

# The Fate of Helminth eggs during the Co-composting of Faecal Sludge with Chicken Feathers and Market waste

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## Abstract

Faecal Sludge (FS) contains high concentrations of pathogenic micro-organisms 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 ways of sanitising FS in Urban Africa. However, it is associated with longer pathogen inactivation periods, which makes 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 helminth eggs (viable *Ascaris* eggs) 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 (3m<sup>3</sup> each), composted and monitored weekly for a viable helminth eggs survival for a period of 15 weeks. The results suggest that the organic waste types have a significant effect on the temperature change and pathogen inactivation efficiency while their mixing ratios do not. Piles containing CF achieved the shortest pathogen survival period of 4 weeks compared to 6-8 weeks for those with MW. A temperature-time factor was found to be responsible for helminth eggs inactivation. However, other mechanisms such as microbial antagonistic mechanisms or antibiotic action induced by indigenous microorganisms and toxic by-products such as NH<sub>3</sub>-N were found to have also played an important role in helminth eggs inactivation. All piles attained 100% helminth 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 helminth 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

## INTRODUCTION

Generally, in urban Africa sanitation service delivery in form of sustainable Faecal Sludge (FS), treatment facilities has not been harmonised with the needs of the increasing population. Consequently, FS is 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. Faecal sludge contains high concentrations of pathogenic micro-organisms that are 10-100 times higher than those in domestic wastewater (Heinss *et al.* 1998). Proper and sustainable treatment is required to inactivate these pathogenic micro-organisms to below detectable levels if faecal sludge is to be reused in agriculture so as to minimise the public health and environmental risk. 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 (Maya *et al.* 2012; Feachem *et al.* 1983; Wichuk and McCartney 2007). Historical evidence has indicated that upon excretion, helminth eggs can survive for about 10-12 months under tropical climatic conditions (Feachem *et al.* 1983).

Composting is one of the common ways of sanitising faecal sludge in urban Africa. However, it is associated with both insufficient helminth eggs inactivation and longer inactivation periods, which

makes its commercialisation uneconomical. For example, Koné *et al.* (2007) found the helminth eggs to have survived for approximately 80 days during the composting of faecal sludge with organic waste. Such inactivation periods are quite too long for commercialisation of faecal sludge compost. Cabañas-Vargas *et al.* (2013) attained only 81% helminth eggs inactivation by the end of a 30-day composting period, during the composting of sewage sludge and green waste. Moreover, little is known about the fate of helminth eggs during composting of faecal sludge (i.e., especially viable *Ascaris* eggs). Therefore, the aim of this study was to assess the effect of FS co-composting with other organic wastes locally available (i.e., chicken feathers, sawdust and market waste) on the fate of viable helminth eggs (*Ascaris* eggs) during faecal sludge composting.

## METHODOLOGY

### **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 1,223m above sea level. The pilot scale composting facility had a total area of 300m<sup>2</sup>, 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 (Manga *et al.* Accepted).

### **Collection of faecal sludge and organic waste**

Raw faecal sludge (septage and VIP latrine sludge) used in this study was collected from the nearby Kampala informal settlements (i.e., Makerere Kikoni, Bwaise). This was mixed in a ratio of 1:2 by volume (VIP latrine sludge: septage) and pre-treated by dewatering on sand drying beds. Detailed work on faecal sludge dewatering and characterisation is also presented in our previous work (Manga *et al.* Accepted). Market waste and chicken feather waste were collected from Nakasero market and Kalerwe market, with the help of Kampala City Council Authority. Sawdust was obtained from Bwaise sawmill located less than 0.5km from the project site. Organic waste delivered at the composting facility was sorted to remove any inorganics before composting.

### **Construction and Monitoring of Composting piles**

Dewatered faecal sludge of about 27 – 35% total solids content was thoroughly mixed with sorted organic waste and sawdust as the bulking agent. Four types of compost static piles each 3 m<sup>3</sup> were constructed in duplicate: (i) SOS 1 (1:2:1 v/v; dewatered sludge: market waste: sawdust); (ii) SOS 2 (1:3:1 v/v; dewatered sludge: market waste: sawdust); (iii) SCS 1 (1:2:1 v/v; dewatered sludge: chicken feathers: sawdust); (iv) SCS 2 (1:3:1 v/v; dewatered sludge: chicken feathers: sawdust). The composting piles were monitored for a period of 15 weeks, between April 2015 and July 2015. The composting temperature was measured daily at different locations: top (ca. 750mm from the pile base), middle (400mm from pile base) and bottom (200mm from pile base), using a TFA (D-Wertheim, Model 19.2008) stainless steel body compost thermometer. The composting piles were aerated by manual turning using 7 days turning frequency.

### **Sampling methods**

*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. The sludge of equal volume was collected from each sampling point. This was then thoroughly mixed to form a composite sample from which a portion was collected using quarter sampling, and taken for total

solids and viable helminth eggs analysis.

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

### **Analytical methods**

*Total solids.* A sample of 50g was weighed into a previously weighed crucible. This was then dried in an oven at 105<sup>0</sup>C overnight (for 24 hours). It was removed thereafter, allowed to cool for 30 minutes and reweighed. A total solid content (%) was then computed by dividing the sample final weight with the initial weight.

*Viable Helminth eggs (Viable Ascaris eggs) analysis.* Helminth eggs concentrations were analysed according to USEPA (2003) technique with the slight modification made to it based on Moodley *et al.* (2008), Nelson and Darby (2001) and Ayres and Mara (1996) procedures. The method is based on a fundamental principle of recovering helminth eggs from compost or dewatered faecal sludge by floating them from other debris using ZnSO<sub>4</sub> (with comparatively relative density of 1.2) in a supernatant obtained by centrifugation. In this study, using a sample of 400g, a series of steps including washing, sedimentation, filtration, flotation and extraction were conducted so as to achieve a highly concentrated suspension of helminth eggs. A filter of 35µm pore size was used for separation of helminth eggs (*Ascaris* eggs) from the supernatant (Maya *et al.* 2012). For viability assessment, the highly concentrated suspension was then re-suspended in 4 ml of 0.1N solution of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) and then incubated for 21 – 28 days at 26<sup>0</sup>C or until when most of the ova were fully embryonated. Thereafter, the incubated concentrates were examined microscopically (10x or 40x magnification) so as to identify and quantify the viable *Ascaris* eggs. To minimise egg losses and 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 dry weight. For better analysis, these were further expressed as the percentage of initial viable eggs count.

### **Statistical Analysis**

The results were reported as mean values or ± one standard deviation of duplicates and subjected to statistical analysis using IBM SPSS 21.0 software. Data was not normally distributed, and thus analysed using non-parametric Friedman test for 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.

## **RESULTS AND DISCUSSION**

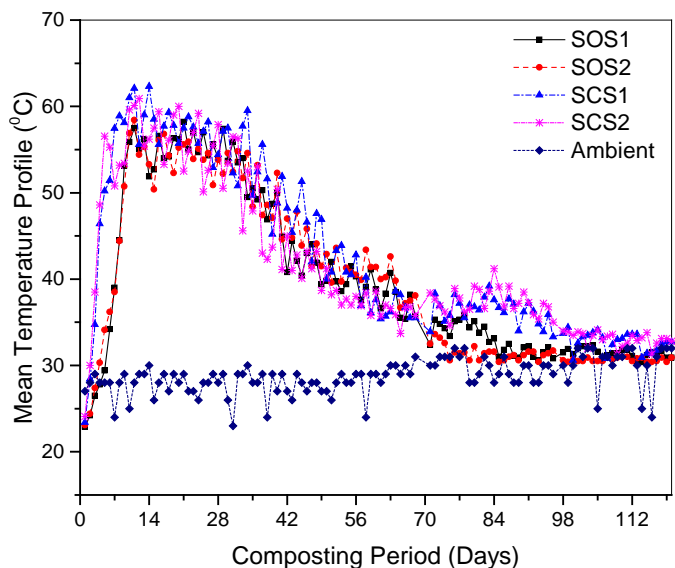
### **Characterisation of raw materials**

In the present study, the viable helminth eggs content of  $37 \pm 16$  eggs/g observed in the dewatered faecal sludge compare well with those published by other authors (Koné *et al.* 2007; Evans *et al.* 2015). However, these concentrations are 10-100 times higher than those found in sewage sludge, especially in developed countries (Déportes *et al.* 1998). This result is not surprising especially in developing countries where helminth eggs are rampant. No viable helminth eggs were detected in the sawdust, market waste and chicken feathers waste samples. Furthermore, the content of dry

solids found in samples of market waste ( $36.6 \pm 3.3$ ), chicken feathers ( $39.3 \pm 0.5$ ) and sawdust ( $68.8 \pm 5.9$ ) compared well with those reported by other authors (Cofie *et al.* 2009; Evans *et al.* 2015).

### Temperature Evolution

All the composting piles attained the composting temperatures ( $\geq 55^{\circ}\text{C}$ ) and conditions suggested by USEPA (2003) for effective pathogen inactivation during composting (Figure 1). SCS1 and SCS2 reached mean temperatures of  $\geq 55^{\circ}\text{C}$  within the shortest composting period of 7 and 5 days, respectively. Such temperatures were sustained within the SCS1 and SCS2 piles for a period of 42 days and 35 days, respectively, before they dropped to  $50^{\circ}\text{C}$ . This implies that the composting feedstock supported the quick establishment of microbial activities within the composting piles, which could have been due to the presence of readily available carbon. In some sections of SOC1 and SOC2 piles, temperatures as high as  $70^{\circ}\text{C}$  and  $67^{\circ}\text{C}$  were reached within a composting period of 11 and 5 days, respectively.

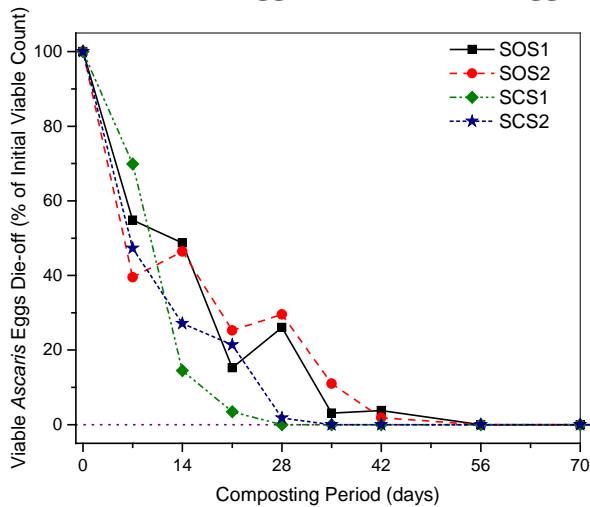


**Figure 1:** Mean temperature profile during faecal sludge co-composting with different organic waste types in varied mixing ratios. Results presented are mean values of the bottom, centre, left side and right side pile temperatures for the duplicated piles.

On the contrary, SOS1 and SOS2 required relatively longer composting periods of approximately 9 and 10 days, respectively, to achieve the mean temperatures of  $\geq 55^{\circ}\text{C}$  (Figure 1). This might probably have been due to the presence of high content of recalcitrant carbon such as lignin in market waste, which is quite hard to biodegrade, and therefore may have limited early microbial activities, thus the low composting temperatures. However, maximum temperatures of  $65^{\circ}\text{C}$  and  $64^{\circ}\text{C}$  were reached in some sections of SOS1 and SOS2 composting piles after 21 and 20 days composting period, respectively. The SOS1 and SOS2 piles maintained the optimum mean temperatures ( $\geq 55^{\circ}\text{C}$ ) for effective pathogen inactivation for a short time of approximately 21 and 25 days, respectively (Figure 1). This could perhaps be attributed to high heat losses to the environment since the piles were observed to have turned porous especially after 6 weeks composting period. It could also have been due to the reduced microbial activities as a result of depletion in the supply of readily available carbon. The temperature profiles of four types of compost 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 difference onto the composting temperatures evolution with  $p = 0.0001$ . As can be seen in Figure 1, SOS1 and SOC1 composting

piles (of 1:2:1 mixing ratios) recorded slightly higher composting temperatures than SOS2 and SCS2 (of 1:3:1 mixing ratio). This implied that such piles contained sufficient readily available carbon sources and favourable conditions for effective composting conditions. Surprisingly, Friedman test results showed that the mixing ratio did not have a significant effect on the temperature evolution.

### Viable Helminth eggs (viable *Ascaris* eggs) Inactivation Efficiency



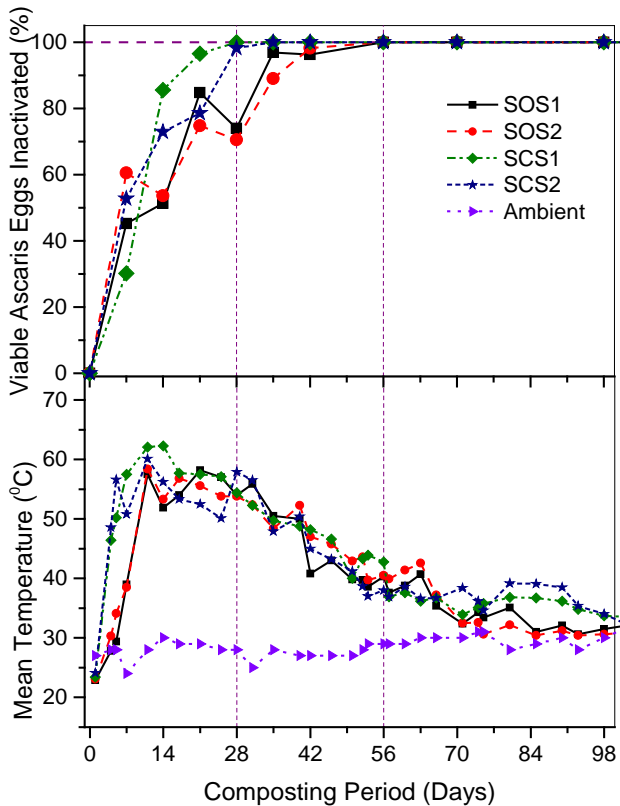
**Figure 2:** Changes in viable Helminth eggs (*Ascaris* eggs) present during the co-composting of faecal sludge with different organic waste. Results presented are mean values of the duplicated piles.

In Figure 2, the helminth 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 feathers) attained 100% helminth eggs inactivation efficiency before the end of the thermophilic phase, within a composting period of about 28 and 35 days, respectively. However, SOS1 and SOS2 piles (containing market waste) attained about 96.3% helminth eggs inactivation efficiency by the end of the thermophilic phase of 42 days, and this improved to 100% inactivation efficiency during maturation, after 56 days composting period. This further reduction in the helminth 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 (Feachem *et al.* 1983). Similar behaviour was observed by Koné *et al.* (2007), who attained 72-88% helminth eggs inactivation during the thermophilic phase and finally 98-100% inactivation during maturation phase, after 80 days composting period. The rapid helminth eggs inactivation efficiency exhibited by SCS piles (containing chicken feathers) may have been due to the comparatively high lethal temperatures reached and sustained for an extended period in such piles. However, longer helminth eggs survival periods in the SOS piles (containing high market waste) may be attributed to the uneven distribution of elevated temperatures within the composting piles. This has also been pointed out by Wichuk and McCartney (2007) as the common explanation of pathogen survival in composting piles.

In the present study, Friedman test results revealed that the different organic waste types had a statistically significant effect ( $p = 0.0001$ ) on to helminth eggs inactivation efficiency during composting of faecal sludge. However, the mixing ratio did not show a significant effect on the helminth eggs inactivation efficiency. From Figure 2, it can be seen that one of the most remarkable feature of the results attained in the present study is the significant fluctuation in viable helminth eggs content especially in the piles containing market waste (SOS). This unusual behaviour may perhaps be attributed to the high heterogeneity nature of the composting material and collected

samples, especially during the early stages of composting. However, as the composting process progressed, the fluctuations in the helminth eggs content considerably reduced, which implies that the composting material had become generally homogeneous, and therefore collected samples were more representative of the actual pathogens within the composting material. Similar behaviour has also been observed by Gallizi (2003) during the composting of similar feedstock.

### Temperatures-Time Relationship



**Figure 3:** Mean temperature evolution (bottom) and Inactivation efficiency of viable helminth eggs (*Ascaris* eggs) during co-composting of faecal sludge with different organic waste types. Results presented are mean values of the duplicated piles.

Figure 3, illustrates the evolution of mean composting temperatures and viable helminth eggs inactivation efficiency of the four composting piles. It can be seen that SCS piles (containing chicken feathers) exhibited generally higher temperatures as well as faster helminth eggs inactivation efficiency compared to SOS piles (containing market waste). This clearly illustrates the importance of high temperature – time factor in inactivation of helminth eggs during composting. Szabová *et al.* (2010) similarly found the high temperatures (of  $> 65^{\circ}\text{C}$ ) to have been responsible for the faster inactivation of *Ascaris suum* within a period of only 4 - 5 days 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 (Wichuk and McCartney 2007; Vinnerås *et al.* 2003; USEPA 2003). However, In Figure 3, it can be clearly seen that although the helminth eggs inactivation efficiency of the four piles differed significantly, there was no significant difference observed in the composting temperatures evolution. Moreover, considering the mean temperatures results of SOS, and SOC piles for the periods when the helminth eggs were inactivated, it was noted that there existed a mean temperature difference of only  $1.3 - 4.1^{\circ}\text{C}$ , which is considerably too small to cause such a significant difference of approximately 2 - 4 weeks in the helminth eggs survival periods. This

strongly confirms that apart from thermal destruction, there are other mechanisms that may have partly been responsible for the significant difference in helminth eggs inactivation as well as their survival periods during faecal sludge composting.

In the present study, it is suspected that toxic by-products such as  $\text{NH}_3$  resulting from the metabolic activities of the composting microorganisms may have partly been responsible for the helminth eggs inactivation during composting. This suggestion was confirmed by highly significant inverse correlation observed between the evolution of  $\text{NH}_4\text{-N}$  content and viable helminth eggs inactivation efficiency in SOS 1 piles ( $p = 0.0001$ ,  $r = -0.832$ ), SOS2 piles ( $p = 0.0001$ ,  $r = -0.848$ ) and SCS 1 piles ( $p = 0.004$ ,  $r = -0.592$ ). This finding is also supported by Pecson *et al.* (2007) who found ammonia (0.2-5.0 g/l) to have been responsible for the significant inactivation of *Ascaris* eggs in sewage sludge at  $\geq 40^\circ\text{C}$  during the lab-scale experiment. They found the inactivation rate to have varied depending on the ammonia concentration, pH value (7 – 12) and temperatures  $\geq 40^\circ\text{C}$ . However, a significant correlation was not observed in SCS2 piles ( $p = 0.084$ ,  $r = -0.378$ ). This could have been due to the high temperature observed in such piles that may have limited the effects of  $\text{NH}_4\text{-N}$  on helminth eggs inactivation efficiency. Similarly, Pecson *et al.* (2007) found the effects of ammonia and pH on *Ascaris* eggs inactivation efficiency in sewage sludge to have been minimal at temperatures of  $50^\circ\text{C}$ .

On the other hand, the inactivation of viable helminth eggs could partly be linked to microbial antagonistic mechanisms or antibiotic action induced by indigenous microorganism 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 helminth eggs inactivation efficiency and microbial competition or activities monitored by  $\text{CO}_2\text{-C}$  respiration rate ( $\text{CO}_2\text{-C}$  evolution data not shown). Similar observation was made by Meekings *et al.* (1996) 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 ( $30^\circ\text{C}$ ) whereas their viability still existed in the microorganism-free compost filtrate aqueous and distilled water at the similar temperatures.

To sum up, the present study, composting of faecal sludge with chicken feathers reduced the helminth eggs inactivation period from 8 weeks to about 4-6 weeks, which represents approximately 42% reduction in the inactivation periods. This study results are consistent with those published by Evans *et al.* (2015) during the composting of similar feedstock, where complete helminth egg inactivation was attained with a composting period of 4 – 6 weeks. The final compost attained in this study was hygienically safe for use in unrestricted agriculture as it was completely free from pathogens.

## CONCLUSION

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

- Regardless of the type of organic waste used, all the composting piles attained the optimum temperatures ( $>55^\circ\text{C}$ ) and conditions specified by USEPA (2003) for effective pathogen inactivation during composting. The composting piles attained and sustained temperatures  $>55^\circ\text{C}$  for a period of more than 4 weeks.
- The organic waste types had a significant effect on the composting temperature evolution

and pathogen inactivation efficiency with  $p = 0.0001$ .

- The mixing ratios of different organic waste with faecal sludge did not have a significant effect on the viable helminth eggs inactivation efficiency and composting temperature.
- The composting period of 4 - 8 weeks is sufficient for complete inactivation of viable helminth eggs (viable *Ascaris* eggs) during the co-composting of faecal sludge with different organic waste. Composting piles containing chicken feathers exhibited the shortest helminth eggs survival periods of only 4 weeks compared to 6 - 8 weeks for those containing market wastes.
- In this study, temperature – time factor was found to be the major 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  $\text{NH}_3\text{-N}$  could have also played an important role in the inactivation of pathogens during composting.
- Co-composting of dewatered faecal sludge with chicken feathers seems to be a promising low-cost faecal sludge treatment options in urban Africa as it reduced the helminth eggs inactivation period by 42%.

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