

ORIGINAL RESEARCH



Inactivation of pathogens in ecological sanitation latrines in Malawi: An observational follow up study

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Abstract

Introduction

In Malawi, EcoSan sludge from ecological sanitation (EcoSan) latrines has been found to contain helminths, *Salmonella* and *E. coli* above WHO recommended levels making sludge unsuitable for direct handling and use on food crops. This research investigated survival of pathogens in EcoSan sludge with time after sealing the pit.

Method

An observational longitudinal follow-up study was conducted where EcoSan latrines were followed from August 2015 to July 2016 in Blantyre and Chikwawa in Southern Malawi. The study enrolled 51 latrines in total with 35 latrines [13 fossa alterna (FAs) and 22 urine diverting dry latrines (UDDLs)] remaining at the end of study. Samples were collected five times from each latrine and examined for helminths, *Salmonella* and *E. coli* in the laboratory. Poisson regression was employed to assess factors that significantly contribute to pathogen die off at $p < 0.05$.

Results

Average concentrations of all pathogens investigated reduced over 12-month follow-up period except for *Salmonella* which increased. *A. lumbricoides*, increased to 2.3 viable eggs during the second sampling and decreased to 0.4 viable eggs per gram after 12 months of follow-up. Time was the only consistent predictor for concentration of helminths. Type of latrine and location were not significant predictors of helminths concentration ($p > 0.05$). However, *Salmonella* and *E. coli* colonies were significantly higher in UDDLs (Blantyre) than FAs (Chikwawa) ($p < 0.05$).

Conclusion

Pathogen concentration was highest after recommended six months of storage posing a public health risk to those handling and using it for agriculture purposes. It is therefore recommended that the current guidelines be reviewed to suit Malawi context. A storage period of one year or more is recommended.

Key Words: sanitation, guidelines, safety, pathogens, sanitation, follow-up, risk, Malawi

Introduction

Human faeces contain different microorganisms including bacteria, viruses, protozoa and helminths which may be pathogenic if passed to a new host¹. Once human faeces are passed in the environment, some organisms survive and the most persistent is *Ascaris lumbricoides* (*A. lumbricoides*) and for this reason, it is commonly used as the main indicator organism for safety of faecal matter²⁻⁵. *Salmonella* and *E. coli* are also used to indicate environmental contamination with faeces⁶. The World Health Organisation (WHO) estimates that about 24% of world's population is infected with soil-transmitted helminths (STHs) and these helminths are transmitted through faeces especially in areas with poor sanitation⁷. In 2010, people were at risk of infection by various parasites, with 5, 346 at risk of hookworm infection, 5, 416 at risk of *Ascaris lumbricoides* (*A. lumbricoides*) infection and 5, 307 at risk of *Trichuris trichiura* infection⁸. STHs are a problem globally especially in Sub-Saharan Africa, America, China and East Asia⁹. In Malawi, in 2013, the average concentration of helminth eggs in one gram of sludge from Fossa Alternas

(FAs) and Urine Diverting Dry Latrines (UDDLs) ranged from 20 to 131 viable ova while total coliforms ranged from 500 to 580 colonies⁴. The recommended number of helminth eggs in Ecological Sanitation (EcoSan) sludge is less than 1 viable egg per gram of EcoSan sludge if the sludge will be used for agriculture purposes¹⁰. According to the United States (US) Environmental Protection Agency (EPA) of 1997, the standard for *Salmonella* and *E. coli* is that there should be no detectable colonies per 50grams and no more than 100 Most Probable Number (MPN) respectively¹¹. EcoSan is an environmentally-friendly sanitation technology that allows economic use of human excreta as manure after decomposition¹². EcoSan promotes recycling of nutrients contained in excreta; thus enhancing food security and reducing need to rely on artificial fertilizers¹³. Most of the nutrients in artificial fertilizers are also present in human excreta and these include nitrogen, urea, potassium and phosphorous^{12,13}. In Malawi, three types of EcoSan latrines are available: FAs, UDDLs, and Arborloos. The UDDL has twin pits which are built about one or more metres above

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ground and has a urine diverting system and a soak away pit while FA has twin pits which are dug up to 1.5metres deep and does not have a urine diverting system. After one pit is full, it is covered with soil and household members start using the other one, making it a permanent latrine¹⁴. By 2001, over 10,000 households in Lilongwe, Salima, Machinga, Nkhatakota, Mzimba and Blantyre were practicing EcoSan. About 0.3% of total latrines in Malawi were EcoSan and promotion of such latrines is increasing^{4,15}.

The Government of Malawi through National Health Research Agenda recognized safety of EcoSan sludge as a priority research area for the country¹⁶. This priority reflected lack of local research on safety of EcoSan humus, as evidence from other studies elsewhere indicate production of unsafe sludge from EcoSan latrines. Studies in rural Panama found that parasites still persisted in sludge from UDDLs after recommended six months of storage^{17,18}. From literature, two different recommended storage times are found with some stating six months while others recommend at least a year^{4,19}. Not only is pathogen die-off dependent on sludge achieving temperatures above 40°C and a moisture content of less than 25%²⁰ but is also dependent on practices during latrine use and other environmental conditions specific to location¹⁴. Effect of temperature and humidity are interdependent, for example, a study in South Africa found that at 60°C, temperature was the only factor while at temperatures below 60°C low humidity became the critical factor for *A. lumbricoides* die-off. At temperatures of 30°C and below, *A. lumbricoides* eggs were not inactivated and at temperatures lower than 20°C, eggs can remain viable for over a year irrespective of humidity²¹.

To investigate factors that lead to pathogen die-off, most studies used to inoculate indicator organism, *Ascaris suum*, into manure and/or faeces while mimicking conditions in latrines²²⁻²⁴. These studies have informed guidelines on operation of EcoSan latrines but few of these have been conducted in real latrine situations^{3,22,23,25,26}. In addition, it is not appropriate to generalize conditions for recommended pathogen die-off due to environmental changes². This research therefore investigated pathogen survival in EcoSan sludge from EcoSan latrines with respect to time. Due to the existing practices of EcoSan users which affect stability of moisture reduction and increase in pH through addition of ash for pathogen die-off⁴, this study concentrated on the storage time after sealing the pit. The target pathogens were *A. lumbricoides*, hookworms, *Trichuris trichiura*, *Taenia spp*, *Salmonella* and *E. coli*. The results of this study will assist in modifying the management of EcoSan latrines and use of sludge which may impact the prevalence of excreta related diseases like STHs infection which have been reported as 16.5% in urban and 3.6% in rural areas among children aged 3 to 14 years in southern Malawi²⁷. The study will also assist in understanding the factors that lead to survival of pathogens in EcoSan and hence assist in reducing the health risk of using EcoSan sludge which have been found to be above WHO recommendation²⁸.

Methods

An observational longitudinal study was done. The observational design helped to understand what happens in

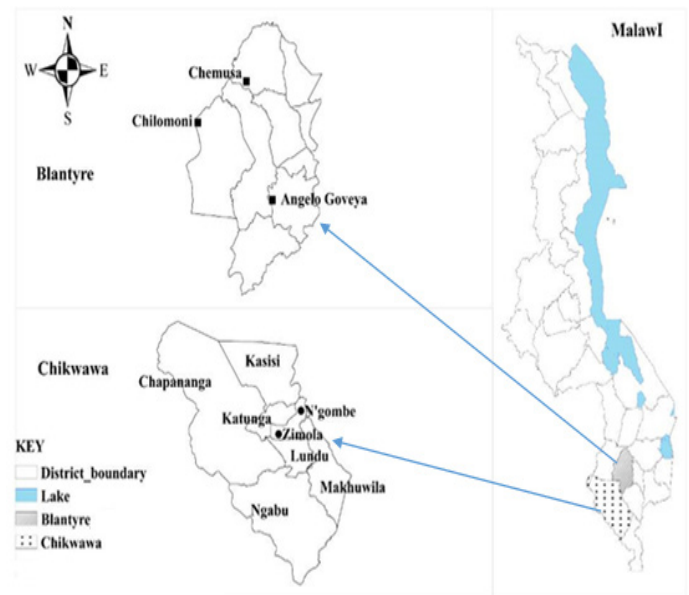


Figure 1: Map of Malawi showing study areas (Map drawn using ArcGIS). Blantyre and Chikwawa offer different climates for this study due to their variations in altitude.²⁹

latrines in real life situations as compared to experimental study. EcoSan latrines were followed for a period of twelve months from August 2015 to July 2016 in Blantyre and Chikwawa Districts respectively (Figure 1).

Sample size estimation

The sample size was estimated based on previous studies done in Malawi which found that the mean helminth concentration was 60 viable eggs per gram of EcoSan sludge (S.D.=38.5 helminth eggs per gram)⁴. Using STATA's test for two means with repeated measures and setting power at 80%, $\alpha = 0.05$, the minimum number of latrines in each location was estimated at 16. Since EcoSan latrines were few in the study areas, all eligible latrines were enrolled. In total, 51 latrines, 18 in Chikwawa and 33 in Blantyre were enrolled. The latrines enrolled had soil and ash for use after defaecation. Owners were encouraged to continue using ash and soil during the study period.

Sample collection

Samples were collected five times in a single latrine during the months of July and November in 2015 and February, May and July in 2016. The samples collected were examined for helminths, *Salmonella* and *E. coli*. The identification of latrines was done with the help of Non-Governmental Organizations (NGOs) who were promoting these latrines in the areas selected for the study. Purposive sampling was done due to the low number of EcoSan latrines in Malawi (0.3%)¹⁵. Eligible latrines were those that were currently in use and due to be sealed within a month from the time of the sampling. Samples during latrine use were collected from the latrine by scooping up to a depth of 30cm. Each sample weighed 200 to 300grams. One sampler was used and disinfected after sampling each latrine. One sample was collected from each latrine at a time. Samples were labelled and transported to laboratory within three hours of collection where they were placed in a refrigerator at 40°C and processed within 24 hours. Follow-up samples were collected after a latrine

was sealed within two weeks after the first sampling. The cover was removed, and the contents were sampled from the middle of pit by digging using a hoe. The follow-up samples were collected after 3, 6, 9 and 12 months of sealing the pit. Concentrations of pathogens were measured at baseline in July 2015 (first month of follow-up) and follow up samples were collected after the pits were sealed in November 2015 (5th month), February 2016 (8th month), May 2016 (11th month) and July 2016 (13th month).

Measurement of the microbiological quality of EcoSan sludge

STHs (*A. lumbricoides*, *Trichuris trichiura*, *Taenia* and hookworms) were measured using a modified triple floatation method³⁰. For each latrine sample collected, 20grams was weighed and washed using the ammonium bicarbonate solution. The final sample after washing was kept in a test tube and read under a microscope. STHs eggs were observed to determine their type and viability using morphological characteristics focussing on the shape, size, external layer and the inside of the ova. For *E. coli* and *Salmonella*, standard culture methods using Xylose cefixime tellurite sorbitol MacConkey and Lysine Deoxycholate agars were used.^{31–33} Moisture content was then measured to determine dry weight of the sample.

Moisture content, ammonia and pH

Moisture content was measured by subtracting the weights of EcoSan sludge after heating in the thermocentre oven at 105°C for 24 hours from the weight of 20g of sludge before heating³⁴. Ammonium content was measured using Palintest 1000 series meter³⁴. For pH, a 20g sample was weighed and put in a 100ml beaker to ensure a standard dilution of 1:5. Fifty milliliters (50ml) of distilled water was added and stirred using a magnetic stirrer for 10 minutes. The pH was then measured using calibrated METROHM 604 pH meter³⁵.

Data analysis

Longitudinal data analysis techniques for panel data were employed. Since main outcomes were count data (number of viable organisms), Poisson regression was used for both bivariate and multivariate analysis. The associations between pathogen die-off and time and place were evaluated. Bivariate Poisson regression was used to determine significant variables to be included in the model. Multivariate Poisson regression was also used to assess the association between pathogen concentration in samples examined after 12 months of follow-up and pH, ammonia and moisture content^{23,36}. Association at $p < 0.05$ were taken to be significant.

Ethical considerations

Ethical approval was obtained from College of Medicine Research Ethics Committee (COMREC) number P.04/14/1565. The approval was obtained in October 2014. A written informed consent form translated in local language (Chichewa) was administered and signed by the household head where a latrine was selected for inclusion in the study.

Results

Description of sample and drop-outs

Fifty-one (51) EcoSan latrines were enrolled (18 FAs in Chikwawa and 33 UDDLs in Blantyre) and sampled at baseline before sealing. Return visits for follow-up samples

was done at 3, 6, 9 and 12 months. A complete data set for all sampling times was achieved for 35 latrines (Chikwawa = 13; Blantyre = 22). Resampling was affected by lack of owner availability, and unwillingness to open sealed latrines for subsequent sampling. Latrines with full data were included in the results. All latrines with missing data were removed and regarded as drop-outs.

Prevalence during the follow up period

Table 1 shows the prevalence in July 2015 (baseline), November 2015, February 2016, May 2016 and July 2016 (end time).

Table 1: Percentage (number) of latrines with *E. coli*, *Salmonella*, *A. lumbricoides*, hookworms and *Taenia* in Chikwawa

Organism	Jul-15	Nov-15	Feb-16	May-16	Jul-16
<i>E. coli</i>	77% (10)	77% (10)	100% (13)	92% (12)	92% (12)
<i>Salmonella</i>	23% (3)	38% (5)	46% (6)	46% (6)	62% (8)
Hookworm	31% (4)	62% (8)	92% (12)	85% (11)	85% (11)
<i>A. lumbricoides</i>	54% (7)	46% (6)	46% (6)	38% (5)	31% (4)
<i>Taenia</i>	8% (1)	15% (2)	38% (5)	23% (3)	23% (3)

Apart from *A. lumbricoides* which showed a decreasing trend in the number of latrines with viable eggs in their sludge, all other organisms did not show any trend. The number of latrines with *E. coli*, *Salmonella*, hookworm and *Taenia* increased if we compare the prevalence at the beginning of the study to the results at the end of the study (Table 1).

Table 2: Percentage (number) of latrines with *E. coli*, *Salmonella*, *A. lumbricoides*, hookworms and *Taenia* in UDDLs

Organism	Jul-15	Nov-15	Feb-16	May-16	Jul-16
<i>E. coli</i>	77% (17)	82% (18)	91% (20)	82% (18)	86% (19)
<i>Salmonella</i>	18% (4)	68% (15)	59% (13)	77% (17)	68% (15)
Hookworm	68% (15)	64% (14)	77% (17)	86% (19)	91% (20)
<i>A. lumbricoides</i>	50% (11)	55% (12)	59% (13)	50% (11)	41% (9)

A decreasing trend was observed in the number of latrines with viable *Taenia* in their sludge in UDDLs while in FAs, no trend was observed. Despite showing a reduction in number of latrines with viable *A. lumbricoides* eggs in their sludge during the last month of follow-up as compared to the first, the number increased in November, 2015 and February, 2016 (Table 2). Combined data for the two geographical locations show an increase of hookworm prevalence from 54% to 89% while those with *A. lumbricoides* in their sludge decreased from 50% to 37%. District specific data show a greater decrease in number of latrines with viable *A. lumbricoides* eggs in Chikwawa than in Blantyre. The number of latrines with colony forming units for *E. coli* and *Salmonella* increased compared to baseline results for July, 2015 (Tables 1 and 2).

Pathogen concentration in EcoSan latrines during the 12 months follow up period

Table 3 show the concentration of pathogens in EcoSan sludge from latrines in both Chikwawa and Blantyre. The units for bacterial pathogens was colony forming units per

gram (cfus/g) while that for helminths was viable eggs per gram (eggs/g) of sludge.

Table 3: Mean pathogen concentration per gram of pit humus

Organism	Jul-15	Nov-15	Feb-16	May-16	Jul-16
<i>E. coli</i> (cfus/g)	2714.9	2031.9	2832.5	1211.2	910.0
<i>Salmonella</i> (cfus/g)	70.7	181.5	150.0	409.5	456.0
<i>A. lumbricoides</i> (eggs/g)	1.5	2.3	1.2	0.9	0.4
Hook-worms (eggs/g)	9.8	8.1	9.4	7.2	7.0
<i>Taenia</i> (eggs/g)	0.7	0.7	0.4	0.3	0.3

The average concentrations of all pathogens investigated reduced over the 12 month follow-up period except for *Salmonella* which increased after the follow-up period. *A. lumbricoides*, increased to 2.3 viable eggs during the second sampling and then decreased to 0.4 viable eggs per gram after 12 months of follow-up. During baseline, 48% (24) of EcoSan latrines had more than 1 viable egg per gram while after 12 months of follow-up; only 12% (6) had more than 1 viable eggs of *A. lumbricoides* per gram (Table 3). The US EPA 1997 standard for Helminths is that pit humus should contain less than 1 viable egg per gram, for *Salmonella* there should be no detectable colonies per 50grams while for *E. coli*, it's no more than 100 MPN.11

Predictors of Pathogen concentration in EcoSan sludge

The dependent variables were concentration of pathogens while type of EcoSan latrine (location) and time in months were the independent variables. All significant dependent variables after a bivariate Poisson regression were put in a multivariate Poisson model and results are presented in Table 4. A p-value of less than 0.05 shows that there is an association between the pathogen concentration and the predictor variable. In case of coefficients, for a one-unit change in the predictor variable, the difference in the logarithm of expected counts is expected to change by the respective regression coefficient, if the other predictor variables in are held constant. Type of latrine and location were not significant predictors of helminths concentration ($p > 0.05$). However, *Salmonella* and *E. coli* colonies were significantly higher in Blantyre (UDDLs) than Chikwawa (FAs) ($p < 0.05$) (Table 4).

A unit increase in storage time in months led to the decrease in log count of *E. coli* colonies, *A. lumbricoides*, hookworms and *Taenia* eggs per gram of sludge. Time in months was the only consistent predictor for the concentration of helminths.

Factors affecting the pathogen levels in the EcoSan sludge after 12 months of follow-up

After twelve months of follow-up, the EcoSan sludge was ready to be applied in the gardens. The laboratory results for the last follow-up sample was analysed separately to see the predictors of the concentration of pathogens. The

Table 4: Multivariate analysis of pathogen concentration, time and type of EcoSan latrine (location) of the EcoSan latrine

Pathogen	Factor	Coefficient	Standard error	P-value
<i>E. coli</i>	Time (months)	-0.478	0.024	<0.001
	EcoSan type (Location)	-0.265	0.099	0.008
<i>Salmonella</i>	Time (months)	0.431	0.060	<0.001
	EcoSan type (Location)	0.937	0.263	<0.001
Hook-worms	Time (months)	-0.046	0.033	<0.001
	EcoSan type (Location)	-0.270	0.142	0.88
<i>A. lumbricoides</i>	Time (months)	-0.567	0.105	<0.001
	EcoSan type (Location)	0.328	0.309	0.288
<i>Taenia</i>	Time (months)	-0.337	0.120	0.005
	EcoSan type (Location)	-0.134	0.572	0.814

mean concentration of pathogens and moisture content, ammonia and pH measured in the sample collected after 12 months of follow-up are summarized in Table 5.

Table 5: Summary table for pathogen levels and mean ammonia, moisture content and pH levels after 12 months of follow-up

Parameter	FA (Chikwawa)	UDDL (Blantyre)	P-value
<i>A. lumbricoides</i> (eggs/g)	0.38	0.41	0.54
Hookworm (eggs/g)	10.15	5.14	0.04
<i>Taenia</i> (eggs/g)	0.31	0.27	0.44
<i>E. coli</i> (cfus/g)	1007.69	859.09	0.36
<i>Salmonella</i> (cfus/g)	346.15	509.09	0.76
Moisture content (%)	13.38	11.91	0.17
Ammonia (mg/l)	84.15	91.86	0.78
pH	8.92	8.68	0.27

The samples had a mean of less than one viable egg per gram for *A. lumbricoides* and *Taenia*. Hookworm concentration were at 10.15 viable eggs per gram in FAs and 5.14 viable eggs per gram for UDDLs (Table 5).

Predictors of pathogen concentration in EcoSan sludge after 12 months of storage

The results from Table 3 show that *Taenia* and *A. lumbricoides* concentration was not related to moisture content, pH,

Table 6: Multivariate analysis for pathogens and moisture content, ammonia and pH for FAs (Chikwawa) and pathogen content in relation to moisture content and pH for UDDLs (Blantyre)

		FAs (Chikwawa)			UDDLs (Blantyre)		
Pathogen	Factor	Coefficient	Standard Error	P-Value	Coefficient	Standard Error	P-Value
<i>E. coli</i>	Moisture content	0.031	0.011	0.004	-0.090		<0.001
	pH	0.220	0.105	0.04	0.388		<0.001
<i>Salmonella</i>	Moisture content	-	-	-	-0.090	0.020	<0.001
	Ammonia	0.052	0.016	0.001	-	-	-
	pH	-	-	-	0.388	0.090	<0.001

ammonia in FAs and moisture content and pH in UDDLs. *E. coli* was predicted by pH and moisture content in both FAs and UDDLs, *Salmonella* was predicted by ammonia in FAs while in UDDLs, it was predicted by moisture content and pH (Table 6). Hookworm, *A. lumbricoides* and *Taenia* concentration were left out because they showed no significant relationships with all predictors under consideration during a bivariate Poisson regression. The residual pH, moisture content and ammonia were unable to predict the concentration of helminths in the sample after 12 months of storage.

Discussion

Despite showing a reduction in mean concentration of hookworm eggs per gram of sludge, the number of latrines with the helminths in their sludge increased. This might be due to high prevalence of hookworms in the population and the poor practices of households members in terms of management of stored sludge leading to contamination¹⁴. *A. lumbricoides* showed a decrease in both number of viable eggs per gram and number of EcoSan latrines with the helminth during the follow-up period. In terms of helminths, it was only the hookworm that was above the recommended guidelines for use of excreta¹⁰. *A. lumbricoides* despite being an indicator organism²⁵, was unable to predict the concentration of hookworm which is less resistant to environmental conditions than *A. lumbricoides*. The absence of *A. lumbricoides* meant that other less resistant helminths were also supposed to be inactivated before *A. lumbricoides* but this was not the case. This could be because of the high levels of environmental contamination due to poor hygiene practices.

It was found that pathogen concentrations were significantly related to time in months. As storage time increased, there was a reduction in log concentration of pathogens per gram of sludge. FA rely on ammonia from urine and pH from the ash added after use to kill pathogens while UDDLs mainly depend on drying the pit contents and increase in pH to sanitize pit contents. Moisture content, pH and ammonia were measured in the samples from FAs while moisture content and pH were determined from samples from UDDLs. The level of moisture content in the last sample taken in July, 2016 was within the recommended level of less than 25% as stipulated in the WHO guideline for use

of excreta after more than 6 months of storage³⁷. However, the moisture content was greater than the United States Environmental Protection Agency standard of 1992 which states that it should be less than 5%.³⁸ The mean pH for Chikwawa and Blantyre respectively were lower than the effective pH of more than 12. Ammonia only reached an average of at most 92mg/l instead of the recommended concentration of

280mg/l.³⁸ Despite finding ammonia content and pH for the two types of latrines (locations) below the effective level for pathogen die-off, we could not tell if levels were high during other months where these parameters were not measured.

A multivariate analysis was performed to assess the factors that predict the concentration of pathogens in the final sample. Moisture content and pH were able to predict concentration of *E. coli* and *Salmonella* while no factor was related to helminths' concentration in the last sample. Though moisture content, pH and ammonia have proved to be important factors for the inactivation of helminths, their effect on hookworms, *A. lumbricoides* and *Taenia* was not significant partly because the recommended minimum levels for rapid inactivation of 5%, 12 and 280mg/l respectively were not reached^{3,38}. Inconsistent use of ash was also suspected to be the main reason for lower pH levels as indicated in an earlier study¹⁴. Environmental factors including pH, temperature, humidity, moisture content and ammonia might not have reached lethal levels for pathogen die-off during the other months where these parameters were not monitored, therefore, it was difficult to conclude any relationship to pathogen concentration.

Since all FAs and UDDLs were from Chikwawa and Blantyre respectively, it was impossible to compare between same types of latrine within the same location. Lack of control made it difficult to explain how the prevalence of viable helminths eggs increased in sludge between November 2015 and February 2016. Observational studies fail to control extraneous factors which may obscure the effect of temperature and humidity³⁹. Despite the limitations, the paper provides important information in the field of ecological sanitation that will help decision makers in their planning process and provides areas for improvements in further research.

Conclusion

In uncontrolled environments where compliance to EcoSan guidelines were not guaranteed, type of EcoSan latrine and time in months were related to the concentration of pathogens in EcoSan sludge. No factor was a consistent predictor for the concentration of helminths in the last sample collected after 12 months of follow-up. The study also concluded that *A. lumbricoides* may not be the

main indicator organism for safety of sludge where other helminths are highly prevalent causing a public health risk and we recommend use of total helminths as outlined in the WHO and US EPA guidelines as a measure of sludge safety. Since EcoSan sludge has been found to still contain high Hookworm eggs, *E. coli* and *Salmonella* even after the waiting period of more than 12 months, protection during handling and use in agriculture is important. Storage time of 12 months or more is recommended.

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Authors' contributions

Save Kumwenda conceptualized the research, took part in data collection, analysed the data and drafted the initial manuscript; Chisomo Msefula revised the research idea, assisted in drafting and revising the manuscript; Wilfred Kadewa assisted in conceptualization of the research idea, assisted in drafting and revising the manuscript; Davis Makupe assisted in data analysis and assisted in writing and revising the manuscript; Bagrey Ngwira took part in conceptualizing the research and revising the manuscript; Tracy Morse took part in conceptualizing the research, drafting and revising the manuscript.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

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