mixed every 2 days for two weeks period, with addition of water to preserve the humidity at 60%. Directly before each mixing, self-heating temperature degree was recorded daily until the end of maturation period. During four months (120 days) after composting, triplicate samples, from different parts of the heap reper enting every treatment were taken for microbiological and chemical determinations at zero, 10, 20, 30, 45, 60, 90 and 120 days.

Physicochemical analyses

Compost and plant samples were dried at 105°C and 70°C respectively to constant weights [12]. The moisture % was calculated as a percentage for each material.

{Moisture content (%) = (wet weight - dry weight / wet weight)*100}

Self-heating temperature was measured by a stem thermometer (testo 925) daily during the first week, then every week during the experimental period in three depths (20, 40 and 60 cm) below the compost top. PH values and Electrical Conductivity (EC) were measured by a pH digital meter (Adwa AD1030) according to Jackson [13]. Organic Matter Content (OM) was determined in materials and compost by glowing (burning) compost samples at 550°C to constant weight, as recommended by Page et al., [12]. Organic Carbon content (OC) was calculated according to Walkley and Black method Black et al., by multiplying the organic matter dry weight by 0.58, as reported by Jackson [13]. Soluble nitrogen was extracted by mixing 10 gram from sample with 100ml. of Page et al., [12]. Nitrite nitrogen (NO_2-N) and Nitrite nitrogen (NO_3-N) ware determined using the method given in Page et al., [12]. Total nitrogen (TN) was determined in plant material and compost, using Kjeldahl digestion method as reported by Jackson [13], While C/N ratio was calculated using values of the (OC) and (TN). On the other hand (TN) of both sugarcane straw samples was determined by micro-kjeldahl method modified by Piper.

Microbiological determinations

Initial suspensions of Ten gram of composing mixtures were prepared according to Vargas - Garica et al., [14]. Suspension was serially diluted, and thereafter 1ml of every dilution was transmitting aseptically to inoculate suitable media in triplicate using the MPN or pour plate method. The total counts of microorganisms were estimated as CFU/g. Aserial dilutions of collected samples of compost were prepared for determining total counts of Mesophilic and Thermophilic bacteria and Actinomycetes as well as Sporeformers using Topping's medium [15].

Triplicate plates for both mesophilic bacteria and actinomycetes were prepared from each dilution and incubated at 30°C for 7 days. Counts were related to one gramme compost. Colonies of mesophilic actinomycetes were distinguished, using a lens, after Ten days of incubation. Lab-lemco Yeast Extract Agar 0.2% was used for the counts of thermophilic actinomycetes [16]. Tri-plicate plates were prepared from each dilution and incubated at 55°C for 3 days. Plates of thermophilic bacteria were incubated at 55°C for 3 days also. To determine the number of sporeformers, the dilutions were pasteurized before planting for 15min, at 80°C. Inoculated plates of sporeformers bacteria were incubated at 30°C for 7 days using Topping's medium [15]. The serial dilution method was also used for counting aerobic-cellulose decomposer; five tubes from each selected liquid medium were incubated with one ml of each dilution. After incubation period, the numbers of microorganisms were obtained from Chochrans [17], and related to one gram compost. For counting aerobic-cellulose decomposers, Dubo's cellulose medium [18] was used. After incubation at 30°C for 21 days, positive tubes were recognized by yellowish-brown colour on the filter paper, which gradually lost its consistency. The microbiological changes of composting at different phase were determined by counting.

Statistical analysis

In this study data presented were the average rate of three replicates. Data were subjected to statistical assessment using one way ANOVA, and differences between average were analyzed using the least significant difference test (LSD) in ($P < 0.05$) together with EXCEL and PRISM software test for various comparisons.

Results and Discussion

Many factors determine microorganism assessment during composting process such as self-heating temperature, Microbiological and biochemical changes of either sugarcane straw differences treatments. Physicochemical and Microbiological changes during composting process in all heaps are presented in (Figure 1-9).

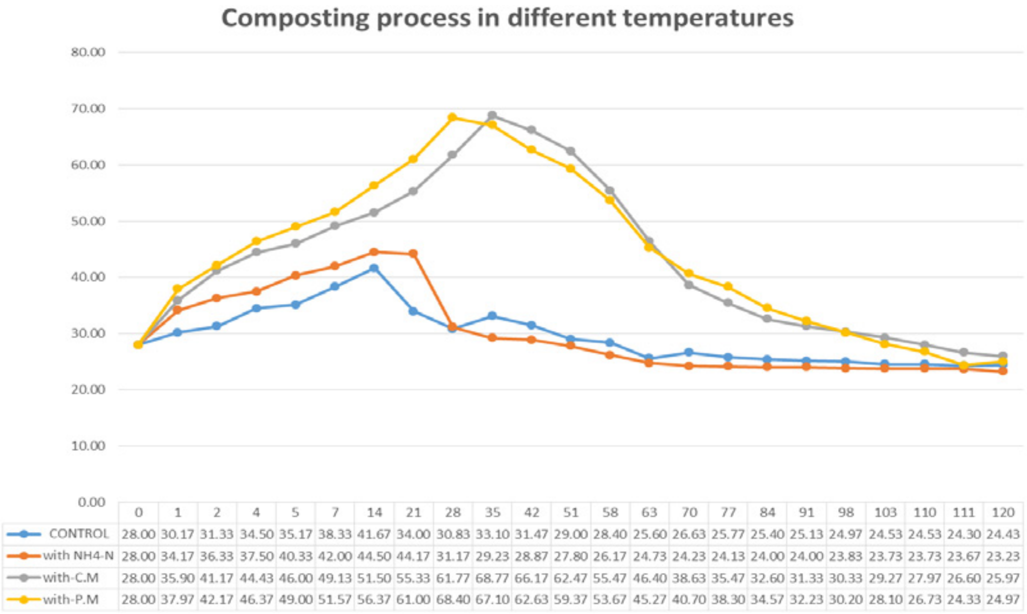
During composting process microorganisms play essential role enhancing the biodegradation of composting mixtures [3].

Physic-chemical parameters changes during composting process

During composting process Self- heating temperature, Humidity, pH and C/N ratio are very significant parameters for composting [19]. Firstly, Moisture content was determined every week for a better control of the composting process at first time at approximately 60-65% and decreased to 32-38%. In some cases moisture content decreased perhaps due to self- heating temperature causing enhanced desiccation [20]. The preferable rang of moisture is 40 to 60% [21]. At the beginning of the composting process an initial humidity of 60-65% is acceptable; then decreased to 30% which inhibits the biological

Table 1: Moisture content during different composting stages for T1: scs only; T2: scs + CM; T3: scs + PM; T4: scs + NH_3 (33.5% N).

Days	Control (T1)	Cow manure (T2)	Poultry manure (T3)	Composted ScS with NH_3 (T4)
0	62	62	65	60
5	58	55	60	53
10	47	48	58	46
15	43	45	55	41
20	41	44	51	40
25	40	40	48	36
30	38	40	44	35
35	35	38	41	33
40	33	36	38	32



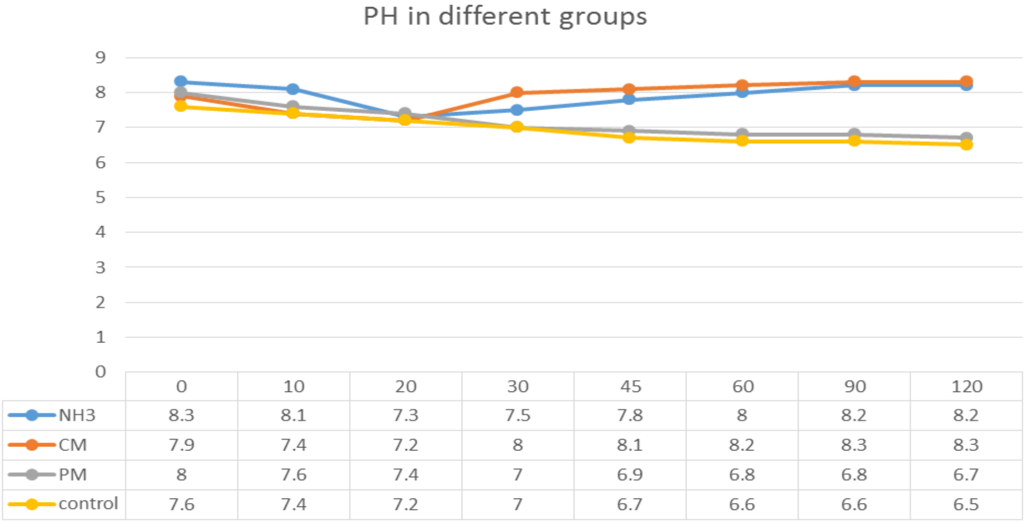
	0	1	2	4	5	7	14	21	28	35	42	51	58	63	70	77	84	91	98	103	110	111	120
CONTROL	28.00	30.17	31.33	34.50	35.17	38.33	41.67	34.00	30.83	33.10	31.47	29.00	28.40	25.60	26.63	25.77	25.40	25.13	24.97	24.53	24.53	24.30	24.43
with NH4-N	28.00	34.17	36.33	37.50	40.33	42.00	44.50	44.17	31.17	29.23	28.87	27.80	26.17	24.73	24.23	24.13	24.00	24.00	23.83	23.73	23.73	23.67	23.23
with-C.M	28.00	35.90	41.17	44.43	46.00	49.13	51.50	55.33	61.77	68.77	66.17	62.47	55.47	46.40	38.63	35.47	32.60	31.33	30.33	29.27	27.97	26.60	25.97
with-P.M	28.00	37.97	42.17	46.37	49.00	51.57	56.37	61.00	68.40	67.10	62.63	59.37	53.67	45.27	40.70	38.30	34.57	32.23	30.20	28.10	26.73	24.33	24.97

Figure 1: Composting process at different Self-heating temperature.

activity in the final product. Humidity progressively decreased with increasing composting age and it was at the ranged from 32% to 60% throughout the composting process (Table 1). When the humidity drops 65%, the oxygen content gradually decreases, and thus the anaerobic conditions prevail. So, in this experiment, the humidity of composts was set at the optimum level to ensure aerobic condition in all heaps in order to perform the proper composting. This agrees with [22,23]. Data in Table 1 presented that the humidity was reached 60 to 65% in all heaps at beginning of composting process, then the humidity was recorded around 30% with the highest values at the end of composting process. The results obtained are also in accordance with those stated by many investigators [19-23].

Secondly, Self-heating temperature is one of the most important factors to track the composting process assessment. Self-heating temperature of heaps was recorded in three depths. The results revealed that increased in different depths in all heaps reaching the maximum during the first month, then gradually decreased thereafter (Figure 1). During composting process, the self-heating temperature increased to above 60°C in the initial month in all heaps, then gradually decreased down to 45-60°C. Self-heating temperature of mixtures thereafter, fluctuated within a range from 25-34°C till the end of composting period. Generally, the self-heating temperature recorded in all treatments seemed to be high in the depths of 60cm as compared with 40 or 20 cm respectively.

Self-heating temperature reached about 71°C in the first month. This may be due to the respiration activity of mesophilic micro-organisms where these micro-organisms found suitable condition of moisture and nitrogen supply. These conditions resulted in an evolution of heat and rise in temperature so that the thermophilic conditions replaced the mesopilic ones. Self-heating temperature higher than 70°C may also inactivate most of the beneficial compost microorganisms. These results agree with those reported by many researchers [24-26]. The highest temperature (45.5°C) was recorded in T3 after 63 days, and the lowest temperature was recorded (27.6°C) in T1 after 51 days, self-heating temperature confirmed statistically ($p = 0.012$) with significant difference between T1 and T3. Lower temperatures are due to lower pH, which can affect microbial activity and growth. This agrees with [27]. But in control (T1), there



	0	10	20	30	45	60	90	120
NH3	8.3	8.1	7.3	7.5	7.8	8	8.2	8.2
CM	7.9	7.4	7.2	8	8.1	8.2	8.3	8.3
PM	8	7.6	7.4	7	6.9	6.8	6.8	6.7
control	7.6	7.4	7.2	7	6.7	6.6	6.6	6.5

Figure 2: Composting process at different hydrogen ion concentrations (pH).

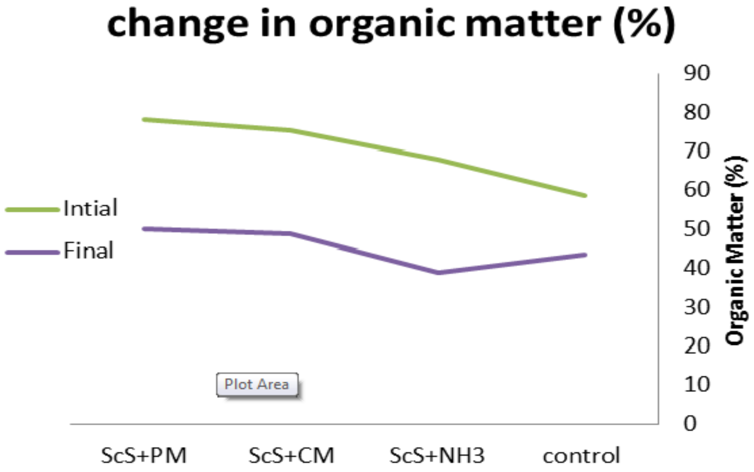


Figure 3: Change in organic matter at initial and final treatments.

is no decrease in pH values, so it recorded a noticeable increase in temperature rise, and this is due to the activity of microorganisms. At the beginning of composting process, decrease pH was due to the decrease pH of the ScS which recorded 6.5 and increased during the initial month (Figure 2). We found a decrease in pH after the first month, so the conditions are acidic in mixed heaps and this is due to the presence of short-chain organic acids, especially acetic and lactic acids. The short-chain organic acids resulted from the activity of acid-forming bacteria that work on decomposing complex carbonaceous material to organic acids as intermediate products [27,28]. In the third month, after the manure was incorporated, the pH increased to 7.0 exactly similar to the ideal neutral final pH with a high natural storage capacity [21]. Thirdly, the pH values of the proton concentration for all heaps express the extent of decomposition in the compost mass. At the beginning of the composting process, we found the pH values in the range from 7 .6 to 8.3 in all heaps and we also obtained that the pH recorded high values and ranged in most cases about 8.0 and above in the initial time of composting (Figure 2). During the composting process, the most appropriate growth of microbes is at pH 6.7-9.0 [30]. Where the pH were decreases to 6.0 or less during this period of their composting systems [31]. The difference in the starting material used in the experiments may be due to its different pH value [32,33]. During the thermophilic phase, we found that the pH levels of all composting heaps decrease to a minimum level of 7.3-7.5, and gradually increased at the range of 7.3-8.3 during 120 days. The pH values are at the same point that is 6.5 for T1, T2 6.7, T3 8.3 and T4 8.2 at the end of composting. The decrease in pH values during the composting process indicates the activity of microorganisms that mineralization and ammonification of organic matter [33,34].

During the 120 days trial period, we recorded the development of the Organic Matter (OM) in all composts heaps and characteristics of final composts are presented in (Figure.3). The Organic Matter (OM) for T3 recorded the highest value between initial and final (83.5- 59.5% OM), followed by compost T2 and compost T4 with 79.2 - 55.5 and 75.4 - 51.4% Organic Matter (OM) loss, respectively. The control compost T1 recorded the lowest percentage of Organic Matter (OM), which ranged between 61.1 and 55.2%, compared to all

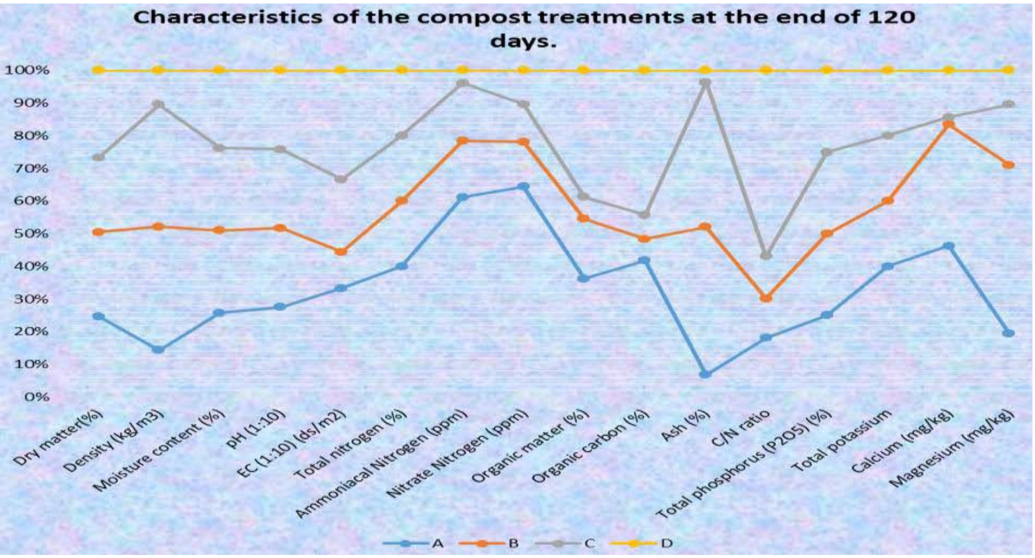


Figure 4: Characteristics of four treatments at the end of 120 days. A): Sugarcane straw + ammonium nitrate (33.5% N); B): Sugarcane straw + Cow manure (2:1); C): Sugarcane straw + poultry manure (1:1); D): Sugarcane straw (Control).

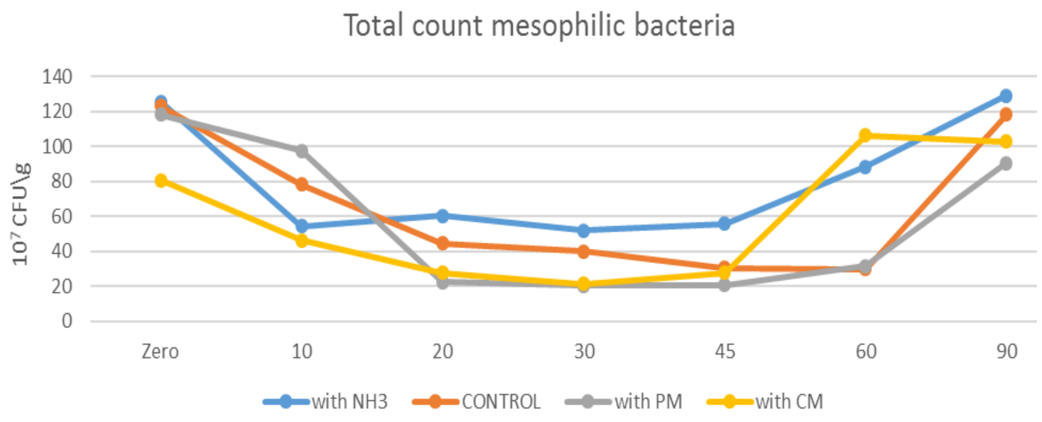


Figure 5: Total counts of mesophilic bacteria at four treatments.

other types of compost. These results agree [35]. Moreover, Carbon/Nitrogen ratio indicated that T1 was less decomposing. The organic matter (OM) and C/N ratio are the most important factors for the maturity of the compost. The Carbon / Nitrogen ratio in the materials used in the compost depends on the chemical composition of the organic matter of these materials [36]. To reach the desired C/N ratio for metabolism, microorganisms go through several life cycles to oxidize excess carbon. So, high Carbon/Nitrogen ratio will increase the composting time [37].

Total counts of mesophilic bacteria during composting process

It is clear from these results that illustrated by Figure 5 the counts of mesophilic bacteria were high in all the heaps, reaching their maximum on 90th day (counts were 129.2 x 10^7 in sugarcane straw with nitrogen and 103.0 x 10^7 sugarcane straw without nitrogen), then decreased gradually till the end experimental period may be due to the elevation of temperature and decreasing pH. Initial counts of mesophilic bacteria were near 80.7 to 125.6x 10^7 CFU/g compost in the different heaps at zero time, then decreases after 15 days and then increases again after 45 days in all heaps (Figure 5). These results show that the mesophilic bacteria play an important in the beginning of the composting as they break down the degradable organic residues. These results are in agreement with the studies presented by [3,19,28,38,39]. Results generally indicate that sugarcane straw with nitrogen contained higher numbers of mesophilic bacteria as compared with sugarcane straw without nitrogen.

Figure 5 Showed that there were significant difference between the 4 groups in 20, 30, 45 and 60 days with p value <0.05 while at zero time, 10, 90 and 120 days there was no significant difference between 4 groups with p value >0.05. In zero time, 20, 30, 45, 90 and 120 days had the highest mean among group 1 was 91.4, 32.4, 29.5, 21.4, 93.1 and 54.1 respectively, while group T3 at 10 days had the highest mean 81.7, while group T4 had the highest mean 20.4 in 60 days

Total counts of thermophilic bacteria during composting process

Data presented in Figure 6 Show that, at zero time, the initial

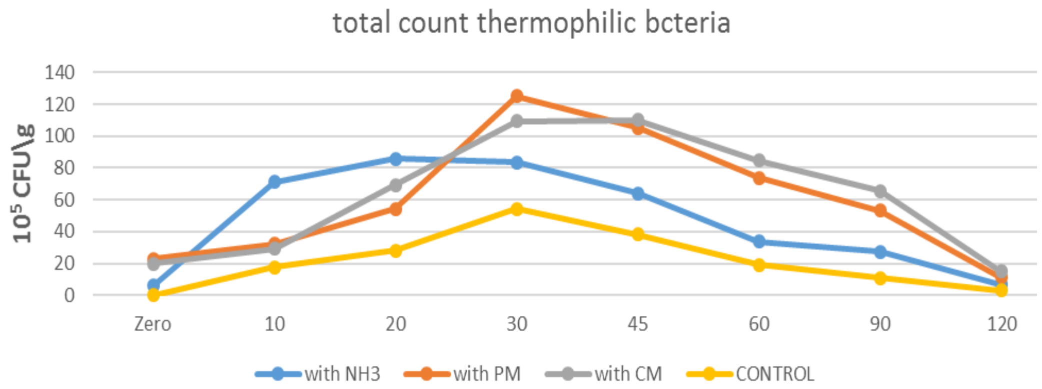


Figure 6: Total counts of thermophilic bacteria at four treatments.

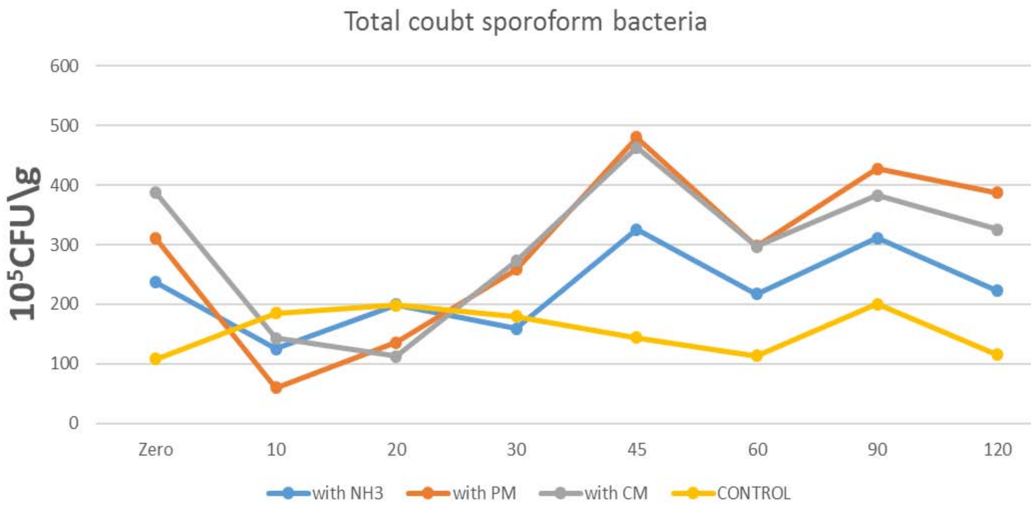


Figure 7: Total counts of Spore-forming bacteria.

densities of total thermophilic bacteria in all heaps ranged from 6.1 to 22.7 $\times 10^5$ CFU/ g compost. The total counts of thermophilic bacteria increased over time during the first few weeks to reach their maximal values in the sixth week in treatments T1, T2, T3 and T4, while treatments T2 and T3 exhibited their maximal counts in the seventh week. During the composting process, the total counts of thermophilic bacteria decreased over time throughout and the till end the composting period. The total counts of thermophilic bacteria in the final stage of composting in all heaps were nearly equal recorded at zero time, being 3.0 $\times 10^5$ to 15.1 $\times 10^5$ CFU/ g compost. These results are in harmony with [40,41].

Figure 6 showed that there were significant difference between the 4 groups in zero time, 10, 20, 30, 45, 60, 90 and 120 days with p value <0.05. In zero time and 30 days had the highest mean among group 2 was 22.7 and 125 respectively, while group 1 at 10 and 20 days had the highest mean 71.1 and 85.5 $\times 10^5$ respectively. While group 3 had the highest mean in 45, 60, 90 and 120 days 109.8, 84.6, 65.5 and 15.1 $\times 10^5$, respectively.

Total counts of spore-forming bacteria during composting process

Data in (Figure 7), show the total counts of spore-forming bacteria increased from 10^3 to 10^5 CFU/g in all heaps during the four weeks. The results we obtained in T3 showed the highest counts of spore-forming bacteria (Figure 7). The apparent increase in the number of spore-forming bacteria due to an increasing the self-heating temperature and a decreasing pH in all heaps. This gradual increase may be due to the phenomenon of competition for available nutrients between microorganisms or that these bacteria unable to degrade cellulosic materials. When the self-heating temperature decreases and the pH increases meaning suitable environmental condition, for the growth of spores and thus a decrease in the number of spore-forming bacteria occurs, These results are agreement with [38,40,41].

Data illustrated in Figure 7 there were significant difference between the 4 groups in zero time, 10, 30, 45, 60, 90 and 120 days with p value <0.05 while at 20 days there was no significant difference between 4 groups with p value >0.05. In zero time had the highest counts of sporoform bacteria in all 3 treatments (236.7), (310.1) and (386.1) $\times 10^5$ respectively, while at 45 days and 120 days had the highest count (325.5), (480) and (462) $\times 10^5$ respectively.

Total counts of actinomycetes during composting process

Actinomycetes play a major role in the reuse organic matters.

Data in Figure 8 show a decrease in counts of actinomycetes

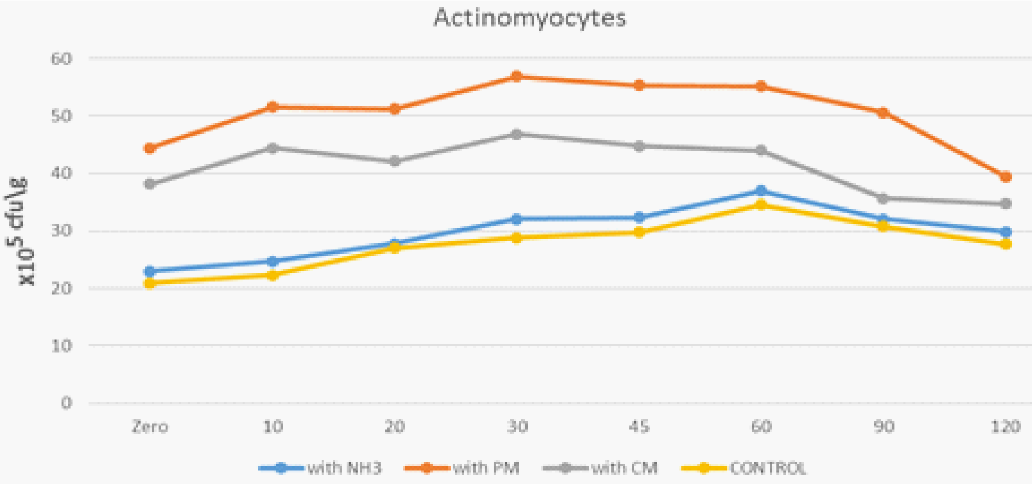


Figure 8: Total counts of actinomycetes.

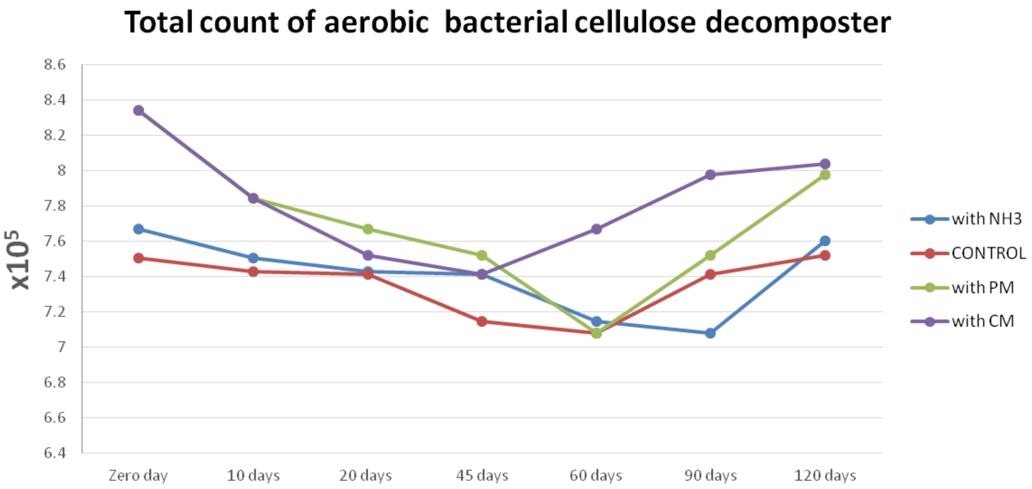


Figure 9: Total counts of aerobic cellulose decomposers.

during the first two weeks of composting followed by increases till the end of the composting period in all heaps. In the 4th week, they appeared and reached 44.0 × 10^5 CFU/g in T3 and the lowest was noted in compost T1 reached 34.5 × 10^5 CFU/g. In the first month, we found that a fast growth in counts of actinomycetes in all heaps, and the highest counts of actinomycetes were obtained after the first 2 months. The highest counts of actinomycetes (56.8 × 10^5 CFU/g) compost T2, compared with counts of actinomycetes followed by compost T3 (46.8 × 10^5 CFU/g) and compost T4 (32.1 × 10^5 CFU/g) (Figure 8). Compost T1 contained the lowest counts of actinomycetes with 28.78 × 10^5 CFU/g. During the thermophilic phase as well as the cooling and maturation phases of composting process, we found that the number of actinomycetes gradually decreased in all heaps. A drastic decrease in counts of mesophilic actinomycetes were observed, this is likely due to the high self-heating temperature effect and decreasing pH, when appropriate environmental condition are available, the number of actinomycetes increases again in the compost heaps. This results are in harmony with [28,39-41].

Figure 8 showed that there were significant difference between the 4 groups in zero time, 10, 20, 30, and 45 and 90 days with p value <0.05 while at 120 days there was no significant difference between 4 groups with p value >0.05. In zero time, 10, 20, 30, 45, 60, 90 and 120 days had the highest counts of *Actinomycetes* sp. in all three treatments was 37.0, 58.8, and 46.8 ×105 respectively.

Total counts of aerobic cellulose decomposers during composting process

Aerobic cellulose decomposers play important role in degradation of complex cellulosic materials [41,42].

Data illustrated in (Figure 9) Showed that, at zero time, the initial densities of cellulose decomposers showed different values in all the tested treatments. The treatment T2 recorded the lowest total count, being 7.5 × 10^5 CFU/ g compost. Aerobic cellulose decomposing bacteria showed a decrease during the first 21 days, and then a progressive increase in their counts was observed reaching to the maximum after 60 days. This might have been due to the reduction in the population of cellulose decomposers during the high temperature phase followed by increase due to the subsequent lower temperature and/or the degradation of the easily decomposable material at the first period of composting leaving the cellulytic-materials which are hardly decomposable to degrade at later stage of composting. This results agree with [40-42].

During the 3rd month as well as the cooling and maturation phases of composting process, we found that the number of aerobic cellulose decomposers gradually decreased in all heaps, and reached 9. 7 × 10^5 to 1.5 × 10^5 CFU/g (Figure 9). A drastic decrease in counts of cellulolytic bacteria were observed, this is likely due to low moisture content or the presence of protective substances, such as lignin. Celluloses enzymes is one of hydrolysis enzymes are cataltic enzymes which are synthesized by microorganisms during their growth on cellulosic materials [43]. Acutely of microbial activity led to mineralization organic materials and water loss resulting in a decrease the C/N ratio and compost heap size; composting additives enhancing the biodegradation of resistive material. The maximum aerobic cellulose decomposers correspond to cellulose activity, Organic Matter (OM) losses, an increase in the amounts of total and available p and k were recorded for heaps received both manure and supplemented PM. These results are agreement with [40-42].

Conclusion

This study aimed to use microbiological studies on the utilization of sugar cane straw and converting it into organic fertilizer in order to reduce environmental pollution and reduce the use of chemical fertilizers. Composting conditions during the bioprocess and the changes in the physicochemical characters confirmed that satisfactory composting occurred after 12 weeks. Although high quality products were obtained from T3 heap supplemented with poultry manure, it is palpable that the cow manure additive was the most potent, so it is recommended to accelerate the bioprocess. Moreover, additives in composting heaps enhanced macro elements availability in the final product by biological methods and enhance its agronomic effectiveness for long period and offering a cheaper source of fertilizer which increase the feasibility of the produced compost. The obtained products would contribute and increase the soil fertility.

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