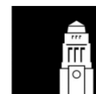

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1 # Fate of faecal pathogen indicators during faecal sludge composting with different bulking agents in tropical
2 climates

3 M. Manga[1,2,3*], M. A. Camargo-Valero[2,4], C. Anthonj[1,5,6], B. E. Evans[2]

4 [1]The Water Institute at UNC, Department of Environmental Sciences and Engineering, University of North Carolina at
5 Chapel Hill, 4114 McGavran Hall, Campus Box # 7431, NC 27599, Chapel Hill, North Carolina, USA.

6 [2]BioResource Systems Research Group, School of Civil Engineering, University of Leeds, Leeds LS2 9JT, UK.

7 [3]Department of Construction Economics and Management, College of Engineering, Design, Art and Technology
8 (CEDAT), Makerere University, P.O. Box 7062, Kampala, Uganda. Mobile: +256-702-965158

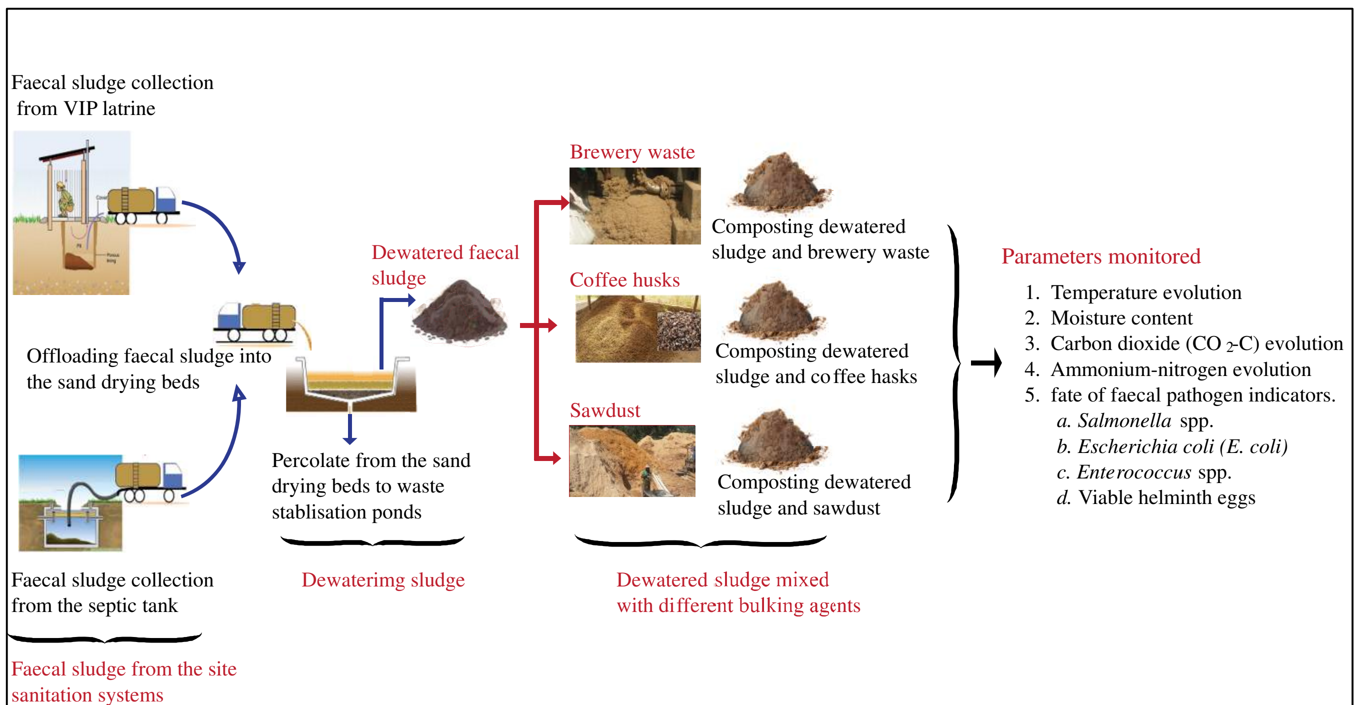
9 [4]Departamento de Ingeniería Química, Universidad Nacional de Colombia, Campus La Nubia, Manizales, Colombia

10 [5]Institute for Hygiene and Public Health, Medical Faculty, University of Bonn, GeoHealth Centre, Bonn, Germany

11 [6] Faculty of Geo-Information Science and Earth Observation, ITC, University of Twente, Enschede, The Netherlands

12 [*]*Corresponding email: musamanga@cedat.mak.ac.ug/ mmanga@email.unc.edu*
13

14 ## Graphic Abstract



15

16 **Figure A:** Graphical Abstract of Faecal Sludge composting with Sawdust - (SSD); Coffee husks - (SCH); Brewery
17 waste – (SBW).

18 ## Abstract

19 In recent years, composting has increasingly been promoted as a reliable method for sanitizing Faecal Sludge (FS)
20 from onsite sanitation systems, particularly where there are opportunities to use the recovered nutrients in agriculture.

21 However, there remain gaps in our understanding of the fate of infectious faecal pathogens during composting,
22 particularly in tropical climates. This study investigated the influence of different locally available bulking agents on
23 the inactivation efficiency of composting by tracking the fate of four key indicator organisms (*E. coli, Salmonella* spp.,
24 *Enterococci* spp., and viable helminth eggs). Dewatered FS was mixed with different bulking agents - i.e. Sawdust
25 (SD), Coffee husks (CH) and Brewery waste (BW). Compost piles of FS:SD, FS:CH, and FS:BW in a volumetric ratio
26 of 1:2 were set-up in duplicate (3 m^3 each), composted on a pilot scale and monitored weekly for the survival of
27 pathogen indicators for a period of 15 weeks. The study findings suggest that the different bulking agents have a
28 statistically significant ($p < 0.05$) effect on the temperature evolution and survival of pathogen indicators in compost.
29 CH was the most suitable bulking agent for composting with FS as piles containing CH exhibited higher pathogen
30 inactivation efficiency and shorter inactivation periods of 6 weeks compared to 8 weeks for SD and BW piles. Time-
31 temperature was the most important factor responsible for pathogen inactivation. However, other mechanisms such as
32 indigenous microbial and toxic by-products such as NH_{4+}-N also played an important role in the inactivation of
33 pathogens. The results suggest that co-composting of FS with a sawdust, coffee husk or brewery waste for 8 weeks
34 with thermophilic temperatures of about 48 – 60 °C sustained in the composting piles for more than 38 days, using 7
35 days turning frequency, is sufficient to ensure complete sanitization of FS before reuse in agriculture.

36 **Keywords:** *Faecal sludge treatment; Composting; Wastewater reuse; Bulking agents; Faecal pathogen indicators;*
37 *Helminth eggs.*

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38 Introduction

39 More than a third of the world's population (~ 2.7 billion people) relies on on-site sanitation facilities (such as septic
40 tanks, aqua privies, unsewered communal and family toilets/ latrines) for their sanitation needs, and this population is
41 anticipated to increase to 5 billion by 2030 (Strande, 2014). In sub-Saharan Africa, about 65–100% of the urban
42 residents are served by on-site sanitation facilities other than water-borne sewered systems (Strande, 2014). These on-
43 site sanitation facilities, when ready to be emptied, require proper management of the resulting Faecal Sludge (FS),
44 including collection, haulage, treatment and reuse or disposal. Unfortunately, adequate FS management is not a
45 common practice in urban Africa where sanitation service delivery in the form of sustainable treatment facilities is still
46 greatly lacking (Manga et al., 2020). As a consequence, untreated FS is often reused or indiscriminately disposed of
47 into the environment (e.g. canals, open drains, surface water bodies, etc.). This results in environmental damage as well
48 as serious public health risks, leading to high occurrences of excreta related diseases, and hence high morbidity and
49 mortality (Peal et al., 2014).

50 FS contains valuable organic matter and plant nutrients, which can be recovered for safe reuse in agriculture. However,
51 FS also contains high concentrations of pathogenic micro-organisms often at concentrations that are a 10-100 factor
52 higher than those found in wastewater (Heinss et al., 1998). The World Health Organization calls for a balance of
53 treatment and on-field interventions to reduce the risk to human health associated with reuse of feacal sludge in
54 agriculture (WHO, 2006). Treatment plays a critical role, particularly where sludge is likely to be used in food
55 production. An understanding of the effectiveness of treatment options is critical for the assessment of appropriate
56 management strategies.

57 Composting is often preferred as a treatment method for FS due to its low energy requirements and efficacy in terms of
58 the recovery of critical nutrients (Nitrogen, Phosphorous and Potassium). However, the effectiveness of FS composting
59 has not been thoroughly explored especially in urban Africa. Few robust research studies on FS composting have been
60 conducted and published in peer reviewed articles to date (Al-Muyeed et al., 2017; Berendes et al., 2015; Cofie et al.,
61 2009; Hashemi et al., 2019; Koné et al., 2007; Mengistu et al., 2017; Nakasaki et al., 2011; Nartey et al., 2017; Oarga-
62 Mulec et al., 2019; Oarga Mulec et al., 2016; Scott, 1952; Thomas et al., 2018), although doubtlessly, composting of FS
63 has been and is extensively being practiced globally, both informally and formally. Raw or dewatered FS is
64 characterized by low Carbon/Nitrogen ratio (C/N) and high moisture content, making it unsuitable for composting
65 alone. Therefore, dewatered FS should be mixed with suitable bulking agents (e.g. sawdust, rice husks, etc.) before
66 composting, which are high in carbon content, and low in moisture content. This can mainly improve the C/N ratio,
67 sustain microbial activity and also absorb the excess moisture content in FS so as to provide a composting feedstock
68 with appropriate support structure (with inter-particle voids) for effective aerobic composting conditions, successful
69 composting process and pathogen inactivation (Eftoda and McCartney, 2004; Tremier et al., 2005).

70 In this study, locally available bulking agents, namely sawdust, coffee husks and brewery waste, were investigated for
71 suitability for composting with FS in Kampala, Uganda. These bulking agents were selected because they are locally
72 available in abundance at low or no cost. Sawdust is a globally abundant organic waste from timber sawmills. It is
73 estimated that about 1,200 tonnes dry mass of sawdust are generated annually by several timber processing companies
74 and carpentry workshops in Kampala suburbs, Uganda (Byrne et al., 2015). Similarly, an estimate of approximately
75 7,440 tonnes dry mass of brewery waste are generated annually by the brewery companies while about 1,200 tonnes
76 dry mass of coffee husks are generated annually by the coffee processing companies in Kampala, Uganda (Byrne et al.,
77 2015). A fraction of generated sawdust, coffee husks and brewery wastes, is currently used to provide energy at
78 household level and industrial scale. It is also used as bedding layer in rearing of chicken, while the other fraction joins

79 the municipal solid waste stream since the demand is still low compared to the supply (Komakech et al., 2014).
80 Therefore, composting with FS has provided a potential approach for the sustainable management of the waste stream
81 fraction of these organic wastes.

82 The type of bulking agent is one of the key factors that affect the aerobic composting process. Considerable literature
83 has been published on co-composting of various feedstock materials such as sewage sludge (Aghili et al., 2019;
84 Banegas et al., 2007; Khadra et al., 2019), farm manures (Nolan et al., 2011; Tiquia and Tam, 2002), and bio-waste
85 materials (Adhikari et al., 2008; Dadi et al., 2019; Khadra et al., 2019) with bulking agents such as rice straw, sawdust,
86 bark, coffee husks, and woodchips. Similarly, studies have been conducted on the composting of FS with different
87 bulking agents such as sawdust (Al-Muyeed et al., 2017; Evans et al., 2015; Hashemi et al., 2019; Nakasaki et al.,
88 2011; Oarga-Mulec et al., 2019; Scott, 1952) oat husks, wheat bran, peat and bark (Oarga-Mulec et al., 2019; Oarga
89 Mulec et al., 2016); rice straw and rice husks (Evans et al., 2015; Hashemi et al., 2019); oil palm empty fruit bunches
90 and cocoa pod husk (Nartey et al., 2017); sugarcane husks (Berendes et al., 2015); and mixed organic waste, (Al-
91 Muyeed et al., 2017; Cofie et al., 2009; Evans et al., 2015; Koné et al., 2007; Mengistu et al., 2017; Thomas et al.,
92 2018). However, the use of other bulking agents has received very little research attention in terms of composting with
93 FS. For examples, the potential of coffee husks and brewery waste as bulking agents in the composting of faecal sludge
94 is still untapped.

95 Although several studies have addressed the optimization of FS co-composting with various organic wastes (Al-
96 Muyeed et al., 2017; Berendes et al., 2015; Cofie et al., 2009; Evans et al., 2015; Hashemi et al., 2019; Koné et al.,
97 2007; Mengistu et al., 2017; Nakasaki et al., 2011; Nartey et al., 2017; Scott, 1952; Thomas et al., 2018), evidence on
98 the effectiveness of the different bulking agents on FS sanitization, during open-air composting system in tropical
99 climate is still scant in published literature (Al-Muyeed et al., 2017; Evans et al., 2015; Mengistu et al., 2017; Nartey et
100 al., 2017; Scott, 1952). Moreover, regarding the sanitization efficiency of FS during composting, contradicting results
101 have been presented in literature. Some studies evaluated the effectiveness of thermophilic composting and time-
102 temperature criteria provided by United States Environmental Protection Agency (USEPA) USEPA (2003) on the
103 survival of pathogens during composting. These studies demonstrated that pathogen concentrations can be reduced to
104 non-detectable limits when the criterion of achieving and maintaining composting temperatures of > 55 °C for 3 days
105 (or 15 days for windrow composting) is fulfilled(Déportes et al., 1998; Evans et al., 2015; Manga et al., 2019; Scott,
106 1952). Evans et al. (2015) showed that all monitored pathogen indicators (*E. coli* and helminth eggs) were completely
107 inactivated after 4-6 weeks composting periods when co-composting FS with rice straw, rice husks, cow dung, and

108 sawdust, where temperatures of > 55 °C were attained and maintained within piles for a period of 26 days, while using
109 3-7 days turning frequency. Similarly, Mengistu et al. (2017) observed that helminth eggs were still detectable in the
110 final compost after 100 days composting period were the time-temperature criteria for pathogen destruction was not
111 attained during the FS co-composting with municipal organic solid waste using windrow composting.

112 On the contrary, some studies have reported that even when the stipulated time-temperature criteria was fulfilled for
113 extended periods during composting, one or more pathogen indicators remained detectable in the final compost
114 (Cabañas-Vargas et al., 2013; Droffner and Brinton, 1995). However, some researchers have observed a reduction in
115 the concentrations of pathogen indicators to non-detectable levels, even when the composting temperatures were much
116 lower than the recommended 55 °C for thermal destruction. For example, Nartey et al. (2017) showed that even when
117 the composting process did not meet the recommended time-temperature criteria for pathogen destruction, all
118 monitored pathogen indicators (*E. coli,* and *Ascaris* eggs) were completely inactivated by the end of 90 days
119 composting period during the co-composting of FS with agricultural wastes in windrows, while using a 3-7 days
120 turning frequency. Therefore, pathogen inactivation during FS composting is more complex, and not well understood
121 due to conflicting information in the literature; the present scenario thus, calls for further exploration in the
122 effectiveness of thermal composting and time-temperature criteria on FS sanitization. Moreover, the effectiveness of
123 sawdust, coffee husks and brewery waste as bulking agents on sanitization of FS using open-air composting system in
124 tropical climate is not yet well understood. In order to fill this knowledge gap, the present study investigated the effect
125 of locally available bulking agents (i.e., sawdust, coffee husks and brewery waste) on the time-temperature relationship,
126 and inactivation efficiency of faecal pathogenic indicators (*E. coli*, *Salmonella* spp., *Enterococcus* spp. and viable
127 helminth eggs) during FS composting on a pilot scale under tropical climate conditions. Improvement in the
128 sanitization efficiency of FS compost could be a driver for its sustained and safe end-use, hence improved sanitation
129 through management of not only FS, but also the sawdust, coffee husks and brewery wastes from the surrounding
130 environment of urban slums.

131 **Material and methods**

132 **Pilot-scale composting facility and raw material collection**

133 The pilot scale composting facility for our study was constructed at National Water and Sewerage Corporation
134 (NWSC) faecal sludge treatment facility at Lubigi, Kampala, Uganda. Kampala, the capital of Uganda with a
135 population of about 1.8 million people, is located at the northern shores of Lake Victoria at an altitude of 1,223 m
136 above mean sea level (geographical coordinates 0°18′58.18″ N latitude, 32°34′55″ E longitude). Kampala's climate is

137 tropical with rainfall throughout the year, mainly concentrated during two rainy seasons between March and May, and
138 in October and November (Fuhrimann et al., 2015). The pilot scale composting facility had a total area of 300 m^2,
139 comprising both dewatering and composting sections. The design and construction details of the dewatering and
140 composting facility are presented in our previous work (Manga et al., 2019; Manga et al., 2016).

141 **Collection of faecal sludge and bulking agents**

142 Raw FS was collected from the nearby Kampala informal settlements (i.e., Makerere Kikoni, and Bwaise). FS from
143 ventilated improved pit latrines (VIP sludge) and septage from vaults and septic tanks was thoroughly mixed in a ratio
144 of 1:2 by volume (VIP latrine sludge: septage) within one big cesspool truck, and pre-treated by dewatering on sludge
145 unplanted sand drying beds. The dewatering cycle was then monitored, and this was considered complete when the
146 dewatering sludge attained total solids content of about 20 - 35%. The dewatered sludge was then removed from the
147 drying beds and transferred to the composting shade. Detailed procedures on FS dewatering and characterisation is also
148 presented in our previous work (Manga et al., 2016). Coffee husks, and brewery waste were collected from Kyagalanyi
149 coffee processing plant and Parambot breweries, respectively. Sawdust was obtained from Bwaise sawmill located less
150 than 0.5km from the project site. Bulking agent wastes delivered at the composting facility were sorted to remove any
151 inorganics before composting. The characteristics of raw materials used in the study are summarised in Table SI.

152 **Construction and monitoring of composting piles**

153 Dewatered FS of about 27–35% total solids content was thoroughly mixed with different sorted bulking agents. Three
154 types of compost static piles each 3 m^3 were constructed, each in duplicate:

155 (i) SSD (1:2 v/v; dewatered sludge: Sawdust);
156 (ii) SCH (1:2 v/v; dewatered sludge: Coffee husks);
157 (iii) SBW (1:2 v/v; dewatered sludge: Brewery waste).

158 The composting piles were aerated by manual turning, with a 3 - 7 days turning frequency. The composting
159 temperature of each pile was measured daily at the: top (ca. 750 mm from the pile base), middle (400 mm from pile
160 base) and bottom (200 mm from pile base), using a TFA (D-Wertheim, Model 19.2008) stainless steel body compost
161 thermometer. The moisture content of piles was monitored weekly and piles wetted to adjust moisture content when
162 necessary to the recommend limits of 50 - 65% for effective aerobic composting conditions throughout the entire
163 composting process. The composting piles were protected from the rain and monitored for a period of 15 weeks,
164 between April and July (air temperature: 19 – 26 ºC; relative humidity: 69 – 80%). The composting piles were
165 protected from the rain using a composting shade roofed with clear polycarbonate roofing sheets.

166 **Sampling methods**

167 Dewatered sludge samples were collected from at least 10 randomly chosen sampling points on each drying bed
168 before sludge removal. To ensure representative sampling, an equal volume of sludge was collected from each
169 sampling point, and all of these were thoroughly mixed to form a composite sample from which a portion was collected
170 using quarter sampling. Detailed procedures for collecting dewatered faecal sludge samples from the sludge drying
171 beds is presented in Manga et al. (2019). Compost samples of about 400 g were collected from the top, middle, and
172 bottom as well as the outer and inner sections of each composting pile. To ensure representative sampling, the collected
173 subsamples were then mixed homogeneously to form a composite sample for each of the three pile types, from which a
174 sample of approximately 500 g was collected using quartering method, and transported to the laboratory for analysis in
175 a cool box containing ice packs. Samples were collected at day 0 and weekly from the composting piles until the end of
176 the composting period. Samples were analysed immediately upon receipt at the laboratory to minimize changes in
177 bacterial population. The analysis was carried out at Bugolobi NWSC central laboratory and Microbiology laboratory
178 in the Faculty of Veterinary Medicine at Makerere University in Kampala.

179 **Analytical methods**

180 **Physical and chemical analyses**

181 **Moisture content (%)** was computed from the difference in the sample initial and final (after oven drying at 105 °C
182 for 24 hours) weights, following the procedure stipulated in Okalebo et al. (2002). Ammonium-nitrogen (NH_4^+-N) was
183 extracted with *0.5M* K_2SO_4 in 1:10 (w/v) from fresh compost samples and determined by spectrophotometric methods
184 according to procedures reported in the literature (Keeney and Nelson, 1982; Okalebo et al., 2002). The microbial
185 respiratory activity in compost samples was measured based on CO_2-C evolution rate conducted in closed bottles
186 according to Öhlinger (1996) soil respiration techniques, but with some modifications made to techniques based on
187 similar soil respiration procedures reported in literature (Anderson, 1984; Tognetti et al., 2007). CO_2-C was trapped in
188 an alkaline solution (KOH), which was then titrated with a HCl solution (0.5*M*). CO_2-C production rate was assessed
189 and expressed as mg CO_2-C per mass of organic matter (as Volatile Solids – VS) per day (Tognetti et al., 2007).

190 **Pathogen analyses**

191 **Sample preparation for culturable pathogen indicators analysis:** The collected bulking agents, dewatered sludge
192 and compost samples were analysed for viable helminth eggs (viable *Ascaris* eggs), *Salmonella* spp., and faecal
193 indicators (*Enterococcus* spp. and *E. coli*). These faecal pathogen indicators were selected because of their widely
194 reported importance when assessing the microbial and public health risks associated with the re-use of various waste

195 streams such as animal manure, FS, sewage sludge, etc. as organic fertilisers (Feachem et al., 1983). Culturable
196 pathogen indicators were determined using dilution plate count method and the resulting counts were expressed as
197 Log_{10} CFU per g of sample dry weight (dwt). A well-mixed sample of about 25 g and 225 ml of peptone H_2O was
198 homogenised in a sterile stomach bag using a stomacher/ pulsifier at 12000 g for 2 minutes (Shepherd Jr et al., 2010).
199 Thereafter, ten–fold serial dilutions of the homogenate up to the 10^{-7} dilutions were prepared, and plated on selective
200 agar (see next section).

201 ***Salmonella* spp. enumeration and identification:** 0.1 mL aliquot of sample from each serial dilution was spread onto
202 sterile Xylose Lysine Deoxycholate Agar (Oxoid, UK) in triplicate (Pant and Mittal, 2006). The inoculated plates were
203 incubated at 37 °C for 24 hours. After incubation, all colonies that were red, medium sized (2-3 mm diameter) with a
204 wholly black centre were counted and suspected to be of *Salmonella* spp. Further examination using biochemical
205 reaction tests (TSI slant, urease, citrate, Lysine decarboxylase broth) was conducted according to APHA-AWWA-
206 WEF. (2005) for the confirmation of *Salmonella spp*. colonies. All the urease negative and citrate positive as well as an
207 alkaline slant and acid butt with the Triple Sugar Iron slant test confirmed presence of *Salmonella* spp.

208 Compost samples, for which *Salmonella spp*. was not detectable, were pre-enriched, with the aim of confirming the
209 complete inactivation of this pathogen during composting. After homogenising of the sample using a stomacher/
210 pulsifier, the homogenate was incubated at 37 °C overnight, to pre-enrich the sample. 1 ml of the enriched homogenate
211 was then transferred into 9 ml Rappaport–Vassiliadis enrichment broth (Oxoid, UK) (Vassiliadis et al., 1984) in
212 triplicates, and then incubated at 42 °C for 24 hours. Using a sterile cotton swab and wire loop, a loopful of each tube
213 was inoculated and streaked in triplicates on plates of Xylose Lysine Deoxycholate Agar (Oxoid, UK). The inoculated
214 plates were then incubated at 37 °C for 24 hours. After 24 hours of incubation, all suspected *Salmonella spp.* colonies
215 that were black-centred red and medium sized were enumerated. Further examination using biochemical reaction tests
216 were conducted for the confirmation of Salmonella spp. colonies. However, based on the results, no suspected colony
217 confirmed *Salmonella spp,* which implies that it was completely absent or inactivated from the composting piles by this
218 composting period.

219 ***E. coli* enumeration and identification:** 0.1 mL aliquot of the sample from each serial dilution was spread onto the
220 sterile *E.coli*-Coliforms Chromogenic Agar (Oxoid, UK) in triplicate. The inoculated plates were incubated at 37 °C for
221 24 hours. After incubation, blue or blue-purple colonies were counted as *E. coli.* Isolates suspected of being *E.coli* were
222 confirmed with IMViC patterns of biochemical tests according to APHA-AWWA-WEF. (2005). All colonies that were

223 Indole positive, Methyl red positive, Voges-Proskauer (VP) negative as well as citrate utilisation negative were
224 confirmed as *E. coli* isolates.

225 ***Enterococcus* spp. enumeration and identification:** 0.1 mL aliquot of the sample from each serial dilution was spread
226 onto the sterile Bile Esculin Azide Agar (Merck, Germany) in triplicates and then incubated at 37 °C for 48 hours.
227 After incubation, all grey-greenish colonies with a black halo around them were considered and counted as
228 *Enterococcus* spp. Isolates suspected of being *Enterococcus* spp. were confirmed with aid of the gram stain and Esculin
229 hydrolysis test (BEA agar slants) according to APHA-AWWA-WEF. (2005). All the positive BEA agar slants test that
230 showed a colour change to dark brown confirmed *Enterococcus* spp.

231 **Viable *Ascaris* eggs analysis:** Viable *Ascaris* eggs concentrations were analysed according to USEPA (2003)
232 techniques. The method is based on a fundamental principle of recovering helminth eggs from compost or dewatered
233 FS by floating them from other debris using $ZnSO_4$ (with a comparatively relative density of 1.2 – 1.3) in a supernatant
234 obtained by centrifugation. Detailed procedure for viable *Ascaris* eggs analysis used in this study is presented in our
235 previous work (Manga et al 2019). The average counted viable eggs were then expressed as *Ascaris* eggs/ g dry weight.
236 For better comparison between composting piles, these values were further expressed as the percentage of initial viable
237 eggs count.

238 **Statistical analysis**

239 Laboratory results were reported as mean values or ± standard error of duplicates, and subjected to statistical analysis
240 using IBM SPSS 21.0 software. Data was analysed using non-parametric Friedman test. The significance of differences
241 amongst the mean values was tested at a level of $p = 0.05$, with 95% confidence level. Spearman's rho test was also
242 used for examining the correlation coefficient between parameters based on a > 95% confidence level. $p \leq 0.05$ was set
243 as the statistical significance criterion. Standard multiple regression analysis was conducted according to Pallant
244 (2013), to determine the most important factors responsible for viable *Ascaris* eggs inactivation during composting.

245 **Results and discussion**

246 **Characterisation of raw materials**

247 *Salmonella* ssp., *E. coli, and Ascaris* eggs were not detected in all the three bulking agents (sawdust, coffee husks and
248 brewery waste) used in this study. This implies that bulking agents used were generally free from faecal contamination.
249 *Enterococci* spp. was detected in brewery waste samples. This faecal contamination may have occurred at the open

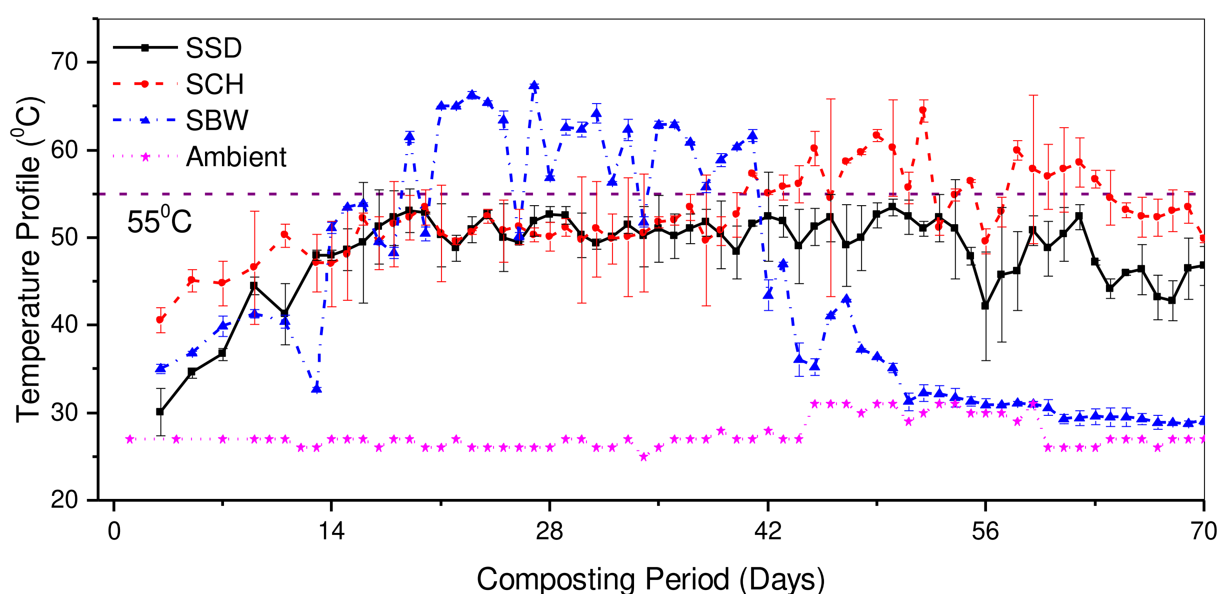
250 dump site where such waste was stored before being transferred to the composting facility, since these storage sites are
251 easily accessible to dogs, pigs and other animals. The average *Salmonella.* ssp. *Enterococci* spp. and *E. coli.* content
252 found in dewatered FS was 7.3, 6.6 and 7.9 log_{10} CFU/g dwt, respectively, whilst viable *Ascaris* eggs count was 37 ±
253 16 eggs/g dry weight; our results were in line with data reported in the literature (Evans et al., 2015; Koné et al., 2007).
254 However, these concentrations are 10 - 100 times higher than those found in sewage sludge (Strauss et al., 1997). This
255 result is not surprising especially in developing countries where these faecal pathogens are rampant (Barda et al., 2014;
256 Yajima and Koottatep, 2010). Detailed work on characterisation of raw FS from VIP latrines and septic tanks as well as
257 FS dewatering is presented in our previous work (Manga, 2017; Manga et al., 2016). Further, the moisture content
258 found in samples of sawdust (31.20% ± 5.94), coffee husks (11.40% ± 2.40), brewery waste (67.55% ± 8.14) and
259 dewatered FS (68.71% ± 3.80) compared well with 13.71% - 64.2% (sawdust), 13% (coffee husks), 51.4% (brewery
260 waste), and 70% (dewatered FS) reported previously (Evans et al., 2015; Leconte et al., 2009; Sánchez-Monedero et al.,
261 2001; Shemekite et al., 2014). Other physical and chemical characteristics of the dewatered FS and bulking agents used
262 in this study are presented in Table S1, and discussed in our other work (Manga et al., 2016; Manga et al., n.d.).

263 **Evolution of composting temperature**

264 Figure 1 presents the evolution of temperature in the composting piles of the three bulking agents. The SCH and SBW,
265 piles attained average temperatures of > 55 °C within a composting period of 41 days and 16 days, respectively.
266 Although in some sections of the SCH and SBW piles (middle section), reached temperatures > 55 °C within 5 days
267 and 11 days of pile formation, with the maximum temperature of 72 °C and 70.2 °C attained within 52 days and 27
268 days, respectively. These SCH and SBW piles sustained the optimum temperatures (> 55 °C) for effective pathogen
269 inactivation for a period of 8 and 4 weeks respectively, before they dropped to 50 °C. In contrast, the SSD composting
270 piles did not attain average temperatures of > 55 °C. However, in some sections of the SSD composting piles,
271 temperatures > 55 °C were attained within a composting period of 16 days, and these were sustained for a period of
272 approximately 6 weeks. This finding reveals that the different bulking agents have a statistically significant effect (p =
273 0.0001) on the evolution of composting temperature during FS composting.

274 All the composting piles were capable of attaining composting temperatures within the recommended range (55 – 65
275 °C) for effective pathogen inactivation and organic waste decomposition. The SCH and SBW composting piles attained
276 temperatures of ≥ 55 °C within a shorter composting period of 5 and 10 days, respectively (Figure 1). This implies that
277 conditions favourable for quick growth and biological activities of microbes' formation existed within the composting
278 piles. However, the temperatures in the SBW piles were considerably lower at the beginning of the composting process

279 (for about 10 days). This delayed rise in the composting temperatures might have been due to the limited supply of
280 carbon sources resulting from the low C/N ratio (of 17.2) recorded in the starting SBW feedstock (Manga, 2017;
281 Manga et al., n.d.). The limited supply of carbon sources may have limited microbial activities at the beginning of the
282 composting process, and thus low temperatures. In contrast, SSD piles required considerably longer composting
283 periods to attain the same temperatures (> 55 °C) within some pile sections. This could have been due to greater heat
284 losses from the composting piles to the environment because of the high porosity nature of SSD feedstock, which may
285 have allowed for convective air flow through the composting piles. A similar effect was reported by Michel et al.
286 (2004) during the composting of dairy manure with straw.



287

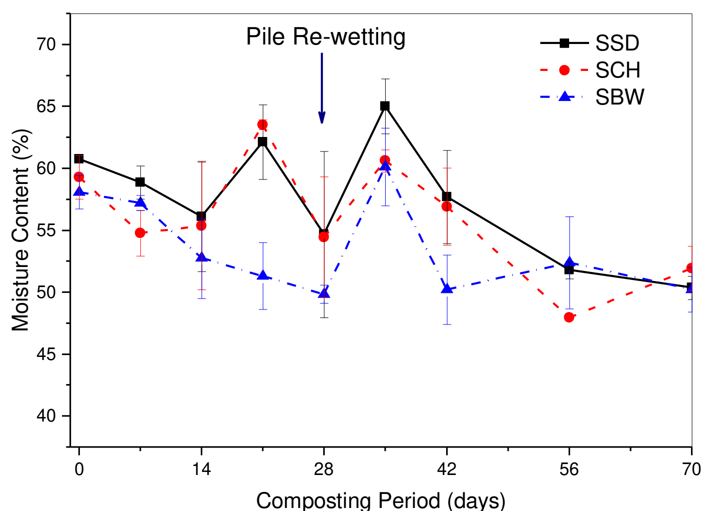
288 **Figure 1:** Temperature evolution during co-composting of Faecal sludge with (A) Sawdust – SSD; (B) Coffee husks –
289 SCH; (C) Brewery waste – SBW. Error bars represent the standard deviation of average bottom, center, left
290 side and right side pile temperature of the duplicated composting piles of each of the three bulking agents.
291

292 Figure 1 shows that SBW composting piles exhibited shorter composting periods than SSD and SCH piles, as their
293 composting temperatures dropped rapidly reaching the ambient temperature at 52 days. This may have been due to
294 greater heat losses from the porous SBW composting material. On the other hand, it can partly be attributed to the
295 reduced supply of easily biodegradable carbon source in the initial SBW feedstock, which may have been consumed by
296 microbial activities during the early intensive decomposition phase. The temperature profiles observed in the present
297 investigation were similar to those observed in other works (Alburquerque et al., 2006). It is important to note that all
298 the composting piles heated up fairly slowly. This may be attributed to their relatively small size (3.0 m^3), which may
299 have contributed to the heat losses during the early composting stages.

300 **Moisture content evolution**

301 Figure 2 illustrates the changes in the moisture content during the composting of FS with different bulking agents. The
302 initial average moisture content of SSD, SCH and SBW composting piles of 60.7%, 59.2%, and 58.1% gradually
303 decreased as the composting process progressed to a final moisture content of 54.8%, 45.8%, and 44.1%, respectively
304 at the end of the 15 weeks composting period. It is important to note that SSD and SCH piles exhibited 8.0% and 8.2%
305 respectively, increase in the moisture content between week 2 – 3. This might have been due to the heavy rainfall that
306 hit the piles from the sides during unusual heavy storms. The moisture content dynamics observed in this study are
307 similar to those documented by Changa et al. (2003) during the composting of dairy manure with sawdust.

308 All composting piles exhibited a high moisture content reduction during the early composting periods. This might be
309 attributed to the high composting temperature (>55 °C) reached by the piles, which may have enhanced moisture loss in
310 the form of water vapour from the piles to the atmosphere especially during the thermophilic phase. Further, moisture
311 content reduction observed especially during the mesophilic phase may be attributed to the porosity of the composting
312 piles, which may have facilitated moisture content losses by means of air drying or convective air flow through the
313 porous structure. In Figure 2, it can be observed that SBW piles exhibited the highest moisture content reduction,
314 especially during the thermophilic phase. It was observed that shortly after the formation of SBW piles, leachate started
315 draining from the bottom of piles. This implied that the composting feedstock had low water holding capacity for
316 retaining the moisture produced during the active decomposition process.



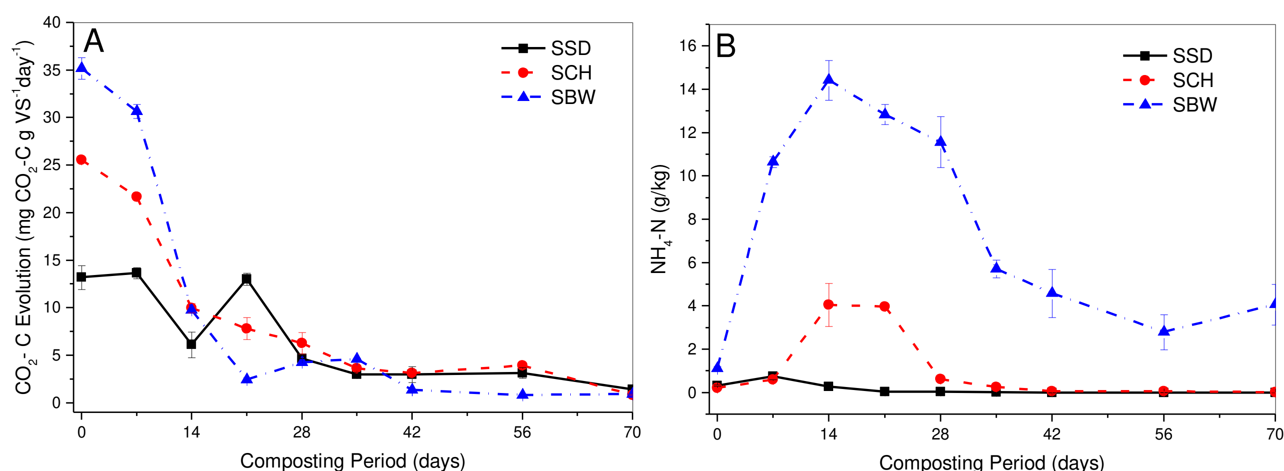
317

318 **Figure 2:** Evolution of Moisture Content (MC) during the co-composting of Faecal Sludge with (A) Sawdust - SSD;
319 (B) Coffee husks - SCH; (C) Brewery waste – SBW. Error bars represent the standard error for the duplicated
320 piles.

321 **Evolution of carbon dioxide (CO_2-C)**

322 The CO_2-C evolution dynamics of SBW, SSD, and SCH piles reduced from the original average values of 35.2, 25.6
323 and 13.2 to 0.9, 1.4 and 0.8 mg CO_2-C $gVS^{-1}day^{-1}$ respectively, within a composting period of 10 weeks. This revealed
324 substantial variations in the average respiration rate of the composting piles over the entire composting process (Figure
325 3 A).

326 In Figure 3, it can be seen that the CO_2-C concentration decreased drastically in all the composting piles during the first
327 two weeks of composting, and thereafter steadily decreased reaching stable values within 8 - 10 weeks composting
328 period. This response can presumably be explained by the decrease in metabolic activities linked to the rapid
329 decomposition and depletion of easily biodegradable carbon sources. In the same vein, all composting piles exhibited
330 fluctuations in the CO_2-C respiration rate, especially during the early stages of composting (Figure 3 A). This perhaps
331 can be explained by the heterogeneity of the starting feedstock, where it was quite hard to select representative sample
332 from the starting feedstock (of only 10 g) for respiration analysis. However, the variation in the CO_2-C respiration rate
333 reduced as the composting process progressed after the composting material had gradually lost its physical structure,
334 and thus turned more homogeneous. Detailed discussion on CO_2-C evolution results observed in these composting piles
335 is being presented in our work elsewhere (Manga et al., n.d.). The CO_2-C dynamics observed in the present study
336 compare well with those found by Wang et al. (2004) during the composting of dairy and pig manure.



337

338 **Figure 3:** Changes in (A) Carbon dioxide respiration rate (mg CO_2-C g $VS^{-1}day^{-1}$) and (B) Ammonium-nitrogen (NH_4-
339 N) during the composting of FS with (A) Sawdust in a ratio of 1:2 – SSD; (B) Coffee husks in a ratio of 1:2 –
340 SCH; (C) Brewery waste in a ratio of 1:2 – SBW. Error bars represent the standard error for the duplicated piles.

341

342

343 **Ammonium-nitrogen (NH_4^+-N) evolution**

344 As shown in Figure 3 B, the average NH_4^+-N concentrations of SCH, SBW and SSD composting piles increased
345 rapidly during the early composing periods, reaching peak values of 4.04 g/kg, 14.42 g/kg, and 0.74 g/kg respectively,
346 within 1 to 2 weeks of composting; and these then decreased to mean low values of 0.018, 4.071 and 0.003 g/kg,
347 respectively after 10 weeks composting period.

348 Several composting studies have documented the increase in ammonium-nitrogen (NH_4^+-N) concentration to occur
349 during the early stages of composting (Wong et al., 2001), and this has similarly been observed in the present study.
350 This rise in NH_4^+-N content has been mainly attributed to the degradation of organic N via ammonification, especially
351 during active composting stages, which are characterised by intensive microbial activities linked to rapid organic matter
352 degradation. All piles exhibited a gradual decrease in the NH_4^+-N concentrations as the decomposition process slowed
353 down (Figure 3 B). This decrease can be explained by the high rates of NH_4^+-N losses via volatilization and
354 nitrification process (NO_3^--N), which were greater than ammonification rates. Similar behaviour has also been observed
355 by several authors during composting (Hao et al., 2004). Detailed discussion on NH_4^+-N evolution results observed in
356 these composting piles is being presented in our work elsewhere (Manga et al., n.d.). The NH_4^+-N evolution trend and
357 final concentrations observed in this study are in line with those published by other authors (Cayuela et al., 2012).

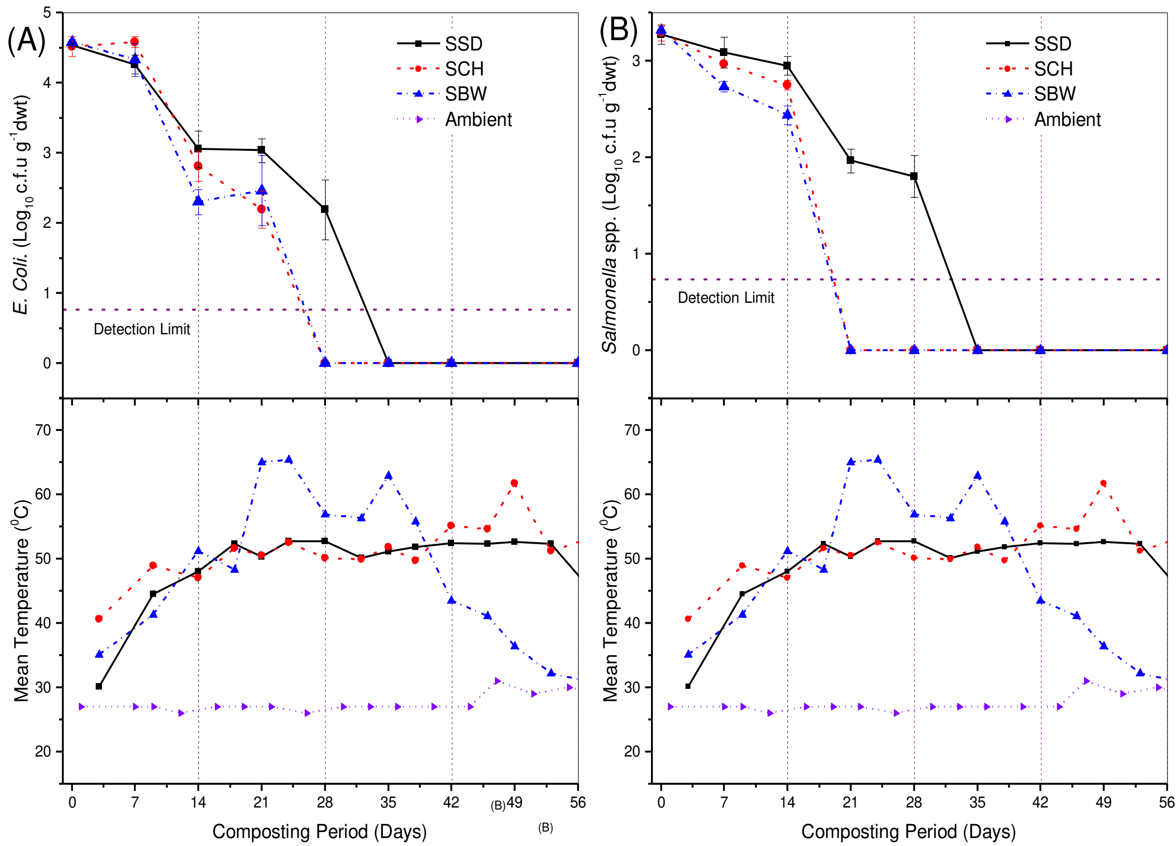
358 ***Escherichia coli (E. coli)***

359 All the three bulking agent compost types recorded similar populations of *E.coli* in the starting feedstock, in the range
360 of 4.53 to 4.58 $\log_{10}$ CFU/g dwt. (Figure 4, A - Top). Within the first week of composting, SSD and SBW piles
361 showed a very slow decline in the *E. coli* population; and this was followed thereafter by a significant decrease,
362 reaching 3.05 and 2.30 $\log_{10}$ CFU/g dwt., respectively by the 14th day of composting. This slow decrease in *E. coli*
363 content exhibited during the first week synchronized with the low temperatures recorded during this composting period,
364 which was in the range of 30.1 to 36.7 °C and 35.1 to 39.9 °C, respectively (Figure 1 and Figure 4, A- Bottom). In the
365 third week, SBW piles showed a slight increase in the *E. coli* population from 2.30 to 2.46 $\log_{10}$ CFU/g dwt. during the
366 thermophilic phase; and this was followed by a rapid decline, reaching undetectable limits after a composting period of
367 approximately 4 weeks. However, SSD piles exhibited a slowed decrease in *E. coli* population during the third week of
368 composting, and this was similarly followed by a rapid decline to undetectable limits by the 5th week of composting.

369 In contrast, the composting temperatures of SCH piles (40.6 - 44.8 °C) were slightly higher than those of SSD piles
370 (30.1 - 36.7 °C) and SBW piles (35.1 - 39.9 °C) during the first week of composting (Figure 1 -A); SCH piles,
371 exhibited a slight increase in the *E. coli* population during the first week from 4.52 to 4.58 $\log_{10}$ CFU/g dwt. (Figure 4

372 A - Top). Hess et al. (2004) similarly observed an increase in *E.coli* content in a laboratory-scale bioreactor with
373 temperature ≥ 50 °C. This response might have been due to the uneven distribution of high temperatures within the
374 composting piles. It may also have been due to the recontamination phenomena of sanitised compost with the
375 pathogens that survived in the cooler zones especially the outer and bottom layers, which recorded the lowest
376 temperatures. Similar evidence was found by other researchers during windrow or pile composting experiments
377 (Chroni et al., 2009). However, the SCH piles exhibited a rapid decrease in the *E. coli* content thereafter, reaching
378 undetectable levels by the 4th week of composting.

379 Further, as can be seen in Figure 4-A, SCH and SBW piles recorded shorter *E. coli* survival periods than SSD piles.
380 This can probably be attributed to the high elevated temperatures (> 55 °C) reached and sustained for an extended
381 period in these piles during those composting periods (Figure 1). This finding is in line with previous studies finding
382 high temperatures responsible for the rapid inactivation of *E.coli* in composting piles (Christensen et al., 2002).
383 Interestingly, SSD piles exhibited a decrease in the inactivation rate of *E. coli* between 14 - 21 days (Figure 4). This
384 behaviour was also observed by Chroni et al. (2009) during the composting of source-separated bio-waste. This could
385 be due to the sub-lethal temperatures especially in the outer layers, which may have induced *E.coli* microorganisms to
386 produce heat shock proteins, so as to increase their protection against thermal destruction during composting (Shepherd
387 Jr et al., 2010). *E.coli* evolution and inactivation periods attained in the present study are in line with those published by
388 previous researchers (Shepherd Jr et al., 2010). Overall, this study results reveal that the different bulking agents had
389 no statistically significant effect (P = 0.186) on the *E. coli* inactivation during FS composting.



390

391 **Figure 4:** Mean composting temperatures (bottom) and changes in (A) *E.coli* population (top) and (B) *Salmonella* spp.
392 population (top), during the composting of FS with Sawdust - (SSD); Coffee husks - (SCH); Brewery waste –
393 (SBW). Error bars represent the standard error for the duplicated piles.
394

395 ***Salmonella* spp.**

396 Figure 4 (B) illustrates the evolution of *Salmonella* spp. during the composting of FS with different bulking agents. The
397 *Salmonella* spp. content ranged from 3.27 to 3.32 log_{10} CFU/g dwt. in the initial feedstock of the three bulking agent
398 compost types. In the SBW and SCH composting piles, the population decreased from 3.29 to 2.75 log_{10} CFU/g dwt.
399 and 3.32 to 2.44 log_{10} CFU/g dwt. respectively, within the first two weeks; and it was undetectable by the end of the
400 third week of composting. However, during the composting of SSD piles, *Salmonella* spp. content decreased from 3.27
401 to 1.80 log_{10} CFU/g dwt. by the fourth week, before it reached undetectable levels by the fifth week of composting.
402 SCH and SBW piles recorded shorter *Salmonella* spp. survival periods than SSD piles where it survived for two weeks
403 longer. In the present study, statistical test results revealed that the different bulking agents had a significant effect (p =
404 0.008) on the inactivation efficiency of *Salmonella* spp., during FS composting.

405 A significant decline in *Salmonella* spp. content was observed in SCH and SBW composting material within 7 days of
406 pile formation (Figure 4, B) where composting temperatures were still quite low in the range of 30.1 - 39.9 °C (Figure

407 1). This result is not surprising, since *Salmonella* is well known for being less resistant to high temperatures and harsh
408 environmental conditions than other microorganisms (Déportes et al., 1998). Previous researchers have also found
409 *Salmonella* to have been eliminated from composting piles at low temperatures (> 45 °C) (Lung et al., 2001). Similarly,
410 Déportes et al. (1998) observed *Salmonella* to have survived for only less than 21 days during the composting of
411 municipal solid waste compost at temperatures less than 40 °C. However, Droffner and Brinton (1995) found some
412 *Salmonella* strain (serotype *Typhimurium Q*) to have survived the composting process longer (ca. 59 days) at high
413 temperatures (60 °C) during the composting of separated household waste at industrial scale. This discrepancy in
414 results suggests that the elimination of *Salmonella* during composting is dependent on the characteristics of the starting
415 feedstock, composting process and *Salmonella* strain monitored. It can also be attributed to the analytical and detection
416 method used for monitoring the pathogenic microorganisms. For example, Droffner and Brinton (1995) used DNA
417 gene probes for monitoring these pathogens rather than the culturing-based analytical methods of viable organisms
418 used in this study and several other studies, which may have accounted for prolonged detection of these pathogens. The
419 former may have detected naked DNA or dead cells while the later may have underestimated stressed microorganisms,
420 thus the variation in results.

421 Generally, *Salmonella* spp. was completely inactivated in all piles after a short composting period of 3 - 5 weeks, and
422 this coincided with the high temperatures recorded during these periods, clearly illustrating the importance of high
423 temperature in inactivation of such pathogens in the present study. Similar response was observed by Hassen et al.
424 (2001) who found *Salmonella* inactivation from municipal solid waste piles by the 25th day, once the composting
425 temperatures had reached 60 °C. In Figure 4 (B), SCH and SBW piles exhibited a rapid die-off of *Salmonella* spp.
426 content between 2 – 3 weeks composting period, and this may be attributed to the higher temperatures observed in such
427 piles. However, it is important to note that the data presented in Figure 4-(B) is the average temperature calculated from
428 all the measurement points. Therefore, this may not clearly show the higher temperatures recorded in the different piles
429 at specific locations, and therefore, may not fully account for the observed die-off. On the other hand, Figure 1, shows
430 the temperature data measured at the individual locations within the piles, and this may accurately account for the
431 observed die-off since high temperatures were observed in the composting piles at specific locations.

432 SSD piles exhibited a reduction in *Salmonella* spp. inactivation rate during the fourth week of composting (Figure 4-
433 B). This odd response can be explained by metabolic adaption of surviving *Salmonellae* strains to the harsh conditions
434 responsible for their inactivation (Erickson et al., 2009). Our results especially at low temperatures suggest that not
435 only time-temperature relationship was the major factor responsible for *Salmonella* spp. inactivation but also

436 indigenous microbial activities may have partly been responsible. This suggestion was confirmed by a significant
437 positive correlation (SSD (p = 0.0001, r = 0.859), SCH (p = 0.0001, r = 0.785), SBW (p = 0.0001, r = 0.783)) observed
438 between *Salmonella* inactivation rate and microbial activities monitored by CO_2-C respiration rate. Similar behaviour
439 was observed by Sidhu et al. (2001) who found indigenous microorganisms responsible for the suppression of
440 *Salmonella* regrowth in non-sterilized compost.

441 ***Enterococcus* spp.**

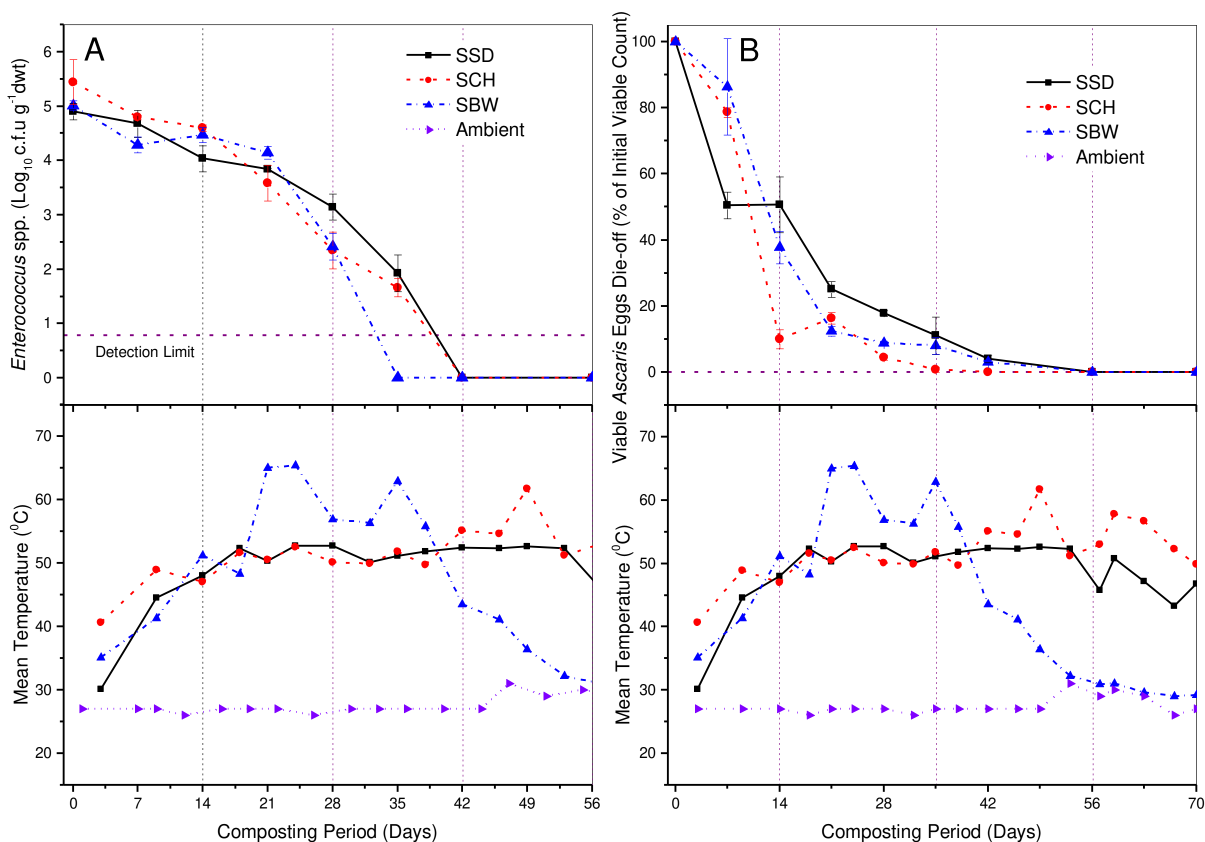
442 In the present study, the *Enterococcus* spp. pathogens proved more resistant compared to *E. coli* and *Salmonella* spp.
443 during the thermophilic composting phase. The SBW, SSD and SCH piles initially exhibited a slow decrease in the
444 *Enterococcus* spp. populations from 5.01 to 4.14 log_{10} CFU/g dwt, 4.90 to 3.84 log_{10} CFU/g dwt. and 5.44 to 3.58 log_{10}
445 CFU/g dwt. within a composting period of 21 days; thus representing log reduction of 0.87, 1.06 and 1.86, respectively
446 (Figure 5, A). This was then followed by a more pronounced decrease reaching undetectable levels in SBW piles by the
447 35th day of composting, whereas in the SSD and SCH piles, *Enterococcus* spp. survived up to day 42. Interestingly, the
448 one-way repeated-measure ANOVA test results showed that the different bulking agents did not have a statistically
449 significant effect (p = 0.758) onto the inactivation efficiency of *Enterococcus* spp. during FS composting.

450 In the present study, *Enterococcus* spp. population reduced drastically to undetectable levels during thermophilic phase
451 composting (Figure 5, A). However, the inactivation rate of *Enterococcus* spp. was relatively low compared to *E. coli*
452 and *Salmonella* spp. in all the composting piles. This is because such microorganisms are knowingly more resistant to
453 die-off, and can easily withstand the elevated temperatures within composting piles for an extended period of time. The
454 comparison of survival of *E. coli* and *Salmonella* spp. with *Enterococcus* spp., revealed that the latter survived about 7
455 - 21 days longer than the former in the composting pile, which confirms that such pathogens are more resistant to die-
456 off during composting (Figure 4 and Figure 5). This confirms a study by Pereira-Neto et al. (1986) who found
457 *Enterococcus* (faecal streptococci) to survive about 16 days longer than *E.coli* and *Salmonella* during the composting
458 of sewage sludge with refuse.

459 As shown in Figure 5 (A), the evolution of *Enterococcus* spp. was similar in the three bulking agent composting piles,
460 with a slow decrease during the initial three weeks of composting, followed by a rapid decrease in the succeeding
461 weeks. This behaviour may be attributed to the composting temperatures evolution exhibited by the composting piles
462 (Figure 1 and Figure 5-B). The rapid decrease in the *Enterococcus* spp. population was observed to synchronize with
463 the high temperatures recorded for an extended time in these piles and vice versa was true for low temperatures. This
464 clearly confirms the effect of high time-temperature factor on the survival of these microorganisms in piles during

465 composting. In this study, SBW piles recorded the shortest *Enterococcus* spp. inactivation period of five weeks
466 compared to SSD and SCH piles which recorded six weeks (Figure 5 -A). This might have been still due to the higher
467 temperatures observed in SBW piles, which may have been responsible for the rapid inactivation of such pathogens.
468 This finding corresponds to Pereira-Neto et al. (1986) and Vuorinen and Saharinen (1997).

469 Surprisingly, a secondary increase in *Enterococcus* spp. population was observed in the SBW piles during the early
470 stages (between the 7th and 14th days) of composting (Figure 5 A). This may be explained by the regrowth of
471 *Enterococcus* spp. linked to the presences of cool zones or pockets within the composting piles, which may have
472 contained conducive environment (such as low temperatures and high moisture) that support their regrowth in
473 composting piles. Such cool zones may have existed in the bottom sections of the composting piles where
474 comparatively low temperatures and relatively high moisture content (from leachate draining from the composting
475 material) were observed. This supports Soares and Cardenas (1995) who found a positive correlation between pathogen
476 regrowth and moisture content as well as re-moisturisation.



477

478 **Figure 5:** Mean composting temperatures (bottom) and changes in (A) *Enterococcus* spp. population (top) and (B)
479 *Viable Ascaris eggs* Inactivation efficiency (top), during the composting of FS with Sawdust - (SSD); Coffee
480 husks - (SCH); Brewery waste – (SBW). Error bars represent the standard error for the duplicated piles.

481 **Viable helminth eggs (Viable *Ascaris* eggs)**

482 Most studies on composting of human waste have focused more on monitoring the survival of *E. coli*, *Salmonella* and
483 *Enterococcus* spp. rather than viable helminth eggs. However, helminth infections are endemic in urban Africa, and
484 understanding the performance of the proposed composting approach in terms of helminth inactivation, is essential.
485 Figure 5 (B) shows the evolution of viable *Ascaris* eggs and the mean composting temperatures in the three bulking
486 agent compost types; and this illustrates that the pathogen die-off rate of the three composting piles differed
487 significantly depending on the bulking agent type, especially-during the early composting periods (3 weeks). In SSD,
488 SBW and SCH piles, the initial viable *Ascaris* eggs content reduced by approximately 49.5%, 21.34% and 13.65%
489 respectively, during the first week of composting. This was followed by 50.5%, 78.7%, and 83.3% reduction during the
490 thermophilic phase on the 8th, 5th and 6th weeks of composting, respectively. The statistical test results revealed that the
491 bulking agent type had a significant effect ($p = 0.001$) on the survival of viable *Ascaris* eggs during FS composting.

492 It is important to note that all the composting piles exhibited a considerable decrease in the viable *Ascaris* eggs content
493 during the first week of composting; where mesophilic conditions with relatively low composting temperatures (30.1 –
494 45.0 °C) were observed within the composting piles (Figure 5 B). This response was also reported by Gallizzi (2003)
495 who observed a reduction in the *Ascaris* eggs of about 40% - 80% within a composting period of less than seven days.
496 Further, during the thermophilic phase, a more pronounced reduction in the viable *Ascaris* eggs content was observed,
497 with SSD and SCH piles exhibiting complete inactivation by the end of the thermophilic phase. This significant
498 reduction in viable *Ascaris* eggs content can mainly be linked to the effects of elevated temperatures reached for an
499 extended period of time in these piles during the thermophilic phase. This result is in agreement with previous research
500 revealing high time-temperature as a major factor responsible for the rapid inactivation of *Ascaris* eggs during
501 thermophilic composting (Koné et al., 2004).

502 Surprisingly, by the end of the thermophilic phase, SBW piles had only attained about 96.9% total viable *Ascaris* eggs
503 inactivation, and this improved to 100% during the maturation, after 56 days composting period (Figure 5 B). This
504 further inactivation attained during maturation phase could be attributed to the positive consequences of damages
505 triggered previously by higher composting temperatures during the thermophilic phase (Feachem et al., 1983).
506 Alternatively, this further inactivation may have been due to high NH4+-N concentrations observed in such piles. This
507 hypothesis was emphasised by the Spearman's rho test ($p \leq 0.05$) results, which showed a meaningful positive
508 correlation between viable *Ascaris* eggs inactivation rate and the evolution of NH4+-N concentration (Figure 3 B)
509 during the composting of SBW piles ($p = 0.004$, r = 0.586). This result is also in agreement with Reimers et al. (1998)

510 and Manga et al. (2019), showing that high NH_3-N concentrations were significantly responsible for the effective
511 inactivation of *Ascaris* eggs. The results also compare well with those published by Scott (1952) during thermophilic
512 composting (at > 60 °C) of night soils with sawdust in stack using a turning frequency of 5-10 day, where 95% *Ascaris*
513 eggs inactivation was attained after three weeks and 100% reached after seven weeks composting period.

514 SCH piles attained complete viable *Ascaris* eggs inactivation after a short composting period of six weeks, while in the
515 SSD and SBW piles; they survived up to the 8th week. This might have been due to the higher temperatures attained
516 and sustained for an extended period within these composting piles, since time-temperature relationship is a vital factor
517 in destruction of such pathogens (USEPA, 2003). Interestingly, SSD and SCH piles exhibited a slight increase in viable
518 *Ascaris* eggs during the 2nd and 3rd week of composting, respectively (Figure 5 B). This may be attributed to analytical
519 problems, which may have been accelerated by the high heterogeneity nature of the composting materials, since
520 *Ascaris* eggs can only reproduce inside their hosts but not outside. This effect has also been observed by other
521 researchers (Koné et al., 2004).

522 **Implication of the study finding**

523 Composting of FS with different bulking agents can produce pathogen free first class compost after 6 - 8 weeks
524 composting periods, depending on the bulking agent type used. The study findings suggest coffee husks as an excellent
525 bulking agent for sanitizing FS in the tropical climate - as FS composting piles containing coffee husks exhibited higher
526 pathogen inactivation efficiency, and generated pathogen free compost in about 6 weeks. This is a significant finding
527 especially for communities in urban Africa where FS and coffee husks wastes are generated in large quantities - as this
528 may be a technically feasible way of promoting decentralised FS treatment and sustainable reuse of FS in urban Africa.

529 The continued monitoring of *E. coli, Salmonella* spp. and *Enterococcus* spp. for more 7 weeks during the maturation
530 phase, showed that there was no regrowth of these micro-organisms during maturation, which confirmed their complete
531 inactivation during composting. Considering the survival of all monitored pathogenic microorganisms, it can be noted
532 that *Ascaris* eggs survived for about 7 - 35 days longer than all other monitored pathogens during composting. This
533 confirms that *Ascaris* eggs are more resistant to die-off, and therefore, can be used as pathogen indicators for
534 monitoring the sanitisation of the composting feedstock containing faecal sludge. This result is consistent with the
535 literature that has reported helminth eggs especially *Ascaris* eggs to be more resistant to die-off than other pathogens
536 during composting (Wichuk and McCartney, 2007). However, in cases where the starting feedstock does not contain
537 viable *Ascaris* eggs, *Enterococcus* spp. can then be used instead of the former, since the latter proved to be
538 comparatively resistant to thermal destruction during composting.

539 The time-temperature relationship has been well documented in the literature as a major factor responsible for thermal
540 destruction of pathogens during composting. Day and Shaw (2001) reported thermal destruction of all pathogens
541 including the most resistant *Ascaris* eggs to occur when exposed to temperatures of 55 °C for 60 minutes. However, in
542 the present study, the temperature and time exposure at which their destruction occurred (ca. 56 days with temperatures
543 >55 °C maintained in some sections of the piles for about 14 - 56 days) was relatively longer than those documented in
544 the literature. It is possible that the time-temperature requirement for pathogen inactivation in composting piles at pilot
545 and full-scale trials might differ from that performed in the laboratory-scale trials, where the pathogen die-off
546 experiments are conducted in a well-controlled environment with a uniform distribution of temperatures in the
547 composting material. Moreover, in laboratory scale trials, pathogen die-off is monitored by exposing the compost
548 samples to different time-temperature relationship inside the incubators.

549 Figure 5 (B) illustrates a virtual impression of the correlation between viable *Ascaris* eggs (selected pathogen indicator)
550 die-off and the mean temperature evolution of the three bulking agent composting piles. It can be noted that significant
551 discrepancies existed in the response of the viable *Ascaris* eggs decay rate and the mean composting temperature
552 evolution/ thermophilic conditions. This implies that, besides time-temperature relationship, other factors may equally
553 have been responsible for the inactivation of these pathogens during composting. In this study, three parameters (i.e.
554 time-temperature factor; indigenous microbial activities; toxic by-products (such as NH_4^+-N) produced during
555 composting) were found to have equally played a significant role in the inactivation of viable *Ascaris* eggs during FS
556 composting (See Table S2). This phenomenon has been observed before (Manga et al., 2019). Based on a multiple
557 regression analysis, the effect of each mechanism on viable *Ascaris* eggs inactivation during composting was assessed
558 (see Table S3). Interestingly, the regression results revealed indigenous microbial activities (measured by CO_2-C
559 respiration rate) that may have induced lethal conditions as one of the most important factors that were responsible for
560 the inactivation of viable *Ascaris* eggs during composting (with $Beta$ = -0.566, p = 0.0001, and R^2 = 0.151). This was
561 followed by the time-temperature relationship (with $Beta$ = -0.348, p = 0.007, and R^2 = 0.031). This finding is of
562 remarkable significance as it reinforces the hypotheses that have been continuously reported in literature reviews
563 (Stentiford and de Bertoldi, 2010) without confirmatory or substantial robust evidence from real field composting trials
564 that apart from time-temperature factor, other factors are equally responsible for pathogen die-off during composting.

565 Although, other mechanisms have played an equally significant role in pathogen inactivation, time-temperature
566 mechanism remains as the primary factor responsible for pathogen inactivation during composting. Therefore, this
567 study results suggest that composting of FS with a suitable bulking agent for a period of eight weeks with temperatures

568 of around 48 – 60 °C sustained in the composting piles for more than 38 days, using 5 - 7 days turning frequency
569 especially during the thermophilic phase, is sufficient to ensure effective thermal destruction of pathogens in faecal
570 sludge. The time-temperature criteria attained in the present study is a significant finding for updating the existing
571 literature on time-temperature requirement for pathogen destruction, and it can be used by practitioners in the tropical
572 climate as an appropriate policy guideline for monitoring FS composting facilities to generate pathogen free compost
573 for use in agriculture. Importantly, complete inactivation of the pathogens in the composting piles will additionally
574 depend on exposing all the composting material to such temperatures for the recommended time, which implies that
575 thorough mixing or sufficient turning of the piles is a very important factor for proper sanitisation of faecal sludge
576 before re-use in agriculture.

577 **Conclusion**

578 This study aimed at investigating the fate of faecal pathogen indicators during faecal sludge composting with different
579 bulking agents in tropical climate. Based on the study findings, the following conclusions can be drawn:

580 - Regardless of the type of bulking agent composted with FS, all composting piles attained and sustained
581 optimum temperatures (> 55 °C) and conditions suggested for effective pathogen inactivation during
582 composting.

583 - The different bulking agents had a statistically significant effect on the evolution of composting temperatures
584 and inactivation efficiency of some pathogens (*Salmonella* spp. and viable helminth eggs) during FS
585 composting.

586 - Coffee husks was found to be the most suitable bulking agent for composting with FS - as it improved the
587 pathogen inactivation efficiency, and reduced the inactivation periods from eight weeks (for sawdust and
588 brewery waste) to six weeks.

589 - The time-temperature relationship was the most important factor responsible for pathogen inactivation.
590 However, other mechanisms such as indigenous microbial activities, and toxic by-products such as $NH4^{+}$-N
591 also played an important role in the inactivation of pathogen indicators during composting.

592 - Each of the three bulking agents composting piles attained 100% faecal pathogen inactivation from FS, and
593 therefore, the compost was pathogen free. Based on faecal pathogen inactivation, the compost was safe for
594 use in agriculture, without public health or environmental risk. It is important to mention, however, that other
595 risks relating to toxicity or antibiotic presence in human excreta may still be existent.

596 • Composting of FS with coffee husks, sawdust or brewery waste for a period of eight weeks with temperatures
597 of around 48 – 60 °C sustained in the composting piles for more than 38 days, using 7 days turning frequency
598 in tropical climate, is sufficient to ensure complete sanitization of FS before reuse in agriculture.

599 • This study suggests composting of FS with coffee husks, brewery waste, or sawdust bulking agents as a
600 technically feasible method for sanitising FS under tropical climate conditions.

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817 **Supplementary Information**
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820 **Table S1:** Characteristics of raw material used‡

	Dewatered FS	*Sawdust*	*Coffee husks*	*Brewery Waste*
Moisture (%)	68.71 ± 3.80	31.20 ± 5.94	11.40 ± 2.40	67.55 ± 8.14
Ammonium-N* (mg/kg)	0.53 ± 0.39	0.00 ± 0.01	0.02 ± 0.01	0.02 ± 0.00
Escherichia coli (log_{10}/cfu/g dwt)	6.6 ± 0.1	ND	ND	ND
Salmonella spp. (log_{10}/cfu/g dwt)	7.3 ± 0.0	ND	ND	ND
Enterococci spp. (log_{10}/cfu/g dwt)	7.9 ± 0.0	ND	ND	3.5 ± 0.2
Helminth (Viable *Ascaris* Eggs) (eggs/ g)	37 ± 16	ND	ND	ND

821 ‡Mean ± Standard deviation (SD) of triplicates; * dry base; ND = no detectable

822 **Table S2:** Spearman's rho test results between viable *Ascaris* eggs survival and other mechanisms responsible for
823 pathogen die-off during FS co-composting with different bulking gents

	CO2-C evolution (n=22)		**NH_4-N (n=22)**	
	p	r	*p*	r
SSD	0.0001	-0.899	0.0001	-0.848
SCH	0.0001	-0.897	0.0001	-0.745
SBW	0.0001	-0.932	0.004	-0.586

824 * Not Significant

825 **Table S3:** Mechanisms responsible for the inactivation of selected pathogen indicator (viable helminth eggs) during FS
826 composting with different bulking agents

Pathogen Inactivation Mechanisms in the order significance effect on pathogen inactivation	**Standardized Coefficient (Beta)**	**Significant**	**R-Square Value**
Microbial activities measured as CO_2-C respiration rate	-0.566	0.0001*	0.151
Temperature	-0.348	0.007*	0.031
NH_4-N Concentrations	-0.067	0.439	0.0024
MC	-0.016	0.845	0.00014

827 * Significant ($P < 0.05$)

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