Original Research Article

Effect of temperature changes on the bacterial and fungal succession patterns during composting of some organic wastes in greenhouse

ABSTRACT

Aim: Organic wastes were composted and the effect of temperature on the bacterial and fungal succession patterns studied.

Study Design: The wastes which included cow dung (CD), pig waste (PW), poultry litter (PL) and source-separated municipal solid waste (MSW) and their combinations: PL+MSW, PW+MSW and CD+MSW were allowed to decompose for 70 days in a greenhouse.

Place and duration of study: This study was carried out in the greenhouse of the Agricultural Research Farm of the Federal University of Technology, Owerri, Nigeria.

Methodology: The wastes were allowed to decompose for 70 days in a greenhouse using the modified windrow method of composting. Standard microbiological and biochemical methods were used to monitor temperature changes in compost piles as well as changes in bacterial and fungal populations

Results: Results revealed that changes in temperature affected microbial composition in the compost piles. The highest temperature recorded was 60^{o}C for cow dung (CD) compost pile while at maturity the temperature in all the compost piles ranged between 27^{o}C to 30^{o}C. Different bacterial and fungal populations were isolated during the thermophilic and mesophilc phases of composting. Bacteria isolates included species of *Staphylococcus, Proteus, Klebsiella, Salmonella, Alcaligenes, Serratia ,Lactobacillus* and *Pseudomonas*. Others included *Enterobacter, Bacillus, Streptococcus, Corynebacterium* and *Micrococcus* spp. Fungal species isolated included *Candida, Saccharomyces, Rhizopus, Aspergillus, Mucor* and *Fusarium.*

Conclusion: The presence of some plant growth promoting (PGP) bacteria at the end of composting qualifies organic waste composts as effective nutrient sources for crop production and can be considered as potential alternatives to chemical fertilizers.

Keywords: composting, thermophilic, mesophilic, bacterial and fungal populations, Plant growth promoting.

1. INTRODUCTION

Composting is an age long process. Before the introduction of inorganic fertilizers in Nigeria, peasant farmers used composts to fertilize their soils. Composting is a preferred and environmentally sound method whereby organic waste is reduced to organic fertilizer and soil conditioners through biological processes [1,2].However, the process is laborious, practically slow and it takes about 6 months to complete. During this period, labile carbon (C) compounds are lost, while more complex substances, such as humic acids, are synthesized [3].Once the microbial degradation has been stimulated to a certain level, the faunal effect will become quantitatively important [4].

Adequate knowledge of microbial succession is, therefore, very important in any chosen composting method. Various biological studies have been carried out to identify the major microbiological agents responsible for biodegradation. A researcher reported that the composting process may also involve invertebrates such as nematodes, pot worms, earthworms, mites and various other organisms [5], however, Youssef et al [6] noted that the sole agents of decomposition of carbonaceous materials are the heterotrophic microorganisms.

The composting process consists of three phases and requires diverse microflora such as bacteria, fungi and mesophilic and thermophilic actinomycetes eventually converting organic waste to humus [7, 8, 9, and 10]. During the first phase there is an increase in carbon dioxide along with the temperature. The substrate is reduced due to the degradation of sugar and proteins by the action of mesophilic organisms [9, 10, 11,12].The second phase is marked by increase in temperature in compost piles from 45 °C to approximately 70 °C. During this phase, mesophiles are replaced by thermophiles [8,9] and large numbers of pathogens are degraded [12]. The third phase begins with decrease in the temperature of compost piles.

The quality and stability of compost is entirely dependent on its raw materials [13, 14, and 15]. During the composting process, various parameters including the C:N ratio, composting temperature, pH of the finished product, moisture content, and the presence of potential pathogens such as coliforms are used to assess the quality and stability of the compost[16,17,18,19,20].The composition of active microflora of composting wastes normally shifts from predominantly mesophiles in the early stages of composting to one of predominantly thermophiles at the peak of the heating cycle [21].

In the traditional method of composting, the influence of the listed factors had been largely ignored and the final composts obtained from such unimproved method are poor in quality. It has therefore become highly imperative to develop an alternative technique for the needed good quality compost. The present investigation studied the effect of temperature changes on the microbial succession pattern during the composting of some organic wastes using the modified windrow method.

2. MATERIALS AND METHODS

2.1 Location of the Study Area

This study was carried out at the Centre for Agricultural Research, Federal University of Technology, Owerri (FUTO),Imo State – Nigeria.

2.2 Climate of the Study Area

The major rainy season runs from March to July and the minor rainy season starts from September and ends around November. There is a short dry period in August. The major dry season occurs between the end of the minor wet season and the next major wet season (November to March). Rainfall distribution is bimodal with peaks in June and October with annual rainfall varying between 1,500mm to 2,200mm (60 to 80 inches).Temperatures are generally high and uniform throughout the year and the mean monthly temperatures range from 24 – 28^{o}C. The hottest months are between January and March.

2.3 Composting of organic wastes

The organic wastes used in this study included Poultry Litter (PL), Pig waste (PW), Cow dung (CD) and Source-Separated Municipal Solid Waste (MSW).

MSW was obtained from a dumpsite located at Ikenegbu, Owerri while PL, PW and CD were obtained from the research farm of the School of Agriculture, FUTO.

The organic wastes were composted/co-composted as following:

a) Pig waste (PW) only

b) Poultry litter (PL) only

c) Cow dung (CD) only

d) Municipal solid waste (MSW) only

e) Pig waste + MSW

e) Poultry litter + MSW

f) Cow dung + MSW

Sixty kilograms (60) each of PW, PL, CD and MSW were introduced respectively into 100-litre(L) buckets that had previously been perforated at several points. For the co-composted systems, 30kg of both samples were introduced into the same 100L bucket that had previously been perforated and mixed thoroughly. The windrow method of composting as modified by Malone [22] was employed. The compost bins were left open and its contents turned at weekly intervals i.e. every seven days. The organic wastes were allowed to decompose at room temperature in a corner of a greenhouse. At intervals of three weeks, the contents of the composting bins were watered with 200mls of sterile distilled water until the compost samples matured. Composting was done for a period of 70 days (10 weeks).

2.4 Determination of temperature of composting piles

The temperature of the composting piles and that of the environment were monitored daily during the entire period of the composting i.e. for 70 days. Process temperatures were determined by taking the average readings from the two thermometers that were inserted 5 cm deep into each pile at different spots. The ambient temperature was continuously monitored by taking average reading of the two different thermometers (Salmoiraghi Co. thermometer model, 1750) fixed permanently at two different spots in the green house.

2.5 Isolation and identification of isolated bacteria.

The media employed included Nutrient Agar, Potato Dextrose Agar ,Mackonkey Agar, Eosine Methylene Blue Agar and Salmonella- Shigella Agar. They were all prepared according to manufacturer's guideline (Oxoid, England). Compost suspensions were prepared by the addition of 10 g compost samples to 90 ml of normal saline (0.85% w⁄v). Serial dilutions of these initial suspensions were made in normal saline. Aliquot (0.1 ml) of each appropriate dilution was inoculated in duplicate and spread with sterile rod spreader in the Petri plates containing the required medium.

Biochemical characterization and identification of various bacterial isolates were carried out according to the standard methods [23, 24].

2.6 Isolation, enumeration and identification of isolated fungi

Potato dextrose agar (PDA) to which Chloramphenicol (30 µg/ml) was added as an antimicrobial agent to inhibit all bacteria growth and to facilitate selective isolation of yeasts and molds was used. Aliquots (0.1 ml) each of the appropriate dilution of the compost suspension was introduced into 2 replicate plates of well-dried PDA plates and separately spread plated with flame-sterilized glass spreader. The cultured plates were inverted and incubated at room temperature for 3-5 days. The colonies that developed on the PDA plates were counted and recorded as counts of total viable fungi.The colour and colonial characteristics of the colonies were also observed and recorded. Discrete colonies were sub-cultured onto freshly prepared medium for the development of pure isolates, which were stored on potato dextrose agar slants for subsequent characterization and identification.

Identification procedures were performed as described [25, 26, and 27]. Pure Fungal cultures were observed while still on plates and after wet mount in lacto-phenol on slides under the compound microscope. Observed characteristics were recorded and compared with the established identification key [28].

3. RESULTS AND DISCUSSION

Changes in temperature during compositing

Fig 1 represents the changes that occurred in the temperature of the composting piles during composting. Initial temperature of the compost piles after piling ranged from 28 – 30^{O}C while the ambient temperature was 24^{O}C. The temperature of the piles increased at different rates. For cow dung the temperature increased to 46^{O}C after two weeks while it took the poultry litter and pig waste compost piles 18 days to attain a temperature of 45^{O}C. The highest temperature of 62^{O}C was recorded for cow dung compost on the 29th day. However, as composting proceeded the temperature of the compost piles began to drop. By the 7th week (day 49) the temperature of the compost piles dropped to between 34 – 40^{O}C but stabilized at between 27 – 30^{O}C by the 9th week (day 63). During the cooling stage that lasted for about 21 days (i.e. day 50 – day 70), the pile temperatures remained in the range of 27 – 37^{O}C in all the composts, however, room temperatures were within the range 22^{O}C – 28^{O}C during the composting period.

As temperature increased, the microbial populations increased until a peak was depending on the type of organic waste involved. Faecal coliforms and Salmonellae were not detected in some of the compost bins when temperatures as high as 47 ^{o}C – 60 ^{o}C were recorded.

The temperature changes in the compost piles, indicated that the organic materials passed through different phases viz mesophilic, thermophilic, cooling and maturation as already reported [29]. Aeration of the compost piles by turning was very essential [30]. Turning was performed weekly from the beginning of process until maturity and it enhanced the decomposition process. Moreover, if the turning process failed to reheat the composting pile, it showed that the composting material was biologically stable [31]. Temperature is one of the key indicators of composting. It determines the rate at which many of the biological processes take place and played a selective role on evolution and succession of microbiological communities [32].

High temperature maintained for long periods in the compost piles during composting served to promote the efficiency and effectiveness of the process by accelerating it and also by destroying pathogenic microorganisms.

The critical temperature which can limit composting is yet to be defined [33], however a scholar [34] suggested that a temperature of 55 ^{O}C – 60^{O}C should be maintained for up to three days for efficient composting.

Temperature increases resulting from microbial activity were noticeable soon after the compost feedstocks were piled together. The rate of increase varied with the compost piles and depended on the microbial populations and other environmental conditions such as nutrient availability, aeration and moisture content in the compost piles [30].

Immediately temperatures in the compost piles rose above 45^{o}C, thermophilic microbial populations such as *Bacillus* sp, *Serratia*s sp and *Streptococcus* sp that could survive high temperatures began to increase while mesophilic populations like *Salmonella* sp, *Enterobacter* sp and *Micrococcus* sp, etc, disappeared. The results further revealed that the cow dung compost pile attained thermophilic temperatures after 2 weeks (day 14) while the source–separated municipal solid waste compost piles attained thermophilic temperatures after 3 weeks, probably due to lower microbial activity.

The organic materials used in this study varied in nutrient composition and after composting they yielded different concentrations of different plant nutrients. [30][35] had reported that attainment of thermophilic temperatures was probable as a result of the rapid mineralization of organic carbon and nitrogen in the presence of adequate aeration required by the microbes responsible for the breakdown of organic compounds. This probably would have generated reaction whereby CO_2 and heat were released into the compost system. The temperature pattern in the various composts indicated that the organic materials passed through almost similar degradation processes and the rise and fall in temperature have been reported to correlate with the increase and decrease of microbial activities [36, 37, 38, and 39].

Generally, temperatures in the compost piles began to decrease around the 30th day of composting. This decrease may be as a result of depletion of organic matter and invariably a reduction in the activity of the microorganisms involved in the composting process.

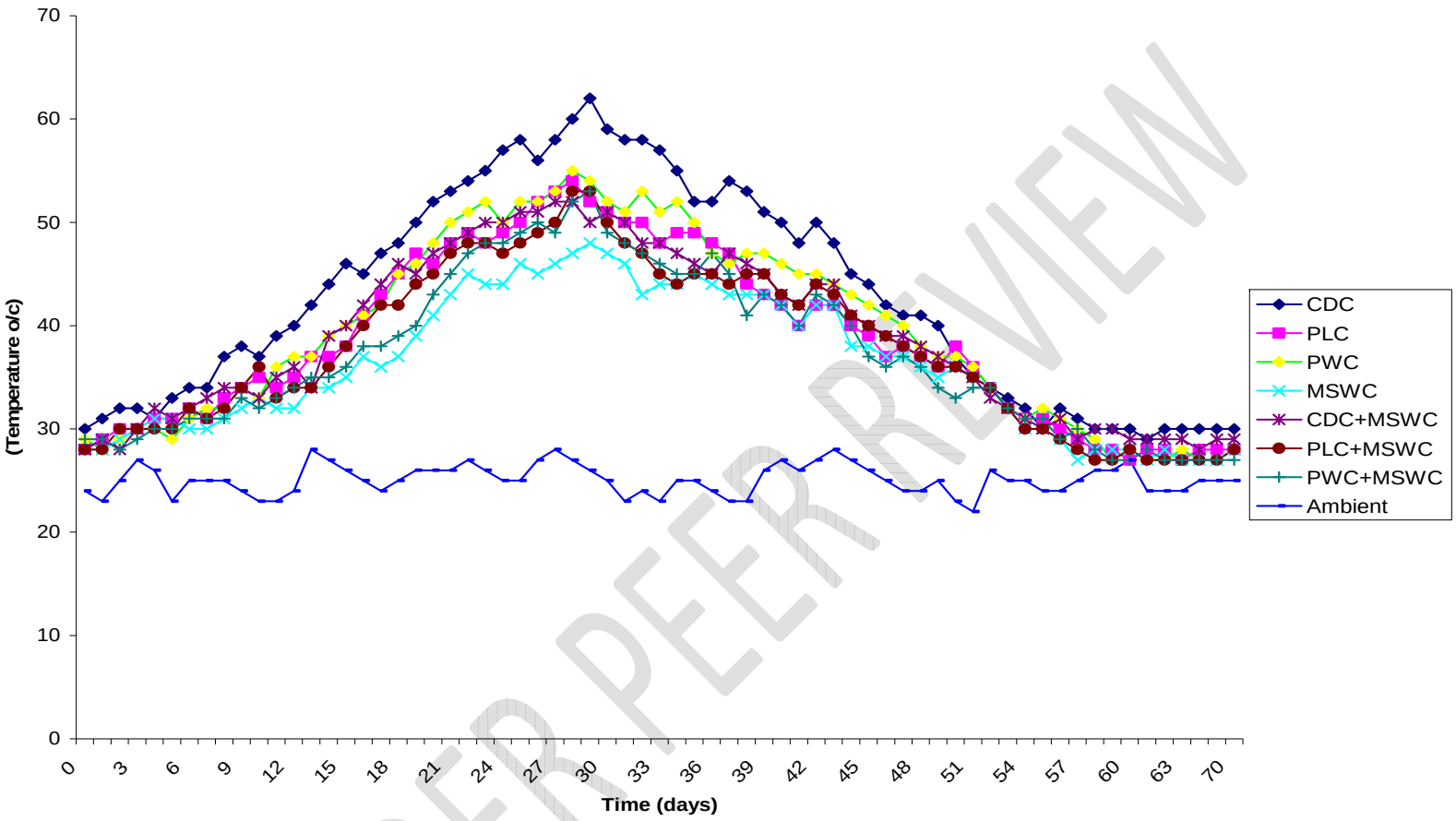

Fig. 1: Changes in the temperature of the compost piles during composting

By the end of the composting process, average temperatures inside the different compost piles marked a real fall in values to approximately 27 – 30^{O}C and the temperatures remained stable in spite of the turning of compost piles.

The succession patterns of different microorganisms involved in the composting of different organic wastes are summarized in Table 1 to 6. The temperature within the composting piles greatly influenced the types and numbers of microorganisms present. During the first mesophilic stage,14 bacterial genera were isolated in all compost piles.These included species of *Staphylococcus* ,*Proteus*, *Klebsiella*, *Pseudomonas* and *Alcaligenes*. Others were *E. coli*, *Enterobacter* ,*Serratia* , *Streptococcus*, *Corynebacterium* as well as *Bacillus* and *Micrococcus* (See Table 1). Table 2 presents the bacterial populations isolated or absent during the thermophilic stage of composting (i.e. between 45 – 65^{O}C). Species of *Serratia* and *Bacillus* were isolated in all samples.

Table 1: Bacteria Isolated or Absent during the First Mesophilic Phase i.e. 20 – 40^{O}C (Day 1 to Day 18)

Isolates	CD	PL	PW	MSW	CD+MSW	PL+MSW	PW+MSW
S. aureus	+	+	+	+	+	+	+
Proteus sp.	+	-	-	-	+	-	-
*Klebsiella*sp.	-	+	-	-	-	+	-
Salmonella sp.	+	+	+	+	+	+	+
Lactobacillus sp.	+	-	+	-	+	-	-
Pseudomonas sp.	-	+	-	+	+	+	+
Alcaligenes sp.	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+
Enterobacter sp.	+	+	+	-	+	+	+
*Serratia*sp.	+	+	+	+	+	+	+
Streptococcus sp	-	+	+	-	-	+	+
Corynebacterium sp.	-	-	-	+	+	+	+
Bacillus sp.	+	+	+	+	+	+	+
Micrococcus sp.	+	-	+	+	+	+	+

Key

+	Present
-	Absent
CDC	Cow dung Compost
PLC	Poultry Litter Compost
PWC	Pig Waste Compost
MSWC	Municipal Solid Waste Compost

Most of the microbial types could not survive the high temperatures of the thermophilic phase of composting, however, *Serratia* and *Bacillus* sp were able to survive the high temperature of the thermophilic phase in all compost piles but *Streptococcus* sp was isolated only in poultry and pig wastes compost piles.

Tables 4 to 6 represent fungal isolates present or absent during the different phases of composting in the different compost pile. Fungi isolated included *Candida albicans*, *Aspergillus, Mucor, Fusarium, Rhizopus and Saccharomyces* spp. Their presence in the compost piles depended on the temperature of the pile as well the type of organic waste involved. Table 4 represent the fungal genera isolated or absent during the 1st mesophilic stage which included *Candida albicans*, *Aspergillus* sp, *Mucor* sp, *Fusarium* sp, *Saccharomyces* sp and *Rhizopus* sp. Of all the fungal species present during the mesophilic phase, only *Aspergillus* and *Fusarium* spp. survived high temperatures above 45^{O}C (Table 5).Table 5 present the fungal population isolated or absent during the thermophilic stage of composting i.e. between 45 - 65 ^{O}C

During the cooling down stage, the occurrence of *Bacillus, Streptococcus*, *Serratia* and *Staphylococcus* spp were observed again (Table 3). These results, as presented in Table 3, show that *Staphylococcus* sp which were not present at the thermophilic phase were later isolated at the curing/maturation phase of composting. This can be seen as a case of ecological succession, and could rightly be attributed to contaminations from the external environments [40]. The fungal populations did not change during the cooling down phase as only *Aspergillus* and *Fusarium* sp were isolated in all the compost piles except cow dung compost in which *Fusarium* sp was absent.

The bacterial and fungal species isolated during the different phases of composting indicates that there was greater microbial diversity at the mesophilic phase of composting than at the thermophilic and cooling phases. *Bacillus* sp, *E. coli*, *Salmonella* sp and *Alcaligenes* sp were present in all the compost piles during the mesophilic stage. Some researchers [41,31] had reported the occurrence of *Bacillus* sp, some Faecal coliforms, *Pseudomonas, Streptococcus, Proteus* and *Serratia* during the mesophilic stage of composting urban refuse and poultry manure.

Table 2: Bacteria Isolated or absent during the thermophilic Phase i.e. 45 – 65^{O}C (Day 16 to Day 49)

Isolates	CD	PL	PW	MSW	CD+MSW	PL+MSW	PW+MSW
S. aureus	-	-	-	-	-	-	-
Proteus sp.	-	-	-	-	-	-	-
Klebsiella sp.	-	-	-	-	-	-	-
Salmonella sp.	-	-	-	-	-	-	-
Lactobacillus sp.	-	-	-	-	-	-	-
Pseudomonas sp.	-	-	-	-	-	-	-
Alcaligenes sp.	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-
Enterobacter sp.	-	-	-	-	-	-	-
Serratia sp.	+	+	+	+	+	+	+
Streptococcus sp	-	+	+	-	-	+	+
Corynebacterium sp.	-	-	-	-	-	-	-
Bacillus sp.	+	+	+	+	+	+	+
Micrococcus sp.	-	-	-	-	-	-	-

Key

+	Present
-	Absent
CDC	Cow dung Compost
PLC	Poultry Litter Compost
PWC	Pig Waste Compost
MSWC	Municipal Solid Waste Compost

Results as shown in Table 2 and Table 5 reveal that *Bacillus* sp, *Serratia* sp, *Streptococcus* sp and *Aspergillus* sp survived the high temperatures in almost all the compost piles and may have been responsible for carrying the process to the cooling down stage. The other microorganisms that were not isolated at high temperatures between 45 – 65^{o}C might have been affected by the heat generated by microbial activities [42, 43, 8].

Table 3: Bacteria Isolated or Absent during the Cooling Phase i.e. 2nd Mesophilic stage (Day50 to Day 70)

	CD	PL	PW	MSW	CD+MSW	PL+MSW	PW+MSW
S. aureus	+	+	+	+	+	+	+
Proteus sp.	-	-	-	-	-	-	-
Klebsiella sp.	-	-	-	-	-	-	-
Salmonella sp.	-	-	-	-	-	-	-
Lactobacillus sp.	-	-	-	-	-	-	-
Pseudomonas sp.	-	-	-	-	-	-	-
Alcaligenes sp.	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-
Enterobacter sp.	-	-	-	-	-	-	-
Serratia sp.	+	+	+	+	+	+	+
Streptococcus sp	-	+	+	-	-	+	+
Corynebacterium sp.	-	-	-	-	-	-	-
Bacillus sp.	+	+	+	+	+	+	+
Micrococcus sp.	-	-	-	-	-	-	-

Key

+	Present
-	Absent
CDC	Cow dung Compost
PLC	Poultry Litter Compost
PWC	Pig Waste Compost
MSWC	Municipal Solid Waste Compost

Table 4:Fungi Isolated or absent during the First Mesophilic Phase i.e 20-40^{O}C (Day 1 to Day 18)

Isolates	CDC	PLC	PWC	MSWC	CDC+MSWC	PLC+MSWC	PWC+MSWC
C. albicans	+	-	-	+	+	+	+
Aspergillus sp	+	+	+	+	+	+	+
Mucor sp	-	-	+	+	-	+	+
Fusarium sp	-	+	+	+	+	+	+
Rhizopus sp	+	+	+	+	+	+	+
Saccharomyces sp	+	+	+	+	+	+	+

Key

+	Present
-	Absent
CDC	Cow dung Compost
PLC	Poultry Litter Compost
PWC	Pig Waste Compost
MSWC	Municipal Solid Waste Compost.

Table 5: Fungi Isolated or absent during the thermophilic Phase i.e. 45 – 65^{O}C (Day 16 to Day 49)

Isolates	CDC	PLC	PWC	MSWC	CDC+MSWC	PLC+MSWC	PWC+MSWC
C. albicans	-	-	-	-	-	-	-
Aspergillus sp	+	+	+	+	+	+	+
Mucor sp	-	-	-	-	-	-	-
Fusarium sp	-	+	+	+	+	+	+
Rhizopus sp	-	-	-	-	-	-	-
Saccharomyces sp	-	-	-	-	-	-	-

Key

+	Present
-	Absent
CDC	Cow dung Compost
PLC	Poultry Litter Compost
PWC	Pig Waste Compost
MSWC	Municipal Solid Waste Compost

Table 6: Fungi Isolated or absent during the Cooling Phase i.e. 2nd Mesophilic stage (Day 50 to Day 70)

Isolates	CDC	PLC	PWC	MSWC	CDC+MSWC	PLC+MSWC	PWC+MSWC
C. albicans	-	-	-	-	-	-	-
Aspergillus sp	+	+	+	+	+	+	+
Mucor sp	-	-	-	-	-	-	-
Fusarium sp	-	+	+	+	+	+	+
Rhizopus sp	-	-	-	-	-	-	-
Saccharomyces sp	-	-	-	-	-	-	-

Key

+	Present
-	Absent
CDC	Cow dung Compost
PLC	Poultry Litter Compost
PWC	Pig Waste Compost
MSWC	Municipal Solid Waste Compost

The isolation of mesophiles during the thermophilic phase of composting strongly indicates that some mesophiles have mechanisms for survival and perhaps replication at elevated temperatures [44]. During the cooling down phase, *Serratia, Staphylococcus, Bacillus* and *Aspergillus spp* were isolated in all the compost piles. [41] had reported that at temperatures below 40^{o}C several mesophiles are capable of recolonizing the compost piles and this depended on nutrient availability and other environmental factors. Awashti et al.[45] had suggested that mesophilic

organisms are responsible for the initial decomposition of organic wastes and the generation of heat responsible for the increase in the compost temperature.

Most of the fungal isolates were saprophytes which obtained energy by breaking down the decomposing material during the composting process. Pandey et al [46] had reported *Aspergillus* sp as among the predominant fungi in compost and classified them as thermophilic fungi capable of surviving high temperatures above 45^{O}C. *Rhizopus* sp were isolated during the mesophilic stage (25 – 45^{O}C) and they are typical early colonizers of the compost capable of exploiting simple sugars and amino acids that were initially present in the organic wastes as sources of energy.The effective action of the different microorganisms resulted to the decomposition of the organic wastes and consequently reduced the volumes of the organic wastes.

CONCLUSION

The results indicated that the microbial composition of the matured composts depended on the raw wastes used to develop the compost, on microbial competitiveness and on the duration of composting; however, the significance of these factors varied at different sampling times. The initial composition of both bacterial and fungal communities differed significantly from the subsequent communities as revealed by changes in the microbial population though the dynamics of the bacterial and fungal communities in the composts were similar. Composting of organic wastes could be adopted to recycle/reuse organic residue as a solid waste management option. The presence of some plant growth promoting (PGP) bacteria at the end of the composting process makes organic waste composts effective nutrient sources for crop production and can be considered as potential alternatives to chemical fertilizers.

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