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On-Site Faecal Sludge Treatment on Raised Latrines during Emergency Situations

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Master of Science Thesis
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Abstract

Sanitation is regarded as a basic human right as it saves lives and eradicates poverty. It is for this reason that there is a need to provide adequate sanitation even during emergency situations; as affected people are vulnerable and more susceptible to high health risks, since they are confined in extreme conditions. However, faecal sludge management is as equally important as the provision of sanitation system with faecal sludge treatment as the most important element as it reduces environmental pollution and health risks.

The aim of this study was to investigate the effect of lime on the microbiological quality of faecal sludge prior disposal to the environment. Lime stabilization is a low cost, simple and easy process that is easy to apply. Its primary role is to eliminate pathogens while reducing the smell in faecal sludge. The results of this study indicate that effective treatment is achieved by increasing the pH of the treated sludge to ≥ 12 and maintain this pH for at least two hours. As a result, the study established a relationship between pH and lime dosage in order to investigate the effectiveness of lime treatment on the inactivation of pathogens in faecal sludge.

The study was conducted in two phases, namely, laboratory scale and field scale. The black water sample for laboratory analysis was obtained from Sneek wastewater treatment plant in the Netherlands. For field-scale analysis, the faecal sludge was collected from households and public latrines in Malawi. Various lime dosage ranges were tested, which resulted in the optimal range between30-60% CaO/TS (w/w). During laboratory scale experiments, *E. coli*, total coliforms and *Salmonella* were analysed and complete inactivation was observed in 5 minutes for dosages 50-60% CaO/TS. In samples treated with 40% CaO/TS, 3 log reductions in *Salmonella*, total coliforms and *E. coli* after 5, 15 and 30 minutes respectively were observed. Moreover, variations in log removal of pathogens were observed in samples treated with 30% CaO/TS; where 1 log reduction of *E. coli* after 5 minutes, 2 log reductions of total coliforms after 15 minutes and 3 log reductions of *Salmonella* after 2 hours were observed. All dosages below 30% CaO/TS could not reach the minimum recommended standards set by WHO for microbiological quality of the sludge before disposal.

During field experiments, the microbiological quality of faecal sludge from pit latrines in Malawi was analysed after treatment with hydrated and quick lime. The lime addition not only increased the pH of the sludge from 7 to > 12 but also increased the temperature of the sludge from 22-24^{o}C. The smell of the sludge also reduced after lime addition. The effect of lime on microbiological quality was carried out after 5,15,30,60 and 120 minutes. *Salmonella* was not detected in any of the samples collected from the latrines. *E. coli* and total coliforms were completely inactivated after 5 minutes of treatment.

Keywords: On-site, Faecal Sludge, Treatment, Raised Latrines, Emergency situations

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Table of Contents

List of Figures

List of Tables

Abbreviations

$CaCO_3$	Calcium Carbonate
CaO	Calcium Oxide
$Ca(OH)_2$	Calcium Hydroxide
CFU	Colony Forming Unit
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
E. coli	*Escherichia coli*
FS	Faecal Sludge
HTH	High test hypochlorite
RNA	Ribonucleic Acid
KOH	Potassium Hydroxide
LA	Lactic Acid
MgO	Magnesium Oxide
$Mg(OH)_2$	Magnesium Hydroxide
NaOH	Sodium Hydroxide
NH_3	Ammonia
NH_4OH	Ammonium Hydroxide
$N\text{-}NH_3$	Ammonia Nitrogen
NL	Netherlands
PAA	Peracetic Acid
PO_4^{3-}	Phosphate ion
TC	Total coliforms
TS	Total Solids
UV	Ultra Violet
VS	Volatile Solids

CHAPTER 1

1.0. Introduction

1.1. Faecal sludge management in emergency situations

Emergency situations require immediate response. This is due to the fact during disaster, affected people become vulnerable and are more susceptible to high health risks as they are confined in extreme conditions where access to sufficient sanitation is essential (Bouwinnovatie and Societies, 2012). As a result, provision and the speed of sanitation technology installation to contain and collect excreta is of a higher priority (Harvey, 2007); especially when the sanitation infrastructure in the affected area has collapsed (Bouwinnovatie & Societies 2012). Generally, emergency interventions often results from natural disasters such as floods, earthquakes, storms, droughts and conflicts in conjunction with physical and social factors. These interventions are rapidly implemented and designed for short-term use in order to prevent and/ or minimize the spread of faecal-oral diseases and to protect the environment (www.sswm.info). However, it will be more beneficial if sanitation interventions are approached in phases (immediate, stabilization, and recovery) to ensure sustainable alternatives in case the emergency prolongs. In other words, during immediate emergency phase consideration has to be given to improve the system for long-term use.

Sanitation systems to be deployed during emergency must be robust enough to withstand harsh environmental conditions (Johannessen et al., 2012)**.** Conditions such as rocky grounds, unstable soil, high water table, floods, shortage of water, spatial constraints, and space limitations can be challenging at times and therefore require suitable options like raised latrines or urine diversion toilets (Ruberto and Johannessen, 2009). However, the choice of a particular toilet system depends entirely on the prevailing conditions taking into account the shortfalls and benefits of each system. For example, the choice of on-site systems such as terra preta or urine diversion toilets is not only influenced by the agricultural re-use of by-products, but also by their ability to produce no or negligible green house gases (e.g. methane), no odour, no ventilation required hence no loss of ammonia to the atmosphere, low cost and does not require water (Johannessen and Bikaba, 2009). Additionally, even though the use of raised latrines for instance may be a suitable alternative as mentioned earlier, limitations such as slow and expensive installation as well as the need for frequent desludging are anticipated (www.sswm.info).

Consequently, the management of faecal sludge must be carefully considered when choosing an on-site toilet system or any other system for that matter. This includes management of the entire faecal sludge treatment process which range from the storage, collection, treatment to disposal (Agyei et al. 2011). In contrast, faecal sludge treatment is usually ignored if not forgotten as most sanitation planners focus more on its collection and disposal (Koné et al. 2010). The treatment of the collected excreta prior transport and disposal is often a challenge. However, there are multiple on-site treatment solutions that can be applied to alleviate health risks associated with faecal contamination. These treatment solutions include chemicals such as ammonia (Fidjeland et al. 2013), urea (Fidjeland 2010), lime (Capizzi-Banas et al. 2004), alkaline (Celoria et al. 1994) , and lactic acid (Alakomi et al. 2005); the use of bio predating methods like black soldier flies (Lalander et al. 2013) and house flies (Zheng & Zhou 2013); drying methods such as gamma radiation (de Souza et al. 2011), UV radiation (Aladawi et al. 2006), ozone (Mun et al. 2009) and solar drying (Belessiotis & Delyannis 2011), thermal drying (Vaxelaire et al. 2000); as well as off-site treatments

such as co-composting (Strauss, 2003) and vermi-composting (Ferris Amanda, Jackson Mark and Campbell Angus, 2002). The mechanism, impact, limitations and the conditions at which these treatment methods inactivate the pathogenic organisms was briefly discussed in the literature review.

Similar to the toilet system, the faecal sludge treatment option to be employed also depends on certain conditions. These conditions include the characteristics of the sludge generated which differs from district to district, town to town, city to city or household to household; the treatment objectives such as agricultural reuse, land filling of biosolids, or discharge of treated liquids into receiving water bodies; and the type of on-site sanitation systems like raised latrine, terra preta, urine diversion toilets, ventilated pit latrine and etc. (Koné et al. 2010).

Faecal sludge from on-site sanitation contains large number of nutrients and organic matter which makes it a useful resource in agriculture, as a fertilizer and soil conditioner. However, untreated sludge also contains large quantities of pathogens. As a result it is essential to treat it prior application on land in order to protect public health and the environment (Fidjeland et al. 2013). The pathogens of concern in faecal sludge are bacteria, viruses, protozoa and helminths (Decrey et al. 2011). Koné et al. (2007) mentioned that in time all pathogens die-off upon excretion except those that multiply in intermediate hosts. For instance, bacteria such as *Salmonella* have a potential to multiply outside the host provided they have sufficient nutrients and high enough temperature, whereas viruses can hardly survive without their host. In addition, it is important to note that the reuse of sludge treated with lime is not only limited to agriculture, but can also be reused as landfill cover and in green areas and forestry (Kelessidis and Stasinakis, 2012).

The study conducted by Koné et al., (2010), reports that the resistance of pathogens against die-off varies depending on factors such as temperature, pH, moisture content, and exposure to sunlight or UV. However, helminth eggs are mostly resistant hence they need a specific treatment that is able to inactivate their viability. Another important factor is the infective dose of a pathogen which determines the intensity of the infection and an extent at which the human host can acquire an infective disease. Infective dose for helminth eggs, protozoa such as amoeba and viruses is less than $1x10^2$ which is low as compared to that of bacteria which is medium to high, i.e. greater than $1x10^4$ to $1x10^6$ respectively. In addition, if the treatment objective is to reuse the sanitation by-products in agriculture, then helminth eggs' viability must be reduced to $\leq$ 1egg/ℓ in order to meet the guidelines set by WHO (2006).

Furthermore, other regulations such as EPA specify requirements for the safe reuse or disposal of sludge. In this regulation the sludge is classified in to either class A or class B. These classifications specify when should the treated sludge be reused or disposed. Class A sludge has no or few restrictions as it contains negligible concentrations of pathogens, whereas Class B sludge can be applied on land with restrictions as it contains high pathogen concentrations than Class A. However, Class B still meets pathogen destruction requirement and therefore can be reused in agriculture and land reclamation, as it poses no threat to public health and the environment (www.lime.org). EPA approved sludge treatment with lime as one of the treatment technologies that are capable of greatly reducing pathogens and has the ability to meet Class requirements as set out in EPA guidelines (USEPA, 1982) cited in Bina et al. (2004).

Recently, studies were conducted on the sanitization of faecal sludge with chemicals in emergency situations. On the contrary, previous studies focused more on off-site treatments such as anaerobic digestion, planted or unplanted drying beds, co-composting, waste stabilization ponds and constructed wetlands. Koné et al. (2010) consider these off-site treatment systems as low-cost options. However, the treatments are not suitable for emergency as they require secondary treatment, take longer periods and also require large footprint which may be problematic during emergency situations. For the purpose of this study, focus was given to lime as a chemical treatment in an endeavour to deepen understanding and to demonstrate its effect on the inactivation of pathogens. Pathogens of interest were enteric bacteria such as *Escherichia coli* (*E. coli*), total coliforms and *Salmonella* as indicator organisms in faecal sludge from raised latrines. These organisms are perceived to persistently adapt to environmental changes to survive and resist traditional faecal sludge treatments commonly applied (Arthurson 2008).

1.2. Problem Statement

In most developing countries, there are no regulations that guide the management of faecal sludge. As a result, this causes high health risks and contamination of the environment (Koné et al. 2010). On-site sanitation systems are mainly practiced in most cities of developing countries such as Malawi. These cities are categorized as "latrine-based cities" as they depend on such infrastructure for excreta disposal. As a result, there are ongoing programs which target such cities in an attempt to achieve goals such as decrease in open defecation, increase to improved sanitation, coverage and use of safe hygiene practices as set out in their national and MDGs sanitation targets (Malawi Ministry of Irrigation and Water Development, 2010). However, service provision for the collection or emptying, transport, safe disposal, reuse or treatment of faecal sludge produced by on-site sanitation infrastructures, is still a challenge (Koné et al. 2007). Hence, many urban residents and urban farmers are at high risk of infection in poor sanitation settings.

Similarly, in any emergency situation, the provision of the best toilet and excreta disposal system does not exist. The choice for a particular system relies on specific conditions and thereby influencing the treatment option required for faecal sludge (Ruberto and Johannessen, 2009). As a result there is a need to find feasible solutions that are economical and easy to manage as these systems require frequent desludging, and handling of untreated excreta thus causes higher health risks.

The health risks of concern which affects almost all developing countries due to lack or poor sanitation is diseases such as typhoid, salmonellosis, gastroenteritis, cholera and diarrhoea. These infectious diseases are associated with enteric pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Arthurson 2008). The infection of these diseases is mainly via oral-faecal route, as they are contained in the faeces that are mostly discharged to the environment untreated. Such inadequate practices of handling and/or disposing faecal sludge, pose a threat to public health and the safety of the environment. Furthermore, enteric bacteria are resistant to ordinary treatments (Sahlstrom, 2003) cited in Arthurson (2008), as a result there is a need to investigate the control measures to be taken in order to minimize or eliminate the spread of diseases caused by enteric bacteria globally.

CHAPTER 2

2.0. Literature Review

The literature review was conducted on various methods available for sludge treatment; in order to deepen understanding and expanding the knowledge on faecal sludge treatment technologies available. This was done in an endeavour to discover cheap, simple, easy, environmentally friendly and efficient method that can ensure safe disposal of sanitized sludge. Consequently, the evaluation criterion in Table 2.1 was used to evaluate the suitability of treatment technologies feasible for emergency situations. This criterion was based on requirements for faecal sludge treatment as set out in the study done by Bouwinnovatie and Societies (2012). Based on the results of the evaluation criterion, the following treatment concepts were identified as possible treatment options for treating on-site faecal sludge from raised latrines during emergency situations. However, chemical treatment with lime was outstanding as it is the most promising treatment method since it met all the criteria mentioned above. Therefore, focus of this study will be based on lime treatment.

Table 2.1 Evaluation criteria for treatment technologies suitable for emergency situations

Treatment	Criteria							References
	Simplicity	Efficiency	Effectiveness	Treatment Period	Applicability	Safety	Costs	
Bio-predating	Yes	yes	yes	8 days	no	no	Low	Diener et al., 2011; Lalander et al., 2013.
Anaerobic digestion	No	yes	yes	days	no	yes	High	Koné et al., 2010.
Constructed wetlands	Yes	yes	yes	days	no	yes	Low	Koné et al., 2010.
Waste stabilization ponds	Yes	yes	yes	3-20 days	no	yes	Low	Koné et al., 2010.
Co-composting	Yes	yes	yes	months	yes	yes	low	Koné et al., 2010.
Drying beds	yes	yes	yes	months	no	yes	high	Koné et al., 2010.
Solar radiation	yes	yes	Not defined	10min-3hours	yes	no	low	VijayaVenkata Raman et al., 2012; Purohit et al., 2006; Midilli and Kucuk 2003
UV radiation	yes	no	no	6-12 hours	yes	yes	medium	Aladawi et al., 2006.
Gamma irradiation	no	yes	yes	< 1hour	yes	yes	High	de Souza et al., 2011; Shamma & Al-Adawi, 2002.
Lime	yes	yes	yes	≤ 2hours	yes	yes	low	Burnham and Nicholsen, 1990; Willford et al., 2007; Capizzi et al., 2004.
Peracetic acid (PAA)	yes	yes	yes	1hour-5days	yes	no	Low	Vinnerås et al., 2003.
Lactic acid (LA)	yes	yes	yes	1hour-8days	yes	yes	low	John et al., 2007; Wang et al., 2013.
Urea	yes	yes	yes	5-50days	yes	yes	low	Vinnerås et al., 2003.
Intrinsic Ammonia (NH_3)	yes	yes	yes	< 2months	yes	yes	low	Fidjeland et al. 2013.

2.1. Comparison of faecal sludge treatment technologies

2.1.1. Mechanical treatment

2.1.1.1. Solar radiation

Solar radiation is the thermal energy derived from the sun. This method of using the sun as a source of energy has been used for centuries in both developing and developed countries. As a result; due to its efficiency for drying food, wood, fruits, other agricultural products and other material, developing countries are still practicing it and consider it as a good preservation method. However, the method is more appropriate for tropical areas (Belessiotis & Delyannis 2011).

Solar radiation can be derived either directly by exposing the treated material to the sun or indirectly by convective solar drying. However, despite the technique used to derive solar radiation, temperature is the most important factor influencing the process. The rapid drying rate is influenced by higher temperatures. In addition, based on sensitivity of some material, drying conditions that suit the material to be dried must be taken into consideration. For example, fruits are more suitable for sun drying as they are rich in sugar and acid which increase their safety; whereas vegetables are vulnerable to rot if exposed to the sun since they have sugar levels and acid (Belessiotis & Delyannis 2011). On the other hand, similar conditions for sun drying of fruits will also apply for faecal sludge as it is rich in organic matter and has the ability to produce acids due to chemical reactions that occur during storage. However, this does not guarantee its safety as it also contains many infectious pathogens.

The mechanism of solar drying does not depend on the type of energy used. However, it operates in such a way that hot air circulates through the treated material. This can be greatly achieved by mechanical methods as a source for indirect solar drying. Belessiotis & Delyannis (2011) also reported that in order to convert water to vapour during drying process, 2258 kJ/kg of energy is required at 101.3 kPa. Additionally, in faecal sludge for example, parameters such as temperature and moisture content must be closely monitored for the determinination of the rate of drying. However, determination of moisture content must be performed either on dry or wet basis depending on the quality and characteristics of the sludge.

VijayaVenkata Raman et al. (2012) considers solar radiation as the best alternative method for drying crops for natural drying systems which uses the sun as the source of energy and artificial drying which uses fossil fuels. The author evaluated variety of solar drying systems. In this study the special attention was given to desiccant based solar drying system which facilitated drying of crops in the off-sunshine hours. However, other types of dryers such as natural and forced convective dryers were also evaluated. As a result, shortfall such as the overall efficiency of the systems was identified. For natural convective dryers, the overall efficiency range between 10-15% whereas forced convective dryers range between 20-30% (Mrema et al., 1987) cited in Purohit et al. (2006). As previously mentioned, there are several factors governing the efficiency of the solar drying system, namely, type of dryer, product dried, weather conditions and moisture content.

In summary, there is limited literature available with regard to the use of solar radiation as a treatment for faecal sludge. However, the treatment has been popular in agriculture as it is simple, cheap, saves energy and time as well as improving agricultural returns. In addition, the studies conducted on this system only focused on the drying efficiency required for food and crop preservation in order to enhance storage and reduce transportation costs of agricultural products. It is therefore recommended that more studies should be conducted on solar drying for faecal sludge treatment for inactivation of pathogenic organisms. This may be a possible treatment option for treating faecal sludge during emergency situations.

2.1.1.2. Ultraviolet (UV) radiation

UV is a well known disinfecting agent in water industry as an alternative to chlorine and its compounds (Aladawi et al. 2006). However, the success rate of its application to effectively inactivate *Ascaris* eggs in wastewater effluent was limited (Keller et al. 2004; Orta de Velasquez et al. 2004; Aladawi et al. 2006) cited by Mun et al. (2009).

Mun et al. also reports that there are conflicting reports as far as inactivation of *Ascaris* eggs is concerned. Some studies reported that 2log inactivation was achieved by UV doses below 400mJ cm^{-2} while other studies reported that UV was totally ineffective even at UV doses as high as 45 792 mJ cm^{-2}. However, in his study (Mun et al), he investigated the inactivation of *Ascaris* eggs in soil by microwave in comparison to UV and ozone treatment. The results revealed that microwave was more effective in that it was able to inactivate about 2.5 log within 60s whereas UV barely inactivated the eggs between 0.01 and 0.32log within 60min. On the other hand ozone was completely unsuccessful in the inactivation of *Ascaris* eggs.

This study, proved that UV is not effective enough to inactivate *Ascaris* eggs in soil as doses as high as 10 800mJ cm^{-2} were applied. This could be due to the fact that eggs hide themselves behind the soil particles thereby shielding them from the UV radiation. However, when similar tests were conducted using water instead of soil, UV managed to achieve 2log inactivation at dosages less than 5000 mJ cm^{-2} (Mun et al, 2009). This confirms that effectiveness of UV depends entirely on the treated medium, the rate at which UV radiation penetrates and the dosage required to inactivate the pathogenic organisms (Aladawi et al. 2006).

On the other hand, while Mun et al. (2009) reports inactivation of *Ascaris* eggs by UV even at negligible amounts, Aladawi et al. (2005) contradicts with this study. The study actually confirmed that UV is completely ineffective in *Ascaris* eggs inactivation; instead it accelerates the development of larvae. It is assumed that UV radiation was unable to penetrate hard and many layers of the egg, but created favourable conditions for early development of larvae. As a result the treatment is not suitable for disinfecting faecal sludge containing high levels of helminth eggs and other spore forming bacteria.

2.1.1.3. Gamma irradiation

Unlike other pathogenic organisms, *Ascaris* eggs are more resistant to other treatment methods especially chemicals and radiation by ultraviolet. However, due to their size and weight, they are easily enumerated and isolated as they have the ability to settle out of water and thereby accumulating in waste sludge. This makes it easier for treatment methods such as Gamma radiation to effectively kill or reduce their viability in the treated sludge.

Inactivation of *Ascaris* eggs by Gamma radiation is well documented by various authors cited in de Souza et al. (2011). These authors confirms that despite the high cost of the treatment, it is highly effective in the removal of pathogenic organisms such as *Ascaris* eggs and can retain nutrients essential for agricultural use. Gamma radiation interrupts the development of the viable egg by penetrating the inner layer of the egg and damaging its DNA and other essential organs responsible for embryonic development. Once the embryonic development has been damaged, the egg become inactive and growth deficiency of larvae occurs (Shamma & Al-Adawi, 2002).

de Souza et al. (2011) reports that the studies done on inactivation of *Ascaris* eggs by Gamma radiation differs in terms of the indicator of Helminth eggs used, their origin or source, quantity of viable eggs inoculated in controls and the type of inoculums used. However, despite all the differences and/or contradictions, the treatment seem to be the best of them all as it is able to achieve 100% inactivation of *Ascaris* eggs. Considering the history of *Ascaris* eggs for surviving in harsh environmental conditions, this was a major breakthrough. The inactivation was achieved even at concentrations as low as 1.5 kGy (Shamma & Al-Adawi, 2002) with higher concentration not more than 5 kGy (de Souza et al. 2011).

Based on literature, it can then be concluded that Gamma radiation will effectively inactivate pathogens found in faecal sludge due to its success rate on the disinfection of wastewater as reported by de Sousa et al. (2011) and other authors mentioned in his report as well as waste sludge as documented in Shamma and Al-Adawi, 2002. This treatment method has a potential to be used during emergency situations.

2.1.1.4. Thermal drying

Large quantities of sludge are produced daily due to domestic and industrial activities. Consequently, the produced sludge should be dealt with in a safe manner by reducing its volume and/or treating it before disposal. One way of achieving this is by thermal drying. Thermal drying can be defined as the process in which water is removed from the solid matters by evaporation. The process is influenced by operating conditions such as temperature, humidity and velocity as well as the nature and texture of the treated material. As a result the drying behaviour is characterized by estimating the evaporation mass flux density (Fm):

$$Fm.A(W) = m_{s} \cdot \frac{dw}{dt} \quad \text{..... (1)}$$

Where *A(W)* is the transfer area which decrease with time during the drying process as the material shrink upon drying. The drying kinetics is characterized by mass flux density as well as the moisture content of the treated matter (Vaxelaire et al., 2000).

Additionally, thermal drying is recognized as a potential faecal sludge sanitizer due to its ability to reduce the volume of the sludge while stabilizing the sludge by inactivating pathogens. However, most thermal dryers are expensive as they consume high energy. The mechanism of thermal dryers applied in sewage sludge operates in such a way that they produce vapour which contains volatile organic compounds and thereby creating unpleasant smell, toxic aerosols and sometimes explosions (Peregrina et al., 2008).

Studies regarding drying of sludge thermally have been reported by several researchers using different approaches. One of the approaches is immersion frying. Peregrina et al. (2008) reported that da Silva et al. (2005, 2003) conducted the first study of frying sewage sludge in soybean oil. The sludge was put in a cylinder and immersed in oil for about 10 min at temperature ranging between 168 and 213°C in order to dry the sludge by reducing its moisture to less than 5%. Similarly, Peregrina et al. (2006a) also performed the same study and achieved similar results but using waste cooking oil. The principle behind fry-drying involves four stages; namely, initial heating of the sample, boiling as the temperature rises, penetration of oil into the sludge particles and finally the sludge changes its composition.

On the other hand, Vaxelaire et al. (2000) investigated the drying potential of a convective dryer on activated sludge from secondary clarifier. The moisture content of the sludge was also monitored in order to characterize the drying process and identify potential crust risks. In this study, the activated sludge was first conditioned to ensure quick filtration before it was placed in a convective dryer that operated with wet air. During the experiment, laboratory software was developed in order to control and regulate operating conditions. The observed results were that activated sludge did not respond well to the treatment as it developed a hard dry layer on the surface (crust) and remained wet in the centre. Therefore, the drying period required to achieve desirable results was longer as the sludge was very hard to dry. Additionally, the development of the crust denotes the kinetic of activated sludge which is associated with weak constant rate period as the drying potential increases.

2.1.2. **Chemical Treatment**

Pathogen inactivation in faecal sludge can also be achieved by using Chemicals (acids and bases). According to Jimenez-Cisnero and Maya-Rendon (2007) treatment of sludge by Acid has been in existence for almost 30 years. Its results were very good in that acids, with a specific reference to organic acids, have the ability to interfere with cellular reactions by inactivating the DNA or RNA due to their toxicity and effect on pH. Acids such as sulphuric, hydrochloric, propionic, acetic and peracetic are the most commonly used to inactivate the pathogens. However, the last two were proven to produce the best results. Lactic acid has also been used as it is also a good disinfectant. As a result, in this study focus will be on peracetic acid and lactic acid since they are notorious of being the best amongst others.

In addition, it has been mentioned that Helminth eggs are more resistant to treatments and harmful than other pathogens since they have multiple layers which provide protection against any intrusion. However, Jimenez-Cisnero et al. (2007) reveals that acids are capable of penetrating the egg and damaging nuclei and thereby completely destroying helminth ova. Furthermore, acid treatment is not only a good sanitizer but can also remove metals, control bad odours and oxidizes organic matter without any increase in the quantity of the sludge.

On the other hand strong inorganic bases such as NaOH, KOH, NH_4OH, $Ca(OH)_2$, CaO, PO_4^{3-}, MgO, $Mg(OH)_2$, NH_3 are widely used to treat sludge. Their application is influenced by the fact that they are cheap, simple and easy to operate (Vinnerås et al. 2003). They can be applied on a small or big scale as well as on on-site sanitation systems. Any alkaline, more especially Lime, have the ability to reduce moisture content and increase the pH of the sludge and thereby increasing the inactivation potential. Alkaline can also inactivate helminth ova, but there are chances of re-growth in sludge after treatment and up to 40% increase in sludge quantity (Jimenez-Cisneros & Maya-Rendon, 2007).

Moreover, there are various factors to consider when choosing chemical as a treatment. Factors such as agronomic value of the substance in the disinfectants, rapidity of the treatment, efficiency of the treatment, physical parameters like pH and temperature of the faecal sludge, and the buffering capacity of the mixture (Vinnerås et al. 2003). As mentioned in chapter 1, the mechanism, impact, limitations and the conditions at which these chemical treatment methods inactivation the pathogens are outlined below.

2.1.2.1. Peracetic acid (PAA)

In most developing countries, ash was a notorious treatment to eliminate bad odour and to reduce the moisture content of the sludge in the latrine. However, its sanitizing effect was not as good. PAA on the other hand has been very effective in the inactivation of pathogens over the years. It is an unstable organic acid which explodes at higher concentrations (Vinnerås et al., 2003). Consequently, extreme care must be taken when handling this acid. The acid occurs in aqueous mixture with acetic acid, hydrogen peroxide, and water under equilibrium. Its advantages are that it is a rapid treatment which is efficient in inactivation of bacteria; it penetrates the cell wall and disrupts sulphydril and sulphur within the enzyme; it is reasonably cheap and easy to apply; it is scale independent and can be applied even at low concentrations of about 0.15 ppm.

On the contrary, the downside of the treatment is that it is not efficient against viruses and some parasites and the increase in organic content of the treated material leads to higher concentration of PAA required to inactivate the pathogens (Vinnerås et al. 2003).

Vinnerås et al. (2003) investigated the potential of using peracetic acid as a chemical treatment for disinfection of faecal matter. The study revealed that PAA has the potential to effectively inactivate *E. coli*, *Salmonella spp*., Enterococcus spp. and Clostridia spp. within 1hour to 5days in concentrations between 0.15% and 1.5%. On the other hand, viruses were rapidly reduced with no significant difference between 1h and 5 days; whereas parasites were only reduced by 10%. It has been observed that the highest

concentration rapidly inactivated and/ or reduced the pathogens within 1 hour and the lowest concentrations between 5 days to even up to 21 days.

2.1.2.2. Lactic acid (LA)

Lactic acid is an organic acid that can be derived from kitchen food waste and garden waste (Jones et al. 2008) as well as carbohydrates, alcohol, filamentous fungi and Lactic acid bacteria (LAB) with the last two being the biggest microbial source. The production of LA occurs due to natural processes such as fermentation and synthesis of chemicals. However, fermentation of LA is perceived economical and efficient in various industries as it is used for the production of polymers, solvents, cleaning and oxygenated chemicals (John et al. 2007).

The production of LA by LAB has been well documented and is widely used in food industries to produce fermented food (Yang et al. 2006) and to prevent the microbial growth of food spoiling bacteria (Alakomi et al., 2005), although there is limited knowledge on its application on faecal sludge sanitization. Since pathogens resulting from faecal sludge can find their way into the environment and even on food, it is therefore extremely important to examine food-borne pathogens.

Wang et al. (2013) investigated the reduction of food-borne pathogens such as *E. coli*, *Salmonella typhimurium* and Listeria monocytogenes by using LA as a chemical treatment in lotus sprout at different concentrations ranging from 0.25% to 2% v/v. The samples contained microbial load of above 7 log cfu/g after 5 minutes inoculation. The results indicated the reduction of microbial load at 0.5 % and 2 % by 1.5 and 2.3 log respectively when using LA. The reduction rate was higher compared to samples with just tap water and NaOCl which were <0.5 and 1.3 log respectively. Furthermore, the results depict the efficiency of LA in determining the microbial quality of lotus sprout by reducing its microbial load thereby improving its colour and ensuring its safety.

Since Lactic Acid was successful in reducing the pathogens such as *E. coli* and *Salmonella* which can also be found in faecal sludge, the treatment can be considered in faecal sludge treatment as well. However, the conditions under which these pathogens were reduced were not clear, as a result, the prevailing conditions must be taken into consideration and the concentrations at which these pathogens can be inactivated in faecal sludge.

In addition, the study recently conducted under supervision of UNESCO-IHE (2014) is in agreement with the fact that LA can really be employed in faecal sludge as a sanitizing agent. The study investigated the effect of LA on the inactivation of *E. coli* in faecal sludge. The results obtained from the study shows that after addition of LA the pH of the faecal sludge reduced from 7 to between 3.8 and 4.2. As a result, this extreme pH reduction led to 7 log reduction of *E. coli* after 9 days with concentrations between 20-30 g/l lactic acid. The precultures used in this study were 10% w/w of both milk and molasses which resulted in total sugar of 1.5-2g/l. Furthermore, the study reveals that not only does LA inactivate *E. coli*, but can also suppress bad smell. The study was concluded by indicating the potential of LA as faecal sludge sanitizer for on-site treatment as well as emergency situations.

2.1.2.3. Intrinsic Ammonia (NH_3)

Literature reveals that faecal sludge contains large quantities of pathogens. As a result, it must be sanitized before disposal or application on land. The intrinsic ammonia has the potential to sanitize faecal sludge if the sludge is not too diluted with flush water or evaporated due to ventilation. Fiejeland et al. (2013) evaluated its potential for the inactivation of pathogens and also determined the manner in which the composition of the sludge and surrounding temperatures affect pathogenic inactivation. Enterococcus faecalis, *Salmonella typhimurium* were used as indicators for bacteria whereas *Ascaris* was used as indicator for Helminth eggs.

In this study, self-sanitization of faecal sludge by intrinsic ammonia was achieved due to the fact that urine can rapidly be converted to ammonia which is effective in the inactivation of pathogens. However, factors such as faeces/urine ratio, infiltration, flush water volume and ventilation have great influence on its sanitizing effect. Therefore, the treatment is even much more suitable for faecal sludge generated from the dry toilets or ordinary latrine since the sludge is rich in ammonia concentrations. If the toilet is ventilated, then large quantities of ammonia might escape to the atmosphere through ventilation (Fidjeland et al., 2013).

In addition, the loss of ammonia can be prevented by containing the faecal sludge in an airtight storage; or utilizing the pit itself as storage provided it is lined and there's another pit to be used during treatment. The results in the study illustrated that intrinsic ammonia was able to inactivate all pathogens except *Ascaris* eggs which were more persistent. However, the viability of *Ascaris* eggs was reduced by $3\log_{10}$ at temperatures between 23-28^{o}C within 1- 1.5 months. The two major contributing factors for effectiveness of the inactivation were ammonia concentration and temperature. On the other hand, *Salmonella spp*., *E. faecalis* and A. *suum* were successfully inactivated by ammonia; whereas Entrococcus spp. was also persistant as it survived under conditions toxic to *E. faecalis*.

In summary, the findings demonstrate that flush water volumes had no relation with ammonia concentration in the pit as inactivation of pathogens favoured faecal sludge with lower flush water and higher urine content. Therefore it can be concluded that the ammonia content in the pit dependent on the rate of urination than defecation in order to enhance self-sanitization. However; factors such as loss of ammonia by ventilation, variations in the ammonia content in urine due to different diets, and the chemical properties of the sludge also played a major role (Fidjeland et al. 2013).

In conclusion, the limitation of the study is that self-sanitization of faecal sludge from dry pit latrines may not be sufficient even though there is no flush water. In addition, the loss of ammonia due to ventilation cannot be avoided as long as the toilet is in operation since ventilation is necessary to eliminate bad odour. Lastly, the pH ranges of the faecal sludge for inactivation of pathogens were not highlighted. As a result it is not clear as to at what pH was the treatment effective.

2.1.2.4. Urea

As mentioned before, chemical disinfectants are beneficial due to their short treatment period and their agricultural value. Chemicals mainly inactivate the DNA or RNA of the bacterial cell (Vinnerås et al., 2003). Urea is known as a Nitrogen fertilizer in agriculture and it has been used continuously. Its mechanism works in such a way that when it comes into contact with the treated matter (e.g. soil or faeces), the enzyme urease degrade it to ammonia and CO_2. Like any other chemical, urea relies on factors such as pH, temperature, time, salinity, free ammonia and moisture content which greatly affects the die-off of pathogens during treatment. In addition, urea does not only disinfect the sludge but it also stabilizes it and ensuring its safety to be reused in agriculture (Ottoson et al. 2008).

Many studies have been done on the effectiveness of urea in inactivating pathogens. As a result, urea was found to be effective in inactivating bacteria such as *E. coli* and *Salmonella spp*. within short period of time (5days). However, spore-forming bacteria are not affected by the treatment (Vinnerås et al., 2003). The free ammonia produced during urea treatment can penetrate through the bacterial cell membrane and causes rapid alkalinization of the cytoplasm or decrease intracellular K^+ concentrations and thereby destroying or damaging the cell (Ottoson et al. 2008). Urea can be applied in its natural form as it is simple, cheap, safe and easy to handle. However, the treatment period for effective inactivation of pathogens range from 5 to 50 days (Vinnerås et al., 2003).

Additionally, the study conducted by Vinnerås (2007), validate the effectiveness of urea treatment. In this study, 3% N-NH_3 added to the treated material was able to raise the pH to 9.2 within 1 hour. *Salmonella spp*. and faecal coliforms were not detected after 5days of treatment, whereas Enterococcus spp. was not detected after 20 days. On the other hand, spore-forming Clostridia spp. was not affected by the treatment.

The inactivation rate was influenced by the amount of free ammonia available in the treated material which depended greatly on the increase in temperature, pH and concentration of total ammonia. In conclusion, urea treatment is effective if ammonia produced remains in the treated material for at least 2 months until application to the soil in order to prevent re-growth of pathogens and reducing the risk for contamination.

Recently, the study on sanitizing faecal sludge with urea has been conducted under management of UNESCO-IHE (2014). The aim of the study was to investigate the effect of urea on the microbiological quality of faecal sludge. The results of the study reveal that urea was able to achieve 3log reduction of *E. coli* and *Salmonella* and 2log reduction of total coliforms within 8 days at pH 9.4. The concentrations tested were 1% and 3% (w/w) urea/kg of faecal sludge. However, insignificant differences were observed in terms of pH increase and reduction of pathogens between the two concentrations. The study further emphasize that temperature can enhance the treatment. As a result, exposure of reactors to the sun during field experiments, might improve the sanitizing effect of urea.

2.1.2.5. Lime

Globally, a typical limestone or calcium carbonate ($CaCO_3$) occurs naturally as a stone which reacts very slow. The limestone differs from region to region based on its composition and physical characteristics; however, in some instances the difference is within the veins of limestone in the same region. This differences result in differences in the quality of the calcium hydroxide to be produced (Hassibi, 1999). Furthermore, the use of lime is not only popular in water and wastewater treatment where it is used as a coagulant which concentrates the impurities or contaminants present in water or wastewater (Polprasert and Valencia, 1981); but it is also utilized in the form of calcium hydroxide in industries to control pollution (Hassibi, 1999).

The manufacturing of lime and its slaking process is well documented in the study made by Hassibi (1999), where he mentioned that calcium hydroxide ($Ca(OH)_2$) results from the limestone ($CaCO_3$) which has been converted to calcium oxide (CaO) in the presence of heat. However, due to the fact that CaO in the presence of moisture and CO_2 is unstable, it is then mixed with water to produce Ca(OH)2 which is a more stable form of lime. This process is called hydration or lime slaking and is well summarized in the following chemical formulae:

$$CaCO_3 + HEAT \rightarrow CaO + CO_2 \quad (2)$$

$$CaO + H_2O \rightarrow Ca(OH)_2 + HEAT \quad (3)$$

The studies done by Burnham and Nicholsen (1990) and Willford et al (2007) are in agreement by reporting that alkaline or lime stabilization is a low cost, simple process that it is easy to apply. The primary role of lime stabilization is to eliminate pathogens while reducing smell in sludge or biosolids (Wong and Selvam, 2009). However this can be achieved by increasing the pH of the treated sludge to $\geq$ 12 and maintaining the pH levels for at least 2 hours (Williford et al., 2007). The sludge that has been stabilized by lime treatment in sufficient quantity is often free from pathogenic organisms which make it safer to be reused or returned to the environment. Christensen (1987) describes sludge stabilization as sludge disinfection, from the disposal point of view. On the contrary, the addition of lime will not serve its purpose of stabilizing the sludge if applied in small quantity thereby resulting in pH less than 11(Nicholson et al., 1990).

Literature reveals that chemical stabilization of sludge by lime has been applied due to its ability to inactivate spores and bacteria forming spores at a very low cost (Loehr, 1968) cited from Polprasert et al., (1981). The other competitive advantage of using lime over other disinfectants could be that it is feasible for developing countries to use methods available for determining pathogens when treating sludge with lime due to their simplicity and user-friendliness. Several studies on lime stabilization were conducted under different conditions.

Polprasert et al. (1981) investigated the effect of lime in inactivating faecal coliforms and *Ascaris ova* in faeces. The study focused on the relationship between lime dosage, contact time and die-off of faecal coliforms and *Ascaris ova*. The findings of the study confirmed that during lime treatment, the hydroxide alkalinity or high pH from $Ca(OH)_2$ has the antimicrobial effect as it inactivated pathogens studied. However, the inactivation efficiency was less at pH < 11 and re-growth of bacteria was observed at pH 9.6. The results also indicated low efficiency of lime in the inactivation of *Ascaris ova*. Due to the complexity of the inactivation mechanism of *Ascaris ova* by lime, it is assumed that the inactivation of the ova occurs due to slow reaction between (OH^-) or high pH and the ova. Similarly, faecal coliform also undergoes the same inactivation process. In conclusion, the study also reveals that there is no correlation between contact time and inactivation effect. However the relationship between lime dosage and die-off of faecal coliforms and *Ascaris* ova was established as the higher the dosage, the greater the inactivation effect.

Furthermore, the kinetics of *Ascaris* eggs in sludge treated with lime (slaked or quick lime) was investigated by Capizzi et al. (2004), in order to determine the time required to inactivate *Ascaris* eggs with the influence of different temperature ranging from 50^{o}C to 60^{o}C. The study was performed in a small-scale (laboratory) comparing inactivation effect of quick lime (CaO) and slaked lime ($Ca(OH)_2$) and on full-scale using only CaO. The findings also confirmed the effectiveness of lime in destroying the pathogens found in sludge by not only increasing the pH but also increased temperature. Less inactivation threshold (5min) was observed with quick lime at 60^{o}C in both laboratory scale and full scale, than the combination of slaked lime and heat (8min). However, longer inactivation threshold of approximately 120min and 128 min was observed at 50^{o}C for quick lime and slaked lime respectively, with exception of full scale which took about 75min at 55^{o}C.

Moreover, the study did not cover the inactivation of protozoa such as Cryptosporidium oocysts and Giardia cysts. These indicator organisms for reduction of protozoan oocysts are also resistant to certain treatment methods applied to treat sludge. As a result, the treatment conditions under which these protozoa are inactivated should be the same as that of *Ascaris* eggs inactivation. The viability of Giardia cysts is greatly affected by higher pH which leads to complete inactivation if exposed to lime treatment at 46^{o}C for 10min. However, inactivation of Cryptosporidium oocysts with lime occurs at 45^{o}C for 20min.

In conclusion, as mentioned before, the effect of inactivation kinetics of *Ascaris* eggs has been established with the homogeneity of temperature and hydroxyl concentration. As a result, the study managed to clearly define an inactivation kinetic with respect to the inactivation threshold as the treatment of time which resulted to negligible level of *Ascaris* eggs. There is limited information on the application of lime as a disinfectant for faecal sludge resulting from on-site sanitation systems. Therefore, this paper aims at investigating the effectiveness of lime in inactivation of pathogens in particular enteric bacteria present in faecal sludge.

2.2. Characterization of faecal sludge

Faecal sludge can be described as the undigested or partially digested sludge from either latrines or septic tank. However, it should be noted that there is a huge difference between faecal sludge and wastewater as 1ℓ of faecal sludge is equivalent to 100ℓ of wastewater. Furthermore, faecal sludge can be characterized as high strength (from latrines) as it is more concentrated and low strength (septage) which is diluted. According to Roland Schertenleib, factors influencing the quality of the faecal sludge include the equipment used for emptying the pit, performance of the sanitation system, intrusion of groundwater or wash water from the kitchen and laundry, temperature, foreign objects in the faecal sludge, and storage duration (weeks, months, and years).

There is limited literature available with regard to the composition of human faeces. However, in the study conducted by Torondel (2010) several authors confirm that human stools are characterized by roughly 70-80% water and 20-30% solid matter. Nevertheless, the composition varies from individual to individual and

from country to country depending on the diet, water intake and digestive function. Furthermore, 84% of the solid matter in faeces is organic in nature which composed mainly bacteria of about 55% and residual dietary fibre of approximately 17%. Additionally, the study also reports that characterization of faeces can be describe in terms of their biodegradability related to chemical oxygen demand (COD, i.e. organic matter). For instance, 80% of human faeces are made up of slowly biodegradable organic matter and the other 20% is unbiodegradable soluble organic matter (Torondel 2010).

In this study, the composition of sewage sludge for laboratory analysis and faecal sludge for field analysis, will be characterized by performing physicochemical and microbiological analysis (see table 2 below).

2.3. Research Objectives

The main objective of this study is to investigate the effectiveness of lime treatment for the inactivation of pathogens present in faecal sludge from raised latrines during emergency situations.

Specific objectives are outlined as follows:

- To examine the impact of lime treatment on the inactivation of pathogens;
- To determine time period required to inactivate pathogens during lime treatment;
- To determine the relationship between pH, temperature, moisture content and concentration of lime in Inactivation of pathogens;
- To sanitize the faecal sludge to achieve at least < 1000 *E. coli*/ 100mℓ of sludge; and
- To stabilize the faecal sludge by maintaining higher pH ≥ 12.

2.4. Research Questions

- Is faecal sludge treated with lime safe to be reused or disposed?
- Does faecal sludge treated with lime has an agronomic value?
- What is the removal efficiency of lime treatment on pathogens?
- What is the optimum time period for the reduction of pathogens during lime treatment?
- Can lime treatment able to sanitize and stabilize faecal sludge?
- Can lime treatment applied in emergency situations?
- Can lime treatment categorized as an on-site treatment method?

2.5. Significance/Justification of the Research

As mentioned before, faecal sludge management challenges are closely related to large number of on-site sanitation systems, such as latrines, unsewered public toilets or septic tanks, used by the majority of the population for disposal of black water in densely populated cities of developing countries. Few of faecal sludge collected from on-site sanitation systems is transported to sludge treatment facilities, whereas most are dumped untreated in receiving water bodies and on the environment (Jiménez et al., 2010). Consequently, these practices pose a serious public health risk as inhabitants of the affected area become more vulnerable to diseases.

In Malawian townships such as Bangwe, the most basic sanitation facilities are still in practice where pit latrines are used by the large population of such communities. Upon the filling of the pit, faecal sludge is desludged by local desludging companies at the owner's expense and the sludge is dumped to the inlet of the nearest wastewater treatment plant (Zingwangwa). Sadly the treatment plant is not designed to treat faecal sludge and it is almost non-functional. As a result, the supposedly treated waste water is being discharged to the river and no strict regulations are in place to prevent such situations.

Similarly, emergency situations also undergo same problems. As a result there is a great need to explore different treatment methods to mitigate the said challenges. Hence the use of lime treatment to stabilize sludge and make it sanitized before disposal and reuse.

CHAPTER 3

3.0. Research Design and Methodology

Microbiological analyses were performed to quantify the initial population of pathogens in the black water sample and of the removal of bacteria during treatment. However, since it is impractical to analyze all possible pathogenic organisms, the need for indicators of faecal contamination was perceived. As a result, *E. coli*, total coliforms and *Salmonella* were chosen as representatives of faecal contamination. *E. coli* can survive for up to 2 months and has a potential to re-grow, and replicate after treatment thereby contaminating the environment. *Salmonella* can also survive in soil for up to 3-5weeks and can potentially re-grow after treatment; whereas coliforms such as faecal coliforms can withstand high temperature of up to 45^{o}C for 48 hours (Estrada, et al. 2004).

The experiments were carried out in two phases, laboratory phase and field phase. The laboratory experiments were carried out at IHE-UNESCO in Delft on the black water collected from Sneek waste water treatment plant (Friesland, NL) of a small community. Field experiments were conducted with faecal sludge collected from latrines (Household and public) in Bangwe Township Malawi

The following table summarizes all materials used in the laboratory and the field. This overview was useful to update all the resources required for the field phase in Malawi. It was necessary to generate the inventory list of all the material and equipment needed in order to ensure smooth operation of the field work. As outlined on table 3.1, equipment such as kitchen and bathroom scales were used as contingency plan to carryout analysis due to shortage of resources. This was mainly because most of equipment and chemicals were not locally available; hence they had to be imported from the Netherlands and Malawi's neighbouring countries. The logistical part of the project was challenging as most the material needed in order to resume field experiments were held in customs for some time. That really affected the time scheduled (4 weeks) for the field phase. As a result, the entire field phase had to be conducted in a period of two weeks only.

Table 3.1 materials used during experimental procedure

Item	Purpose	Laboratory	Field
Equipment			
pH meter	Measure pH	✓	✓
Conductivity meter	Measure Electrical conductivity	✓	✓
Infrared thermometer	Measure temperature		✓
Weighing balance	Weigh chemicals	✓	✓
Kitchen scale	Weigh lime		✓
Bathroom scale	Weigh faecal drums containing faecal sludge		✓
Oven (muffle)	Dry samples to obtain TS/VS	✓	✓
Autoclave	Sterilize samples and glassware	✓	✓
Water bath	Boil media	✓	✓
Incubator	Incubate agar plates	✓	✓
Apparatus/Glassware			
Spatula	Measure chemicals	✓	✓
Glass beakers	Served as batch reactors where black water samples were added	✓	
Volumetric flasks	Prepare agar media	✓	✓
Test tube rack	Hold test tube	✓	✓
Test tube	Serial dilutions	✓	✓
Pipettes	Transfer diluted samples	✓	✓
Syringe	Transfer raw samples	✓	✓
Measuring cylinder	Measure liquid samples and water	✓	✓
Aluminium foil	Cover flasks containing liquid media	✓	✓
Aluminium cups	Collecting sludge	✓	✓
Cotton wool	Seal the flask containing liquid media	✓	✓
Crucible tong	Takeout samples from the oven	✓	✓
Petri dishes	Plate the media	✓	✓
Glass spreading rod	Spread the sample on the media	✓	✓
Gas burner	Provide flame for sterilization	✓	✓
Lighter	Ignite the gas burner	✓	✓
Plastic sampling bottles	Collect samples	✓	✓
Plastic sampling buckets	Collect samples	✓	
Plastic drums	Served as reactors to contain faecal sludge during field experiments		✓
Chemicals/Reagents			
Distilled water	Prepare serial dilutions and media	✓	✓
70% Alcohol	Sterilize the working area (bench)	✓	✓
Chromocult coliform agar	Growth media for organisms	✓	✓
Sodium Chloride	Serial dilutions	✓	✓
Laboratory grade lime	Treat black water and faecal sludge	✓	✓
Hydrated lime	Treat faecal sludge		✓

3.1. Laboratory-scale experiments

3.1.1 Sample preparation

Six sets of experiments were conducted in the laboratory with black water sample. The raw black water was taken from Sneek decentralized waste water treatment plant of a small community in the Friesland, NL. Initially, for safety reasons the sample was sterilized in an autoclave at 121^{o}C for two hours before use to kill all organisms. Sterilization was initially required because of laboratory safety regulations. Then the sample was spiked with E. coli bacteria with an end concentration of 1x108 CFU/100mℓ. After gaining experience with working with non-pathogenic material it was decided to use non-autoclaved samples, in order to mimic field conditions. The samples were then treated with quick lime to inactivate pathogens. Prior to the treatment with lime, the black water was analyzed for microbiological quality expressed as CFU/100mℓ as well as physical parameters such as pH, TS, VS, temperature, moisture content and organic matter.

3.1.2 Test organisms

The microbial culture of ATCC 25922 *E. coli* was obtained from IHE Laboratory to spike an autoclaved sample. A working culture of this organism was maintained on slant of peptone agar sloped of 35^{o}. A loop of organism was then taken from peptone agar slant, transferred into a nutrient broth and incubated at 37^{o}C on the shaker for 24 hours. After the incubation period the organism was stored in a refrigerator at 4^{o}C.

The raw black water samples (non-autoclaved) were tested for *E. coli*, total coliforms and *Salmonella* prior and after treatment. The average initial concentrations of these indicator organisms were 1x10^{7}, 1x10^{6} and 1x10^{7} CFU/100ml respectively.

3.1.3 Growth media

A laboratory *Chromocult*® coliform agar manufactured by Merck was selected as a nutrient media to provide optimum growth conditions for the test organisms. The selection of the medium was guided by the fact that Chromocult media not only rapidly enumerate enteric bacteria but also clearly distinguish *E. coli* from total coliforms and non-coliform by the colour of the colony (Finney et al. 2003a). The agar media was prepared according to the manufacturer's instructions. During preparation, 26g of Chromocult agar powder was diluted in 1ℓ of distilled water and boiled for 1hour in a water bath at temperature of 100^{o}C. The media was then cooled to 50^{o}C under room temperature before pouring into the Petri dishes or plates. After the plates solidified, they were turned upside down to avoid contamination of water formed by steam in the plates. The plates were then stored for five days under room temperature before use to dry.

3.1.4 Experimental design and protocol

This small-scale experimental protocol was designed to quantify the survival or monitor the die-off of *E. coli*, total coliforms and *Salmonella* which were exposed to chemical treatment with CaO at different dosages. The aim was to study the physical characteristics influencing the inactivation kinetics of these indicator organisms to assist the scientific rationale of full-scale processes for faecal sludge treatment. The procedure was carried as follows:

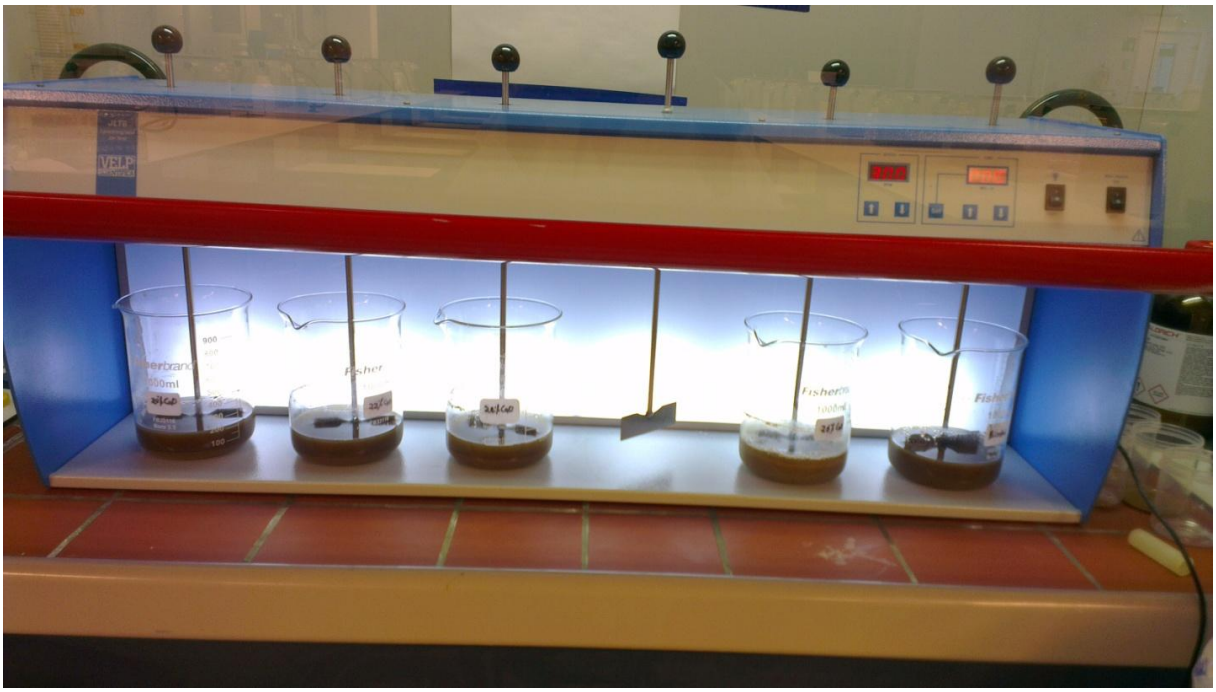

Figure 3.1 Jar test instrument with 5 sludge samples during treatment with CaO

- Five sterile 1000ml beakers were placed under a jar test instrument
- The beakers were then filled with 200ml black water sample each
- Quick lime (CaO) was added to the first four beakers and the fifth beaker served as a control
- The lime dosages were 20, 22, 24, 26, 28, 30, 40, 50 and 60% per total solids
- The lime was thoroughly mixed with the sludge sample under jar test instrument at 300rpm.
- pH was monitored throughout the experiment and 10ml samples were withdrawn from each beaker after 5, 15, 30, 60 and 120 minutes to examine the die-off of *E. coli*, total coliforms and *Salmonella*
- The maximum contact time for lime treatment was 2 hours
- 25 samples were collected per set of experiment and serial dilutions of each sample were prepared
- The dilutions were 1:10, 1:100, 1:1000 and 1:10 000. However, the optimum dilution factor was 1:100. This dilution range was initially chosen as it was not known which dilution factor would be best in terms of quantifying the colonies grown on the plate. However, after many trials and errors, 1:100 was found to be the best dilution factor as it showed colonies that can be easily counted. Colonies on dilutions below this were not countable (too many) whereas on dilutions above were too few to be counted.
- 0.1ml was withdrawn from each diluted sample and spread on chromocult media plates
- The plates were then incubated for 24 hours at 37^{o}C
- Purple colonies were observed on the media and then counted as viable *E. coli*, pink colonies as total coliforms and blue colonies as *Salmonella*. White colonies which represented other enteric bacteria were also observed but never counted as they were not bacteria of interest (Figure 3.2).
- The colonies were counted using a digital colony counting machine (Figure 3.2).
- The experiment was performed under room temperature and plated in duplicate per dilution factor

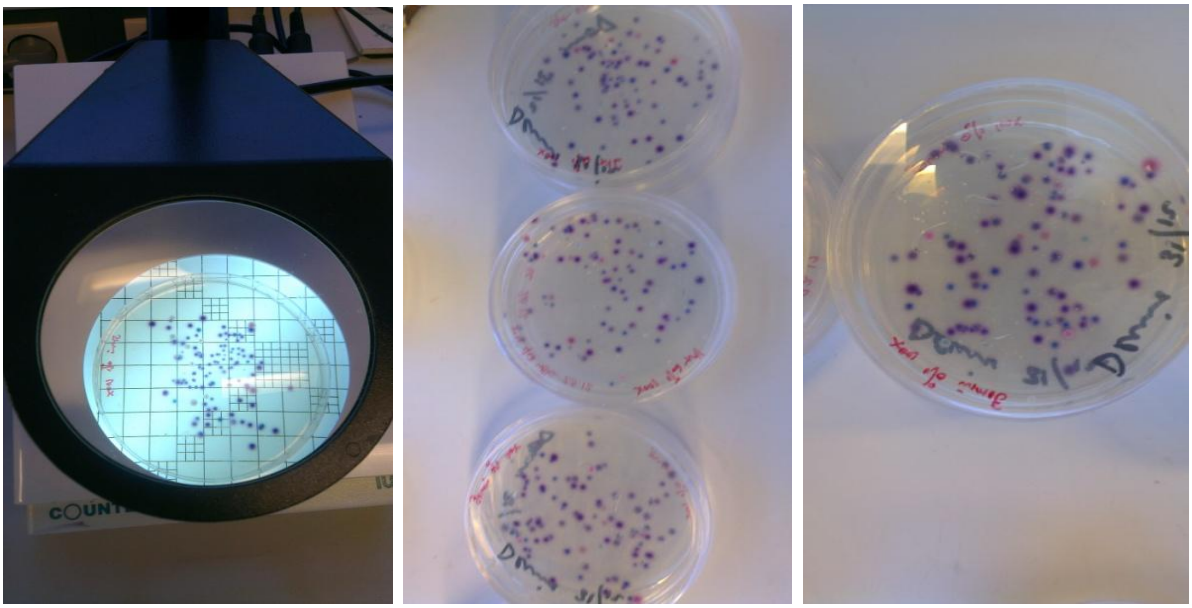

Figure 3.2 Colony counter and agar plates with blue, pink and purple colonies

3.2. Field-scale experiments

3.2.1 Sample preparation

Faecal sludge samples were collected from two household latrines and one public latrine in Bangwe Township, Malawi using mechanical desludging equipment depicted in figure 3.3. Due to time constraints, only 1 set of experiments per latrine was conducted.

a b c

Figure 3.3 Desludging equipment (a), water as a fluidizing agent (b), Desludging pipe (c)

The age of the faecal sludge samples collected and treated was 1 month (public), 1 year and 7 years (household). The samples were characterized by analyzing for pathogenic organisms, pH, TS, VS, organic content and moisture content prior treatment. The samples were then treated with the known pathogenic concentration expressed as CFU/100mℓ.

3.2.2 Test organisms

The organisms under study were the same as those tested in laboratory. As a result faecal sludge samples were also tested for *E. coli*, total coliforms and *Salmonella* prior and after treatment. However, *Salmonella* was not detected in any of the three pit latrines. Therefore, only *E. coli* and total coliforms were analyzed.

3.2.3 Growth media

A laboratory *Chromocult*® coliform agar manufactured by Merck was selected as a nutrient media to provide optimum growth conditions for the test organisms. The agar media was prepared according to manufactures' instructions as already mentioned above.

3.2.4 Experimental design and protocol

This pilot-scale experimental protocol was designed to quantify the survival or monitor the die-off of test organisms whereby faecal sludge was exposed to chemical treatment with CaO and $Ca(OH)_2$ at different dosages for the duration of 2 hours. The aim of the experiment was to study the physicochemical characteristics influencing the inactivation kinetics of these indicator organisms. The procedure outlined on Figure 3.4 below was carried as follows:

Figure 3.4 50ℓ plastic drums filled with 25ℓ faecal sludge samples from various latrines during treatment with CaO and $Ca(OH)_2$.

- Five 50 litre plastic drums filled with faecal sludge from pit latrine were placed on the floor in a shed
- Three set of experiments were carried out
- The first and third batch which contained faecal sludge aged 1 year and 1 month respectively were treated with hydrated lime ($Ca(OH)_2$); and the second batch which contained 7 years old faecal sludge was treated with quick lime (CaO). This was done due to availability of resources as only 2kg of quick lime was available; hence it was used for only 1 set of experiment. The other reason

was to check the efficiency of lime in two different forms, CaO (unstable) and $Ca(OH)_2$ (stable). It was desirable to treat each sample with both CaO and $Ca(OH)_2$, however due to time constraints and shortage of chemicals, particularly CaO, this was not possible.

- Quick/hydrated lime was added to the first four drums and the fifth drum served as a control.
- The lime dosages were 30, 40, 50 and 60% CaO / $Ca(OH)_2$ per total solids.
- The lime was thoroughly mixed with the sludge sample for 1 minute using a mechanical drill mixer (see Figure 3.5 below).

Figure 3.5 Mechanical drill mixer in sludge and a drill bit

- pH was monitored throughout the experiment and 10g samples were withdrawn from each drum after 5, 15, 30, 60 and 120 minutes to examine the die-off of pathogens.
- The maximum contact time for lime treatment was 2 hours.
- 25 samples were collected and serial dilutions of each sample were prepared
- The dilutions were 1:10, 1:100, 1:1000 and 1:10 000 with the optimal dilution of 1:100.
- 0.1ml was withdrawn from each diluted sample and spread on chromocult media plates
- The plates were then incubated for 24 hours at 37^{o}C.
- Purple colonies were observed on the media and then counted as viable *E. coli* and pink colonies as total coliforms, see Figure xx below (white colonies which represented other enteric bacteria were also observed but never counted as they were not bacteria of interest).
- The colonies were counted manually using magnifying glass.
- The experiments were performed under room temperature and plated in duplicate per dilution factor (Figure 3.6).

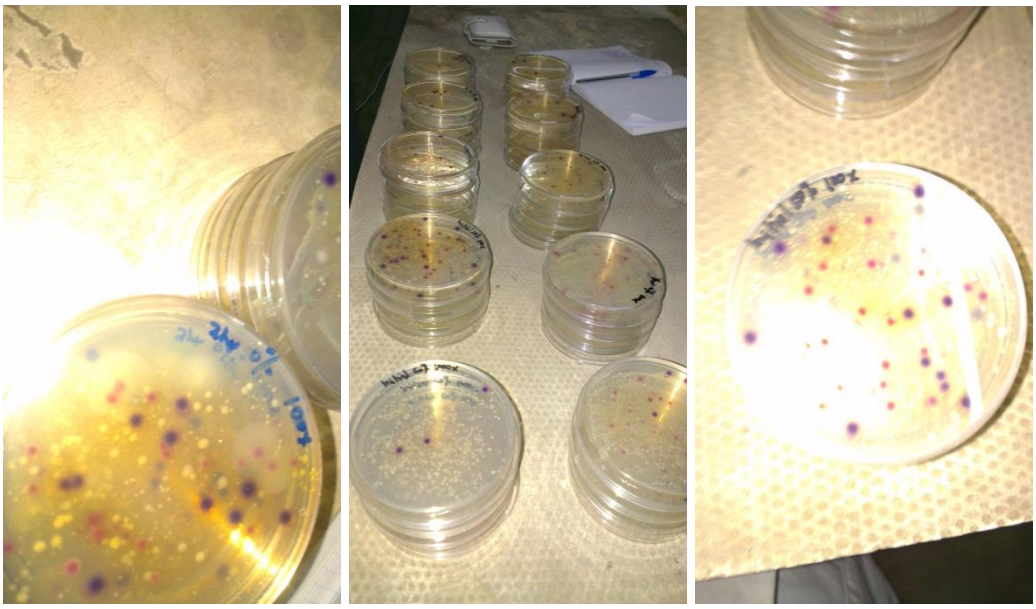

Figure 3.6 Colonies of *E. coli*, total coliforms and other enteric bacteria

CHAPTER 4

4.0. Results

4.1. Laboratory-scale results

4.1.1 Physical and microbiological Characterization

The black water was characterized by analyzing several physical parameters such as pH, conductivity, moisture content and organic matter, see Table 4.1, before treatment with quicklime (CaO). In the raw black water samples, *E. coli*, total coliforms and *Salmonella* were analyzed as the microbiological content of the samples. However, the first sample was exempted from this analysis since it was spiked with a known concentration of *E. coli* after sterilizing in an autoclave for 1 hour at 121^{o}C. The black water samples had a high moisture content which was expected since they are a mixture of water, urine and faeces. However, the black water had low organic content which indicates little decay to take place during the sanitization process. The pH of the sample ranged between 6 and 8.

For the samples which were slightly acidic (pH around 6), it was difficult to attain the recommended pH levels ($\geq$ 12) required for inactivation of pathogens; whereas samples with pH around 8 could easily reach the recommended pH levels. The results are depicted in figure 4.1, 4.2 and 4.3 below.

Initially, the sterile black water sample was spiked with 1x10^{8} laboratory cultured *E. coli*. Sterilization was initially required because of laboratory safety regulations. After gaining experience with working with pathogenic material it was decided to use non-autoclaved samples, in order to mimic field conditions. As a result, microbiological analyses were performed to quantify the initial concentration of above-mentioned indicator organisms' prior to treatment with lime. The concentrations of *E. coli* and *Salmonella* in the raw black water sample were similar, whereas that of total coliforms was 1 log lower (see Table 4.1 below).

Table 4.1 Physical and microbiological characterization of black water

Parameter	Measured values	Method
PH	6.8-8.6	The initial pH was determined by using a digital pH meter.
Conductivity (µS/cm)	11.93-13.30	Electrical conductivity was measured using digital Conductivity meter.
Temperature (^{o}C)	21-22	The sample temperature was measured by using digital thermometer ranging from 0-100^{o}C.
Total solids (%)	1.3-1.5	The TS was obtained by drying the sample in an oven for 2 hours at 105^{o}C.
Volatile solids (%)	26-54	The VS was obtained by further drying the sample at 55^{o}C.
Moisture content (%)	90-92	The moisture content was derived from difference between wet and dry sample (after drying at 105^{o}C)
Organic matter (%)	6-13	The organic content was derived from difference between dry sample after loss at 105^{o}C and loss at 550^{o}C.
E. coli (cfu/100ml)	$1x10^{7}$ - $1x10^{8}$	Plate count method using *Chromocult*® Coliform Agar.
Total coliforms (cfu/100ml)	$5x10^{6}$	Plate count method using *Chromocult*® Coliform Agar.
Salmonella (cfu/100ml)	$1x10^{7}$	Plate count method using *Chromocult*® Coliform Agar.

4.1.2 Changes in pH measurements

The contaminated black water samples were treated with quicklime (CaO) targeting pH levels high enough to inactivate pathogens. The pH was monitored throughout the entire treatment process. The average monitored pH ranged between 9.4 -9.8 at dosages between 20 to 26% CaO/TS respectively (see Figure 1). The quicklime dosages were based on the total solids and the volume of the sample. It was observed that the higher the lime dosage, the higher the pH. However, it was also observed that the initial pH of the sample determined to which extend the pH rose after lime addition. Additionally, alkalinity of the black water also played a huge role in that it determined the buffering capacity of the sample and thus determined how much CaO to dose in order to achieve desired pH levels. The black water alkalinity was 22960 mg $CaCO_3/\ell$, which may suggests higher buffering capacity which determines the resistant of the black water sample to change its initial pH to higher levels.

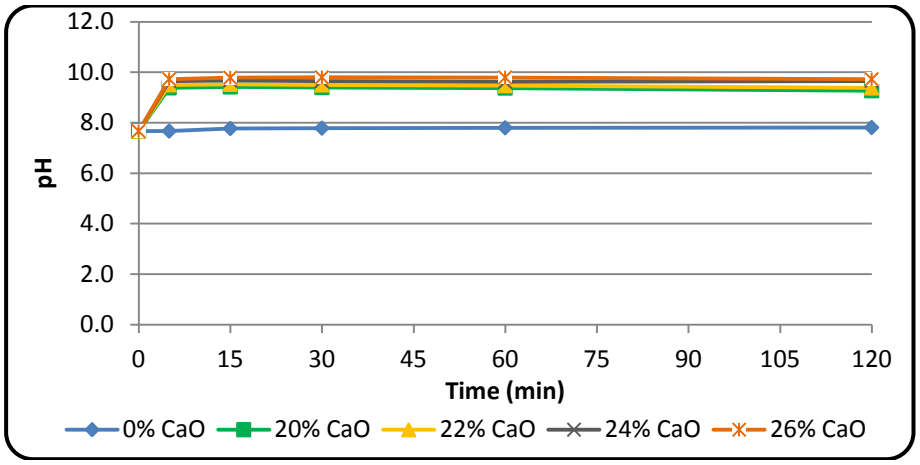

Figure 4.1 Duplicate experiment on changes in pH after CaO addition in black water

Prior the lime treatment, the average initial pH of the black water was 7.7; however, after treatment, the pH slightly increased but could barely reach pH 10. As a result this was not effective in inactivating pathogens. The objective of the experiment was to obtain pH ≥ 12; however this was not achieved at lower dosages of CaO. In an attempt to reach the desired pH levels, the dosages were then slightly increased and ranged from 24 to 30% CaO/TS (Figure 2). However, even at this range, the desired pH levels could still not be achieved. As a result, drastic measures were taken in order to attain the desired pH levels.

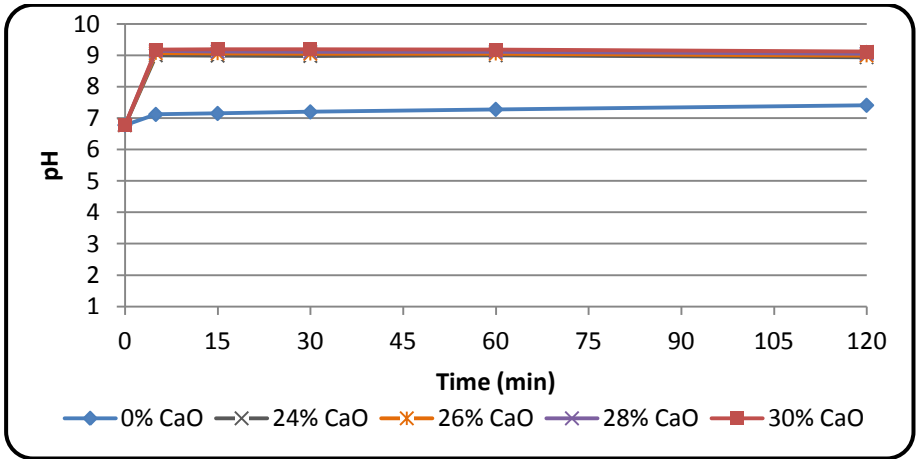

Figure 4.2 single experiments on changes in pH after CaO addition in black water

CaO dosages were then increased to up to 60% CaO/TS with lower dosage being 30% CaO/TS (Figure 3 below). At this range, pH levels between 11 and 12 were obtained in samples dosed with 50 and 60% CaO/TS.

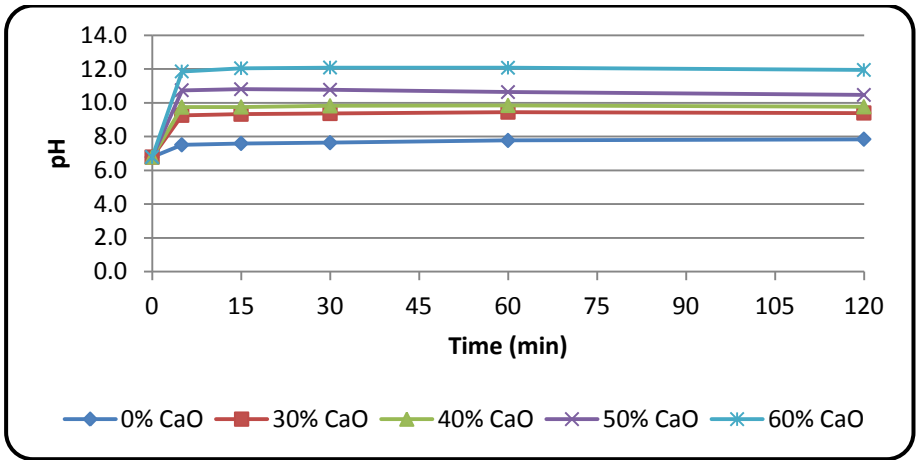

Figure 4.3 Triplicate experiments on changes in pH after CaO addition in black water

4.1.3 Effect of lime on pathogen die-off

Bina et al. (2004) reported that the effect of lime on microbiological quality is not related to the percentage of lime added to the sludge but to the pH obtained. However, the percentage of lime added, alkalinity and pH determines the extent to which pH increases. In this study, the addition of lime (CaO) led to the reduction of E. coli, total coliforms and Salmonella concentrations depending on the dosage applied. A strong relationship between lime dosage, pH and pathogens die-off was observed. Higher lime dosages led to higher pH levels and thus higher reduction of pathogens at a specific time interval. In the control samples, insignificant reduction of pathogens was observed. Whereas in lime treated samples, complete inactivation of pathogens was observed at the dosages of 30% and 60% CaO/TS within 5 minutes. Similar trends were also observed at dosage of 26% CaO/TS but within 15 minutes of treatment. This was mostly influenced by the high pH of the treated sample and the initial pH of the sample. As mentioned earlier, the extent to which pH will increase to levels higher enough for pathogen inactivation, is greatly influenced by the initial pH of the sample. As a result, the optimum dosage of lime for inactivation of pathogens depends entirely on the high levels of pH achieved. Even though pH $\geq$ 12 is recommended for pathogen inactivation; it was observed that pH 10 and above is enough for the inactivation.

4.1.3.1 *E. coli*

Figure 4.4 shows results of the black water after treatment with 20-26% CaO/TS (w/w). At this dosage range, up to 5 log reduction of *E. coli* was observed after two hours. In control experiment, a reduction of about 4 log was observed. WHO standards recommend that prior to disposal, *E. coli* concentration of the sludge should not exceed 1000 cfu/100ml. This is indicated by the red line on the graph which serves as a detection limit and colony counts above the line indicates that standards have not been met, whereas colonies below were either negligible or non-detected. Dosages between 20-24% CaO/TS did not meet the standard whereas 26% CaO/TS met the required standard after two hours of treatment.

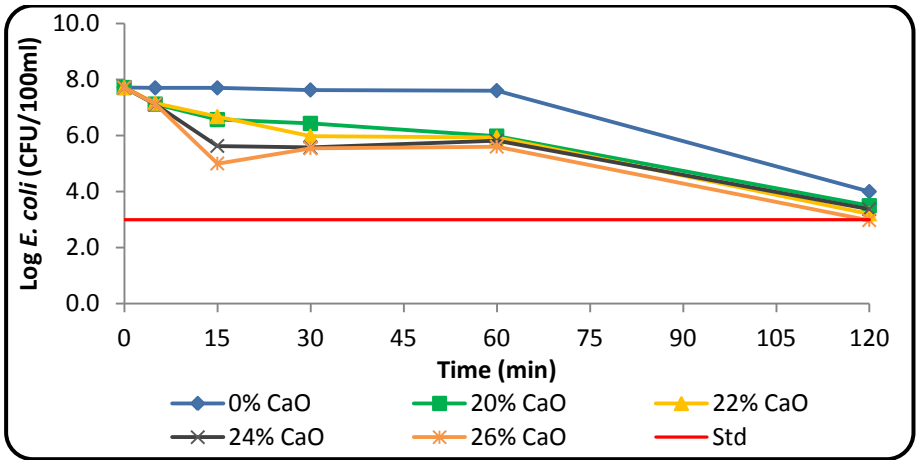

Figure 4.4 Duplicate experiment on survivability of *E. coli* during black water treatment with 20-26%CaO/TS per unit time.

Moreover, dosage range between 24-30% CaO/TS were also tested. However, at this range none of the dosages met WHO standard. This may seem strange since dosages below this range were able to achieve 5 log reductions. Hence the effect of lime on microbiological quality is greatly influenced by pH not percentage of lime dosed. In addition, the initial pH of the sample also influences the extent to which lime will increase. During this experiment, the initial pH of the sample was slightly acidic (>6).

The results in figure 5 depict insignificant reduction of *E. coli* between the 24% CaO/TS dosage and the control experiment, whereas only 1 log reduction of *E. coli* was observed in dosages 26-30% after 5 minutes . The graph also shows three points that were not connected to the reduction profile of *E. coli* in dosages 26-30% CaO/TS. This points suggest complete inactivation after 5 (28 and 30% CaO/TS) and 15 (26% CaO/TS) minutes, respectively, and re-growth was observed immediately afterwards. The re-growth increased to 6log per 100ml and it is not possible for re-growth to rise to 6 log in few minutes. As a result, the points were overruled and considered as outliers. However, this may suggest that during sampling at that particular time, no *E. coli* was present at that specific sampling point. The absence of *E. coli* at that point is not well understood but insufficient mixing could have been a contributing factor.

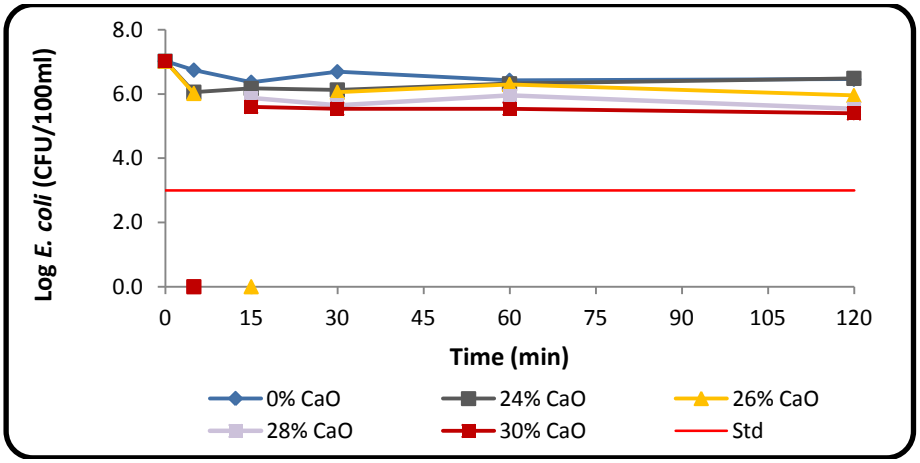

Figure 4.5 single experiments on survivability of *E. coli* during black water treatment with 24-30% CaO/TS per unit time

Since the objective of this experiment was to obtain pH ≥ 12 in order to achieve the antimicrobial effect, the lime dosages were then increased to higher dosages (30-60% CaO/TS) as none of the dosage ranges mentioned above could reach pH 12 (see Figure 6 below).

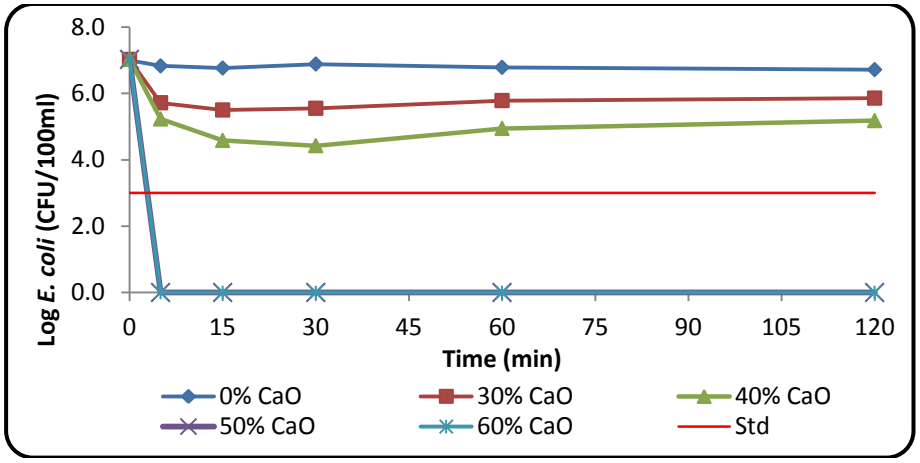

Figure 4.6 triplicate experiment on survivability of *E. coli* during black water treatment with 30-60%CaO/TS per unit time

At this range, complete inactivation with no re-growth was observed with 50-60% dosages; whereas 1log and 2log reductions were observed at 30% and 40% within five minutes respectively. The control experiment was stable and no significant reduction was observed. Consequently, dosages between 30-40% CaO/TS could not meet the standard recommended by WHO which specifies that upon disposal to any land, the concentration of *E. coli* should not exceed 1000 CFU/100ml.

4.1.3.2 Total coliforms

In the raw black water sample, coliforms were also enumerated. Coliforms were easily identifiable due to their distinctive pink colour (Figure 3.4). This made it possible to detect and quantify its survivability after treatment. Similarly to *E. coli*, coliforms were also enumerated after treatment with various dosage ranges of lime (CaO).

Figure 4.7 below shows results of dosage range between 20-26% CaO/TS. At 20% CaO/TS no coliforms were detected after 2 hrs; whereas at 24% and 26% CaO/TS, coliforms were not detected between 15 -30 minutes but re-growth occurred after 30 minutes of the treatment and escalated to almost its original concentration before treatment. As mentioned earlier, it is not possible for the re-growth to occur and rise to that level in less than an hour. Additionally, the complete inactivation of 20% CaO/TS at the end of treatment period may suggest natural die-off of coliforms which may have been influenced by unfavourable conditions. However, it is highly unlikely for 20% CaO/TS to reach complete inactivation considering insignificant reduction of coliforms in higher dosages. As a result, the points that indicated complete inactivation were considered outliers and therefore excluded from the profile. Furthermore, the control experiment was slightly stable throughout the entire treatment period and no significant reduction was observed.

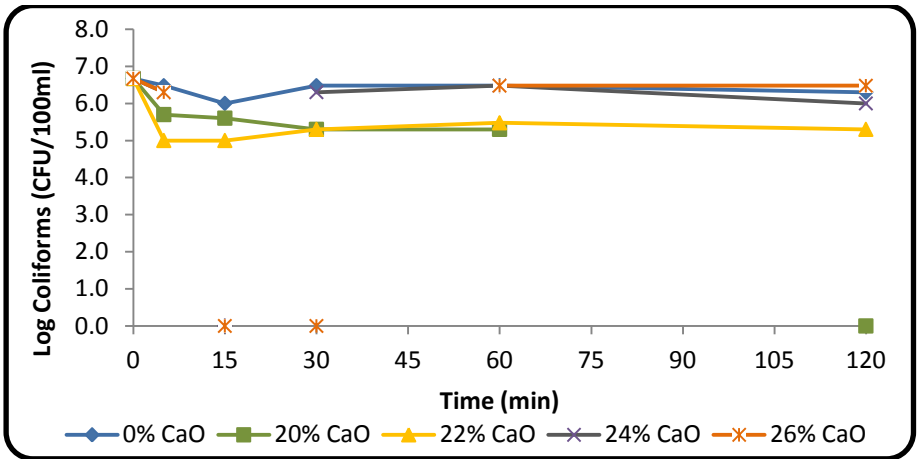

Figure 4.7 single experiments on survivability of coliforms during treatment with 20-26% CaO/TS

For the dosage range between 24-30% CaO/TS, surprisingly similar trends mentioned with 26% CaO/TS dosage were also observed with the dosage of 30% CaO/TS. However, there was insignificant reduction of Coliforms between dosages 24-26% CaO/TS and the control experiment was slightly stable (figure 8). This may suggest that the dosages were not sufficient to increase the pH to levels lethal to these coliforms.

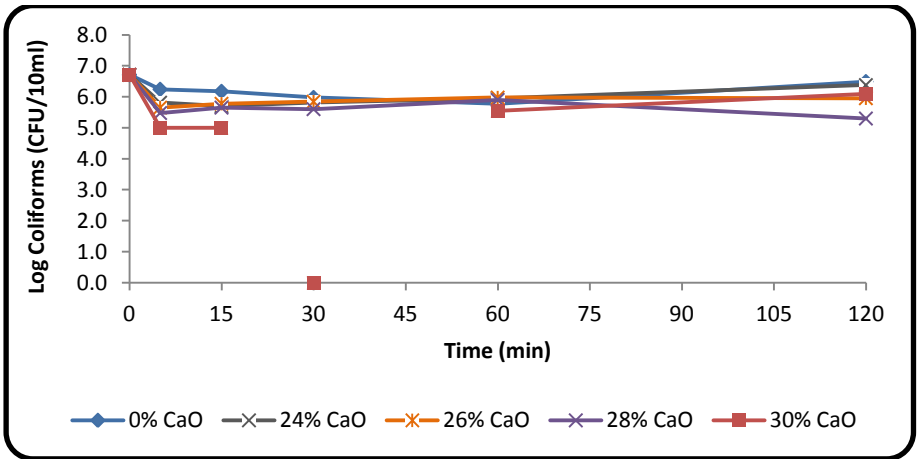

Figure 4.8 single experiments on survivability of coliforms during treatment with 24-30% CaO

Finally, in the dosage range between 30-60% (figure 9) complete inactivation of coliforms was observed between 50-60% CaO/TS and 2 log reductions was observed in samples dosed with 40% CaO/TS. In samples dosed with 30% CaO, 1 log reduction was observed within 5 minutes and was stable for the entire treatment period. Control showed similar trend as well.

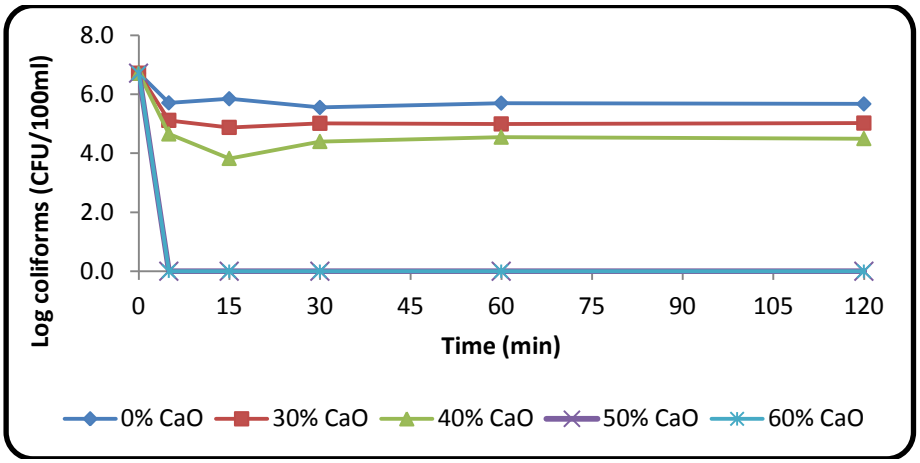

Figure 4.9 Triplicate experiments on survivability of coliforms during treatment with 30-60% CaO/TS

4.1.3.3 *Salmonella*

Unlike coliforms, *Salmonella* was not easily identifiable. It possesses similar characteristics as *E. coli* ((Estrada, et al. 2004) as a result, it was mistaken for *E. coli* as the colours were not fully blown, meaning the colour was neither purple (for *E. coli*) nor blue (*Salmonella*). In these instances, thorough counting was done with assistance of a qualified Laboratory technician. Figure 10 below shows the results on survivability of *Salmonella* during treatment with 24-30% CaO/TS conducted in a single experiment.

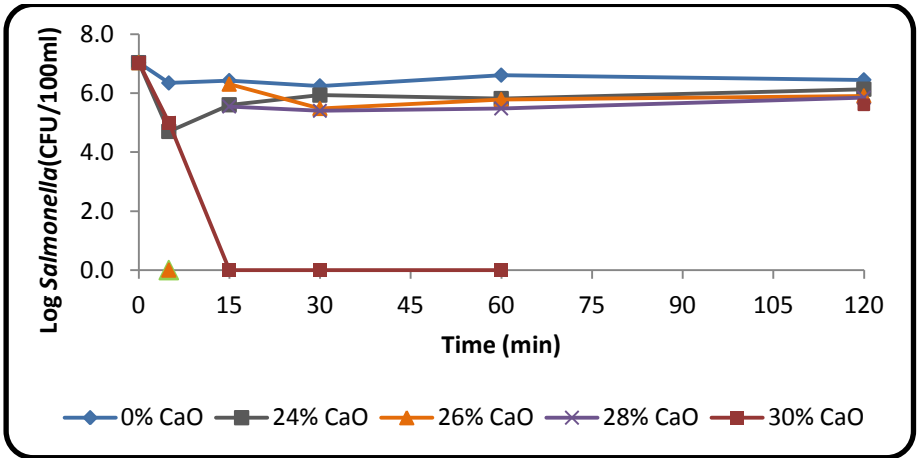

Figure 4.10 Single experiments on survivability of *Salmonella* during treatment with 24-30% CaO/TS

Complete inactivation was observed after 15 minutes with 30% dosage. However, after 1 hour re-growth occurred and rose to 5 log per 100ml. It is not common to observe such a trend as normally re-growth is encouraged by a drop in pH. However, in this case pH dropped from 9.2 to 9.1; therefore this minor decrease could not have affected the increase in the concentration of *Salmonella*. The point was then considered an outlier and could not be included in the profile. Similar trends were also observed 26 and 28% CaO/TS where complete inactivation occurred within 5 minutes of treatment and immediately re-growth occurred. The reduction profile of the dosages was presented without the 5 minute points. Moreover, in a sample dosed with 24% CaO/TS, over 2 log reduction was observed within 5 minutes but re-growth occurred to levels 1 log lower than the initial concentration. Insignificant reduction was observed in a control experiment. Due to the fact that the above-mentioned dosages could not reach pH 12 and

thereby were unsuccessful in complete inactivation of *Salmonella* or to lower levels, further dosage range was tested (Figure 4.11).

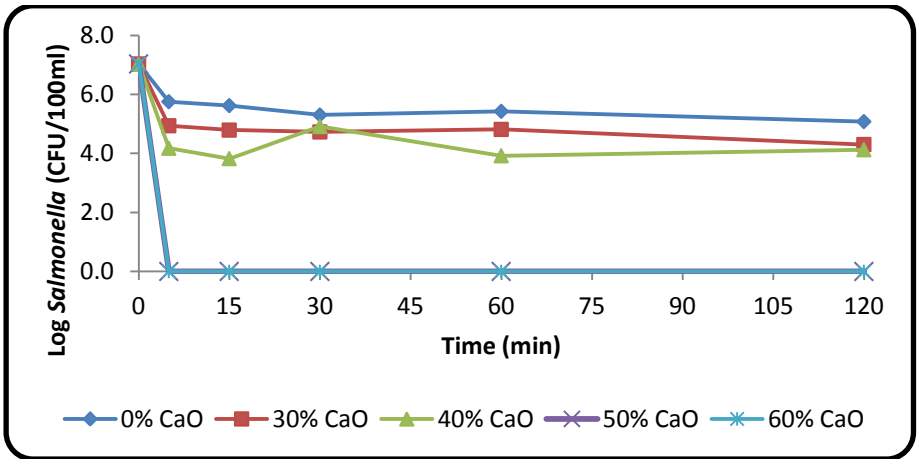

Figure 4.11 Triplicate experiments on survivability of *Salmonella* during treatment with 30-60%CaO

Dosages ranging from 30-60% CaO/TS resulted in complete inactivation between 50-60%, whereas 1, 2 and 3 log reductions were observed in samples dosed in the control, 30% and 40% respectively. In this dosage, 3 log reductions were achieved in the two experiments despite the re-growth; whereas complete inactivation was observed in the other experiment. On the other hand, samples dosed with 30% also varied in inaction of *Salmonella* as 3 log reduction was observed after two hours in one experiment; 2 log reduction in the second experiments; and fluctuations between complete inactivation and re-growth were observed in the last experiment, where *Salmonella* was not detectable within 15 minutes of the treatment but re-growth occurred after 30 minutes and an hour later complete inactivation was observed.

In summary, the overall results of the laboratory experiments are outlined on table 4.1 below. The table illustrates the comparison in log reduction between the three test organisms. *E. coli* has the highest log reduction, followed by *Salmonella* then total coliforms. Total coliforms seemed to be persistent in black water sample treated with lower concentrations of CaO (20-40% CaO/TS). However, the results also show that at lime concentrations between 50-60% CaO/TS complete inactivation of all organisms was achieved or the organisms were below detection.

Table 4.2 Overall results of laboratory experiments comparing the test organisms

% CaO	Initial pH	pH after 2 hours	Initial concentration of organisms (Log CFU/100ml)			Log reduction after 2 hours (CFU/100ml)		
			E. coli	*Salmonella*	TC	*E. coli*	*Salmonella*	TC
0	7.3	7.8	7.5	7.7	7.0	2.0	1.3	1.0
20	7.7	9.3	8.0	ND	7.0	4.0	ND	0.5
22	7.7	9.4	8.0	ND	7.0	4.0	ND	0.5
24	7.3	9.7	8.0	7.0	7.0	5.0	1.0	1.0
26	6.8	9.4	7.0	7.0	7.0	5.0	1.0	1.0
28	6.8	9.0	7.0	7.0	7.0	5.0	1.0	1.5
30	6.8	9.3	7.0	7.0	6.7	1.0	2.3	2.0
40	6.8	9.8	7.0	7.0	6.7	2.0	3.0	2.0
50	6.8	10.5	7.0	7.0	6.7	7.0	7.0	6.7
60	6.8	11.5	7.0	7.0	6.7	7.0	7.0	6.7

ND: not detected
TC: total coliforms

4.2. Field-scale results

4.2.1 Physical and microbiological characterization

Unlike wastewater, there are no specific standardized methods prescribed for characterizing faecal sludge. Not even guidelines for characterization with a limited set of parameters to significantly describe faecal sludge have been developed (Straus and Heinss, 1995). As a result, it is important to identify parameters relevant to the targeted treatment technique in order to assess characteristics of faecal sludge. However, the selected parameters as well as related methods of analysis should be well defined and adjusted to suit the capability of available resources. Additionally, the choice of parameters should allow reasonable judgment about the re-usability or disposability of the treated sludge.

Faecal sludge was characterized by analyzing physical parameters such as pH, conductivity, moisture content and organic matter before treatment with quicklime (CaO) and hydrated lime ($Ca(OH)_2$), see Table 2. The microbiological content of the samples was also analyzed. The high moisture content of the samples could have been caused by the addition of water during the fluidization process as well as the already decomposed organic matter. On the other hand, the organic content of the faecal sludge was low. This suggests that since the sludge has been kept in the latrine for over a month, decomposition of organic matter took place way before desludging process; or simply because the sludge was more concentrated and did not contain detergents or soaps, hence it was subjected to faster anaerobic digestion. The pH of all the samples was neutral, ranging between 7.3-7.6 and the alkalinity was about 10g $CaCO_3/\ell$. After addition of lime, pH significantly increased to recommended pH levels (≥ 12) required for inactivation of pathogens.

Microbiological analyses were also performed were *E. coli* and total coliforms were enumerated. *Salmonella* was not detected in any of the faecal sludge samples collected from different latrines. As a result, discussed in this section are *E. coli* and total coliforms only. The absence of *Salmonella* in the sludge sample suggests the possibility that the users of the latrines were not infected with *Salmonella;* or possibly due to processes that occur in the pit itself, which may have initiated partial treatment of the sludge. The other possibility is that during the collection process, there may have been microbial competition; or microorganisms were too diluted to be detected since the sludge was mixed with large volumes (200 litres) of water during the fluidization process. Prior the desludging process, it was observed that the desludging equipment was not completely emptied. As a result the fresh sludge was mixed with the old sludge from other latrines where HTH chlorine granules were used as an additive prior desludging. Even though the aim of this procedure was to reduce the smell, chlorine has the ability to reduce the population of pathogens. However, its antimicrobial effect depend on the concentration of chlorine that encounter the microbial cells (Beuchat & Ryu 1997). In addition, Mazollier, studied the effect of chlorine concentration on aerobic microorganisms and faecal coliforms. The study reveals that chlorine concentrations as high as 50 mg/l can remarkably reduce the microbial count (cited from Beuchat and Ryu, 1997). Nevertheless, during desludging of the three latrines, the desludging company was requested not to add chlorine in the pit as this might have affected the microbiological population of the faecal sludge and thereby compromising the quality of the analysis.

The following table depicts the characterization of faecal sludge prior lime treatment. Microbiological and physical parameters such as pH, temperature and conductivity were measured upon arrival of the sludge. However, due to shortage of an oven at wastewater treatment where analyses were carried out, TS and VS were analysed during weekends at the University of Malawi.

Table 4.2 selected physical and microbiological characteristics of faecal sludge

Parameter	Measured values			Method
Sludge age	1 year	7 years	1 month	Information provided by house owners and desludging company.
Location collected	Household latrine	Household latrine	Market public latrine	Mechanical pressurized desludging equipment
Date collected	25/01/2014	31/01/2014	07/02/2014	Calendar
Volume of water added to sludge (ℓ)	200	200	180	Tap water was collected with 25 of jerry cans which were counted.
Level of sludge (m)	0.4	0.5	0.7	General knowledge of a standard pit latrine
pH	7.3	7.6	7.3	The initial pH was measured by using a digital pH meter
EC (µS/cm)	5,374	8,653	2,600	Electrical conductivity was measured using digital EC meter
Temperature (^{o}C)	25.5	21.0	26.0	The sample temperature was measured by using digital thermometer ranging from 0-100^{o}C
Total solids (%)	8.6	5.6	8.2	The TS were obtained by drying the sample in an oven for 1 hour at 105^{o}C
Volatile solids (%)	45	55	59	The VS were obtained by further drying the sludge at 550^{o}C
Moisture content (%)	80	86	86	Moisture content was derived from difference between wet and dry sample (after drying at 105^{o}C)
Organic matter (%)	20	21	18	Organic matter was derived from difference between dry sample after loss at 105^{o}C and loss at 550^{o}C.
E. *coli* (CFU/100ml)	3x10^{6}	3x10^{6}	4x10^{7}	Plate count method using *Chromocult*® Coliform Agar.
Total Coliforms (cfu/100ml)	3x10^{6}	4x10^{6}	2x10^{6}	Plate count method using *Chromocult*® Coliform Agar.
Salmonella (cfu/100ml)	Not detected	Not detected	Not detected	Plate count method using *Chromocult*® Coliform Agar.

4.2.2 Changes in pH measurements

Similarly to laboratory scale experiments, faecal sludge samples were treated with quicklime (CaO) but also with hydrated lime (Ca(OH)2) targeting pH ≥ 12 in order completely to inactivate pathogens and thereby stabilizing the sludge. Additionally, alkalinity of the faecal sludge also played a role in determining the buffering capacity of the sample and thus determining the amount of CaO and Ca(OH)2 required to dose in order to achieve desired pH levels. The average alkalinity of the faecal was 10 g CaCO3/ℓ, which may suggests lower buffering capacity that determined the acceptance of the faecal sludge sample to change its initial pH to higher levels.

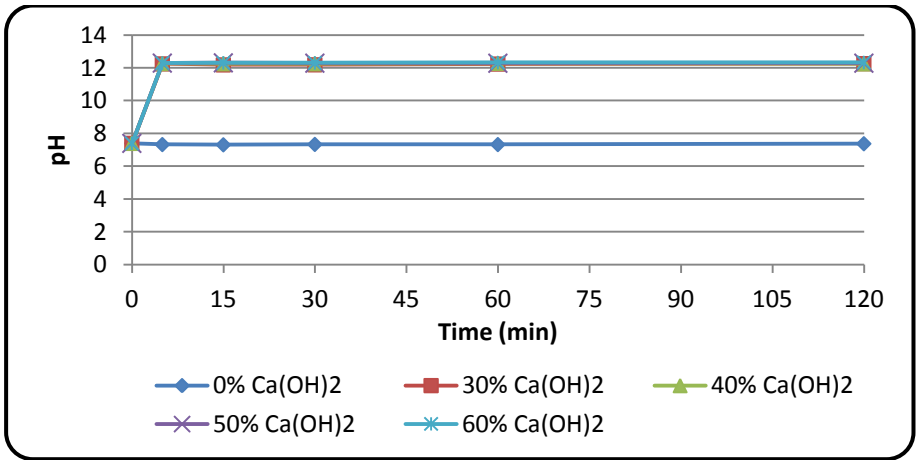

Figure 4.12 Average changes in pH measurements of faecal sludge collected from household (aged 1 year) and public (aged 1 month) latrines conducted in duplicate experiment.

The pH was monitored throughout the entire treatment process. The monitored pH ranged between 12.2 - 12.4 at dosages between 30 to 60% respectively. Since CaO is unstable in the presence of moisture and CO_2 (Systems, 2009) hydrated lime was expected to be more efficient. However, no significant difference between quick and hydrated lime dosages in terms of increase in pH and pathogen die-off. They both maintained pH > 12 for period of two hours and thus achieved complete inactivation of pathogens (figure 7). The dosages were based on the total solids and the volume of the sample.

Additionally, it was observed that after lime addition, the temperature of the sludge increased from 22 to 24^{o}C and the smell of sludge was less offensive. However, no odour tests were done to distinguish the intensity of the smell before and after treatment.

4.2.3 Effect of lime on pathogen die-off

The study conducted by Estrada *et.al* (2003) indicated that most enteric bacteria are greatly affected by extreme acidity (pH<6) and alkalinity (pH>8), whereas neutral pH (pH 7) aggravates their survival and growth. The addition of quick and hydrated lime increased the pH to >12 and thereby led to 7 log reduction in *E. coli* and Total coliforms. Figure 5 and 6 presents the results which indicate complete inactivation of *E. coli* and Total coliforms. In control samples, insignificant reduction of pathogens was observed. Whereas in lime treated samples, complete inactivation of pathogens was observed at the dosages of 30% and 60% CaO/TS and ($Ca(OH)_2$) within 5 minutes. This suggests that the pathogens were subjected to unfavourable conditions because the pH of the sludge was extremely high.

4.2.3.1 *E. coli*

Expected results were obtained in all faecal sludge samples even with lower lime dosages. Rapid inactivation of *E. coli* was observed within 5 minutes of the treatment in either CaO or $Ca(OH)_2$ dosed samples (Figure 13,14 and 15). The initial concentration of *E. coli* in 1 year, 7 years was $3x10^6$ whereas in 1 month faecal sludge was $4x10^7$. The faecal sludge samples treated with 30-60% CaO or $Ca(OH)_2$ was

stable, sanitized and free of *E. coli* and thus suitable for disposal as it meets recommended WHO standards of less than 1000 CFU/100ml (WHO, 1996).

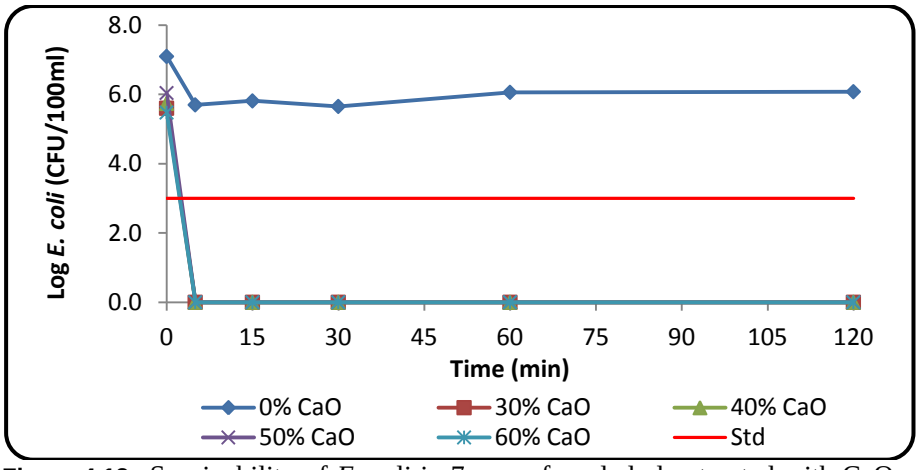

Figure 4.13 Survivability of *E.* coli in 7 years faecal sludge treated with CaO with dosage range between 30-60% CaO/TS.

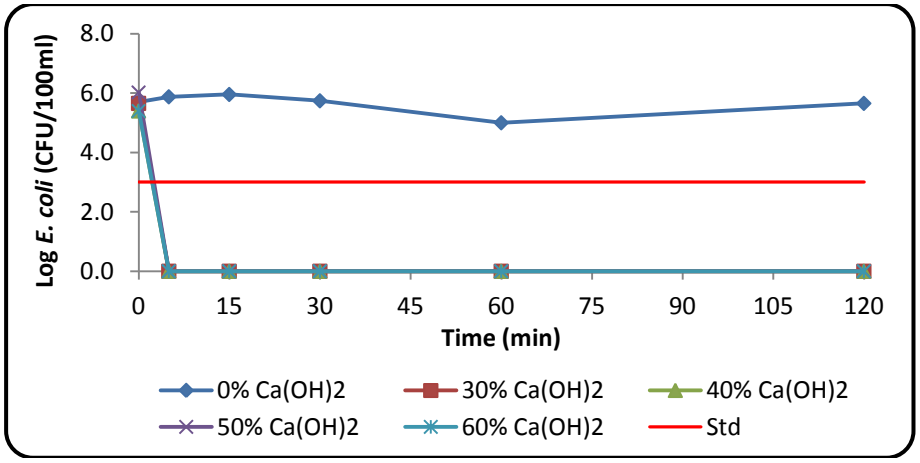

Figure 4.14 Survivability of *E.* coli in 1 year faecal sludge treated with $Ca(OH)_2$ with dosage range between 30-60% CaO

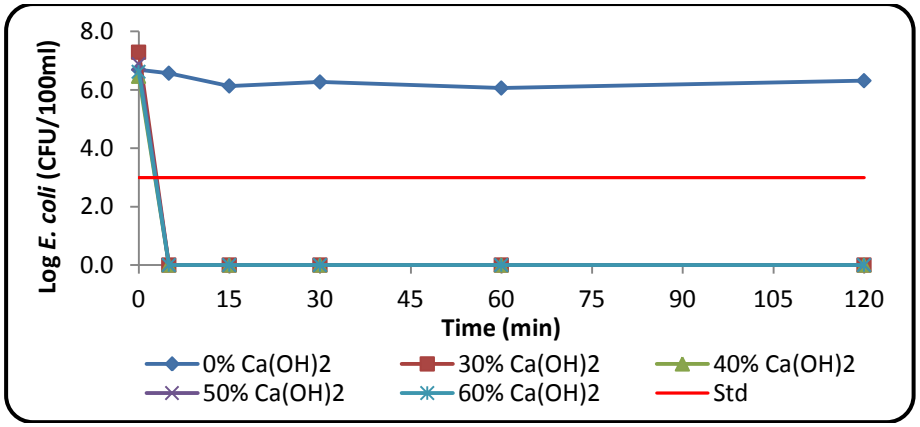

Figure 4.15 Survivability of *E.* coli in 1 month faecal sludge treated with $Ca(OH)_2$ with dosage range between 30-60% CaO

4.2.3.2 Total coliforms

Trends similar to those observed with *E. coli* were also observed with total coliforms where rapid inactivation was observed within 5 minutes of the treatment (Figure 16, 17 and 18). The initial concentrations of Coliforms were $3x10^6$, $4x10^6$ and $2x10^6$ in faecal sludge aged 1 year, 7 years and 1 month respectively.

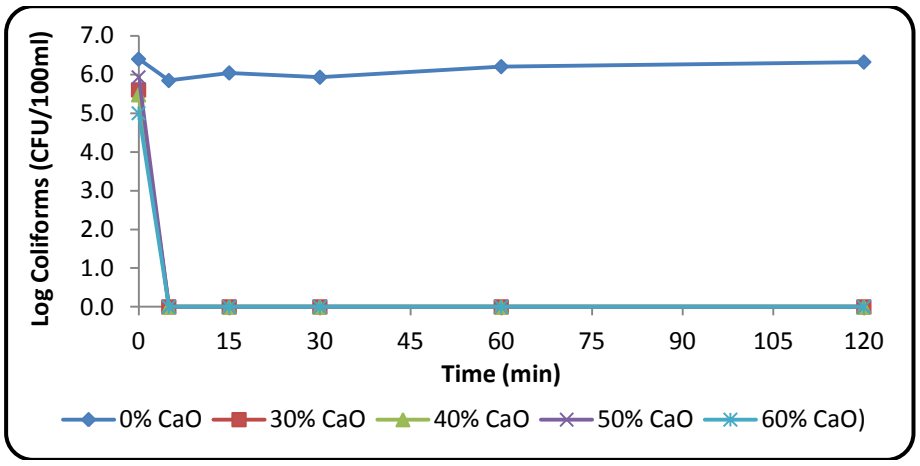

Figure 4.16 Survivability of total coliforms in 7 years faecal sludge treated with CaO with lime dosage range between 30-60%

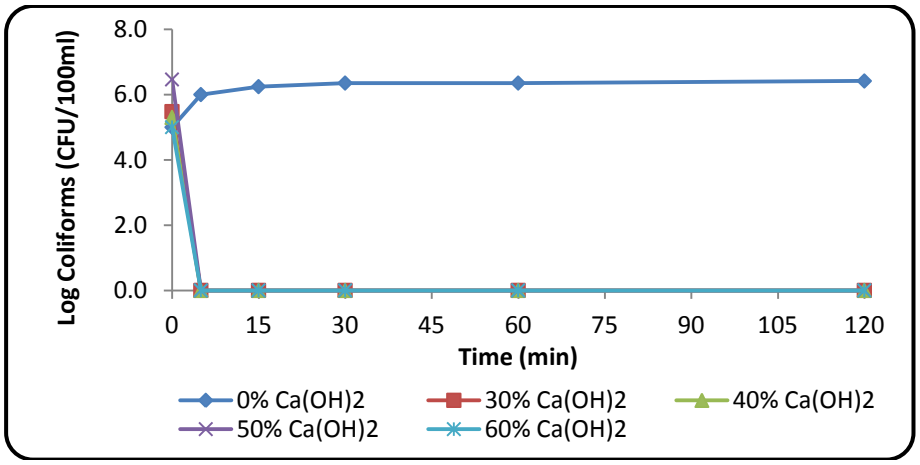

Figure 4.17 Survivability of total coliforms in 1 year faecal sludge treated with $Ca(OH)_2$with lime dosage range between 30-60%

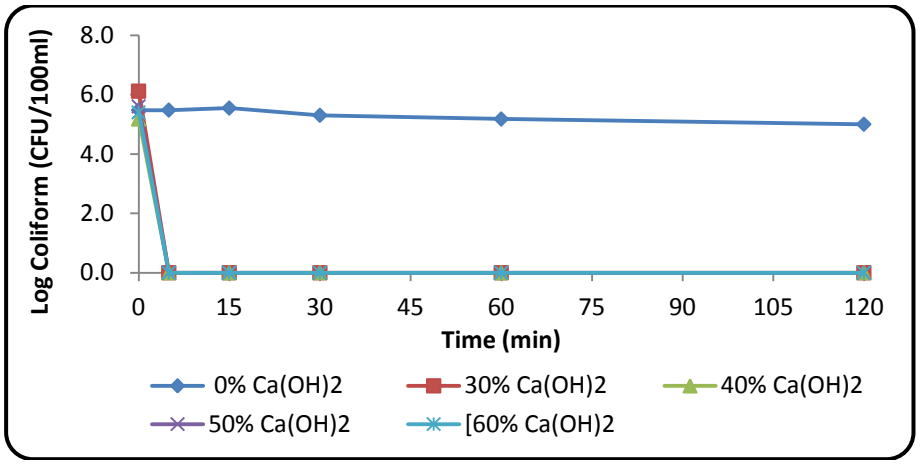

Figure 4.18 Survivability of total coliforms in 1 month faecal sludge treated with $Ca(OH)_2$with lime dosage range between 30-60%

CHAPTER 5

5.0. Discussion

Several studies perceive lime treatment as an interesting alternative to other faecal sludge treatment techniques, as also observed in this study. Apart from its cost-effectiveness, lime has the ability to destroy pathogens in a short period of time (Arthurson, 2008; Capizzi-Banas et al., 2004). In this study, the main objective was to investigate the effectiveness of lime treatment for the inactivation of pathogens in faecal sludge. The experiments were carried out under similar conditions but in two different scales, laboratory and field. In both scales quick lime was added to black water during laboratory experiment and faecal sludge during field experiment at concentrations between 30-60% CaO/TS with the intention to increase the pH to $\geq$ 12 for the duration of 2 hours. Both experiments were conducted under room temperature. Furthermore, an additional experiment was carried out in the field where hydrated lime (($Ca(OH)_2$) was added to faecal sludge in order to obtain pH $\geq$ 12 and maintain it for two hours. The concentration of Ca $(OH)_2$ also ranged between 30-60% $Ca(OH)_2$/TS.

The laboratory experiments were initially subjected to trials and errors, in an attempt to attain optimum CaO dosage range that is sufficient to raise the pH and inactivate pathogens. The initial dosage ranged between 20-26% CaO/TS (Figure 4.1) and 24-30% CaO/TS (Figure 4.2) with the average pH ranges between 9.4-9.8 and 7.1-9.2 respectively. Even though the first dosage range (20-26% CaO/TS) was low compared to the second dosage range (24-30% CaO/TS), it achieved pH higher than the latter dosage range. The difference in pH is not well understood but could be influenced by the initial pH of the black water sample. It was observed that the extent to which pH increased depended on the initial pH of the sample. For example, the addition of CaO in slightly acidic samples (pH 6.8) increased the pH up to 9.2, whereas in slightly alkaline samples (7.7) pH increased to 9.8. Polprasert and Valencia (1981) studied inactivation of faecal coliforms and *Ascaris* ova in faeces by lime. The results of the study show that rapid inactivation of faecal coliform was greatly influenced by the initial pH of the sample and the concentration of CaO added. In addition, the inactivation was obtained at pH 12 whereas pH between 10 and 11 were less effective in inactivation. These results validate the outcomes of the study conducted by Bina et al. (2004) which concludes that the pH obtained has greater impact on the inactivation of microorganisms than the percentage of lime added.

Furthermore, it is extremely important to note that the addition of lime will not serve its purpose of stabilizing the sludge if applied in small quantity thereby resulting in pH less than 11(Nicholson et al., 1990). For this reason, the lime dosage range was increased to higher concentrations of between 30-60% after failure to obtain pH $\geq$ 12 with previously mentioned dosage ranges which were rendered insufficient. The pH values achieved at this range were 9, 10, 11 and 12 respectively.

In contrast, pH of the faecal sludge during field experiments was above 12 at dosage range between 30-60% CaO/TS and/or $Ca(OH)_2$/TSwith insignificant difference in pH values between 30 and 60%. The average initial pH of the faecal sludge from the three pit latrines was 7. This difference in pH values between laboratory and field tests cannot be related to the initial pH of the samples but to differences in buffering capacity. It was observed that the alkalinity of the black water (22g $CaCO_3$/l) was twice as high as that of faecal sludge (10g $CaCO_3$/l). This suggests that black water had a high buffering capacity hence more CaO was needed in order to increase the pH of the sample; whereas the buffering capacity in faecal

sludge was low which resulted in high pH levels after addition of lime. Due to the nature of faecal sludge, it is suspected that even dosages below 30% would have increased the pH to levels appropriate for sanitization of sludge. However, due to time constraints, no further tests were carried out to confirm this.

The effect of lime on the inactivation of pathogens was one of the specific objectives of the study. The study carried out by Polprasert and Valencia (1980) on the inactivation of faecal coliforms and *Ascaris* ova in faeces by lime concluded that in order to achieve at least 5 log reductions of faecal coliforms, the pH of the sample should be raised to 12. This was also the case in this study except that organisms under study were *E. coli*, total coliforms and *Salmonella*; thereby resulting in higher log removal (7 log). During field experiments 7 log removal was achieved within 5 minutes of the treatment even at lower dosages; whereas laboratory results report 7 log removal only in higher concentrations (50 and 60%). Only 1 log and 2 log removal was observed in samples treated with 30% CaO and 40% CaO respectively. The pH in these samples was below 11, which suggests insufficient dosage, hence the less removal efficiency.

There was no correlation between the moisture content and the dosage of lime required for the inactivation of pathogens. However, a strong relationship between pH and pathogen die-off was observed. The higher the pH, the larger the death rate of pathogens and vice versa.

Additionally, factors such as temperature greatly influenced the efficiency of lime treatment on pathogen inactivation. Increase in temperature led to higher log reductions. Moreover, after the lime treatment the smell of the faecal sludge was less offensive. Unfortunately, odour tests were not conducted due to time constraints and for the laboratory experiments it was impractical to observe any changes in the smell of the sample since all the experiments were run under the hood which extracted the smell away.

The results of this study indicate lime as a possible and interesting chemical treatment technique that can be applied during emergency situations for the inactivation of pathogens in faecal sludge.

CHAPTER 6

6.0. Conclusions

This study is in agreement with various authors which reported that lime stabilization is a low cost, simple process that it is easy to apply (Burnham and Nicholsen, 1990; Willford et al., 2007) and eliminate pathogens while reducing smell in sludge (Wong and Selvam, 2009). However this can be achieved by increasing the pH of the treated sludge to ≥ 12 and maintaining the pH levels for at least 2 hours (Williford et al., 2007).

Strauch (1999), cited in Bina et al. (2004) reported that the removal of pathogens depended on the pH reached by the sludge, the period liming activity and the dryness of the sludge. In contrast, this study observed no relationship between the dryness of the sludge or its moisture content in pathogen removal. Additionally, even though the moisture content of faecal sludge was very high (about 80%), complete inactivation of pathogens was achieved at pH ≥ 12. Furthermore, the addition of lime will not serve its purpose of stabilizing the sludge if applied in small quantities thereby resulting in pH less than 11(Nicholson et al., 1990).

Lime is definitely suitable to be employed during emergency situations since it inactivates pathogens in a very short period of time and also eliminates smell. The reduction of smell is attributed to the reduction in the mass of volatile solids in the sludge. Smelly sludge in most cases attracts vectors which spread diseases. However, the reduction in vector attraction requires volatile solids to be reduced by at least 38% (Bine et al., 2004), or pH to be increased to 12 and maintain it for 2 hours, or 11 and maintained for 22 hours (Mignotte, 2001)

In order to fairly conclude this study, it was perceived crucial to compare lime treatment with other chemical treatments recently studied. This study was part of a three-fold approach by IHE MSc students in collaboration with WASTE and its associates, focusing on sanitizing faecal sludge using also, besides lime, urea and lactic acid as potential treatment methods that can be applied during emergency situations. The effect of these chemicals on the microbiological quality of faecal sludge was investigated. For all the studies, indicator organisms such as *E. coli*, *Salmonella* and total coliforms were chosen as representatives of pathogenic organisms.

The mechanism, impact, benefits, limitations and the conditions at which these treatment methods inactivate pathogens are outlined on Table 6.1 below. The table shows lactic acid, Ammonia and lime as treatment techniques that can be employed during emergency situations. Amongst the three techniques, lime seems to be the best treatment method due to its rapidity in reduction of pathogens (7log) in short period of time. The treatment time for lime was only 2 hours whereas for ammonia was 8 days and lactic acid was 15 days. The other advantage of lime over these treatments is that it is simple, readily useable and can also reduce smell.

Table 6.1 Comparison of various treatment methods for sanitizing faecal sludge during emergency situations

S/N	Attribute	Lactic Acid Bacteria treatment	Ammonia treatment	Lime treatment
1	Sanitization time	7-15 days	8 days	5-120 minutes
2	*E. coli* log removal	6	3	7
3	*Salmonella* log removal	NA	3	ND
4	Total coliforms log removal	NA	2	6
5	Odour suppression	Yes (sour smell)	No	Yes
6	End pH	3.8-4.2	9.4	12.4
7	Effect on environment	Non toxic	Non toxic	Non toxic
8	Effect on ground water	Non contaminant		Non contaminant
9	Energy requirements	mixing	mixing	mixing
10	Sludge disposal after treatment	Drying bed	Drying bed	Sanitary landfill/drying beds
11	Re-use of FS	Yes-Agriculture	yes-Agriculture	yes
12	Chemical use	Sugar additive required	Urea	Quick and hydrated lime
13	Technology	Biological treatment	Bio-chemical treatment	Chemical treatment
14	Treatment cost (Chemicals)	€2/m^3	€1.6/m^3	€0.89/m^3
15	Problems/shortfalls	Temperature dependant (30-40 °C optimum)	Airtight reactors required	Homogenous mixing required
16	O&M	Minimal	Minimal	Minimal
17	Robustness of technology	Yes (mixing required)	Yes	Yes
18	Integration with existing emergency technical option	Yes (Highly recommended)	Yes	Yes

NA: not analyzed
ND: not detected

CHAPTER 7

7.0. Recommendations

Literature reveals that lime treatment has the potential to inactivate spores and spore forming bacteria. However, the inactivation mechanism of *Ascaris* ova by lime is not well understood but it is assumed that it occurs due to slow reaction between (OH^-) or high pH and the ova. It is therefore recommended that further investigations of lime be conducted on the inactivation of spore forming bacteria and helminth eggs(?) in order to understand their inactivation mechanism.

Initially faecal sludge was characterized by analyzing physical parameters such as pH, temperature, moisture content, organic matter, TS and VS before treatment. However, only pH was monitored after the addition of lime. It is highly recommended that further studies analyze this parameter even after treatment in order to understand the effect of lime on the physical parameters or determine any relationship thereof.

Furthermore, since 30% CaO and $Ca(OH)_2$ were able to quickly inactivate enteric bacteria found in faecal sludge, it is recommended to examine dosages lower than that. This might lower the costs of treatment even further and make treated sludge more useable.

During field experiments, *Salmonella* was not detected. However, this may not be related to the fact it was not present in the sludge, but to the detection method used. Plate count is considered reliable, cheap and easy to use. However, its shortfall is that it is unable to detect lower concentrations. On the contrary, other methods such as the most probable number (MPN) detect even the lowest concentration of pathogens present in the sludge. In addition, during laboratory experiments it was sometimes difficult to detect *Salmonella*. Nevertheless, the difficulty in detection and quantification of enteric pathogens is related to low concentrations in the sludge, hence the use of indicator organisms (Finney et al. 2003b). Therefore comparison of the two methods, MPN and plate count should be explored in future studies.

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Appendices

Appendix A Laboratory Results

Table A.1 pH measurements of experiment 1 at dosage range between 20-26% CaO/TS

% CaO	Experiment 1 pH measurements at different lime dosage					
	0	5	15	30	60	120
0%	8.59	8.59	8.59	8.61	8.58	8.5
20%	8.59	9.92	9.95	9.95	9.93	9.78
22%	8.59	10.05	10.07	10.08	10.04	9.91
24%	8.59	10.28	10.3	10.3	10.24	10.36
26%	8.59	10.37	10.46	10.5	10.47	10.36

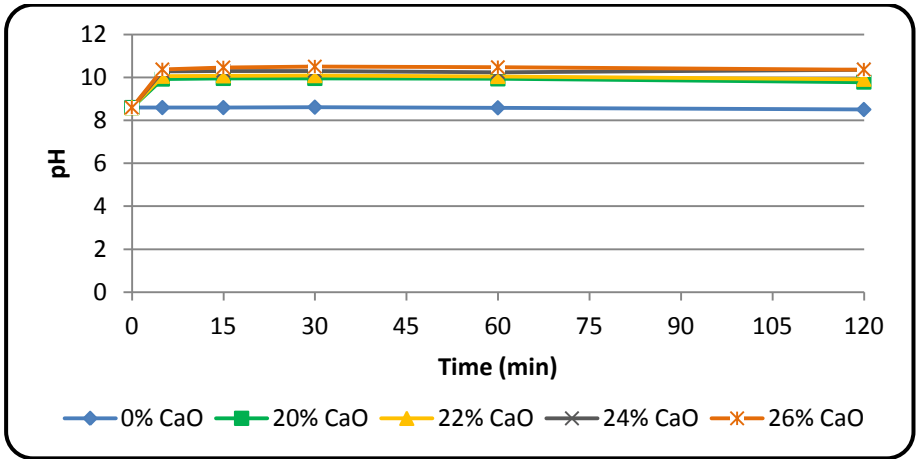

Figure A-1 pH measurements of experiment 1 at lime dosage range between 20-26% CaO/TS

Table A.2 pH measurements of experiment 2 at dosage range between 20-26% CaO/TS

% CaO	Experiment 2 pH measurements at different lime dosage					
	0	5	15	30	60	120
0%	6.75	6.75	6.95	6.96	7.02	7.12
20%	6.75	8.85	8.89	8.84	8.82	8.76
22%	6.75	8.96	8.98	8.92	8.9	8.85
24%	6.75	9.03	9.06	9.01	9	8.95
26%	6.75	9.09	9.11	9.1	9.12	9.09

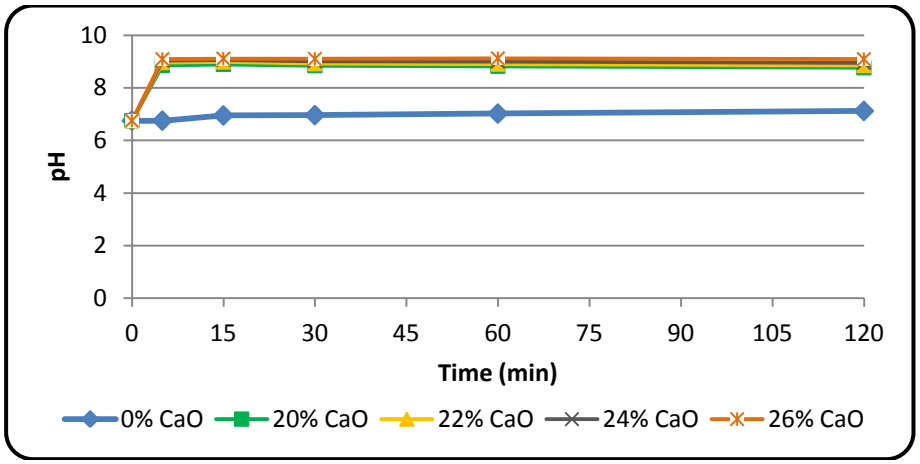

Figure A.2 pH measurements of experiment 2 at lime dosage range between 20-26% CaO/TS

Table A.3 pH measurements of experiment 3 at dosage range between 24-30% CaO/TS

% CaO	Experiment 3: pH measurements at different lime dosage					
	0	5	15	30	60	120
0%	6.78	7.12	7.15	7.2	7.28	7.41
24%	6.78	9	8.99	8.98	9	8.94
26%	6.78	9.07	9.06	9.05	9.05	8.99
28%	6.78	9.14	9.12	9.11	9.11	9.05
30%	6.78	9.18	9.19	9.19	9.18	9.12

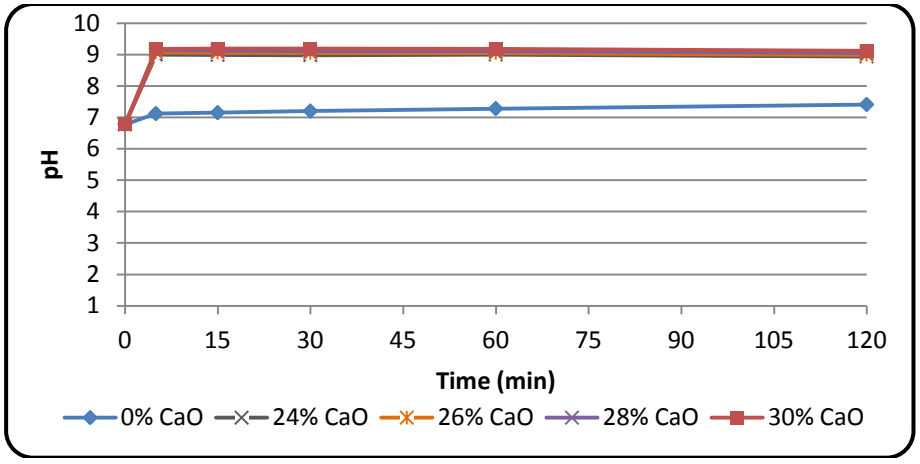

Figure A.3 pH measurements of experiment 3 at lime dosage range between 24-30% CaO/TS

Table A.4 pH measurements of experiment 4 at dosage range between 30-60% CaO/TS

% CaO		Experiment 4: pH measurements				
	0	5	15	30	60	120
0%	6.78	7.18	7.31	7.43	7.59	7.63
30%	6.78	8.71	8.8	8.86	9.14	9.12
40%	6.78	8.97	9.03	9.34	9.51	9.43
50%	6.78	10.12	10.21	10.2	10.07	9.91

60%	6.78	11.12	11.56	11.64	11.62	11.35

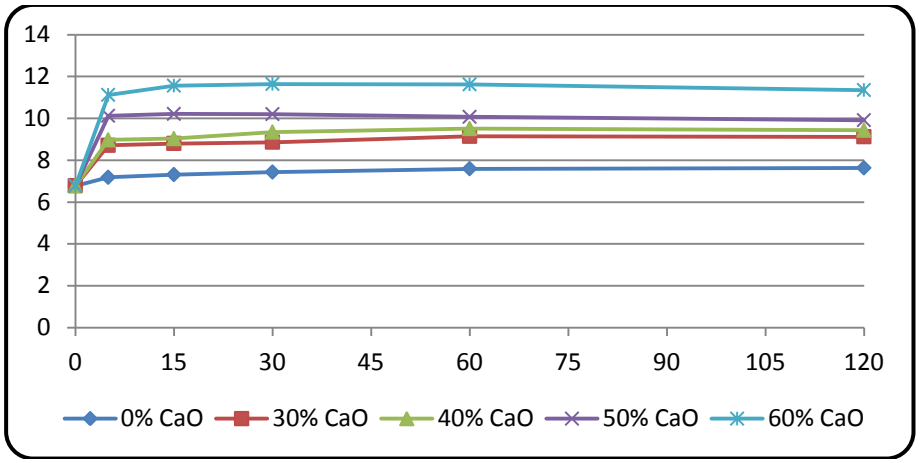

Figure A.4 pH measurements of experiment 4 at lime dosage range between 30-60% CaO/TS

Table A.5 pH measurements of experiment 5 at dosage range between 30-60% CaO/TS

% CaO		Experiment 5: pH measurements				
	0	5	15	30	60	120
0%	6.78	7.91	7.94	7.95	8.09	8.16
30%	6.78	9.88	9.88	9.84	9.83	9.76
40%	6.78	10.33	10.32	10.26	10.23	10.15
50%	6.78	11.19	11.24	11.2	11.08	10.91
60%	6.78	12.5	12.54	12.57	12.54	12.47

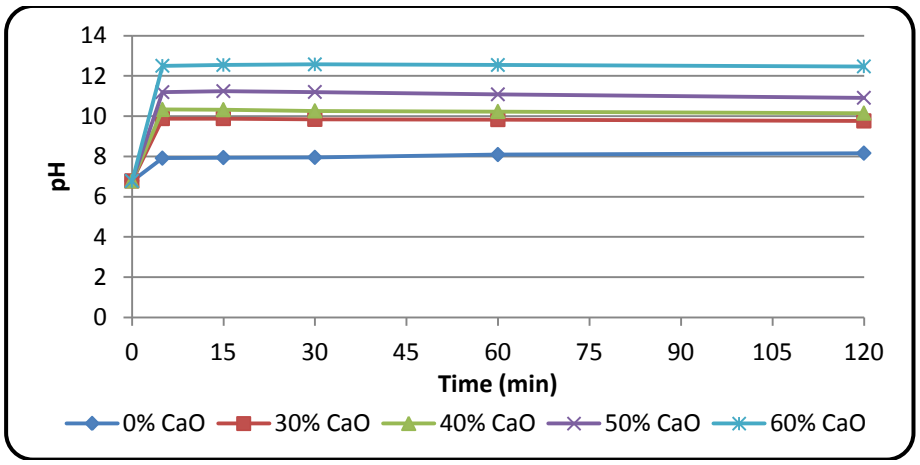

Figure A.5 pH measurements of experiment 5 at lime dosage range between 30-60% CaO/TS

Table A.6 pH measurements of experiment 6 at dosage range between 30-60% CaO/TS

% CaO		Experiment 6: pH measurements (date)				
	0	5	15	30	60	120
0%	6.78	7.43	7.52	7.52	7.62	7.73
30%	6.78	9.18	9.28	9.39	9.36	9.3
40%	6.78	9.92	9.9	9.85	9.78	9.7
50%	6.78	10.91	10.95	10.9	10.74	10.56
60%	6.78	11.94	12.02	12.04	12.05	12

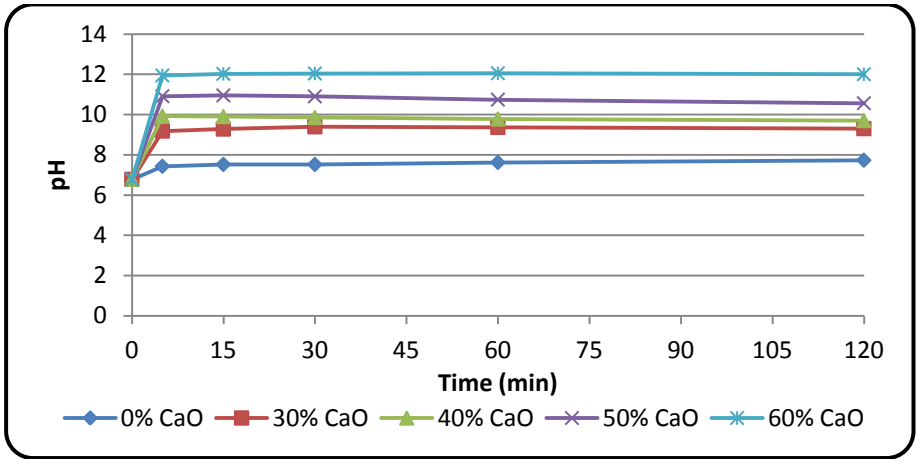

Figure A.6 pH measurements of black water at lime dosage range between 30-60% CaO/TS (Experiment 6)

Table A.7 Log *E. coli* survivability in black water after treatment with 20-26% CaO/TS (Experiment 1)

% CaO	*E. coli* CFU/100ml					
	0	5	15	30	60	120
0%	1.00E+08	9.80E+07	9.80E+07	8.10E+07	7.80E+07	9.70E+07
20%	1.00E+08	2.50E+07	6.00E+06	3.00E+06	1.00E+06	9.00E+06
22%	1.00E+08	2.80E+07	6.00E+06	1.00E+06	0.00E+00	2.00E+06
24%	1.00E+08	2.40E+07	0.00E+00	0.00E+00	0.00E+00	5.00E+06
26%	1.00E+08	2.60E+07	0.00E+00	0.00E+00	0.00E+00	0.00E+00

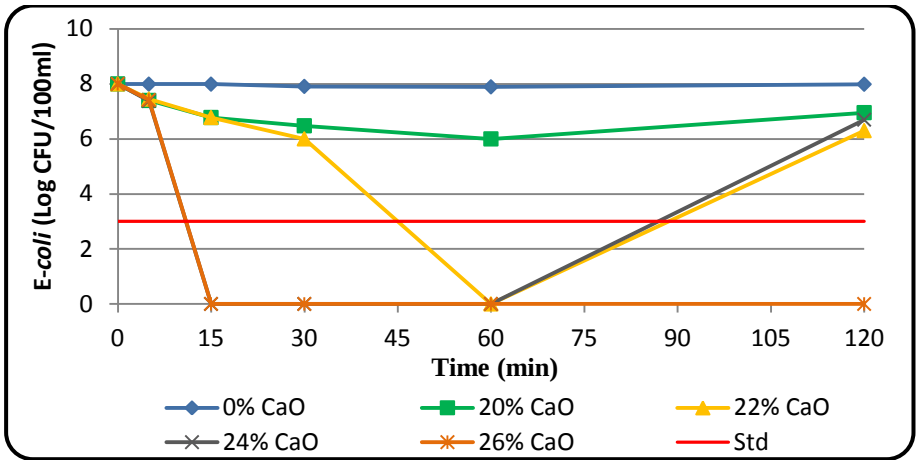

Figure A.7 Log *E. coli* survivability in black water after treatment with 20-26% CaO/TS (Experiment 1)

Table A.8 Log *E. coli* survivability in black water after treatment with 20-26% CaO/TS (Experiment 2)

% CaO	*E. coli* CFU/100ml					
	0	5	15	30	60	120
0%	3.00E+06	2.60E+06	2.50E+06	3.60E+06	2.30E+06	2.30E+06
20%	3.00E+06	1.40E+06	1.40E+06	2.40E+06	9.00E+05	7.00E+05
22%	3.00E+06	8.00E+05	3.50E+06	9.00E+05	1.70E+06	5.50E+05
24%	3.00E+06	1.40E+06	8.50E+05	7.50E+05	1.30E+06	5.50E+05
26%	3.00E+06	6.00E+05	2.00E+05	7.00E+05	8.00E+05	9.00E+05

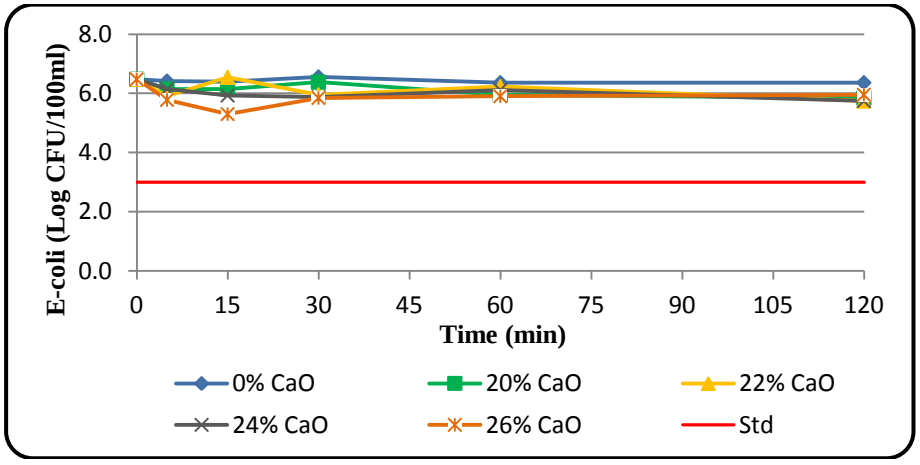

Figure A.8 Log *E. coli* survivability in black water after treatment with 20-26% CaO/TS (Experiment 2)

Table A.9 Log *E. coli* survivability in black water after treatment with 20-26% CaO/TS (Experiment 3)

% CaO	*E. coli* Log CFU/100ml (single)					
	0	5	15	30	60	120
0%	7.03	6.75	6.37	6.70	6.43	6.46
24%	7.03	6.06	6.18	6.13	6.32	6.49
26%	7.03	6.02	0.00	6.06	6.30	5.95
28%	7.03	0.00	5.88	5.65	5.95	5.54

30%	7.03	0.00	5.60	5.54	5.54	5.40

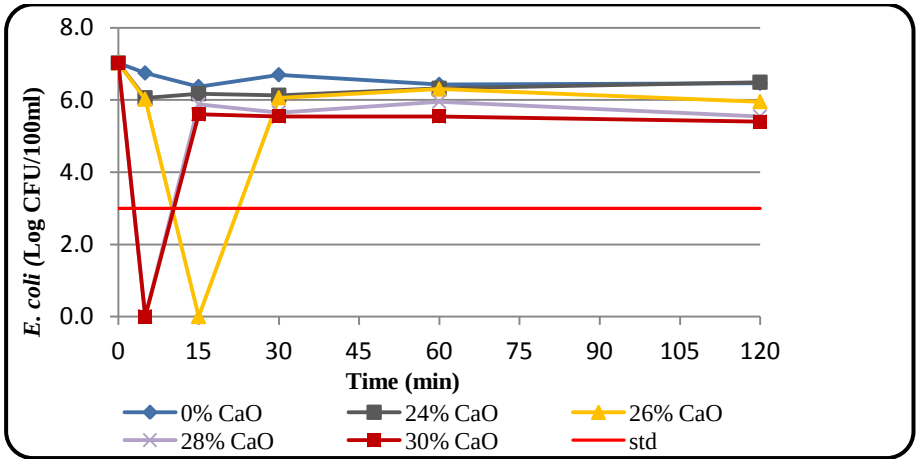

Figure A.9 Log *E. coli* survivability in black water after treatment with 24-30% CaO/TS (Experiment 3)

Table A.10 Log *E. coli* survivability in black water after treatment with 30-60% CaO/TS (Experiment 4)

% CaO	Log *E.coli* CFU/100ml					
	0	5	15	30	60	120
0%	7.0	6.9	7.0	7.0	7.0	6.9
30%	7.0	5.7	5.8	5.8	6.1	6.1
40%	7.0	5.4	4.8	0.0	5.4	5.6
50%	7.0	0.0	0.0	0.0	0.0	0.0
60%	7.0	0.0	0.0	0.0	0.0	0.0

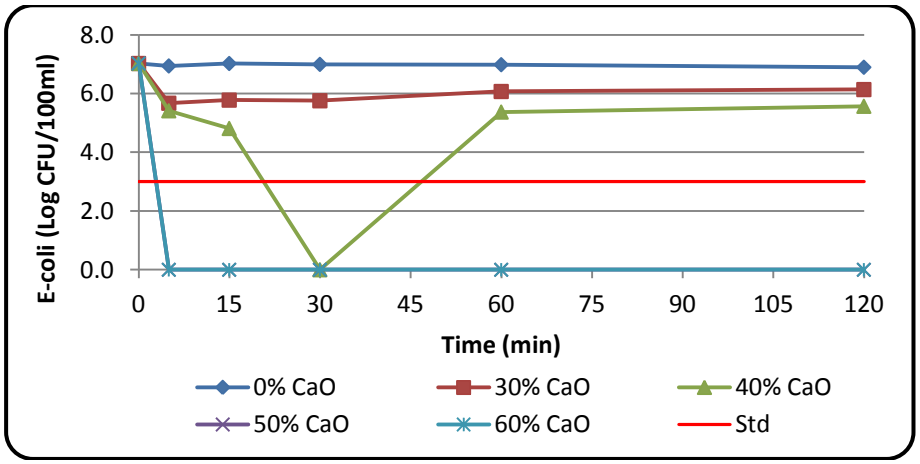

Figure A.10 Log *E. coli* survivability in black water after treatment with 30-60% CaO/TS (Experiment 4)

Table A.11 Log *E. coli* survivability in black water after treatment with 30-60% CaO/TS (Experiment 5)

% CaO	Log *E.coli* CFU/100ml					
	0	5	15	30	60	120
0%	7.0	6.8	6.5	7.0	6.6	6.6
30%	7.0	5.6	0.0	0.0	5.0	5.0
40%	7.0	0.0	0.0	0.0	0.0	0.0
50%	7.0	0.0	0.0	0.0	0.0	0.0
60%	7.0	0.0	0.0	0.0	0.0	0.0

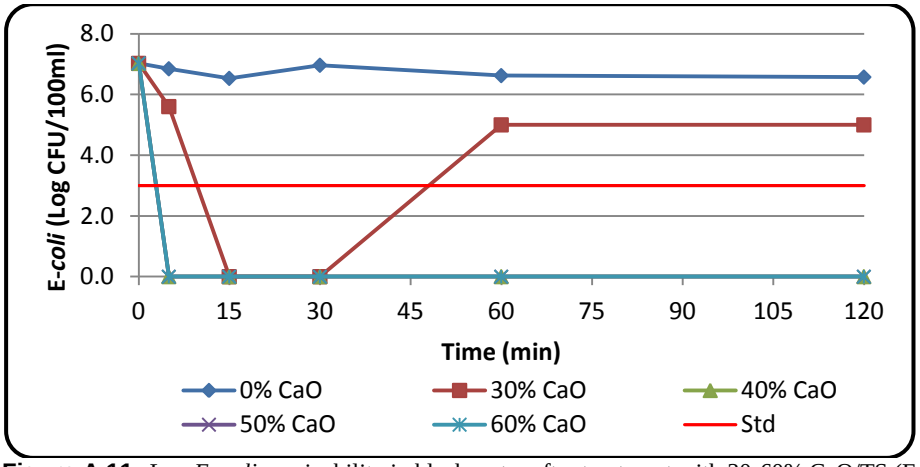

Figure A.11 Log *E. coli* survivability in black water after treatment with 30-60% CaO/TS (Experiment 5)

Table A.12 Log *E. coli* survivability in black water after treatment with 30-60% CaO/TS (Experiment 6)

% CaO	Log *E. coli* CFU/100ml					
	0	5	15	30	60	120
0%	7.0	6.7	6.5	6.6	6.7	6.6
30%	7.0	5.8	5.5	5.7	5.7	5.8
40%	7.0	5.4	4.7	4.9	4.5	4.9
50%	7.0	0.0	0.0	0.0	0.0	0.0
60%	7.0	0.0	0.0	0.0	0.0	0.0

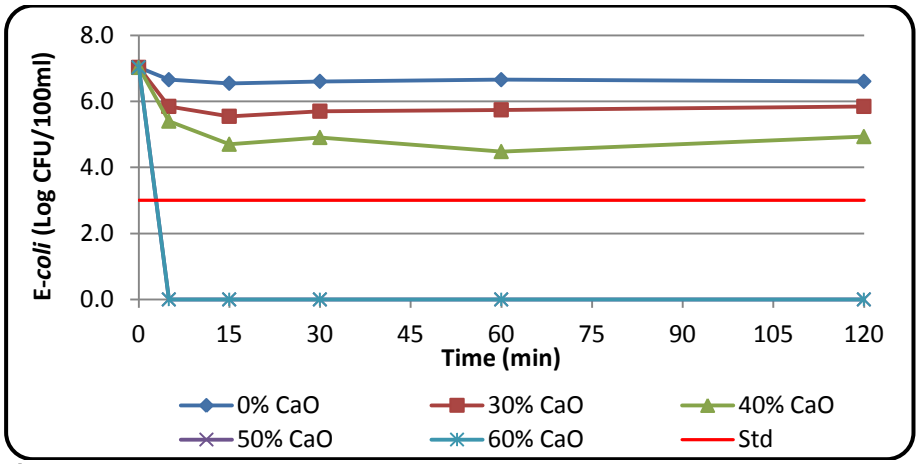

Figure A.12 Log *E. coli* survivability in black water after treatment with 30-60% CaO/TS (Experiment 6)

Table A.13 Log *Salmonella* survivability in black water after treatment with 24-30% CaO/TS (Experiment 3)

% CaO	Log *Salmonella* CFU/100ml					
	0	5	15	30	60	120
0%	7.03	6.34	6.42	6.24	6.61	6.45
24%	7.03	4.70	5.60	5.93	5.81	6.13
26%	7.03		6.30	5.48	5.78	5.90
28%	7.03		5.54	5.40	5.48	5.85
30%	7.03	5.00	0.00	0.00	0.00	5.60

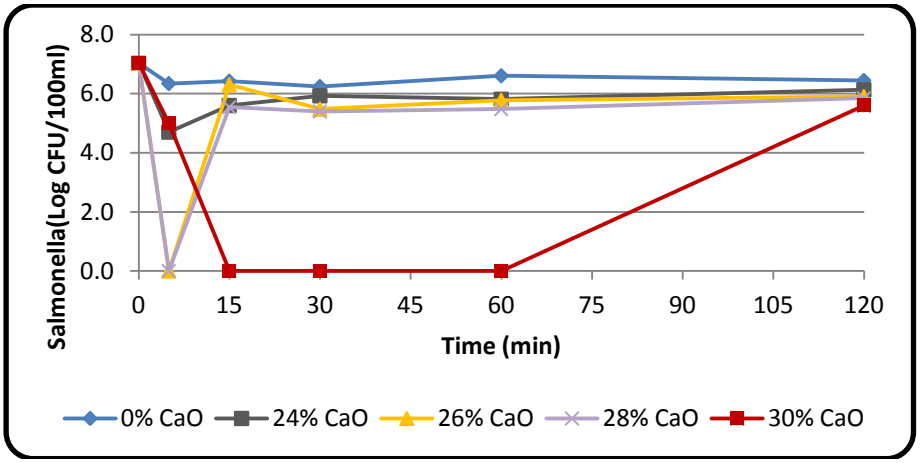

Figure A.13 Log *Salmonella* survivability in black water after treatment with 24-30% CaO/TS (Experiment 3)

Table A.14 Log *Salmonella* survivability in black water after treatment with 30-60% CaO/TS (Experiment 4)

% CaO	Log *Salmonella* CFU/100ml					
	0	5	15	30	60	120
0%	7.03	5.48	5.54	5.54	5.74	4.00
30%	7.03	4.81	4.88	4.48	4.30	4.00
40%	7.03	4.30	0.00	5.35	4.18	4.00
50%	7.03	0.00	0.00	0.00	0.00	0.00
60%	7.03	0.00	0.00	0.00	0.00	0.00

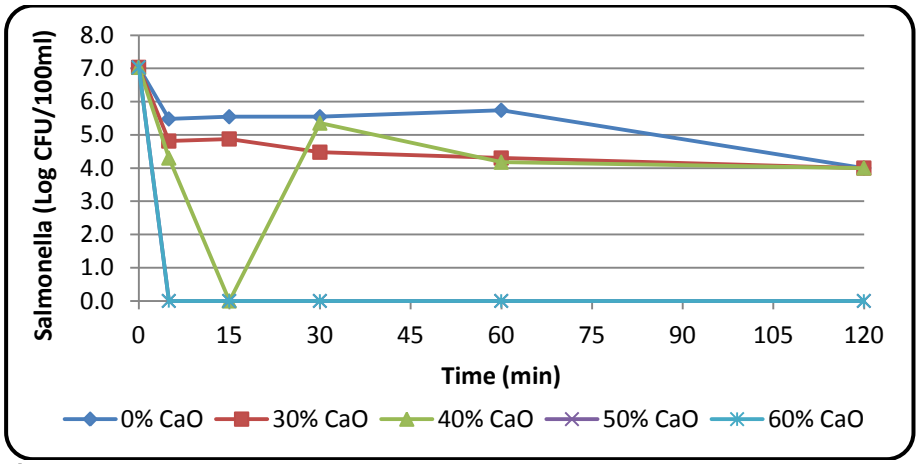

Figure A.14 Log *Salmonella* survivability in black water after treatment with 30-60% CaO/TS (Experiment 4)

Table A.15 Log *Salmonella* survivability in black water after treatment with 30-60% CaO/TS (Experiment 5)

% CaO	Log *Salmonella* CFU/100ml					
	0	5	15	30	60	120
0%	7.0	5.9	5.9	5.0	5.2	5.5
30%	7.0	5.0	0.0	5.0	0.0	0.0
40%	7.0	0.0	0.0	0.0	0.0	0.0
50%	7.0	0.0	0.0	0.0	0.0	0.0
60%	7.0	0.0	0.0	0.0	0.0	0.0

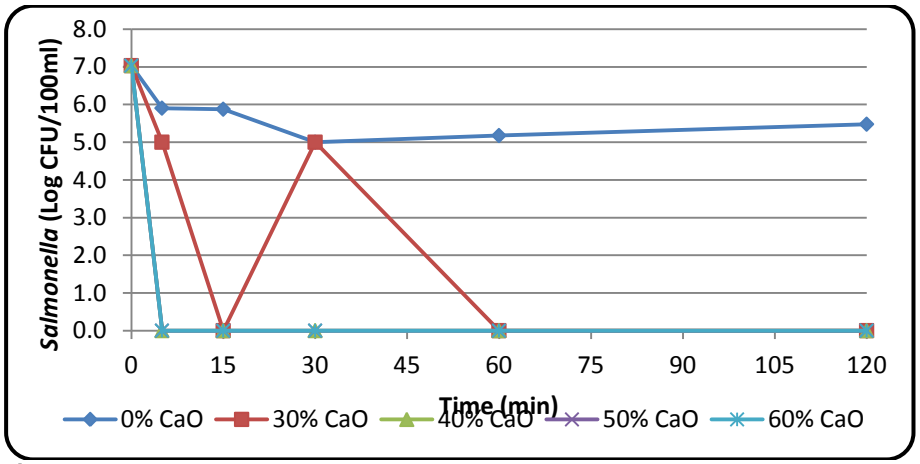

Figure A.15 Log *Salmonella* survivability in black water after treatment with 30-60% CaO/TS (Experiment 5)

Table A.16 Log *Salmonella* survivability in black water after treatment with 30-60% CaO/TS (Experiment 6)

% CaO	Log *Salmonella* CFU/100ml					
	0	5	15	30	60	120
0%	7.0	5.8	5.2	5.2	5.0	4.7
30%	7.0	5.0	5.0	4.5	5.2	4.7
40%	7.0	4.4	4.3	4.3	4.0	4.5
50%	7.0	0.0	0.0	0.0	0.0	0.0
60%	7.0	0.0	0.0	0.0	0.0	0.0

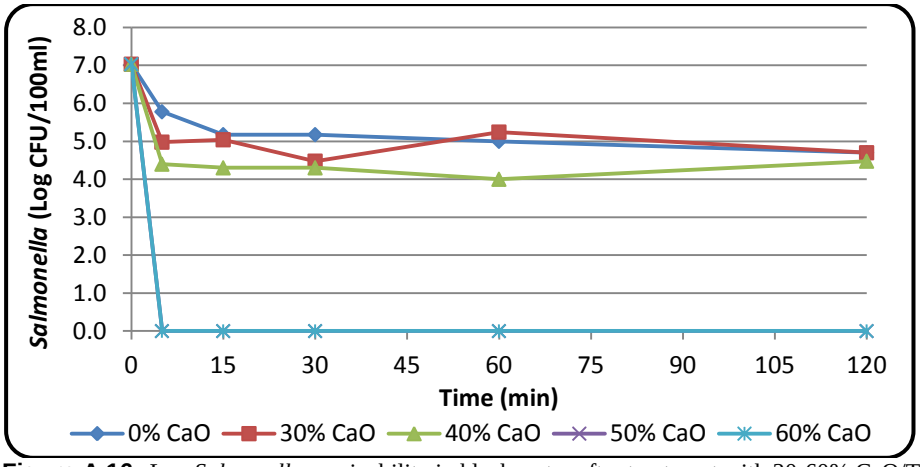

Figure A.16 Log *Salmonella* survivability in black water after treatment with 30-60% CaO/TS (Experiment 6)

Table A.17 Log Total coliform survivability in black water after treatment with 20-26% CaO/TS (Experiment 2)

% CaO	Log total coliforms CFU/100ml					
	0	5	15	30	60	120
0%	6.7	6.5	6.0	6.5	6.5	6.3
20%	6.7	5.7	5.6	5.3	5.3	
22%	6.7	5.0	5.0	5.3	5.5	5.3
24%	6.7	6.3	0.0	6.3	6.5	6.0
26%	6.7	6.3	0.0	0.0	6.5	6.5

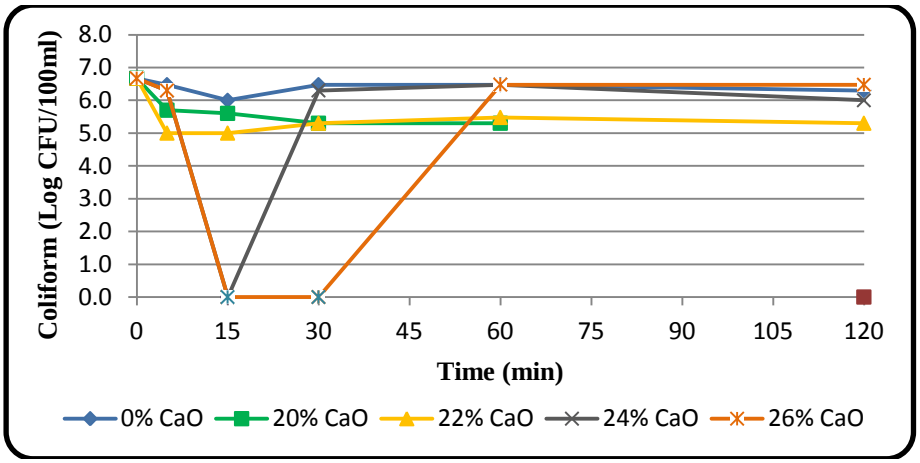

Figure A.17 Log Total coliforms survivability in black water after treatment with 20-26% CaO/TS (Experiment 2)

Table A.18 Log Total coliform survivability in black water after treatment with 24-30% CaO/TS (Experiment 3)

% CaO	Log total coliforms CFU/100ml					
	0	5	15	30	60	120
0%	6.71	6.24	6.18	5.98	5.78	6.49
24%	6.71	5.81	5.70	5.81	5.95	6.38
26%	6.71	5.65	5.78	5.85	5.98	5.95
28%	6.71	5.48	5.65	5.60	5.90	5.30

30%	6.71	5.00	5.00	0.00	5.54	6.10

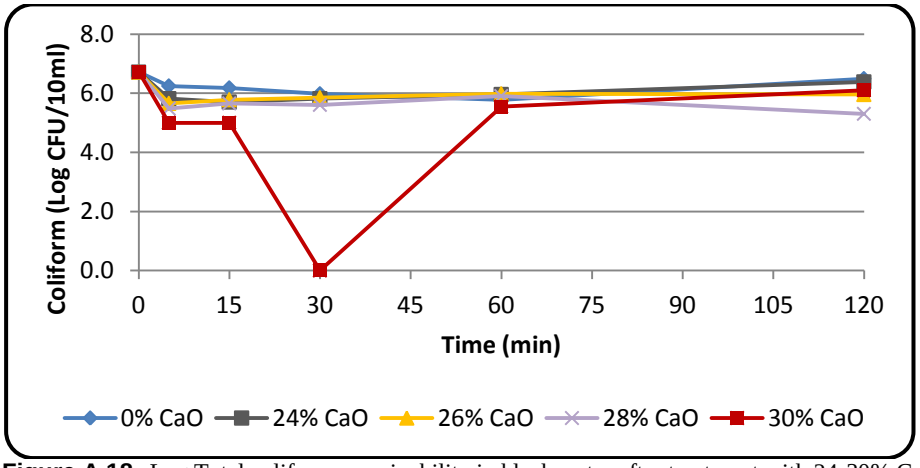

Figure A.18 Log Total coliforms survivability in black water after treatment with 24-30% CaO/TS (Experiment 3)

Table A.19 Log Total coliform survivability in black water after treatment with 30-60% CaO/TS (Experiment 4)

% CaO	Log total coliforms CFU/100ml					
	0	5	15	30	60	120
0%	6.7	5.1	5.9	5.2	5.2	5.9
30%	6.7	5.3	5.3	5.2	5.3	5.5
40%	6.7	5.1	4.3	4.8	5.0	4.9
50%	6.7	0.0	0.0	0.0	0.0	0.0
60%	6.7	0.0	0.0	0.0	0.0	0.0

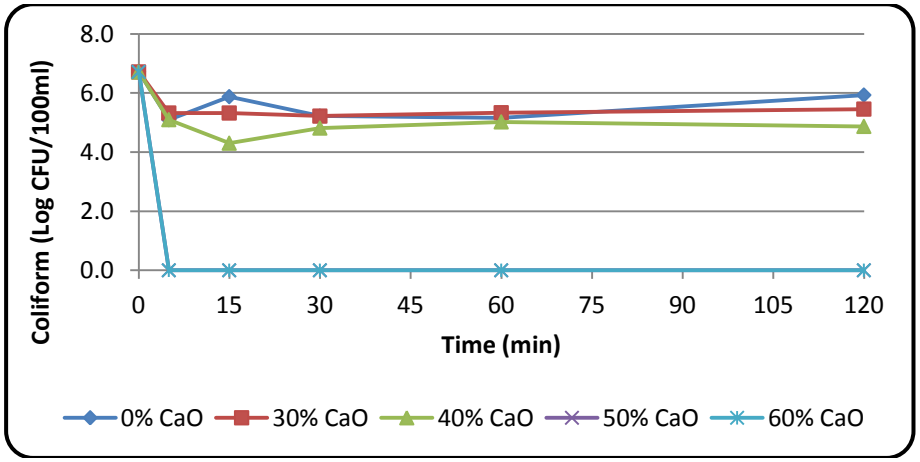

Figure A.19 Log Total coliforms survivability in black water after treatment with 30-60% CaO/TS (Experiment 4)

Table A.20 Log Total coliform survivability in black water after treatment with 30-60% CaO/TS (Experiment 5)

% CaO	Log total coliforms CFU/100ml					
	0	5	15	30	60	120
0%	6.7	5.8	5.6	5.6	5.6	5.4
30%	6.7	5.0	0.0	5.0	0.0	0.0
40%	6.7	0.0	0.0	0.0	0.0	0.0

50%	6.7	0.0	0.0	0.0	0.0	0.0
60%	6.7	0.0	0.0	0.0	0.0	0.0

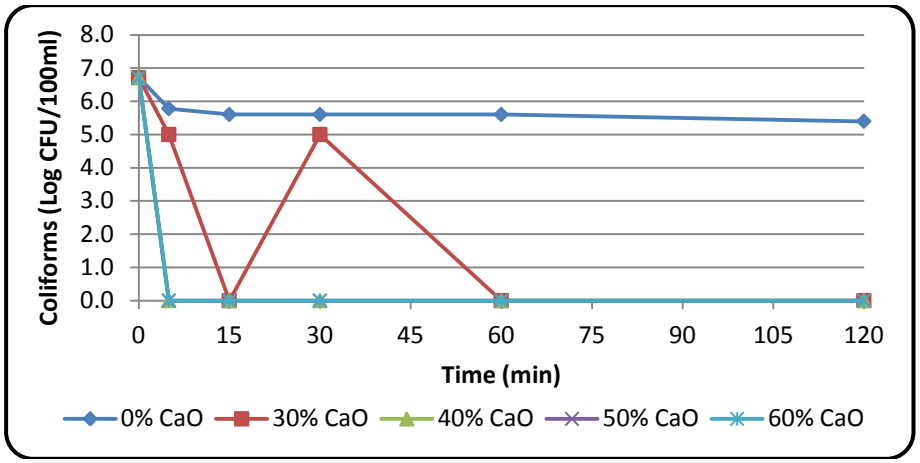

Figure A.20 Log Total coliforms survivability in black water after treatment with 30-60% CaO/TS (Experiment 5)

Table A.21 Log Total coliform survivability in black water after treatment with 30-60% CaO/TS (Experiment 6)

% CaO	Log total coliforms CFU/100ml					
	0	5	15	30	60	120
0%	6.7	5.9	6.0	5.7	6.0	5.5
30%	6.7	4.9	4.2	4.7	4.9	4.5
40%	6.7	4.0	0.0	4.0	0.0	4.3
50%	6.7	0.0	0.0	0.0	0.0	0.0
60%	6.7	0.0	0.0	0.0	0.0	0.0

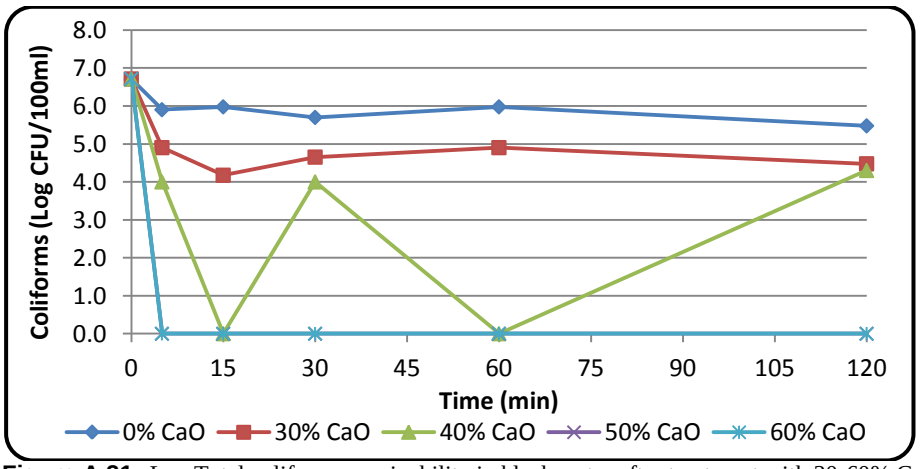

Figure A.21 Log Total coliforms survivability in black water after treatment with 30-60% CaO/TS (Experiment 6)