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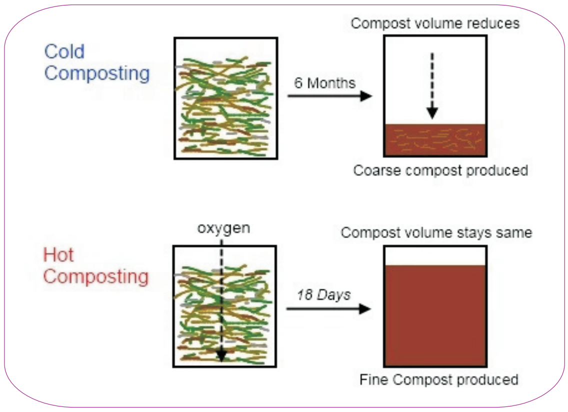
THE MICROBIAL FLUCTUATIONS IN COMPOSTED PLANT RESIDUES UNDER AEROBIC AND ANAEROBIC CONDITIONS



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ABSTRACT

A compost trial was implemented to monitor the microbial fluctuations during composting process under aerobic and anaerobic conditions. Plant residues (grass clippings) were collected from king Abdulaziz University gardens. Plastic barrels of 40cm diameter and 80cm depth were used for composting the plant residues. Four bacterial strains of cellulose decomposers were isolated and identified as Proteus vulgaris, Pseudomonas aeroginosa, Bacillus megaterium and Bacillus cereus. The most efficient two strains in cellulose decomposition (Proteus vulgaris and Bacillus cereus) were used for inoculating plant residues. The microbial populations in the composted plant material were determined at 0, 14, 28, 42 and 60 day of composting. Under aerobic conditions, the total microbial count, cellulose decomposers, actinomycetes and fungi were 1.3×10^8, 1.5×10^7, 5.0×10^6 and 5.8×10^4 CFU/g consecutively at 0 time; these numbers reduced at 14-day-old compost to record 1.1×10^7, 9.9×10^6, 1.1×10^6 and 4.3×10^4 CFU/g respectively. At 28-day-old, the microbial population raised again to record 1.4×10^8, 4.8×10^7, 9.9×10^5 and 4.1×10^4 CFU/g respectively and then decreased again at 42 and 60-day-old to register 5.9×106, 2.1×106, 2.9×104 and 1.2 × 104 at 60-day-old consecutively. Under anaerobic conditions, the same trend was recorded with less numbers in comparison with those found under aerobic conditions. Further, actinomycetes disappeared at 28, 42 and 60-day-old. Regarding temperature of the compost, the highest degree was recorded at 14-day-old (43˚C), under aerobic conditions, then gradually decreased to reach the room temperature (26˚C) in the final product. Under the anaerobic condition, the highest temperature degree (35˚ C) was recorded at 28-day-old then decreased gradually to reach 23˚C at the mature compost. The PH of compost under aerobic conditions tended to the alkaline side (8.90) at the mature compost; while under anaerobic conditions, the pH tended to the neutral side at the final compost (7.55). No great differences were recorded in organic matter content, N-content and carbon content of the mature content under aerobic and anaerobic conditions. The C:N ration of mature compost under aerobic and anaerobic conditions recorded 13 and 16 respectively.

KEYWORDS: *Compost, cellulose decomposers, aerobic and anaerobic conditions.*

INTRODUCTION

Recycling of organic wastes into organic manure is the right way to significantly fulfill the plant's nutritional requirements which has been practiced in organic farming from time to time. Hence recycling of renewable organic waste to meet the challenges of agriculture in the twenty first century is of utmost importance

(Singh *et al.*, 2005). Utilization of organic waste composts is particularly important for unfruitful soils that have low organic matter content. Likewise, many Asian agricultural regions have defective soils since farmers have used inorganic fertilizers for many years without regard to their long-term effect on soil structure and thus greatly need this type of treatment (Saithep *et al.*, 2009). Composting involves the conversion of organic residues of plant and animal origin into manure. It is largely a microbiological process based upon the activities of several bacteria, actinomycetes and fungi (Bharadwaj, 1995). High-quality compost is produced by interaction of many organisms that have suitable properties for the composting processes (Yamada *et al*., 2008). The main product is rich in humus and plant nutrients; the by-products are carbon dioxide, water and heat (Abbasi and Ramasamy, 1999). During composting, compounds containing carbon and nitrogen are transformed through successive activities of different microbes to more stable organic matter, which chemically and biologically resembles humic substance (Pare *et at*., 1998). The rate and extent of these transformations depend on available substrates and process variable used to control composting (Marche et al., 2003). The main concern for composting process is shortening the composting period. So, many efforts have been made to accelerate composting process. Microbial inoculation is one of these attempts where microbial inoculation can increase the microbial population that improves microbiological quality, generate various desired enzymes and thus enhance the degradation of organic materials (Ohtaki *et al*., 1998). Selection of suitable microorganisms is an important factor on effectiveness of inoculation (Ghaffari *et al*., 2011).

The work within hand aims at monitoring the microbiological changes during composting the plant residues under aerobic and anaerobic conditions and how to accelerate the composting process to shorten the composting period.

MATERIALS AND METHODS

Isolation and Purification of cellulose-decomposing microorganisms:

Samples were collected in new clean plastic bags from various rhizosphere soils of lettus (*Lactuca sativa*), rubber tree (*Calotropis procera*), Prosopis or acacia (*Acacia sp*.), aloe Vera (*Aloe barbadensis*) in addition to pigeons waste and previously made mature compost. Microbial strains were isolated from the inoculated tubes that gave positive results (degraded strips of filter papers) in CMC broth medium, incubated for 14 days at 30 oC, by streaking on CMC agar plates that incubated at 30oC for 5 days. The pure isolated colonies were maintained on CMC agar slants at 4oC for further analysis.

Determining the efficiency on CMC agar medium

Microbial isolates were investigated for their biodegradation efficiency of cellulose. The bacterial isolates were inoculated on CMC agar medium then incubated at 30oC for 24 hr. A preliminary qualitative assay for cellulolytic activity was carried out according to (Teather and Wood, 1982), using Congo red stain. At the end of the incubation period, the CMC agar medium was flooded with an aqueous solution of Congo red (0.1% w/v) for 15 min. The excess Congo red solution was poured off and the plates were further treated by flooding with 1M NaCl for another 15 min. The ratio between diameters of the clear zones to colony diameters was measured in order to select the highest cellulase producing microorganism where the largest ratio was assumed to contain the highest activity.

Identification of the isolated microorganisms

The obtained isolates were identified at Macrogen Inc., Seoul, South Korea by using 16S rRNA Sequencing.

Inocula Preparation

Batch cultures of the highest efficient strains of cellulose decomposers were prepared separately and mixed thoroughly before application to the pile of plant residues. In addition, diluted carbohydrate solution was prepared from dates molasses (1:20 wt/v) to be applied to the pile of the residues as an easy nutrient source for the applied cellulose decomposers strains.

Plant residues

Plant residues (Grass clippings) were collected from the gardens of King Abdulaziz University. The material was stockpiled in a safe place, until a sufficient quantity has been collected.

Plate 1: Plant residues stocking in a pile.

Plastic barrels of 40 cm diameter and 80 cm depth (Plate 2) were used for composting the plant residues. One barrel was filled with the plant residues to approximately determine the suitable quantity that will be contained in each barrel. According to the treatment, a little bit more of the approximate quantity of plant residues was spread over a clean plastic sheet for mixing with liquid culture of the cellulose decomposing microbial strain and to moisten the plant residues with water.

Plate 2: Open (aerobic) and closed (anaerobic) plastic containers for composting the grass residues.

A representative sample from each treatment was picked up for analysis at 0 time. Each barrel and according to the treatment was refilled with inoculated and moistened plant residues inside plastic bag. During the composting process, samples were taken after 14, 28, 42 and 60 days where each sample was a mixture of three replicates taken from different depths, to represent the whole quantity of composted plant residues per each barrel, for microbiological and chemical analysis. The barrels containing inoculated plant residues were divided into two groups; the first was left open during the experimental period (to represent aerobic composting) and the second group was tightly closed with the barrels covers (to represent the anaerobic composting) during the composting period (Plate 3).

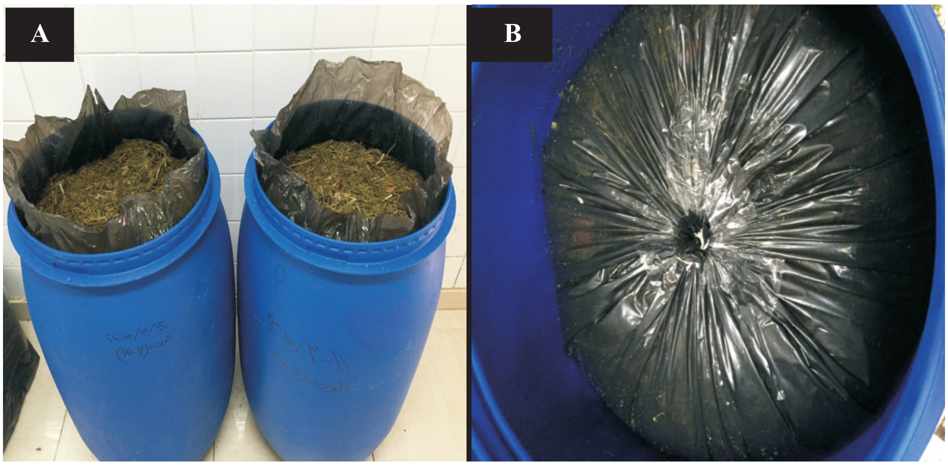

Plate 3: Inoculated grass residues packed in containers. (A), open containers for aerobic degradation; (B), tightly closed plastic bag in a container for anaerobic conditions.

Samples Collection:

The sampling was performed according the method that described by Mahdy *et al.* (2012). The combinations of 3 samples were taken from the whole profile of the pile from the top, middle and bottom after each turning 0, 14, 28, 42 and 60 day. All collected three samples were mixed thoroughly in plastic bags in order to make as one sample of one kilogram.

Samples Analysis:

Samples were collected from the compost during the different decomposition stages for the physico-chemical and microbiological determinations. Chemical and microbiological analyses were performed and results were calculated on dry weight base expect pH. Three replicates were used for physico-chemical analysis expect temperature where five replicates were used; also five replicates were used for microbiological analysis.

Estimation of Organic Matter

Organic matter content was determined via burning at 550 °C for about 3 hour in a muffle furnace then the organic matter was calculated as the difference between ash and dry weight as a percentage according to Tiquia and Tam (1998):

Organic Matter % = (Dry weight – Ash weight) / Dry weight x 100

Biodegradability coefficient (Kb) was calculated using the equation (Diaz *et al.*, 1996):

$$Kb = (OM_i - OM_f) \times 100 / OM_i (100 - OM_f)$$

Where OM_i is the organic matter content at the beginning of the composting process; OM_f is the organic matter content at the end of the composting process.

Estimation of Carbon Content

The organic matter percentage contains 58-60% carbon (Nelson and Sommers, 1996). The amount of carbon in the compost samples was calculated according to the following procedure:

Carbon content (%) = Organic Matter % ÷ 1.7.

Estimation of Nitrogen Content

Nitrogen content of the compost samples was determined at Center of Excellence in environmental Studies using Kjeltec 8420.

Estimation of C: N Ratio

The ratio between nitrogen to carbon content of the compost samples was calculated according to the following procedure:
C : N ratio = Carbon Content (%) ÷ Nitrogen Content (%).

Microbiological Analysis

The total aerobic mesophilic microorganisms were determined by the dilution plate count technique on nutrient agar according to Hassen *et al*. (2001). The number of cellulolytic aerobic microorganisms was determined by plating the appropriate dilutions of samples on CMC agar medium based on clear zones formation after staining with Congo red (0.1% w/v) for 15 min then flooding with 1M NaCl for 15 min. The number of viable fungi was measured by plating appropriate diluted suspensions into Rose Bengal agar according to Smith and Dawson (1944). Starch - Nitrate agar medium was used for actinomycetes according to Atta *et al*. (2011).

RESULTS AND DISCUSSION

Isolation and identification of cellulose-decomposing microorganisms:

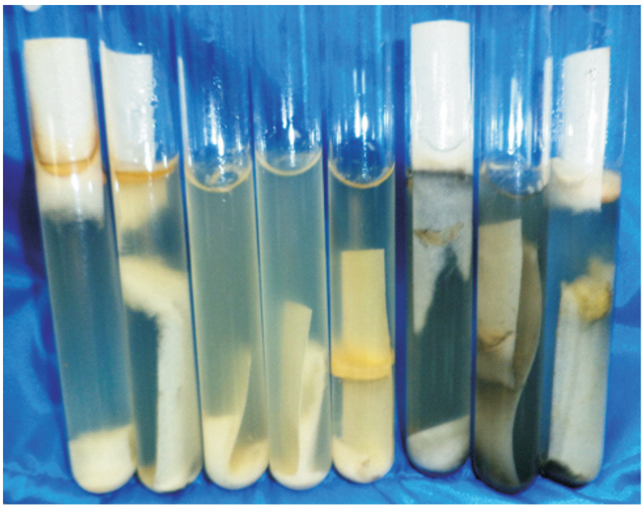

Plate 4: The positive tubes of cellulose-decomposing microorganisms.

The most efficient isolates in cellulose decomposition were shown in Plate (4). Eight positive tubes were selected and streaked on cellulose-decomposing agar medium for purification purposes. Four microbial colonies completely different in morphology were chosen and given the code symbols Rh1, Rh2, Rh3 and C. The efficiency of the four selected isolates in cellulose decomposition was assayed on CMC agar medium. Table (1) and Plate (5) show that the clear zone diameters were increased with increasing the incubation period to reach their maximum after 120 hr at 30oC. The produced clear zones by the four isolates were 4.5, 3.3, 2.9 and 4.9 cm respectively for Rh1, Rh2, Rh3 and C. The highest clear zone was recorded for the isolate C while the lowest clear zone was recorded for the isolate Rh3.

Table 1: Clear zones diameters (cm) of various isolates in CMC agar medium at different incubation periods at 30°C.

Microbial isolates	Incubation period (hr.)				
	24	48	72	96	120
	Clear zones diameter (cm)				
Rh1	2.2	3.1	3.7	4.1	4.5
Rh2	2.1	2.3	2.9	3.1	3.3
Rh3	1.2	1.5	2.1	2.6	2.9
C	2.6	2.8	3.5	4.3	4.9

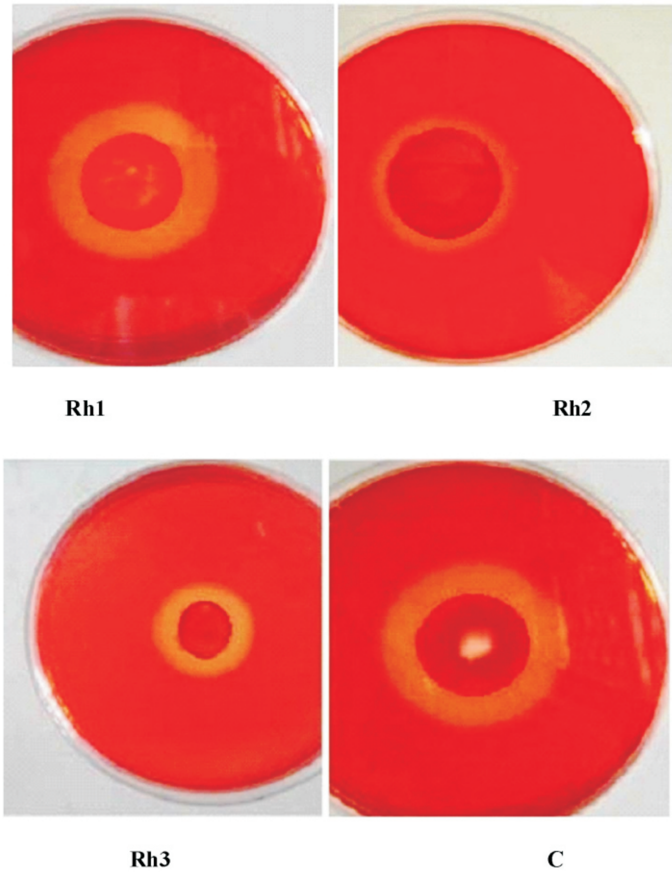

Plate 5: The Cellulose degradation by selected isolates on CMC agar medium after incubation for 120hr at 30°C.

Identification of the isolated cellulose decomposers:

According to the data obtained from Macrogen Inc., Seoul, South Korea by using 16s rRNA Sequencing, the microbial isolates were identified as:

Rh1: identified as ***Proteus vulgaris.***
Rh2: identified as ***Pseudomonas aeruginosa.***
Rh3: identified as ***Bacillus megaterium.***
C : identified as ***Bacillus cereus.***

Table (2) demonstrates the counts of microbial populations in the composted plant materials at 0, 14, 28, 42 and 60 days under aerobic and anaerobic conditions. Under aerobic conditions, the total microbial count ranged from 1.3×10^{8} at 0 time to 5.9×10^{6} CFU/g at the mature compost (60 day). For cellulose decomposers, their count was 1.5×10^{7} CFU/g at 0 time and recorded 2.1×10^{6} CFU/g dry weight of the composted plant materials after 60 day. Regarding actinomycetes, they ranged from 5×10^{6} at 0 time to 2.9×10^{4} CFU/g at the end product of compost. As to fungi count, they recorded 5.8×10^{4} at the beginning of composting (0 time) and 1.2×10^{4} CFU/g in the end product of compost (60 day). It is worthy to mention that a drop in count for both total microbial count and cellulose decomposers were recorded at 14-day-old compost and then these counts rose again at 28 day of composting process. This could be attributed to the turning over process of compost that activated aerobic microorganisms to grow and multiply due to the aeration process resulting from compost turning over. The results obtained by Ebtihal et al. (2016) stated the same trend of microorganisms' depression at 14-day-old compost. With regard to actinomycetes and fungi counts, gradual decreases were found along the composting period where the least count was recorded in the final product of compost. In general, the total counts of tested microorganisms in the mature compost (60-day-old) were the least. This could be ascribed to stability of compost where the C: N ratio reached 13.0.

Under anaerobic conditions, Table (2) also indicates the low numbers of microbial populations in comparison with those recorded under aerobic conditions (except at 0 time). The total count of microorganisms ranged between 1.3×10^{8} CFU/g at 0 time and 1.4×10^{6} CFU/g at 60-day-old compost. Cellulose decomposers recorded 1.5×10^{7} at 0 time and declined to 1.2×10^{5} CFU/g at 60 day (the mature compost).With regard to actinomycetes, they recorded 5×10^{6} CFU/g at 0 time and 4.7×10^{3} CFU/g at 14 day and then disappeared during the rest tested periods, i.e. 28, 42 and 60 day; this is because most of the actinomycetes are strict aerobes (Ref.).Concerning the fungi counts (CFU/g), they ranged from 5.8×10^{4} to 4.3× 102.

In general, Table (2) illustrates that the content of compost from different microorganisms under anaerobic conditions was lower than those found under aerobic conditions at all estimated period of compost age (14, 28, 42 and 60 day). This finding could be attributed to the available oxygen under aerobic conditions for aerobic microorganisms that represent the majority of composting microorganisms.

Table (3) indicates some of the physical and chemical properties of the compost during the various stages of degradation (0, 14, 28, 42 and 60 day). As to temperature under aerobic conditions, it reached its maximum after 14 day of composting process (43°C) and then gradually decreased to record 26°C at 60-day-old compost (Fig. 1). This finding was confirmed by that obtained by Ebtihal *et al.* (2016). Under anaerobic conditions, the compost temperature reached its maximum after 28 day of composting process to record 35°C and then gradually decreased to record 23°C at the final product of compost (Fig. 2). The high temperatures of compost under aerobic conditions could be attributed to the recorded high content of compost from microorganism (Table 2) responsible for degrading plant materials.

Regarding the pH of compost during the various periods of degradation under aerobic condition it was found that it was 8 at 0 time and 8.90 at 60 day. An observed drop in the pH was recorded at 14 day to reach 6; this means that the compost turned out to the acidic side due to the high temperature that recorded at 14 day (43°C) and consequently affect negatively on both the total microbial number (1.1×10^{7} CFU/g) and cellulose decomposers count(9.9×10^{6} CFU/g). This finding was on the same line with that obtained by Ebtihal *et al*. (2016) who stated that the pH value of the composted plant residues decreased during the first 14 days of composting process to reach 5.0 and thereafter it increased to reach 8.6. It is worthy to mention that the pH under aerobic conditions tended to the alkaline side (started from 8 at 0 time and ended with 8.90 at the final compost product). Under anaerobic condition the pH of compost tended to the neutral side (started from 8 at 0 time and recorded 7.55 at the end product of compost).

Table 2: Microbial populations counts (CFU/g) during aerobic and anaerobic composting process.

Microbial population	Composting period (Day)									
	Aerobic					Anaerobic				
	0	14	28	42	60	0	14	28	42	60
Total count (CFU/g)	1.3×10^8	1.1×10^7	1.4×10^8	1.4×10^7	5.9×10^6	1.3×10^8	7.0×10^6	9.8×10^7	1.6×10^6	1.4×10^6
Cellulose decomposers (CFU/g)	1.5×10^7	9.9×10^6	4.8×10^7	9.2×10^6	2.1×10^6	1.5×10^7	3.7×10^6	2.3×10^7	2.7×10^5	1.2×10^5
Actinomycetes (CFU/g)	5.0×10^6	1.1×10^6	9.9×10^5	3.1×10^4	2.9×10^4	5.0×10^6	4.7×10^3	_	_	_
Fungi (CFU/g)	5.8×10^4	4.3×10^4	4.1×10^4	2.1×10^4	1.2×10^4	5.8×10^4	3.8×10^3	2.0×10^3	1.0×10^3	4.3×10^2

Table 3: Physico-chemical parameters of plant residues during various stages composting process.

Parameters	Composting period (Day)									
	Aerobic					Anaerobic				
	0	14	28	42	60	0	14	28	42	60
Temperature (°C)	23	43	39	35	26	23	23	35	28	23
Moisture content (%)	75	77	73	62	30	75	75	75	75	73
pH	8.00	6.00	8.50	8.71	8.90	8.00	6.00	7.00	7.40	7.55
Organic matter (%)	77	69	67	62	62	77	70	64	64	64
Nitrogen content (%)	1.1	1.9	2.4	2.8	2.8	1.1	1.3	1.4	2.4	2.4
Carbon content (%)	45	41	39	37	37	45	41	38	38	38
C:N ratio	41.0	22.0	16.0	13.0	13.0	41.0	32.0	27.0	16.0	16.0
Potassium content (%)	1.43	2.08	2.17	2.38	2.50	1.43	1.49	1.50	1.61	1.63
Phosphorus content (%)	0.28	0.29	0.34	0.35	0.35	0.28	0.28	0.28	0.28	0.28
Lead (ppm)	4	5	5	7	7	4	4	5	5	6
Zinc (ppm)	58	86	96	98	102	58	62	63	64	66
Cadmium (ppm)	<0.5	<0.5	<0.5	<.50	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

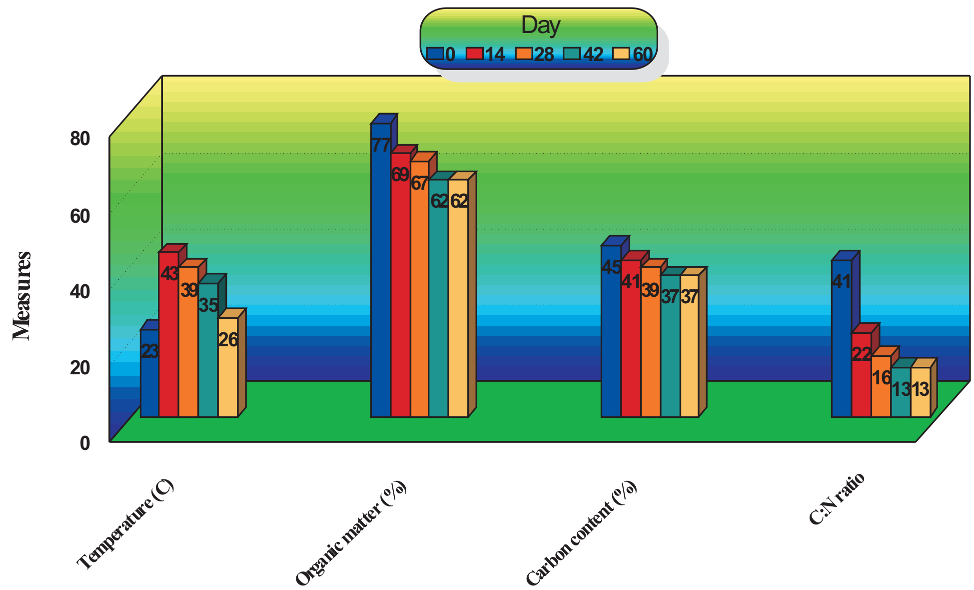

Fig. 1: changes in temperature, organic matter, carbon content and C:N ratio of plant residues during composting process under aerobic conditions.

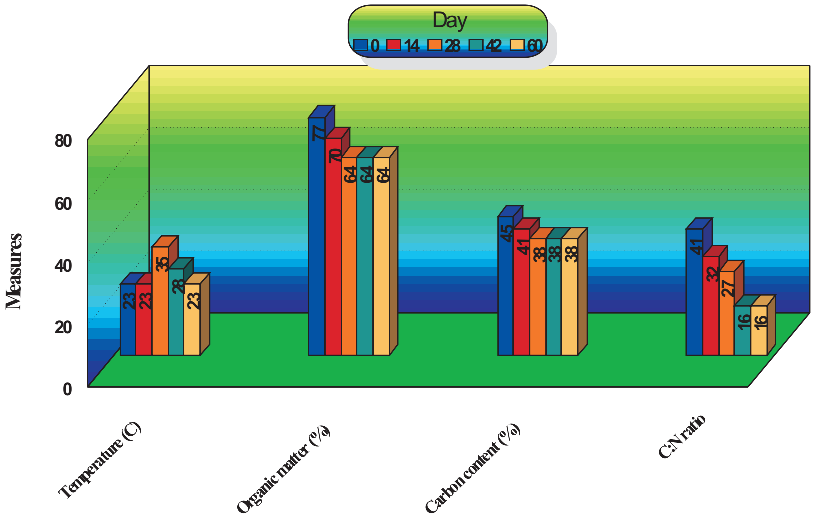

Fig. 2: changes in temperature, organic matter, carbon content and C:N ratio of plant residues during composting process under anaerobic conditions.

For organic matter content of the compost, Table (3) and Figs. (1&2) indicate that no remarkable variations were recorded under aerobic and anaerobic conditions. Under aerobic conditions, the organic matter content of compost ranged from 77% at 0 time to 62% at the final product of compost (at 60-day-old). Further, under anaerobic conditions the percentages of organic matters of compost ranged between 77 at 0 time and 64 at 60 day-old. With regard to compost nitrogen content, it ranged from 1.1% at 0 time to 2.8% at the mature compost (60-day-old) under aerobic conditions meanwhile it was 1.1% at 0 time and reached 2.4% at the mature compost (60-day-old). No great differences were recorded for carbon content of compost under aerobic and anaerobic conditions. Concerning the C:N ratio of compost, Table (3) and Figs. (1&2) show that under aerobic conditions it started with 41:1 at 0 time and ended with 13:1 at the final product of compost {Ebtihal et al. (2016) got a compost of C:N ratio 14:1 at the mature compost of 60-day-old.); while this ratio was 41:1 at 0 time and 16:1 at 60-day-old compost. It is worthy to mention that C:N ratio under aerobic conditions suddenly drop (from 41:1 to 22:1) after 14 day and another drop from 22:1 to 16:1 after another 14 day and the compost started its stability after 42 day; while under anaerobic conditions the drop in C:N ratio was gradually, i.e. 41, 32, 27, 16 and 16 at 0, 14, 28, 42 and 60 day respectively. This means that the maturity stage of compost could be at 42 day under either aerobic or anaerobic conditions but the compost quality under aerobic conditions was better than under anaerobic conditions that showed bad smell.

For macronutrients content of compost (K & P), they recorded higher quantities under aerobic conditions than under anaerobic condition. As to k-content the quantities ranged from 1.43% at 0 time to 2.5% at the mature compost under aerobic conditions; while under anaerobic conditions these quantities were 1.43% at 0 time and 1.63% at final compost product. Concerning P-content of compost, its quantities ranged between 0.28% at 0 time and 0.35% at 60-day-old compost under aerobic conditions. Under anaerobic conditions, no changes were recorded along the various tested periods to record 0.28%. Regarding the trace elements (micro-nutrients), the quantities of lead (Pb) recorded no sensible changes were recorded among either aerobic or anaerobic conditions, i.e. 4-7 and 4-6 ppm respectively under aerobic and anaerobic conditions. As for zinc, its quantities were from 58 ppm to 102 ppm under aerobic conditions and from 58 ppm to 66 ppm under anaerobic conditions. With regard to cadmium content of compost, insensible quantities were measured, <0.5 ppm (Table 3).

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