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Inactivation of viable *Ascaris* eggs during faecal sludge co-composting with chicken feathers and market waste

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ABSTRACT

Faecal Sludge (FS) contains high concentrations of pathogenic microorganisms that are 10–100 times higher than those in domestic wastewater. Proper and sustainable treatment is required to inactivate these pathogens if FS is to be recycled in agriculture, so as to minimise public health and environmental risks. Composting is one of the common low-cost technologies of sanitising FS in Urban Africa; however, it is associated with longer pathogen inactivation periods that make it commercially uneconomical. This study investigated the effect of different organic wastes types and their mixing ratios with FS on the inactivation efficiency of viable *Ascaris* eggs (*suum* and *lumbricoides*) during composting. Dewatered FS was mixed with market waste (MW), chicken feathers (CF) and sawdust (SD) in different ratios. Compost piles of FS:MW:SD and FS:CF:SD both in volumetric ratios of 1:2:1 and 1:3:1 were set-up in duplicate (3 m^3 each), composted and monitored weekly for viable *Ascaris* eggs presence for a period of 15 weeks. The results suggest that the organic waste types have a significant effect on the temperature evolution and pathogen inactivation efficiency while their mixing ratios do not. Piles containing CF achieved the shortest pathogen survival period of 4 weeks compared with 6–8 weeks for those with MW. The temperature–time factor was found to be the most important variable responsible for viable *Ascaris* eggs inactivation. However, other mechanisms such as microbial antagonism or antibiotic action induced by indigenous microorganisms and toxic by-products such as free ammonia were found to have also played an important role in *Ascaris* eggs inactivation. All piles attained 100% *Ascaris* eggs inactivation from FS, and therefore, the compost was safe for use in agriculture. The study findings suggest that composting of FS with CF can reduce *Ascaris* eggs inactivation periods by 42%, which may thus reduce the operational costs of FS treatment facilities.

Keywords: Faecal sludge; Composting; Helminth eggs; Viable *Ascaris* eggs; Chicken feathers

1. Introduction

Sanitation service delivery in urban Africa comprises the use of on-site sanitation facilities together with conventional sewered systems. Indeed, on-site facilities have played a predominant role in the provision of sanitation services in informal urban areas, but unfortunately, faecal sludge (FS) management has not been harmonised with the needs of

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the increasing urban populations. Consequently, FS is often collected from on-site sanitation installations and indiscriminately disposed of untreated into the environment (e.g., wetlands, drainage channels, etc.) leading to severe environmental and public health risks [1]. FS contains high concentrations of pathogenic microorganisms that are 10–100 times higher than those in domestic wastewater [2]. Proper and sustainable treatment is required to inactivate these pathogenic microorganisms to acceptable standards if FS is to be safely reused, for instance in agriculture (≤1 egg per 4 g of total biosolids [3]). Literature has continuously reported helminth eggs especially *Ascaris* eggs as the most resistant excreted pathogen and therefore, it has been selected as the best pathogenic indicator to monitor FS composting processes [4–6]. Historical evidence has indicated that upon excretion, helminth eggs can survive for about 10–12 months under tropical climatic conditions [5].

Composting is one of the widely promoted ways of sanitising FS in urban Africa [7–9]. However, it is associated with both insufficient helminth eggs inactivation and longer inactivation periods, which makes its commercialisation uneconomical. For example, Koné et al. [10] found that helminth eggs (*Ascaris* eggs inclusive) are able to survive for approximately 80 d during the composting of FS with organic waste. Such inactivation periods are too long for commercialisation of FS compost. Cabañas-Vargas et al. [11] attained only 81% helminth eggs inactivation by the end of a 30-d composting period during the composting of sewage sludge and green waste. Moreover, little is known about the fate of *Ascaris* eggs during the composting of FS. Therefore, the aim of this study was to assess the effect of locally available organic wastes (i.e., chicken feathers, sawdust and market waste) and their mixing ratios with FS on the fate of viable *Ascaris* eggs during FS composting.

2. Material and methods

2.1. Pilot-scale composting facility and raw material collection

The study was conducted at a pilot-scale composting facility constructed at National Water and Sewerage Corporation (NWSC) faecal sludge treatment facility at Lubigi, Kampala, Uganda. This is at a geographical location of latitude 0°18′58″ N, longitude 32°34′55″ E and elevation of 1,223 m above sea level. The pilot-scale composting facility had a total area of 300 m^2, which comprised of both dewatering and composting sections. The composting section consisted of a roof covered composting platform sloping gently towards the leachate drainage channel. The detailed design and construction of the dewatering facility are presented in our previous work [12].

2.2. Collection of faecal sludge and organic waste

Raw FS used in this study was collected from the nearby Kampala informal settlements (i.e., Makerere Kikoni and Bwaise). FS from VIP latrines and septage from vaults and tanks was mixed in a ratio of 1:2 by volume (VIP latrine sludge: septage) and pre-treated by dewatering on sludge drying beds. Detailed work on FS dewatering and characterisation is also presented in our previous work [12]. Market waste and chicken feather waste were collected from Nakasero market and Kalerwe market, with the help of Kampala City Council Authority. The market waste consisted of vegetable, fruit and food waste as well as green waste such as twigs with leaves or small tree branches, which are usually used for covering and protecting vegetables, food or fruits from damage or sunrays while in transit. Sawdust was obtained from Bwaise sawmill located less than 0.5 km from the project site. Organic waste delivered to the composting facility was sorted to remove any inorganics and chopped into smaller pieces by hand (about 20–40 mm) prior to mixing with dewatered FS and pile formation.

2.3. Construction and monitoring of composting piles

Dewatered FS of about 27%–35% total solids content was thoroughly mixed with sorted organic wastes and sawdust as the bulking agent. Four types of compost static piles each 3 m^3 were constructed, each in duplicate: (i) SOS1 (1:2:1 v/v; dewatered sludge: market waste: sawdust); (ii) SOS2 (1:3:1 v/v; dewatered sludge: market waste: sawdust); (iii) SCS1 (1:2:1 v/v; dewatered sludge: chicken feathers: sawdust); (iv) SCS2 (1:3:1 v/v; dewatered sludge: chicken feathers: sawdust). The composting piles were aerated by manual turning, with a 7 d turning frequency. The composting temperature of each pile was measured daily at the: top (ca. 750 mm from the pile base), middle (400 mm from pile base) and bottom (200 mm from pile base), using a TFA (D-Wertheim, Model 19.2008) stainless steel body compost thermometer. The composting piles were monitored for a period of 15 weeks (air temperature: 19°C–26°C; relative humidity: 69%–80%).

2.4. Sampling methods

2.4.1. Dewatered faecal sludge sampling

Dewatered sludge samples were collected from at least 10 randomly chosen sampling points on each drying bed before sludge removal. At each chosen location, the sludge was stirred until it was homogeneous prior to sample collection. An equal volume of sludge was collected from each sampling point, and all of these were thoroughly mixed to form a composite sample from which a portion was collected using quarter sampling. This sample was then analysed for total solids and presence of viable *Ascaris* eggs.

2.4.2. Compost sampling

Compost samples of about 400 g were collected from the top, middle and bottom as well as the outer and inner sections of each composting pile. To ensure representative sampling, the collected subsamples were then mixed homogeneously to form a composite sample for each of the four pile types, from which a sample of approximately 500 g was collected using quartering method, and taken for total solids and viable *Ascaris* eggs analysis. Samples were collected at day 0 and weekly from the composting piles until the end of the composting period. The analysis was carried out at Bugolobi NWSC central laboratory in Kampala.

2.5. Analytical methods

2.5.1. Total solids and moisture content

A sample of 50 g was weighed into a previously weighed crucible. This was then dried in an oven at 105°C for 24 h. It was removed thereafter, allowed to cool for 30 min and reweighed. A total solid and moisture content (%) were then computed by using the sample initial and final weights [13].

2.5.2. Ammonium

Ammonium (NH_4–N) was extracted with 0.5 M K_2SO_4 in 1:10 (w/v) from fresh compost samples and determined by spectrophotometric methods according to procedures reported in the literature [13,14].

2.5.3. Respiration rate

The microbial respiratory activity in compost samples was measured based on CO_2–C evolution rate conducted in closed bottles according to Öhlinger [15] and Alef [16] soil respiration techniques, but with some modifications made to techniques based on similar soil respiration procedures reported in literature [17–21]. CO_2–C was trapped in an alkaline solution (KOH), which was then titrated with HCl. CO_2–C production rate was assessed and expressed as mg CO_2–C per mass of organic matter (as volatile solids – VS) per day [22,23].

2.5.4. Viable Ascaris eggs analysis

Viable *Ascaris* eggs concentrations were analysed according to USEPA [3] technique with slight modifications made to it by other authors [24–26]. The method is based on a fundamental principle of recovering helminth eggs from compost or dewatered FS by floating them from other debris using $ZnSO_4$ (with a comparatively relative density of 1.2–1.3) in a supernatant obtained by centrifugation. The relative density of 1.2 was used so that the *Ascaris* eggs with relative density of about 1.13 are able to float in the solution [24,25]. In this study, using a sample of 400 g, a series of steps including washing, sedimentation, filtration, flotation and extraction were conducted so as to increase concentration of *Ascaris* eggs in the suspension. A filter of 35 μm pore size was used for separation of helminth eggs (*Ascaris* eggs) from the supernatant [4]. For viability assessment, the highly concentrated suspension was then re-suspended in 4 mL of 0.1 N solution of sulphuric acid (H_2SO_4) and then incubated for 21–28 d at 26°C or until when most of the ova were fully viable (embryonated) [3]. Thereafter, the incubated concentrates were examined microscopically (10× or 40× magnification). All the viable and non-viable (unembryonated) *Ascaris* eggs observed within the grid in both chambers of the McMaster slide were counted. Ova, where the larva was observed, were considered and counted as viable. In some cases, the larva was observed to be moving in viable *Ascaris* ova. The images in Fig. 1 were used as a guide in the identification of the viable and non-viable *Ascaris* eggs [24,26]. To minimise errors during egg counting, each sample was counted in triplicate and average values reported. The average counted viable eggs were then expressed as *Ascaris* eggs g^{-1} dry weight. For better analysis, these were further expressed as the percentage of initial viable eggs count.

2.6. Statistical analysis

Laboratory results were reported as mean values or ± standard error of duplicates, and subjected to statistical analysis using IBM SPSS 21.0 software. Data were analysed using non-parametric Friedman test. The significance of differences amongst the mean values was tested at a level of $p = 0.05$, with 95% confidence level. Spearman's rho test was also used for examining the correlation coefficient between parameters based on a >95% confidence level. $p \leq 0.05$ was set as the statistical significance criterion. Standard multiple regression analysis was conducted according to Pallant [27], to determine the most important factors responsible for viable *Ascaris* eggs inactivation during composting.

3. Results and discussion

3.1. Characterisation of raw materials

In the present study, the average viable *Ascaris* eggs content of 37 ± 16 eggs g^{-1} dry weight observed in the dewatered FS compare well with those published by other authors [10,28]. However, these concentrations are 10–100 times higher than those found in sewage sludge [9]. This result

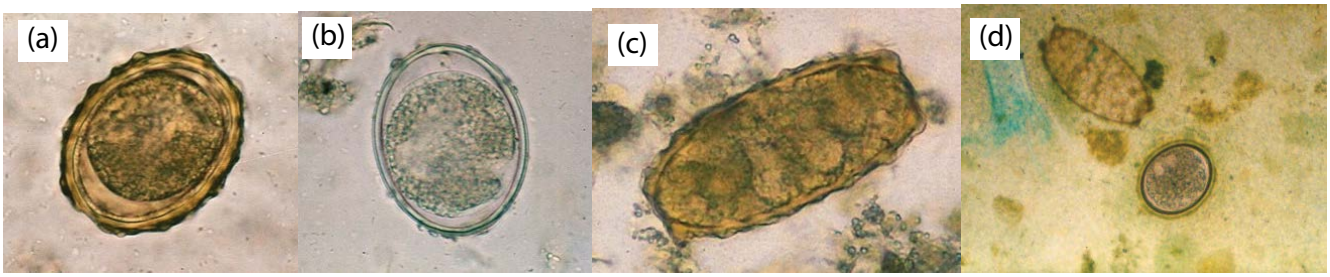

Fig. 1. (a) Normal fertile *Ascaris lumbricoides* ovum showing brownish coloured mamillated outer shell. Measures size: 55–75 × 35–50 μm, (b) normal fertile *Ascaris lumbricoides* ovum but lacking mamillated layer referred to as "decorticated" egg, (c) infertile *Ascaris* ovum (these are longer and thinner than fertile eggs. They have brownish coloured grossly irregular mamillated layer and the egg contents are granular and disorganised [size: 85–95 × 43–47 μm]), and (d) fertile and infertile eggs of *Ascaris lumbricoides* in a Kato-Katz preparation.

is not surprising especially in developing countries where helminth eggs are rampant [29]. Detailed work on characterisation of raw FS from VIP latrines and septic tanks as well as FS dewatering is presented in our previous work [12]. No *Ascaris* eggs were detected in the sawdust, market waste and chicken feathers waste samples. This implies that the solid wastes used in this study were free from *Ascaris* eggs or faecal contamination. Furthermore, the content of dry solids found in samples of market waste (36.6% ± 3.3%), chicken feathers (39.3% ± 0.5%) and sawdust (68.8% ± 5.9%) compared well with those reported by other authors [7,28]. Other physical and chemical characteristics of the organic wastes used in this study are presented in Table S1, and these have been extensively discussed in our previous work [30].

3.2. Temperature evolution

All the composting piles attained the composting temperatures (≥55°C) and conditions suggested by USEPA [3] for effective pathogen inactivation during composting (Figs. 2a–d). Composting trials using SCS1 and SCS2 blends reached mean temperatures of ≥55°C within the shortest composting period of 7 and 5 d, respectively (Figs. 2a and b). These temperatures were sustained within the SCS1 and SCS2 piles for a period of 42 and 35 d, respectively, before they dropped to 50°C. This implies that the composting feedstock supported the quick establishment of microbial activities within the composting piles, which could have been due to the availability of easily biodegradable organic materials (such as fats, starch and proteins) commonly found in chicken feather waste [31]. This also suggests that chicken feather waste, FS and sawdust are excellent complementary organic substances for co-composting. In some sections of SCS1 and SCS2 piles, temperatures as high as 70°C and 67°C were reached within a composting period of 11 and 5 d, respectively. This implies that the composting material containing chicken feather wastes had an excellent self-insulating characteristic. Self-insulating phenomenon is an important factor for the temperature increase in piles especially during the early composting stages, where the heat generated as a result of microbial activities is trapped within the composting piles [32,33].

In contrast, SOS1 and SOS2 piles required relatively longer composting periods of approximately 9 and 10 d, respectively, to achieve the mean temperatures of ≥55°C (Figs. 2c and d). This might have been due to the presence of high proportion of recalcitrant carbon (e.g., lignin) and a low proportion of easily biodegradable carbon in the composting material, which may have limited the early microbial activities, and thus the rapid rise in the composting temperatures. Some previous studies have similarly found market waste/green waste to have contained low proportions of easily biodegradable carbon [34,35]. The shredded twigs, leaves and wood added to these composting piles may have contained high proportions of recalcitrant carbon (e.g., lignin). However, maximum temperatures of 65°C and 64°C were reached in some sections of SOS1 and SOS2 composting piles after 21 and 20 d composting period, respectively. The SOS1 and SOS2 piles maintained the optimum mean temperatures (≥55°C) for effective pathogen inactivation for a short time of approximately 21 and 25 d, respectively (Figs. 2c and d). This could perhaps be attributed to high heat losses from the composting piles to the environment as a result of energetic flux since these piles

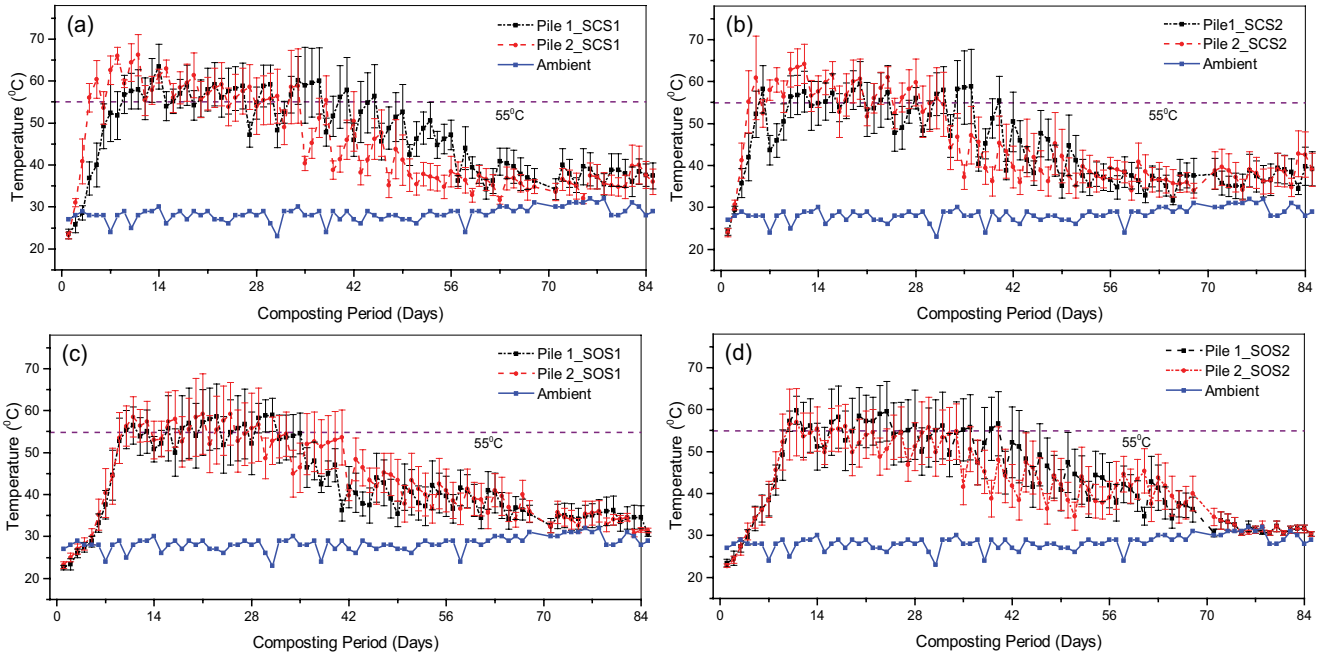

Fig. 2. Temperature evolution during co-composting of faecal sludge with (a) chicken feather waste and sawdust in a ratio of 1:2:1 – SCS1, (b) chicken feather waste and sawdust in a ratio of 1:3:1 – SCS2, (c) organic market waste and sawdust in a ratio of 1:2:1 – SOS1; and (d) organic market waste and sawdust in a ratio of 1:3:1 – SOS2. Error bars represent the standard deviation of the bottom, center, left side and right side pile temperatures for each composting pile.

were observed to have turned more porous, especially after 6 weeks composting period.

The temperature profiles of four types of composting piles suggest that the different organic waste types had an influence on the composting temperatures. This was confirmed by the Friedman test results at 95% confidence level, which indicated that the organic waste types had a statistically significant effect on the composting temperatures evolution with $p = 0.0001$. This could be due to the difference in the characteristics of the biodegradable organic materials contained in the feedstock materials used. It is important to note that the SCS piles generally exhibited higher composting temperatures and longer thermophilic phase duration than the SOS piles during composting (Figs. 2a–d). This may have been due to the labile nature of the organic materials present in the SCS composting piles and their high thermal inertia [36].

As can be seen in Figs. 2a–d, SOS1 and SCS1 composting piles (of 1:2:1 mixing ratios) recorded slightly higher composting temperatures than SOS2 and SCS2 (of 1:3:1 mixing ratio). This clearly indicates that such piles contained more proportions of easily biodegradable organic materials (e.g., fats, sugars, proteins and starch) which may have been present in the dewatered FS, and favourable conditions for effective composting conditions. The increase in the mixing ratio of organic waste/chicken feathers to faecal sludge from 1:2:1 to 1:3:1; (a) may have led to a dilution effect of readily available organic matter sources which may have been present in FS; (b) may have negatively affected the structure of the composting material by turning it extremely loose or porous, and thus leading to the heat diffusion to the environment. Lu et al. [37] similarly found low mixing ratios (1:3) of sewage sludge to municipal solid waste to have attained higher composting temperatures than higher mixing ratios (1:5 and 1:9). Surprisingly, Friedman test results at 95% confidence level showed that the mixing ratio did not have a significant effect on the temperature evolution.

3.3. Moisture content evolution

All the four organic waste compost types recorded high initial moisture content in the range of 62.0% and 66.0% (Fig. 3). However, this was within the suggested optimum moisture content range of 55%–70% for effective composting [38]. SCS1 and SCS2 composting piles exhibited a significant drop of approximately 16.5% and 17.7%, respectively, in the initial moisture content (MC), within the early composting periods of 6 weeks (Fig. 3). This was then followed by an increase from 48.8% and 45.4%, on week 6 to 52.2% and 50.0%, respectively, on week 8 after re-wetting of the piles, and finally a gradual decrease to the final mean MC values of 44.0% and 43.0%, respectively, at the end of the composting period. In contrast, SOS1 and SOS2 piles had different MC evolution trend, with significant fluctuations especially during the thermophilic phase. The MC descended sharply in the first week, then rose in the second week; then descended, and that behaviour continued until reaching the final MC of 39.0% and 44.0% at the end of the composting periods.

The decrease in moisture content exhibited by all the composting piles could be attributed to the high initial composting temperatures (Figs. 2a–d), that may have resulted into water vaporisation from the composting piles especially during the thermophilic phase. Similar behaviour has been reported by other authors [39]. However, moisture loss during composting has been suggested by Finstein et al. [40] as an index for assessing the decomposition rate of organic matter, since heat generated as a result of the decomposition process stimulates vaporisation. The increase in the MC may be attributed to moisture generated as a by-product of the intensive decomposing process of organic substances, and the water holding capacity of the composting material. It is important to note that the SOS piles (containing only organic market waste) exhibited higher moisture content than SCS piles, especially during the intensive composting periods. This could be attributed to the high biomass of the organic market waste in the composting feedstock, which may have resulted into the release of high water content as a metabolic end-product of the decomposition process of the most labile organic matter fractions. The final moisture content and dynamics observed in this study are comparable with those observed by other authors during the composting of different feedstock [18].

3.3.1. Evolution of carbon dioxide (CO_2–C)

Fig. 4a illustrates the evolution of CO_2–C respiration rate exhibited by all composting piles during the FS composting with different organic waste. During the composting of SCS1 and SCS2 piles, the high initial CO_2–C respiration rate mean values of 11.5 and 10.2 mg CO_2–C g VS^{-1} d^{-1} decreased but with oscillations to mean values of 0.75 and 0.60 CO_2–C g VS^{-1} d^{-1} by 14 weeks composting periods, respectively (Fig. 4a). In contrast, a sudden increase was observed in the respiration rates of SOS1 and SOS2 composting piles

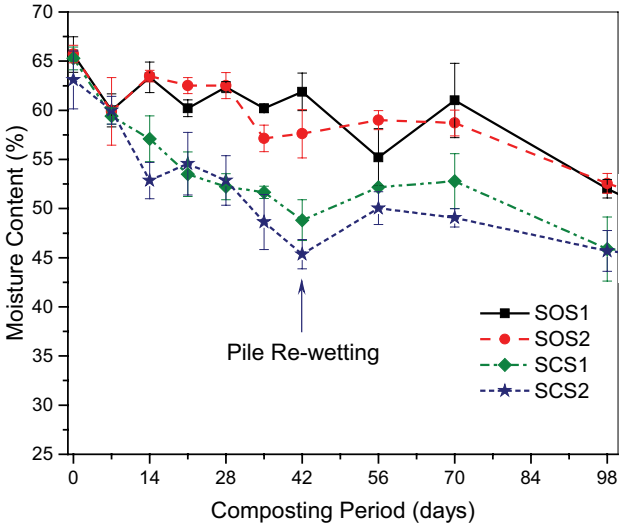

Fig. 3. Evolution of moisture content (MC) during the co-composting of faecal sludge with: (a) chicken feather waste and sawdust in a ratio of 1:2:1 – SCS1, (b) chicken feather waste and sawdust in a ratio of 1:3:1 – SCS2, (c) organic market waste and sawdust in a ratio of 1:2:1 – SOS1, and (d) organic market waste and sawdust in a ratio of 1:3:1 – SOS2. Error bars represent the standard error for the duplicated piles.

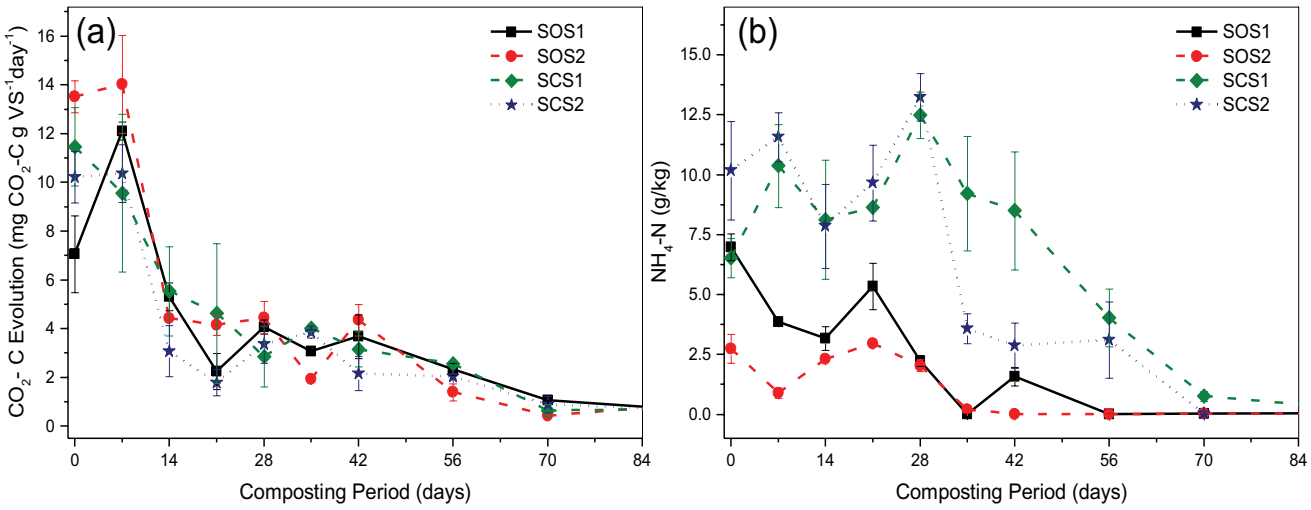

Fig. 4. Changes in (a) carbon dioxide respiration rate (mg CO_2–C g VS^{-1} day^{-1}) and (b) ammonium-nitrogen (NH_4–N) during the composting of FS with different organic waste type during the co-composting of faecal sludge with (a) chicken feather waste in a ratio of 1:2 – SCS1, (b) chicken feather waste in a ratio of 1:3 – SCS2, (c) organic market waste in a ratio of 1:2 – SOS1, and (d) organic market waste in a ratio of 1:3 – SOS2. Error bars represent the standard error for the duplicated piles.

from initial values of 7.1 and 13.5 mg CO_2–C g VS^{-1} d^{-1} to peak values on the 7th day. These then decreased gently but with slight fluctuations to final and stable values of 0.4 and 0.38 mg CO_2–C g VS^{-1} d^{-1}, respectively (Fig. 4a).

The initial increase in the CO_2–C respiration rate exhibited by the SOS piles can be attributed to the existence of favourable conditions within these piles that may have favoured the growth, multiplication and activities of microorganisms [41]. It is important to note that all the composting piles recorded a sharp decrease in the CO_2–C respiration rate during the early composting period of 1–2 weeks, followed by a gentle decrease reaching stable values within 10–12 weeks composting period (Fig. 4a). This behaviour could be attributed to the rapid decomposition and depletion of organic carbon substances because of the early intensive microbial activities. The gradual and faster drop in the CO_2–C respiration rate content to low concentrations also indicates that the organic matter had stabilised into humic-like substances and that it could no longer support the growth and activities of microorganisms [42]. The CO_2–C results and evolution trends observed in this study are in accordance with those obtained in previous assays [43,44]. Detailed discussion on the evolution of CO_2–C respiration rates observed in composting piles processing FS is presented in our previous work [30].

3.4. Ammonium-nitrogen (NH_4–N) evolution

In Fig. 4b it can be noted that NH_4–N concentrations of SCS1 and SCS2 compost piles increased from initial values of 11.9 and 7.5 g kg^{-1} reaching peak values of 19.3 and 16.9 g kg^{-1}, at day 7 during the thermophilic phase, respectively. These NH_4–N concentrations remained considerably high in these piles for a period of approximately 56–70 d. Afterwards, they decreased sharply reaching generally stable final values of 0.05 and 0.06 g kg^{-1}, respectively, after 98 d composting period. In contrast, the NH_4–N concentration of SOS1 and SOS2 dropped initially from 6.98 and 2.74 g kg^{-1} to 3.17 and 0.89 g kg^{-1} at day 14 and 7, respectively; this then rose to mean values of 5.40 and 2.96 g kg^{-1}, before it declined to low stable values of 0.01 and 0.02 g kg^{-1}, after 56 and 42 d composting period, respectively.

All composting piles exhibited a rapid increase and high NH_4–N concentrations, especially during the early composting stages. This can be attributed to the intensive microbial activities responsible for the rapid decomposition of nitrogen-containing organic molecules, leading to the production of NH_4–N. However, as the composting process progressed, all composting piles exhibited decreases in the NH_4–N content, and finally, a gradual decrease reaching stable values in the range of 0.01–0.06 g kg^{-1} at the end of the composting period. This gradual decrease of NH_4–N, especially during the last stages of composting, could have been due to subsequent oxidation to NO_3–N [32]. Detailed discussion on NH_4–N evolution results observed in composting piles processing FS is presented in our previous work [30]. The NH_4–N evolution trends observed in this study are similar to those published by Tam and Tiquia [45] during the composting of spent pig litter-sludge with sawdust.

3.5. Viable Ascaris eggs inactivation efficiency

All piles met the time–temperature criterion suggested by USEPA [3] for pathogen inactivation during composting. This criterion recommends that compost in windrows or piles should be subjected to temperatures ≥55°C for a minimum of 15 d; with at least five turnings of the windrow during these high-temperature periods. In Fig. 5 it can be noted that the *Ascaris* eggs inactivation efficiency of the four types of composting piles differed significantly depending on the organic waste type and their mixing ratio. SCS1 and SCS2 piles (containing chicken feather waste) attained 100% *Ascaris* eggs inactivation efficiency before the end of the thermophilic phase, within a composting period of about

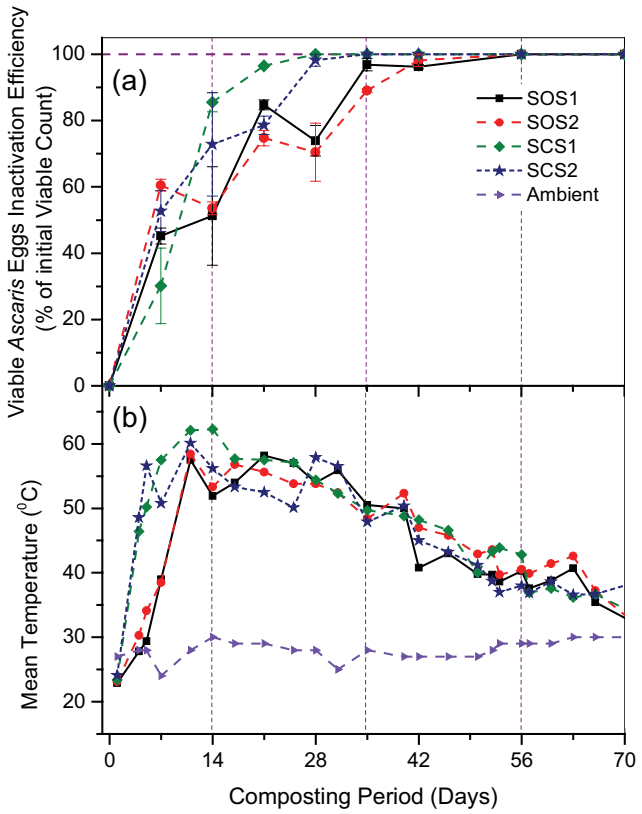

Fig. 5. Mean temperature evolution (b) and inactivation efficiency of viable *Ascaris* eggs (a) during co-composting of faecal sludge with different organic waste types. Error bars represent the standard error of $n = 2$.

28 and 35 d, respectively. However, SOS1 and SOS2 piles (containing organic market waste) attained about 96.3% viable *Ascaris* eggs inactivation efficiency by the end of the thermophilic phase of 42 d, and this improved to 100% inactivation efficiency during maturation, after 56 d composting period. This further reduction in the viable *Ascaris* eggs during the maturation phase can be attributed to the positive consequences of damages triggered previously by higher composting temperature values during the thermophilic phase [5]. A similar behaviour was observed by Koné et al. [10], who attained 72%–88% helminth eggs inactivation during the thermophilic phase and finally 98%–100% inactivation during maturation phase, after 80-d composting period.

The rapid viable *Ascaris* eggs inactivation efficiency exhibited by SCS piles (containing chicken feather waste) may have been due to the comparatively high lethal temperatures reached and sustained for an extended period in such piles (Figs. 2a and b). However, the longer viable *Ascaris* eggs survival periods exhibited by the SOS piles (containing organic market waste) may be attributed to the non-uniformity or uneven distribution of elevated temperatures within the whole mass of the composting piles especially during the early composting stages, given the heterogeneity nature of their composting feedstock that contained relatively large particles. This has also been pointed out by Wichuk and McCartney [6] as the common explanation of pathogen survival in composting piles. This study results suggest that co-composting of faecal sludge with chicken feather waste (SCS) and organic market waste (SOS) for a period of 5 and 8 weeks with temperatures of around 53°C–62.1°C and 50.7°C–58.7°C sustained in the composting piles for more than 3 and 5 weeks, respectively, using 7-d turning frequency, are sufficient for ensuring complete inactivation of viable *Ascaris* eggs in FS.

In the present study, Friedman test results revealed that the different organic waste types had a statistically significant effect ($p = 0.0001$) on viable *Ascaris* eggs inactivation efficiency during FS composting. However, the mixing ratio of organic waste to faecal sludge did not show a significant effect on the *Ascaris* eggs inactivation efficiency. For comparison purposes, no publication could be found in literature that specifically evaluated the effect of organic waste mixing ratios on the inactivation efficiency of viable *Ascaris* eggs during composting. From Fig. 5 it can be seen that one of the most remarkable features of the results attained in the present study is the significant fluctuation in viable *Ascaris* eggs content especially in the piles containing organic market waste (SOS). Similar behaviour has been observed by Bean and Brabants [46] during the composting of similar feedstock. This odd phenomenon can be attributed to the high heterogeneity nature of the composting material and collected samples, especially during the early stages of composting where the composting material was observed to be very clumpy. It can be noted that FS did not mix thoroughly well with the organic market waste/chicken feather waste, despite the efforts made when setting up the composting piles. Therefore, depending on the samples collected and analysed, the viable *Ascaris* eggs content may have been considerably high if the sample consisted of high proportions of FS, and very low if it contained a high proportion of organic market waste or chicken feather waste. However, as the composting process progressed, the fluctuations in the *Ascaris* eggs content considerably reduced, which implies that the composting material had become generally homogeneous and therefore, the collected samples were more representative of the actual pathogens within the composting material.

3.6. Temperature–time relationship

Fig. 5 illustrates the evolution of mean composting temperatures and viable *Ascaris* eggs inactivation efficiency of the four composting piles. It can be seen that SCS piles (containing chicken feather waste) exhibited generally higher temperatures as well as faster *Ascaris* eggs inactivation efficiency compared with SOS piles (containing organic market waste). This clearly illustrates the importance of high temperature–time factor in inactivation of *Ascaris* eggs during composting. Szabová et al. [47] similarly found the high temperatures (of >65°C) to have been responsible for the faster inactivation of *Ascaris suum* within a period of only 4–5 d during the composting of sewage sludge with different organic waste on an industrial composting scale. Several studies have also reported temperature–time relationship as the major factor for pathogen die-off during composting [3,6,48]. However, it can be seen from Fig. 5 that

although the *Ascaris* eggs inactivation efficiency of the four piles differed significantly, there was no significant difference observed in the composting temperatures evolution. Moreover, considering the mean temperature results of SOS and SCS piles for the periods when the *Ascaris* eggs were inactivated, it was noted that there existed a mean temperature difference of only 1.3°C–4.1°C, which is considerably too small to cause such a significant difference in the *Ascaris* eggs survival periods of approximately 2–4 weeks. This strongly confirms that apart from thermal destruction, there are other mechanisms such as indigenous microbial activities, microbial antagonism, antibiotic action induced by certain actinomycetes and fungi, toxic by-products (mainly NH_4–N) produced during composting, etc. that may have partly been responsible for the significant difference in *Ascaris* eggs inactivation as well as their survival periods during FS composting.

In the present study, the potentially toxic by-products especially high NH_4–N concentrations (Fig. 4b) resulting from the metabolic activities of the composting microorganisms were found to have partly been responsible for the inactivation of viable *Ascaris* eggs during FS composting. This observation was confirmed by highly significant inverse correlation observed between the evolution of NH_4–N content and viable *Ascaris* eggs inactivation in SOS1 piles ($p = 0.0001$, $r = –0.832$), SOS2 piles ($p = 0.0001$, $r = –0.848$) and SCS1 piles ($p = 0.004$, $r = –0.592$). It can be noted that the inactivation rate of viable *Ascaris* eggs varied depending on the level of NH_4–N concentrations observed in the composting material, with SOC1 piles (containing chicken feather waste) which exhibited higher NH_4–N concentrations attaining faster *Ascaris* eggs inactivation than SOS (market waste) piles. This implies that high NH_4–N concentrations may lead to the release of free ammonia and other toxic by-products to *Ascaris* eggs in composting piles. This finding is also supported by Pecson et al. [49] who found ammonium concentrations (0.2–5.0 g L^{-1}) to have been responsible for the significant inactivation of *Ascaris* eggs in sewage sludge at ≥40°C during the lab-scale experiment. They found the inactivation rate to have varied depending on the total ammonium concentration, pH value (7–12) and temperatures ≥40°C. However, a significant correlation between *Ascaris* eggs inactivation and NH_4–N evolution was not observed in some chicken feathers piles – that is, SCS2 piles ($p = 0.084$, $r = –0.378$). This could be attributed to the high temperatures observed in these piles, which may have masked the effects of NH_4–N on the survival of *Ascaris* eggs in composting piles. Similarly, Pecson et al. [49] also found the effects of ammonium and pH on *Ascaris* eggs inactivation efficiency in sewage sludge to have been minimal at temperatures of 50°C.

On the other hand, the inactivation of viable *Ascaris* eggs could also be explained by microbial antagonistic mechanisms or antibiotic action induced by indigenous microorganisms due to microbial competition or activities. This hypothesis was supported by Spearman's rho test, which revealed a statistically significant negative correlation (SOS1 ($p = 0.0001$, $r = –0.899$), SOS2 ($p = 0.0001$, $r = –0.837$), SCS1 ($p = 0.0001$, $r = –0.727$) and SCS2 ($p = 0.003$, $r = –0.605$)) between viable *Ascaris* eggs inactivation efficiency and microbial competition or activities monitored by CO_2–C respiration rate evolution (Fig. 4a). This study results show that the *Ascaris* eggs inactivation rate varied depending on the level of microbial activities observed in the composting piles. SCS piles (containing chicken feather waste) where higher microbial activities were observed, attained faster *Ascaris* eggs than SOS (market waste piles). This suggests that the microbial antagonistic mechanisms or antibiotic action induced by indigenous microorganisms due to microbial competition or activities may have been responsible for *Ascaris* eggs inactivation in the composting material. Similar observations were made by Meekings et al. [50] who found the antagonistic mechanisms and antibiotic action resulting from the activities of indigenous microorganisms to have been responsible for the inactivation of *Ascaris* eggs in the compost aqueous. In their study, they found *Ascaris* eggs inoculated in the sewage sludge compost aqueous destroyed at very low temperatures (30°C) whereas their viability still existed in the microorganism-free compost filtrate aqueous and distilled water at the similar temperatures.

In this study, three parameters were found to have equally played a significant role in the inactivation of viable *Ascaris* eggs during FS composting. However, using a multiple regression analysis, the effect of each mechanism on viable *Ascaris* eggs inactivation during composting was assessed (Table S2). On the whole, the regression results revealed temperature–time relationship as the most important factor that was responsible for the inactivation of viable *Ascaris* eggs during composting (with Beta = –0.581, $p = 0.0001$ and $R^2 = 0.052$). This was followed by the indigenous microbial activities (measured by CO_2–C respiration rate) that induce antibiotics or antagonistic effects (with Beta = –0.427, $p = 0.0001$ and $R^2 = 0.072$); and lastly, high concentrations of NH_4–N (measured as NH_4–N evolution) released as a result of intensive microbial activities during composting (with Beta = –0.253, $p = 0.003$ and $R^2 = 0.033$). This finding is of remarkable significance as it confirms the hypotheses frequently reported in literature review studies [51,52] without substantial evidence from real field composting trials that pathogen inactivation during composting is not solely dependent on the temperature–time relationship factor. In the same vein, this study results suggest, therefore, that there is no single or universal mechanism that is solely responsible for pathogen inactivation during composting.

Overall, composting of FS with chicken feather waste reduced the viable *Ascaris* eggs inactivation periods from 8 weeks to about 4–5 weeks, when compared with piles containing organic market waste. This represents approximately 42% reduction in the inactivation periods. This finding is of remarkable significance in the treatment of FS using composting in urban Africa, as it can reduce the capital and operational costs as well as the duration for treating a cubic meter of FS at the FS treatment facility by 42%, which thus increase the capacity of the treatment plant by 42%. The *Ascaris* eggs inactivation periods attained in this study are consistent with those published by Evans et al. [28] during the composting of similar feedstock, where complete helminth egg inactivation was attained with a composting period of 4–6 weeks. This study results suggest that co-composting of FS with a suitable organic waste for a period of 8 weeks with temperatures of around 50.7°C–58.7°C sustained in the composting piles for more than 31 d, using 7 d turning frequency, is sufficient to

ensure complete inactivation of viable *Ascaris* eggs in FS. Importantly, complete inactivation of the *Ascaris* eggs in the composting piles will additionally be dependent on exposing all the composting material to such temperatures for the recommended time, which implies that thorough mixing or sufficient turning of the piles is a very important factor for proper composting and compost sanitisation. The final compost attained in this study was hygienically safe for use in unrestricted agriculture as it was completely free from pathogen indicators.

4. Conclusion

This study aimed at investigating the fate of viable *Ascaris* eggs during the co-composting of FS with chicken feathers or organic market waste. Based on the study findings, the following conclusions can be drawn:

- Regardless of the type of organic waste co-composted with FS, all composting piles attained and sustained optimum temperatures >55°C of effective pathogen inactivation for a period of more than 4 weeks.
- The organic waste types had a significant effect on the composting temperature evolution and viable *Ascaris* eggs inactivation efficiency. However, the mixing ratios of different organic waste with FS did not have a significant effect on the viable *Ascaris* eggs inactivation efficiency and composting temperature.
- The temperature–time factor was found to be the most important factor responsible for pathogen inactivation. However, other mechanisms such as microbial antagonistic mechanisms or antibiotic action induced by indigenous microorganisms and toxic by-products such as NH_4–N also played an important role in the inactivation of viable *Ascaris* during composting.
- Composting piles containing chicken feathers exhibited the shortest *Ascaris* eggs survival periods of only 4 weeks compared with 6–8 weeks for those containing market wastes. Co-composting of dewatered FS with chicken feathers seems to be a promising low-cost FS treatment method in urban Africa, as it reduced the *Ascaris* eggs inactivation period by 42%. This could thus increase the capacity of FS treatment plants or reduce their required capital investment, operational costs by 42%.
- Co-composting of FS with a suitable organic waste for a period of 8 weeks with temperatures of around 50.7°C–58.7°C sustained in the composting piles for more than 31 d, using 7 d turning frequency, is sufficient to ensure complete inactivation of *Ascaris* eggs in FS.

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Supplementary Information

Table S1
Characteristics of raw material used[a]

	Dewatered FS	Chicken feathers	Market waste	Sawdust
pH (1:10)	7.96 ± 0.15	7.50 ± 0.43	8.25 ± 0.49	5.66 ± 0.44
EC 27°C (Sm cm^{-1})[b]1:10	2.12 ± 0.42	1.20 ± 0.50	3.08 ± 0.32	0.40 ± 0.09
Moisture (%)	68.71 ± 3.80	60.67 ± 0.52	63.35 ± 3.32	31.20 ± 5.94
Organic matter[b] (%)	62.17 ± 3.75	56.50 ± 4.95	51.50 ± 3.82	94.30 ± 2.97
Total organic Carbon[b] (%)	33.83 ± 1.41	32.77 ± 2.87	31.25 ± 3.89	51.44 ± 2.08
Total Kjeldahl N[b] (%)	3.35 ± 0.29	4.50 ± 0.71	1.67 ± 0.33	0.39 ± 0.03
Nitrate–N[b] (mg kg^{-1})	0.05 ± 0.03	0.04 ± 0.01	ND	ND
Ammonium–N[b] (mg kg^{-1})	0.53 ± 0.39	0.38 ± 0.11	0.08 ± 0.00	0.00 ± 0.01
C/N ratio	10.11 ± 4.94	7.28 ± 4.06	18.77 ± 11.70	132.25 ± 69.90

[a]Mean ± Standard deviation (SD) of triplicates.
[b]Dry base.
ND = no detectable.

Table S2
Spearman's rho test results between viable *Ascaris* eggs survival and other mechanisms responsible for pathogen die-off during FS co-composting with different organic waste types

	CO_2–C evolution (n = 22)		NH_4–N (n = 22)	
	p	r	p	r
SOS1	0.0001	–0.899	0.0001	–0.832
SOS2	0.0001	–0.834	0.0001	–0.848
SCS1	0.0001	–0.727	0.004	–0.592
SCS2	0.003	–0.605	0.084[a]	–0.376

[a]Not significant.

Table S3
Mechanisms responsible for the inactivation of selected pathogen indicator (viable helminth eggs) during FS composting

Pathogen Inactivation Mechanisms in the order significance effect on pathogen inactivation	Standardised Coefficient (Beta)	Significant	R-Square Value
Temperature	–0.581	0.0001[a]	0.052
Microbial activities measured as CO_2–C respiration rate	–0.427	0.0001[a]	0.072
NH_4–N Concentrations	–0.253	0.003[a]	0.033

[a]Significant ($p < 0.05$).