

Physico-Chemical Analysis and Microbial Diversity during Windrow Pile Composting In Nile Delta Ecosystem

Mahdy A. Abd-El-Kader, Amr M. Mohamed Abd- Elall, Hesham Dahshan*, Ayman Mohamed Megahed

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia governorate, Egypt.
dr_hisham2001@yahoo.com

Abstract: The aim of this study was to investigate the composting of cattle manure with or without a variety of bulking agents. Four piles formed by cattle manure, blended with rice straw (CP2), banana leaves (CP3), maize straw (CP4). However, the first pile (CP1) composted without a bulking agent. All blends were composted for 60 days. During composting, the piles were monitored for the main physical-chemical characteristics: temperature, moisture%, pH value, total organic matter, total organic carbon, total nitrogen, C/N ratio, and $\text{NH}_4^+\text{-N}$. The majority of the studied parameters were influenced by the bulking agents rather than composting of cattle manure solely. Furthermore, temperature, pH, C/N ratio, and nitrogen retention were more valuable in composting pile blended with, rice straw and maize straw. Therefore, in another experimental trial of 10 weeks duration, due to its availability, rice straw was used as a bulking agent to investigate the prevailing microbial communities. The indigenous population of total mesophilic and thermophilic bacteria increased after two and three weeks, respectively and then the mesophilic decreased rapidly and the thermophilic stabilized or increased. Besides, the average number of total coliforms, fecal coliforms, and fecal enterococci showed decrement with time. In conclusion, the addition of a bulking agent was necessary to compost cattle manure in Nile Delta ecosystem. Specially, rice straw as it produced compost with an organic matter, total nitrogen, and C/N ratio content suitable for use as soil amendment and also more sanitary from the microbial counts view. Furthermore, this is the first report determining the influence of bulking agent addition to cattle manure on performance of composting process in the continent of Africa, Egypt.

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Key words: Composting, cattle manure, physical-chemical characteristics, bulking agent, Microbial diversity.

1. Introduction

Nowadays, there are many food-producing animals, including cattle farms in many countries throughout the world which daily produce huge amounts of manure, which must be managed under appropriate disposal practices to avoid a negative impact on the environment (**Burton and Turner, 2003**). Although, Composting cannot be considered a new technology amongst the waste management strategies, it is gaining interest as a suitable option for treatment of animal manure with economic and environmental profits (**Larney and Hao, 2007**). Certain physical and chemical characteristics of food-producing animals manure, including cattle manure are not adequate for composting and could limit the efficiency of the process; excess of moisture, low porosity, low C/N ratio, and in some cases high pH values.

Amongst the controllable factors which influence manure composting, the selection of appropriate bulking agent that will modify manure composting substrate properties (moisture, porosity, C/N ratio, pH) (**Petric et al., 2009**). **Barington et al. (2002)** noted that the moisture and the aeration regime had no consistent significant effect on adequate composting process. Only the type of bulking agent had a significant effect. There are a lot of bulking agents that could be used as a

carbon source. Very often, Farmers make their choice on the basis of the availability of specific bulking agent. However, some of the available agents have less readily degradable carbon source.

To-date, a majority of conducted studies have mainly focused on monitoring the influence of bulking agent types on the composting of poultry manure (**Petric et al., 2009**). However, there are scanty reports describing the effect of bulking agents on the physico-chemical characteristics of composting manures produced from cattle farms worldwide. So far, data about the factors affecting the composting of animal manure, including bulking agents was completely absent in Egypt. Thus, considering the adverse effects food-producing animal manures can have on public and animal health, this study was conducted to monitor the chemical and biological changes during composting of cattle manure with various bulking agents in order to get high quality stabilized product.

2. Experimental studies**Biological composting treatment of cattle manure****Input composting materials**

The experiment was performed at El-Salheya district of Sharkia governorate, Egypt. Compost materials were the cattle manure and three different

bulking agents (rice straw, banana leaves, and maize straw). Prior to composting, all compost materials were triplicate analyzed to determine the Physico-chemical

parameters including, moisture %, pH value, total organic matter%, total organic carbon%, total nitrogen%, and C/N ratio (**Table 1**).

Table 1: Input composting materials physico-chemical analysis.

Physico-chemical parameter	Cattle manure	Bulking agents		
		Rice straw	Banana leaves	Maize straw
Moisture %	44	12.7	30.9	20.3
pH value	8.4	6.4	6.8	6.9
Total organic matter %	76	82.4	75.5	93.5
Total organic carbon %	44.1	47.8	43.8	54.2
Total nitrogen %	0.95	0.76	1.13	1.24
C/N ratio	46.4	62.9	38.8	43.7

*Analysed physico-chemical parameters are presented as a mean values.

1.1.2 Composting piles preparation

The experiment was conducted for two months from February to April in 2011 on an open site at El-Arabia Factory, Salheya district of Sharkia governorate, Egypt. Weather temperature was measured during the whole period of composting trial. Four composting windrow piles were investigated. The second pile was prepared from a mixture of cattle manure and rice straw as bulking agent. However, banana leaves and maize straw were used as a bulking agent in the third and fourth composting piles, respectively. Besides, the first one was prepared from solely cattle manure. Cattle manure was mixed with bulking agents at a ratio of 4:1 (v/v) in all composting trials.

Prior to composting, the bulking agents were passed through a cutting macerating machine to yield a 10-20 cm particle size, and then were homogenized with cattle manure using a front end loader. The four

piles of cattle manure were built. Each pile had a pyramidal shape of size 2 m X 12 m square base and a height of 2 m (**Figure 1**). Initial moisture content of the mixtures was adjusted to about 50-60%, and kept within this range along the composting period by moistening the mixtures during turning process. To get the goal of composting piles moisture adjustment, the moisture content was determined immediately before every turning using an infra red moisture determinator balance (FD- 610, Kell 610).

After Windrow preparations, composting piles mixtures were initially turned for further homogenization. Furthermore, the piles were turned during the whole period of treatment once/ a week using Backhus backward windrow turning machine (Backhus, Germany), that is provided by a water tank and pipe distribution for moistening the piles during turning, if necessary.

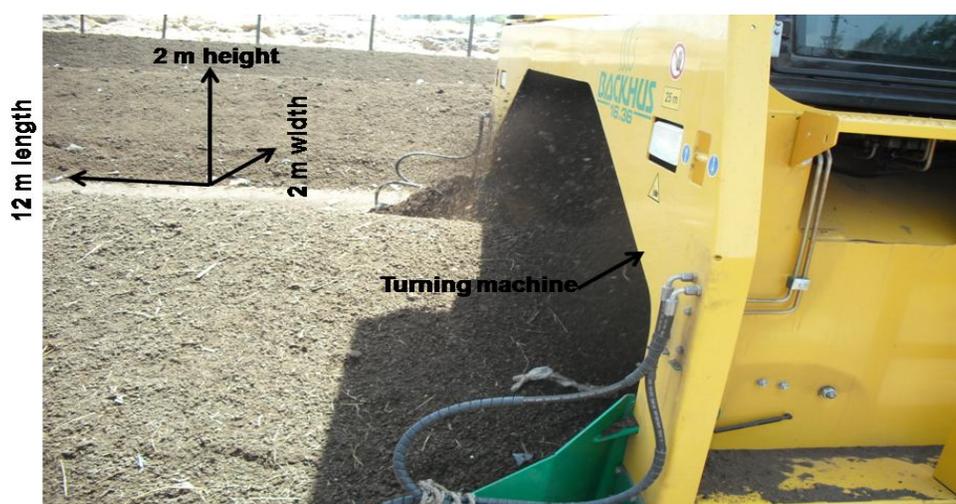


Figure1. Pictorial view of windrow composting cattle manure piles during turning

Sample regime

Compost samples were gathered using compost sampler from three different locations of the piles; top (150 cm from the base of the pile), middle (100 cm from the base of the pile), and bottom (25 cm from the base of the pile). One kilogram of cattle manure compost was collected from the presented locations in triplicate manner at day zero (initial), and then every two weeks until the end of the composting process (two months).

Analysis of Physico-chemical parameters

The compost cattle manure samples were analyzed for the following parameters; temperature, moisture content, pH value, total organic matter, total organic carbon, total nitrogen, C/N ratio, and (NH₄⁺-N) throughout the composting period. Temperature of the piles was monitored twice a week using a digital compost thermometer at the mid-way point of the pile. Ten temperature degrees were recorded per pile and the mean was obtained. The moisture content was determined by weight loss of compost sample (105 °C for 24 h) using the gravimetric method (**Tiquia and Tam, 1998**). Stirred 5 g of the sub-sample in 50 ml distilled water and pH value was measure using a pH digital meter with a glass electrode, previously calibrated and corrected in **Zhu (2007)**. Moreover, total organic matter (expressed in %) was determined by the difference between ash and dry weight (**Tiquia and Tam, 1998**) as the ash was calculated by determining the weight of compost sample after burning in the hot air oven at 550°C for 8 hrs. But, total organic carbon (%) was estimated from total organic matter using the conventional Van Bermelem factor of 1.7241 (**Tiquia and Tam, 1998**). Moreover, total nitrogen was analyzed using regular Kjeldal method (**Bremner, 1996**). Meanwhile, NH₄⁺-N was extracted using 2N KCl. Then, estimated by the Steam Distillation Kjeltic method in alkaline media (MgO) (**Lu, 2000**).

Screening of microbial counts on rice straw composting pile

Experimental design and sample collection

Cattle manure and rice straw were used as experimental materials and were homogenized into two composting windrow piles for ten weeks from the 1st of December, 2011 to the end of January, 2012 on an open site at El-Arabia Factory, Salheya district of Sharkia governorate, Egypt. Composting piles set-up (construction and management) were prepared as previously presented in the first experiment. Compost samples were collected at day zero (Initial) and then every week until the end of composting trial. Samples were taken using sterilized compost sampler, at three locations as described previously in the first experiment. Furthermore, all collected three samples were mixed thoroughly in order to make as one sample of one kilogram. For our point of view, compost samples were collected in triplicate manure from each

pile and sealed in a plastic bags, which was kept in a cooler and carried to the laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University. All samples were investigated bacteriological in our laboratory for various indicator bacteria including, mesophilic and thermophilic bacterial count, total coliform count, faecal coliform count, and faecal enterococci count within few hours after collection.

Microbial counts

Sample preparation for microbiological assays

Ten grams of cattle manure compost was added into 90 ml of sterile distilled water and shaken mechanically for 2 hrs, in order to remove the maximum of microorganisms from their organo-mineral substrates. After that, the compost suspensions prepared was used for microbial counts.

Total mesophilic and thermophilic bacterial count

The total bacteria (mesophilic and thermophilic) were determined by the dilute plate counts method (DPC) according to **ISO 6887-1, (1999)**. Eight aliquots serial dilutions of each suspension (10⁻¹ to 10⁻⁸) were inoculated on triplicate plates of Plate Count Agar (Hi Media, India) then, incubated at 30 and 55 °C/ 72 hrs for determination the total mesophilic and thermophilic count, respectively. Plates containing 25 to 250 colonies were enumerated and recorded as colony forming units (CFU) per gram of the dry weight.

Enumeration of indicator organisms

Indicator organisms including; total coliform, fecal coliform and fecal enterococci count were monitored in all compost samples using the most probable number method (MPN) according to **ISO 6887-1, (1999)**. Eight aliquots (1 ml) of each dilution (10⁻¹ to 10⁻⁸) were inoculated in lauryl sulfate tryptose broth (Hi Media, India) and then incubated at 37°C for 24 hrs for counting of total coliforms and 44°C for 24 hrs for enumeration of fecal coliforms. However, for counting of fecal enterococci, azide dextrose broth (Oxoid) was used under incubation conditions of 37°C for 22 hrs.

3. Results and discussion

Analyses of physico-chemical parameters

Input composting materials

Analysis of input composting materials on the base of physico-chemical characteristics are shown in **table (1)**. Moisture content was recorded in a descending manner as following; 44%, 30.9%, 20.3%, and 12.7% in cattle manure, banana leaves, maize straw, and rice straw, respectively. Besides, pH values were acidic in all bulking agents and alkaline only in cattle manure. Total organic matter was 93.5%, 82.4%, 76%, and 75.5% in maize straw, rice straw, cattle manure, and banana leaves, respectively. Moreover, total organic carbon content was highest in maize straw and rice straw (54.2% and 47.8%), followed by cattle

manure and banana leaves (44.1% and 43.8%), respectively. Total nitrogen content was 1.24%, 1.13%, and 0.76% in maize straw, banana leaves, rice straw, respectively but, in cattle manure was 0.95%. Regarding C/N ratio, the initial estimation was 62.9, 43.7, and 38.8 in rice straw, maize straw, and banana leaves, respectively. While, C/N ration was 46.4 in cattle manure.

According to the majority of published data, all physico-chemical parameters of analyzed input materials are ideal to achieve excellent quality matured compost (Haug, 1993; Barington *et al.*, 2002; Bernal *et al.*, 2009). Meanwhile, we noted low level of nitrogen content in the used manure in contrast with available data (Barker, 1990; Mathur *et al.*, 1990; Haug, 1993), which might be attributed to the storage of cattle manure inside farms before composting, resulting in the loss of its valuable nutrients such as nitrogen.

Composting piles analyses

Temperature

During the study, temperature gradually increased from 24.7°C, 27.3°C, 24.9°C, and 34°C in day zero (initial temperature) in composting pile1(CP1), composting pile2 (CP2, cattle manure with rice straw, composting pile3 (CP3, cattle manure with banana leaves, and composting pile4 (CP4, cattle manure with maize straw) to 38.1°C, 57°C, 43.9°C, and 55.4°C, respectively within 45 days attributed to the higher microbial activity (Table 2 and Figure 2). However, as microbial activity decreased owing to the lesser availability of nutrients, temperature finally dropped to 48.7°C, 40.8°C, and 53.2°C in CP2, CP3, and CP4, respectively. In contrast, temperature in CP1 showed continuous increasing to 48.7°C owing to the absence of bulking agents and consequently increasing the time of composting by gradually increasing of temperature for long period.

Table (2): Physico- chemical analysis of composting piles

Composting pile type	Sample collection (day)	Physico-chemical parameters							
		Temperature (°C)	Moisture %	pH value	Total organic matter%	Total organic carbon%	Total nitrogen%	C/N ratio	NH4 ⁺ -N (mg/kg)
I. Cattle manure without bulking agent ^a	Initial	24.7	51.8	8.3	78.9	45.8	0.95	49.6	144
	15	27.4	50.7	8.9	48.4	28.1	0.5	55.7	199
	30	32.8	51.1	8.5	31.5	18.3	0.8	21.2	135
	45	38.1	43	8.1	29.9	15.6	0.8	22.2	175
	60	48.7	45.2	8.2	23.3	13.5	0.5	27.4	138
II. Cattle manure with rice straw	Initial	27.3	56.8	7.7	75.1	43.5	0.9	47.5	55
	15	34.1	52.6	8.3	44.2	25.6	0.5	51.6	71
	30	40.1	50.8	8.1	30.7	17.8	0.8	22	115
	45	57	49.5	7.4	34.1	19.8	0.8	21.6	174
	60	48.7	38.7	7.1	34.5	20	0.9	22.6	165
III. Cattle manure with banana leaves	Initial	24.9	59.2	7.9	75.3	43.6	0.1	44	64
	15	28.5	55.8	8.4	52	30.2	0.6	55.3	72
	30	36.8	55.3	8.4	34.3	19.9	0.6	29.4	101
	45	43.9	49.9	7.9	25.4	14.7	0.8	17.2	126
	60	40.8	40.7	7	34.1	19.7	0.9	21.2	110
IV. Cattle manure with maize straw	Initial	34	57.7	8.1	75.8	44.0	1.0	43.4	165
	15	38.9	51.9	8.6	41.5	24.1	0.8	31.7	188
	30	47.8	49.9	8.0	38.4	22.9	0.8	27.8	149
	45	55.4	46	7.4	36.1	20.9	0.9	23.1	368
	60	53.2	35.7	7.3	35.7	20.7	0.95	21.9	251

Analysed physico-chemical parameters are presented as a mean values

Temperature is one of the key indicators of composting. It determines the rate at which many of the biological processes takes place and plays a selective role on evolution and succession on the microbiological communities (Hassen *et al.*, 2001).

Besides, Strauch and Ballarini (1994) presented temperature within 55°C for 3 consecutive days is sufficient to destroy pathogenic bacteria. Therefore, CP2 and CP4 are the best time-temperature criteria for composting sanitation process in this study.

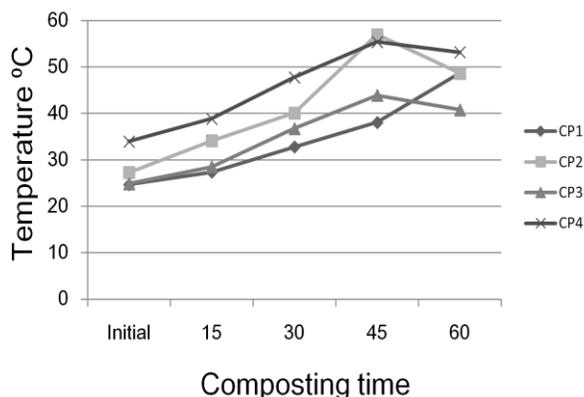


Figure 2: Temperature profile during composting time.

Moisture content

An optimum moisture content of the compost mixture is important for the microbial decomposition of the organic waste. Initial moisture content was 50 - 60% in all composting piles and according to **Gajalakshmi and Abbasi (2008)**, this range is fit for ideal composting process. However, by the end of composting time the moisture content dropped to 45.2%, 38.7%, 40.7%, and 35.7% in CP1, CP2, CP3, and CP4, respectively (**Table 2**). Moisture loss at the end of composting might be attributed to leachate formation and direct exposure to the surrounding air temperature. For our point of view, the moisture content of the CP2 and CP4 is the approved compost texture for Egyptian farmers as it facilitates compost transportation, compost handling, and spreading as a fertilizer.

pH

Initial pH of 8.3, 7.7, 7.9, and 8.1 increased to 8.9, 8.3, 8.4, and 8.6 in CP1, CP2, CP3, and CP4, respectively within 15 days of composting (**Table 2**). Increase in the pH level during composting resulted from increase in volume of ammonia released due to protein degradation (**Liao et al., 1996**). At the later stages of composting, pH decreased to 8.2, 7.1, 7.0, and 7.3 in CP1, CP2, CP3, and CP4, respectively, owing to the aerobic composting process. Similar pattern of pH change was recorded by **Kalamdhad and Kazmi (2009)**. Our findings are coincides with **Young (2005)** who stated as long as the pile stays aerobic, the buffering has been proved sufficient within the compost to allow the pH to stabilize at an alkaline level.

Total organic matter

Total organic matter reduced rapidly in all composting piles from 78.9, 75.1, 75.3, and 75.8 to 23.3, 34.5, 34.1, and 35.7 in CP1, CP2, CP3, CP4, respectively (**Table 2**). The reduction in total organic matter during composting process, mostly due to the degradation of easily degradable compounds such as proteins, cellulose and hemi-cellulose, which are

utilized by microorganisms as carbon and nitrogen sources (**Barington et al., 2002**).

Total nitrogen

Initially, total nitrogen values rapidly decreased from 0.95, 0.9, 0.1, and 1.0% at the first day of composting to 0.5, 0.5, 0.6 and 0.8% at 15 days in CP1, CP2, CP3, and CP4, respectively (**Table 2**). The process of volatilization at pH above 7 and increased temperature could be the reason for observed decrement in nitrogen values (**Bishop and Godfrey, 1983; Tiquia and Tam, 2000**). While, gradual increase in total nitrogen was noticed from the day 15 till the end of composting period in CP2 (0.9%), CP3 (0.9%), and CP4 (0.95%). However, total nitrogen in CP1 was firstly slightly increased then decreased (0.5%) in the end of composting (**Table 2**). Re-increasing in total nitrogen content after the first two weeks may be related to microorganisms nitrification and nitrification process (**Zhu, 2007**).

C/N ratio

Initial C/N ratio ranged from 43.4 to 49.6 was estimated in all composting piles (**Table 2**). A relative increase was detected in CP1, CP2, and CP3 at 15 days of composting period. While, decreased in CP4. Increase in C/N ratio in represented piles might be attributed to rapid decrease in total nitrogen in comparable to total organic carbon losses (**Eghball et al., 1997; Tiquia et al., 2001**). However, decrease in C/N ratio in case of CP4, owing to rapid onset of decomposition and consequently high carbon losses comparable to the losses in total nitrogen (**Goyal et al., 2005**). After that, the C/N ratio decreased to 27.4, 22.6, 21.2, and 21.9 in CP1, CP2, CP3, CP4, respectively. According to the CCQC maturity index (**TMECC, 2002**), excellent quality matured compost generally contain C/N ratio ≤ 25 which match with our finding results in case of CP2, CP3, and CP4 (cattle manure mixed with bulking agents). On the other aspect, CP1 (cattle manure only), the final C/N ratio is slightly higher than CCQC maturity index. According to the C/N ratio results, we highlight the importance of bulking agents in composting performance.

NH₄⁺-N

NH₄⁺-N of CP2, CP3, and CP4 increased from the initial value of 55, 64, and 165 to 165, 110, and 251, respectively (**Table 2**). Elevation of NH₄⁺-N in composting piles mixed with bulking agents indicates successful mineralization of organic matter. However, NH₄⁺-N in CP1 decreased from 144 to 138 by the end of composting period. The increment in the process of volatilization at pH above 7 and high temperature (>40) could be the reason for observed decrement in NH₄⁺-N values (**Barington et al., 2002**).

According to aforementioned physico-chemical results, was can concluded that bulking agents have a significant effect on the composting time and quality. Besides, maize straw and rice straw was the best

bulking agent amongst the three used agents due to its great influence on composting performance (physico-chemical characteristic). Concerning the selection of rice straw for analysis of microbial counts during composting of cattle manure, the main reason is its availability for cattle farms since huge amount of rice straw exist in Egypt.

Microbial diversity during composting of cattle manure with rice straw.

Mesophilic and thermophilic bacteria count

Indigenous population of mesophilic and thermophilic bacteria in the composting pile was 0.27×10^7 CFU/g and 0.03×10^7 CFU/g, respectively. The average mesophilic bacterial number increased within initial two weeks to 1.46×10^7 CFU/g. However, thermophilic number increased to 0.26×10^7 CFU/g within three weeks (**Table 3 and Figure 3**). Increasing of mesophilic and thermophilic bacteria might be attributed to the availability of large amount of organic matter/energy sources. Finally, the number reduced to 0.44×10^7 CFU/g for mesophilic bacteria and to

0.02×10^7 CFU/g for thermophilic bacteria. For our point of view, mesophilic and thermophilic bacteria count curve (**Figure 3**) indicates the succession of composting process and consequently hygienic mature compost.

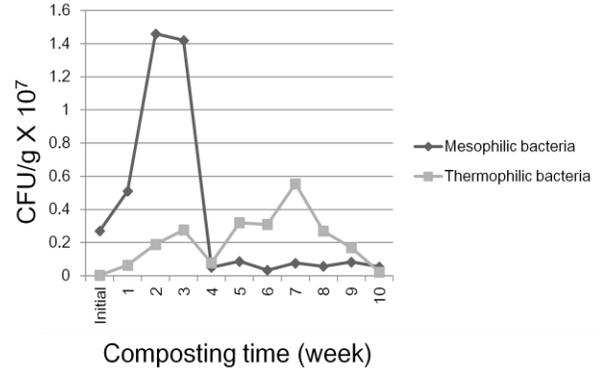


Figure 3: Mesophilic and thermophilic bacteria count during composting time

Table (3): Microbial diversity during cattle manure pile composting.

Bacterial population	Sample collection time (week)										
	Initial	1 st week	2 nd week	3 rd week	4 th week	5 th week	6 th week	7 th week	8 th week	9 th week	10 th week
Mesophilic bacteria (CFU/g X10 ⁷)	0.27	0.51	1.46	1.42	0.06	0.09	0.03	0.08	0.06	0.08	0.44
Thermophilic bacteria (CFU/g X10 ⁷)	0.03	0.06	0.20	0.26	0.28	0.32	0.31	0.56	0.27	0.17	0.02
Total Coliform (MPN/g)	36300	53000	7000	1800	50	1800	20	70	9	4	60
Fecal Coliform (MPN/g)	29700	43000	300	100	90	900	30	50	5	20	10
Fecal Enterococci (MPN/g)	7600	20000	100	700	90	2600	60	50	50	20	20

Indicator microorganisms count

Presence of coliforms is often used as an indicator of overall sanitary quality of the compost. For compost hygenisation, the recommended Total coliform, Fecal Coliforms, and Fecal enterococci densities are less than 500 MPN/g (**Vuorinen and Saharinen 1997**). The average number of total coliforms, fecal coliforms, and fecal enterococci showed a decrement with time from 36300, 29700 and 7600 MPN/g to 60, 10, and 20 MPN/g, respectively (**Table 3 and Figure 4**). Reduction in the number of indicator organisms was presumably the result of high temperature and unfavorable conditions established during thermophilic phase (**Liao, 1996**).

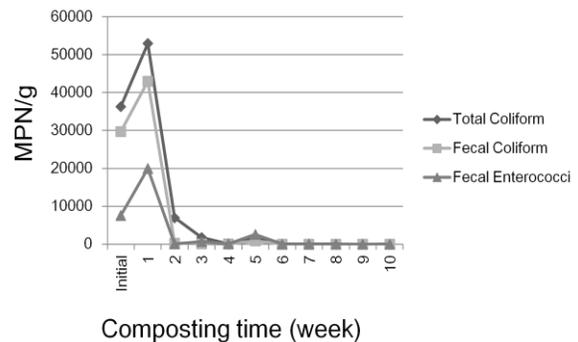


Figure 4: Indicator microorganisms count during composting time.

Conclusions

Composting of cattle manure with different bulking agents in Nile Delta ecosystem was characterized by determinations of such parameters as temperature, moisture content, pH value, total organic matter, total organic carbon, total nitrogen, C/N ratio, and (NH₄⁺-N). Our results highlighted the great influence of maize straw and rice straw on composting performance. According to the experimental results, temperature, pH, C/N ratio, and nitrogen retention were the best for cattle manure composting in CP2 and CP4. Moreover, physico-chemical characteristics of CP2 provided conditions for sanitation by reducing indicator pathogens. Further studies should be undertaken to standardize the role of bulking agents with other controllable factors on performance of composting process.

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Corresponding author

Hesham Dahshan

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Sharkia governorate, Egypt.

Dr_hisham2001@yahoo.com

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