

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 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 in the Agricultural Research Centre of the Federal University of Technology, Owerri, Nigeria.

Methodology: The organic wastes namely Poultry Litter (PL), Pig waste (PW), Cow dung (CD) and Source-Separated Municipal Solid Waste (MSW) were composted/co-composted using the windrow method as modified. 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. In the co-composted piles, 30kg of both samples were introduced into the same 100L bucket that had previously been perforated and then mixed thoroughly. The organic wastes were allowed to decompose at room temperature at a corner of the greenhouse. and initial microbial populations as well as subsequent populations in the compost bins were studied using standard microbiological methods

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 but at maturity the temperature in all compost piles ranged between 27°C to 30°C. The bacterial colony forming units were higher than fungal colony forming units throughout the composting period for both mesophilic

34 and hemophilic microorganisms. The population of mesophilic organisms increased in the first
35 14 – 15 days; for cow dung, the initial total heterotrophic bacteria count (THBC) and total
36 coliform count (TCC) were 2.4×10^7 cfu/g and 5.0×10^5 cfu/g respectively and increased to
37 2.5×10^8 cfu/g and 1.7×10^7 cfu/g for THBC and TCC, respectively, after the 14th day.
38 Thermophilic bacteria dominated all the composting systems after the 21st day and lasted to the
39 35th day except for cow dung compost where thermophilic temperatures were still observed on
40 the 45th day with a THBC of 6.3×10^6 cfu/g on the 49th day. Faecal coliforms and *Salmonella*
41 were completely eliminated in all the compost systems after the 28th day with temperature
42 values between 47°C – 60°C.

43

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

46

47 **Keywords:** composting, mesophilic organisms, thermophilic microorganisms, colony forming
48 units, total heterotrophic bacteria count, total coliform count

49

50 **1. INTRODUCTION**

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

60 The use of organic waste materials as soil amendment is one important approach to
61 sustainable agriculture. To an extent, organic wastes are utilized as nutrient sources in
62 agriculture, however, some of them are not suitable to be applied directly to the soil to improve
63 plant growth [7, 8, and 9]. In some countries, like Pakistan, where sewage sludge is directly
64 used as manure without any treatment, the heavy metals and other toxic substances contained in
65 it usually gain entry into the food chain producing serious human health issues [10,6].

66 Moreover, the availability of organic materials could be limited if it is used in huge bulk
67 volumes, as in the conventional practice where organic wastes are used at several tons per
68 hectare of land for the improvement of crop productivity [11, 12]

69 Composting offers a remedy and a sensible way to avoid wasting of useful natural
70 resources and creating environmental problems. It is a recycling process in which organic
71 materials are biologically converted into stable humus-like substances under controlled
72 conditions of temperature, moisture and aeration [13] The composting process involves mixed
73 populations of microorganisms e.g. bacteria, fungi and actinomycetes that are indigenous to the
74 waste being converted and transforms the waste into a nutrient-rich amendment capable of
75 improving the nutrient level of depleted farmland soils. During composting, the kinds and
76 numbers of microorganisms that develop are usually affected by temperature and nutrient
77 availability.

78 Initially, mesophiles predominate and proceed to decompose the readily degradable sugars,
79 proteins, starches, and fats typically found in undigested feed stocks and the availability of
80 easily usable organic substances enables the proliferation of the fast-growing microorganisms
81 [14]. At higher temperatures, thermophilic microorganisms dominate the microbial community
82 and continues generating more heat as a result of the decomposition of more organic matter.
83 The higher temperatures will ensure rapid organic matter processing while simultaneously
84 providing optimal conditions for the destruction of human and plant pathogens [15].

85 Composting has resolved problems associated with the use of raw organic wastes as soil
86 amendments, which include malodors, human pathogens, toxic heavy metals, toxic organic
87 compounds and other undesirable physical and chemical properties [16, 9, and 17]. It also
88 provides a way to manage big volumes of organic wastes in environmentally sound manners
89 [13,18].

90 The present investigation studied the changes on the microbial population numbers during the
91 composting of some organic wastes using the modified windrow method

92

93 **2. MATERIALS AND METHODS**

94

95 **2.1 Location of the Study Area**

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

98

99 **2.2 Duration of the Study**

100 **This** study was done between September 2017 and January 2018.

101

102 **2.3 Composting of Organic Wastes**

103 The organic wastes used in this study included Poultry Litter (PL), Pig waste (PW), Cow
104 dung(CD) and Source-Separated Municipal Solid Waste (MSW). MSW was obtained from a
105 dumpsite located at Ikenegbu, Owerri while PL, PW and CD were obtained from the research
106 farm of the School of Agriculture, FUTO.

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

- 108 a) Pig waste (PW) only
- 109 b) Poultry litter (PL) only
- 110 c) Cow dung (CD) only
- 111 d) Municipal solid waste (MSW) only
- 112 e) Pig waste + MSW
- 113 e) Poultry litter + MSW
- 114 f) Cow dung + MSW

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

124

125 **2.4 Determination of Temperature of Composting Piles**

126 **The temperature of the composting piles were monitored daily during the entire period of**
127 **composting** i.e. for 70 days. Process temperatures were determined by taking the average
128 readings from the two thermometers that were inserted 5 cm deep into each pile at different
129 spots. The ambient temperature was continuously monitored by taking average reading of the

130 two different thermometers (Salmoiraghi Co. thermometer model, 1750) fixed permanently at
131 two different spots in the green house.

132

133 **2.5 Isolation and Enumeration of Isolated Bacteria**

134 The media employed included Nutrient Agar, Mackonkey Agar, Eosin Methylene Blue Agar
135 and Salmonella-Shigella Agar and were all prepared according to manufacturer's guideline
136 (Oxoid, England). The initial microbial populations as well as subsequent populations in the
137 compost bins were studied using standard microbiological methods as described by Harley-
138 Prescott [20]. The Total Heterotrophic Bacteria Count (THBC), Fecal Coliform count (FCC),
139 Salmonella Count(SC), Total Coliform Count (TCC) and Total Fungal Count(TFC) of
140 composting organic wastes were determined on day 0, day 4, day 7, day 10, day 14, day 21, day
141 28, day 35, day 42, day 49, day 56, day 63, and day 70 on the appropriate growth medium.

142 Compost suspensions were prepared by the addition of 10 g compost samples to 90 ml of
143 normal saline (0.85% w/v). Serial dilutions of these initial suspensions were made in normal
144 saline. Aliquot (0.1 ml) of each appropriate dilution was inoculated in duplicate and spread with
145 sterile rod spreader. Fecal coliforms were counted on Eosin Methylene Blue Agar plates
146 incubated at 44.5°C while *Salmonellae* were counted on Salmonella-Shigella agar plates
147 incubated the at 37°C according to the method described by APHA [21]. The colonies that
148 developed on the plates were counted and recorded as colony forming units using standard
149 methods [19, 22].

150

151 **3. RESULTS AND DISCUSSION**

152

153 Table1 represents changes in the temperature of the composting piles during composting.
154 Initial temperature of the compost piles ranged from 28 – 30°C. The temperature of the
155 piles increased at different rates. For CD the temperature increased from 30°C to 46°C
156 after two weeks while it took PL, MSW and PW+MSW 21 days to attain a temperature of
157 45°C. The highest temperature of 60°C was recorded for CD compost on the 28th day.
158 However, by the 7th week (day 49) the temperature of the compost piles dropped to
159 between 34°C – 40°C and stabilized at between 27°C – 30°C by the 9th week (day 63).

160 During the cooling stage that lasted for about 21 days (i.e. day 50 – day 70), the pile
 161 temperatures remained in the range of 27°C – 37°C in all the compost piles.

162 Figures 1 to 5 show the changes in the microbial populations of the different
 163 organic wastes. The same pattern was observed for Total Fungal Count (TFC), Total
 164 Coliform Count (TCC) and Total Heterotrophic Bacteria Count (THBC). As temperature
 165 increased, the microbial populations increased until a peak was attained as determined
 166 by the type of organic waste. Faecal coliforms and *Salmonellae* were not detected in
 167 some of the compost bins when temperatures as high as between 47°C – 60°C were
 168 recorded., however, THBC as high as 7.2×10^9 cfu/g was recorded for CD on day 28 when pile
 169 temperature was 60°C. PL had the lowest THBC throughout the composting period, from day
 170 zero to maturity, when compared to the others. Meanwhile, fungal counts were lower than
 171 THBC when compared and the lowest fungal count of 1.0×10^3 cfu/g was recorded for PW.

172
 173 **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

174 **Key**

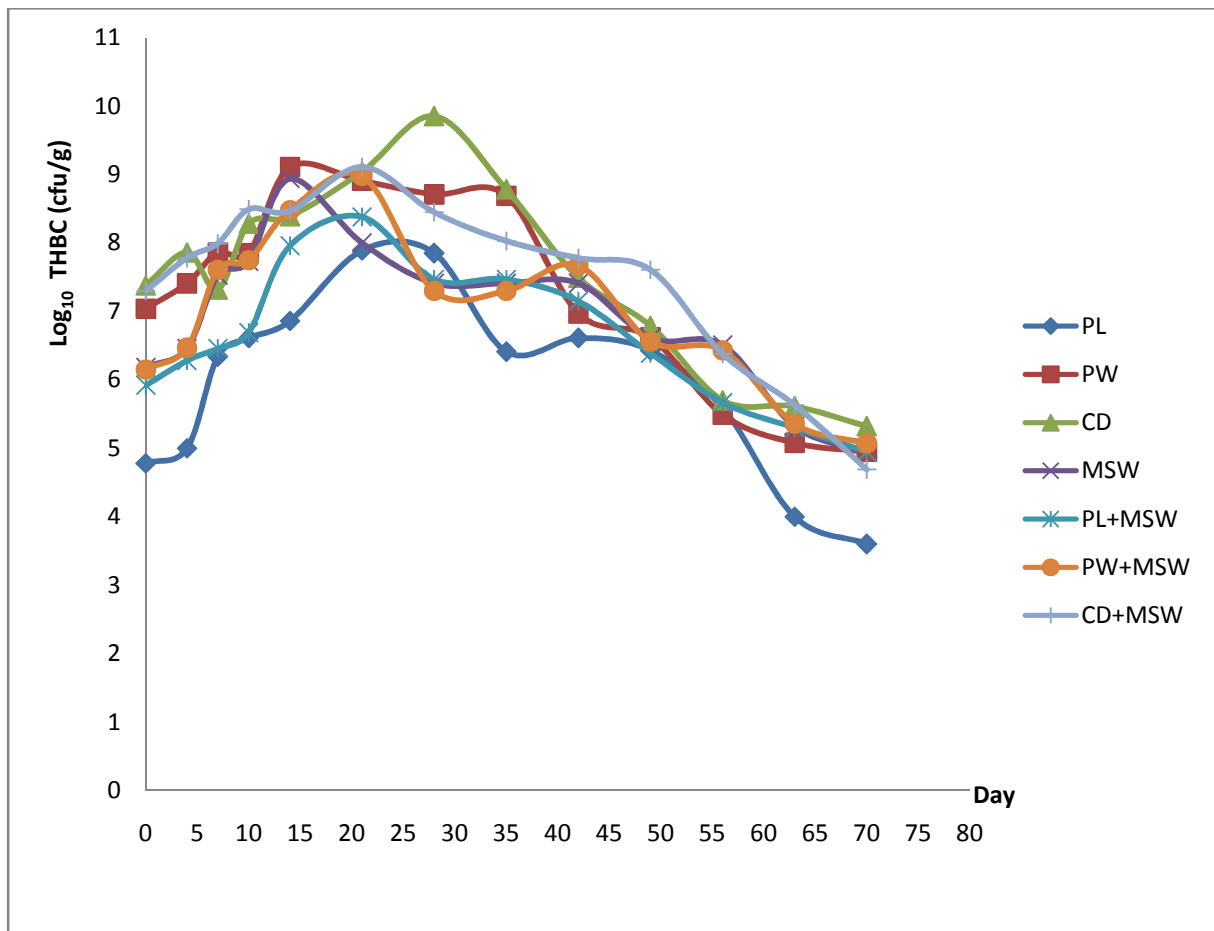
175 PLC = Poultry Litter Compost

176 PWC = Pig Waste Compost
177 CDC = Cow dung Compost
178 MSW = Municipal Solid Waste Compost
179 T = Temperature ($^{\circ}\text{C}$)

180

181

UNDER PEER REVIEW



182

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

185 Key

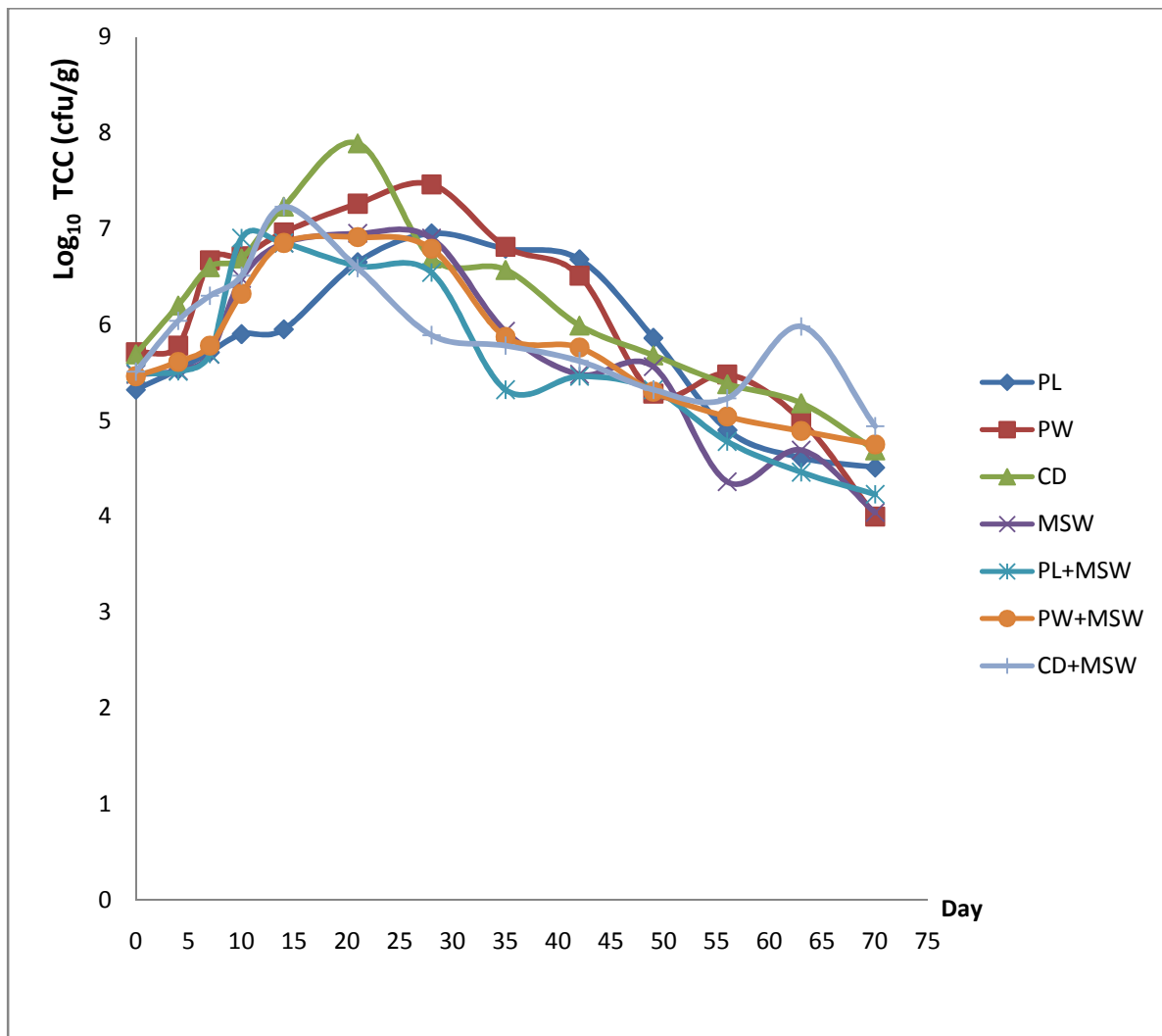
- 186 CDC = Cow dung Compost
- 187 PLC = Poultry Litter Compost
- 188 PWC = Pig Waste Compost
- 189 MSWC = Municipal Solid Waste Compost

190

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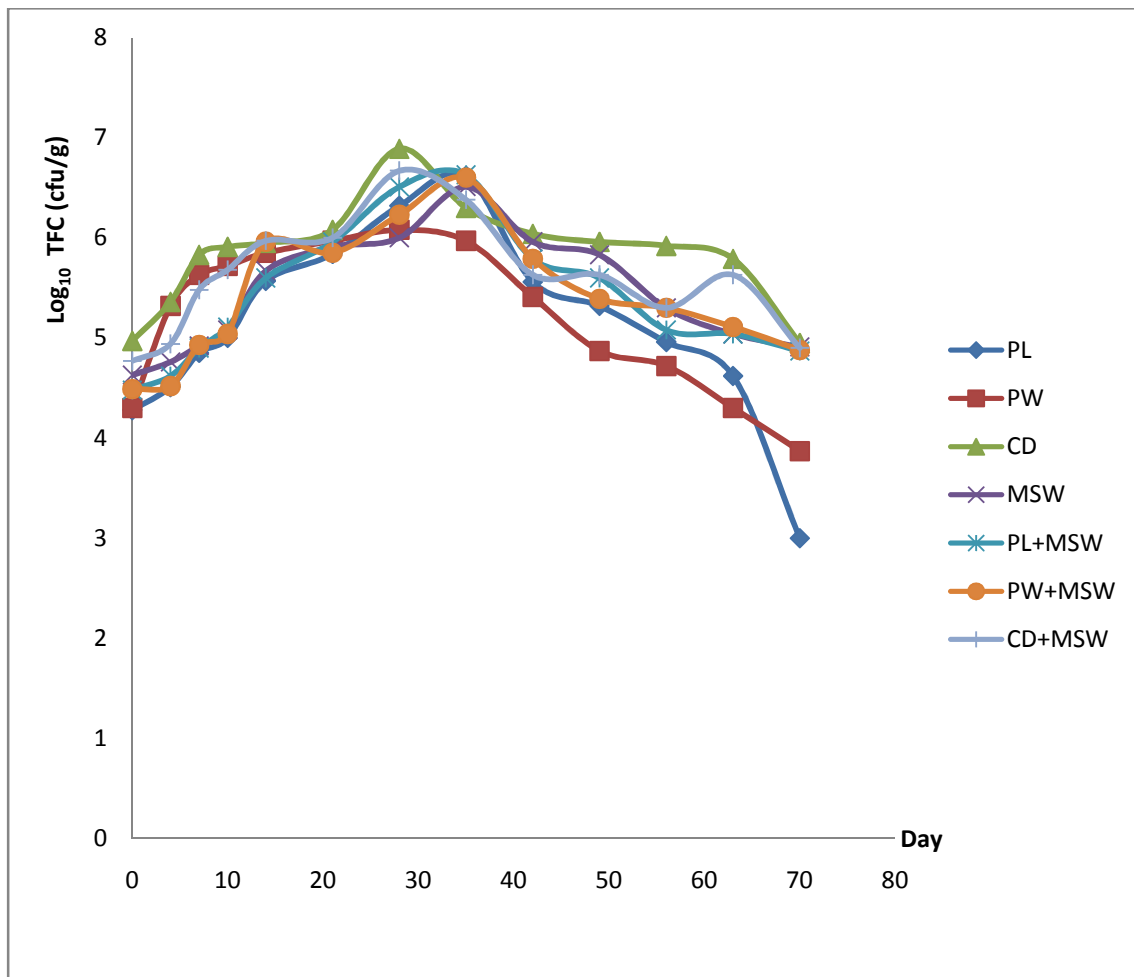
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194
 195 **Fig. 2: Changes in the Total Coliform Count (TCC) of the organic wastes during the**
 196 **composting period.**

197 Key
 198 CDC = Cow dung Compost
 199 PLC = Poultry Litter Compost
 200 PWC = Pig Waste Compost
 201 MSW C = Municipal Solid Waste Compost
 202



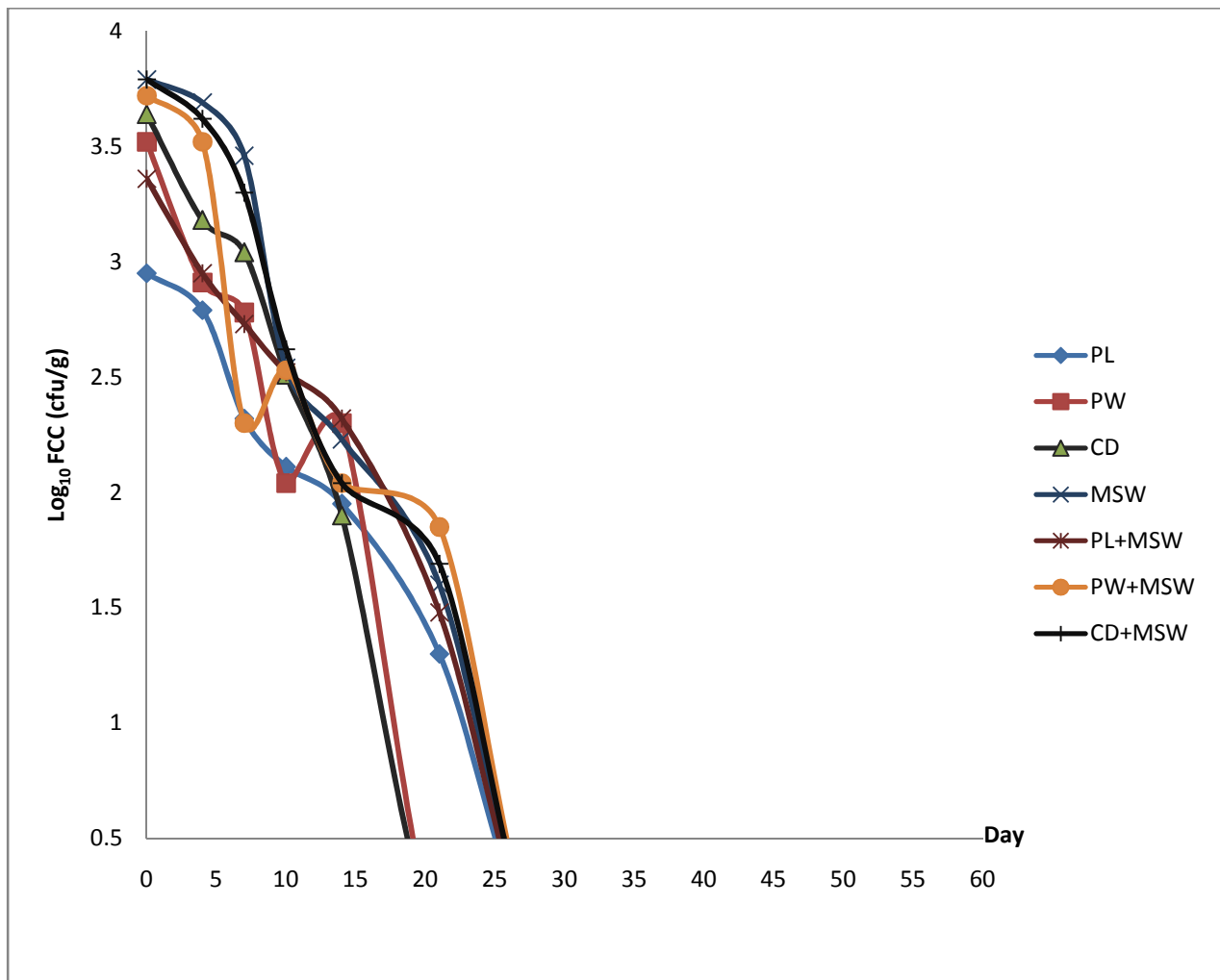
203

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

206 Key

- 207 CDC = Cow dung Compost
- 208 PLC = Poultry Litter Compost
- 209 PWC = Pig Waste Compost
- 210 MSWC = Municipal Solid Waste Compost

211



212

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

215

216 Key

217 CDC = Cow dung Compost

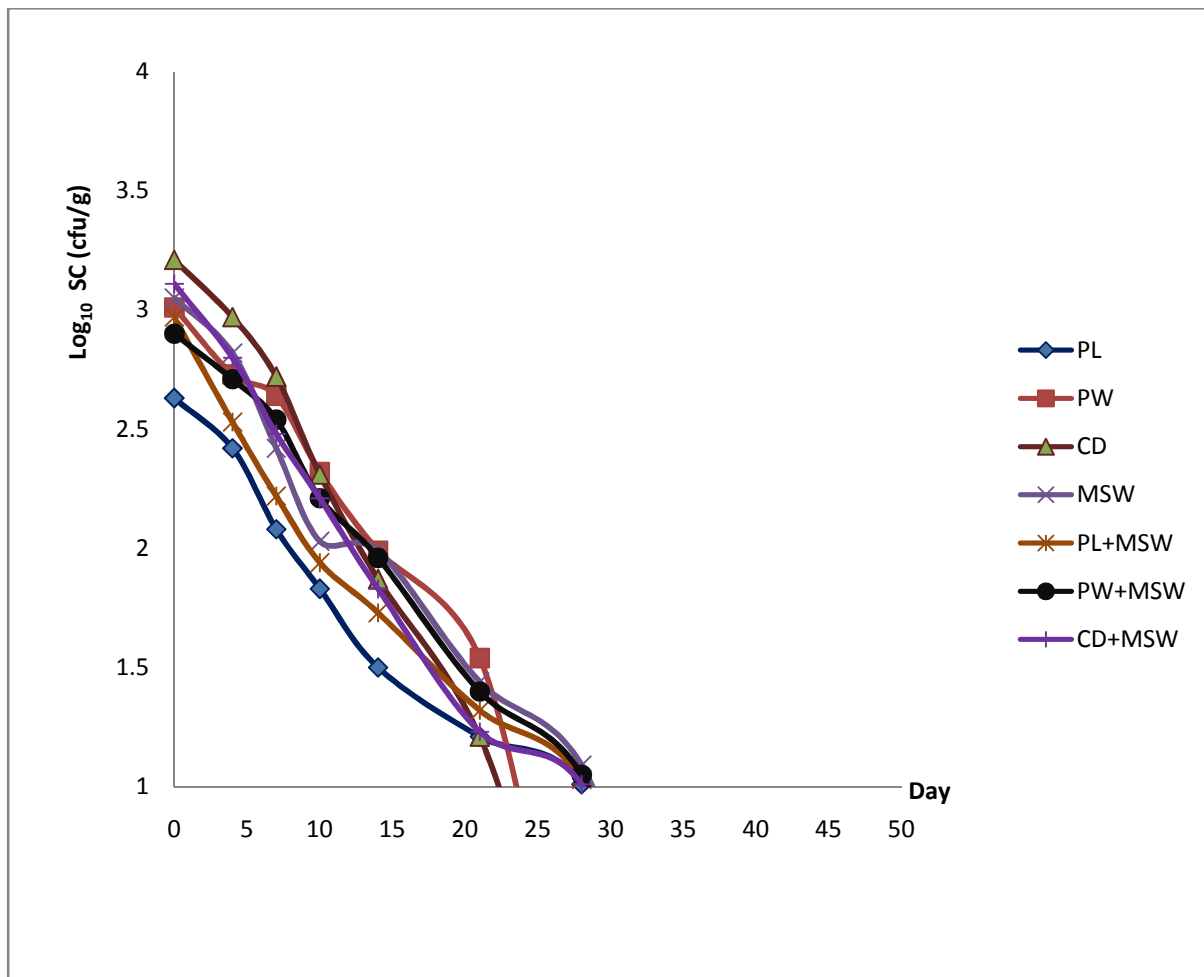
218 PLC = Poultry Litter Compost

219 PWC = Pig Waste Compost

220 MSW C = Municipal Solid Waste Compost

221

222



223

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

226 Key

227 CDC = Cow dung Compost

228 PLC = Poultry Litter Compost

229 PWC = Pig Waste Compost

230 MSWC = Municipal Solid Waste Compost

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

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

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

269 During the mesophilic stage, lots of pathogenic organisms proliferated, however, the
270 thermophilic stage is considered important for destroying thermo-sensitive pathogens [27,28].
271 Most human pathogens eg *Salmonella* and faecal coliforms etc. that dominated the mesophilic
272 phase were eradicated from the composts when temperature reached 45^oC. Previous studies had
273 indicated that temperatures between 45 – 55^oC for three consecutive days is sufficient to
274 destroy pathogenic bacteria [29, 30, and 31]. Liao *et al.* [32] had also reported that reduction in
275 the number of fecal coliforms and *Salmonella* was due probably to high temperatures and
276 unfavourable conditions.

277
278 The Fungal Counts(FC) showed slight increases in the first 28 days of composting from a
279 range of 1.9x10⁶ – 5.9x10⁶ cfu/g to a range of 1.2x10⁶ – 7.8x10⁶ cfu/g. After the 35th day, the
280 fungal counts began to decrease until cooling and maturation phase (table 4.1c). Insam *et al* [33]
281 had earlier reported that mesophilic bacteria and fungi were the dominant active degraders of
282 the organic wastes, and the interaction between the various groups of microorganisms depended
283 on the nutrient resources and the biochemical mechanisms of organic and inorganic matter
284 transformation changes. Microorganisms play key roles in the composting process and the
285 presence of some microorganisms reflects the quality of the maturing compost. Ryckeboer *et al.*
286 [34] further reported that bacterial and fungal populations were fundamentally influenced by
287 temperature, pH and the nutritional composition of the organic wastes.

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

294

295 4. CONCLUSION

296

297 Microorganisms play key roles in the composting process and the number of microorganisms
298 were fundamentally influenced by the temperature of the compost piles.

299

300

301 **COMPETING INTERESTS**

302

303 Authors have declared that there are no competing interests .

304

305

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