Research Paper

Estimation of *Ascaris lumbricoides* egg inactivation by free ammonia treatment of ash-amended UDDT vault products using stored urine in Uganda

John T. Trimmer, Neema Nakyanjo, Robert Ssekubugu, Marc Sklar, James R. Mihelcic and Sarina J. Ergas

ABSTRACT

Urine-diverting dry toilets (UDDTs) are designed to recover nutrients and organic matter from human excreta for use as agricultural amendments, and have been promoted in many developing countries, including Uganda. Wider UDDT implementation could help address problems in areas where water scarcity limits sanitation coverage and/or declining soil fertility jeopardizes growing populations' nutritional security. However, concerns have been raised regarding the safety of recovered UDDT vault products, which may contain persistent pathogens such as *Ascaris lumbricoides* eggs. *A. lumbricoides* eggs can be inactivated through elevation of free ammonia levels. This study assessed the feasibility of a secondary ammonia treatment strategy for UDDT ash-amended vault products using urine. Treatment parameters were measured in mixtures of urine, ash-amended vault products, and wood ash, a model was developed to account for temperature fluctuations, and *A. lumbricoides* egg inactivation times were estimated using a previously published model. A mixture containing two parts urine and one part ash-amended vault products was estimated to provide 2-$\log_{10}$ inactivation after 3 months of indoor storage (daily mean temperatures: 22.8 ± 0.3 °C) or 2 months of outdoor storage (25.9 ± 1.3 °C). This strategy could improve the safety of recovered products for agricultural use to improve the nutritional security of vulnerable populations.

Key words | ecological sanitation, helminth, resource recovery, sanitation, sub-Saharan Africa, urine-diverting dry toilet

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INTRODUCTION

Ecological sanitation (Eco-San) systems are designed to recover nutrients and organic matter from human excreta for agricultural use (Esrey *et al.* 2001). Wider implementation of these systems could help to address sanitation and food security problems in countries where water scarcity limits improved sanitation coverage, and/or declining soil fertility jeopardizes growing populations' nutritional security (NEMA 2010). For example, water availability is a barrier to as many as 46 million people living without improved sanitation (Fry *et al.* 2008). Furthermore, if collected, the phosphorus in urine and feces could account for 22% of the total global phosphorus demand (Mihelcic *et al.* 2011). However, hygienic quality must be ensured to protect the safety of agricultural workers and consumers.

Urine-diverting dry toilets (UDDTs) are a type of Eco-San system designed to treat feces through alkaline desiccation without water. They separate urine from feces, and desiccants are added to fecal vaults to increase pH (>9.0) and decrease moisture content (<25%). Given sufficient time, conditions in UDDT vaults have been shown to inactivate numerous

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pathogens (Stenström 2001). The World Health Organization (WHO 2006) recommends that at least 1 year of storage is needed at temperatures of between 20 and 35 °C. However, this timeframe may be insufficient to eliminate more resistant pathogens (Hawksworth *et al.* 2010; Mehl *et al.* 2011), such as *Ascaris lumbricoides* eggs (WHO 2006).

Soil-transmitted helminths (STHs), including *A. lumbricoides*, are intestinal worms that cause infection (PAHO 2014). Globally, up to 5.3 billion people are at risk of STH infection (Pullan & Brooker 2012), with an estimated 1.3 billion infected with *A. lumbricoides* (Hawksworth *et al.* 2010). Ascariasis is endemic in areas of Latin America, Asia, and Africa, with children being greatly affected (Hawksworth *et al.* 2010). In Rakai District, Uganda (this study's location), Kabatereine *et al.* (2001) reported that 28.8% of tested schoolchildren were infected with *A. lumbricoides*, with a mean egg count of 2,289 eggs per gram feces, representing infections of light to moderate intensity. Heavy infections can exceed 50,000 eggs per gram (Smith *et al.* 2001). The WHO (2006) has set a tolerable additional disease burden of 10^{-6} disability-adjusted life years regarding agricultural application of UDDT products. To remain below this level, treated feces must contain below one egg per gram (WHO 2006). To achieve this concentration in the worst cases of infection, a 5-$\log_{10}$ overall inactivation of *A. lumbricoides* eggs is required.

In most UDDT vaults, conditions are inadequate to provide 5-$\log_{10}$ inactivation of *A. lumbricoides* eggs after 1 year. A moisture content below 5% is required for complete inactivation (WHO 2006), and some eggs can survive for 700 days when exposed to pH levels between 9 and 11 (Moe & Izurieta 2003). *A. lumbricoides* egg inactivation rates have been shown to be highly dependent on temperature. For example, eggs were inactivated in a matter of weeks when peak temperatures in solar toilets exceeded 36 °C (Moe & Izurieta 2003). In contrast, Brownell & Nelson (2006) reported that eggs survived for over 1 year at 40 °C but were destroyed in minutes above 60 °C. Furthermore, in mesophilic anaerobic digesters operated at 35 °C, approximately 65% of *A. lumbricoides* eggs remained viable for 16 days, after which inactivation increased until nearly all eggs were nonviable by day 24 (Manser *et al.* 2015). In most instances, the required temperatures are not achieved in UDDTs (Hawksworth *et al.* 2010; Mehl *et al.* 2011). Accordingly, based on an evaluation of the treatment effectiveness of alkaline desiccation (WHO 2006), 2-$\log_{10}$ inactivation was assumed to occur in fecal vaults. An additional 1-$\log_{10}$ decrease due to die-off in soil after agricultural application can further reduce risk (WHO 2006); however, an additional 2-$\log_{10}$ reduction is still required to achieve 5-$\log_{10}$ inactivation. Therefore, a secondary treatment step is required after vault desiccation and prior to agricultural application.

A number of options for secondary treatment of UDDT vault products exist, including the elevation of free ammonia levels. Free ammonia's destructive effect on *A. lumbricoides* eggs is related to the molecule's small size and high solubility in water and lipids, enabling it to pass through cellular barriers and disrupt internal chemistry (Nordin 2010). Elevated free ammonia can be achieved by adding urea (Nordin *et al.* 2009; Cruz-Espinoza *et al.* 2012). Urea hydrolysis, catalyzed by urease-positive bacteria ubiquitous in sanitation systems, produces ammonia and increases pH (O'Neal & Boyer 2013).

Several laboratory studies have demonstrated that adding urea can inactivate *Ascaris suum* eggs, which infect swine. These eggs are used in place of *A. lumbricoides* in laboratory studies because of their relative availability and safety (Nordin *et al.* 2009; Manser *et al.* 2015). Inactivation rates of both species are similar when subjected to ammonia treatment (Nordin *et al.* 2009). Vinnerås *et al.* (2004) observed no viable eggs after 50 days when 30,000 mgN/L urea was added to source-separated fecal matter. Pecson *et al.* (2007) added ammonia at concentrations of 1,000 and 5,000 mgN/L to municipal sludge and found that temperature, pH, and ammonia all influenced die-off rates. Nordin *et al.* (2009) found that approximately 800 mgN/L of free ammonia achieved 2-$\log_{10}$ inactivation in fecal matter after 35 days at 24 °C. At 34 °C, 1,000 mgN/L caused 2-$\log_{10}$ inactivation in 4 days. Cruz-Espinoza *et al.* (2012) found that, under conditions simulating dry toilet vaults in El Salvador, free ammonia concentrations of 1,110 mgN/L achieved complete inactivation after 14 days at 28 °C.

While urea can be obtained as fertilizer, chemical fertilizers are too expensive for many farmers in sub-Saharan Africa (Wambui 2011). A potential alternative for secondary treatment of vault products is stored urine. Urine is high in nitrogen, 75% to 90% of which is present as urea, with the remainder being mostly ammonium ion (Mihelcic *et al.* 2011). Two studies (McKinley *et al.* 2012; Fidjeland *et al.* 2013) have investigated ammonia treatment of

A. lumbricoides eggs using urine, but this strategy has not been studied previously using ash-amended vault products from Uganda. McKinley *et al.* (2012) considered dry toilets in Bolivia, but the use of sawdust as a desiccant produced pH levels lower than those observed in locations where highly-alkaline wood ash is used, such as Panama (Mehl *et al.* 2011) and Uganda (Kamuteera *et al.* 2013). Due to the acid-base equilibrium of ammonia, pH is critical in determining achievable free ammonia concentrations. McKinley *et al.* (2012) found that a mixture including vault products (feces and sawdust), stored urine, and supplementary ash resulted in free ammonia concentrations between 2,200 and 2,800 mgN/L and achieved 2-$\log_{10}$ inactivation after 8 weeks at 19.5 °C. Fidjeland *et al.* (2013) simulated conditions in pit latrines, vacuum toilets, and pour-flush latrines using un-amended feces and urine collected in Sweden. At 23 °C, 3-$\log_{10}$ reductions were achieved after 1–6 months depending on the free ammonia concentration, which ranged from 620 to 4,760 mgN/L.

This study, conducted in Uganda, investigated urine treatment of ash-amended UDDT vault products. Due to the use of wood ash as the desiccant in UDDTs investigated in this research, the composition and pH differed from those of prior studies. Additionally, urine from Uganda is likely to contain less total ammonia than urine from other locations, due to low protein availability (FAOSTAT 2015). Protein consumption affects the amount of nitrogen excreted in urine (Jönsson *et al.* 2004), and treatment with stored urine from protein-limited populations may be less effective for *A. lumbricoides* inactivation. Nordin *et al.* (2013) reported total ammonia concentrations in undiluted urine in Uganda to be 4.0 ± 1.5 g N/L, whereas typical global values are calculated to be 8.3 ± 3.5 g N/L (Friedler *et al.* 2013). Therefore, this study assessed free ammonia treatment using urine from an area with protein-related nutritional insecurity (FAOSTAT 2015) and provides a conservative assessment of this strategy on a global level.

Accordingly, the objective of this study was to estimate the potential for free ammonia treatment of ash-amended vault products using urine to achieve 2-$\log_{10}$ inactivation of *A. lumbricoides* eggs in Uganda. If this strategy is estimated to be successful in Uganda, where total ammonia concentrations in urine are low, free ammonia treatment could represent a globally applicable secondary treatment alternative able to reduce health risks associated with persistent pathogens and improve the safety of reusing recovered products in agriculture.

METHODS

This study, performed in Kalisizo, Uganda, evaluated mixtures of ash-amended vault products (including feces, wood ash, and toilet paper; pH = 10.5), stored urine (pH = 9.0), and wood ash (pH = 10.5). Materials were collected from a UDDT that had been operating for approximately 6 months at a primary school (Trimmer *et al.* 2016). Students using the UDDT had been instructed to add 1–2 cups (200–500 ml) of ash after use (Mihelcic *et al.* 2006). Two stages of experiments are summarized in Table 1.

Stage 1: supplementary wood ash to elevate pH

Stage 1 focused on adding supplementary wood ash to raise pH. One volumetric mixture contained two parts stored urine (U), one part ash-amended vault products (V), and one part supplementary ash (A) (*2U:1V:1A*), while a second mixture contained half the volume of supplementary ash (*2U:1V:0.5A*). A third mixture contained only urine and ash-amended vault products (*2U:1V:0A*). All mixtures were set up in triplicate in closed, half-liter plastic bottles. The volume of each mixture was approximately 400 mL, leaving approximately 100 mL of headspace. Mixtures were stored indoors for 113 days at ambient temperature.

Temperature, pH, and total ammonia concentration ($NH_3 + NH_4^+$) were measured in each mixture at the beginning of the testing period and then once per week. pH was measured using indicator strips (Machery-Nagel), and temperature was measured according to Standard Method 2550-B (APHA 2012). Using samples collected and diluted after mixing each bottle, total ammonia was measured using Seachem Multitest: Ammonia Test Kits. The final moisture content of each mixture was measured after the testing period ended according to Standard Method 2540-G (APHA 2012); however, samples were dried using a solar oven, and extended drying times were required (5–8 hours/day for 5–10 days). Additional details regarding pH and total ammonia measurements, and results of quality assurance/quality

Table 1 | Average treatment conditions for all mixtures in Stages 1 and 2 (mean and standard deviations reported for triplicate treatments)

		Average results ± Standard deviation				
Stage	**Volumetric mixture (parts urine:parts ash-amended vault products : parts supplementary wood ash)[a]**	**Final moisture content**	**pH**	**Temperature (°C)**	**Total ammonia (mg/L NH_3)**	**Free ammonia (mg/L N)**
1[b]	*2U:1V:0A*	84 ± 0%	9.6 ± 0.2	22.2 ± 0.6	2,800 ± 400	1,200 ± 300
	2U:1V:0.5A	76 ± 1%	9.8 ± 0.3	22.2 ± 0.6	2,300 ± 300	1,300 ± 300
	2U:1V:1A	70 ± 4%	10.3 ± 0.3	22.3 ± 0.6	1,700 ± 200	1,200 ± 200
2[c]	*2U:1V:0A* - in	83 ± 3%	9.6 ± 0.2	22.4 ± 0.9	2,800 ± 200	1,300 ± 300
	2U:1V:0A - out	82 ± 1%	9.6 ± 0.2	35.0 ± 9.7	2,800 ± 200	1,700 ± 400
	2U:1V:0.5A - out	70 ± 3%	10.0 ± 0.2	35.3 ± 10.1	2,300 ± 200	1,700 ± 300

[a]U: urine; V: ash-amended vault products; A: supplementary wood ash.
[b]Stage 1 focused on adding supplementary ash to elevate pH; mixtures were stored indoors for 113 days; pH, temperature, and total ammonia were measured (and free ammonia was calculated) weekly for triplicate mixtures ($n = 3 * 17$ weeks $= 51$); moisture content was measured after the stage had been completed ($n = 3$).
[c]Stage 2 focused on outdoor storage to elevate temperature; one mixture (*2U:1V:0A*) was stored indoors and two mixtures (*2U:1V:0A; 2U:1V:0.5A*) were stored outdoors for 80 days; pH, temperature, and total ammonia measurements were collected (and free ammonia was calculated) weekly for triplicate mixtures ($n = 3 * 12$ weeks $= 36$); moisture content was measured after the stage had been completed ($n = 3$).

control tests assessing the accuracy of total ammonia measurements, are available in the supplementary material (this can be found in the online version of this paper). Using measured pH, temperature, and total ammonia values, with acid-base relationships for non-dilute solutions (Mihelcic & Zimmerman 2014), free ammonia concentrations were calculated for each sampling day.

Stage 2: outdoor storage to elevate temperature

Stage 2 compared indoor and outdoor storage, to determine how mixture temperatures changed. Two mixtures (*2U:1V:0A, 2U:1V:0.5A*) were stored outside on an iron roof, with a control mixture (*2U:1V:0A*) located indoors to determine whether indoor conditions were significantly different between Stages 1 and 2. This stage lasted for 80 days, and temperature, pH, and total ammonia were measured as in Stage 1. Given that all treatment conditions measured for the indoor *2U:1V:0A* control mixture were not significantly different from the Stage 1 *2U:1V:0A* mixture (all $p \geq 0.744$), indoor mixture conditions in both stages were assumed to be equivalent, allowing for direct comparisons.

Additional temperature measurements: the 'typical day' model

As most mixture temperatures were measured during daylight, calculated average temperatures were higher than actual daily mean temperatures (i.e., averages over the entire day and night). Therefore, over a period of 291 days (23 January, 2014–9 November, 2014), outdoor and indoor ambient temperatures were measured ($n = 165$) at varying times throughout the day and night, and were used to develop a sinusoidal ambient temperature model over a 'typical day'. Linear relationships were developed between mixture and ambient temperatures using another set of outdoor ($n = 15$) and indoor ($n = 29$) measurements.

To quantify the uncertainty surrounding this model and estimate the expected distributions of daily mean mixture temperatures, 100,000 Monte Carlo simulations were performed. The fitted constants of the ambient temperature equations were treated as normally distributed random variables, with expected values and standard deviations taken from the regression analysis. Outdoor and indoor daily mean mixture temperatures could then be estimated.

Estimating *A. lumbricoides* egg inactivation potential

Due to resource limitations, *A. lumbricoides* inactivation could not be measured directly in the field. Instead, a model developed by Fidjeland *et al.* (2015) was used to estimate potential inactivation resulting from treatment conditions in Uganda. This model, created and validated using various literature data, estimates *A. lumbricoides* egg

inactivation as a function of temperature and free ammonia (Equation (1)):

$$t = \frac{3.2 + LRV}{10^{(-3.7+0.062T)}\mathrm{NH}_3^{(0.7)}}(1.14) \quad (1)$$

where t is the time (days) to achieve the inactivation level defined by LRV, representing $\log_{10}$ reduction in egg viability, T represents temperature (°C), NH_3 represents the free ammonia concentration (mmol/L), and the safety factor (1.14) accounts for the 99% confidence interval from error propagation (Fidjeland *et al.* 2015). Total ammonia and pH are indirectly incorporated through their effects on free ammonia concentration.

To calculate the 2-$\log_{10}$ inactivation time for each mixture, the daily mean mixture temperatures from the 'typical day' model were used instead of the measurements, since the modeled values are more conservative and likely more representative of overall conditions. Free ammonia concentrations were recalculated for each mixture and used in Equation (1). All treatment conditions fell within the valid ranges for Equation (1), as defined in Fidjeland *et al.* (2015).

To assess the level of uncertainty surrounding the calculated times, 100,000 Monte Carlo simulations were performed, treating total ammonia, pH, and daily mean temperature as normally distributed random variables. For an additional factor of safety when developing treatment recommendations, the 99th percentile values from the resulting inactivation time distributions were used. Finally, the individual impact of each input parameter (pH, total ammonia, temperature) was assessed through a one-at-a-time sensitivity analysis. Due to varying scales and uncertainty levels surrounding the parameters, each parameter's 1st, 5th, 25th, 75th, 95th, and 99th percentile values were used while holding other parameters at their 50th percentile values, so that the effects of various changes to one parameter could be determined.

Statistical analyses

Significance testing of physical and chemical measurements was performed using one-way analysis of variance tests followed by Holm-Sidak tests for multiple comparisons, conducted in SigmaPlot 13.0 (Systat Software Inc.). p values below 0.05 were considered statistically significant. Linear and non-linear temperature modeling was also performed in SigmaPlot. Monte Carlo simulations associated with the temperature and inactivation models, as well as the sensitivity analysis, were conducted in Matlab R2015a (Mathworks, Inc.).

RESULTS

Treatment conditions in stages 1 and 2

A summary of treatment conditions in all Stage 1 and 2 mixtures is provided in Table 1. Mixtures with different mix ratios exhibited total ammonia and pH levels that were significantly different ($p < 0.05$). In contrast, no mixtures stored in the same location (indoors or outdoors) exhibited statistically significant temperature differences, suggesting that the mix ratio does not significantly impact mixture temperature. Similarly, calculated free ammonia levels for mixtures in the same location were statistically equivalent. Mixtures with higher supplementary ash fractions exhibited lower total ammonia concentrations but higher pH values, meaning that a larger percentage of total ammonia was present as free ammonia. For a given location, this 'balancing' pushed all free ammonia concentrations into a similar range.

However, while free ammonia levels in outdoor mixtures (1,700 mgN/L) balanced with one another, they differed from those in indoor mixtures (1,200–1,300 mgN/L), because higher outdoor temperatures shifted equilibrium toward free ammonia. Stage 2 free ammonia concentrations over time are shown in Figure 1. All mixtures exhibited decreasing trends, resulting from total ammonia losses caused by periodic opening for sampling. These losses should not be problematic in actual systems, where periodic opening would not be recommended (Nordin *et al.* 2009).

Figure 1 also shows greater variability in outdoor mixtures. For example, when comparing actual data points with regression lines, the outdoor *2U:1V:0A* mixture's sum of squared residuals (1.2×10^6) was higher than the indoor mixture's (3.0×10^5). Especially on colder days, outdoor concentrations decreased substantially. On day 9, the outdoor *2U:1V:0A* mixture's free ammonia concentration fell below that of the indoor mixture. *A. lumbricoides* egg inactivation

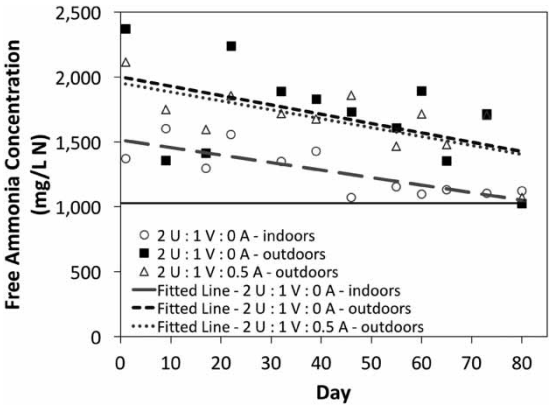

Figure 1 | Average free ammonia concentrations measured over time in Stage 2 mixtures and associated fitted lines. The horizontal solid line represents the concentration required to achieve 2-$\log_{10}$ inactivation of *A. lumbricoides* eggs in 60 days at the measured indoor average temperature of 22.4 °C, as calculated using Equation (1) (Fidjeland *et al.* 2015).

by ammonia treatment depends highly on temperature, possibly because the shell lipid layer exhibits increased permeability at higher temperatures (Nordin *et al.* 2009). For example, using Equation (1) (Fidjeland *et al.* 2015), a concentration of 1,030 mg/L NH_3-N at 22.4 °C (the measured average indoor mixture temperature) results in 2-$\log_{10}$ inactivation after 60 days. At 35.0 °C (the outdoor *2U:1V:0A* mixture's measured average temperature), the same concentration results in 2-$\log_{10}$ inactivation after 10 days. Higher overall temperature is beneficial, but the effect of variations is uncertain. McKinley *et al.* (2012) found that ammonia treatment was less effective when free ammonia concentration was not sustained over time, which could be caused by a temperature decrease. However, Nordin *et al.* (2013) reported that, for urine stored at equal average temperatures, samples exposed to greater daily temperature fluctuations exhibited higher inactivation.

Additional temperature measurements and modeling

Although outdoor mixtures seemed to exhibit higher average temperatures than indoor mixtures, it is important to note that the temperature datasets in Table 1 were collected during daylight hours. To more fully consider daily temperature variations, additional ambient and mixture temperature measurements were collected throughout the day and night (Figure 2). Indoor ambient temperatures ($n = 165$) were relatively consistent, ranging from 20.6 to 25.1 °C and averaging 22.8 °C, while outdoor ambient temperatures ($n = 165$) varied considerably, ranging from 16.1 to 32.9 °C and averaging 23.1 °C. Indoor mixture temperatures ($n = 29$) were similar to indoor ambient temperatures, while outdoor mixture temperatures fluctuated more widely than ambient temperatures, ranging from 15.9 to 48.1 °C and averaging 31.5 °C.

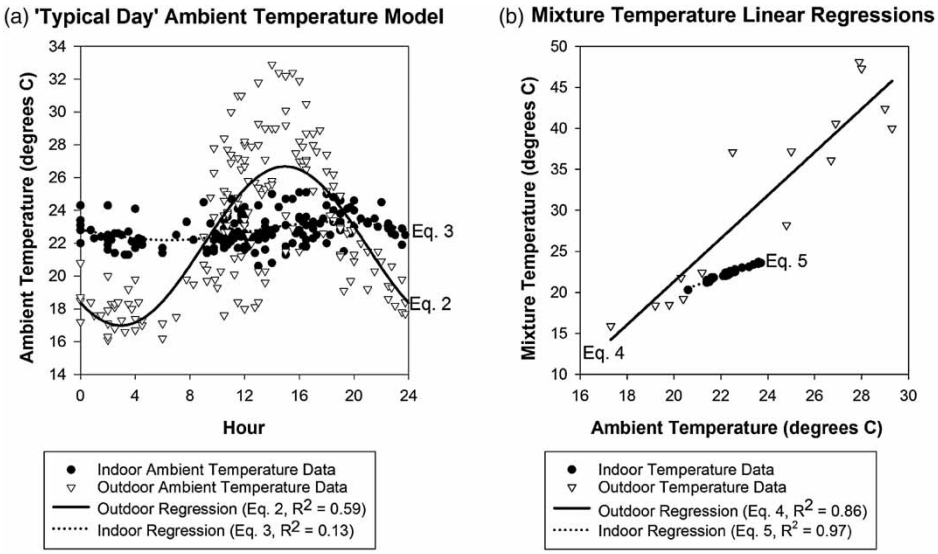

Figure 2 | 'Typical day' ambient and mixture temperature modeling: (a) ambient temperature measurements with sinusoidal regression models over a 24-hour period; (b) linear regression model relating ambient temperatures to mixture temperatures.

While these additional datasets provide a fuller picture, they were still skewed, since most measurements occurred during daylight hours. To evenly represent temperature variations throughout an entire day, all ambient temperature measurements were plotted over a single 24-hour period (Figure 2(a)). Sinusoidal regressions (Equations (2) and (3)) were fitted to the outdoor and indoor datasets, portraying a 'typical day' in Kalisizo, Uganda:

$$T_{outdoor-amb} = 21.8^{\circ}\mathrm{C} + \left[(4.85^{\circ}\mathrm{C}) * \sin\left(\left(\frac{2\pi}{24}\mathrm{hour}^{-1}\right) * t_h + 3.94\right)\right] \quad R^2 = 0.59 \tag{2}$$

$$T_{indoor-amb} = 22.8^{\circ}\mathrm{C} + \left[0.559^{\circ}\mathrm{C} * \sin\left(\left(\frac{2\pi}{24}\mathrm{hour}^{-1}\right) * t_h + 3.00\right)\right] \quad R^2 = 0.13 \tag{3}$$

where t_h is hourly time (e.g., 1:30 PM is 13.5), while $T_{outdoor-amb}$ and $T_{indoor-amb}$ are outdoor and indoor ambient temperatures (°C), respectively. Linear regressions were developed relating ambient temperatures to mixture temperatures as follows (Figure 2(b)):

$$T_{outdoor-mix} = 2.63 * T_{outdoor-amb} - 31.3^{\circ}\mathrm{C} \quad R^2 = 0.86 \tag{4}$$

$$T_{indoor-mix} = 0.872 * T_{indoor-amb} + 2.83^{\circ}\mathrm{C} \quad R^2 = 0.97 \tag{5}$$

where $T_{outdoor-mix}$ and $T_{indoor-mix}$ represent outdoor and indoor mixture temperatures (°C), respectively. Using Equations (2) through (5), outdoor and indoor mixture temperatures were modeled over an entire 'typical day' to more fully represent daily temperature cycles.

Running this model over 100,000 Monte Carlo simulations estimated daily mean outdoor and indoor mixture temperatures of 25.9 ± 1.3 and 22.8 ± 0.3 °C, respectively. The indoor mean is similar to measurements from Stages 1 and 2, while the outdoor mean is considerably lower than measured outdoor mixture temperatures. In place of measured mixture temperatures, the more conservative model values were used when estimating inactivation.

Estimating *A. lumbricoides* egg inactivation potential

To estimate the potential for *A. lumbricoides* egg inactivation, free ammonia concentrations were recalculated using modeled daily mean mixture temperatures combined with measured total ammonia and pH values. These free ammonia and temperature values were input into Equation (1) (Fidjeland *et al.* 2015) to estimate 2-$\log_{10}$ inactivation. 100,000 Monte Carlo simulations were employed to account for uncertainty in the input parameters.

Results for indoor and outdoor mixtures are presented in Figure 3, which shows the 50th, 95th, and 99th percentiles of the inactivation time distributions. For indoor mixtures, the estimated 50th percentile inactivation times fall between 45 and 48 days, while 99th percentile values (representing near-worst cases) range from 71 to 88 days. The estimated inactivation times for outdoor mixtures were faster. For the *2U:1V:0A* and *2U:1V:0.5A* mixtures, 50th percentiles were 27 and 26 days, respectively, while 99th percentiles were 51 and 43 days, respectively. Outdoor treatment may be more effective than predicted by the model because fluctuating temperatures were shown to provide move efficient treatment by Nordin *et al.* (2013). In all cases, 2-$\log_{10}$ inactivation is estimated to be much shorter than the 12-month treatment period recommended for alkaline desiccation of vault products (WHO 2006).

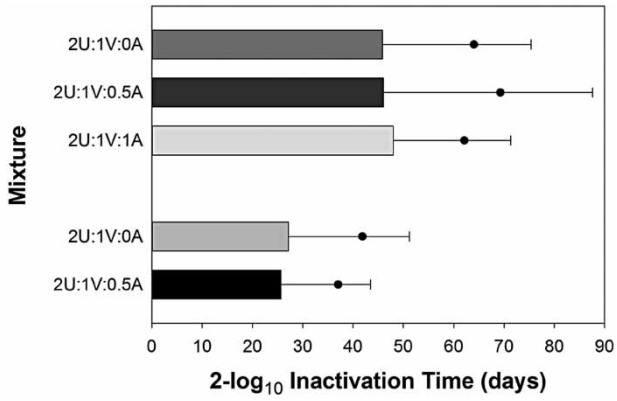

Figure 3 | Estimated treatment times (in days) required for 2-$\log_{10}$ inactivation of *A. lumbricoides* eggs in test mixtures of stored urine, ash-amended vault products, and supplementary wood ash. Each filled bar represents the 50th percentile inactivation time from 100,000 Monte Carlo simulations. Each black point represents the 95th percentile, and the end of each whisker represents the 99th percentile.

DISCUSSION

Treatment recommendations

Given the estimated inactivation times in Figure 3, the mixtures and treatment conditions most likely to be feasible and effective were identified. When developing treatment recommendations, 99th percentile values of inactivation time were used for an additional factor of safety, and values were rounded up to the next full month to simplify communication. The following summarizes the recommendations of this study.

Supplementary ash

Stage 1 experiments showed that similar free ammonia concentrations were achieved in treatments with varying amounts of supplementary wood ash. Given that ash availability has been reported as an issue in some locations (e.g., Mehl *et al.* 2011; Kamuteera *et al.* 2013; Trimmer *et al.* 2016), mixtures requiring little or no supplementary ash are likely to be more feasible.

Urine addition

A volumetric mixture containing two parts urine to one part ash-amended vault products is recommended. Using estimated generation rates at school-based UDDTs in Uganda (Trimmer 2015), the maximum ratio achievable at the study location was calculated to be *2.1U:1 V*. Therefore, enough urine would be available for the recommended mixture.

Storage location and time

Using 99th percentile inactivation times for the *2U:1V:0A* indoor and outdoor mixtures, 3 months of indoor storage or 2 months of outdoor storage were estimated to provide at least 2-$\log_{10}$ inactivation of *A. lumbricoides* eggs. Outdoor storage may inactivate eggs faster than the estimated times presented here, given that fluctuating temperature patterns may be more effective than constant temperatures (Nordin *et al.* 2013). However, indoor storage might provide a greater degree of security, in that a container stored in a closed room would present less of a potential hazard to curious children or animals. The decision regarding whether to employ outdoor or indoor storage is likely best left to users.

Sensitivity analysis

Finally, a sensitivity analysis was performed to evaluate how changing each individual input parameter (pH, total ammonia, temperature) affected the 2-$\log_{10}$ inactivation time estimate for the *2U:1V:0A* mixture. Indoor and outdoor storage conditions were both considered. The results of this sensitivity analysis are shown in Figure 4. Note that all parameters affect inactivation time through an inverse relationship, such that a higher value of a given parameter results in a lower estimated inactivation time.

In Figure 4, inverse relationships define all effects on inactivation time of the parameters. The left and right edges of each box represent inactivation when using that parameter's 75th

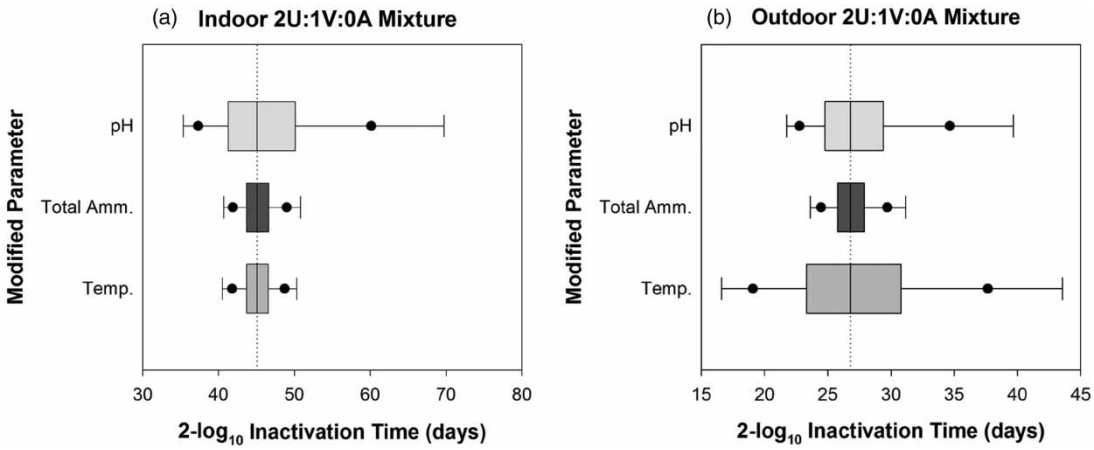

Figure 4 | Sensitivity analysis assessing the individual impact of each input parameter on the 2-$\log_{10}$ inactivation time (in days) estimated for the (a) *2U:1V:0A* indoor and (b) outdoor mixtures, relative to the expected value when all of the parameters' 50th percentile values are used.

and 25th percentile values, respectively. Black points represent inactivation times when the 95th and 5th percentiles are used, and the ends of the lower and upper whiskers represent times when using the 99th and 1st percentiles, respectively.

Indoors, pH has the largest individual effect on inactivation time, likely because the average pH value is only slightly above ammonia's pK_a value at the daily mean indoor temperature. If the pH is reduced, equilibrium shifts away from free ammonia and its concentration becomes considerably lower, leading to longer inactivation times. Outdoors, pH remains important, but temperature changes have a greater impact. At higher temperature, the pK_a value is further from the mixture's average pH, meaning that a change in pH has a diminished effect. Given that outdoor temperatures are more variable, however, a significant temperature decrease would directly increase inactivation time (through the inclusion of temperature in Equation (1)), while also indirectly increasing it by bringing the pK_a value closer to the system's pH, thereby reducing the free ammonia concentration. Overall, maintaining high pH levels is most crucial for indoor storage, while ensuring high temperatures is critical for outdoor storage. However, all changes in estimated inactivation time remain well within the treatment times recommended above.

CONCLUSIONS

Based on our estimates, free ammonia treatment with stored urine should inactivate *A. lumbricoides* eggs in UDDT ash-amended vault products in Uganda. A mixture of two parts urine and one part ash-amended vault products with no supplementary ash (*2U:1V:0A*) was found to be most appropriate and was estimated to provide 2-$\log_{10}$ inactivation after 3 months of indoor storage ($22.8 \pm 0.3\ ^{\circ}C$) or 2 months of outdoor storage ($25.9 \pm 1.3\ ^{\circ}C$).

This study shows that free ammonia treatment could be successful as an appropriate secondary treatment option in Uganda. Since Uganda functions as a conservative case regarding ammonia levels in urine, it also implies that free ammonia treatment could function effectively in other contexts with or without protein-related nutritional insecurities. This possibility of a low-cost, readily-available secondary treatment technique that efficiently inactivates persistent pathogens could represent a globally significant improvement to the UDDT system. Free ammonia treatment using urine leverages resources already present in human waste to increase the safety of recovered products, which can be agriculturally applied to improve the nutritional security of vulnerable populations.

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REFERENCES

APHA 2012 *Standard Methods for the Examination of Water and Wastewater*. 22nd edn. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, DC.

Brownell, S. A. & Nelson, K. L. 2006 Inactivation of single-celled *Ascaris suum* eggs by low-pressure UV radiation. *Applied and Environmental Microbiology* **72**, 2178–2184.

Cruz-Espinoza, L. M., Yeh, D., Vinnerås, B., Rajaram, L., Whiteford, L., Corvin, J. & Izurieta, R. 2012 Inactivation of *Ascaris suum* by ammonia in feces simulating the parameters of the solar toilet. *Journal of Applied Sciences in Environmental Sanitation* **7**, 173–182.

Esrey, S. A., Andersson, I., Hillers, A. & Sawyer, R. 2001 *Closing the Loop: Ecological Sanitation for Food Security*. Swedish International Development Cooperation Agency, Mexico.

FAOSTAT 2015 Food Balance Sheets. Food and Agricultural Organization of the United Nations, Statistics Division. Available from: http://faostat3.fao.org/home/E. (accessed 30 June 2015).

Fidjeland, J., Magri, M. E., Jönsson, H., Albihn, A. & Vinnerås, B. 2013 The potential for self-sanitization of faecal sludge by intrinsic ammonia. *Water Research* **47**, 6014–6023.

Fidjeland, J., Nordin, A., Pecson, B. M., Nelson, K. L. & Vinnerås, B. 2015 Modeling the inactivation of Ascaris eggs as a function of ammonia concentration and temperature. *Water Research* **83**, 153–160.

Friedler, E., Butler, D. & Alfiya, Y. 2013 Wastewater composition. In: *Source Separation and Decentralization for Wastewater Management*. (T. A. Larsen, K. M. Udert & J. Lienert, eds.). IWA Publishing, London, UK, pp. 241–258.

Fry, L. M., Mihelcic, J. R. & Watkins, D. W. 2008 Water and non-water-related challenges of achieving global sanitation coverage. *Environmental Science & Technology* **42**, 4298–4304.

Hawksworth, D., Archer, C., Rajcoomar, K., Buckley, C. & Stenström, T. A. 2010 The effect of temperature and relative humidity on the viability of Ascaris ova in urine diversion waste. *Cell* **72**, 8033491.

Jönsson, H., Stinzing, A. R., Vinneras, B. & Salomon, E. 2004 *Guidelines on the use of Urine and Feces in Crop Production*. EcoSanRes Programme, Stockholm Environmental Institute, Stockholm, Sweden.

Kabatereine, N. B., Tukahebwa, E. M., Brooker, S., Alderman, H. & Hall, A. 2001 Epidemiology of intestinal helminth infestations among school children in southern Uganda. *East African Medical Journal* **78**, 283–286.

Kamuteera, E., Trimmer, J., Carpenter, J. & Girod, C. 2013 Identifying challenges in the use of urine-diverting toilets: a case study from Rukungiri Municipality (S.W. Uganda). In: *36th WEDC International Conference: Delivering Water, Sanitation, and Hygiene Services in an Uncertain Environment*, Nakuru, Kenya.

Manser, N. D., Wald, I., Ergas, S. J., Izurieta, R. & Mihelcic, J. R. 2015 Assessing the fate of *Ascaris suum* ova during mesophilic anaerobic digestion. *Environmental Science and Technology* **49**, 3128–3135.

McKinley, J. W., Parzen, R. E. & Guzman, A. M. 2012 Ammonia inactivation of Ascaris ova in ecological compost by using urine and ash. *Applied and Environmental Microbiology* **78**, 5133–5137.

Mehl, J., Kaiser, J., Hurtado, D., Gibson, D., Izurieta, R. & Mihelcic, J. R. 2011 Pathogen destruction and solids decomposition in composting latrines: study of fundamental mechanisms and user operation in rural Panama. *Journal of Water and Health* **9**, 187–199.

Mihelcic, J. R. & Zimmerman, J. B. 2014 *Environmental Engineering: Fundamentals, Sustainability, Design*. 2nd edn. John Wiley & Sons, Inc., New York, NY, p. 656.

Mihelcic, J. R., Phillips, L. D. & Watkins, D. W. 2006 Integrating a global perspective into engineering education & research: engineering international sustainable development. *Environmental Engineering Science* **23**, 426–438.

Mihelcic, J. R., Fry, L. M. & Shaw, R. 2011 Global potential of phosphorus recovery from human urine and feces. *Chemosphere* **84**, 832–839.

Moe, C. & Izurieta, R. 2003 Longitudinal study of double vault urine diverting toilets and solar toilets in El Salvador. In: *The Proceedings of the 2nd International Symposium on Ecological Sanitation*, April 2003, Lubeck, Germany.

NEMA 2010 *State of the Environment Report for Uganda 2010*. National Environment Management Authority (NEMA), Kampala.

Nordin, A. 2010 *Ammonia Sanitization of Human Excreta – Treatment Technology for Production of Fertilizer. Doctoral Thesis*, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Nordin, A., Nyberg, K. & Vinnerås, B. 2009 Inactivation of Ascaris eggs in source-separated urine and feces by ammonia at ambient temperatures. *Applied Environmental Microbiology* **75**, 662–667.

Nordin, A., Niwagaba, C., Jönsson, H. & Vinnerås, B. 2013 Pathogen and indicator inactivation in source-separated human urine heated by the sun. *Journal of Water Sanitation and Hygiene for Development* **3**, 181–188.

O'Neal, J. A. & Boyer, T. H. 2013 Phosphate recovery using hybrid anion exchange: applications to source-separated urine and combined wastewater streams. *Water Research* **47**, 5003–5017.

Pan American Health Organization 2014 Factsheet: Soil-Transmitted Helminthiasis. Available from: http://www.paho.org/hq/index.php?option=com_content&view=article&id=5747&Itemid=4138&lang=en. (accessed 29 June 2015)

Pecson, B. M., Barrios, J. A., Jimenez, B. E. & Nelson, K. L. 2007 The effects of temperature, pH, and ammonia concentration on the inactivation of Ascaris eggs in sewage sludge. *Water Research* **41**, 2893–2902.

Pullan, R. L. & Brooker, S. J. 2012 The Global Limits and Population at Risk of Soil-Transmitted Helminth Infections in 2010. *Parasites and Vectors* **5**, 81–95.

Smith, H. M., DeKaminsky, R. G., Niwas, S., Soto, R. J. & Jolly, P. E. 2001 Prevalence and intensity of infections of *Ascaris lumbricoides* and *Trichuris trichiura* and associated socio-demographic variables in four rural Honduran communities. *Memórias do Instituto Oswaldo Cruz* **96**, 303–314.

Stenström, T. A. 2001 Reduction efficiency of Index pathogens in dry sanitation compared with traditional and alternative wastewater treatment systems. Internet Dialogue on Ecological Sanitation (15 Nov. – 20 Dec. 2001).

Trimmer, J. T. 2015 *Ecological Sanitation in Uganda: Promotion Through Demonstration Facilities and Potential for Ascaris Reduction by Free Ammonia Inactivation Using Stored Urine. Master's Thesis*, University of South Florida, Civil and Environmental Engineering, Tampa, Florida.

Trimmer, J. T., Nakyanjo, N., Ssekubugu, R., Sklar, M., Mihelcic, J. R. & Ergas, S. J. 2016 Assessing the promotion of Urine-Diverting Dry Toilets through school-based demonstration facilities in Kalisizo, Uganda. *Journal of Water, Sanitation, and Hygiene for Development*. In press.

Vinnerås, B., Jönsson, H. & Albihn, A. 2004 Comparison of composting and urea treatment for sanitizing of faecal matter. In: *The Proceedings of the 11th International Conference of the FAO ESCORENA Network for Recycling of Agricultural, Municipal, and Industrial Residues in Agriculture (RAMIRAN)*, Murcia, Spain, 6–9 October, 2004.

Wambui, T. 2011 *Uganda: Declining Soil Fertility Threatens Food Security*. The Monitor, 8 February, 2011.

WHO 2006 *Guidelines for the Safe use of Wastewater, Excreta and Greywater: Volume 4: Excreta and Greywater use in Agriculture*. World Health Organization, Geneva, Switzerland.

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