

Fecal indicator and *Ascaris* removal from double pit latrine content

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ABSTRACT

Since May 2006, the BRAC Water, Sanitation and Hygiene (WASH) Programme in Bangladesh has enabled more than 30 million people to achieve hygienic sanitation, contributing to an increase in sanitation coverage from 33 to 83% in programme areas and rapid progress towards universal access. In rural areas, most families have single pit latrines that need to be emptied when full. Since 2007, BRAC has promoted the use of hygienic double-pit latrines. Use of double-pit latrines, where appropriate, is also recommended in the Bangladeshi Draft National Water Supply and Sanitation Strategy. More than 800,000 double-pit latrines are in use in BRAC WASH areas, delaying the need for emptying and allowing time for the fecal matter to decompose while the resting pit is sealed. This paper focuses on a study undertaken by BRAC WASH to treat and safely use fecal material from double pit latrines as an organic fertilizer for rice and other crops. The study investigated the removal of pathogens from pit waste through simple solar drying and conducted analysis on nutrient properties of fecal sludge. The study showed a significant positive impact on developing organic fertilizer from fecal sludge.

Key words | double-pit latrine, fecal sludge, market, organic fertilizer

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INTRODUCTION

In 2006, BRAC initiated a comprehensive intervention on Water, Sanitation, and Hygiene (WASH). The programme originally covered 150 upazilas (sub-districts) and subsequent phases expanded to 250 upazilas out of the total of 488 upazilas of Bangladesh. The programme promotes the use of sanitary latrines that separate feces from human contact and do not cause contamination of water sources. At the outset, the programme promoted single-pit latrines but since 2007 has championed double pit models, with the preferred option now being a double-pit latrine where the superstructure remains in place and a switching system directs material to one of the two pits (Figure 1) (BRAC 2013a). By the end of 2013, more than 800,000 households had received a double-pit latrine with support from BRAC. Latrine provision alone is not sufficient to ensure safe sanitation. Promoting latrine use and

good hygiene practices in the community have been major focal areas of the BRAC WASH programme. In addition, action is required to deal with human waste once hygienic latrines fill up. Environmentally safe collection, transport, treatment, and productive reuse of treated human waste within a well-constructed and well-managed sanitation service delivery chain has the potential to safeguard the environment, improve public health, and provide financial benefits to users and service providers (Graham & Polizzotto 2013).

This study looked at fecal sludge recovered from typical double-pit latrines in rural Bangladesh. Typically these pits comprise three concrete rings to form the pit lining, and a top slab with an integral plastic pan and 'goose-neck' style water seal. Most slabs are made of concrete but it is possible to use a smaller concrete or plastic 'SanPlat', laid on top of a

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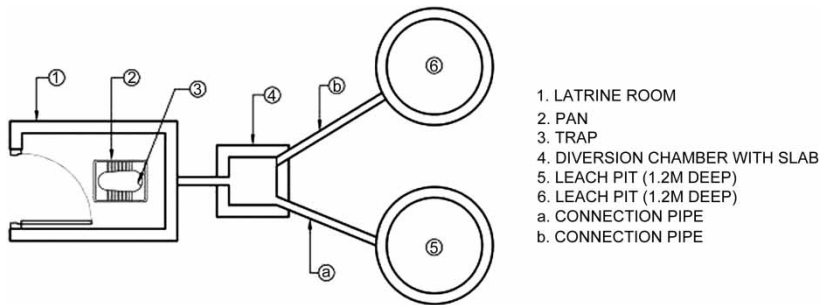


Figure 1 | Double-pit toilet model.

latrine cover made from wood and other ‘natural’ materials (Figure 1).

Usually the first pit is covered with a lid and sealed by cement for a year or more before emptying. Although it is usually assumed that disease-causing organisms will be destroyed by natural processes during this time, concerns remain about the risk of contamination from the fecal sludge of these latrines (Tilley *et al.* 2014).

It is assumed that during this composting process temperatures are sufficiently elevated for an adequate amount of time to destroy pathogens. However, according to previous studies the majority of composting latrines in developing countries do not reach high enough temperatures for complete pathogen inactivation. These studies suggest that desiccation at high pH may be the primary mechanism for pathogen inactivation (Kone *et al.* 2007; Mehl *et al.* 2011).

The implication of pathogen removal from the fecal sludge of a double pit latrine is connected with the scope to reuse it as organic fertilizer. Bangladesh soil is highly depleted in organic content. Status of organic matter is below 1% in 0.76 million ha and between 1 and 1.7% in 2.87 million ha of land (Akter *et al.* 2012). The risk of using fecal sludge as organic fertilizer is the pathogen content of it. Fecal sludge contains pathogens that can be of great risk to the users such as the pit emptier, transport person and farmers. It is of high risk to the farmers because of their mode of application of the sludge as organic fertilizer in the field (Singh & Agrawal 2008). Farmers have direct contact with the sludge when spreading it in the soil; they collect raw fecal sludge from pits and use it in the field without any safety gear such as gloves, masks, etc.

Calculations based on United Nations (UN) Food and Agriculture Organization (FAO) figures suggest that the

average family in Bangladesh produces the fertilizer equivalent of 25 kg of urea, 10 kg of triple super phosphate and about 13 kg of muriate of potash (potassium chloride) yearly (Jonsson *et al.* 2004; FAO 2013). Bangladeshi farmers have traditionally used fecal sludge from livestock and humans as a fertilizer or soil conditioner because of its positive impact on yields. The reuse of treated fecal waste in the agricultural sector has potential but there are constraints; notably any product has to be safe for human use. The safe use of composted fecal sludge as organic fertilizer would not only have a positive impact on the agriculture sector, but would also provide a sustainable solution to the increasing accumulation of fecal sludge in rural pit latrines.

This paper describes technical research carried out as part of the BRAC WASH programme to explore the potential of agricultural re-use of human fecal waste derived from double-pit latrines in Bangladesh.

MATERIAL AND METHODS

Pathogen inactivation in composting latrines is dependent on temperature, pH, moisture, and storage time. However, the conditions required to inactivate pathogens are specific to different pathogens. The main categories of pathogens found in human excrement in composting latrines can be categorized as: viruses, bacteria, protozoa and helminths. Viruses were not analyzed in this research because this research aimed to reuse fecal sludge from a double pit latrine. In double pit latrines, users rotationally use the two pits. When the first one fills up they use the second. When the second one fills up they use the first one again. Meanwhile, the content of the first pit has dried up and

can be used as fertilizer if it is scooped out before being used again. Samples analyzed in this research were collected after storage of 14–18 months, which is much greater than the survival period of viruses in feces (Strauch 1991). *Escherichia coli* was chosen as indicator bacteria because of its capacity to survive in different conditions (Edberg et al. 2000). Helminths are of special consideration, especially the eggs of *Ascaris lumbricoides*. This is because *A. lumbricoides* eggs are very persistent and not easily inactivated (Feachem et al. 1983). *Ascaris* ova are also considered the appropriate organism for evaluating treatment effectiveness of a sanitation technology that is to protect public health because their thick shells are very resistant to environmental stressors. *Ascaris* is the most resistant ova among helminths (Pike & Carrington 1986; Jimenez & Wang 2006), hence it can be used as marker of inactivation of helminth eggs in general.

For testing the pathogen content of the sludge, samples were collected from different parts of Bangladesh. Double pit latrines were selected in different climatic zones. The selection of latrines was based on the criteria of having one pit sealed for at least 12 months. There are seven climatic zones in Bangladesh (Figure 2). Ten samples were collected from each climatic zone, therefore there were 70 samples in total. One hundred grams of sludge sample was collected from each pit using a sterile soil scooper. The samples were collected and stored in sterile screw-cap containers and were transported on ice, stored below 4 °C and processed within 24 hours (Clesceri et al. 1998). Moisture content, pH, and temperature of the samples were also measured. Fecal indicator analysis was done on all three samples assessing *E. coli*, and *A. lumbricoides* as indicator bacteria and helminth. For enumeration of *E. coli*, sludge samples of three different dilutions were inoculated onto m-TEC agar (membrane-Thermotolerant *Escherichia coli* Agar) plate using spread plate technique. The m-TEC agar plates were then incubated at 35 + 0.5 °C for 2 h followed by further incubation at 44.5 + 0.2 °C for 22–24 h to enumerate the *E. coli*. Characteristic red or magenta colonies were then counted as *E. coli*. All samples were expressed as colony forming units (cfu) present per g of sludge sample (EPA 2002). The presence of *Shigella* and *Salmonella* was also tested in the samples collected from each pit. *Salmonella* and *Shigella* were

analyzed using *Salmonella/Shigella*[®] agar (Pond et al. 2000), however no presence of *Shigella* and *Salmonella* was found.

To identify, quantify and determine the nematode eggs' viability, the suspension was incubated at 26 °C for 20 days in a 0.1 N solution of sulfuric acid. Each species was then identified and quantified using an optical microscope, and the presence/absence of the larvae was confirmed to determine viability (Maya et al. 2012).

For pathogen removal from the digested sludge a separate trial was carried out. Three different zones were selected and 10 mature pits (14–18 months old) from each zone were selected. Samples from each zone were mixed properly to prepare three composite samples. All three samples were analyzed for the presence of microbial and helminth parameters, and were then subjected to sun drying. The drying was carried out in summer (April–May) and the temperature was 30–32 °C (Weatheronline 2013). The drying continued for two months and the samples were analyzed at regular intervals. The sludge remained inside the pit for more than 12 months under anaerobic conditions. Then, in this study, the sludge was subjected to sun drying which is called aerobic digestion. This combination of aerobic and anaerobic digestion removed pathogens. Anaerobic digestion can reduce pathogens but does not completely eliminate such organisms (Farrah & Bitton 1984).

RESULTS

The presence of *E. coli* and *A. lumbricoides* was analyzed as indicator pathogen of the 70 samples (Table 1). In four of the 70 samples, 0 cfu/g of *E. coli* was found. In the other 66 samples, the *E. coli* content was ≥ 1 cfu/g. The segregation of *E. coli* is described in Figure 3. Among the 70 samples, 48 samples had levels of *E. coli* of $\leq 1,000$ cfu/g. No *Salmonella* or *Shigella* was found in the samples.

Out of 70 samples, 21 samples were found to be negative with regards to *Ascaris* ova. In 38 samples the load was ≤ 100 *Ascaris* ova. The maximum number of ova found in one sample was 6,000. Figure 4 illustrates the range of *Ascaris* ova found in the samples.

The three composite samples subjected to sun drying were monitored at regular intervals. An *E. coli* content of

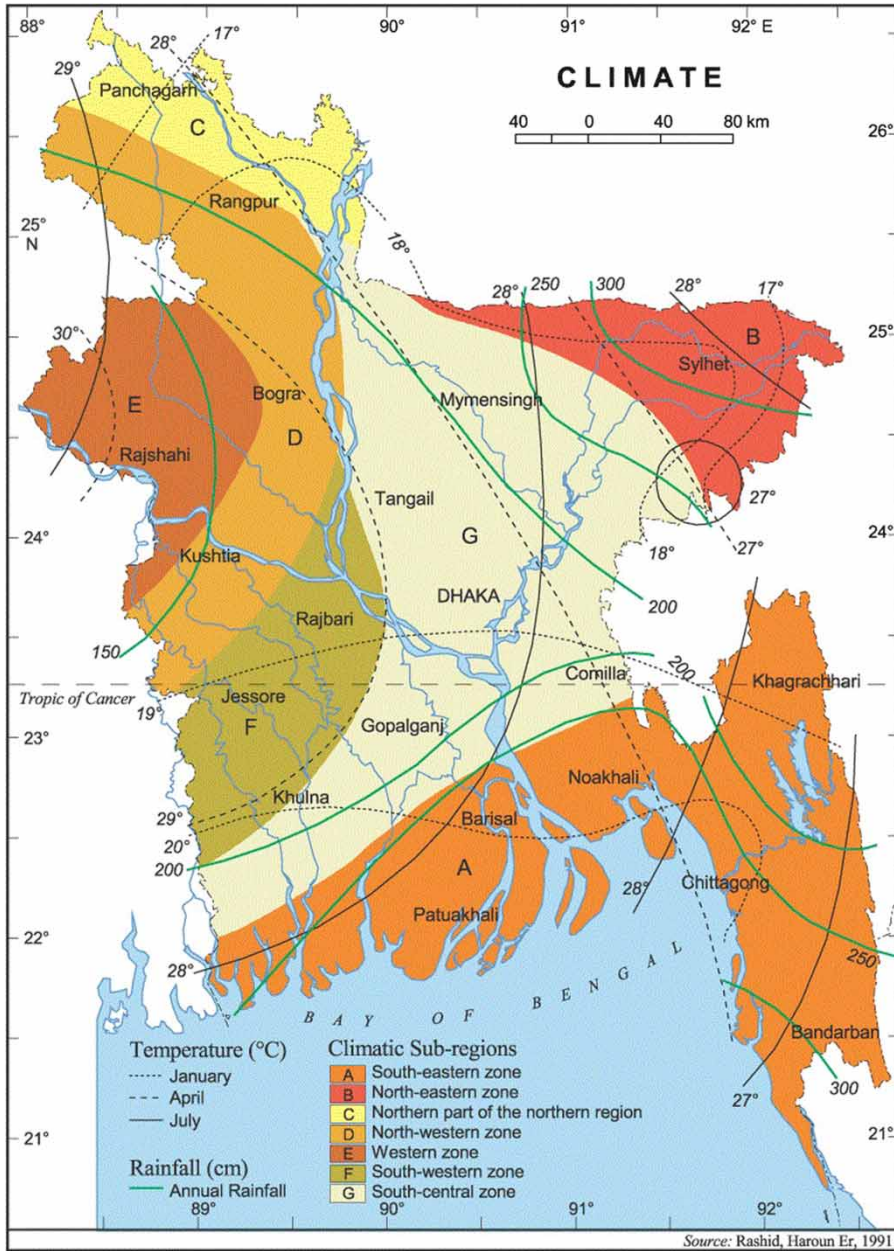


Figure 2 | Seven climatic zones of Bangladesh (Source: Prime Minister’s Office database, Bangladesh, <http://lib.pmo.gov.bd/maps/images/bangladesh/Climate.gif>).

Table 1 | Average value of the physical and chemical parameters of the sludge tested

Parameter	pH	Moisture content (%)	Organic carbon	Organic nitrogen (%)	Phosphorus %	Potassium %	Sulphur %
Average value of sample	5.1	35.6	13.4	1.3	1.2	0.4	0.3
Parameter	Zinc %	Copper %	Arsenic (ppm)	Chromium (ppm)	Lead (ppm)	Nickel (ppm)	Mercury (ppm)
Average value of sample	0.05	0.03	10	19	12	15	0.05

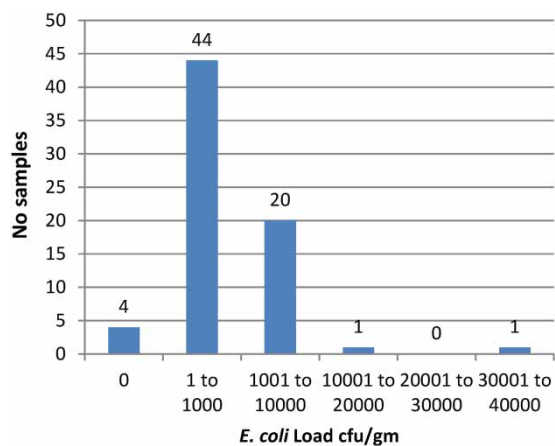


Figure 3 | *E. coli* content of the sludge.

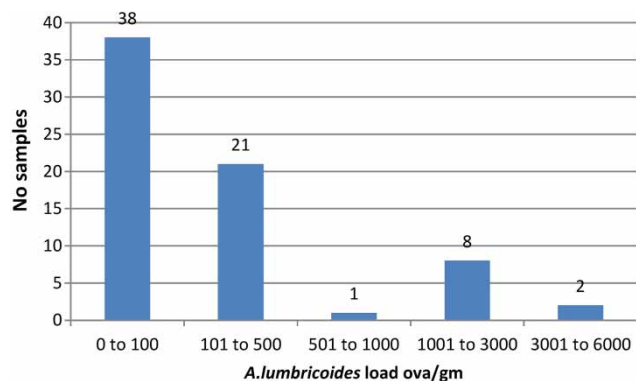


Figure 4 | *A. lumbricoides* content of the sludge.

0 cfu/gm was found after 144 days of drying. Figure 5 shows the rate of reduction of *E. coli*.

Table 2 shows the T90 (90% reduction time) values of *E. coli* reduction during sun drying with time.

Figure 6 illustrates the reduction of *Ascaris* ova viability over time in the composite samples. The viability of ova gradually reduced and one sample was found to be negative for viable *Ascaris* ova after 45 days. Two others were found to be negative after 60 days. Gradual reduction of helminth ova viability is presented by a trend line. A trend line shows the tendency of gradual inactivation of *Ascaris* ova.

Table 3 shows the T90 (90% reduction time) values of *A. lumbricoides* ova inactivation during sun drying with time.

Figures 7 and 8 show the physical (pH and moisture) and chemical (NPK) parameters of the three subsamples before and after drying, respectively. Figure 7 shows that

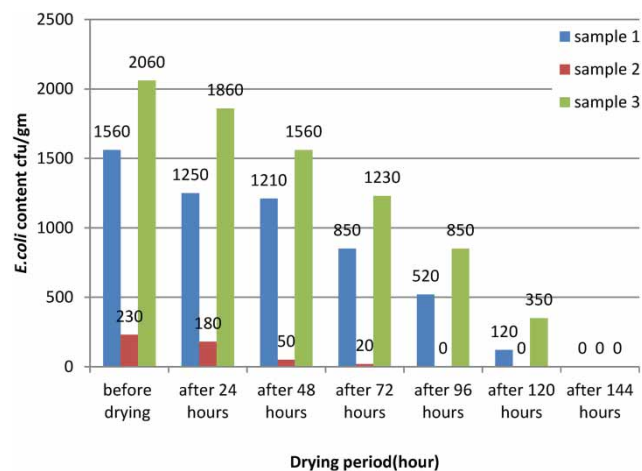


Figure 5 | *E. coli* content before and after drying.

Table 2 | T90 value of *E. coli* reduction

Test organism	T90-values (hour)
<i>E. coli</i>	
Sample 1	120
Sample 2	72
Sample 3	144

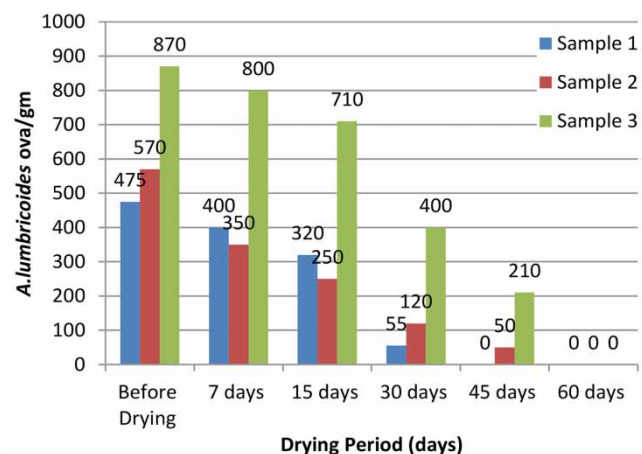


Figure 6 | Gradual reduction of *A. lumbricoides* ova viability in the trial sludge.

Table 3 | T90 value of *A. lumbricoides* inactivation

Test organism	T90-values (days)
<i>A. lumbricoides</i>	
Sample 1	35
Sample 2	45
Sample 3	55

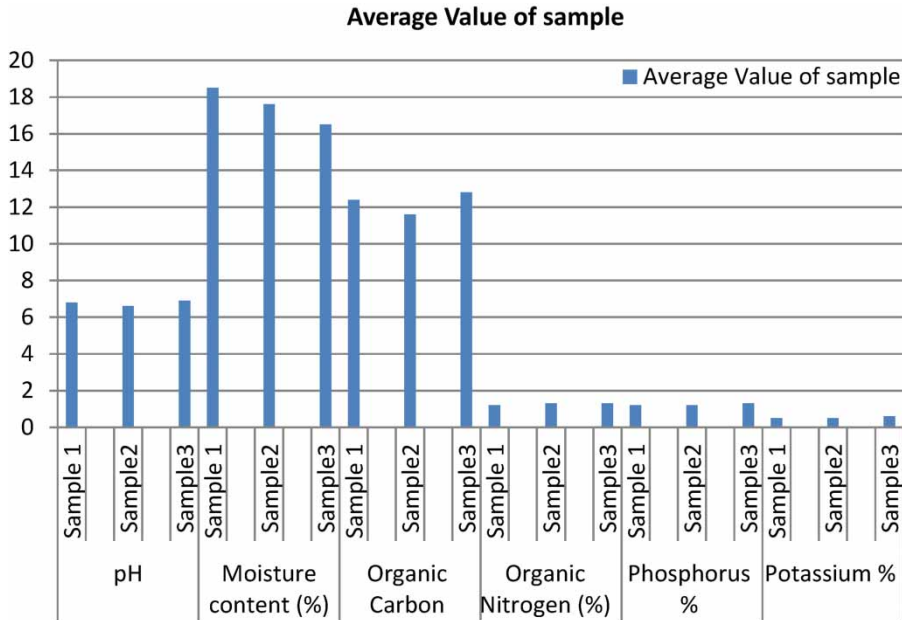


Figure 7 | NPK value of the composite samples before drying.

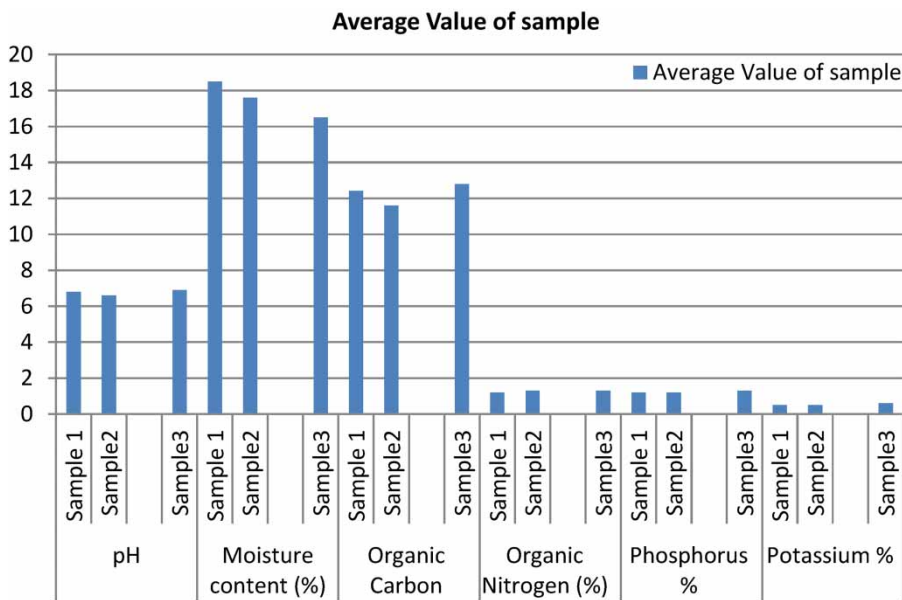


Figure 8 | NPK value of the composite samples after drying.

moisture is relatively high and the pH value is less than the characteristics of the Bangladesh organic fertilizer standard (BARC 2012), whereas Figure 8 shows that moisture reduced and pH increased significantly after drying in all three sub-samples in a uniform manner.

DISCUSSION

This research started with a view to produce organic fertilizer from the digested fecal sludge of a double pit latrine. The BRAC WASH programme has provided this latrine to

ultra-poor people of the community (BRAC 2013b), thus reuse of fecal sludge was of much importance to ensure sustainable use of the double pit latrines. All the users of those latrines belong to a village community, which is an added advantage because most of the rural people in Bangladesh are linked to agriculture and have a higher scope to use this fertilizer. According to the concept of double pit latrines, the sludge is digested after a certain period of undisturbed storage inside the pit. Usually, it took 14–18 months for one pit to be filled up. Thus a countrywide study was launched, which analyzed the sludge samples collected from pits that were under undisturbed storage for at least 14 months and at most 18. Along with the chemical and physical parameters of organic fertilizer, pathogen content was also analyzed. *E. coli* as indicator bacteria and *A. lumbricoides* as indicator helminth were taken for analysis. Figures 1 and 2 show that most of the samples were found to be positive for the presence of *E. coli* and *A. lumbricoides* after the digestion period inside the pit. Figure 1 shows that most of the samples contained *E. coli* within the range of 0–1,000 cfu/g. Thirty-eight out of 70 samples contained ova of *A. lumbricoides* within the range of 0–100 gm. Twenty-one of the samples were within the range of 101–500 ova/gm. The presence of *E. coli* and *A. lumbricoides* indicated that other pathogens might also be present in the sludge fertilizer. Thus it was a challenge for the researchers to remove the pathogen from the sludge to ensure its safe reuse. Considering the existing protocols for pathogen removal from sludge, researchers have chosen the sun drying method for it. The sludge was already under anaerobic digestion for at least 14 months, thus sun drying was chosen as a better method for pathogen removal than subjecting it to any other digestion process. Researchers involved in this research set some rules for a sun drying experiment. Three composite samples prepared from sludge of 30 pits collected from different areas of Bangladesh, the three samples were subjected to drying under the sun on an open ramp during summer at temperatures of 30–32 °C. The samples were continuously monitored during the day and were covered with a plastic sheet overnight and during rainfall. Continuous monitoring was carried out for observation of pathogen removal. Seven days of drying showed 0 cfu/gm of *E. coli* in all three samples. In the case of helminth, viability and presence of the ova was analyzed because dead ova may remain in the sludge. Seven days of drying showed a

reduction in helminth viability but a significant amount of helminth were still there. Thus drying continued with helminth ova viability analysis at regular intervals. After 45 days drying, sample 1 showed a presence of 0 viable *Ascaris* ova. Samples 2 and 3 showed 0 *Ascaris* ova viability after 60 days of drying. The three samples were also analyzed for physical and chemical parameters. Most of the parameters remained the same as before apart from moisture and pH. Moisture reduced significantly with drying whereas pH increased, which improves the quality of the sludge as organic fertilizer. The whole process was repeated and the same pattern of results during the repetition period was found. This indicates that the process can be performed as a means of pathogen removal from pit latrine contents and it has an important role in reducing moisture and increasing pH of the sludge. Thus, it can be used as a protocol to develop pathogen free organic fertilizer from double pit latrine contents. Previous studies on solar drying as a means of pathogen removal have proven successful to reduce the content but they did not reach a level allowing unlimited use of fecal sludge in agriculture (Sypula et al. 2013). This study has combined the anaerobic digestion of fecal sludge with solar drying. The increase of pH has also played a role in the pathogen removal process. pH of the three samples raised during the sun drying process is about 1.8–1.9 which is sufficient to meet the standard in this process. The combination of anaerobic digestion with aerobic drying was a new initiative and was found to be successful. BRAC WASH has provided 1.1 million double pit latrines to the community. Following the same method, many individuals have constructed this type of latrine in their households. As most of the double pit latrines are in the rural areas of Bangladesh, these pits can serve the farmers with a huge amount of organic fertilizer per year. Bangladeshi soil is highly depleted of organic content and needs organic supplements (Akter et al. 2012). Thus safe reuse of fecal sludge can be a good source of organic content for the soil. Traditionally farmers of Bangladesh uses raw pit latrine content in their soil as fertilizer, especially during the cultivation of vegetables. This is unsafe for the farmer and also for the consumer. Eight hundred thousand latrines may produce approximately 16,000 Mg of organic fertilizer which can play an important role in enriching the organic content of the soil. The financial return of safe sanitation will greatly encourage households in rural areas.

CONCLUSIONS

The objective of this research was to find a simple solution for pathogen removal of fecal sludge in double pit latrines in the rural areas of Bangladesh. *E. coli* and *Ascaris* ova were selected as indicators in this research. The presence of *Shigella* and *Salmonella* was also tested as part of the research. Partial application of anaerobic digestion and desiccation through sun drying has proven successful to remove those indicators. This revealed a possibility to widely use this technique for pathogen removal from fecal sludge and ensure safe reuse of it as organic fertilizer in the field. Further research needs to be carried out to improve the procedure and its efficiency. Application of the organic fertilizer also needs to be tested in crops to determine its optimum dose.

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