SURVIVAL OF COMPOST MICROBIAL COMMUNITY IN TWO COMPOSTING SYSTEMS

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ABSTRACT

Temperature development during composting was studied in Turned windrow and Dome Aerated Technology composting systems to ascertain their effectiveness in the reduction or elimination of pathogens in the final product. Also, an assessment of potential health hazard was investigated. Temperature, moisture and pH development in the two composting systems were monitored for thirteen weeks to assess their effects on total viable count, total coliform count, total fungi count and helminth eggs count. The Hydrogen ion concentration (pH) and moisture measured in both systems gave a p-values of 0.25 and 0.68 respectively at $\alpha = 5\%$, indicating existence of no significant difference between these parameters in both systems. Generally, the total viable count reduced while total fungi increased at the end of week 12 in all the systems. However, total coliform reduced to 0 during week 4. Microorganisms such as *Listeria spp., Penicillium spp.* and *Mucor spp.* survived the process in the Dome Aerated Technology system.

Keywords: Composting, microorganism, maturity, decomposition, composting systems.

INTRODUCTION

Composting is one of the widely used methods for treating biodegradable waste. It is increasingly gaining attention compared to the other known waste disposal methods such as incineration, landfilling and anaerobic digestion because of its environmental friendliness as well as the commercial benefits derived from its final product as soil conditioner and fertilizer (Eriksson, 2003).

Composting is a self-heating, aerobic microbial decomposition of organic materials. A typical composting process goes through a series of phases, including a rapid temperature increase, sustained high temperatures and a gradual cooling of the composting mass (Ryckeboer et al., 2003). Different microbial communities dominate during these various composting phases, each adapted to a particular environment (Ryckeboer et al., 2003). As a process involving microorganisms; it is affected by all factors that affect the lives of microorganisms such pH, temperature, moisture, air and nutrients (Miller, 1993). Compost microbial community comprises beneficial and harmful microbes that are active in varied conditions and are classified as such: mesophilic and thermophilic; in terms of temperature; aerobic and anaerobic, in terms of utilisation of oxygen (Madigan et al., 2000; Miller, 1993).

Heat energy released by the microbes during composting, as a result of their metabolic activities, increases the

temperature of compost masses and consequently help inactivates pathogens present (Miller, 1993). However, when excessive heat generated is not dissipated quickly, it leads to the drving of compost masses and killing of beneficial microbes, and thus, resulting in complete failure of the process (Miller, 1993). Different systems would allow dissipation of heat generated during the process at different rates depending on their designs; and this, affect the dynamics of compost microbial community. In the selection of composting systems for organic waste treatment, there is a need to consider systems' effectiveness in reducing or eliminating pathogens in the final product via temperature development: in order to prevent the penetration of pathogens into the food chain (Droffner and Brinton, 1995). Many countries have developed regulations to further reduce pathogens prior to land application of compost materials using temperature as the cardinal factor. The US Environmental Protection Agency stipulates that, to further reduce pathogens, a minimum activation of 55°C must be achieved for a minimum of 3 days during in-vessel and aerated static pile composting. The minimum conditions for windrow composting are 55 °C for 15days and five minimum turnings during the composting period. The regulation requires the quantification of the faecal coliforms and Salmonella *spp*. in the finished product. The recommended values for final counts of faecal coliforms and Salmonella spp. are < 1000 most probable number (MPN/g[dry] and < 3

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MPN/4g[total solids] respectively (Cekmecelioglu *et al.*, 2005). The regulations for biowaste composts in Europe shows slight variations: the regulations require >55 °C for at least 3 weeks or 60 for at least 1 week in Switzerland; >55 for at least 2 weeks in Denmark; >60 for 1 week for in-vessel composting and >55 for at least 2 weeks or > 65 for at least 1 week in Germany (Cekmecelioglu *et al.*, 2005).

Mesophilic and thermophilic microorganisms are involved in composting and their succession is important in the effective management of composting process (Beffa et al., 1996; Ishii et al., 2000). Since plant and animal waste possibly contains viral, bacterial, and protozoan zoonotic pathogens, the application of untreated livestock wastes to plants could be a hygienic risk for humans (Dai et al., 2005). Several pathogens known to cause diseases and death in humans have been identified in manure and foodwaste. Some of these microorganisms include Samonella spp., Listeria spp. Clostridium spp. Staphylococcus spp., Escherichia spp., Aspergillus spp. and Penicillium spp. For instance, Listeria monocytogenes, Salmonella spp. and Escherichia coli O157:H7 infections are important to food safety as classes of pathogens combine to these cause approximately 1.5 million illnesses and 60% of all deaths related to foodborne illnesses (Mead et al., 1999). Solomon et al. (2002) reported that failure to inactivate pathogens in the compost microbial community can allow their penetration into the food chain via the use of final compost on farm and consequently, result in serious public health problems. Esherichia coli O157:H7 is responsible for 20000 cases of infection and 250 deaths per year in the US as a result of diarrhoea and haemorrhagic colitis while Salmonella spp. caused human salmonellosis, which is associated with 2,000,000 infection cases and 50 to 2000 deaths per year (Lung et al., 2001). Since these microorganisms are found during composting, it becomes important to treat animal wastes and plant materials harbouring these pathogens, to limit the risks of pathogenic microorganisms entering the food chain via the use of the compost on the farms.

Literature indicates the regulation of temperatures developed in different composting systems as the most used indicator of compost safety but there is however lack of enough data on the inactivation of pathogenic microorganisms in different systems. This thus has necessitated the study, to ascertain the effects of temperatures developed in two different composting systems: dome aerated technology, passive system; and turned windrow, an active system, on the survival of compost microbial community and safety of their final products vis-à-vis microbial hazards.

The objectives of this study are to quantify and identify the various microorganisms present in both systems from the start to the end of the composting process. Result obtained would allow one ascertain and compare the systems' effectiveness and efficiency in inactivating pathogens present; and the possible health hazards likely to be presented to the public by the survived pathogens. The findings would also help farmers and compost manufacturers to consider choosing appropriate composting systems to safeguard the safety of finished compost from pathogen penetration into the food chain.

MATERIALS AND METHODS

The study was carried out at Volta River Estate Limited Farms (VREL), one of the leading organic banana and pine apple producing farms in Ghana. Compost materials included: River reed, harvested into canoes and brought to the site; banana stock, obtained from VREL Farms; cocoa seed husk, rice husk, cow and poultry manure, obtained from farms and households nearby. The composition of the feed stocks are given as follows: River Reed (RR)-75%, Clay(C)-10%, Banana Stalk/Stem (BS)-5%, Cow Manure/Dung (CM)-4%, Rice Chaff (RC)-4%, Cocoa Seed Husk (CSH)-1% and Poultry Manure (PM)-1%. A starter containing genetically modified organisms such as *Bacillus spp.* and *Corynebacterium spp.* was mixed in the proportion 500g: 40L (m/v) water and added to the feed stocks to facilitate the decomposition process.

Description of Compost Systems Understudy

Dome Aerated Technology (DAT)

The DAT is a passive aeration system that utilizes thermal convection to drive the aeration process within a windrow of waste. The principle of the DAT method is the creation of large voids in a windrow of waste (Trois and Polstera, 2007), using in this case bamboo structures, called domes and channels. Domes are positioned centrally in the windrow to allow for venting of the hot gases generated by the degradation reactions through the channels and chimneys. The layout of the DAT system is shown in figure1. This pile is composed of 4 bamboo domes and 4 chimneys (4 in. dia., and 2.5m high). Additionally, 10 pieces of perforated uPVC (4 in. dia.) used as channels, were inserted into the compost pile to promote the chimneys effect and the chimney pipes supported by cables. Dimensions for the construction of the pile and triangular bamboo dome were 13.7m (L) ×2.7m (B) $\times 1.8$ m (H) and 1.4m(H) x 0.75m equilateral base respectively.

Turned Windrows

As regard the turned windrow system, the feedstock was initially turned four times and used to form long piled compost rows of dimensions 35m (L) $\times 2m$ (B) $\times 0.9m$ (H).A front-loader (165 HP) was used to reshape the pile after which a Toptex (fleece) sheet was used to cover the windrow. Subsequent turnings were conducted with Sandberger ST 300 pulled by a 90 HP tractor.

Sampling of Compost Mass for Physico-chemical and Microbiological Analysis

Compost masses were sampled at the top, middle and bottom locations in the different systems mounted weekly for laboratory study on moisture, pH and microbial analysis. However, temperature measurements of compost masses were done *in situ* with a long-stem thermometer. The samples taken were bulked to obtain a representative sample, packed with ice cubes in an ice chest and transported weekly to the laboratory. The samples were kept in a refrigerator at a temperature of 4°C for a day before microbial analysis performed.

Physico-chemical parameters Analysis

Temperature Determination

Temperature readings from three different locations; The top, middle and bottom of compost masses from the different systems understudy were taken daily using the long stem thermometer (Salmoiraghi Co. thermometer model 17506) at the site. The daily ambient temperatures were also determined.

pH Determination

The representative samples from each system were thawed. Three sub samples of 10g were taken from the representative sample of each system and poured into labelled beakers for the pH determination. The triplicated sub samples were suspended in distilled water in the ratio (1:10) and shaken on a rotary shaker for 30mins. The supernatant was then poured into a beaker and pH determined using a pH meter (Scientific Instruments Co., Italy., model 9000/3). The pH of the triplicated samples for each system were averaged to represent pH of compost mass in each system.

Moisture Determination

A well mixed sample of 10g each of from the different systems were weighed in triplicates for moisture content determination using the oven method. Samples were kept in the oven at 105°C for 24hrs and changed in weight of samples were averaged and used as the measure of moisture content of compost mass in each system.

Microbiological Analysis

Serial Dilution for Total Viable, Coliform and fungi Count

Representative samples of 1g are taken from each of the systems mounted for study was weighed into 9ml of 0.1% peptone water contained in 4 different McCartney bottles and incubated at 37°C for 15minutes.They were well mixed and 1ml of the supernatant was drawn from each of the bottles and diluted using 10-fold dilution into 4 other McCartney bottles each containing 9ml of sterile 0.1% blank peptone water. Different pipettes were used for each of the dilution. 1ml of the diluents taken from dilution factors: 1:10³, 1:10⁴ and 1:10⁵ were transferred into 2 sets of 3 different McCartney bottles one set

containing 9ml of molten Plate Count Agar(PCA) for viable count and the other set 9ml of Violet Red Bile Agar for coliform count. Both sets were kept in water bath at 45°C to prevent solidification (Collins and Lyne, 1983). They were mixed by swirling and then poured into sterile Petri-dishes aseptically and allowed to set. For fungi enumeration, 1ml of the neat representing compost mass taken from each system was transferred onto the Sabouraud agar in labelled Petri-dishes. The whole set was incubated at 37°C for 24hours and at 30°C for 2-7 days respectively for bacteria and fungi. Cultures showing between 30-300 colonies were counted using the colony counter (AOAC, 1983).

Cultures and subcultures

Cultures were made from the neat dilutions onto Blood agar, MacConkey agar and Sabouroud agar using plateout technique as described by Heritage *et al.* (1996).The cultures were incubated for 24h at 30°C made on Blood agar and MacConkey plates, and 2-7 days at 30°C for those made on Sabouraud agar. After growth was observed, identified colonies were purified by subculturing on Blood agar, MacConkey agar and Sabouroud agar. Subcultures were made on Brilliant Green agar and Eosin Methylene Blue agar in order to isolate the *Salmonella spp.* and *coli* forms present

Isolation and Identification

The colonial morphology and cultural characteristics were studied for size, shape and colour on the different media used for the subcultures. Bacteria were Gram stained and their reactions examined by using the light microscope at x100 magnification with oil immersion. The colonial morphology and cultural characteristics of bacteria identified were compared with characteristics of bacteria outlined in Cheesbrough (1984) for confirmation. Fungi were stained with lactophenol cotton blue stain and examined at x10 magnification. They were identified using microscopy and colonial morphology as used in Schneierson (1960). This was because the API (Analytical Profile Index) for bacteria and fungi were not available.

Helminths Eggs Analysis

This was done using a modified McMaster technique of that described in Murray *et al.* (1983). 3g of sample obtained from each system was weighed into labelled plastic container. The samples were emulsified with 45ml of distilled water and sieved with a wire mesh of 0.15 apertures into a bowl. The sieved solution was poured into claytone-lane test tube to 15.5ml mark and centrifuged at 1500 rpm for 3 minutes. The supernatant was poured off and the suspended sediment mixed with saturated saline solution. The samples were replicated for each system and resulting solution was loaded into a McMaster egg counting chamber with dropping pipette and examined under the light microscope with x10 eyepiece.

RESULTS AND DISCUSSION

The hydrogen ion (pH) concentration of the compost masses in the two systems were measured for the 13 weeks with their average weekly readings determined and used in plotting the graph in figure 1.

The graph shows that the pH values in both systems ranged from 7-8.5 during the process. The pH values rose from 7.69 and 7.79 in the DAT and T-W systems respectively during week 0 to a peak of 8.18 in DAT during week 2 and 8.16 in T-W during week 3. However falls in pH values were observed in both systems during week 5. During subsequent weeks of compositing, the patterns observed were similar with values recorded in DAT systems being higher. There was a gradual fall in the pH values towards neutrality in both systems at the end of the process. ANOVA performed on the pH values of compost masses in both systems yielded a p-value of 0.25 at $\alpha = 0.05$, indicating that no significance difference existed in both systems in terms of the pH values. Eklind et al. (1997) revealed that organic acids such as lactic and acetic acid are frequently produced during the initial microbial degradation of food waste which reduces the pH of compost materials to between 4-5. The peculiar trend taken by the pH values recorded in the both systems may be due to the inclusion of 500g/40L of starter containing genetically modified microorganisms such as Corynebacterium spp. and Bacillus spp. that might have eliminated the organic acids, thus preventing the characteristic initial low pH of 4-5 reported by Eklind et al. (1997). This confirms the result from Nakasaki et al.

(1996) that the degradation rate at the initial stage of composting can be significantly increased by inoculation of acid-tolerant thermophilic bacteria. Choi and Parks (1998) also reported the use of microorganisms such as thermophilic yeast to eliminate the organic acids produced initially during composting to stimulate the growth of thermophilic bacteria, hence preventing the low pH inhibiting factor associated with the transition from the mesophilic to the thermophilic phase of composting. Sundberg (2005) reported that during successful and fully developed composting, the pH often rises to 8-9. Hydrogen ion concentration (pH) values in both systems ranged from 7-8.5 during the process.

ANOVA performed on the average moisture content of compost masses in both systems gave a p-value of 0.68 at $\alpha = 0.05$, indicating the existence of no significant differences in moisture contents recorded in both systems. The patterns of the moisture values are illustrated in figure 2.

The rise and fall moisture patterns illustrated by the curves in figure 3 was as a result of the variations in the temperature and aeration in the different systems causing different rates of evaporation of moisture from compost mass in each system. The correlation between temperature and moisture in both systems were r = 0.86 and r = 0.29 in T-W and DAT respectively. The higher positive correlation observed in T-W was due to the lower temperatures recorded in that system as a result of better aeration, hence low water evaporation as compared to the DAT system. However, it must be noted that, the positive

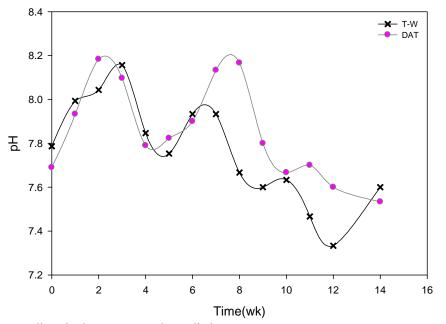


Fig. 1. Average pH readings in the systems understudied.

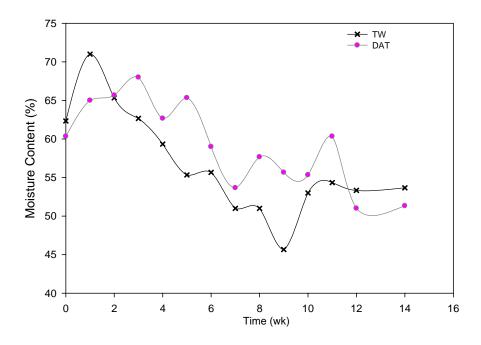


Fig. 2. Average moisture contents in the composting systems understudied.

correlation coefficient values achieved in both systems were as a result of regular rehydration of compost mass to avoid drying up by the heat produced during the process. The moisture contents were high initially in both systems but reduced at the end of the process. The moisture values recorded during the process ranged from 40% - 70% in both systems with values from the last 6 weeks ranging between 46 - 60%.

The average temperature values recorded in both systems were significantly different as ANOVA performed on such values yield a p-value of 0.01 at $\alpha = 0.05$. Temperature distribution patterns as recorded in both systems during the process are show in figure 3.

The peak temperatures recorded were 63.24 and 62.56°C in DAT and T-W systems respectively. It was observed that temperatures fell gradually in both systems but at different rates and to different extent. Temperature values recorded were higher in the DAT system than the T-W as a result of better aeration in T-W. Temperature values recorded in the DAT system ranged between 63.24°C recorded in week1 to 48.14°C recorded in week 12 whereas those in T-W ranged between 62.56°C in week1 to 37.89°C in week 11. Thus, the variations in temperature in both systems implied the succession of different microbial communities in all the systems as reported in Miller (1993) and hence different rate of decomposition rate as indicated by Sundberg, (2005). However, the ambient temperature took nearly a linear pattern ranging from 27 °C in week 0 to 24.33°C in week12.

The distribution of microorganisms in the systems in relation to temperature variation is shown in figures 5 and 6. While figure 4 shows that every temperature change recorded in the DAT system affected the total viable count. Rise in temperature from 53.20°C to 63.24°C (week 0 to week1); saw a slight decline in total viable count from 7.31logCFU/g of compost to 7.21logCFU/g of compost. Every rise and fall in the temperatures recorded in the DAT during the subsequent weeks resulted in the decrease and increase respectively in the total viable count until week 12 when it reduced to 7.08logCFU/g of compost. The rises and falls in the total viable count show that temperature inactivated some of the microorganisms while others increased during the process. However, the final total viable count (7.08logCFU/g of compost) was lower than the initial total viable count (7.31logCFU/g of compost) indicating a decrease in microbial population as the process came to an end.

Bacillus spp., Staphylococcus spp., Streptococcus spp., Clostridium SDD., Campylobacter SPD., Listeria *spp.,Corynebacterium* spp., Yersinia spp. and Enterobacter spp. were the bacteria identified during the composting process. At the end of week 12, only Bacillus (33.33%), Listeria (8.33%)spp. spp. and Corynebacterium spp. (58.33%) survived. Bacillus spp. and Corynebacterium spp. were the genetically modified microorganisms in the starter used to aid the decomposition process. Listeria spp. are found in the soil and known to be zoonotic. Banwart (1989) reported that Listeria spp. causes listeriosis which has several

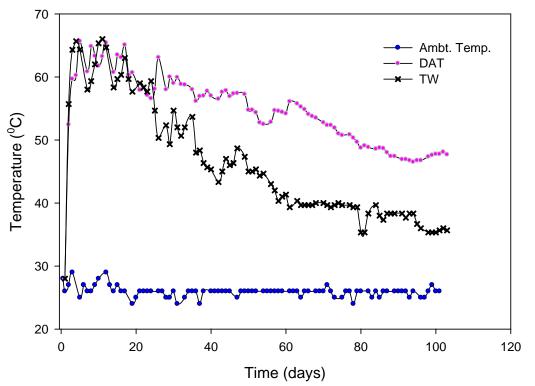


Fig. 3. Average Temperature Readings Recorded in the Systems Understudied.

manifestations including septicaemia (leading to abortion or stillbirth in women), endocarditis, pneumonia, conjunctivitis, pharyngitis, urethritis and meningitis.

Total coliform count in the DAT system decreased from 7.21logCFU/g of compost to 6.26logCFU/g with rise in temperature from week 0 to week 1. Fall in temperature during week 2 resulted in increased total viable counts from 6.26logCFU/g of compost to 6.82logCFU/g of compost. *Enterobacter spp.* was the only coliform that was identified and did not survive the process. The total coliform count was reduced to 0 in week 4 as seen in fig.5 when temperature was at 59.08°C, indicating the reduction in faecal contamination.

The total fungi count increase from 1.11logCFU/g of compost in week 0, with slight rises and falls in numbers during the subsequent weeks, until finally reaching 2.13logCFU/g of compost in week 12. The increase recorded in fungi population towards the end of the process in the DAT system was due to the temperature decrease which presented favourable conditions for them to decompose the cellulose, chitin and lignin known to be prevalent at the latter stages of composting as reported in Compost Microbiology and Soil Food Web (2008). The

fungi identified during the process were Aspergillus spp., Penicillium spp., Mucor spp. and Rhizopus spp. with Penicillium spp. (97.79%) and Mucor spp. (2.21%) surviving the process. Penicillium spp. is reported to produce mycotoxins such as citrinin, luteoskyrin, ochratoxins and rubratoxin which cause illnesses in humans. Luteoskyrin is associated with high incidence of liver cancer in humans (Banwart, 1989). However, Mucor spp. is not known to cause diseases in human (Banwart, 1989).

The total viable count decreased from 7.79logCFU/g of compost to 7.40logCFU/g of compost with a rise in temperature from 57.60°C to 62.56°C during week 0 to week 1 in the T-W system. Temperature fell gradually from week 2 to week 7 but was within the thermophilic range. This temperature range caused almost uniform rises and falls in the total viable count during week 2 to week 7. As the temperature fell to 41.17°C (mesophilic condition) during week 8, total viable count decreased to 7.19logCFU/g of compost. There was a rise in the total viable count from week 10 to 11 and a slight fall in week 12 when temperature fell within the mesophilic range. It was noted also that the percentage frequencies of *Corynebacterium spp.* increased from week 9 to week 12.

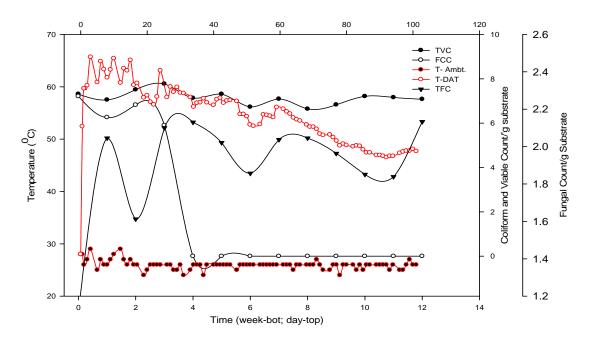


Fig. 4. Temperature and microbial survival in DAT System.

The fall in the total viable count during week 12 might be due to nutrient depletion. The bacteria identified during the process were *Bacillus spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, *Clostridium spp.*, *Campylobacter spp.*, *Listeria spp.*, *Corynebacterium spp.*, *Yersinia spp.* and *Enterobacter spp.* with *Bacillus spp.*(28.57%) and *Corynebacterium spp.*(71.43%) surviving the process. Regular turning of compost masses made in T-W system might have aided the inactivation of the Listeria *spp.* as it helped uniform distribution of heat through the compost mass.

The total coliform count decreased from 6.88logCFU/g of compost to 6.56logCFU/g of compost with rise in temperature from 57.60°C at start-up of pile to 62.56°C at week 1 as seen in Figure 5. The total coliform count decreased until the week 4 when the total coliform count yielded 0logCFU/g of compost at a temperature of 51.83°C.The total coliform count of 0logCFU/g of compost obtained in week 4 indicates the reduction of faecal contamination. *Enterobacter spp.* was the only coliform identified during the process.

Total fungi count in T-W system decreased from 1.68logCFU/g of compost to 1.45logCFU/g of compost with increased in temperature from 57.60°C start-up of pile to 62.56 °C at week 1.The total fungi count increased as temperature fell from week 1 to week 4 and subsequently decline in week 5.The total fungi count further rose to values ranging between 1.90logCFU/g of compost to 2.15logCFU/g of compost during week 6 to week 11. A fall was recorded in the total fungi count

during week 12. This distribution of fungi during 6 to 11 confirms the fact that they tolerate low temperature and largely present at the latter stages of composting to decompose cellulose, chitin and lignin. The fungi identified during the process were *Penicillium spp., Aspergillus spp., Mucor spp.* and *Rhizopus spp.* The only fungus that survived the process was *Penicillium spp.*

It must be noted that helminths egg was not found in both systems for the duration of the experiment.

CONCLUSION AND RECOMMENDATIONS

Data collected on temperature, moisture content, pH, total viable count, total coliform count, total fungi count and Helminths eggs determined during composting in two different systems at VREL Farms for a period of thirteen weeks were analysed to ascertain the effects of temperature, moisture and pH on the microbial survival.

Moisture content and pH values ranged between 40 - 70% and 7-8.5 respectively during the thirteen weeks monitoring period. There were no significant differences in pH and moisture content values for both systems. Temperature values recorded however were significantly different in both systems and affected the microbial distribution during the process. Temperature values recorded in the DAT system ranged between 48 - 64°C whereas those in T-W ranged between 37 - 63°C.

Enterobacter spp. was the only coliform identified in the systems and was inactivated in week 4. *Bacillus spp.*,

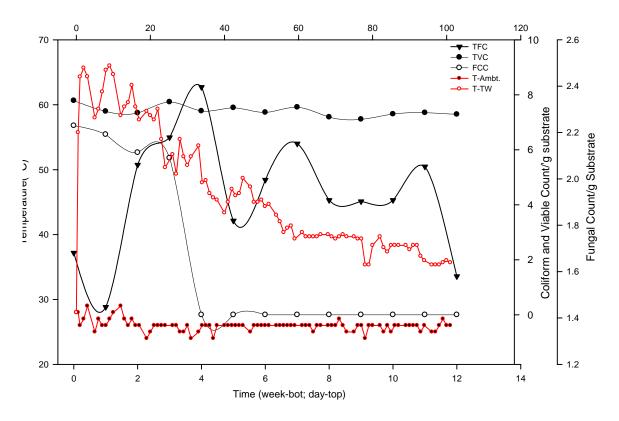


Fig. 5. Temperature and microbial survival in T-W System.

Staphylococcus spp., Streptococcus spp., Clostridium spp., Campylobacter spp., Listeria spp., Corynebacterium spp., Yersinia spp. and Enterobacter spp. were the bacteria identified during composting process in the systems. Bacillus spp. (33.33%), Listeria spp.(8.33%) and Corynebacterium spp.(58.33%) survived the process in the DAT system while Bacillus spp.(28.57%) and Corynebacterium spp.(71.43%) survived in T-W system. Bacillus spp. and Corynebacterium spp. were the genetically modified microorganisms present in the starter used to aid the decomposition process.

Aspergillus spp., Penicillium spp., Mucor spp. and Rhizopus spp. were the fungi identified during the composting process in the different systems. Penicillium spp. (97.79%) and Mucor spp. (2.21%) survived the process in DAT system with only Penicillium spp. (100%) surviving in T-W system. However, no helminths egg was found during the process.

Some members of the compost microbial community identified in compost masses in the two different systems are responsible for causing diseases in humans. Banwart (1989) reported that *Listeria spp.* causes listeriosis, which has several manifestations including septicaemia (leading

to abortion or stillbirth in women), endocarditis, pneumonia, conjunctivitis, pharyngitis, urethritis and meningitis. *Penicillium spp.* is reported to produce mycotoxins such as citrinin, luteoskyrin, ochratoxins and rubratoxin which cause illnesses in humans. Luteoskyrin is associated with high incidence of liver cancer in humans. Since Listeria *spp.*, known to be zoonotic survived in compost processed in DAT system and penicillium *spp.* in both, protectives need be worn by compost manufacturers and farmers to safeguard their health. Also, regular turning of compost masses must be done as it helped in uniform distribution of heat through compost masses; hence, the inactivation of Listeria spp. in the T-W system.

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