

Changes in Microbial Population Numbers During Composting of Some Organic Wastes in Greenhouse

ABSTRACT

AIM: The study identified and enumerated microorganisms associated with the composting of some organic wastes using the plate count method

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

Place and duration of study: This study was carried out at an agricultural research farmland in the Federal University of Technology, Owerri, Nigeria.

Methodology: Seeds of *Capiscum chinense* were planted in the soil samples in a greenhouse. Rhizosphere soil was collected for analysis to identify the bacterial composition of the rhizosphere soil

Results: Microbial populations increased concurrently with temperature during the first 3 – 4 weeks of composting except however for faecal coliforms and *Salmonella*. The highest temperature recorded was 60°C for cow dung (CD) compost pile while at maturity the temperature in all the compost piles ranged between 27°C to 30°C. The bacterial colony forming units were higher than fungal colony forming units throughout the composting process for both mesophilic and thermophilic microorganisms. The population of mesophilic organisms increased in the first 14 – 15 days; for cow dung, the initial total heterotrophic bacteria count (THBC) and total coliform count (TCC) were 2.4×10^7 cfu/g and 5.0×10^5 cfu/g respectively and increased to 2.5×10^8 cfu/g and 1.7×10^7 cfu/g for THBC and TCC, respectively, after the 14th day. Thermophilic bacteria dominated all the composting systems after the 21st day and lasted till the 35th day except for cow dung compost where thermophilic temperatures were still observed on the 45th day with a THBC of 6.3×10^6 cfu/g on the 49th day. Faecal coliforms and

35 *Salmonella* were completely eliminated in all the compost systems after the 28th day at
36 temperatures between 47°C – 60°C..

37

38 **Conclusion:** Organic wastes when managed properly through the application of knowledge of
39 composting can be transformed into beneficial materials for human and agricultural use.

40

41 **Keywords:** composting, mesophilic organisms, thermophilic microorganisms, total
42 heterotrophic bacteria count, total coliform count

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45 **1. INTRODUCTION**

46

47 Composting is the process whereby organic wastes are reduced to organic fertilizers and soil
48 conditioners through biological processes [1,2]. Organic wastes are potential sources of
49 macronutrients and large quantities of micronutrient required by plants for growth and
50 improvement of soil health [3]. These nutrients are available in huge amounts in farmyard
51 wastes (e.g. cow dung, pig waste and poultry waste), domestic wastes, agricultural wastes,
52 municipal wastes and industrial wastes. Most rural, semi-urban and urban areas of Nigeria lack
53 proper waste collection and disposal system, hence the continuous accumulation of these wastes
54 which presents many unpleasant environmental consequences including land, water and air
55 pollution [4, 5, 6]

56 The use of organic waste materials as soil amendment is one important approach to
57 sustainable agriculture. To an extent, organic wastes are utilized as nutrient sources in
58 agriculture, however, some of them are not suitable to be applied directly to the soil to improve
59 plant growth [7, 8, 9]. In some countries, like Pakistan, where sewage sludge is directly used as
60 manure without any treatment, the heavy metals and other toxic substances contained in it
61 usually gain entry into the food chain producing serious human health issues [10,6]. Moreover,
62 the availability of organic materials could be limited if it is used in huge bulk volumes, as in the
63 conventional practice where organic wastes are used at several tons per hectare of land for the
64 improvement of crop productivity [11, 12]

65 Composting offers a remedy and the most sensible way to avoid wasting of useful
66 natural resources, and creating environmental problems. It is a recycling process in which
67 organic materials are biologically converted into stable humus-like substances under controlled

68 conditions of temperature, moisture and aeration [13] The composting process involves mixed
69 populations of microorganisms e.g. bacteria, fungi and actinomycetes that are indigenous to the
70 waste being converted and transforms the waste into a nutrient-rich amendment capable of
71 improving the nutrient level of depleted farmland soils. During composting, the kinds and
72 numbers of microorganisms that develop are usually affected by temperature and nutrient
73 availability.

74 Initially, mesophiles predominate and proceed to decompose the readily degradable sugars,
75 proteins, starches, and fats typically found in undigested feed stocks and the availability of
76 easily usable organic substances enables the proliferation of the fast-growing microorganisms
77 [14]. At higher temperatures, thermophilic microorganisms dominate the microbial community
78 and continues generating more heat as a result of the decomposition of more organic matter.
79 The higher temperatures will ensure rapid organic matter processing while simultaneously
80 providing optimal conditions for the destruction of human and plant pathogens [15].

81 Composting has resolved problems associated with the use of raw organic wastes as soil
82 amendments, which include malodors, human pathogens, toxic heavy metals, toxic organic
83 compounds and other undesirable physical and chemical properties [16, 9, 17]. It also provides
84 a way to manage big volumes of organic wastes in environmentally sound manners [13,18].

85 The present investigation studied the changes on the microbial population numbers during the
86 composting of some organic wastes using the modified windrow metho

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88

89 **2. MATERIALS AND METHODS**

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91 **2.1 Location of the Study Area**

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

94

95 **2.2 Duration of the Study**

96 The study was done between between September 2017 and January 2018.

97

98 **2.3 Composting of Organic Wastes**

99 The organic wastes used in this study included Poultry Litter (PL), Pig waste (PW), Cow dung

100 (CD) and Source-Separated Municipal Solid Waste (MSW). MSW was obtained from a
101 dumpsite located at Ikenegbu, Owerri while PL, PW and CD were obtained from the research
102 farm of the School of Agriculture, FUTU.

103 The organic wastes were composted/cocomposted as following:

- 104 a) Pig waste (PW) only
- 105 b) Poultry litter (PL) only
- 106 c) Cow dung (CD) only
- 107 d) Municipal solid waste (MSW) only
- 108 e) Pig waste + MSW
- 109 e) Poultry litter + MSW
- 110 f) Cow dung + MSW

111 The windrow method of composting as modified by Malone [19] was employed. Sixty
112 kilograms (60) each of PW, PL, CD and MSW were introduced respectively into 100-litre(L)
113 buckets that had previously been perforated at several points. For the co-composted piles, 30kg
114 of both samples were introduced into the same 100L bucket that had previously been perforated
115 and then mixed thoroughly. The compost bins were left open and contents turned every seven
116 days. The organic wastes were allowed to decompose at room temperature in a corner of the
117 greenhouse. The contents of the composting bins were watered with 200 mls of sterile distilled
118 water at intervals of three weeks until the compost samples matured. Composting was done for
119 a period of 70 days (10 weeks).

120

121 **2.4 Determination of Temperature of Composting Piles**

122 The temperature of the composting piles and that of the environment were monitored daily
123 during the entire period of the composting i.e. for 70 days. Process temperatures were
124 determined by taking the average readings from the two thermometers that were inserted 5 cm
125 deep into each pile at different spots. The ambient temperature was continuously monitored by
126 taking average reading of the two different thermometers (Salmoiraghi Co. thermometer model,
127 1750) fixed permanently at two different spots in the green house.

128

129 **2.5 Isolation and Enumeration of Isolated Bacteria**

130 The media employed included Nutrient Agar, Mackonkey Agar, Eosine Methylene Blue Agar
131 and Salmonella- Shigella Agar. They were all prepared according to manufacturer's guideline

132 (Oxoid, England). The initial microbial populations as well as subsequent populations in the
133 compost bins were studied using standard microbiological methods as described by Harley-
134 Prescott [20]. The Total Heterotrophic Bacteria Count (THBC), Fecal Coliform count (FCC),
135 Salmonella Count(SC), Total Coliform Count (TCC) and Total Fungal Count(TFC) of
136 composting organic wastes were determined on day 0, day 4, day 7, day 10, day 14, day 21, day
137 28, day 35, day 42, day 49, day 56, day 63, and day 70 on the appropriate growth medium.
138 Compost suspensions were prepared by the addition of 10 g compost samples to 90 ml of
139 normal saline (0.85% w/v). Serial dilutions of these initial suspensions were made in normal
140 saline. Aliquot (0.1 ml) of each appropriate dilution was inoculated in duplicate and spread with
141 sterile rod spreader in the Petri plates containing the required medium. Fecal coliforms were
142 counted on Eosin Methylene Blue Agar plates incubated at 44.5°C while *Salmonellae* were
143 counted on Salmonella-Shigella agar plates incubated the at 37°C according to the method
144 described by APHA [21]. The colonies that developed on the plates were counted and recorded
145 as colony forming units using standard methods [19, 22].
146

147 **3. RESULTS AND DISCUSSION**

148

149 Table1 represents changes in the temperature of the composting piles during composting.
150 Initial temperature of the compost piles ranged from 28 – 30°C. The temperature of the
151 piles increased at different rates. For CD the temperature increased from 30°C to 46°C
152 after two weeks while it took the PL, MSW and PW+MSW 21 days to attain a
153 temperature of 45°C. The highest temperature of 60°C was recorded for CD compost on
154 the 28th day. However, by the 7th week (day 49) the temperature of the compost piles
155 dropped to between 34°C – 40°C and stabilized at between 27°C – 30°C by the 9th week
156 (day 63). During the cooling stage that lasted for about 21 days (i.e. day 50 – day 70),
157 the pile temperatures remained in the range of 27°C – 37°C in all the compost piles.

158 Figures 1 to 5 show the changes in the microbial populations of the different
159 organic wastes. The same pattern was observed for Total Fungal Count (TFC), Total
160 Coliform Count (TCC) and Total Heterotrophic Bacteria Count (THBC). As temperature
161 increased, the microbial populations increased until a peak was attained as determined
162 by the type of organic waste. Faecal coliforms and *Salmonellae* were not detected in

163 some of the compost bins when temperatures as high as between 47°C – 60°C were
164 recorded.

165 THBC as high as 7.2×10^9 cfu/g was recorded for CDC on day 28 when pile temperature was
166 60°C and PL had the lowest THBC throughout the composting period, from day zero to
167 maturity, when compared to the others. Meanwhile, fungal counts were lower than THBC when
168 compared and the lowest fungal count of 1.0×10^3 cfu/g was recorded for PW.

169

170 **Table 1: Changes in Temperature (°C) during composting of organic wastes**

Day	PLC T	PWC T	CDC T	MSWC T	PLC +MSWC T	PWC+MSWC T	CDC+MSWC T
0	28	29	30	28	28	29	28
4	31	30	31	31	30	30	32
7	31	32	34	30	31	31	33
10	35	33	37	33	36	33	33
14	37	39	44	34	36	35	39
21	45	50	53	45	47	45	48
28	54	55	60	47	53	52	52
35	50	49	52	45	45	45	46
42	45	42	50	42	44	43	44
49	37	36	40	35	36	44	37
56	31	31	32	29	29	29	31
63	28	27	30	27	27	27	29
70	28	28	30	28	28	27	28

171 **Key**

172 PLC = Poultry Litter Compost

173 PWC = Pig Waste Compost

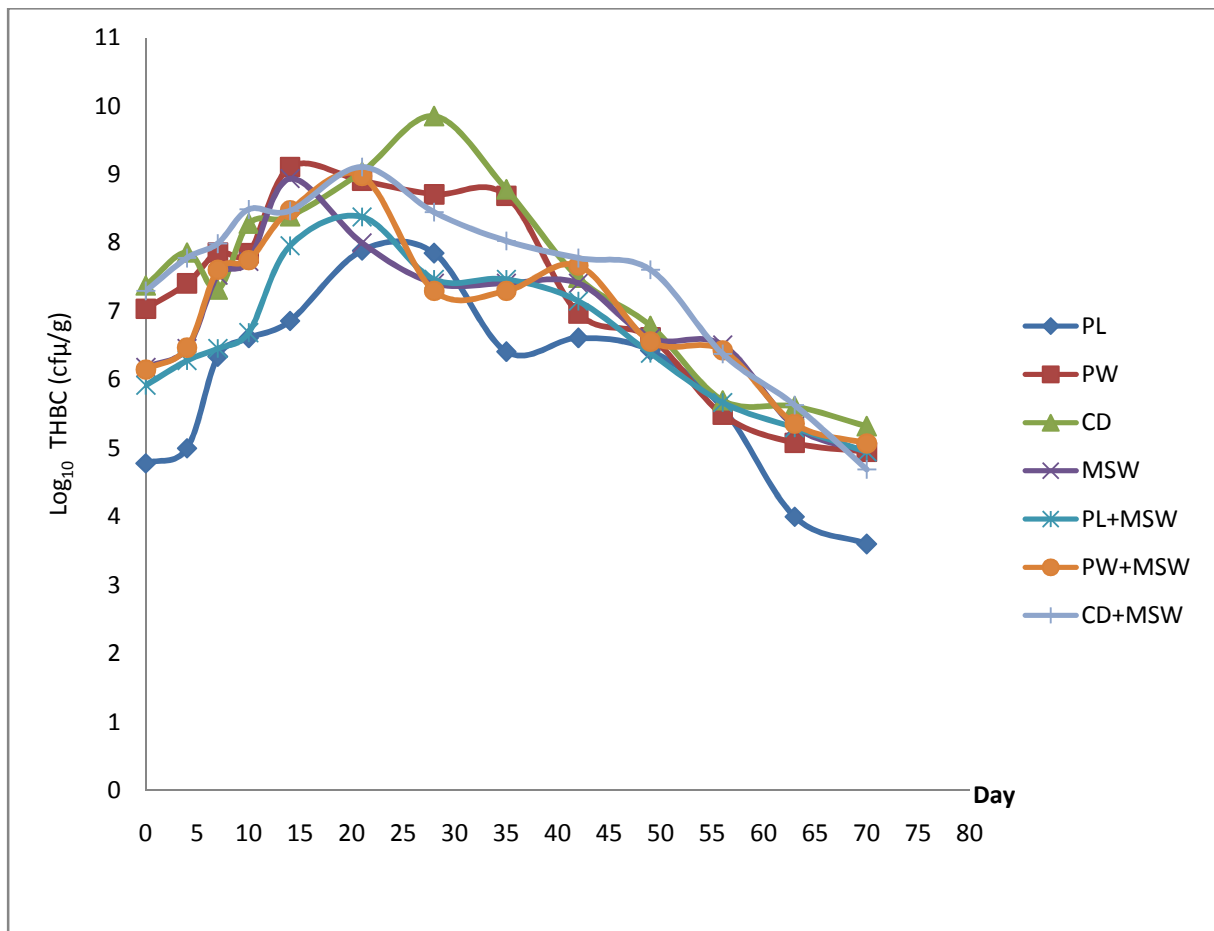
174 CDC = Cow dung Compost

175 MSW = Municipal Solid Waste Compost

176 T = Temperature (°C)

177

178



179

180 **Fig. 1: Changes in the Total Heterotrophic Bacteria Count (THBC) of the organic wastes**
 181 **during the composting period.**

182 Key

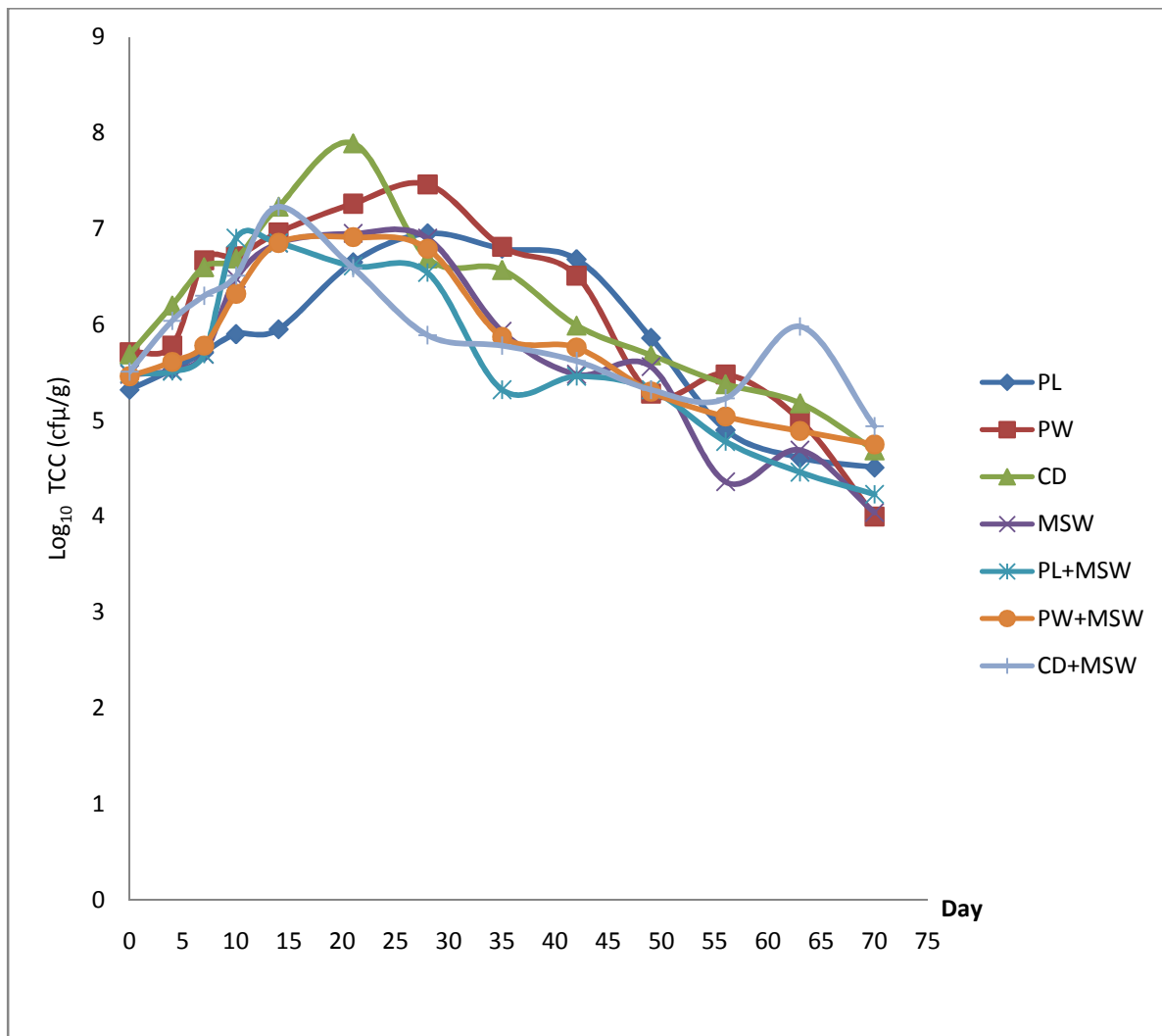
- 183 CDC = Cow dung Compost
- 184 PLC = Poultry Litter Compost
- 185 PWC = Pig Waste Compost
- 186 MSWC = Municipal Solid Waste Compost

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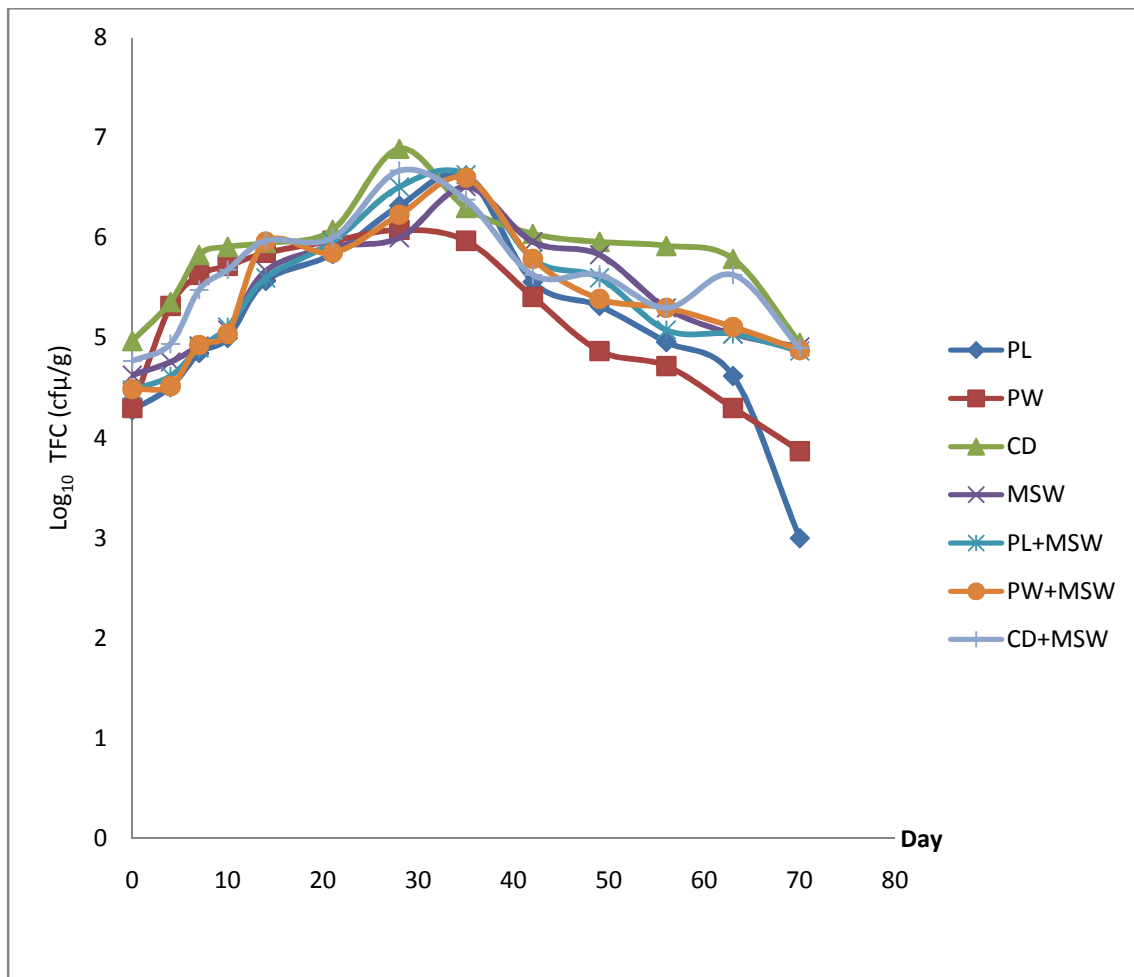
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 192 **Fig. 2: Changes in the Total Coliform Count (TCC) of the organic wastes during the**
 193 **composting period.**

194 Key
 195 CDC = Cow dung Compost
 196 PLC = Poultry Litter Compost
 197 PWC = Pig Waste Compost
 198 MSW C = Municipal Solid Waste Compost
 199



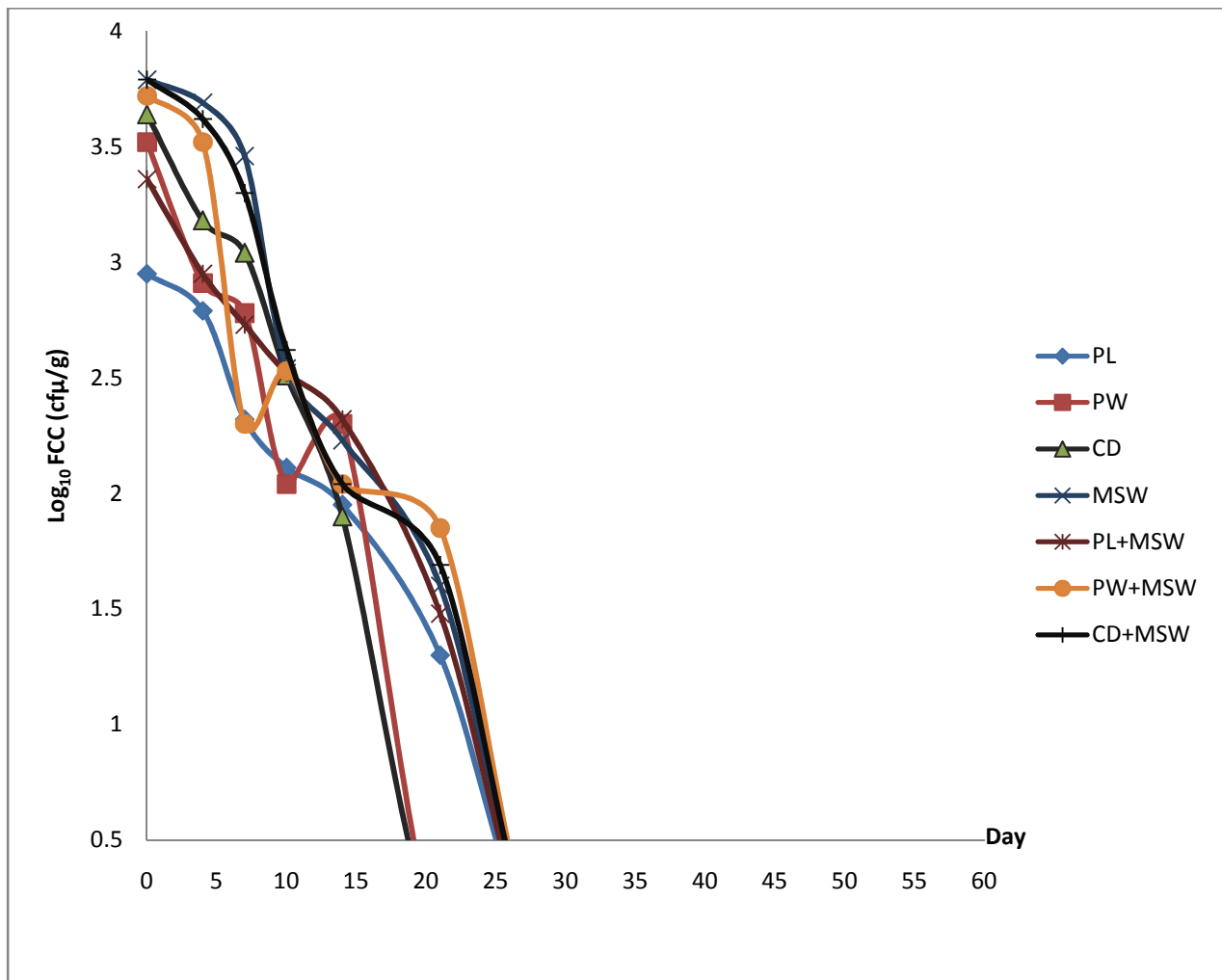
200

201 **Fig.3: Changes in the Total Fungal Count (TFC) of the organic wastes during the**
 202 **composting period.**

203 Key

- 204 CDC = Cow dung Compost
 205 PLC = Poultry Litter Compost
 206 PWC = Pig Waste Compost
 207 MSWC = Municipal Solid Waste Compost

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209

210 **Fig. 4: Changes in the Fecal Coliform Count (FCC) of the organic wastes during the**
 211 **composting period.**

212

213 Key

214 CDC = Cow dung Compost

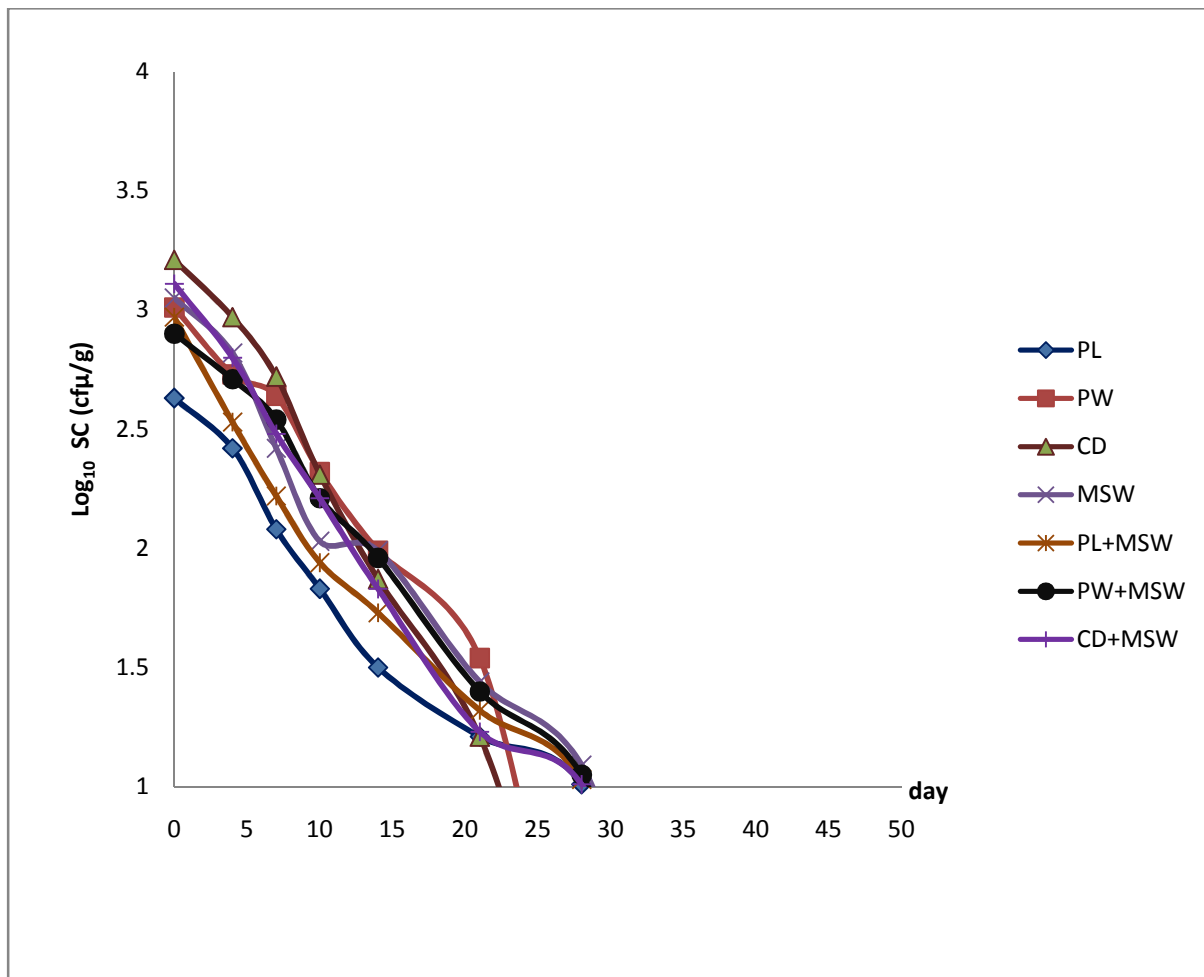
215 PLC = Poultry Litter Compost

216 PWC = Pig Waste Compost

217 MSW C = Municipal Solid Waste Compost

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220

221 **Fig. 5: Changes in the Salmonella Count (SC) of the organic wastes during the**
 222 **composting period.**

223 Key

224 CDC = Cow dung Compost

225 PLC = Poultry Litter Compost

226 PWC = Pig Waste Compost

227 MSWC = Municipal Solid Waste Compost

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234 Generally, the microbial population of the different composting systems increased during the
235 first 3 – 4 weeks of composting except however, faecal coliforms and salmonella. This could be
236 attributed probably to the utilization of the various nutrients available to the microorganisms in
237 the compost due to vigorous microbial activity during this period. The mesophilic population
238 starts the process, oxidizing readily available substrates such as
239 proteins, sugars, starch. As temperature increased, thermophilic microbes developed. This is the
240 period of fastest decomposition, and more resistant compounds such as lignin are degraded to
241 form humus [23]. The microorganisms make use of the organic matter in the compost as food
242 source and this process generates heat, water vapor and humus as a result of the growth and
243 activities of microorganism [24]. Hargerty *et al.* [25] reported that there is usually maximum
244 increase in the microbial population of composts during the first 4 weeks of composting
245 provided all other environmental conditions are favourable.

246
247 During composting, the population of mesophilic bacteria increased rapidly for the first 14 – 15
248 days for cow dung. The initial THBC and TCC for cow dung compost were 2.4×10^7 cfu/g and
249 5.0×10^5 cfu/g respectively. Meanwhile, after the 14th day the THBC and TCC increased to
250 2.5×10^8 and 1.7×10^7 respectively. For the other wastes namely; poultry litter, pig waste and the
251 co-composted wastes, mesophilic temperature still manifested between days 14 and 21 of
252 composting. Thermophilic bacteria became dominant in all the composting systems after the
253 21st day and lasted till the 35th day except for cow dung compost where thermophilic
254 temperature were still observed on the 45th day with a THBC of 6.3×10^6 cfu/g on the 49th day.
255 Mesophilic populations were again noticed after the thermophilic phase and this lasted for
256 between 21 to 30 days.

257 The Faecal Coliform Count (FCC) and Salmonella Count (SC) decreased as the
258 composting process progressed. After 21 days, faecal coliforms were completely eliminated in
259 the pig waste and cow dung composts but it took 28 days of composting to completely eliminate
260 *Salmonella* sp in the same compost systems i.e PW and CD. There was complete elimination of
261 faecal coliforms and *Salmonella* in all the compost systems after the 28th day with temperature
262 range between 47°C – 60°C . This was probably due to the high temperatures generated in the
263 different compost bins. Many pathogenic bacteria carried via animal are found in high
264 concentration in their waste and the numbers and types depend on the source of the waste and
265 the physico-chemical composition of the wastes [26].

266 During the mesophilic stage, lots of pathogenic organisms proliferated, and so, the
267 thermophilic stage is considered important for destroying thermo-sensitive pathogens [27,28].
268 In the cause of this study, it was observed that most common human pathogens, like fecal
269 coliforms and *Salmonella* spp. etc. that dominated the mesophilic phase were eradicated from
270 the composts when temperature reached 45^oC. Previous studies had indicated that temperatures
271 between 45 – 55^oC for 3 consecutive days is sufficient to destroy pathogenic bacteria [29, 30,
272 and 31]. Liao *et al.*[32] had also reported that reduction in the number of fecal coliforms and
273 *Salmonella* was due probably to high temperatures and unfavourable conditions.

274

275 The Fungal Counts(FC) showed slight increases in the first 28 days of composting from a
276 range of 1.9x10⁶ – 5.9x10⁶ cfu/g to a range of 1.2x10⁶ – 7.8x10⁶ cfu/g. After the 35th day, the
277 fungal counts began to decrease until cooling and maturation phase (table 4.1c). Insam *et al* [33]
278 had earlier reported that mesophilic bacteria and fungi were the dominant active degraders of
279 the organic wastes, and the interaction between the various groups of microorganisms depended
280 on the nutrient resources and the biochemical mechanisms of organic and inorganic matter
281 transformation changes. Microorganisms play key roles in the composting process and the
282 presence of some microorganisms reflects the quality of the maturing compost. Ryckeboer *et al.*
283 [34] further reported that bacterial and fungal populations were fundamentally influenced by
284 temperature, pH and the nutritional composition of the organic wastes.

285 During the first 4 weeks of composting diverse populations of mesophilic fungi proliferated and
286 degraded the readily available nutrients and raised composting system temperatures to above
287 45^oC The microbial counts showed a decline during the later weeks of composting and at
288 maturity. Reasonable numbers of microorganisms were still present in all the composts at
289 maturity and these depended on the nutrients available and other environmental factors such as
290 temperature, pH, aeration and moisture content [25,35].

291

292 **4. CONCLUSION**

293

294 Microorganisms play key roles in the composting process and the presence of microorganisms
295 were fundamentally influenced by the temperature of the compost piles. The microorganisms
296 made use of the organic matter in the compost as food source and this process generated
297 beneficial materials for agricultural usage.

298

299 **COMPETING INTERESTS**

300

301 Authors have declared that there are no competing interests .

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