SANITIZING ON-SITE PIT SLUDGE DURING EMERGENCY SITUATIONS USING VERMICOMPOST, TERRA PRETA (ANAEROBIC DRY TOILET) AND ANAEROBIC DIGESTER TOILETS

MASTER OF PHILOSOPHY IN APPLIED SCIENCES (ENVIRONMENTAL SANITATION) DISSERTATION

FLAVIUS MAGEDE KAMWANI

UNIVERSITY OF MALAWI
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Master of Philosophy in Applied Sciences (Environmental Sanitation) Dissertation

By

FLAVIUS MAGEDE KAMWANI

BEd (Science)-University of Malawi

Submitted to the Department of Physics and Biochemical Sciences, Faculty of Applied Sciences, in partial fulfilment of the requirements for the degree of Master of Philosophy in Applied Sciences (Environmental Sanitation)

University of Malawi

The Polytechnic

September, 2017

DECLARATION

I, Flavius Magede Kamwani declare that this thesis is my own original work. Where other sources of information have been used, they have been acknowledged. I hereby certify that this work has not been submitted before in part or full for any other degree or examination.

SIGNATURE :

DATE : 04/09/2017

CERTIFICATE OF APPROVAL

We, the undersigned, certify that we have read and hereby recommend for acceptance by the University of Malawi a thesis entitled *'Sanitizing on-site pit sludge during emergency situations using Vermicompost, Terra Preta (anaerobic dry toilet) and Anaerobic Digester toilets.'*

Dean – Postgraduate :

Signature :

Date :

Main Supervisor :

Signature :

Date :

Co-Supervisor :

Signature :

Date :

Head of Department :

Signature :

Date :

DEDICATION

I dedicate this project to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my wife: Blessings, who has encouraged me all the way and whose encouragement has made sure that I give it all it takes to finish that which I had started

Thank you. My love for you can never be quantified. God bless you.

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ABSTRACT

Reaponse to emergency sanitation faces faecal sludge containment and treatment challenges. As a result in emergency camps frequently experience faecal-oral related disease outbreaks. In search of possible solutions the Anaerobic Digester, Terra Preta and Vermicompost toilets were placed under observations as they either lacked on-site scientific evidence, or there was contradicting literature regarding their performance during emergency situations.

Using randomly selected grab samples, the sanitation systems were assessed for faecal sludge stabilization (Temperature, pH, and Chemical Oxygen Demand), pathogen reduction (*Escherichia coli* and Total Coliforms) and useful agricultural by-product generation (Total Ammonia Nitrogen).The results indicated that Anaerobic Digester sanitised faecal sludge in summer (*E. coli*/100ml $<10^3$/100ml) but not in winter (7.96 x 10^5 *E. coli*/100ml). In both seasons, faecal sludge never got stabilised (110.08mg/l in winter and 278.20mg/l in summer for COD) although it produced fertiliser rich by-product (15.17mg/l in winter and 25.58mg/l in summer for TAN). The Anaerobic Digester also harvested 5m^3/day biogas against the designed 10m^3/day biogas volume due to its observed limited capacity of converting Chemical Oxygen Demand from faecal sludge to methane (CH_4) as evidenced by the 17% COD removal difference in the collected data. The LAB led TPS system harvested rich in Total Ammonia (16.58mg/l) and pathogen free (*E. coli*/100ml $<10^3$/100ml) urine but got challenged in reducing Lacto-Fermented Sludge pathogens (1.05 x10^7 *E. coli*/100ml and 2.18 x 10^7 TCFU/100ml) to below Malawi Standard ($<10^3$CFU/100ml) and stabilising faecal sludge to 60mg/l. The worms showed the capacity of increasing Total Ammonia Nitrogen concentration of faecal sludge by 14.38% in winter and 27.37% in summer. However they got challenged in producing pathogen free vermicompost (7.72 x 10^5 *E. coli*/100ml in winter and 1.53 x 10^7 *E. coli*/100ml in summer and 9.42 x 10^5 TCFU/100ml in winter and 5.33 x 10^7 TCFU/100ml in summer) and stabilized vermicompost (348.31mg/l winter and 534.85mg/l summer).

In conclusion, the results observed under this study suggest that the three proposed sanition systems should not be recommended for use during an immediate phase of an emergency situations as they have demonstrated inconsistences in as far as pathogen reduction and faecal sludge stabilization is concerned. However, further studies in actual emergency situations and improvements of the sanitation systems could help in coming up with an informed decision on their functionality.

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ABBREVIATIONS

ABR : Anaerobic Baffled Reactor
AD : Anaerobic Digestion
ADE : Amazon Dark Earth
APHA : American Public Health Association
C/N : Carbon/Nitrogen Ratio
CH_4 : Methane
CHP : Combined Heat and Power
CO_2 : Carbon Dioxide
COD : Chemical Oxygen Demand
CSTRs : Continuously Stirred Reactors
Dr. : Doctor
E : East
E. coli : *Escherichia coli*
g : Gram
GPS : Geographical Position System
H_2S : Hydrogen Sulphide
HS : High Solids
IFRC : International Federation of Red Cross and Red Crescent Societies
LAB : Lactic Acid Bacteria
LS : Low Solids
MBS : Malawi Bureau Standards
MS : Multi Stage
MSHS : Multi Stage High Solid
n.d : No Date
NH_3 : Ammonia
OLR : Organic Loading Rate
PFR : Plug Flow Reactors
S : South
SO_2 : Sulphur Dioxide
SOWTech : Sustainable One World Technology
SSLS : Single Stage Low Solids
TAN : Total Ammonia Nitrogen
TCFU : Total Colony Forming Units
TOC : Total Organic Carbon
TPS : Terra Preta Sanitation
TS : Total Solids
UK : United Kingdom
USAB : Upflow Sludge Anaerobic Baffled reactors
WHO : World Health Organisation

LIST OF SYMBOLS

%.	: Percentage
˚C	: Degree Celsius

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CHAPTER ONE
INTRODUCTION

1.1 Background

Malawi experiences natural disasters that arise from weather related events such as winds, hailstorms and heavy rains, which result in floods. Amongst these events, floods are the most common and have impacted Malawi more than 157 times since 1946 (Misomali, 2009). The districts which are often hit by these floods include Nsanje, Chikwawa, Blanytre, Phalombe, Mulanje, Zomba, Machinga, Chiladzulu, Thyolo, Mangochi, Salima, Karonga, Balaka, Rumphi and Lilongwe (European Report, 2015; Misomali, 2009; International Federation of Red Cross and Red Crescent Societies[IFRC], 2015). Natural disasters such as these often lead to unforeseen and sudden emergency situations that cause great damage, destruction and human suffering such as reduction in the abilities to sustain normal living conditions for good health, life and, livelihoods (Wisner & Adams, 2002; Harvey, Baghri & Reed, 2002; Guha-Sapir, Vos, Below, & Ponserre, 2011).

For quite a long time, Malawi's response to natural disasters, just like the rest of the world, has not been addressed as a whole due to limited resources, instead it has been to put provision of medical care, shelter, water supply and food as a priority at the expense of sanitary facilities (Misomali, 2009; Malambo, 2014; Spit et al., 2014). This approach puts aside proper management of rapidly produced wastewater and faecal sludge in emergency camps. The poorly managed faecal sludge is often disposed of untreated either at the shortest possible distance, on open grounds, into drainage ditches, or into water courses (Strauss, Larmie, & Heinss, 1997).

Sanitation becomes more challenging especially when there is flooding and the evacuation sites have unstable soils, high water tables and rocky soils (Wisner & Adams, 2002; Brown, Jeandron, Cavill, & Cumming, 2012). Literature explains that poor sanitation and hygiene practices leads to food contamination, outbreaks of faecal-oral related diseases such as diarrhoea, cholera and typhoid (Wisner & Adams, 2002; IFRC, 2010, The Johns Hopkins and the International Federation of Red Cross and Red Crescent Societies, 2008). Such disease outbreaks have been order of the day in the above mentioned districts (IFRC, 2015). Disease outbreak incidences in emergency situations do not only expose the emergency response gap of not properly managing sanitation at an early stage of an emergency, but also points to the fact that, when the population is more vulnerable, containment and treatment of faecal matter

is a vital barrier against the spreading of faecal oral related diseases (Johannessen, 2011; Bastable & Lamb, 2012, Fenner, Guthrie, & Piano, 2007)

Looking at how challenging faecal sludge management could become, emergency response organizations, in particular WASTE-Malawi and Sustainable One World Technology(SWOTech) with funding from International Federation of Red Cross and Red Crescent Societies, embarked on an investigation of low-cost faecal sludge treatment technologies that could not only be rapidly deployed and effectively work under challenging physical conditions (Spit et al., 2014), but also sanitize faecal sludge and recycle human excreta by-products for possible soil fertility improvement, water conservation, and on-site reduction of huge volumes of faecal sludge (Esrey et al., 1998; Mnkeni & Austin, 2009). The recycling of sanitized faecal sludge would ensure protection of the environment, natural resources and people that are in emergency situations.

Three sanitation systems were identified for possible use during emergency situations namely Anaerobic Digester, Terra Preta and Vermicompost toilets. The Anaerobic Digester is a water tight Anaerobic Digestion (AD) system that sanitises and stabilises organic waste (human, animal or vegetable) through solar energy pasteurisation to recover the energy and nutrients in it, and produce a non-fossil fuel derived biogas for cooking and a pasteurised fertiliser for improved crop growth. The digester uses a single stage AD system as all the stages (hydrolysis, acidogenesis, acetogenesis and methanogenesis) of AD take place in one digestion chamber. It has a reinforced black rubber body with solid plastic turrets, discharge pipe, orca valves and biogas storage bags. Design specifications include faecal sludge treatmen at thermophilic temperatures of 55oC, 38 days faecal sludge retention time and capacity of being used by 200 people. A key advantage of the Anaerobic Digester is that no electricity of external power is required for the system to operate and can be rapidly deployed to emergency sites. In addition, the system suites emergency situations as it is not only delivered complete and ready to use with minimal installation using hand tools but also placed above ground with only a shallow trench that does not require concrete nor bricks to lay which makes it ready for use in hours and not weeks. However, despite the Anaerobic Digester being recommended for use during emergency situations, it lacked evidence on whether the anaerobic digestion processes taking place in it could effectively and efficiently stabilize, and sanitize faecal sludge and generate useful by-product while on-site. Hence this study sought to assess the Anaerobic Digester's functionality and applicability in treating Faecal Sludge on-site, during emergency situations,

by quantifying the process efficiency in terms of stabilisation, sanitization and useful by-product generation.

Terra Petra toilet is a sanitation system that involved urine diversion away from faeces through a specially designed pedestal, addition of a charcoal mixture and replaced vermicomposting process with Lactic Acid Bacteria inoculum. Lactic Acid Bacteria (LAB) are a heterogeneous group of Gram positive, non-motile, non-spore-forming rod-shaped or coccoid bacteria which do, through fermentation of carbohydrates, produce lactic acid as their major end product (Mahony & Sinderen, 2014; Khalid, 2011). The produced lactic acid causes acidification and undissociation of the pathogens' cell cytoplasm which, being lipophilic, diffuse passively across the membrane thereby either collapsing the electrochemical proton gradient, or altering the cell membrane permeability which results in disruption of substrate transport systems (Snijders, Logtestijn, Mossel & Smulders, 1985; Kashket, 1987; Beasley, 2004). LAB also produces bacteriocins which are ribosomally synthesized antimicrobial peptides that are active against other bacteria, either of the same species (narrow spectrum), or across genera. Bacteriocins kill microorganisms by causing disruption of the cytoplasmic membrane-potential through the formation of pores in the phospholipids bilayer (Montville, Winkowski, & Ludescher, 1995) and/or leakage of cellular solutes that eventually leads to cell death. Integration of the anaerobic dry toilet and vermicomposting promised to be an ideal approach for managing wastes generated in emergency situations as it could make the product Terra Preta address problems of soil degradation and food insecurity common in many emergency camps. However, the challenge for emergency situations is how to make TPS sanitise, and stabilise faecal sludge and generate useful by-products, which are acceptable, affordable and sustainable for an early phase of an emergency as it takes too long to be completed. To address this challenge, and for the purpose of this study, the final stage of vermicomposting was left out and replaced by the addition of Lactic Acid Bacteria (LAB) inoculum(see Figure 10) which according to Malambo, (2014), while carrying off-site batch experiments, successfully sanitized and stabilized faecal sludge. The reduction of faecal sludge pathogens to safe levels is the key issue, in as far as the choice of sanitation systems to be deployed to emergency sites is concerned. However, despite Malambo, (2014) demonstrating that antimicrobial actions of both bacteriocins and lactic acid successfully sanitized faecal sludge, the study was done off-site and not on-site. Hence this study sought to determine if the procedure for lactic acid treatment of faecal sludge established through off-site small scale experiments could be up scaled to on-site treatment in a pit latrine. In addition, the research sought to determine the safety and usefulness

of by-products generated from the separation of urine and Lacto-Fermented Sludge for possible sustainable agriculture in emergencies.

Vermicompost toilet is a sanitation system that used earthworms called Tiger Worm (*Eisenia foetida*) to treat faecal sludge. The earthworms, in a combined action with microorganisms, convert faecal sludge into nutrient-rich humus and produce 'antibiotics' onto the ingested faecal sludge thereby killing the pathogenic organisms in faecal sludge to safe levels. Literature outlines that worms have the potential of reducing both faecal sludge volume to almost half its original size and pathogens to safe levels. The reduction of faecal sludge pathogens to safe levels and volume of faecal sludge to almost half its original size is key, in as far as the choice of sanitation systems to be deployed to emergency sites is concerned. As the increase in number of people that are often taken to emergency evacuation sites leads to increase in faecal sludge production rates such that the installed sanitation systems get filled up so quickly which in turn increases the frequency of desludging for possible off-site treatment at designated sites. The more the sanitation systems are desludged, the faster the resources are depleted, and the more challenging the management of sanitation in emergency sites becomes. Therefore, if the earthworms could be used to treat faecal sludge on-site during emergency situations, it could take time before the sanitation systems get full, in so doing the resources that are spent on managing faecal sludge could be used for other equally important things. Literature also indicates that the worms have the capacity of increasing the fertilizer content of faecal sludge. This character of worms could help improve agricultural productivity in emergency situations. However, despite the worms having such interesting characters, most of the studies investigated were done in faecal sludge that was treated off-site and not on-site. Secondly, the efficiency of earthworms in treating faecal sludge lacked locally tested scientific evidence. Thirdly, there is contradicting literature on the efficiency of the worms, in as far as pathogen reduction is concerned, suggesting the need for piloting Vermicompost toilet efficiency in treating faecal sludge and improving urban agriculture in emergency situations. Hence this study, using a pilot Vermicompost emergency sanitation toilet planted in Blantyre, Malawi, sought to provide evidence based information regarding the functionality and applicability of earthworms to treating on-site Faecal Sludge, by quantifying the process efficiency in terms of stabilisation, sanitization and useful by-product generation.

1.2. Problem Statement

In Malawi whenever an emergency occurs, people dwelling in emergency evacuation sites produce huge volumes of faecal sludge which has cost implications in terms of money spent on operations and maintenance and failure in recycling nutrients which happens when is dislodged for possible off-site treatment. Even when the technology for pit emptying is available the emergency sites have existed where there are no proper disposal sites. Generally, response to emergency situations has prioritized food and shelter and not sanitary facilities. Even where the provision of sanitary facilities is available evacuation camps have had unstable soils, high water tables and rocky soils, conditions which have prohibited sinking of pit latrines. Unsafe faecal sludge disposal during such emergencies has resulted in outbreaks of faecal-oral related diseases such as diarrhoea, and cholera. Commonly, not everything is provided for by emergency response organisations as they usually have limited resources. Basic things such as fuel for both cooking and lighting and inorganic fertilizer for urban agriculture (growing food, in any manner, for markets around the perimeter of densely populated communities) are not provided for. Such types of challenges have demanded sanitation technologies that would sanitise faecal sludge, harvest useful faecal sludge by products such as biogas and fertiliser, within an early stage of an emergency, while placed aboveground. In search of solutions WASTE-Malawi identified three sanitation systems that could be used during emergency situations. However it was not known whether the proposed sanitation systems would effectively sanitise and stabilise faecal sludge, and harvest by-products that are rich in fertilizer for possible agriculture in emergency sites. Hence this study sought to test the functionality and applicability to emergency situations of the proposed sanitation systems as well as the process efficiency regarding stabilisation, sanitisation and useful by-product generation.

1.3.0. Research Objectives

1.3.1. General objective

The main objective of the research was to investigate the functionality and applicability to emergency situations, of the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria (Terra Preta) in treating Faecal Sludge on-site, by quantifying the process efficiency in terms of stabilisation, sanitization and useful by-product generation.

1.3.2. Specific Objectives

The specific objectives of this research were;

- To determine the feasibility of deploying the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as a sanitation system that will treat faecal sludge to meet Malawi Standards of pathogen free sludge during challenging conditions common in emergency situations.
- To assess the efficiency of Anaerobic Digester, Vermicompost and Terra Preta toilets in converting fresh faecal sludge on-site into safe and useful by-products for possible sustainable agriculture during emergency situations.
- To evaluate the Anaerobic Digester, Vermicompost and Terra Preta toilets for their suitability in stabilising faecal sludge during challenging conditions common in emergency situations.

1.4.0. Hypothesis

It was hypothesised that the use of Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as an on-site faecal sludge sanitation systems during emergency situations could reduce faecal sludge pathogens to acceptable Malawi standards. It was also hypothesised that the use of Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as on-site faecal sludge sanitation systems during emergency situations could stabilize faecal sludge to acceptable Malawi standards. It was further hypothesised that the use of Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as on-site faecal sludge sanitation systems during emergency situations could increase the concentration of faecal sludge by-products for possible use in emergency situations.

1.5.0 Significance of the Research

Emergency response organizations spend a lot of resources in responding to emergency situations every year. However, literature indicates that these organizations experience challenges in the area of containment and treatment of faecal sludge. The researcher realized that these challenges are as a result of not properly containing and treating faecal sludge on-site during emergency situations. Therefore, the findings of this study will help emergency response organizations treat faecal sludge on-site and not off-site thereby reducing operations and maintence costs that are associated with treating off-site treatment of faecal sludge commonly practiced during emergency situations. The recommendations derived from the study will provide the emergency response sector with scientific information that could serve as a basis for deploying the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as emergency sanitation systems to areas that have unstable soils, high water tables and rocky soil.

Chapter Summary

Conclusively, this chapter has outlined the frequency of occurrence of emergencies situations in Malawi. It has been highlighted that on-site faecal sludge treatment has not received the much needed attention as evidenced by reoccurrence of faecal oral related disease outbreaks in emergency sites. Three sanitation systems namely Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets have been proposed as possible on-site emergency sanitation systems that can contain and treat faecal sludge during challenging conditions common in emergency situations. The next chapter outlines the literature behind the proposed sanitation systems.

CHAPTER TWO

LITERATURE REVIEW.

2.0 Introduction

In this chapter, review of pertinent literature is presented on factors surrounding emergency situations, anaerobic digestion, Terra Preta, Lactic Acid Bacteria, and Vermicomposting. General information on phases/stages of emergency sanitation and pathogens found in faecal sludge is presented. Finally, review of empirical literature presents a critical analysis of other research findings, observations, gaps as well as conclusions related to the topic under study.

2.1. 0. Emergency

An emergency, as outlined in Chapter one, is “an unforeseen and often sudden event that causes great damage, destruction and human suffering” (Guha-Sapir et al., 2011, p.7). Emergencies could be complex especially if they overwhelm local capacity, necessitating a request to a national or international level for external assistance. Events such as warfare, civil disturbance, large scale movement of people and natural disasters end up putting people in situations that are emergency in nature (Brown et al., 2012; The Johns Hopkins and the International Federation of Red Cross and Red Crescent Societies, 2008, Malambo, 2014; Wisner & Adams, 2002).

Over the past decades, the world has experienced an increase in natural disasters (EM-DAT, 2011). Figure 1 below shows the trend of number of natural catastrophes worldwide from 1980 to 2010.

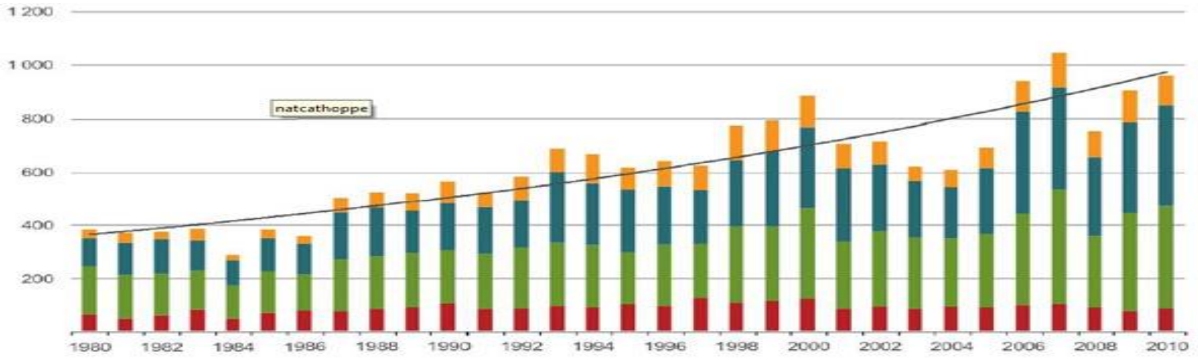


Figure 1: Number of Natural catastrophes Worldwide (1980-2010)

Source: Emergency Events Database (EM-DAT, 2011)

In 2010, 64 natural disasters were reported for Africa and these disasters affected 15 million people (Guha-Sapir et al., 2011). Malawi, just like other African countries experiences recurrence of natural disasters such as tropical storms, floods, earthquakes, and landslides (Plan of Action for Malawi 2012 - 2016). It should be mentioned that floods often occur in districts

such as Nsanje, Chikwawa, Phalombe, Mulanje, Zomba, Machinga, Chiladzulu, Thyolo, Mangochi, Salima, Karonga, Balaka, Rumphi and Lilongwe (Misomali, 2009; IFRC, 2015). The deadliest recorded floods were experienced in March 1991 and January 2015 in which 500 and 104 people were reported dead, respectively (Hay et al., 2010; IFRC, 2015). These experienced natural disasters have continuously demanded special attention from both government and emergency response organizations. One of the focal areas, as regards responding to these emergency situations, is the provision of safe sanitary facilities. However, literature indicates that provision of sanitary facilities is more challenging during emergency situations as the evacuation sites often have unstable soils, high water tables and rocky soils (Wisner & Adams, 2002; Brown, Jeandron, Cavill & Cumming, 2012).

2.1.1. Stages of an Emergency

Different authors have presented various stages of an emergency (Harvey et al., 2002; Davis & Lambert 2002; Sustainable Sanitation Alliance 2009). Shown in Table 1 below is the adapted information that is outlining brief description, duration and the reason behind making interventions at each respective stage. In this research the three sanitation technologies mentioned in chapter one were assessed in order to see if they were feasible for either the immediate, short term or long term stsges of an emergency situation.

2.1.2. Emergency Sanitation

Sanitation is described as the efficient collection and disposal of excreta, urine, refuse, and silage so as not to endanger the health of individuals and the entire community (WHO, 1987; Harvey et al., 2002). Unlike general sanitation, emergency sanitation involves control and management of excreta, solid waste, medical waste, dead bodies, wastewater, and promotion of best hygiene practice with an aim of creating a safer environment and minimizing the spread of disease in a disaster affected area. (Harvey et al., 2002). In this research, the faecal sludge management aspect of an emergency sanitation was explored.

Table 1: Phases of an Emergency

Stages of an Emergency	Description	Duration	Aim of Intervention
Immediate	• Initial stage of an emergency. • Occurs immediately after the impact phase of a disaster. • Characterized by great instability and often high mortality.	1-2 Months	Containment and localization of sources of sanitation related disease in order to create a safer environment and minimize the spread of disease
Short Term	• Period of stabilization following the immediate phase	≤6 Months	Reduction of morbidity and mortality rates (where appropriate) and prevention of any further spread of disease.
Long Term	• Recovery and settlement stage, affected population returning to their homes or settling in a new area.	≥Several years.	Sustenance of health and wellbeing of affected population, and promotion of self-sufficiency.

Adapted From: Harvey et al., 2002

2.2.0. Faecal Sludge

Faecal sludge in its simple term means human waste that contains faeces. Faecal sludge contains pathogens that cause diseases that quickly affect people especially the vulnerable children and elderly. Pathogens found in faecal sludge if not treated may cause millions of deaths every year (World Health Organisation [WHO], 2004). The microorganisms contained in faecal sludge may enter the body through contaminated food, water, eating and cooking utensils and by contact with contaminated objects. The major groups of organisms that can be found in faecal sludge include protozoa, helminthes in the form of eggs, viruses and bacteria (Jiménez 2009; Sinha, Herat, Bharambe & Brahambhatt, 2009). Therefore the paragraphs below outline brief insights of each group of pathogens found in faecal sludge.

2.2.1. Protozoa

Protozoa are unicellular microorganisms whose cell walls are surrounded by a cytoplasmic membrane covered by a protective structure called a pellicle (Bitton, 2005; Hartsock, 2010.) Protozoa reproduce mainly by binary fission, although a few species reproduce sexually. Some protozoa form cysts that contain one or more infective forms. Cysts passed in faeces have a protective wall, enabling the parasite to survive in the outside environment for a period ranging from days to a year, depending on the species and environmental conditions (Yaeger, 1996). Protozoa do exist in two stages namely cyst stage and trophozoite stage. The cyst stage is dormant and highly resistant to environmental stress. They can survive in water bodies for long periods, especially in winter. Their sizes measure between 10 and 16 µm (Jiménez et al., 2009).

Infection into human beings is by means of feacal-oral contamination (Hartsock, 2010). Unlike cyst stage, trophozoite stage is the active, reproductive, pathogenic and feeding stage of the protozoa. Their sizes range between 8-40 µm long and 7-10 µm wide (Jiménez et al., 2009; Hartsock, 2010). Protozoa such as ciliates/ciliophora, move by waving short cilia that line the cell. Because of this kind of movement the protozoa have the capacity to move quickly, make sudden stoppage, and sharp turn while hunting for their prey such as bacteria, fungi, or other protozoa.

Table 2: Classification of Parasitic Protozoa and Associated Diseases

Phylum	Subphylum	Representative Genera	Major Diseases Produced in Human Beings	Chapter
Sarcomastigophora (with flagella, pseudopodia, or both)	Mastigophora (flagella)	*Leishmania*	Visceral, cutaneous and mucocutaneous infection	82
		Trypanosoma	Sleeping sickness Chagas' disease	
		Giardia	Diarrhea	80
		Trichomonas	Vaginitis	
	Sarcodina (pseudopodia)	*Entamoeba*	Dysentery, liver abscess	79
		Dientamoeba	Colitis	
		Naegleria and *Acanthamoeba*	Central nervous system and corneal ulcers	81
		Babesia	Babesiosis	
Apicomplexa (apical complex)		*Plasmodium*	Malaria	83
		Isospora	Diarrhea	80
		Sarcocystis	Diarrhea	
		Cryptosporidium	Diarrhea	
		Toxoplasma	Toxoplasmosis	84
Microspora		*Enterocytozoon*	Diarrhea	—
Ciliophora (with cilia)		*Balantidium*	Dysentery	80
Unclassified	—	*Pneumocystis*	Pneumonia	85

Source: Yaeger, (1996)

Many protozoa cause diseases in both animals and humans. For instance, the Flagellates/Mastigophora, cause diseases that are characterized by extremely liquid, odorous and explosive diarrhoea, stomach and intestinal gases, nausea and loss of appetite (Jiménez et al., 2009); Amoebas/sarcodina, a huge group of protozoa that is characterized by having a trophozoite stage, has no structural components on its membrane to maintain a shape resulting in an amorphous blob that moves by pseudopod projections, causes amoebic dysentery, a

potentially deadly disease characterized by painful ulcers in the large intestine and diarrhea. The disease amoebic dysentery is as a result of drinking water contaminated with Entamoeba cysts, usually present in areas with poor sanitation. (Jiménez et al., 2009). Other diseases caused by protozoa can be seen in Table 2.

2.2.2. Helmithis

The term helminth simply means worm. In general helminths are multicellular eukaryotic animals that generally possess digestive, circulatory, nervous, excretory, and reproductive systems. There are three different kinds of helminthes namely plathelminths or flat worms, nemathelminths (Aschelminths) or non-segmented round worms, and Annelida or segmented round worms. Plathelminths and nemathelminths infect humans through wastewater, sludge or faecal sludge (Jimenez & Maya, 2007; Maizels et al., 2004). Helminth eggs are discharged to the environment in faeces and the oral-faecal route is the main dissemination pathway of the disease. The inadequate management and disposal of wastewater, sludge and faecal sludge pollutes crops, water and food that when ingested serve as vehicles for transmitting the disease.

2.2.3. Viruses

Viruses are the obligate intracellular parasites that cannot multiply unless they invade a specific host cell and instruct its genetic and metabolic machinery to make and release quantities of new viruses (CDC, 2004.). According to Stanier, (1987) they have no cytoplasm or metabolism of their own and reproduce only within a host cell where their nucleic acid guides their replication. Viruses occur in different shapes and consist of nucleic acid surrounded by a protein layer and sometimes a lipid membrane (see Figure 8 below). There are more than 150 types of enteric viruses capable of producing infections that multiply in the intestine and get expelled in faeces (Jiménez et al., 2009). These enteric viruses have been detected in the drinking water supply systems, often in large numbers, despite the fact that those waters have received conventional water treatment where chlorination is part of the treatment process (Melnick & Gerba, 1980). Examples of the enteric viruses common to humans include enteroviruses, rotaviruses, reoviruses, caliciviruses, adenoviruses and hepatitis A viruses. Out of these enteric viruses, rotaviruses are the main cause of diarrhea, a common disease in emergency situations, entailing that the discharge of sludge into the environment is potentially hazardous to human health. In fact, several outbreaks of infectious hepatitis and viral gastroenteritis have been traced to sewage contamination of water and food (Baron et al., 1982; Gerba & Goyal, 1978; Gunn, Janowski, Lieb, Prather & Greenberg, 1982).

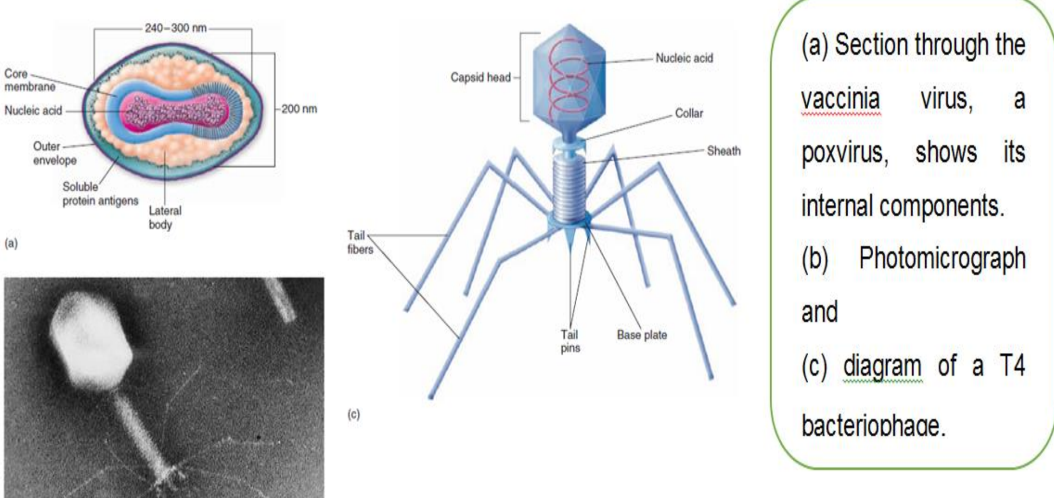


Figure 2: Detailed Structure of Complex Viruses

Source: CDC. (2004)

Determination of enteric viruses is achieved if methods such as *plant leaf local lesion assay* and *the plaque assay* are carried out. Briefly *plant leaf local lesion assay* is a method that involves applying a suspension of virions, previously concentrated from a sample, onto the surface of a leaf together with an abrasive material that tears small holes in the walls of the plant cells. Unlike *plant leaf local lesion assay,* the *plaque assay* involves infecting host cells growing in a thin layer on a medium partially solidified by agar. In both cases a local infection is initiated by each virion that enters a host cell, creating a region that becomes discolored and easily noticeable. These infections are made more visible by applying a dye that stains live cells and not those killed by the viruses. (Stanier, 1987).

2.2.4. Bacteria

Bacteria are single celled microorganisms ranging in size from 0.2 - 10 µm. They have a complex structure and their morphology shows a wide range of shapes and sizes. The most common shapes are rod-like called bacillus, spherical and coccus. The rod form varies considerably from very short rods that almost look like cocci, to very long filaments, thousands of microns in length (see Figure 3 below). Bacteria also form spirals and corkscrews, ovals (coccoid), commas, and elaborately branched structures. The cocci often take on multi-cell forms known as diplococci, streptococci, and tetrads (Thiel, 1999) (see Figure 4 below).

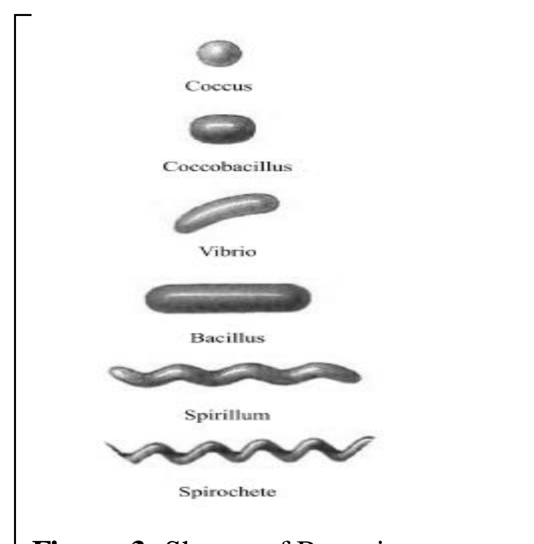


Figure 3: Shapes of Bacteria
Source: Thiel, (1999)

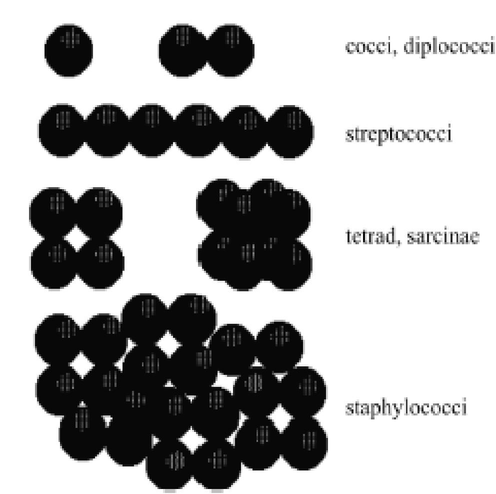


Figure 4: Arrangement of Cocci
Source: Thiel, (1999)

Bacteria reproduce and grow in an appropriate environment at defined temperature ranges of -15°C to 120°C and pH (6-8) (Jiménez et al., 2009). The extreme temperature favoring bacteria such as psychrophiles and extremophiles grow best at temperature ranges of about 0°C to -15°C and 100°C to 120°C, respectively. In this research, while observing the Anaerobic Digester, a particular interest was put on the thermophiles as the system's expected temperatures were around 55°C.

2.2.4.1. Growth of bacteria

Despite having many variations in their morphology, bacteria have a common characteristic of multiplying by simple binary fission (Thiel, 1999) (see Figure 5 below*)*. By undergoing binary fission, bacteria exhibit an exponential growth that rapidly increases its population. When the log of the cell number is plotted against time, a curved graph having four phases (lag phase, exponential (log) phase, stationary phase and death phase) is produced (see Figure 6 below)

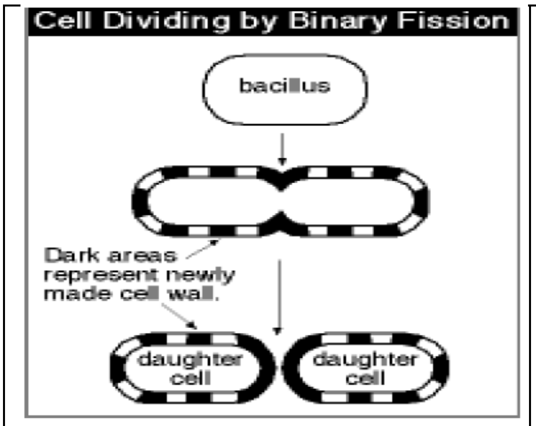


Figure 5: Cell Division by Binary Fission. **Source:** Thiel, (1999)

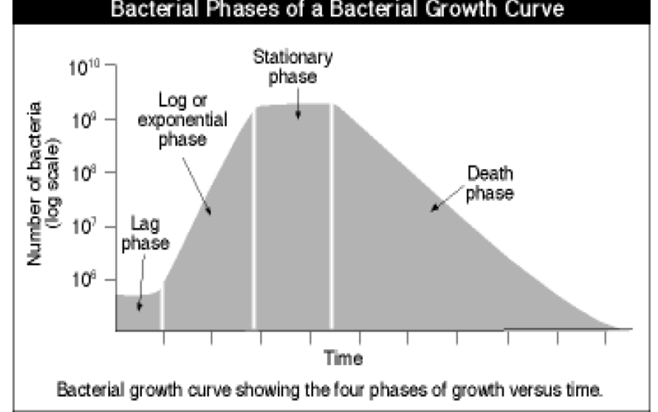


Figure 6: Bacteria Phases of a Bacterial Growth Curve
Source: Thiel, (1999)

Briefly during the lag phase no increase in cell number is observed as the cells are actively metabolizing in preparation for cell division. During the exponential (log) Phase cells divide and grow very fast, multiplying at a directly proportional constant rate. At stationary phase, both metabolism and cell division slow down and then eventually stop completely. Finally bacteria undergo the death phase of their growth cycle where cells quickly give up in as far as cell division is concerned.

One of the methods used in identifying bacteria is by Gram staining of their cell wall structure, a classification system that has withstood the test of time (Lowy, 2009). Bacteria are said to be Gram negative when their thin wall layer and outer membrane stain red and Gram positive when their thicker wall layer, lacking the outer membrane, stain violet (Thiel, 1999) (see Figure 7 below). Between the two classes of bacteria, it is the Gram-negative bacteria, such *Escherichia coli,* which are responsible for the faecal oral disease outbreaks in emergency camps (Hanna, 2007).

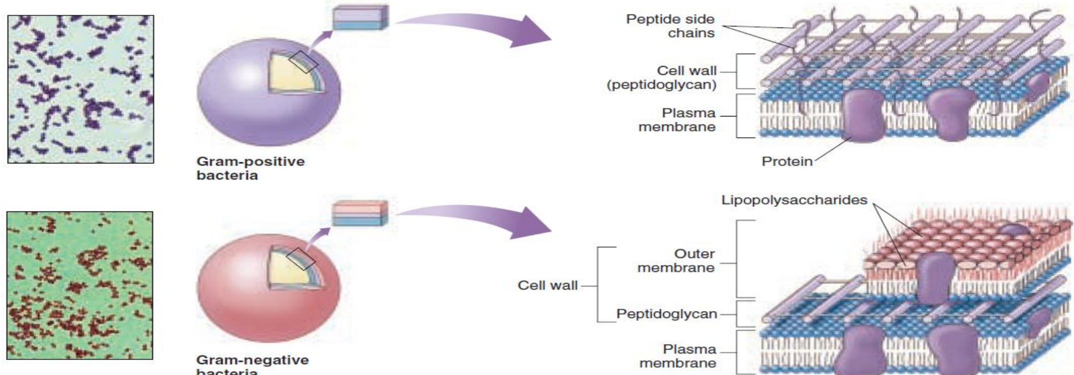


Figure 7: Gram Negative and Gram Positive Bacteria
Source: Raven & Johnson, (2001)

As outlined in the foregone paragraphs, this study had an interest in what happens to faecal sludge after defecation, as this is key to prevention of faecal-oral disease outbreaks during emergency situations. Interestingly, in the previous paragraphs, it is noted that the Gram-negative bacteria require special handling as they are responsible for the faecal-oral disease outbreaks. Hence for the purpose of this literature review and research, focus shall thus be on the Gram-negative bacterial pathogens that are associated with the oral-faecal route. Figure 2 below shows disease transmission routes, from faecal sludge, of disease such as diarrhea, cholera and typhoid, just to mention but a few, which happen to be major causes of sickness and death in disasters and emergencies.

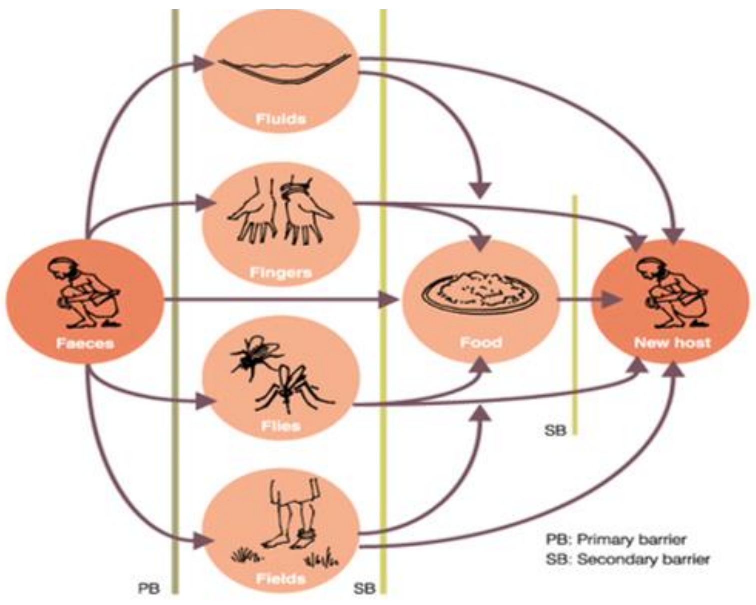


Figure 8: Transmission of Diseases from Faecal Sludge

Source: Kawata, (1978) as cited by Harvey et al., (2002)

2.3. Indicator organisms/Biological indicators

Detection of Gram-negative bacterial pathogens in faecal sludge is best done when analysis targets indicator organisms. By description, these are a group of organisms that indicate the presence of faecal contamination (Stephen, Odonkor, Joseph & Ampofo, 2013). Examples of these biological indicators include bacterial groups such as total coliforms, faecal coliforms, faecal streptococci, *Escherichia coli, Clostridium perfringens*, bifidobacteria and thermotolerant Coliforms (Bitton, 2005; Arthurson, 2008; Jiménez et al., 2009). In this research *Escherichia coli,* the predominant member of the facultative gram-negative, non-spore forming, rod-shaped anaerobic bacteria, and total coliforms were used as indicator organisms because of the following reasons: (1) their abundance is easy to detect; (2) a relatively rapid, accurate, and cost effective analytical method for their enumeration exists; (3) a reasonably strong correlation exists between their presence/absence and a particular pathogen or group of pathogens; and (4) indicator organisms behave in a similar way to most pathogenic bacteria in the environment and during treatment (Environmental Fact Sheet 2003; Stephen et al., 2013) (5) *E. coli* has most of the characteristics of an indicator organism which include its only natural habitat being the large intestine of warm-blooded animals; it does not survive well outside of the intestinal tract; it is easily detected using quick, easy, and inexpensive means; it has comparable or slightly better resistance than target organisms when subjected to harsh environmental conditions or lethal parameters of sludge treatment (Bitton, 2005; Arthurson, 2008; Stephen et al., 2013).

It is worth mentioning that the presence of *E. coli* fails to indicate the presence of pathogenic protozoa and helminth eggs (WHO, 2004) suggesting the need for additional indicators of faecal sludge pathogens. In this research enumeration of Total Coliforms and *Escherichia coli* used Chromocult Coliform Agar because it is a selective and differential chromogenic culture medium which contains Tergitol 7, as an inhibitor of Gram-positive bacteria which has no negative effect on the growth of the targeted coliforms/*E. coli* (ISO 9308-1, 2014), the character which makes it an ideal medium for the detection of coliforms/*E. coli* in wastewater.

2.4. Anaerobic Digestion

Anaerobic Digestion (AD) is a psychrophilic, mesophilic and thermophilic biological decomposition and stabilisation of biodegradable waste, in the absence of oxygen, which results in a stable sanitised biogas and digestate material that can be applied as a fertiliser and soil conditioner to an agricultural land to improve the soil structure or nutrients (Bywater, 2010; Zhang, 2010; Wikipedia, 2014; Omolola, 2007). Exceptional in its characteristics, AD is not only a cost effective proven technology for handling and treating biological wastes and effluents, but also a reliable solution for generation of electricity and household heating gas, as well as maintenance of clean environment (Viswanath et al., 1992). Its main features include mass reduction, biogas production and improved dewatering properties of the treated sludge (Zhang, 2010).

The anaerobic digestion process involves a series of distinct stages namely hydrolysis, acidogenesis, acetogenesis and methanogenesis (Bywater, 2010; Sansalone & Srinivasan, 2004; Michigan Department of Environmental Quality, n.d.). Briefly, hydrolysis involves breaking down of insoluble complex organic polymers, such as carbohydrates, proteins, lipids, and phosphorylated organics into soluble organics, such as glucose, amino acids and fatty acids that become available for use by other bacteria. Several enzymes, such as lipases, proteases, cellulases, and amylases, secreted by microbes, are involved in this stage of AD. Hydrolysis is a critical stage of AD, as it limits the rate of reactions especially when the raw materials have high organic waste. The products of hydrolysis undergo the second stage of AD known as acidogenesis. At this stage fermentative acidogenic bacteria convert the sugars and amino acids into carbon dioxide, hydrogen, ammonia, and organic acids (Volatile fatty acids) such as acetic acids and propanoic acids just to mention but a few. The third step of AD is acetogenesis, at this stage, the carbonic acids and alcohols are further digested by acetogens to produce mainly acetic acids as well as hydrogen and carbon dioxide. Microbes such as s*yntrophobacter wolinii*, a propionate decomposer and s*ytrophomonos wolfei*, a butyrate decomposer play a very important

role at this stage (Verma, 2002). The final stage of AD is called methanogenesis. This stage produces methane using methanogenic bacteria such as *methanobacterium, methanobacillus, methanococcus and methanosarcina*. It is the methanogenic bacteria that either split acetate into methane and carbon dioxide or uses hydrogen as electron donor and carbon dioxide as acceptor to produce methane (Zhang, 2010; Bywater, 2010; Wikipedia, 2014; Verma, 2002). These reactions use any substrate of organic or biological origin, whereas the metabolic products of each stage act as food for the bacteria in the next stage (Bywater, 2010; Verma, 2002).

Depending on the content of the total solids (TS), AD would take place in either single stage(SS) or multistage(MS) digesters. In SS digesters, all the four stages of AD take place in one reactor separated with time lapse. This digestion could be low solid (LS) commonly called SSLS or high solid (HS) also called SSHS depending on the total solids content in a reactor. SSLS processes are preferred because of their operational simplicity, existence for a much longer time than high solids systems, use of less expensive equipment for handling slurries and high yield of biogas as heavy fractions or the scum layer is not removed during the digestion. In multi-stage (MS) digesters, two or more reactors are used to separate the stages of AD. Stages such as hydrolysis, acidogenesis and acetogenesis take place in the first reactor while methanogenesis takes place in the second reactor. Just like SS digesters, MS digesters are also grouped into MSLS and MSHS with an aim of providing some improvements on the SS digesters. Generally MS digesters, unlike SS digesters, have high organic loading rate (OLR) (Verma, 2002; Massimo & Giordano, 2014).

During AD, operating parameters such as carbon to nitrogen ratio(C/N), total solids(TS)/organic loading rate, retention time, mixing, pH and temperature just to mention but a few are very important in as far having a successful AD system is concerned. Rajeshwar et al., (2000) reviewed the suitability and the status of development of anaerobic reactors for the digestion of selected organic effluents from sugar and distillery, pulp and paper, slaughterhouse and dairy units. In their review they noted that temperature ranges that influence anaerobic digestion can be categorized as psychrophilic (0-20°C), mesophilic (20-42°C) and thermophilic (42-75°C). Amongst the categories thermophilic anaerobic fermentation were reported to have reduced process stability and reduced dewatering properties of the fermented sludge and the requirement for large amounts of energy for heating, whereas the thermal destruction of pathogenic bacteria at elevated temperatures is considered a big advantage. Labatut & Gooch (2014) while monitoring anaerobic digestion process to optimize performance and prevent system failure found that operating anaerobic digesters at temperatures outside the normal range

results in decreased biogas production and organic matter stabilization. Maintenance of the system's pH in the optimal range of 5.5 and 8.5 is required for efficient anaerobic digestion (RISE-AT, 1998). Changes in digester operating conditions or introduction of toxic substances may result in process imbalance and accumulation of volatile fatty acids (VFA) that inhibits the digestion process.

RISE-AT, (1998) conducted a review of current status of anaerobic digestion technology for treatment of municipal solid waste where they also looked at important operating parameters in AD process. In their review they noted that feeding the AD system above its sustainable OLR results in low biogas yield due to either accumulation of inhibiting subatances such as fatty acids or inadequate mixing of the waste with slurry. This observation suggests that when monitoring the performance of AD system there is need to put much focus on the feeding rate as it is an important control parameter in continuous systems. Verma, (2002) examined in depth anaerobic digestion (AD) technologies in order to determine their economic and environmental competitiveness, as one of the options for processing the biodegradable organic materials in Municipal Solid Waste (MSW) and noted that there are differences in retention times for wastes treated at various temperature. The study reported that retention time for mesophilic digesters ranged from 10 to 40 days while as low as retention time of 14 days were required for thermophilic digesters. The study conducted by Verma, (2002) further reported that mixing in a digester in order to blend the fresh material with digestate containing microbes prevents scum formation and avoids temperature gradients within the digester while overmixing disrupts microbes. Verma, (2002) also repoted that optimum C/N ratios in anaerobic digesters are between 20-30 and rapid consumption of nitrogen by methanoges indicates a high C/N ratio which results in lower gas production while lower C/N ratio causes ammonia accumulation and pH values exceeding 8.5, which is toxic to methanogenic bacteria.

There are two major by-products of AD namely biogas and a mixture of both faecal sludge and water called digestate. In this research both biogas and digestate production were areas of interest hence the paragraph below will shed more light on these two AD by-products. Digestate is a mixture of both faecal sludge and wastewater which can be put on crops and is a valuable nutrient for recycling back to land. Where appropriate, this digestate can also be separated into a liquid fraction and a fibre which can be used as a soil conditioner (Bywater, 2010). Biogas is an odourless gas that is produced as a result of bacteria degrading biological and organic matter in the absence of oxygen through a process called anaerobic digestion (Kigozi, Aboyade & Muzenda, 2014; Omolola, 2007). This process of generating biogas occurs in an oxygen free

environment where complex biological and organic wastes get converted to methane through a series of stages of AD that have been explained above (Omolola, 2007). Depending on the feedstock, biogas is principally a mixture of methane $(CH_4)_g$ (55-70%), Carbon dioxide $(CO_2)_g$ (30-45%) and minute traces of hydrogen sulphide $(H_2S)_g$, hydrogen, nitrogen (0-1%), ammonia $(NH_3)_g$ and sulphur dioxide $(SO_2)_g$. (Omolola, 2007; Kigozi et al., 2014; Kengne, Moya, Diaz & Strande, 2014; Verma, 2002). Zhang, (2010) carried out a review of the main faecal sludge pretreatment methods which have the potential to increase biogas production in anaerobic digestion process. The methods included thermal, oxidative, thermochemical, mechanical (ultrasonic, grinding, high pressure homogenization) as well as other methods such as enzymic hydrolysis. Emphasis was mainly put on their impact on biogas production. The review was concluded by recommending further research for the better option as the reviewed methods could not lead to a conclusion of which method was best in as far treating faecal sludge and enhancing biogas production is concerned.

Anaerobic digestion has been widely applied in centralised wastewater treatment facilities for the digestion of primary sludge and waste activated sludge, typically with plug flow reactors (PFR) or continuously stirred reactors (CSTRs). In relation to AD in treating human waste off site, efforts to adapt AD to treat human waste have been documented by many authors over the last years 50 years. Lettinga et al., (1995) studied anaerobic treatment of domestic wastewater in small scale Upflow Sludge Anaerobic Baffled (USAB) reactors. The USAB was sunk in the soil and monitored for its effectiveness in treating wastewater. Their study revealed that anaerobic treatment using the UASB-system was one of the promising technologies with effective treatment of sludge, limited maintenance and sludge disposal and some recovery of biogas. However, they recommended that the USAB Reactor still required post-treatment of effluent for it to effectively remove pathogens.

Barber & Stuckey (1999) reviewed the use of the anaerobic baffled reactor (ABR) for wastewater treatment and found that anaerobic baffled reactors have several advantages (better resilience to hydraulic and organic shock loadings, longer biomass retention times, lower sludge yields, and the ability to partially separate between the various phases of anaerobic catabolism) over well-established systems such as the upflow anaerobic sludge blanket and the anaerobic filter. However, in their review it is reported that ABR requires expert design and construction and produces effluent and sludge that has high concentrations of pathogens requiring further treatment and/or appropriate discharge. Tilley et al., (2014) compiled an overview of Sanitation

Systems and Technologies that described a wide range of available low-cost sanitation technologies. Amongst the reviewed technologies was an anaerobic filters. In their review it is reported that, despite having an improved capacity, over septic tanks and anaerobic baffled reactors, of removing organic matter and solids as wastewater passes through the filters, they require piped water and expert design and construction, produce effluents that have high concentrations of pathogens requiring further effluent and sludge treatment and/or appropriate discharge. The review also reports that anaerobic filters are only suitable for low-density housing areas with low water table and not prone to flooding.

The reviewed literature, in the foregone paragraphs, on AD indicates that treatment of faecal sludge using AD during emergency situations requires some modifications of the existing digesters as most them require sinking of the digesters in soil and pretreatment of effluent which is not feasible during emergency situations. The conditions that are encountered during emergency situations are so challenging that one would want to deploy sanitation systems that do not require either sinking or expert design and construction as there is no such time and resources to carter for that. While searching for sanitation systems that can easily be deployed during emergency situations and as part of the Emergency Sanitation Project, IFRC working on wastewater treatment and WASTE working on research of innovative and creative sanitation systems applicable in the development stage of an emergency, recommended that Anaerobic Digester be used as a sanitation system during emergency situations (Spit, 2013).

Briefly, the Anaerobic Digester is a water tight Anaerobic Digestion (AD) faecal sludge treatment technology that is claimed to have the capability of sanitizing and stabilizing organic waste (human, animal or vegetable) through solar energy pasteurisation to recover the energy and nutrients in it, and produce a non-fossil fuel derived biogas for cooking and a pasteurised fertiliser for improved crop growth (Spit 2013). The Anaerobic Digester is designed for use in emergency aid situations, temporary camps and medium sized communities or institutions. It has a reinforced black rubber body with solid plastic turrets, discharge pipe, orca valves and biogas storage bags. It is believed that when exposed to solar energy it has the capacity, taking advantage of the black rubber, of raising the temperatures of its contents to thermophilic levels of 55°C. The main processes involved in the Anaerobic Digester are stabilisation and gas production through anaerobic digestion and sanitisation through pasteurisation utilising solar thermal heating. The system also provides gas storage for the produced methane gas as well as a digestate evaporation unit (see Appendix 9). A key advantage of the Anaerobic Digester is that no electricity of external power is required for the system to operate and can be rapidly

deployed to emergency sites. In addition, the system suites emergency situations as it is not only delivered complete and ready to use with minimal installation using hand tools but also placed above ground with only a shallow trench that does not require concrete nor bricks to lay which makes it ready for use in hours and not weeks. However, despite the Anaerobic Digester being recommended for use during emergency situations, it lacked evidence on whether the anaerobic digestion processes taking place in it could effectively and efficiently stabilize, and sanitize faecal sludge and generate useful by-product while on-site. Hence this study sought to assess the Anaerobic Digester's functionality and applicability in treating Faecal Sludge on-site, during emergency situations, by quantifying the process efficiency in terms of stabilisation, sanitization and useful by-product generation.

2.5.0. Vermicomposting

2.5.1. Earthworms

Earthworms were described by Aristotle as the intestines of earth (Fraser-Quick, 2002 as cited by Sinha et al. 2009) because they have the capacity of digesting a variety of organic materials. Literature outlines that there are different types of earth worms that decompose human waste. (See Table 3 Below) such as Tiger Worm (*Eisenia foetida*), Red Tiger Worm (*Eisenia andrei*), the Indian Blue Worm (*Perionyx excavatus*), the African Night Crawler (*Eudrilus euginae*), and the Red Worm (*Lum-bricus rubellus*) *Pseudomonas, Mucor, Paenibacillus, Azoarcus, Burkholderia, Spiroplasm, Acaligenes,* and *Acidobacterium* (Singh, Saxena, Shivay & Nain, 2014; Sinha et al., 2009; Mehali, Mehta, Karishma & Chorawala, 2014). However, a number of researchers recommend the use of *Eisenia foetida* due to its excellent performance in as far as removal of pathogens, faecal coliforms (*E. coli*), *Salmonella* spp., enteric viruses and helminth ova from human waste is concerned.

It is reported in literature that worms' survival is subject to different environmental conditions such as adequate moisture, soil texture, pH, electrolyte concentration, temperature, sludge age and nutrient content, adequate aeration, appropriate carbon/nitrogen (C/N) ratio of the feed material, adequate supply of calcium, multiplication of earthworms and food source regardless of the presence of toxic chemicals such as heavy metals. Neuhauser et al. (1988) and Naddafi et al., (2004) studied the potential of several earthworm species to grow in sewage sludge and the effect of temperature, dry solids and C/N ratio on vermicomposting of waste activated sludge, respectively. The two studies concluded that earthworms' optimal growth temperature falls within the range of 15°C to 25°C. Edwards (1988) studied the life cycle and optimal conditions for survival and growth of *E. fetida*, D. Veneta, E. Eugeniae, and P. excavates. The

study found that there were differences in terms of response(E. fetida (25°C), E. Eugeniae, and P. excavates(25°C) and tolerance (*E. fetida* 0°C - 35°C, E. eugeniae and P. excavates 9°C - 30°C) of worms to various temperatures. In extreme temperature conditions earthworms tend to hibernate and migrate to deeper layers of the windrow for protection. In terms of moisture content there exists a strong relationships in as far as growth rate of earthworms is concerned. Domínguez and Edwards (2011) reported that the optimum range of moisture contents for species such as *Eisenia fetida* and E. Andrei is between 50% and 90%.

Aleagha & Ebadi (2011).studied heavy metals bioaccumulation in the process of vermicomposting and found that earthworms, despite favoring more acid material, with a pH preference of 5.0, can survive the pH that is within 5-9. High levels of ammonia tend to kill earthworms as such for successful studies it is recommended that organic wastes containing high levels of ammonia be pre-treated either by precomposting or by leaching with water before use. Sinha et al., (2008) studied sewage treatment by vermifiltration with synchronous treatment of sludge by earthworms: a low-cost sustainable technology over conventional systems with potential for decentralization and found that earthworms' body works as a 'biofilter' that can remove the 5 days' BOD (BOD5) by over 90%, COD by 80–90%, total dissolved solids (TDS) by 90–92%, and the total suspended solids (TSS) by 90–95% from wastewater thereby improving the turbidity of wastewater and the hydraulic conductivity, and natural aeration. Singh et al., (2014) studied the potential of two epigeic earthworms (*Perionyx excavatus* and *Eisenia foetida*) for composting of crop residues (wheat straw and paddy straw) amended with farm yard manure and found that vermicomposting significantly increased total nitrogen (71 - 150%), phosphorus (49 %–116%) and potassium (26.3–142%).

Table 3: Categories of earthworms in temperate ecosystems

Groups	Leaf litter dwellers	Shallow burrowers	Deep burrowers
	Surface dwellers, epigeic species	Shallow burrowing, endogeic species, horizontal burrowers	Vertically burrowing, anectic species
Representatives			
Examples	› Redworm (*Eisenia fetida*) › European earthworm (*Lumbricus rubellus*)	› *Octolasion lacteum* › Common field worm (*Allolobophora caliginosa*)	› Nightcrawler (*Lumbricus terrestis*) › Black-headed worm (*Allolobophora longa*)
Colour	Brownish-red overall	Pale	Reddish-brown, head darker
Habitat	› In litter layers, especially in grasslands, forests, and compost › Rarely found in cropland due to lack of permanent litter layers	› Topsoil (5–40 cm), humic mineral soil › Mostly horizontal, unstable burrows › Juveniles are generally found in the upper layers in the tangle of roots	› All soil layers, 3–4 m deep › Spend their entire lives in vertical, stable dwelling tubes (Ø 8–11 mm) › Important in agricultural soils
Size	Small, generally 2–6 cm long	Small to up to 18 cm long	Generally large, 15–45 cm long
Feeding behavior	› Feed on small plant parts on the surface of the soil	› Feed on plant parts incorporated in the topsoil	› Pull large plant parts into their dwelling tubes
Reproduction	› Vigorous › 100 cocoons per year	› Limited › 8–12 cocoons per year	› Limited › 8–12 cocoons per year
Lifespan	Short, 1–2 years	Medium, 3–5 years	Long, 4–8 years
Sensitivity to light	Weak	High	Moderate

Source: Majlessi et al., (2012)

There is also documented information that explain that earthworms produce 'antibiotics' and kill the pathogenic organisms in their surroundings to safe levels through a process known as vermicomposting (a low cost technology system that involves a combined action of earthworms and microorganisms in the conversion of organic wastes into nutrient-rich humus called vermicompost) (Ninawe, 2008; Fox, Halpin & Rose, 2009; Eastman, 1999). Edwards & Fletcher, (1988) described vermicomposts as finely divided nonthermophilically stabilized mature fertilizer-like materials that is high in porosity, aeration, drainage and water-holding capacity and microbial activity that acts as a soil conditioner. Sinha et al., (2009) studied vermistabilization of sewage sludge (biosolids) by earthworms and found that while in the process of producing vermicompost, earthworms fed on and/or breakdown sludge, triggered microbial activity, raised the rates of mineralisation, reduced the pathogens (bacteria, fungi, nematodes and protozoa) to safe levels and ingested the heavy metals. In their study they noted that vermicomposting process significantly reduced volume of sludge from 1 m^3 of wet sludge (80% moisture) to 0.5 m^3 of vermicompost (30% moisture) and concluded that earthworms had

real potential of increasing the rate of aerobic decomposition, composting of organic matter, stabilizing the organic residues in the sludge and removing the harmful pathogens and heavy metals. Key advantages of this biological treatment are the reduction in sludge volume as well as pathogen inactivation (Furlong, 2013).

The findings by Sinha, et.al., (2009) were also echoed by Kalmath et al., (2012) who noted that activity of earthworms rapidly converted faecal sludge into finer structured humus that was richer in fertiliser content than faecal sludge itself to such an extent that calcium, nitrogen, phosphorus and potassium were three times, five times, seven times, and eleven times more, respectively. According to Eastman, (1999), while studying pathogen stabilization using vermicomposting, earthworms demonstrated a wide variety of benefits such as nearly odour-free process, destruction of pathogens, removal of heavy metals and toxic chemicals, mineralisation of nutrients from the sludge and formation of a more nutritive end-product rich in macro- and micronutrients, reduction of total organic carbon (TOC), lower C/N ratio of 20-30 and volatile solids from sludge, emission of low greenhouse gas (methane) and production of worm biomass: a nutritive meal for the fishery, poultry and dairy industries.

Hill & Baldwin (2012) conducted research with Source separating vermicompositing toilets (SSVCs) and indicated that SSCV outperformed mixed latrine microbial composting toilets (MLMCs) and provided a superior end-product. The SSCVs were recorded to have lower maintenance costs and risks compared to MLMCs, adequate worm density for pathogen destruction (0.03g worm/g-material), ability to reduce the pathogenic indicator *E. coli* to below WHO guideline limits at 200 CFU/g in neutral (pH7.4) conditions and produce a stable (60% Volatile solids) and mature end product (Hill & Baldwin, 2012). The work of Eastman et al., (2001) and Rodríguez-Canché et al., (2010) demonstrate the applicability of vermicomposting to sanitize multiple sludge types (both solids and liquid sludge). Eastman et al., (2001) seeded Class B biosolids with earthworms (*Eisenia fetida*) at a ratio of 1:1.5 wet weight earthworm biomass to biosolids ratio. The biosolids were heavily inoculated with four human –pathogen indicators, fecal coliforms, *salmonella spp*, enteric viruses and helminth ova. The results indicated that Eisenia fetida ably removed pathogens to below WHO standards after 144 hours. The work of Rodriguez-Canche et al (2010) evaluated the effectiveness of vermicomposting using Eisenia fetida to remove pathogens from septic tank sludge and found that a sanitized sludge, compliant with the Mexican standards, could be achieved after 60 days of treatment. Although the literature reviewed above inidicate that earthworms reduce pathogen concentrations in faecal sludge there is contradicting literature that showed that when worms

ingest a material, the number of bacteria and actinomycetes contained in the ingested material increases up to 1000-fold while passing through the gut. (Edwards et al., 1988; Morgan & Burrows; 1982, Sinha et al., 2009).

The literature reviewed, in the foregone paragraphs regarding the activity of earthworms, outline some features that are of interest to this study. Firstly, the worms have been portrayed to have the potential of reducing faecal sludge pathogens to safe levels. The reduction of faecal sludge pathogens to safe levels is key, in as far as the choice of sanitation systems to be deployed to emergency sites is concerned. The idea behind such a choice is to contain faecal oral related disease outbreaks common in emergency situations. Secondly, the literature has hinted that worms also have the potential of reducing the volume of faecal sludge to almost half its original size. Due to increased number of people that are often taken to emergency evacuation sites, faecal sludge production is done at a faster pace such that the installed sanitation systems get filled up so quickly which in turn increases the frequency of desludging for possible off-site treatment at designated sites. The more the sanitation systems are desludged, the faster the resources are depleted, and the more challenging the management of sanitation in emergency sites becomes. Therefore, if the earthworms could be used to treat faecal sludge on-site during emergency situations, it could take time before the sanitation systems get full, in so doing the resources that are spent on managing faecal sludge could be used for other equally important things. Lastly the worms are said to have the capacity of increasing the fertilizer content of faecal sludge. This character of worms could help improve agricultural productivity in emergency situations. However, despite the worms having such interesting characters, most of the studies investigated were done in faecal sludge that was treated off-site and not on-site. Secondly, the efficiency of earthworms in treating faecal sludge lacked locally tested scientific evidence. Thirdly, there is contradicting literature on the efficiency of the worms, in as far as pathogen reduction is concerned, suggesting the need for piloting Vermicompost toilet efficiency in treating faecal sludge and improving urban agriculture in emergency situations. These reasons made local emergency response organizations not to use them when treating faecal sludge on-site during emergency situations. Hence this study, using a pilot Vermicompost emergency sanitation toilet planted in Blantyre, Malawi, sought to provide evidence based information regarding the functionality and applicability of earthworms to treating on-site Faecal Sludge, by quantifying the process efficiency in terms of stabilisation, sanitization and useful by-product generation.

2.6.0. Terra Preta

Terra Preta do Indo is the anthropogenic black soil that was produced by ancient cultures of the Amazonian through the conversion of biowaste and faecal matter into long-term fertile soils (Michalovic, 2009; De Gisi et al., 2014). An exploration of an ancient Amazon/Brazil, exposes the benefits of an efficient handling of organic wastes. (Lehmann et al., 2003). It should be mentioned that the Amazonian dark earth (ADE) or Terra Preta (black earth) is a critical research topic for contemporary archaeology and historical ecology of Amazonia and potential strategies for sustainable development in tropical regions. The idea of Terra Petra Sanitation (TPS) is to produce fertile soils of human excreta. The production of fertile soils involves a series of activities such as urine diversion away from faeces, addition of a charcoal mixture, lactic-acid-fermentation and vermicomposting (De Gisi et al., 2014; Factura et al., 2010; Schmidt, 2013). The first step in TPS system is lactic acid fermentation (or lacto-fermentation) followed by a second step of vermicomposting (De Gisi et al., 2014) (see Figure 9 below).

In TPS systems urine and feces are collected in 2 separate compartments. Urine is collected in a jerry can and feces fall into a bucket that is placed airtight underneath the toilet bowl to allow for anaerobic conditions in the bucket. After each defecation, a mix of charcoal powder together with a finely cut wood source and some limestone/volcanic soil needs to be added to cover the feces. In addition, a few dashes of a lacto-bacilli containing microbial mix is added (Factura et al., 2010; Brown et al., 2012; De Gisi et al., 2014). Immediately after filling up of collection chamber Lacto-Fermented Sludge is vermicomposted in order to further reduce the concentrations of pathogens.

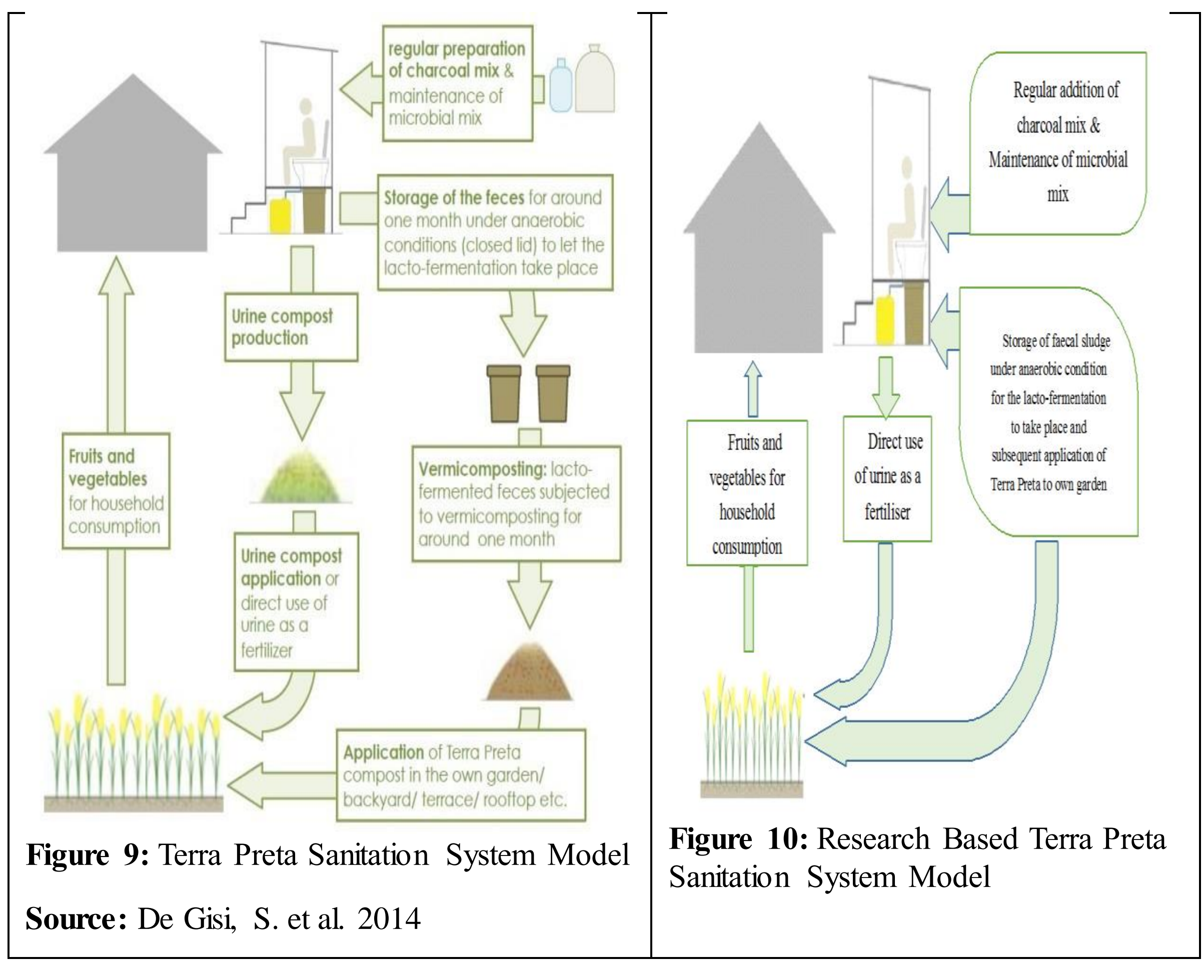


Figure 9: Terra Preta Sanitation System Model

Source: De Gisi, S. et al. 2014

Figure 10: Research Based Terra Preta Sanitation System Model

In this study TPS was preferred because of the following reasons; (1) it uses little or no water and the excreta is not discharged or buried in deep pits thus making it better than the conventional latrine-based systems commonly used in most emergency camps as it enables the hygienic recovery of faeces and urine for possible use as soil amendments (Mnkeni & Austin, 2009); (2) it produces no gas and odour in so doing reducing vector attraction in emergency camps; (3) it transforms the carbon and nutrients into the deep black, fertile and stable soil that can be utilized in agriculture; (4) no ventilation or external energy is required. (De Gisi et al., 2014); (5) urine can be collected separately and used to increase the production of green vegetables, maize, pumpkin and other valuable food items; (6) the Lacto-Fermented faecal sludge are far more easily handled and dehydrated, as they are not mixed with urine.

Integration of the anaerobic dry toilet and vermicomposting promises to be an ideal approach for managing wastes generated in emergency situations as it may make the product Terra Preta address problems of soil degradation and food insecurity common in many emergency camps. However, the challenge for emergency situations is how to make TPS sanitise, and stabilise faecal sludge and generate useful by-products, which are acceptable, affordable and sustainable for an early phase of an emergency as it takes too long to be completed. To address this

challenge, and for the purpose of this study, the final stage of vermicomposting was left out and replaced by the addition of Lactic Acid Bacteria (LAB) inoculum(see Figure 10 above) which according to Malambo, (2014), while carrying off-site batch experiments, successfully sanitized and stabilized faecal sludge. Section 2.7.0 below discusses the theory behind Lactic Acid Bacteria as regards faecal sludge treatment.

2.7.0. Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) are described as a heterogeneous group of Gram positive, non-motile, non-spore-forming rod-shaped or coccoid bacteria which do, through fermentation of carbohydrates, produce lactic acid as their major end product (Mahony & Sinderen, 2014; Khalid, 2011).These bacteria can be found in certain foods, in the mouth, in the gastrointestinal and urogenital tracts of humans and animals in soil, water, manure, sewage and fermented products such as meat, milk products, vegetables, beverages and bakery products (Holzapfel et al., 2001; Aukrust & Blom, 1992; Caplice & Fitzgerald, 1999; Harris et al., 1992; Gobbetti & Corsetti, 1997; Jay, 2000; Lonvaud, 2001; O'Sullivan, Ross & Hill. 2002).

LAB are classified into four main genera namely *Lactobacillus, Leuconostoc, Pediococcus and Streptococcus.* Recent taxonomic revisions have proposed several new genera, the additional ones being *aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Globicatella, Oenococcus, Tetragenococcus, Vagococcus, Weissella Carnobacterium, Lactococcus, Lactosphaera, Melissococcus, Tetragenococcus, Vagococcus , Microbacterium*, *Propionibacterium,* and *Bifidobacterium* (Jin et al., 2009; Ercolini et al., 2001; Jay, 2000; Holzapfel et al., 2001; Stiles & Holzapfel, 1997; Sneath & Holt, 2001; Gibson & Fuller, 2000).

LAB ferments carbohydrates into energy and lactic acid (Jay, 2000) either through homofermentative or heterofermentative metabolic pathways. Homofermentative pathway yields two lactates from one glucose molecule whereas the heterofermentative pathway transforms a glucose molecule into lactate, ethanol and carbon dioxide (Caplice & Fitzgerald, 1999; Jay, 2000; Kuipers et al., 2000; Derek et al., 2009). The succession of specific lactic acid bacteria during the natural fermentation is dependent on the chemical and physical environments such as pH and temperature (Harris et al., 1992.). Malambo (2014) while carrying out off-site faecal sludge treatment batch experiments, indicated that lactic acid bacteria effectively reduced pathogens at pH as low as 4.2 and temperature of 25°C. Sanders, Venema & Kok, (1999) pointed out that lactic acid, being a weak organic acid that is not charged at low

pH, can easily pass through the cell membrane of pathogens found in faecal sludge while in its protonated form thereby emphasizing that the antimicrobial effect of lactic acids on pathogens is dependent on the reduction of pH. It is reported in literature that the reduction of pH of lactic acid causes acidification of the cell cytoplasm and undissociation of the molecules which, being lipophilic, diffuse passively across the membrane thereby either collapsing the electrochemical proton gradient, or altering the cell membrane permeability which results in disruption of substrate transport systems (Snijders, Logtestijn, Mossel & Smulders, 1985; Kashket, 1987; Beasley, 2004). Gram et al., (2003) while studying utilization of various starter cultures in the production of Amasi, a Zimbabwean naturally fermented milk product, found that, at pH 4.2±0.12, LAB reduced numbers of *E. coli* in milk products. The findings led to the suggestion that LAB could be used as a sanitizing inoculum to the milk by-products. According to Abdel-Rahman et al., (2013) the optimal growth conditions of LAB vary depending on the producers, since these bacteria can grow in the pH range of 3.5-10.0 and temperatures of 5-45°C.

The Lab are also particularly unique from other bacterial species in that they are capable of surviving without iron (Helander et al., 1997), an essential element for the growth of all microorganisms. As a result of this unique capability of surviving without iron and also because of the production of lactic acid and other metabolites, particulary the heterofermentative LAB, which are antimicrobial in nature, LAB thus become perfect candidates whose characteristics can be used as sanitizing agents against pathogens found in faecal sludge.

Belfiore et al., (2007) suggests that unlike Gram positive bacteria, the inhibition of Gram negative enteric bacteria such as *E. coli* is especially problematic due to their resistance to antimicrobials. The reasons suggested for this resistance is the inability of the antimicrobials to penentrate the protective outer membrane of the Gram negative bacteria made up of glycerophospholipids and lipopolysaccharides (LPS) molecules. Several reports suggest that the synergetic use of chelators (outer membrane disrupting agents) and antimicrobials produced by LAB extends the antimicrobial spectrum to include the Gram negative bacteria as well (Helander et al., 1997; Belfiore et al., 2007). Helander et al., (1997) explains that the treatment which chelators such as ethylenediaminetetraacetate (EDTA) results in the removal by chelation of divalent cations from lipopolysaccharide molecules of the other membrane of the Gram negative bacteria thus permiabilising it and allowing for antimicrobial action. However, Alakomi et al., (2000), argues that lactic acid itself, is capable of permibilising Gram negative bacteria. He demonstrates that LPS release is substantially observed in a sample of Gram

negative bacterial species treated with lactic acid only, even more than in EDTA treated samples.

LAB also produces bacteriocins which are described as ribosomally synthesized antimicrobial peptides that are active against other bacteria, either of the same species (narrow spectrum), or across genera (broad spectrum) (Bowdish et al., 2005; Cotter et al., 2005). Bacteriocins are classified as antibiotics (Class I), the most documented and industrially exploited, nonantibiotics, small heat-stable peptides (Class II) and large heat-labile protein (Class III) (O'Sullivan et al., 2002). Caplice & Fitzgerald, 1999 reviewed the role of lactic acid bacteria in many fermentation processes of milk, meats, cereals and vegetables and the mechanisms of antibiosis with particular reference to bacteriocins. Their review indicated that bacteriocins, produced by LAB, ensure not only increased shelf life and microbiological safety of a food but also make some foods more digestible. While in their cationic property, bacteriocins kill target cells by causing disruption of the cytoplasmic membrane-potential through the formation of pores in the phospholipids bilayer (Montville, Winkowski & Ludescher, 1995) and/or leakage of cellular solutes that eventually leads to cell death (Arief, Jenie, Suryati, Ayuningtyas & Fuziawan, 2012). Having seen that LAB has the potential of inhibiting Gram negative bacteria, Malambo, (2014) conducted off-site faecal sludge treatment batch experiments using LAB and found that LAB could reduce the concentrations of faecal sludge pathogens to safe levels.

The literature reviewed in the foregone paragraphs, regarding the antimicrobial actions of both bacteriocins and lactic acid, the products of LAB, is of interest to this study. Firstly, LAB have been portrayed to have the potential of reducing faecal sludge pathogens to safe levels. The reduction of faecal sludge pathogens to safe levels is the key issue, in as far as the choice of sanitation systems to be deployed to emergency sites is concerned. The idea behind such a choice is to contain faecal oral related disease outbreaks common in emergency situations. However, despite the LAB having such interesting characters, the study by Malambo, (2014) found that antimicrobial actions of both bacteriocins and lactic acid could successfully sanitize faecal sludge were done off-site and not on-site. Hence this study sought to determine if the procedure for lactic acid treatment of faecal sludge established through off-site small scale experiments could be up scaled to on-site treatment in a pit latrine. In addition, the research sought to determine the safety and usefulness of by-products generated from the separation of urine and Lacto-Fermented Sludge for possible sustainable agriculture in emergencies.

Chapter Summary

In conclusion, this chapter has outlined the literature behind emergency situations, faecal sludge, anaerobic digestion, vermicomposting, Terra Preta and lactic acid bacteria. It has been shown that the trend of emergency situations is increasing and yet containment of faecal sludge still remains a challenge. The chapter has also outlined that there is no documented evidence on the performance of the three studied sanitation systems in as far as their on-site pit sludge treatment applicability and functionality is concerned. Outlined in the next chapter, is the methodology used towards the actualisation of the proposed faecal sludge treatment options.

CHAPTER THREE
RESEARCH METHODOLOGY

3.0 Introduction

This chapter discusses the methods that were used in carrying out this study. It explains and discusses the Literature that has been reviewed, the research philosophy, approach, strategy, data collection and analysis methods, sample Size, experimental site, sampling site preparation, sample Collection, ethical considerations and the limitations experienced during the study. It aims to give an outline of how the whole research project was carried out in order to achieve the stated objectives. This ensured analysis of findings and drawing of conclusions in an objective manner. It should be mentioned that sample collection and analysis was done concurrently with course work.

3.1 Literature Review

Literature search involved a collection of information regarding the following specific areas;

- General information on emergency situations,
- Information on faecal sludge management in emergency situations,
- Specific information on pathogens found in faecal sludge,
- Specific information on Lactic Acid Bacterium, Terra Preta, Urine Diversion, Anaerobic Digestion and Vermicomposting not necessarily in this order.

3.2 Research Philosophy

Positivism Research Philosophy was adopted in this study. Information was acquired through observations and experiments. It adopted scientific methods as a means of generating knowledge by using highly structured methodology that aimed at facilitating replication and quantification of observations, leading themselves to statistical analysis. The data was collected and analysed and the results were interpreted to draw conclusions and some recommendations based on achievement or non-achievement of the research objectives.

3.3 Research Approach

Deductive Research Approach was adopted in this study. The researcher begun with thinking up a theory about a research project, then narrowed down into more specific hypothesis that was tested. The hypothesis directed the data collection and/or literature review in the research project.

3.4 Research Strategy

An Experimental Research Strategy was used in this study. The researcher objectively observed phenomena that occurred in a strictly controlled situation where one or more variables were varied and others kept constant.

3.5 Sample Size

Block randomization was used in determining the selected days of the study period (May, 2014 to December, 2014). The study period was divided by the number of seasons (winter and summer) that fell within it. In total, 24 samples, 13 in winter and 11 in summer, were collected from the Anaerobic Digester and Vermicompost toilets while 19 samples, 13 in winter and 6 in summer were collected from the Terra Preta toilet.

3.6 Experimental site

The Anaerobic Digester was placed at Aquaid Life Line orphanage village (GPS coordinates: Latitude 15.62285°S, Longitude 35.055672°E). The Terra Preta (TP) and Vermicompost toilets were built at Crown Ministries in Chigumula (GPS coordinates: Latitude 15.882023°S, Longitude 35.066946°E). All the three sanitation systems were installed in Blantyre, Malawi. The specific locations are shown in Figures 11, 12, 13 and 14 below.

3.7 Sampling site Preparation

3.7.1 Anaerobic Digester

This sanitation system was placed above ground and then connected to a pour flush toilet (see Figure 15 below. The toilet was installed at an orphanage that had 500 orphans. However, the Anaerobic Digester only served 200 orphans and an average of 37 orphans patronized the toilet on daily basis. The amount of water that was used for both individual and general toilet cleaning was 55.7L per day. To enhance biogas production, 10% w/v of cow dung was added to the Anaerobic Digester. In addition to the cow dung, kitchen waste was put into the Anaerobic Digester to boost up the concentration of carbohydrates, lipids and fats which are central to the anaerobic digestion stage called hydrolysis. In winter temperatures were very low and in order to raise the temperature, a plastic paper cover was placed on top of the pasteurisation tubes. Faecal sludge was retained in the whole system for 38 days before being discharged into the three $1m^3$ soil made donuts. The 38 faecal sludge retention time included 36 days of keeping sludge in the digestion bag and 2 days in the pasteurisation tubes. The digestate flowed from the digestate output tank by gravity through the solar Pasteurization tube into the three $1m^3$ soil made donuts. Mixing of sludge in the digester was done manually using a specially designed roller.

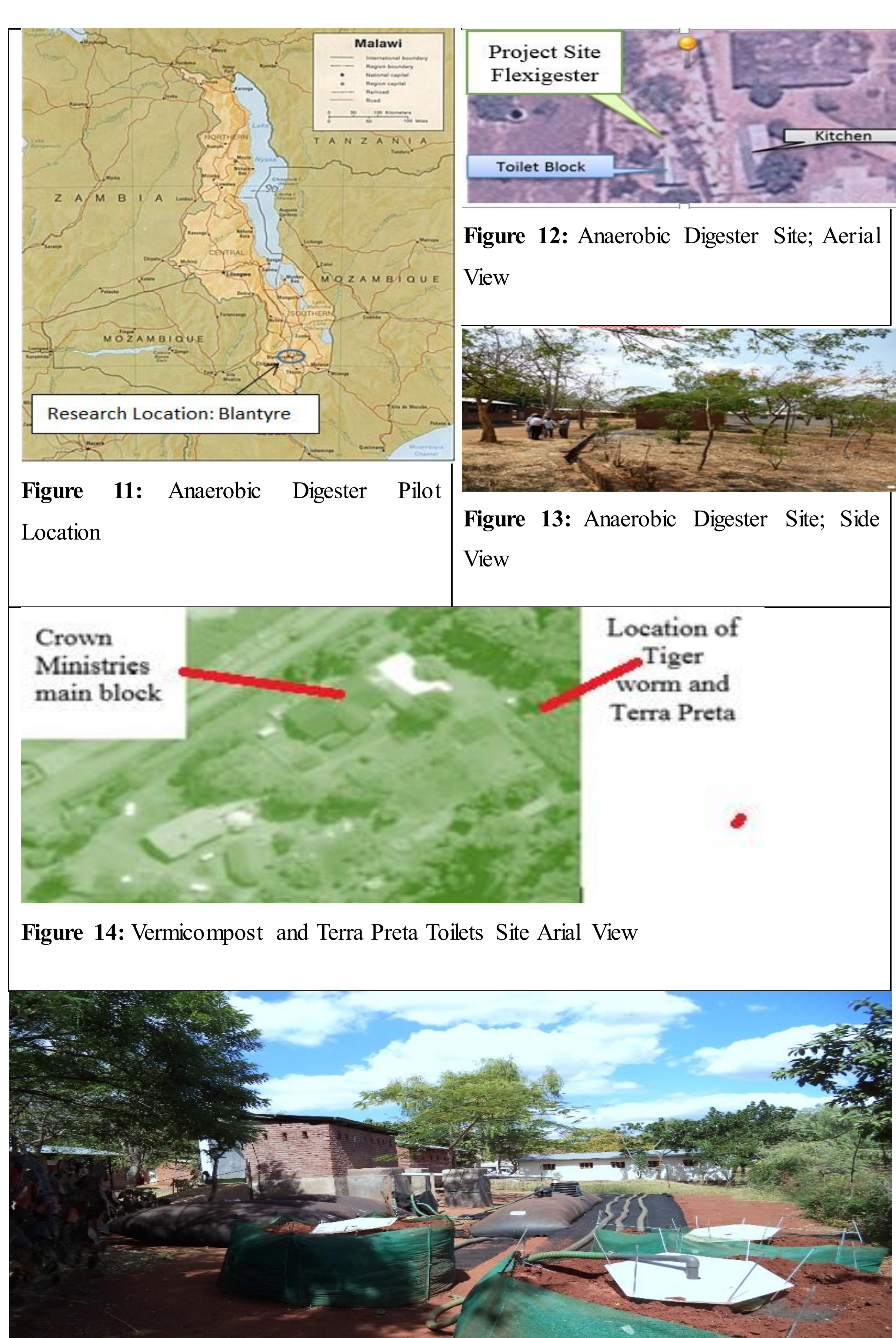


Figure 11: Anaerobic Digester Pilot Location

Figure 12: Anaerobic Digester Site; Aerial View

Figure 13: Anaerobic Digester Site; Side View

Figure 14: Vermicompost and Terra Preta Toilets Site Arial View

Figure 15: Anaerobic Digester Connected to Pour Flush Toilet

3.7.2 Terra Preta

Physically, the TP toilet was raised and consisted of four elements: (1) toilet superstructure, which provided shelter for the user and the toilet itself; (2) a urine diversion seat placed on a slab; (3) a 50L urine collection Drum and (4) a 200L faecal sludge collection Drum (see Figures 16, 17, 18, 19 and 20 below). This toilet involved the addition of Lactic Acid Bacteria inoculum. Lactic Acid Bateria (LAB) inoculum was prepared using the procedure found in Malambo, (2014). 15L pasteurized milk was fermented by mixing with 30ml of Yakult (a readily available probiotic diary product) and 1.5g of cane molasses. The fermentation process was done at room temperature till the pH reached 4.2. The fermentation process almost took 48 hours. The fermented LAB inoculum was added to a 200L drum before the toilet was in use.

Approximately 100ml of Charcoal, made from corncobs, bamboo and firewood, was added to the Lacto-Fermented Sludge inside the 200L drum after each defecation using a 100ml plastic cup in order to increase the carbon content of faecal sludge (O'Grady & Rush, 2007). The charcoal was prepared by using a specially made pyrolysis Stove(see Figure 21) which works anaerobically under the following parameters: temperature range of 350–800 °C, heating rate less than 10 °C min^{-1}, atmospheric pressure, hours-days as residence time and char as primary product (Brewer & Brown, 2012 as cited by De Gisi et al., 2014). In addition to charcoal, 2g of cane molasses were also added soon after defecation. The Terra Preta toilet diverted urine from faecal sludge using a specially designed pedestal (see Figures 17 and 18), via a 20mm internal diameter plastic pipe which was connected to a 50L plastic drum (see Figures 20). Faecal sludge fell into a 200L plastic drum, placed airtight underneath the toilet seat's slab (see Figure 19). Both urine and faecal sludge were kept under anaerobic conditions in the drums. The toilet seat's lid was left covered and only made open during use. In order to make TPS sanitise, and stabilise faecal sludge and generate useful by-products, which are acceptable, affordable and sustainable for an early stage of an emergency, and for the purpose of this study, the final stage of vermicomposting was left out and replaced by the addition of LAB inoculum which according to Malambo, (2014)**,** while carrying off-site batch experiments, successfully sanitised and stabilised faecal sludge.

Figure 16: TPS Toilet Structure

Figure 17: TPS Pedestal Inside

Figure 18: TPS Toilet Inside

Figure 19: TPS Urine Diverting Pipe Network

Figure 20: TPS Urine and Faecal Sludge

Figure 21: TPS Toilet Model Figure

3.7.3 Vermicompost Toilet

The Vermicompost Toilet comprised of a raised on-site toilet that had a superstructure, a protruded 110cm x 100cm x 70cm deep manhole covered with a rectangular cast iron lid (see Figures 22 and 24). The superstructure and the manhole were connected using a bucket led pour flush system. The 60g Tiger Worm (*Eisenia foetida*) earthworms were introduced in the manhole before the toilet was in use. The other contents of the manhole included, from bottom going upwards, stones, sand, wood chips and avocado pair peels. The layout of these manhole contents were as shown in Figure 23 below.

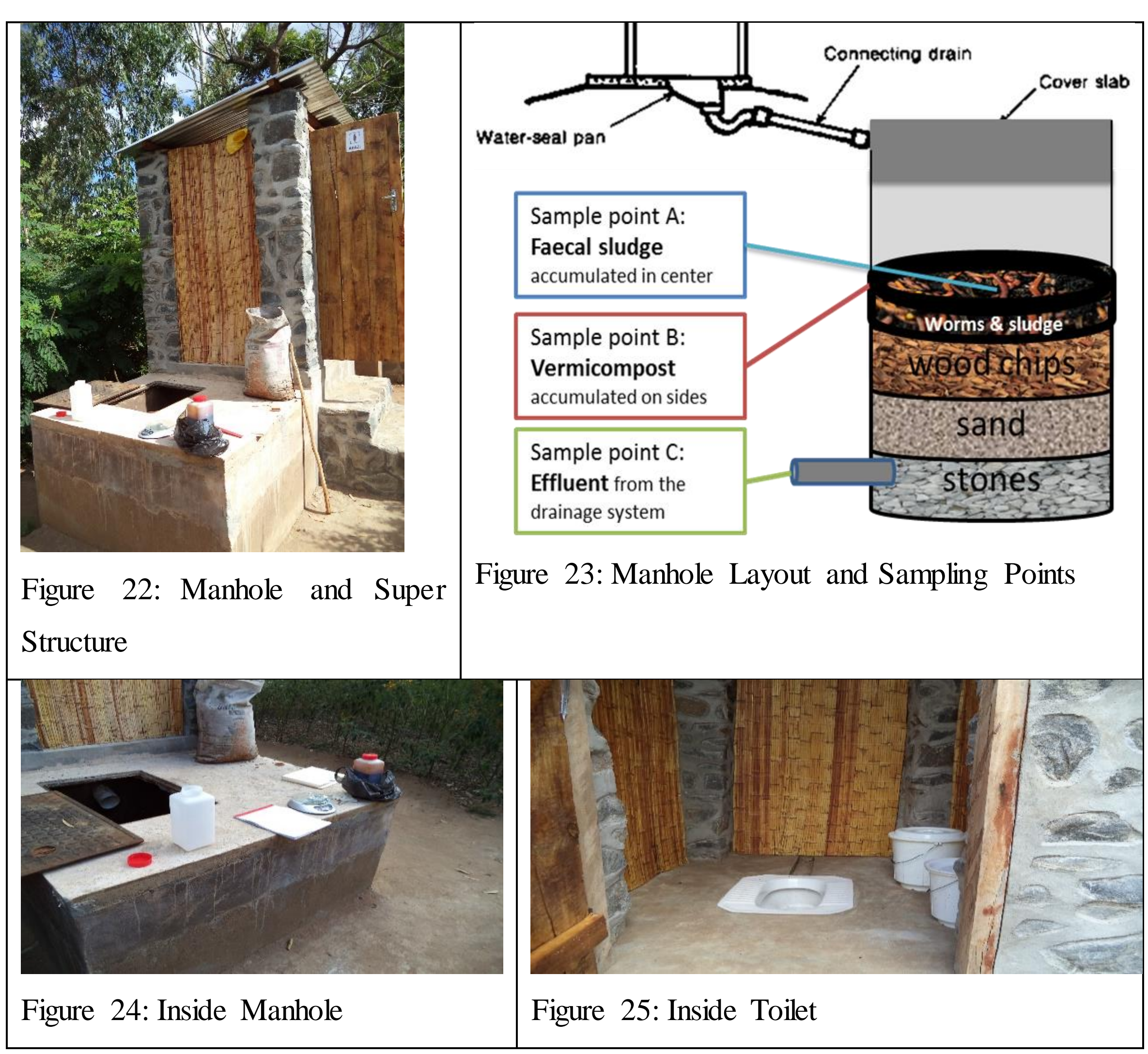


Figure 22: Manhole and Super Structure

Figure 23: Manhole Layout and Sampling Points

Figure 24: Inside Manhole

Figure 25: Inside Toilet

3.8 Sample Collection

3.8.1 Anaerobic Digester

Grab samples, in 1 litre sterilized sampling bottles, were taken from three strategically chosen sampling points (see Figure 26 below) and then transferred from the sampling site (Aquaid Lifeline) to Soche Pollution Control Laboratory for analysis. Before the samples were taken,

faecal sludge was mixed using a manually driven roller in order to ensure uniformity of the samples collected.

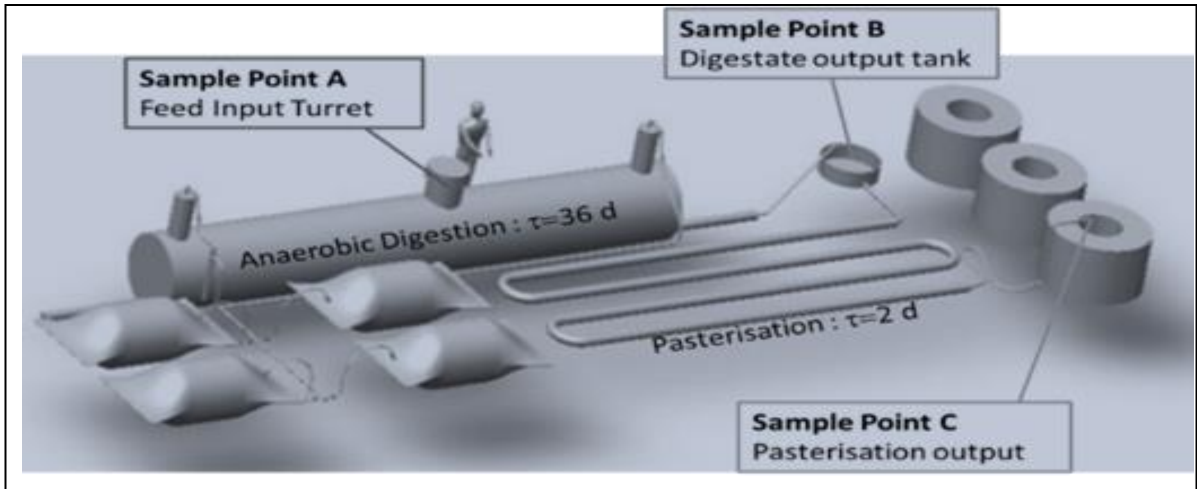


Figure 26: Anaerobic Digester 3D Showing Sampling Points

Source: Emergency Sanitation Project: Malawi Field Testing Proposal (2014)

3.8.2 Terra Preta

Grab samples, in 1 litre sterilized plastic sampling bottles, were taken from both urine and Lacto-Fermented Sludge, as indicated in Figure 21, and then transferred from the sampling site (Crown Ministries) to Soche Pollution Control Laboratory for analysis. Unlike the Anaerobic Digester a manually driven stick was used for mixing both urine and sludge. The stick used for mixing both urine and sludge was sterilized by washing, first, with clean water, then chlorinated water and finally clean water before switching between urine and sludge in order to avoid cross contamination. The mixing was done before the samples were taken to ensure uniformity of the samples collected.

3.8.3 Vermicompost

Randomly selected grab samples, within the selected days of the study period (May, 2014 to December, 2014), of fresh faeces and vermicast were taken from sampling points A and B of the manhole (see Figure 23) using 60ml sterilised plastic sampling bottles, and then transferred from the sampling site (Crown Ministries) to Soche Pollution Control Laboratory for analysis. No samples were collected from sampling point C as there was no effluent from the manhole.

3.9 Sample Analysis

3.9.1 Microbial Analysis

Microbial Analysis of *Escherichia coli* (*E. coli*) and Total Coliforms were analysed to determining the feasibility of deploying the Anaerobic Digester, Vermicompost and Terra Preta toilets as a sanitation system that will treat faecal sludge to meet Malawi Standards of pathogen free sludge during challenging conditions common in emergency situations and to examine possibility of treating on-site faecal sludge using Lactic Acid Bacteria inoculum. Most of the analysis for *E. coli* and Total Coliforms were done within six hours from the time samples were taken and those that were not analysed within the six hours were refrigerated at 4°C till the next day. Chromocult Coliform Agar was used for the enumeration of Total Coliforms and *Escherichia coli* in all samples from the three sanitation systems according to the APHA 2012 standard method SM-9020 indicated as 3 and 4 in Table 4 below. The detailed experimental procedure for APHA 2012 standard method SM-9020 is outlined section 3.9.1.1 below. Chromocult Coliform Agar was used because it is a selective and differential chromogenic culture medium which contains Tergitol 7, as an inhibitor of Gram-positive bacteria, which has no negative effect on the growth of the targeted coliforms/ *E. coli*, the character which makes it an ideal medium for the detection of coliforms/ *E. coli* in wastewater.

Table 4: Analyzed parameters and the respective methods for analysis

No.	Parameter	Method
1	pH	Potentiometric SM-4500-H+
2	Temperature (^{o}C)	SM-2550B
3	*Escherichia coli*	Pour plate SM-9020
4	Total Coliforms	Pour plate SM-9020
5	Total Ammonia Nitrogen (TAN)	Indophenol blue method Hach LR/HR TNTN tube test
6	Chemical Oxygen Demand (COD)	Hach tube test HR Oxidation by Potassium dichromate

3.9.1.1 APHA 2012 standard method SM-9020 Enumeration of *E. coli* and Total Colony Forming Units

Step 1: Preparing the plates

NOTE: Plates need to be poured approximately 5 days in advance of plating the samples.

EQUIPMENT	CONSUMABLES
• Electric Balance • Water Bath • Measuring cylinder • 2L Volumetric Flask • 250ml Volumetric Flask • Spatula	• Chromocult Agar • Petri dishes • Cotton wool • Aluminum foil • Weight tray • Distilled water

Method

1. Measure 1.5L in measuring cylinder
2. Put 1.5L of distilled water into the 2L Volumetric Flask
3. Fill the Water Bath with distilled water to a level that ensures that the 1.5L of water in the flask is covered
4. Pre-heat water bath
5. Using the Electric Balance weigh 39.8g Chromocult Agar (26.5g per L of distilled water)
6. Add the 39.8g of Chromocult Agar to the 1.5L of distilled water in the 2L Flask and stir until dissolved
7. Seal the flask with cotton wool and cover the tip with Aluminum foil
8. Place the 2L flask into the water bath
9. Boil in water bath for 1h (start timer only once boiled)
10. Cool medium to 45 – 50 deg. C (just cool enough to be able to touch)
11. Heat the top of the 2Lvolumetric flask using the Bunsen burner flame
12. Pour the heated agar solution into the 250ml volumetric flask
13. Heat the top of the 250ml volumetric flask (Constantly heat after every 3 plates) – work close to the flame to prevent cross contamination
14. Using the 250ml volumetric flask pour the sterile plates, pour just enough to cover the surface and cover with the lid a soon as possible after pouring
15. Wash flask immediately to avoid the chromocult agar solidifying
16. Leave the plates for 24hours to cool, then turn the plates upside down to avoid contamination and moisture ruining the agar
17. Leave the plates for a total of 5 days before using to ensure that they are dry.

Step 2: Preparing Dilution Water

Materials required.

EQUIPMENT	CONSUMABLES
• Electric Balance • Autoclave • Measuring cylinder • 1L Volumetric Flask • Spatula	• Sodium Chloride • Cotton wool • Aluminum foil • Weight tray • Distilled water

Method

1. Using an electronic balance, weigh 8g of Sodium Chloride with a spatula and a weight tray
2. Place the Sodium Chloride in a 1L volumetric flask
3. Measure 1L of distilled water using a measuring cylinder
4. Pour 1L of distilled water into the 1L volume flask
5. Mix to dissolve the Sodium Chloride solution
6. Place cotton wool into the top of the 1L volumetric Flask to ensure that it is sealed
7. Cover the top of the 1L volumetric flask with aluminum foil
8. Pour distilled water into the autoclave – ensure that the water comes just above the bottom plate
9. Place the 1L volumetric flask into the autoclave
10. Turn on autoclave at the wall and once the temperature has reached 121 deg. C, time for 15minutes (i.e. autoclave the solution at 121 ^{0}C, 1KPa pressure for 15 minutes)
    a. Note: Time for Autoclaving is volume dependent – large volumes will require more time
11. Turn off after 15 minutes. Wait until the temperature is 85 degrees before opening the Autoclave (0 pressure)

Step 3: Preparing Dilution Samples

For a single sample set, prepare 10, 100, 1000, 10,000 and 100,000 dilutions

Materials.

EQUIPMENT	CONSUMABLES
• Electric Balance • Spatula • Vortex Mixer • Test tube rack • 4 test-tubes per sample • 250ml volumetric Flask • Pen • Pipette (1 - 50ml) • Bunsen burner	• Syringe • Pipette tips (1 – 5ml) • Test tubes caps or (Cotton wool and aluminum) • Sterilized (Autoclave) Sodium chloride solution • Sludge sample • 60ml plastic sampling container

Method

1. Label each of the dilutions (e.g. Sample 1 10x, Sample 1 100x, Sample 1:1000x etc.)
2. Pour 250ml of the sterile sodium chloride solution from the 1L volumetric flask into the 250ml volumetric flask
3. Using the syringe, extract 9ml of sterile sodium chloride solution and insert into a test tube
4. Cover the test tube with either a cap or (insert cotton wool and cover with aluminum foil)
5. Repeat for the required number of test tubes (approximately 5 per sample)
6. Sterilize the test tubes in the autoclave for 15 minutes at 121 deg. C and 1kPa

For 10x Dilution

1. Place the 60ml sampling bottle on the electronic balance
2. Take 10x Dilution sample, mix using vortex mixer and heat on the flame
3. Using the spatula for solid samples and syringes for liquid samples to place 1g or 1ml of the sample into the sample bottle
4. Add 9ml of the sterile sodium chloride solution
5. Mix well using the vortex mixer

For 100x Dilution

1. Label the test tube 100x Dilution
2. Take 10x Dilution sample, mix using vortex mixer and heat on the flame
3. Turn on gas and create flame on Bunsen burner
4. Set the Pipette to 1ml and extract 1ml from the 10x Dilution sample bottle
5. Remove cap/or (cotton wool and aluminum foil) from the test tubes
6. Using the Bunsen burner flame, heat the top of the 100x test tube
7. Insert the 1ml 10x Dilution sample into the 100x Dilution test tube

8. Replace cap (or cotton wool and aluminum foil) and mix well using the vortex mixer

For 1000x Dilution

1. Label the test tube 1000x Dilution
2. Take 100x Dilution test tube, mix using vortex mixer and heat on the flame
3. Extract 1ml from the 100x Dilution test tube
4. Remove cap/or (cotton wool and aluminum foil) from the test tubes
5. Using the Bunsen burner flame, heat the top of the 1000x test tube
6. Insert the 1ml 100x Dilution sample into the 1000x Dilution test tube
7. Replace cap (or cotton wool and aluminum foil) and mix well using the vortex mixer

For 10,000x Dilution

1. Label the test tube 10,000x Dilution
2. Take 1000x Dilution sample, mix using vortex mixer and heat on the flame
3. Extract 1ml from the 1000x Dilution test tube
4. Remove cap/or (cotton wool and aluminum foil) from the test tubes
5. Using the Bunsen burner flame, heat the top of the 10,000x test tube
6. Insert the 1ml 1000x Dilution sample into the 10,000x Dilution test tube
7. Replace cap (or cotton wool and aluminum foil) and mix well using the vortex mixer

Step 4: Plating Samples

Label all test tubes and plates in duplicates or triplicates.

Batch	Sample ID	Dilution Rage	Duplicate ID	Date

Label dishes on the lid

Example of plate label: Sample 1, 10x Dilution Plate A, 3rd February

EQUIPMENT	CONSUMABLES
• Vortex Mixer • Test tube rack • Test tubes with Dilution • Pen • Pipette (1 - 50ml) • Bunsen burner • Glass Spreader • Incubator	• Pipette tips (1 – 5ml) • Labels • Matches • 70% Alcohol solution in Petri Dish • Waste bin for used pipette tips

Method

1. Place the first test tube on the Vortex Mixer and mix thoroughly
2. Remove the cap and heat the top of the tube with the Bunsen burner flame
3. Using the pipette, extract 0.1ml of the diluted sample
4. Remove the Petri dish lid and Pipette the 0.1ml sample into the centre of the dish, repeat for the no. of duplicates
5. Soak the tip of the glass spreader in alcohol
6. Pass the tip of the glass spreader in the flame of the Bunsen burner and wait for all the alcohol to combust and cool down
7. Using the glass spreader ensure that the sample is equally spread over the plate using a zigzag pattern
8. Sterilize the glass spreader using the flame and then placing the spreader in alcohol
9. Turning the plates upside down
10. Incubate the plates (upside down) for 24 hours at 37 deg. C.
11. Count the colonies
    a. Blue /Aqua – *Salmonella*
    b. Purple – E-coli
    c. Pink – Coliforms

Colour of colony	Organism
Dark – blue to violet	*Escherichia coli*
Salmon to red	Enterobacter, Citrobacter, Klebsiella
Light – blue	*Salmoenella*
Colourless	Other Enterobactriaceae

*1 CFU = colony forming units=colonies
2 From CFU to Bacteria per 100ml: A 1000*1/d
A=CFU.
d= dilution (example: dilution 1/10, d=0.1)
*Removal efficiency (%): (In –Out)/In * 100

3.9.2 Chemical analysis

Chemical analysis of Total Ammonia Nitrogen (TAN) was conducted in order to assess the efficiency of Anaerobic Digester, Vermicompost and Terra Preta toilets in converting fresh faecal sludge on-site into safe and useful by-products for possible sustainable agriculture during emergency situations while pH, Temperature, and Chemical Oxygen Demand (COD) were done to evaluate the Anaerobic Digester, Vermicompost and Terra Preta toilets for their suitability in stabilising faecal sludge during challenging conditions common in emergency situations. Temperature and pH were measured in situ immediately after collecting the samples from the above mentioned sampling points. Total Ammonia Nitrogen and Chemical Oxygen Demand were analysed according to the APHA 2012 Indophenol blue method 10023-Hach LR/HR TNT tube test and TNT 822-Hach tube test HR Oxidation by Potassium dichromate, respectively, indicated as 5 and 6 in Table 4 below. The detailed experimental procedure for methods 5 and 6 in Table 4 is outlined in sections 3.9.2.1a and b and 3.9.2.2, respectively. Section 3.9.2.3 outlines a list of consumables and non-consumables used in this study. Total Ammonia Nitrogen and Chemical Oxygen Demand were analysed in triplicates at Soche Pollution Control Laboratory.

3.9.2.1a Determination of Total Ammonia Nitrogen (Low Range Vials)

Method 10023

NITROGEN, AMMONIA, Low Range, Test 'N Tube (0 to 2.50 mg/L NH_3-N)

Salicylate Method* **For water, wastewater, and seawater**

1. Enter the stored program number for low range nitrogen, ammonia Test 'N Tube.

Press: **PRGM**

The display will show:

PRGM ?

2. Press: **66 ENTER**

The display will show **mg/L, NH3-N** and the **ZERO** icon.

Note: For alternate forms (NH_3), press the **CONC** *key.*

3. Insert the COD/TNT Adapter into the cell holder by rotating the adapter until it drops into place. Then push down to fully insert it.

Note: For increased performance, a diffuser band covers the light path holes on the adapter. Do not remove the diffuser band.

4. Remove the caps from 2 AmVer Diluent Reagent vials. Add 2 mL of sample to one vial (the sample). Add 2 mL of deionized water to the other vial (the blank).

Note: Adjust the pH of stored samples before analysis. See Interferences on page 247.

5. Using a funnel, add the contents of one Ammonia Salicylate Reagent Powder Pillow for 5 mL sample to each vial.

6. Using a funnel, add the contents of one Ammonia Cyanurate Reagent Powder Pillow for 5 mL sample to each vial.

7. Cap the vials tightly and shake thoroughly to dissolve the powder.

Note: A green color will develop if ammonia is present.

8. Press:

TIMER ENTER

A 20-minute reaction period will begin.

9. Wipe the outside of the vials with a towel. After the timer beeps, place the blank into the adapter. Tightly cover the vial with the instrument cap.

Note: Wipe with a damp cloth followed by a dry one to remove fingerprints and other marks.

10. Press: **ZERO**

The cursor will move to the right, then the display will show:

0.00 mg/L NH3-N

11. Place the prepared sample in the adapter. Push straight down on the top of the vial until it seats solidly into the adapter.

Note: Do not move the vial from side to side as this can cause errors.

12. Tightly cover the sample cell with the instrument cap.

Press: **READ**

The cursor will move the right, then the resu in mg/L ammonia nitrogen will be displayed.

Note: Standard Adjust m be performed using a prepared standard (see Standard Adjust (Adjust the Standard Curve) on page 47).

Sampling and Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a one liter sample. Preserve the sample by reducing the pH to 2 or less with hydrochloric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5.0 N sodium hydroxide. Correct the test result for volume additions. See *Correcting for Volume Additions on page 22* for more information.

Accuracy Check

Standard Additions Method

a) Snap the neck off a Nitrogen, Ammonia Ampule Standard Solution, 50 mg/L NH_3-N.

b) Use the TenSette Pipet to add 0.1, 0.2, and 0.3 mL of standard to three 25 mL samples. Mix thoroughly.

c) Analyze each sample as described above. The nitrogen concentration should increase 0.20 mg/L for each 0.1 mL of standard added.

d) If these increases do not occur, see *Standard Additions, Section 1*, for more information.

Standard Solution Method

To check accuracy, use a 1.0 mg/L Nitrogen, Ammonia Standard Solution listed under Optional Reagents. Or, dilute 1 mL of solution from a
50 mg/L Ampule Standard for Nitrogen, Ammonia to 50 mL with deionized water using a 50-mL volumetric flask.

Method Performance

Precision

In a single laboratory, using a standard solution of 1.0 mg/L ammonia nitrogen and two representative lots of reagent with the instrument, a single operator obtained a standard deviation of ±0.02 mg/L NH_3-N.

Estimated Detection Limit

The estimated detection limit for program 66 is 0.08 mg/L NH_3-N. For more information on the estimated detection limit, see *Section 1*.

Interferences

Interfering Substance	Interference Level and Treatment
Calcium	2500 mg/L as $CaCO_3$
Iron	1. Determine the amount of iron present in the sample following one of the total iron procedures. 2. Add the same iron concentration to the deionized water in step 4. The interference will then be successfully blanked out.
Magnesium	5000 mg/L as $CaCO_3$
Nitrite	30 mg/L as NO_2^--N
Nitrate	250 mg/L as NO_3^--N
Orthophosphate	250 mg/L as PO_4^{3-}-P
pH	Acidic or basic samples should be adjusted to about pH 7. Use 1 N Sodium Hydroxide Standard Solution for acidic samples and 1 N Hydrochloric Acid Standard Solution for basic samples.
Sulfate	300 mg/L as SO_4^{2-}

Interfering Substance	Interference Level and Treatment
Sulfide	1. Measure about 350 mL of sample in a 500 mL erlenmeyer flask. 2. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix. 3. Filter the sample through a folded filter paper. 4. Use the filtered solution in step 4.
Other	Less common interferences such as **hydrazine** and **glycine** will cause intensified colors in the prepared sample. **Turbidity** and **color** will give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set. See Optional Apparatus at the end of this procedure.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to [illegible] 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow colc from the excess reagent present to give a final green-colored solution.

Pollution Prevention And Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >**11** to prevent release of hydrogen cyanide gas. See *Section 3* for further information in proper disposal of these materials.

REQUIRED REAGENTS

Cat. N

AmVer Reagent Set for Nitrogen, Ammonia, Low Range TNT (25 tests).................... 26045–

Includes: (1) 23952-66, (1) 23954-66, (1) 272-42, (50) AmVer Low Range Vials

Description	Quantity Required Per Test	Unit	Cat. N
AmVer Diluent Reagent, Low Range Test 'N Tube	2 vials	50/pkg	
Salicylate Reagent Powder Pillows, 5 mL sample	2 pillows	50/pkg	23952-(
Cyanurate Reagent Powder Pillows, 5 mL sample	2 pillows	50/pkg	23954-(

3.9.2.1b Determination of Total Ammonia Nitrogen (High Range Vials)

Method 10031

NITROGEN, AMMONIA, High Range, Test 'N Tube

(0 to 50 mg/L NH_3-N)

Salicylate Method*

For water, wastewater, and seawater

1. Enter the stored program number for nitrogen, ammonia, high range Test 'N Tube (NH_3-N) method.

Press: **PRGM**

The display will show:

PRGM ?

2. Press: **67 ENTER**

The display will show **mg/L, NH3-N** and the **ZERO** icon.

Note: For alternate forms (NH_3), press the **CONC** *key.*

Note: For proof of accuracy, use a 10-mg/L nitrogen, ammonia standard in place of the sample.

3. Insert the COD/TNT Adapter into the cell holder by rotating the adapter until it drops into place. Then push down to fully insert it.

Note: For increased performance, a diffuser band covers the light path holes on the adapter. Do not remove the diffuser band.

4. Remove the caps from 2 AmVer Diluent Reagent High Range Vials. Add 0.1 mL of sample to one vial (the sample). Add 0.1 mL of deionized water to the other (the blank).

5. Add the contents of 1 Ammonia Salicylate Reagent Powder Pillow for 5 mL Sample to each vial.

6. Add the contents of 1 Ammonia Cyanurate Reagent Powder Pillow for 5 mL Sample to each vial.

7. Cap the vials tightly and shake thoroughly to dissolve the powder.

Note: A green color will develop if ammonia is present.

8. Press:

TIMER ENTER

A 20-minute reaction period will begin.

9. Clean the outside of the vial with a towel. After the timer beeps, place the blank into the vial adapter. Tightly cover the vial with the instrument cap.

Note: Wipe with a damp cloth and follow with a dry one to remove fingerprints and other marks.

10. Press: **ZERO**

The cursor will move to the right, then the display will show:

0 mg/L NH3-N

11. Place the prepared sample in the adapter.

Push straight down on the top of the vial until it seats solidly into the adapter.

Note: Do not move the vial from side to side as this can cause errors.

12. Tightly cover the vial with the instrument cap.

13. Press: **READ**

The cursor will move to the right, then the result in mg/L NH_3-N will be displayed.

Note: Standard Adjust may be performed using a prepared standard (see Standard Adjust in Section 1).

Sampling and Storage

Collect samples in clean plastic or glass bottles. Best results are obtained with immediate analysis. If chlorine is known to be present, add one drop of 0.1 N sodium thiosulfate for each 0.3 mg/L Cl_2 in a one liter sample. Preserve the sample by reducing the pH to 2 or less with hydrochloric acid (at least 2 mL). Store at 4 °C (39 °F) or less. Preserved samples may be stored up to 28 days. Before analysis, warm samples to room temperature and neutralize with 5.0 N sodium hydroxide. Correct the test result for volume additions.

Accuracy Check

Standard Additions Method

a) Snap the top off an Ammonia PourRite Ampule Standard, 150 mg/L NH_3-N.

b) Use the TenSette Pipet to add 0.2, 0.4 and 0.6 mL of standard to three 25-mL samples. Swirl to mix.

c) Analyze each sample as described above. The ammonia concentration should increase approximately 1.2 mg/L NH_3-N for each 0.2 mL of standard added.

d) If these increases do not occur, see *Standard Additions* in *Section 1* for more information.

Standard Solution Method

To check accuracy, use a 10 or 50 mg/L Nitrogen, Ammonia Standard Solution or use a Nitrogen, Ammonia Voluette Ampule Standard, 50 mg/L.

Method Performance

Precision

In a single laboratory, using a standard solution of 50 mg/L ammonia nitrogen (NH_3-N) and two representative lots of reagent with the instrument, a single operator obtained a standard deviation of ± 5 mg/L NH_3-N.

Estimated Detection Limit

The estimated detection limit for program 67 is 1 mg/L NH_3-N. For more information on the estimated detection limit, see *Section 1*.

NITROGEN, AMMONIA, High Range, Test 'N Tube, continued

Interferences

The following ions may interfere when present in concentrations exceeding those listed below.

In some lab environments, airborne cross contamination of the blank is possible. Complete preparation of the blank before opening or handling any samples or standards to avoid transfer of ammonia. If sample or standard containers have already been open, move to a separate area of the lab to prepare the blank.

Substance	Concentration and Suggested Treatments
Acidic or basic samples	Adjust to approximately pH 7. Use 1 N Sodium Hydroxide Standard Solution for acidic samples and 1 N Hydrochloric Acid Standard Solution for basic samples.
Calcium	50,000 mg/L as $CaCO_3$
Glycine, hydrazine	Will cause intensified colors in the prepared sample.
Magnesium	300,000 mg/L as $CaCO_3$
Iron	Eliminate iron interference as follows: 1. Determine the amount of iron present in the sample using one of the total iron procedures. 2. Add the same iron concentration to the deionized water in step 4. 3. The interference will then be successfully blanked out.
Nitrite	600 mg/L as NO_2^--N
Nitrate	5,000 mg/L as NO_3^--N
Orthophosphate	5,000 mg/L as PO_4^{3-}-P
Sulfate	6,000 mg/L as SO_4^{2-}
Sulfide	Sulfide will intensify the color. Eliminate sulfide interference as follows: 1. Measure about 350 mL of sample in a 500 mL Erlenmeyer flask. 2. Add the contents of one Sulfide Inhibitor Reagent Powder Pillow. Swirl to mix. 3. Filter the sample through folded filter paper. Use the filtered solution in step 4.
Turbidity and color	Give erroneous high values. Samples with severe interferences require distillation. Hach recommends the distillation procedure using the Hach General Purpose Distillation Set.

Summary of Method

Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a green-colored solution.

Safety

Good safety habits and laboratory techniques should be used throughout the procedure. Consult the *Material Safety Data Sheets* for information specific to the reagents used. For additional information, refer to *Section 3*.

Pollution Prevention And Waste Management

The ammonia salicylate reagent contains sodium nitroferricyanide. Cyanide solutions are regulated as hazardous wastes by the Federal RCRA. Collect cyanide solutions for disposal as reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section 3* for further information in proper disposal of these materials.

REQUIRED REAGENTS

AmVer™ Reagent Set for Nitrogen, Ammonia, High Range, TNT (25 tests) 26069-45

Includes: (1) 23952-66, (1) 23954-66, (1) 272-42, *(50) AmVer HR Vials

Description	Quantity Required Per Test	Unit	Cat. No.
AmVer™ HR Reagent Test 'N Tube™ Vials	2 vials	50/pkg	*
Ammonia Salicylate Reagent Powder Pillows	2 pillows	50/pkg	23952-66
Ammonia Cyanurate Reagent Powder Pillows	2 pillows	50/pkg	23954-66

REQUIRED APPARATUS

Description	Quantity Required Per Test	Unit	Cat. No.
COD/TNT Adapter	1	each	48464-00
Pipet, TenSette®, 0-1 mL	1	each	19700-01
Pipet Tips for 19700-01	varies	50/pkg	21856-96
Test Tube Rack	1-3	each	18641-00
Funnel, micro (for reagent addition)	1	each	25843-35

NITROGEN, AMMONIA, High Range, Test 'N Tube, continued

OPTIONAL REAGENTS

Description	Quantity Required Per Test	Unit	Cat. No.
Nitrogen, Ammonia Standard Solution, 50 mg/L NH_3-N		500 mL	14791-50
Nitrogen, Ammonia Standard Solution, 10 mg/L NH_3-N		500 mL	153-49
Ammonia Standard Solution, PourRite™ ampules, 150 mg/L NH_3-N, 2 mL		20/pkg	21284-20
Hydrochloric Acid, ACS		500 mL	134-49
Sodium Hydroxide Standard Solution, 5.0 N		50 mL	2450-26
Sodium Hydroxide Standard Solution, 1.0 N		100 mL	1045-32
Sodium Thiosulfate Standard Solution, 0.1 N		100 mL	323-32
Sulfide Inhibitor Powder Pillows		100/pkg	2418-99
Sulfuric Acid, 1.00 N		100 mL MDB	1270-32
Wastewater Influent Standard, Inorganic (NH_3-N, NO_3, PO_4, COD, SO_4, TOC)		500 mL	28331-49
Water, deionized		4 L	272-56

OPTIONAL APPARATUS

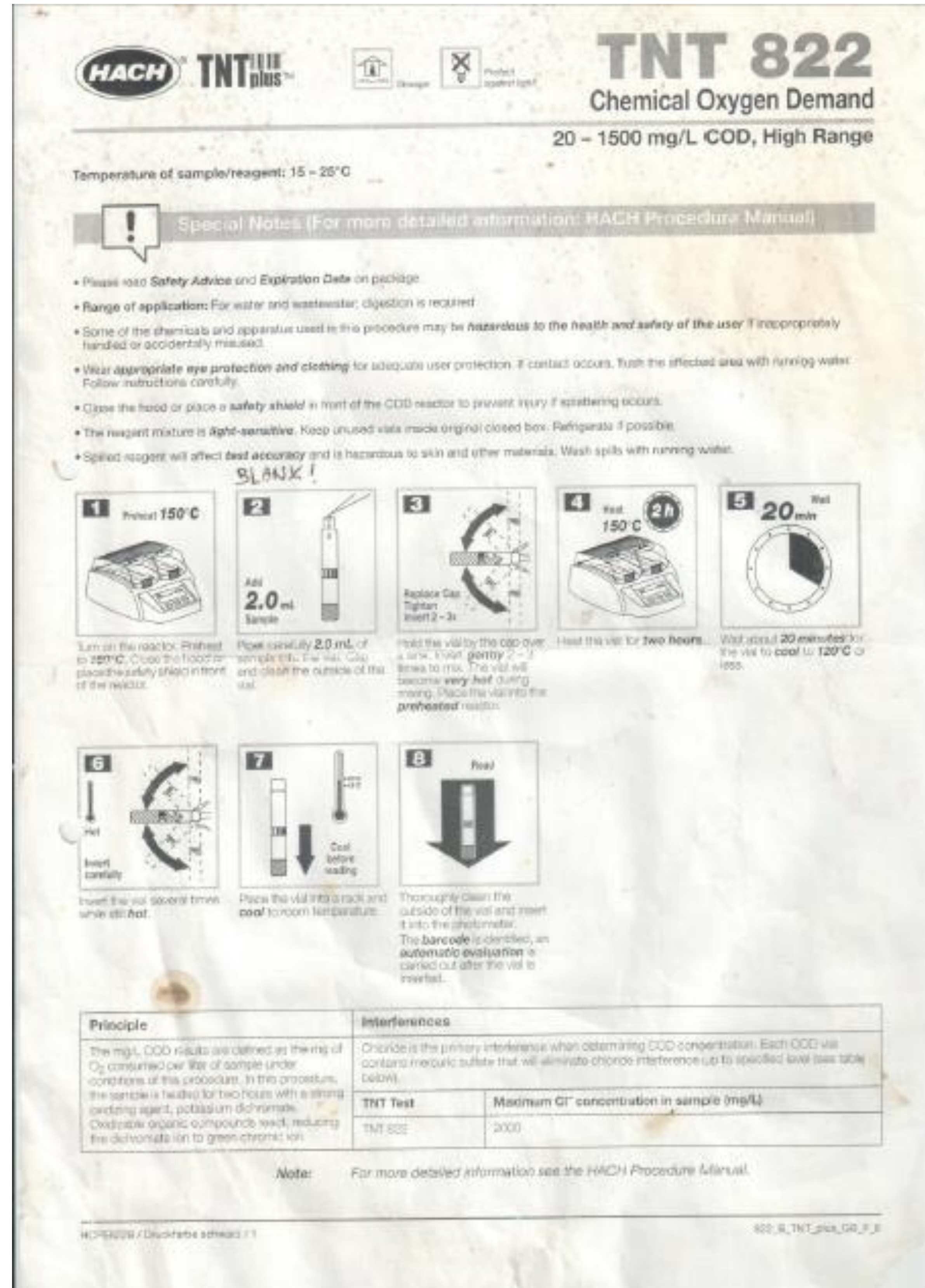
Description	Unit	Cat. No.
Cylinder, 25 mL, graduated, mixing	each	20886-40
Distillation Apparatus Set, general purpose	each	22653-00
Heater and Support Apparatus (for distillation), 115 VAC	each	22744-00
Heater and Support Apparatus (for distillation), 230 VAC	each	22744-02
Filter Paper, folded	100/pkg	1894-57
Flask, Erlenmeyer, 500 mL	each	505-49
Funnel, analytical (for filtering)	each	1083-68
Jack, laboratory (use with distillation apparatus)	each	22743-00
pH Indicator Paper, 1 to 11 pH	5 rolls/pkg	391-33
Pipet Tips, for 19700-01 TenSette Pipet	1000/pkg	21856-28
PourRite™ Ampule Breaker	each	24846-00
Sample Cell, 10-20-25 mL, w/cap	6/pkg	24019-06

For Technical Assistance, Price and Ordering

In the U.S.A.—Call 800-227-4224

Outside the U.S.A.—Contact the Hach office or distributor serving you.

3.9.2.2 Determination of Chemical Oxygen Demand (High Range Vials)



HACH TNTplus

TNT 822

Chemical Oxygen Demand

20 – 1500 mg/L COD, High Range

Temperature of sample/reagent: 15 – 25°C

Special Notes (For more detailed information: HACH Procedure Manual)

- Please read **Safety Advice** and **Expiration Date** on package.
- **Range of application:** For water and wastewater; digestion is required.
- Some of the chemicals and apparatus used in this procedure may be ***hazardous to the health and safety of the user*** if inappropriately handled or accidentally misused.
- Wear ***appropriate eye protection and clothing*** for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.
- Close the hood or place a ***safety shield*** in front of the COD reactor to prevent injury if spattering occurs.
- The reagent mixture is ***light-sensitive***. Keep unused vials inside original closed box. Refrigerate if possible.
- Spilled reagent will affect ***test accuracy*** and is hazardous to skin and other materials. Wash spills with running water.

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1. Turn on the reactor. Preheat to 150°C. Close the hood or place the safety shield in front of the reactor.
2. Pipet carefully **2.0 mL** of sample into the vial. Cap and clean the outside of the vial.
3. Hold the vial by the cap over a sink. Invert **gently** 2 – 3 times to mix. The vial will become ***very hot*** during mixing. Place the vial into the ***preheated*** reactor.
4. Heat the vial for **two hours**.
5. Wait about ***20 minutes*** for the vial to **cool** to **120°C** or less.
6. Invert the vial several times while still ***hot***.
7. Place the vial into a rack and **cool** to room temperature.
8. Thoroughly clean the outside of the vial and insert it into the photometer. The **barcode** is identified, an **automatic evaluation** is carried out after the vial is inserted.

Principle	Interferences	
The mg/L COD results are defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion to green chromic ion.	Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to specified level (see table below).	
	TNT Test	**Maximum Cl^- concentration in sample (mg/L)**
	TNT 822	2000

Note: *For more detailed information see the HACH Procedure Manual.*

HCPE[illegible] / Druckfarbe schwarz / 1

822_[illegible]_TNT_plus_[illegible]

3.9.2.3 Consumables and Non-consumables

CONSUMABLES	NON-CONSUMABLES
TNTplus™COD Digestion Reagent Vial	Vortex Mixer
Low Range 3 to 150mg/l COD	Test Tubes
High Range, 20 to 1500mg/l COD	Test Tube Rack
TNT plus™ Reactor/Cuvette Tubes	Pipette (1-5ml)
AmVer ™ High Range Ammonia 0-50mg/l N Reagent Set 26069-45	Gas Burner
Ammonia Salcylate Reagent	Glass Spreader
Ammonia cyanurate Reagent	Incubator
Deionized (Demineralised) Water, 100ml	Analytical Balance
Distilled Water	Water Bath
Oxoid CM1046 Brilliance ™ E. Coli/Coliform Selective Medium	2L Volumetric Flask
Pipette tips (1-5ml)	Spatula
Labels	Measuring cylinder
Matches	Autoclave
70% alcohol solution	pH Meter
Petri dishes	COD Heating Unit RD 125
Cotton Wool	Lovibond Photometer MD 600
Aluminum Foil	200L Plastic Drums (2)
Sodium Chloride	50L Plastic Drums (2)
Weigh tray	Pyorolysis Stove
Syringe	Gas Cylinder
Reference Buffer	
Molasses	
Milk	
Yalkut	
Fuel Wood	

3.10 Data Analysis

Data analysis was done by conducting an independent samples t-test using SPSS. One-way analysis of variance was used to check if there was a statistically significant difference in the average concentrations of the test parameters of the samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets and the safe average concentrations outlined in Malawi standard 539, (2013). The significance level used in this research was 0.05 implying that the researcher rejected the null hypothesis that the average concentrations of the test parameters of samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets were equal to average Malawi

standard concentrations when *p*-value was less than or equal to 0.05 and failed to reject the null hypothesis when *p*-value was greater than 0.05. The means for each analysis were calculated and graphs were produced using excel.

Data collected on both *E. coli* and Total Coliforms were statistically analysed to test the hypothesis that the use of Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as an on-site faecal sludge sanitation systems during emergency situations could reduce faecal sludge pathogens to acceptable Malawi standards levels. One-way analysis of variance was used to determine if there was a statistically significant reduction in the mean *E. coli* and Total Coliforms of the samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets and the set mean values in Malawi standards.

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Data collected on pH, Temperature, and Chemical Oxygen Demand was also statistically analysed to test the hypothesis that the use of Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as an on-site faecal sludge sanitation systems during emergency situations could stabilize faecal sludge to acceptable Malawi standards. One-way analysis of variance was used to evaluate if there was a statistically significant difference in the mean pH, Temperature and Chemical Oxygen Demand of the samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets and the set mean values in Malawi standards. The significance level used was 0.05 implying that the researcher rejected the null hypothesis that the mean pH, Temperature and Chemical Oxygen Demand of the samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets were equal to average Malawi standard pH, Temperature and Chemical Oxygen Demand when *p*-value was less than or equal to 0.05 and failed to reject the null hypothesis when *p*-value was greater than 0.05. Data on Chemical Oxygen Demand was analysed in triplicates, with averages of the triplicates analysed reported.

Data collected on Total Ammonia Nitrogen was statistically analysed to test the hypothesis that the use of Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as an on-site faecal sludge sanitation systems during emergency situations could harvest faecal sludge by-products that are rich in fertilizer for possible agriculture in emergency situations. One-way analysis of variance was used to assess if there was a statistically significant difference in the mean Total Ammonia Nitrogen of the samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets and the set Malawi standards Total Ammonia Nitrogen. The significance level used was 0.05 implying that the researcher

rejected the null hypothesis that the mean Total Ammonia Nitrogen of the samples taken from the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets were equal to average Malawi standard Total Ammonia Nitrogen when p-value was less than or equal to 0.05 and failed to reject the null hypothesis when p-value was greater than 0.05. Data on Total Ammonia Nitrogen was analysed in triplicates, with averages of the triplicates analysed reported.

3.11 Limitations of the study

This research had the following limitations;

- This was a funded research as such the scope of work was predetermined by the funding agency both in terms of time and scope.
- The study was conducted in an abstract emergency situation when it should have been conducted in the actual emergency situation.
- Although the study is expected to have a bearing on decision making regarding emergency sanitation response of different organizations, the results of this study may ably apply for Blantyre but may not ably apply to other districts in Malawi as the study was only conducted in Blantyre.

3.12 Ethical Considerations

The goal of ethics in this research was to ensure that none of the participants was harmed or suffered adverse consequences from the research activities (Shahnazarian, Hagemann, Aburto & Rose, 2013). As such only the number of visits of the people that used the toilets were recorded. Furthermore, all users of the toilets were discouraged from writing their name on the attendance and comments register that was placed behind the door of the toilet. Participants' voluntary consent was sought before commencement of the study.

Chapter Summary

In conclusion, this chapter has outlined the methodology used during the study. It has been stated that APHA 2012 methods were used for analysing samples for pH, Temperature (^{o}C), *Escherichia coli,* Total Coliforms, Total Ammonia Nitrogen (TAN), and Chemical Oxygen Demand (COD). The chapter has also addressed areas such as data analysis, limitations of the study, and ethical considerations. Outlined in the next chapter, are the results and their respective discussions.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 INTRODUCTION

This chapter presents and discusses the findings of the study. It describes the characteristics of the Anaerobic Digester, Vermicompost and Terra Preta toilets in as far as faecal sludge pathogen reduction to safe levels, converting fresh faecal sludge on-site into safe and useful by-products for possible sustainable agriculture and stabilising faecal sludge during challenging conditions, common in emergency situations, is concerned. Due to the nature of this study the objectives of the study will guide the presentation and discussion of the results obtained in this research but not necessarily in the order the objectives are presented in chapter one.

4.1.0 PATHOGEN REDUCTION

The study sought to determine the feasibility of deploying the Anaerobic Digester, Vermicompost and Terra Preta toilets as a sanitation system that could treat faecal sludge to meet Malawi Standards of pathogen free sludge during challenging conditions common in emergency situations. As outlined in chapter three determination of faecal sludge sanitisation was done by analysing the concentration of *E. coli* and Total Colony Forming Units from the samples collected from the three sanitation systems mentioned above. Outlined in Table 5, is a summary of the pathogen concentrations found in this the study.

4.1.1 Anaerobic Digester

The results for *E. coli* indicate that the average winter *E. coli* were 3.04 x 10^{6} CFU/100ml, 1.57 x 10^{6} CFU/100ml and 7.96 x 10^{5} CFU/100ml for the feeding point, digestate point and pasteurization tubes, respectively. The *E. coli* results for samples collected in summer indicate that the average summer *E. coli* were 1.13 x 10^{6} CFU/100ml , 1.02 x 10^{6} CFU/100ml, and <1000 CFU/100ml for the feeding point, digestate point and pasteurization tubes, respectively. Additionally, the results for Total Colony Forming Units (TCFU) indicate that the average winter TCFU were 5.60 x 10^{6} CFU/100ml, 2.46 x 10^{6} CFU/100ml and 9.76 x 10^{5} CFU/100ml for the feeding point, digestate point and pasteurization tubes, respectively. The TCFU results for samples collected in summer indicate that the average summer TCFU were 4.45 x10^{6} CFU/100ml , 1.02 x10^{6} CFU/100ml, and <1000 CFU/100ml for the feeding point, digestate point and pasteurization tubes, respectively. The results also indicated that, in both summer and winter, there was a reduction in the concentration of pathogens as faecal sludge passed

Table 5: Pathogen Reduction Analysis Results

	Winter			**Summer**		
	N	**Mean**		**N**	**Mean**	
	Statistic	**Statistic**	**Optimal Limit**	**Statistic**	**Statistic**	**Optimal Limit**
Pathogen Reduction						
Anaerobic Digester Toilet						
E. coli (CFU/100ml)						
Feed	13	3.04×10^{6}	**<10³**	11	1.13×10^{6}	**<10³**
Digestate	13	1.57×10^{6}		11	1.02×10^{6}	
Pasteurised	13	7.96×10^{5}		11	$<10^{3}$	
Total Coliforms (CFU/100ml)						
Feed	13	5.60×10^{6}	**<10³**	11	4.45×10^{6}	**<10³**
Digestate	13	2.46×10^{6}		11	1.02×10^{6}	
Pasteurised	13	9.76×10^{5}		11	$<10^{3}$	
Terra Preta Toilet						
E. coli (CFU/100ml)						
Lacto-Fermented Sludge	13	1.05×10^{7}	**<10³**	11	4.1×10^{1}	**<10³**
Urine	13	$<10^{3}$		11	$<10^{3}$	
Total Coliforms (CFU/100ml)						
Lacto-Fermented Sludge	13	2.18×10^{7}	**<10³**	11	9.0×10^{0}	**<10³**
Urine	13	$<10^{3}$		11	$<10^{3}$	
Vermicompost Toilet						
E. coli (CFU/100ml)						
Faecal Sludge	13	2.38×10^{7}	**<10³**	11	4.67×10^{6}	**<10³**
Vermicompost	13	7.72×10^{5}		11	9.42×10^{5}	
Total Coliforms (CFU/100ml)						
Faecal Sludge		3.26×10^{7}	**<10³**		5.33×10^{8}	**<10³**
Vermicompost		1.53×10^{7}			5.33×10^{7}	

from the feeding point, digestate point and pasteurization tubes of the Anaerobic Digester. However, despite the results indicating a reduction in the concentration of pathogens as faecal sludge passed from the feeding point, digestate point and pasteurization tubes, sanitization of the effluent faecal sludge was only achieved in summer(see Figure 27 below) where the

pathogen concentration was below the Malawi Standard limit of <1000 CFU/100ml (Malawi Standard 359, 2013).

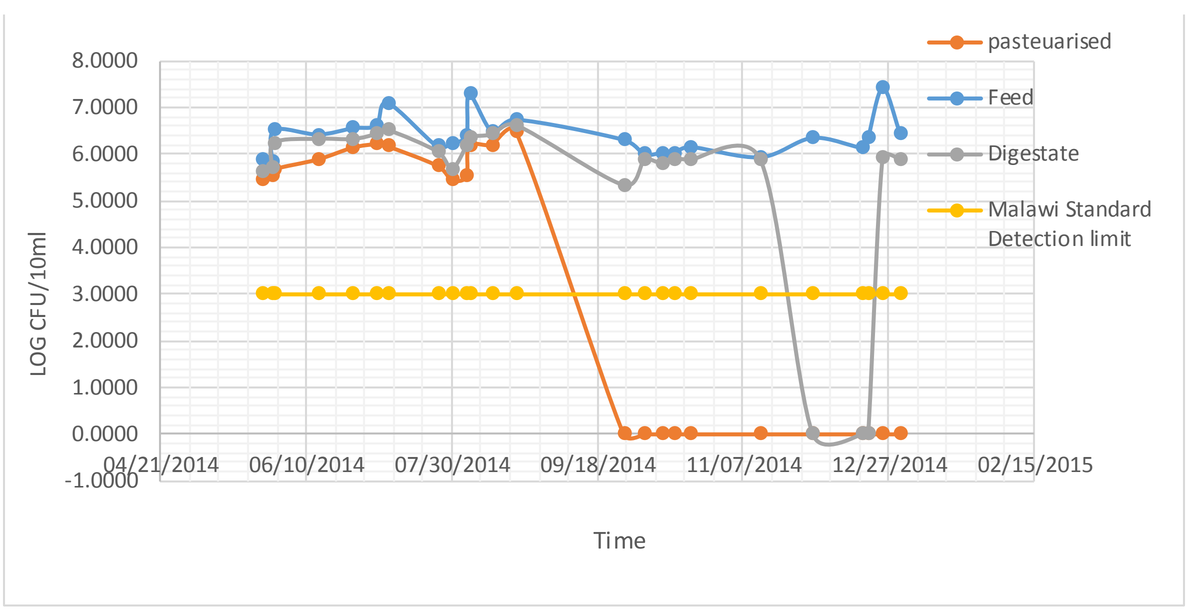

Figure 27: **Graph of *E. coli* against Time**

Unlike the rest of the observed research period, the digestate achieved Malawi Standard pathogen reduction guideline of <1000CFU/100ml from mid-November, 2014 to late-December, 2014 (see Figure 28 below) probably due to reduced number of people using the toilet as observed in the summarised toilet use register (see Figure 29 below), or longer faecal sludge retention times, as during this period the school's orphanage had closed for Christmas Holiday or the raised temperatures effectively pastreurised faecal sludge in the digester.

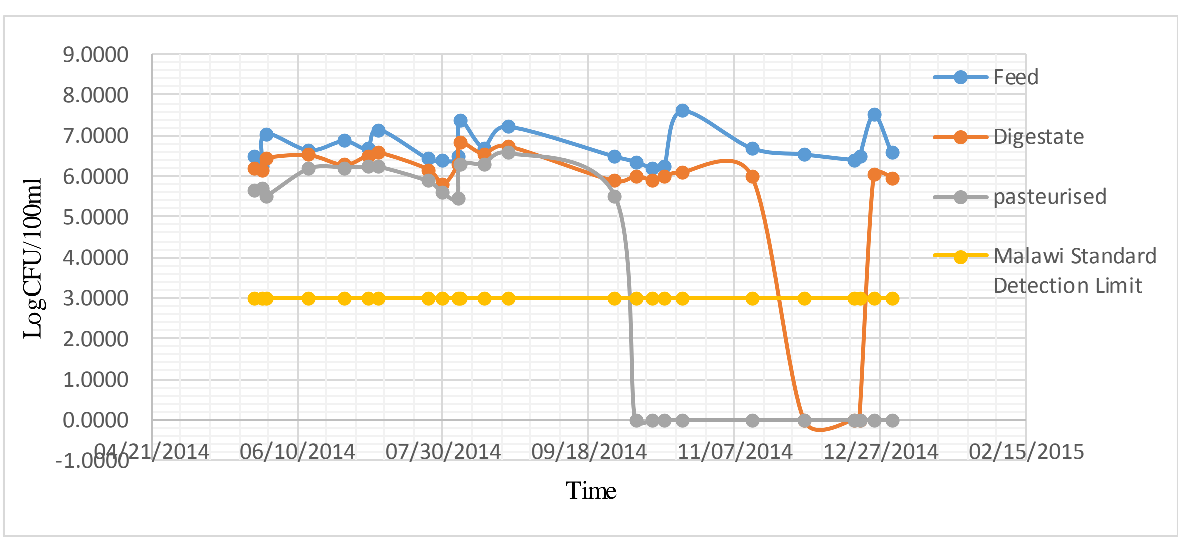

Figure 28: Graph of Total Coliforms against Time

The poor pathogen reduction in winter could be attributed to either the Anaerobic Digester's limited capacity of raising temperatures to optimal themophilic level of 42°C to 75°C, despite putting a cover over the pasteurization tubes. The observed lower than normal thermophilic

temperature ranges that were registered by the Anaerobic Digester resulted in reduction of both process stability and dewatering properties of the fermented sludge that required large amounts of energy for heating which unfortunately the digester failed to provide (Labatut & Gooch (2014). The poor pathogen reduction by the Anaerobic Digester further suggests the need for improving it from being a single stage digester to being a multi-stage digester as this could allow for reactions such as hydrolysis, acidogenesis and acetogenesis take place in the first reactor while methanogenesis takes place in the second reactor (Verma, 2002; Massimo & Giordano, 2014). The observations under this study suggest that the digester operated as a single stage high solid digester that required longer retention time, more complex expensive equipment and removal of heavy fractions or the scum layer during the digestion which did not form part of the design parameters of the digester placed under observation in this study. Further possible reasons to the poor pathogen reduction of the Anaerobic Digester could be attributed to either the possible existence of a very high C/N ratio that resulted in the rapid consumption of nitrogen by the methanogens as they tried to meet their protein requirement thus making the methanogens no longer react on the left over carbon content in the material or a possible existence of a very low C/N ratio that resulted in the liberation and accumulation of nitrogen in the form of ammonia which in turn increased the pH value of the digestrer material that affected the population of the methanogenic bacteria (RISE-AT, 1998). The other possible explanation on poor pathogen reduction could be that faecal sludge had high concentrations of insoluble complex organic polymers, such as carbohydrates, proteins, lipids, and phosphorylated organics which limited the rate of reactions in the digestion chamber. The observed poor pathogen reduction could also suggest that the Anaerobic Digester was fed above its sustainable organic loadng rate (OLR) and this resulted in accumulation of volatile fatty acids (VFA) which inhibited the digestion process of faecal sludge (RISE-AT, 1998).

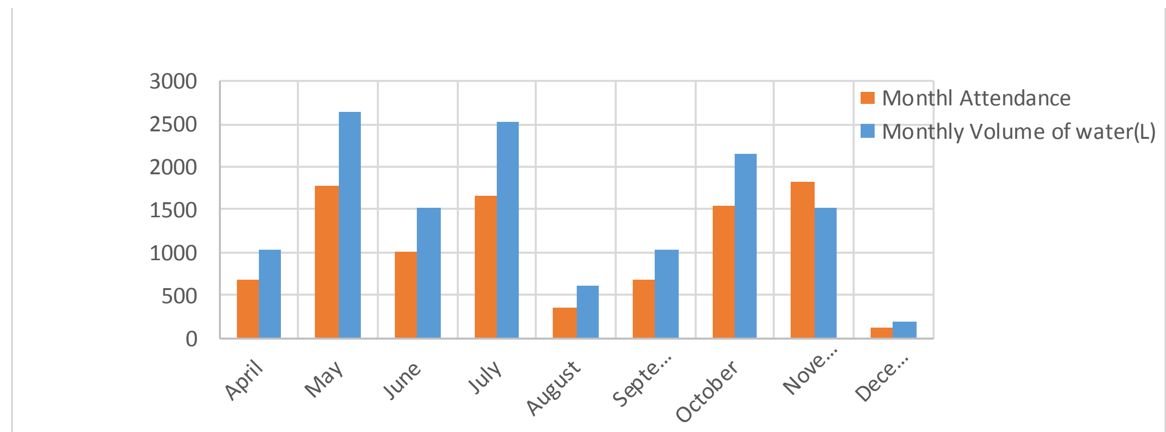

Figure 29: Summarized Anaerobic Digester Usage Register

4.1.2 Terra Preta

Microbial analysis results for Lacto-Fermented Sludge indicated that the average winter *E. coli* was 1.05 x10^7 CFU/100ml while the average summer *E. coli* was 4.1 x 10^1 CFU/100ml. Analysis results for Lacto-Fermented Sludge indicated that the average winter Total Colony Forming Units (TCFU) was 2.18 x 10^7 CFU/100ml while the average summer *E. coli* was 4.1 x 10^1 CFU/100ml (see Table 5 Above). The results for urine indicated that both *E. coli* and TCFU were below detection limits of <1000CFU/100ml in both winter and summer (see Figures 30 and 31 below). The absence of pathogenic micro-organisms in urine indicates that the separation of urine from Faeces was successfully done because detecting *E. coli* in urine would imply that there was either direct or indirect faecal contamination in the collected samples. Non detection of both *E. coli* and TCFU in urine suggests the possibility of directly using urine for agricultural activities without causing faecal oral related disease outbreaks in emergency camps. Unlike urine samples, Lacto-Fermented Sludge samples showed high concentrations of *E. coli* and TCFU suggesting that, despite being successful off-site (Malambo, 2014), the addition of LAB inoculum was challenged in as far as sanitizing on-site faecal sludge was concerned (see Figures 30 and 31 below). However, it is also possible that higher than Malawi Standard pathogen concentrations in the Lacto-Fermented Sludge could have been contributed by the addition of charcoal which was used in order to increase the carbon content of faecal sludge as well as a dehydrating agent while the toilet was in use. The addition of charcoal was meant to increase the carbon content and water holding capacity of faecal sludge which would in turn lead to the possibility of practicing urban agriculture in emergency camps. Unfortunately, the observed results seemed to have been affected by the addition of charcoal. It should be mentioned that the addition of charcoal was not part of the research methodology that led to the conclusion that LAB inoculation could sanitize faecal sludge.

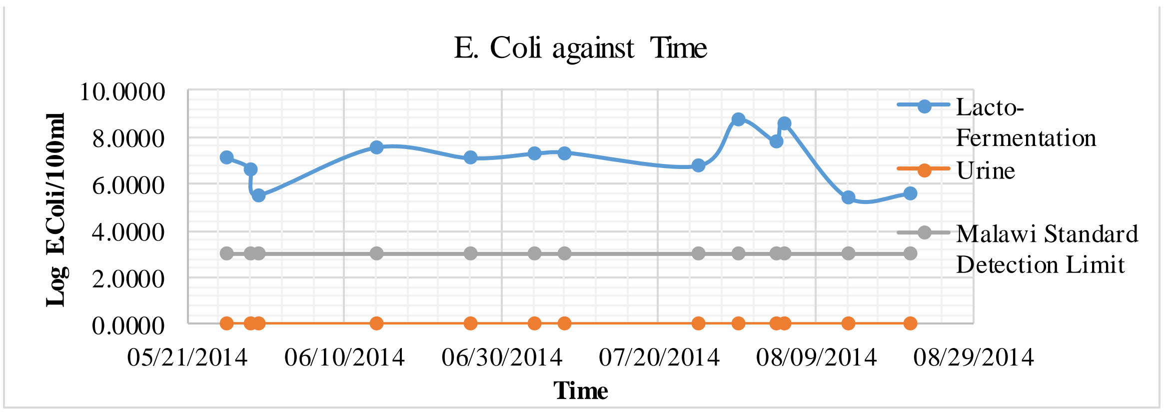

Figure 30: Graph of E. coli CFU/100ml of Lacto-Fermented Sludge and Urine against Time

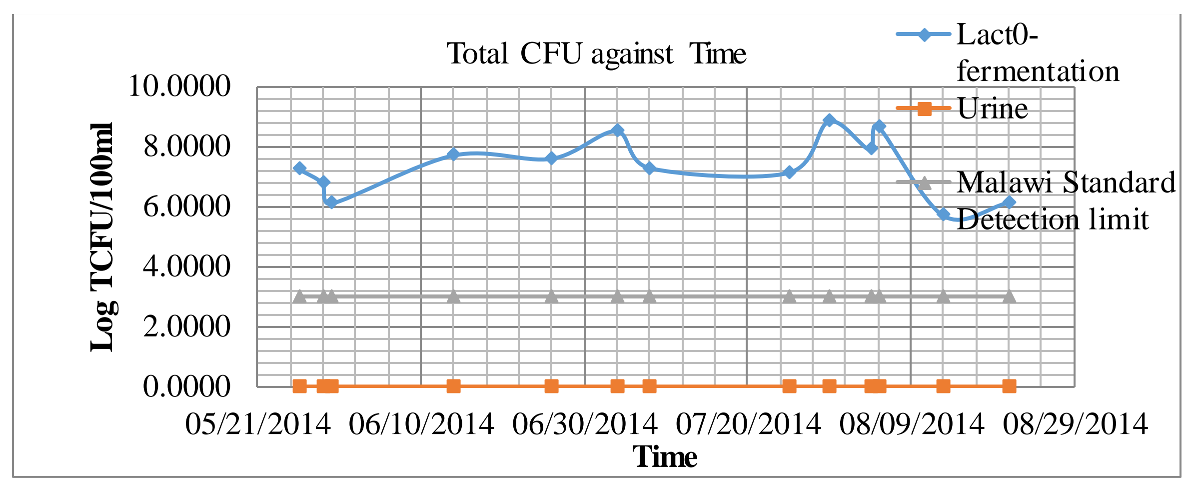

Figure 31: Graph of Log TCFU/100ml of Lacto-Fermented Sludge and Urine against Time

4.1.3 Vermicompost

Microbial analysis results for faecal sludge indicated that the average winter *E. coli* was 2.38 x 10^7 CFU/100ml while the average summer *E. coli* was 4.67 x 10^6 CFU/100ml. Analysis results for faecal sludge indicated that the average winter Total Colony Forming Units (TCFU) was 3.26 x 10^7 CFU/100ml while the average summer TCFU/100ml was 5.33 x 10^8 CFU/100ml. Microbial analysis results for vermicompost indicated that the average winter *E. coli* was 7.72 x 10^5 CFU/100ml while the average summer *E. coli* was 9.42 x 10^5 CFU/100ml. Analysis results for vermicompost indicated that the average winter Total Colony Forming Units (TCFU) was 1.53 x 10^7 CFU/100ml while the average summer TCFU/100ml was 5.33 x 10^7 CFU/100ml. These analysis results suggested that the worms were challenged in as far as reducing pathogens in faecal sludge to safe levels is concerned (see Figures 32 and 33). This observation agrees with Morgan et al., (1982) who showed that the number of bacteria and Actinomycetes contained in the ingested material increased up to 1000-fold while passing through the gut of worms. The other reason behind the failure of Vermicompost toilet in reducing pathogen concentrations to set standards could probably be attributed sludge age, the loading rate and moisture content as literature that demonstrated that worms reduced concentrations of pathogens to safe levels did their observations off-site and not on-site as is the case in this study (Dominguez, Edward & Webster, 2000; Masciandaro, Ceccanti & Garcia, 2000).

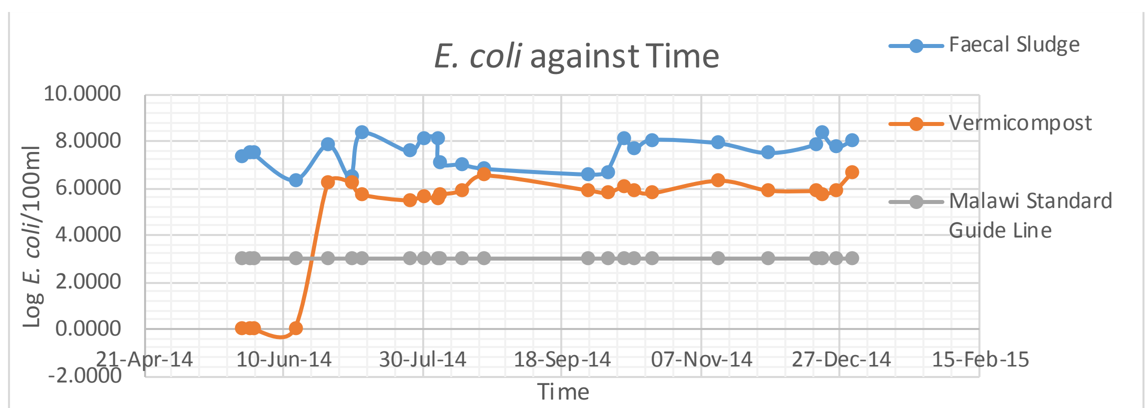

Figure 312: Graph of Log *E. coli* CFU/100ml of Faecal Sludge and Vermicompost against Time

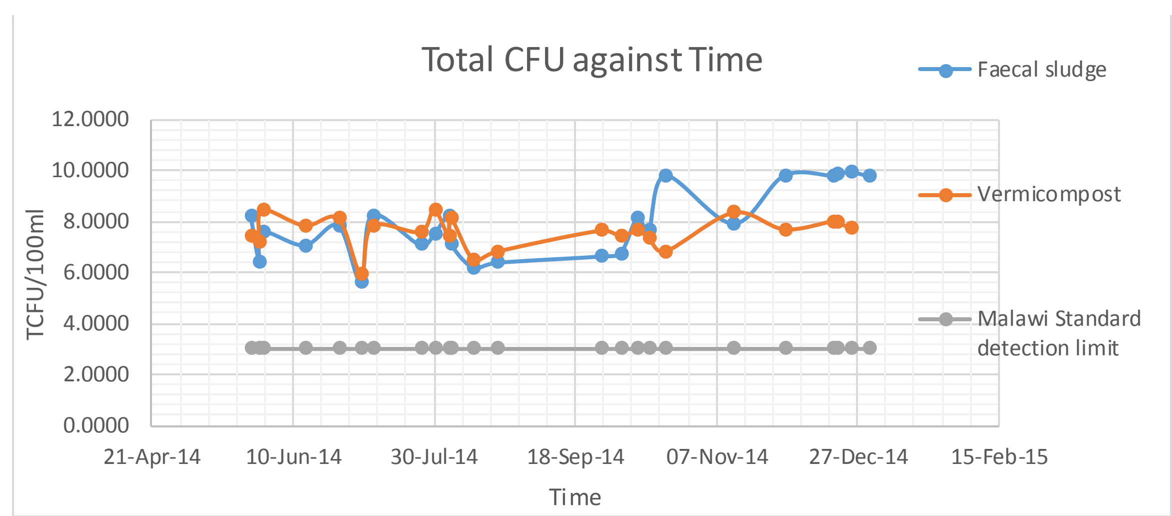

Figure 323: Graph of Log TCFU/100ml of Faecal Sludge and Vermicompost against Time

4.2.0 GENERATION OF USEFUL BY-PRODUCT

Under this section, the results of assessing the efficiency of Anaerobic Digester, Vermicompost and Terra Preta toilets in converting fresh faecal sludge on-site to useful by-products for possible sustainable agriculture during emergency situations are outlined. As highlighted in chapter three assessment of the efficiency of Anaerobic Digester, Vermicompost and Terra Preta toilets in converting fresh faecal sludge on-site to useful by-products was done by analysing the concentration of TAN in the samples collected from the three sanitation systems mentioned above. Further assessment of by-product generation of the Anaerobic Digester done by observing biogas harvesting. Outlined in Table 6, is a summary of the TAN concentrations and volume of biogas observed in this the study.

4.2.1 Anaerobic Digester Toilet

The analysis results for TAN of the samples taken from the Anaerobic Digester toilet tells us that the average winter TAN were 15.387mg/l, 15.738mg/l and 15.172mg/l with standard deviations of 3.817mg/l, 2.527mg/l and 2.598mg/l for the feeding point, digestate point and

pasteurization tubes, respectively. The TAN results for samples collected in summer indicate that the average summer TAN were 25.515 mg/l, 24.970 mg/l, and 25.5766 mg/l with standard deviations of 5.214 mg/l, 4.708 mg/l, and 5.114 mg/l for the feeding point, digestate point and pasteurization tubes, respectively. Regardless of different sampling points, TAN mean values in winter were approximately 40% lower than those in summer. The mean TAN also indicated a strong positive linear relationship at all sampling points (r = 0.714, 0.631, and 0.653) with 51%, 39.8% and 42.7% of TAN from the feeding point sample, digestate sample and pasteurization sample, respectively, being explained by temperature. Further observations indicated that TAN values kept on increasing throughout the study period probably due to their being temperature dependent (Montangero & Strauss, 2002). Further analysis of the recorded data also indicated that the mean TAN obtained in summer were relatively higher than those obtained in winter ($t_{4.119}$= 0.838, p= 0.448>0.05). The observed data for both winter and summer were within optimal range of 15 mg/l to 35 mg/l suggesting that, subject meeting standards of other parameters, the effluent could be used for practicing sustainable agriculture during emergency situations.

In order to assess Anaerobic Digester's capability of harvesting biogas, four biogas collecting bags were connected to the system. By design it was anticipated that the Anaerobic Digester would harvest 10m^3 of biogas per day. However only half of the anticipated volume was harvested per day. The limited capacity of the Anaerobic Digester to harvest the designed biogas volume suggests that part of the COD from faecal sludge was not being converted to methane (CH_4). This is evidenced by the 17% COD removal difference in the collected data. The low methane gas production further suggests the need for improving the Anaerobic Digester from being a single stage digester to being a multi-stage digester as this could boost COD conversion to biogas (Verma, 2002). Further possible reasons to the low gas production of the Anaerobic Digester could be attributed to either the possible existence of a very high C/N ratio that resulted in the rapid consumption of nitrogen by the methanogens as they tried to meet their protein requirement thus making the methanogens no longer react on the left over carbon content in the material or a possible existence of a very low C/N ratio that resulted in the liberation and accumulation of nitrogen in the form of ammonia which in turn increased the pH value of the digestrer material that affected the population of the methanogenic bacteria (RISE-AT, 1998).

Figure 334: Filled Biogas Bags

Figure 345: Methane Burning a Stick

The decreased biogas production could also be due to the observed outside normal thermophilic temperature ranges that were registered by the Anaerobic Digester which reduced both process stability and dewatering properties of the fermented sludge thus requiring for large amounts of energy for heating (Labatut & Gooch, 2014). The other possible explanation on low biogas yield could be that faecal sludge had high concentrations of insoluble complex organic polymers, such as carbohydrates, proteins, lipids, and phosphorylated organics which limited the rate of hydrolysis reactions in the digestion chamber. The observed low biogas yield could also suggest that the Anaerobic Digester was fed above its sustainable organic loadng rate (OLR) and this resulted in accumulation of volatile fatty acids (VFA) which inhibited the digestion process of faecal sludge (RISE-AT, 1998). Figures 34 and 35 show filled biogas bags and a stick being burnt by methane from one of the bags. The burning demonstration of harvested methane supports further consideration of the Anaerobic Digester for use in emergencies as it may provide additional advantage of lighting and cooking (see Figure 35 below).

4.2.2 Terra Preta Toilet

The analysis results for TAN of samples from Terra Preta toilet tells us that the average TAN were 11.778mg/l and 16.579mg/l with a standard deviations of 6.226mg/l and 5.691mg/l for the Lacto-Fermented Sludge and urine, respectively. The average TAN of urine was significantly higher than that of Lacto-Fermented Sludge ($t_{11}= 3.924$, $p= 0.002<0.05$). This is in agreement with observation that urine has a high content of readily available nitrogen to such an extent that its fertilising effect is similar to that of nitrogen rich chemical fertiliser (Kirchmann & Pettersson, 1995). The observed higher TAN in urine justifies the importance of keeping urine under anaerobic conditions. This is because, according De Gisi et al., (2014), keeping urine under such conditions helps to prevent the hydrolysis of urea and its

transformation to volatile ammonia and CO_2, which would result in the loss of nitrogen and CO_2 into the atmosphere and bad odour. The Lacto-Fermented Sludge has, however, lower

Table 6: Useful By-Product Analysis Results

	Winter				**Summer**			
	N	**Mean**		**Std. Dev**	**N**	**Mean**		**Std. Dev**
	Statistic	**Statistic**	**Optimal Limit**	**Statistic**	**Statistic**	**Statistic**	**Optimal Limit**	**Statistic**
Useful By-Product								
Anaerobic Digester Toilet								
TAN (mg/l)								
Feed	13	15.387		3.817	11	25.515		5.214
Digestate	13	15.738		2.527	11	24.970		4.708
Pasteurised	13	15.172		2.598	11	25.577		5.114
Terra Preta Toilet								
TAN (mg/l)								
Lacto-Fermented Sludge	13	11.778	**15 - 30**			6.226	**15 - 30**	
Urine	13	16.579				5.691		
Vermicompost Toilet								
TAN (mg/l)								
Faecal Sludge	14.38	10.2167	**15 -30**	5.325	27.37	8.121	**15 -30**	7.181
Vermicompost		13.6487		7.347		14.242		2.276

TAN than urine because its nitrogen content is slowly released as it is organically bound in undigested food remains of the sludge Mnkeni & Austin, 2009) This implies that urine could be a better by-product for sustainable agriculture as compared to Lacto-Fermented Sludge.

4.2.3 Vermicompost Toilet

The analysis results for TAN tells us that the average TAN in winter were 11.778mg/l and 16.579mg/l with a standard deviations of 6.226mg/l and 5.691mg/l for the faecal sludge and vermicompost, respectively while the average TAN in summer were 11.778mg/l and

16.579mg/l with a standard deviations of 6.226mg/l and 5.691mg/l for the faecal sludge and vermicompost, respectively. Further observations indicate that mean TAN of vermicompost samples fell within the optimal range of 15 mg/l to 35 mg/l. The results also indicate that the worms, while converting fresh faecal sludge to vermicompst, were able to increase TAN of faecal sludge by 14% in winter and 27% in summer. The mean TAN values for vermicompost samples, at $\alpha = 0.05$ level of significance do not statistically suggest enough evidence to conclude that the mean TAN for vermicompost samples taken in summer and samples taken in winter were statistically significant for the two seasons ($t_{8.988} = 0.261$, $p = 0.108 > 0.05$). The behavior, of the worms, of increasing the fertliser content of faecal sludge, is in line with available literature which supports that worms increase the fertiliser content of faecal sludge as it passes through the gut (Sujit, 2012, Fox et al., 2009). The worms increase the fertilizer content by enhancing microbial activity that increase nutrient mineralization rates thus providing greater quantities of TAN in the vermicast. Additionally, the rise in the level of organic TAN during vermicomposting was probably due to mineralization of organic TAN by combined action of faecal TAN of earthworms and microbial activity of the vermicasts (Naddafi et al., 2004). The increased TAN concentrations in vermicompost suggests that vermicompost could be a viable by-product in as far as urban agriculture for emergency situations is concerned.

4.3.0 FAECAL SLUDGE STABILISATION

The study also evaluated the Anaerobic Digester, Vermicompost and Terra Preta toilets for their suitability in stabilising faecal sludge during challenging conditions common in emergency situations. As outlined in chapter three, the evaluation was done by analysing the concentration of Chemical Oxygen Demand, Temperature and pH in the samples collected from the three sanitation systems mentioned above. Outlined below are the findings of the study starting with Chemical Oxygen Demand, Temperature and pH in that order.

4.3.1 CHEMICAL OXYGEN DEMAND

The COD analysis results for the samples taken from the three sanitation systems are outlined in Table 7 below.

4.3.1.1 Anaerobic Digester Toilet

The Anaerobic Digester sanitation system's mean COD values observed in summer were 268.018 mg/l, 423.982 mg/l, and 278.191 mg/l with standard deviations of 276.589 mg/l, 239.076 mg/l and 256.460 mg/l for the feeding point, digestate point and pasteurization tubes, respectively. Whereas, the mean COD values observed in winter were 132.958 mg/l, 134.337 mg/l, and 110.077 mg/l with standard deviations of 86.133 mg/l, 57.654 mg/l and 26.910 mg/l

for the feeding point, digestate point and pasteurization tubes, respectively. Based on the results, we can also state that the average COD for samples observed in summer were 1.5 times more than the average COD observed in winter. Further observations of the results indicate that the summer output COD (278.191 mg/l) from the pasteurization tubes were significantly higher than the input COD (268.018 mg/l) from feeding point ($t_{8.590}=2.922$, $p=0.018<0.05$). On the contrary, in winter it was the input COD (132.958 mg/l) from the feeding point which was higher than the output COD (110.077 mg/l) of the pasteurization tubes. These observed mean COD values, at α= 0.05 level of significance, suggest that there is not enough evidence to conclude that the mean COD for effluent samples (samples taken from pasteurization tubes)

Table 7: Chemical Oxygen Demand Stabilization

	Winter				**Summer**			
	N	**Mean**		**Std. Dev**	**N**	**Mean**		**Std. Dev**
	Statistic	**Statistic**	**Optimal Limit**	**Statistic**	**Statistic**	**Statistic**	**Optimal Limit**	**Statistic**
Stabilisation								
Anaerobic Digester Toilet								
COD (mg/l)								
Feed	13	132.958	60	86.133	11	268.018	60	276.589
Digestate	13	134.337		57.654	11	423.982		239.076
Pasteurised	13	110.077		26.910	11	278.191		256.460
Terra Preta Toilet								
COD (mg/l)								
Lacto-Fermented Sludge	13	431.226	60		11	294.392	60	
Urine	13	868.385			11	248.696		
Vermicompost Toilet								
COD (mg/l)								
	Efficiency (%)				Efficiency (%)			
Faecal Sludge	28.08	195.603	60	99.756	50.78	174.606	60	30.313
Vermicompost		348.308		141.708		534.850		162.637

taken in summer and effluent samples taken in winter are the same for the two seasons (t_9= 0.312, p= 0.762>0.05). With respect to temperature, the COD values for the digestate sample

indicated a stronger positive linear relationship (r = 0.512) than those of both the feeding point (r = 0.338) and pasteurisation tubes (r =0.235) such that 26.2% of the digestate's COD values, unlike 15% and 5% of both the feed and pasteurised, respectively, could be explained by temperature. The graphical representation of COD values (see Figure 36 below) further indicated that the COD for samples obtained in September and October went extremely high as the system was switching from winter season to summer season. The higher COD values for the samples from the digestate sampling point could be either due to the higher concentration of physical biodegradable organic compounds (suspended solids) that were observed in the samples or the use of the Anaerobic Digester by 200 orphans, overloaded the system thereby affecting the flow of the digestate to the point of faecal sludge being washed out of the digester before it were completely digested. This observation of suspended solids was strange because

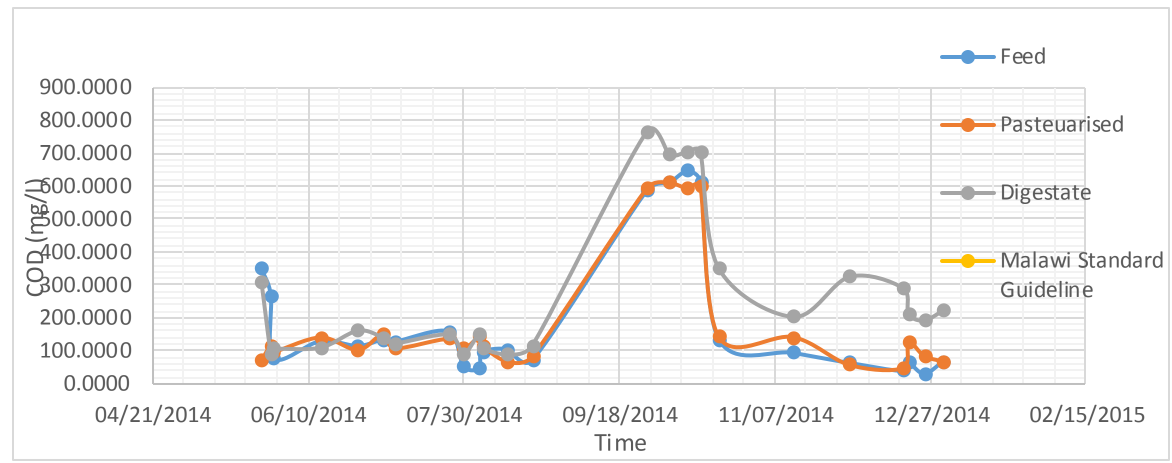

Figure 356: Graph of COD against Time

the Anaerobic Digester, being anaerobic digestion in nature, should have had a steady sludge volume reduction (Wong & Law-Flood, 2011). The observed COD trends also indicate that the Anaerobic Digester did not successfully wash out the suspended solids. Further explanations to the observed COD trends could be attributed to the Anaerobic Digester's response to the temperature changes, from mesophilic to thermophilic ranges, because COD values went down again in early November. This COD sharp rise agrees with Atta, (2011) who observed that a rise in wastewater temperatures demands more oxygen for nitrification process. If we were to use the observed COD trend in projecting the amount of biogas to be produced from the Anaerobic Digester observed in this study, we could not be surprised to see the observed poor biogas volume observed in this study. Overall the Anaerobic Digester, with respect to COD, was challenged in as far as discharging of stabilized faecal sludge is concerned because the observed values hardly went below the standard guideline of 60mg/l (Malawi Standard 539:2013).

4.3.1.2 Terra Preta Toilet

The results indicate that Terra Preta Sanitation System's mean COD values observed in winter were 431.226 and 868.385 with standard deviations of 294.3921mg/l and 248.6960mg/l for the Lacto-Fermented Sludge and urine samples, respectively. Further analysis of the data indicate that the average COD value (868.4mg/l) for urine was two times more than that of Lacto-fermented faecal sludge (431.2mg/l). Further observations of the results indicate that COD values for both Lacto-Fermented faecal sludge and urine were significantly higher than the stabilization COD value of 60mg/l (MS539:2013). The differences in the average COD for Lacto-fermented faecal sludge and urine could attributed to high concentrations of TAN that are found in urine. The higher than the Malawi Standard COD values for Lacto-Fermented faecal sludge indicate that the addition of both charcoal and LAB inoculum did not have a significant impact on the stabilisation of Lacto-Fermented faecal sludge. It is not surprising that the Terra Preta toilet attracted a lot of house flies. The presence of house flies around the Terra Preta toilet defeats the whole purpose of recommending this sanitation system for use in emergency situations.

While Malambo, (2014) and Factura et al., (2010) reported successful treatment/stabilization of faecal sludge using LAB and Terra Preta, respectively, the current study has shown the contrary. This could be attributed to differences in both age and source of sludge. The studies by Malambo, (2014) were conducted off-site and involved desludging of faecal sludge from existing pit latrines while this study was done on-site and targeted flesh faecal sludge. The other possible reason could be that in the study conducted by Malambo, (2014), LAB inoculation was not combined with addition of charcoal which was the case with the current study. The addition of Charcoal was inevitable as it is key to Terra Preta's long organic matter residence times and continuing fertility if emergency dwellers were to practice sustainable agriculture (Glaser, 2007). One would also argue that, on the part of Lacto-Fermented Sludge, the failure could be as a result of frequent opening of the pedestal's lid that may have continuously disturbed the required anaerobic conditions. This would be contradicting literature which says that LAB is aero-tolerant (Malambo, 2014).

4.3.1.3 Vermicompost Toilet

Analysis results of samples taken from Vermicompost Toilet indicate that the mean COD values of vermicompost were 348.32mg/l and 534.85mg/l with standard deviations of 141.708 mg/l and 162.637 mg/l for winter and summer, respectively. The results also indicate that the mean

COD values of faecal sludge samples were 195.60mg/l and 174.61mg/l with standard deviations of 99.756 mg/l and 30.313 mg/l for winter and summer, respectively. These results demonstrated that the worms increased the COD of faecal sludge by 28% in winter and 50% in summer. These mean COD values for vermicompost, at $\alpha = 0.05$ level of significance, suggest that there is not enough evidence to conclude that the mean COD for samples taken in summer and samples taken in winter were the same for the two seasons ($t_9 = 0.085$, $p = 0.934 > 0.05$). Similarly, the mean COD values for Faecal sludge samples, at $\alpha = 0.05$ level of significance, suggest that there is not enough evidence to conclude that the mean COD for samples taken in summer and samples taken in winter were the same for the two seasons ($t_9 = 0.500$, $p = 0.349 > 0.05$). The results showed higher COD values than the stabilisation Malawi Standard value of 60mg/l (MS539:2013). This may be misinterpreted as a failure of the worms to stabilise the faecal sludge. However, despite all COD values being above Malawi Standards, the vermicompost never attracted vectors, which should have been the case if stabilisation had not taken place. Therefore based on this observation, it may be argued that, although the vermicompost did not chemically meet the stabilisation standard, the faecal sludge was physically stabilised. Probably the higher COD values may have resulted from the increased TAN concentrations.

4.3.2 TEMPERATURE

The Temperature analysis results for the samples taken from the three sanitation systems are outlined in Table 8 below.

4.3.2.1 Anaerobic Digester Toilet

The results indicate that the mean winter temperature for the feeding point, digestate point and pasteurization tubes were 22.35°C, 36.79°C and 33.24°C with standard deviations of 2.31°C, 7.01°C and 5.73°C, respectively while the mean summer temperature for the feeding point, digestate point and pasteurization tubes were 34.39°C, 48.58°C and 44.10°C with standard deviations of 2.74°C, 1.63°C and 2.09°C, respectively. There were much variations in temperatures recorded during winter (25–42°C) than those recorded during summer (42–48°C). The temperatures in winter were within mesophilic range (20-42°C) (Kuffour et al., (2013) while those in summer were within thermophilic ranges (42-75°C) (Lettinga, 1995; Rajeshwari et al., 1999). The observed temperatures for winter samples were lower than the optimal thermophilic temperature range of 42°C - 75°C to support the anaerobic conditions that were required by Anaerobic Digester to stabilize and sanitize faecal sludge. The results also indicated that temperatures observed in summer were within the optimal thermophilic temperature range

of 42°C - 75°C that were required for the Anaerobic Digester to stabilise and sanitise faecal sludge. Furthermore the results indicated that, regardless of temperature being mesophilic (22.35, 36.79, and 33.24°C for the feed, digestate, and pasteurised, respectively) in winter and

Table 8: Temperature Stabilization

	Winter				**Summer**			
	N	**Mean**		**Std. Dev**	**N**	**Mean**		**Std. Dev**
	Statistic	**Statistic**	**Optimal Limit**	**Statistic**	**Statistic**	**Statistic**	**Optimal Limit**	**Statistic**
Stabilisation								
Anaerobic Digester Toilet								
Temperature(°c)								
Feed	13	22.35	42- 75	2.31	11	34.39	42 - 75	2.74
Digestate	13	36.79		7.01	11	48.58		1.63
Pasteurised	13	33.24		5.73	11	44.10		2.09
Terra Preta Toilet								
Temperature(°c)								
Lacto-Fermented Sludge	13	17.55	25		11	29.82	25	
Urine	13	16.12			11	28.58		
Vermicompost Toilet								
Temperature(°c)								
	Efficiency (%)				Efficiency (%)			
Faecal Sludge	-2.78	21.89	<35	5.23	-11.88	31.52	35	1.59
Vermicompost		20.70		4.68		24.83		1.21

thermophilic (48.58, 44.10°C, except feed 34.39°C) in summer, throughout the study period, mean temperature values for the digestate sample could not provide enough evidence to suggest that they were statistically significantly higher than those from pasteurisation tubes ($t_9 = 0.075$, $p = 0.942 > 0.05$). The mean temperature values for effluent samples (samples taken from pasteurization tubes), at $\alpha = 0.05$ level of significance, suggest that there is not enough evidence to conclude that the mean temperature for samples taken from pasteurization tubes in summer and samples taken in winter were the same for the two seasons ($t_9 = 0.205$, $p = 0.842 > 0.05$). The variations mentioned actually explain the poor pathogen reductions seen in the samples

collected from all the three sampling points because, in addition to other operating conditions such as pH, organic loading rate and influent strength, the effective performance of AD systems is largely dependent on the sensitivity to temperature of the methane producing bacteria (Rajeshwari et al., 1999)

4.3.2.2 Terra Preta Toilet

The results indicate that the mean winter temperature for both Lacto-fermented faecal sludge and urine were 17.54°C and 16.12°C with standard deviations of 3.55°C and 3.60°C, respectively while the mean summer temperature for both Lacto-fermented faecal sludge and urine were 29.82°C and 28.58°C with standard deviations of 3.53°C and 3.95°C, respectively. The observed temperatures for both winter samples were lower than the optimal temperature of 25°C to support the anaerobic conditions (Malambo, 2014) that were required by both urine and Lacto-fermented faecal sludge to achieve stability. The mean temperature values for both Lacto-Fermented Sludge and urine, at $\alpha = 0.05$ level of significance, suggest that there was a significant difference between the mean temperatures for Lacto-Fermented Sludge and urine samples taken in both summer and winter ($t_{11} = 2.261$, $p = 0.045 < 0.05$).

4.3.2.3 Vermicompost Toilet

The results indicate that mean temperature values for faecal sludge were 21.8°C and 31.5°C with standard deviations of 5.23°C and 4.68°C where as those of vermicompost were 20.7°C and 24.8°C with standard deviations of 4.50°C and 3,35°C for winter and summer, respectively. The observed winter and summer temperatures were within the optimal temperature range of 15°C to 35°C for worms to biodegrade faecal sludge. Further analysis indicated that mean temperature values for the vermicompost were significantly 3% in winter and 12% in summer higher than those of the fresh faecal sludge. These mean temperature values for faecal sludge, at $\alpha = 0.05$ level of significance, could not suggest enough evidence to statically conclude that the mean temperature for faecal sludge samples taken in summer and samples taken in winter were significantly different for the two seasons ($t_{11} = 1.891$, $p = 0.085 > 0.05$). Similarly, the mean temperature values for vermicompost samples, at $\alpha = 0.05$ level of significance, suggest that there is not enough evidence to conclude that the mean temperature for vermicompost samples taken in summer and samples taken in winter were the same for the two seasons ($t_9 = 0.200$, $p = 0.846 > 0.05$). The increased mean temperature values in summer actually explain the increased TAN concentrations of the vermicompost as it largely depends on operating conditions like temperature (Rajeshwari et al., 1999).

4.3.3 pH

The pH analysis results for the samples taken from the three sanitation systems are outlined in Table 9 below.

Table 9: pH Stabilisation

	Winter				**Summer**			
	N	**Mean**		**Std. Dev**	**N**	**Mean**		**Std. Dev**
	Statistic	**Statistic**	**Optimal Limit**	**Statistic**	**Statistic**	**Statistic**	**Optimal Limit**	**Statistic**
Stabilisation								
Anaerobic Digester Toilet								
PH								
Feed	13	7.22	6.5 - 9	0.31	11	7.16	6.5 - 9	0.08
Digestate	13	7.28		0.45	11	7.30		0.12
Pasteurised	13	7.28		0.33	11	7.37		0.09
Terra Preta Toilet								
PH								
Lacto-Fermented Sludge	13	6.65	6.5 – 9		11	0.63	6.5 – 9	
Urine	13	9.58			11	0.16		
Vermicompost Toilet								
PH								
	Efficiency (%)				Efficiency (%)			
Faecal Sludge	0.10	7.20	6.5–9.5	0.87	-1.95	6.38	6.5– 9.5	0.21
Vermicompost		7.21		0.50		6.14		0.43

4.3.3.1 Anaerobic Digester Toilet

The results for pH indicated that mean pH values for both winter and summer for samples taken from feed, digestate and pasteurized faecal sludge were 7.22, 7.28, 7.28 with standard deviations of 0.31, 0.45, and 0.33 for winter and 7.16, 7.30, and 7.37 with standard deviations of 0.08, 0.12 and 0.09 for summer, respectively. The mean pH values for effluent samples (samples taken from pasteurization tubes) recorded for the entire study period were within optimal range of 6.5 to 9 required by microorganisms to biologically degrade the organic matter (Veenstra & Polprasert, 1997 as cited by Kuffour et al., 2013; Rajeshwari et al., 1999; Strauss, Larmie, Heinss & Montangero, 1999). However, despite the results indicating a weak positive

linear relationship (r =0.0617 and 0.088) between pH values and temperature over the observed data with only 0.4% and 0.8% of the pH values being explained by temperature. Although the mean pH values of the Anaerobic Digester' effluent fell within stabilization optimal range of 6.5– 9.5 (Malawi Standard 539:2013), at α= 0.05 level of significance, the results suggest that there is not enough evidence to conclude that the mean pH values for samples taken from pasteurization tubes in summer and samples taken in winter were the same for the two seasons ($t_{1.349}$= 1.060, p= 0.443>0.05). Thus at α= 0.05 level of significance, the results do not provide enough evidence to conclude that the effluent samples were stabilized in both winter and summer

4.3.3.2 Terra Preta Toilet

The results indicate that the mean pH for urine was 9.58 with standard deviation of 0.16 while the mean pH for Lacto-Fermented Sludge was 6.65 with standard deviation of 0.63 (see Table 9). Based on the results, we can also state that the average pH value for urine was 18% higher than that of Lacto-fermented faecal sludge. Further observations indicate that the mean pH for urine was significabtly higher than that of Lacto-Fermented Sludge (t_{11}=2.370, p= 0.037<0.05). The observed on-site pH of 6.6 for Lacto-Fermented Sludge was higher than the reported off-site pH of 4.2 (Malambo, 2014) that is supposed to be maintained for effective stabilisation of faecal sludge. The higher pH for urine could be attributed to the fact that the main proportion of the nitrogen in urine is excreted as urea, which increases the pH to 8.8 - 9.0 during its transformation into ammonia in the collection tank. The alkaline pH of urine is advantageous because it is critical in as far as getting substantial amount of TAN is concerned (Mnkeni & Austin, 2009). The observed pH of Lacto-fermented faecal sludge probably explains why the Terra Preta sanitation system hardly reduced concentrations of pathogen in faecal sludge to safer levels of <10^3CFU/100ml (Malawi Standard 539, 2013). Although the pH of both urine and Lacto-Fermented Sludge fell slightly within the optimal stabilisation limits of 6.5 – 9 (Malawi Standards 539, 2013), it would be misleading to say that the two were stabilised because the toilet still attracted a lot of flies.

4.3.3.3 Vermicompost Toilet

The analysis results for pH tells us that the average pH in winter were 7.20 and 7.21 with a standard deviations of 0.87 and 0.50 for the faecal sludge and vermicompost, respectively while the average pH in summer were 6.38 and 6.14 with a standard deviations of 0.21 and 0.43 for the faecal sludge and vermicompost, respectively. Further observations and analysis indicate that pH of fresh faecal sludge increased by a margin of 0.1% in winter and decreased by 2% in

summer and also that the observed pH values were acidic in summer and alkaline in winter. The low pH might have been either due to production of CO_2 and organic acids by microbial activity during the vermicomposting process or the further processing of the acidic intermediates had a pH shift reversing (Naddafi et al., 2004). Although the mean pH values of both faecal sludge and vermicompost fell within the Tiger Worms' activity optimal pH range of 4.5 to 9 (Sinha, et.al.2009) and stabilization range of 6.5– 9.5 (Malawi Standards 539, 2013), at $\alpha = 0.05$ level of significance, the results suggest that there is a statistically significant difference between the mean pH for faecal sludge of the samples taken in winter and that of the samples taken in summer ($t_{8.947} = 3.742$, $p = 0.005 < 0.05$) whereas vermicompost results also suggest that there is a statistically significant difference between the mean pH for the samples taken in winter and summer ($t_9 = 6.319$, $p = 0.001 < 0.05$).

Chapter Summary

In conclusion, this chapter has presented and discussed the results observed in this study. It has been demonstrated that the Anaerobic Digester produced promising results, in areas such as pathogen reduction, TAN and biogas, in summer and not winter. It did not do well in stabilizing faecal sludge in both seasons under which it was observed. The chapter has also outlined that Vermicompost toilet was challenged in reducing pathogen concentrations found in faecal sludge but did well stabilizing faecal sludge and generating rich in TAN concentrations of vermicompost. The Terra Preta toilet has been observed to have performed badly in all areas. Outlined in the next chapter, are the conclusions and recommendations of each sanitation system monitored in this study.

CHAPTER FIVE

5.0. CONCLUSION AND RECOMMENDATIONS

5.1.0. Conclusions

Based on the findings discussed in Chapter four, this chapter provides conclusions on the major research findings of this study. Due to the nature of this research the conclusions and recommendations for each sanitation system will be presented by answering whether the objectives have been met or not and if not met, the chapter also suggests recommendations for further research and improvement.

The study aimed at determining the feasibility of deploying the Anaerobic Digester, Vermicompost and Lactic Acid Bacteria led Terra Preta toilets as sanitation systems that could treat faecal sludge to meet Malawi Standards of pathogen free sludge during challenging conditions common in emergency situations. The observations from the results of the study have demonstrated that concentrations of faecal sludge pathogens in effluent samples from the Anaerobic Digester toilet were poorly reduced in winter. In summer the Anaerobic Digester managed to discharge effluents that met Malawi Standards of pathogen free sludge. The observations under the Anaerobic Digester suggest that it can be good emergency sanitation system to areas that have tropical climate as the system's performance seemed to be largely dependent on temperature. The study has also demonstrated that Vermicompost Toilet was challenged in reducing pathogen concentrations found in faecal sludge to safe levels in both summer and winter, suggesting that the findings of this study cannot be used as the basis for recommending the toilet for use during the immediate phase of an emegency until further research on their effectiveness is carried out. The Terra Preta toilet successfully harvested pathogen free urine indicating that there was no cross contamination between urine and Lacto-Fermented Sludge. However, the use of Lactic Acid Bacteria and charcoal as sanitizing and stabilizing innoculums was challenged in as far as reducing faecal sludge pathogens to Malawi Standard safe levels is concerned. The research based On-Site Lactic Acid Bacteria led Terra Preta toilet demonstrated that, minus vermicomposting, a lot needed to be done to have a sanitised Lacto-Fermented Sludge. Finally, the poor results obtained from an early emergency stage targeted Terra Preta Sanitation system suggest that the toilet, despite having a lot advantages, can only be adopted when targeting the second and third stages of an emergency situation.

The second specific objective for the study was assessing the efficiency of Anaerobic Digester, Vermicompost and Terra Preta toilets in converting fresh faecal sludge on-site into safe and useful by-products for possible sustainable agriculture during emergency situations. The study has demonstrated that the Anaerobic Digester discharged effluent digestate that had high concentrations of TAN suggesting the possibility of practicing sustainable urban agriculture in emergency situations. Furthermore, the Anaerobic Digester harvested biogas that could clearly burn. The possibility of methane harvesting demonstrated by the Anaerobic Digester toilet suggests further consideration of the toilet for use in emergency sites as it may provide additional advantage of lighting and cooking. The results obtained from the Terra Preta toilet samples demonstrated that urine had higher TAN concentrations than Lacto-Fermented Sludge suggesting that urine could be a better by-product, than Lacto-Fermented Sludge, for possible practice of sustainable agriculture in emergency sites. The study on Vermicompost toilet demonstrated that worms had the capacity of improving the agricultural value of faecal sludge. This was evidenced by higher TAN concentration yield from vermicast than fresh faecal sludge suggesting the possibility of practicing sustainable urban agriculture in emergency situations.

The study also evaluated the Anaerobic Digester, Vermicompost and Terra Preta toilets for their suitability in stabilising faecal sludge during challenging conditions common in emergency situations. The study demontsrted that both the Anaerobic Digester and Terra Preta toilets were challenged in as far as stabilizing faecal sludge was concerned. The COD results obtained from studying the two toilets were obseseved to be above the set Malawi Standard values. The faecal sludge stabilization challenge encountered by the Anaerobic Digester and Terra Preta toilets suggest that the systems required more digestion time as such they could not be used during the immediate stage of an emergency but rather the shot and long term stages of an emergency situation. The study on Vermicompost toilet demonstrated that it stabilized faecal sludge. This was evidenced by absence of vectors such as house flies around vermicast.

In conclusion, the three sanitation systems were observed to have had the potential of being applied to emergency situations in the following ways; The Anaerobic Digester did not require electricity, it was delivered as one complete package and got connected to the housing structure within hours and not weeks with minimal installation using hand tools but also placed above ground with only a shallow trench that does not require concrete nor bricks to lay. The Terra Preta toilet used little water for cleaning the toilet only, excreta was not discharged or buried in deep pits thus enabling hygienic recovery of faeces and urine for possible use as soil amendments, and did not require external energy. The Vermicomposting toilet never got filled

up thus reducing desludging costs and did not attract vectors thereby reducing possibility of faecal related disease transmission. However, during emergency situations, the primary objective is faecal sludge containment and pathogen reduction, therefore based on the results observed in this study we could conclude that the three proposed sanition systems could not be recommended for use during the immediate phase of an emergency situation as they have demonstrated inconsistences in as far as pathogen reduction and stabilization of faecal sludge is concerned. However, further studies in actual emergency situations and improvements of the sanitation systems could help in coming up with an informed decision on their functionality.

5.2.0 Recommendations

5.2.1. Anaerobic Digester Sanitation System.

In order for the Anaerobic Digester to ably meet the pathogen reduction standards, stabilise faecal sludge, generate useful by-products and consistently function regardless of seasonal and environmental variations, it is necessary that the following be done;

Firstly, it has been observed that the Anaerobic Digester requires thermophilic temperatures to achieve faecal sludge pathogen free guidelines. These temperatures were never met in winter despite creating a plastic cover. This means that if the system were to achieve its intended purpose it required raised temperatures. Fortunately in the same season the biogas could still be collected. Taking advantage that the collected biogas ably burnt, faecal sludge temperature could be raised to thermophilic ranges by heating the both the digester and the pasteurisation tubes. The heating would raise faecal sludge temperatures to thermophilic levels which would in turn make the treatment process effective. Secondly, the Anaerobic Digester could be improved by increasing faecal sludge retention time by either modifying the digester from being single stage to multistage or putting digestate retention bags at the end of the pasteurisation tubes or increasing the length of the pasteurization tubes. Modification of the above mentioned sections of the Anaerobic Digester could ensure that faecal sludge matures before the actual disposal into the donuts. The other possible way of improving the effluents from the Anaerobic Digester could be done by subjecting it to vermicomposting. As mentioned above, the Anaerobic Digester failed to meet the $10m^3$ biogas production target. The possible reason could be that the system capacity was overloaded and that resulted in reduction of reactor volume. Further research could be carried out to establish the right loading rates that could produce the designed $10m^3$ biogas volume per day. It is worth mentioning that the designed $10m^3$ is achievable as there is enough evidence in literature that AD registers have high energy

recovery rates. As demonstrated above, biogas from AD systems is a mixture of methane, Carbon dioxide, and hydrogen sulphide to mention but a few. Amongst these gasses methane constitutes approximately 50 - 75%. However, despite the Anaerobic Digester producing biogas it is not known what percentage of the gas is methane suggesting an area of further studies. The Anaerobic Digester got burst eight months down the observation line. The collapsing was attributed to weakening of the rubber as time passed by. Therefore it is recommended that the rubber be made thicker and stronger than the one that was placed under observation. The study did not observe the behavior of the Anaerobic Digester during the rain season due to lack of resources. Therefore it is recommended that further observations be conducted during this season as it is the rainy season that frequently experiences natural disisters.

5.2.2. Terra Preta

The results under this study indicated treating faecal sludge in emergency situations using Lactic Acid Bacteria led Terra Preta toilet was challenged. However, for purposes of reducing pathogens to safe levels, this being the primary objective in faecal sludge treatment, further studies on the use of LAB led Terra Preta on-site toilet, despite not ensuring that the soils have an improved water-holding capacity, increased organic matter content, and increased availability of nutrients, should not involve the addition of charcoal as it was not part of the methodology of the study that reported LAB’s effectiveness on faecal sludge treatment. The performance of LAB depends partly on the availability of enough glucose and and continuous anaerobic digestion process. In order to inmprove the addition of molasses after each defecation and ensure continuous AD process, it is recommended that the toilet be installed with a molasses flushing device and a self closing device (flap) inside the faecal sludge collection part of the toilet seat. Not targeting the immediate phase of an emergency, it is recommended that the LAB led Terra Preta toilet be combined with vermicomposting processes.

5.2.3. Vermicomposting

The study has demonstrated that Vermicompost Toilet was challenged in as far as reduction of faecal sludge pathogen concerntrations to safe levels is concerned. In order to ensure that faecal sludge is successfully rendered pathogen free, it is recommended that Vermicomposting toilet should not be fed with fresh faecal sludge but rather be fed with pretreated faecal sludge. This could be done by combining Vermicomposting with LAB led Terra Prta Toilet. The faecal sludge pathogen reduction challeng demonstrated by Vrmicomposting Toilet suggests

possibility of further studies that could estabilish the right ratio between the amount of Tiger Worms and faecal sludge loading rates.

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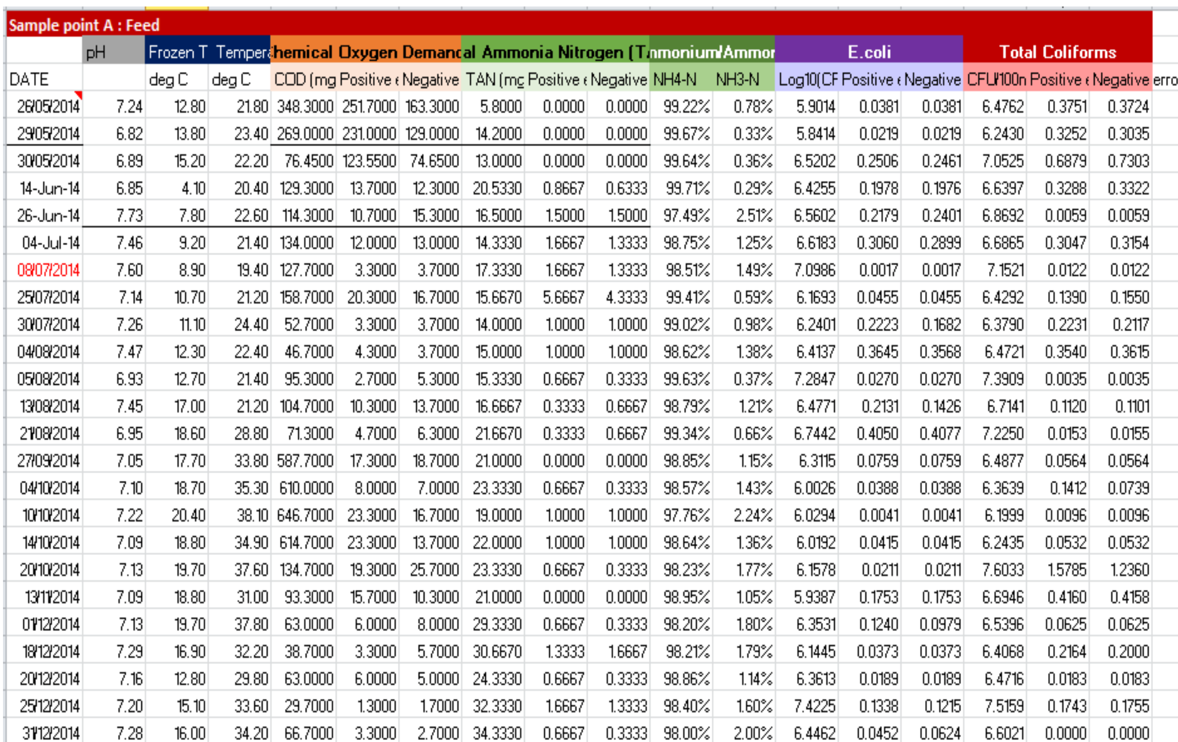
LIST OF APPENDICES

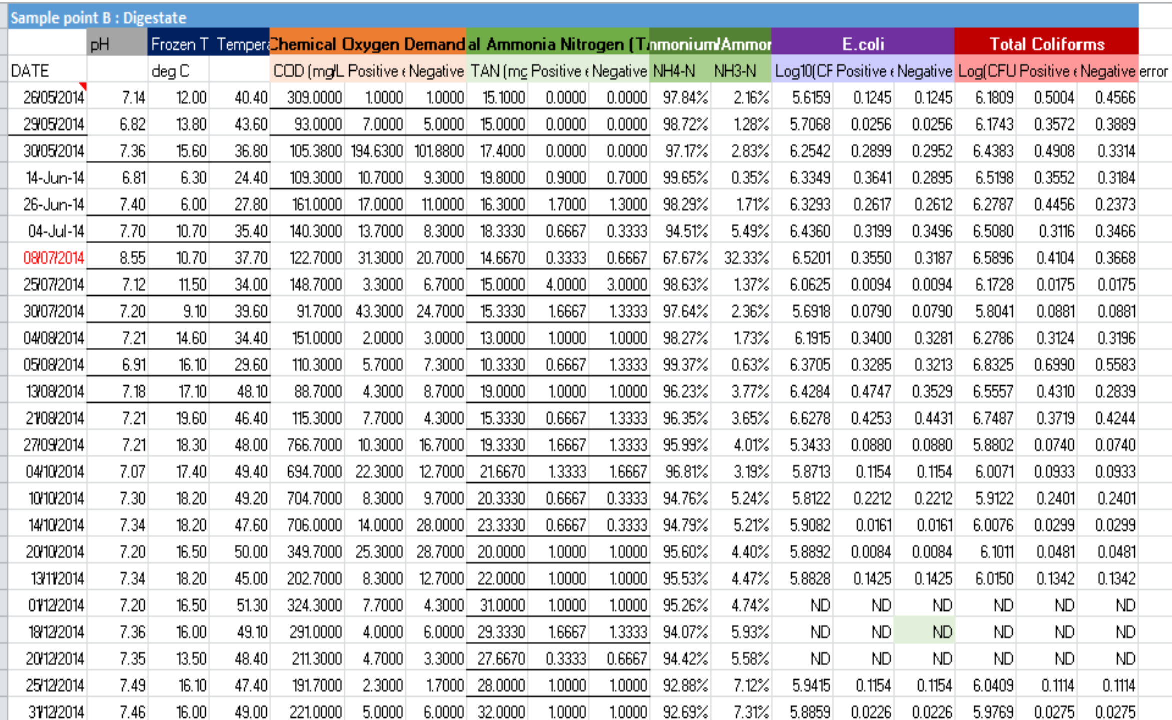
Appendix 1: Processed Data for Samples Collected from Feeding Point



Sample point A : Feed

	pH	Frozen T	Temper	Chemical Oxygen Demand			Total Ammonia Nitrogen (T			Ammonium/Ammor		E.coli			Total Coliforms		
DATE		deg C	deg C	COD (mg	Positive e	Negative	TAN (mg	Positive e	Negative	NH4-N	NH3-N	Log10(CF	Positive e	Negative	CFU/100n	Positive e	Negative error
26/05/2014	7.24	12.80	21.80	348.3000	251.7000	163.3000	5.8000	0.0000	0.0000	99.22%	0.78%	5.9014	0.0381	0.0381	6.4762	0.3751	0.3724
29/05/2014	6.82	13.80	23.40	269.0000	231.0000	129.0000	14.2000	0.0000	0.0000	99.67%	0.33%	5.8414	0.0219	0.0219	6.2430	0.3252	0.3035
30/05/2014	6.89	15.20	22.20	76.4500	123.5500	74.6500	13.0000	0.0000	0.0000	99.64%	0.36%	6.5202	0.2506	0.2461	7.0525	0.6879	0.7303
14-Jun-14	6.85	4.10	20.40	129.3000	13.7000	12.3000	20.5330	0.8667	0.6333	99.71%	0.29%	6.4255	0.1978	0.1976	6.6397	0.3288	0.3322
26-Jun-14	7.73	7.80	22.60	114.3000	10.7000	15.3000	16.5000	1.5000	1.5000	97.49%	2.51%	6.5602	0.2179	0.2401	6.8692	0.0059	0.0059
04-Jul-14	7.46	9.20	21.40	134.0000	12.0000	13.0000	14.3330	1.6667	1.3333	98.75%	1.25%	6.6183	0.3060	0.2899	6.6865	0.3047	0.3154
08/07/2014	7.60	8.90	19.40	127.7000	3.3000	3.7000	17.3330	1.6667	1.3333	98.51%	1.49%	7.0986	0.0017	0.0017	7.1521	0.0122	0.0122
25/07/2014	7.14	10.70	21.20	158.7000	20.3000	16.7000	15.6670	5.6667	4.3333	99.41%	0.59%	6.1693	0.0455	0.0455	6.4292	0.1390	0.1550
30/07/2014	7.26	11.10	24.40	52.7000	3.3000	3.7000	14.0000	1.0000	1.0000	99.02%	0.98%	6.2401	0.2223	0.1682	6.3790	0.2231	0.2117
04/08/2014	7.47	12.30	22.40	46.7000	4.3000	3.7000	15.0000	1.0000	1.0000	98.62%	1.38%	6.4137	0.3645	0.3568	6.4721	0.3540	0.3615
05/08/2014	6.93	12.70	21.40	95.3000	2.7000	5.3000	15.3330	0.6667	0.3333	99.63%	0.37%	7.2847	0.0270	0.0270	7.3909	0.0035	0.0035
13/08/2014	7.45	17.00	21.20	104.7000	10.3000	13.7000	16.6667	0.3333	0.6667	98.79%	1.21%	6.4771	0.2131	0.1426	6.7141	0.1120	0.1101
21/08/2014	6.95	18.60	28.80	71.3000	4.7000	6.3000	21.6670	0.3333	0.6667	99.34%	0.66%	6.7442	0.4050	0.4077	7.2250	0.0153	0.0155
27/09/2014	7.05	17.70	33.80	587.7000	17.3000	18.7000	21.0000	0.0000	0.0000	98.85%	1.15%	6.3115	0.0759	0.0759	6.4877	0.0564	0.0564
04/10/2014	7.10	18.70	35.30	610.0000	8.0000	7.0000	23.3330	0.6667	0.3333	98.57%	1.43%	6.0026	0.0388	0.0388	6.3639	0.1412	0.0739
10/10/2014	7.22	20.40	38.10	646.7000	23.3000	16.7000	19.0000	1.0000	1.0000	97.76%	2.24%	6.0294	0.0041	0.0041	6.1999	0.0096	0.0096
14/10/2014	7.09	18.80	34.90	614.7000	23.3000	13.7000	22.0000	1.0000	1.0000	98.64%	1.36%	6.0192	0.0415	0.0415	6.2435	0.0532	0.0532
20/10/2014	7.13	19.70	37.60	134.7000	19.3000	25.7000	23.3330	0.6667	0.3333	98.23%	1.77%	6.1578	0.0211	0.0211	7.6033	1.5785	1.2360
13/11/2014	7.09	18.80	31.00	93.3000	15.7000	10.3000	21.0000	0.0000	0.0000	98.95%	1.05%	5.9387	0.1753	0.1753	6.6946	0.4160	0.4158
01/12/2014	7.13	19.70	37.80	63.0000	6.0000	8.0000	29.3330	0.6667	0.3333	98.20%	1.80%	6.3531	0.1240	0.0979	6.5396	0.0625	0.0625
18/12/2014	7.29	16.90	32.20	38.7000	3.3000	5.7000	30.6670	1.3333	1.6667	98.21%	1.79%	6.1445	0.0373	0.0373	6.4068	0.2164	0.2000
20/12/2014	7.16	12.80	29.80	63.0000	6.0000	5.0000	24.3330	0.6667	0.3333	98.86%	1.14%	6.3613	0.0189	0.0189	6.4716	0.0183	0.0183
25/12/2014	7.20	15.10	33.60	29.7000	1.3000	1.7000	32.3330	1.6667	1.3333	98.40%	1.60%	7.4225	0.1338	0.1215	7.5159	0.1743	0.1755
31/12/2014	7.28	16.00	34.20	66.7000	3.3000	2.7000	34.3330	0.6667	0.3333	98.00%	2.00%	6.4462	0.0452	0.0624	6.6021	0.0000	0.0000

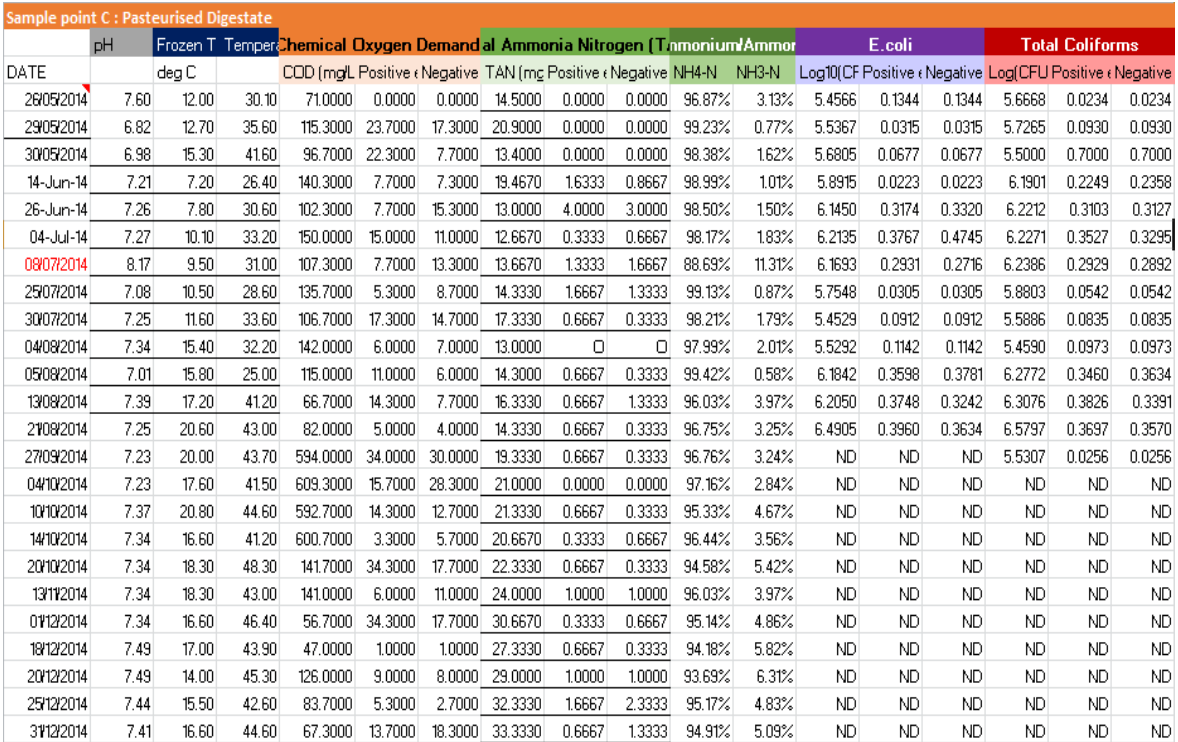
Appendix 2: Processed Data for Samples Collected from Digestate Sampling Point



Sample point B : Digestate

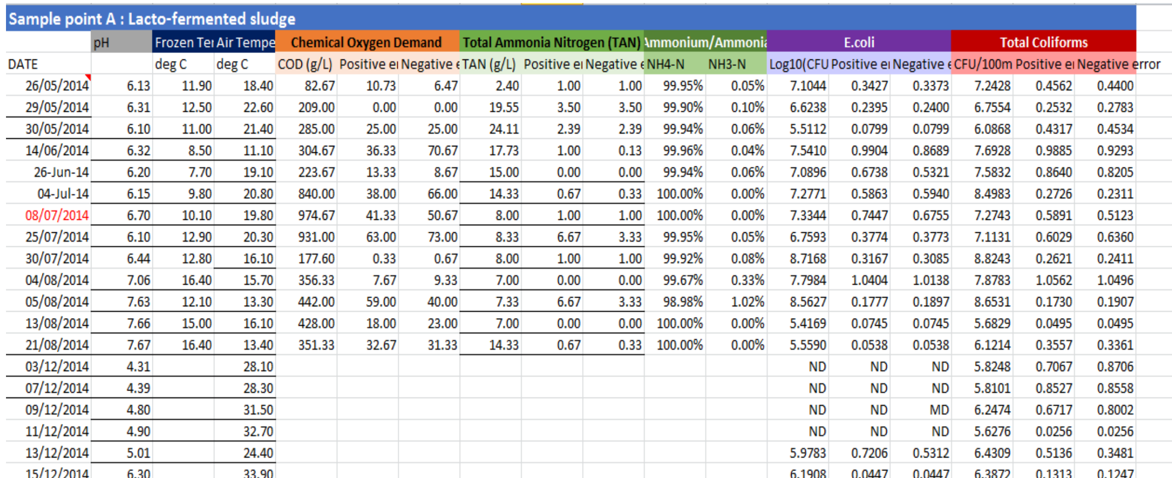
	pH	Frozen T	Temper	Chemical Oxygen Demand			Total Ammonia Nitrogen (T			Ammonium/Ammor		E.coli			Total Coliforms		
DATE		deg C		COD (mg/L	Positive e	Negative	TAN (mg	Positive e	Negative	NH4-N	NH3-N	Log10(CF	Positive e	Negative	Log(CFU	Positive e	Negative error
26/05/2014	7.14	12.00	40.40	309.0000	1.0000	1.0000	15.1000	0.0000	0.0000	97.84%	2.16%	5.6159	0.1245	0.1245	6.1809	0.5004	0.4566
29/05/2014	6.82	13.80	43.60	93.0000	7.0000	5.0000	15.0000	0.0000	0.0000	98.72%	1.28%	5.7068	0.0256	0.0256	6.1743	0.3572	0.3889
30/05/2014	7.36	15.60	36.80	105.3800	194.6300	101.8800	17.4000	0.0000	0.0000	97.17%	2.83%	6.2542	0.2899	0.2952	6.4383	0.4908	0.3314
14-Jun-14	6.81	6.30	24.40	109.3000	10.7000	9.3000	19.8000	0.9000	0.7000	99.65%	0.35%	6.3349	0.3641	0.2895	6.5198	0.3552	0.3184
26-Jun-14	7.40	6.00	27.80	161.0000	17.0000	11.0000	16.3000	1.7000	1.3000	98.29%	1.71%	6.3293	0.2617	0.2612	6.2787	0.4456	0.2373
04-Jul-14	7.70	10.70	35.40	140.3000	13.7000	8.3000	18.3330	0.6667	0.3333	94.51%	5.49%	6.4360	0.3199	0.3496	6.5080	0.3116	0.3466
08/07/2014	8.55	10.70	37.70	122.7000	31.3000	20.7000	14.6670	0.3333	0.6667	67.67%	32.33%	6.5201	0.3550	0.3187	6.5896	0.4104	0.3668
25/07/2014	7.12	11.50	34.00	148.7000	3.3000	6.7000	15.0000	4.0000	3.0000	98.63%	1.37%	6.0625	0.0094	0.0094	6.1728	0.0175	0.0175
30/07/2014	7.20	9.10	39.60	91.7000	43.3000	24.7000	15.3330	1.6667	1.3333	97.64%	2.36%	5.6918	0.0790	0.0790	5.8041	0.0881	0.0881
04/08/2014	7.21	14.60	34.40	151.0000	2.0000	3.0000	13.0000	1.0000	1.0000	98.27%	1.73%	6.1915	0.3400	0.3281	6.2786	0.3124	0.3196
05/08/2014	6.91	16.10	29.60	110.3000	5.7000	7.3000	10.3330	0.6667	1.3333	99.37%	0.63%	6.3705	0.3285	0.3213	6.8325	0.6990	0.5583
13/08/2014	7.18	17.10	48.10	88.7000	4.3000	8.7000	19.0000	1.0000	1.0000	96.23%	3.77%	6.4284	0.4747	0.3529	6.5557	0.4310	0.2839
21/08/2014	7.21	19.60	46.40	115.3000	7.7000	4.3000	15.3330	0.6667	1.3333	96.35%	3.65%	6.6278	0.4253	0.4431	6.7487	0.3719	0.4244
27/09/2014	7.21	18.30	48.00	766.7000	10.3000	16.7000	19.3330	1.6667	1.3333	95.99%	4.01%	5.3433	0.0880	0.0880	5.8802	0.0740	0.0740
04/10/2014	7.07	17.40	49.40	694.7000	22.3000	12.7000	21.6670	1.3333	1.6667	96.81%	3.19%	5.8713	0.1154	0.1154	6.0071	0.0933	0.0933
10/10/2014	7.30	18.20	49.20	704.7000	8.3000	9.7000	20.3330	0.6667	0.3333	94.76%	5.24%	5.8122	0.2212	0.2212	5.9122	0.2401	0.2401
14/10/2014	7.34	18.20	47.60	706.0000	14.0000	28.0000	23.3330	0.6667	0.3333	94.79%	5.21%	5.9082	0.0161	0.0161	6.0076	0.0299	0.0299
20/10/2014	7.20	16.50	50.00	349.7000	25.3000	28.7000	20.0000	1.0000	1.0000	95.60%	4.40%	5.8892	0.0084	0.0084	6.1011	0.0481	0.0481
13/11/2014	7.34	18.20	45.00	202.7000	8.3000	12.7000	22.0000	1.0000	1.0000	95.53%	4.47%	5.8828	0.1425	0.1425	6.0150	0.1342	0.1342
01/12/2014	7.20	16.50	51.30	324.3000	7.7000	4.3000	31.0000	1.0000	1.0000	95.26%	4.74%	ND	ND	ND	ND	ND	ND
18/12/2014	7.36	16.00	49.10	291.0000	4.0000	6.0000	29.3330	1.6667	1.3333	94.07%	5.93%	ND	ND	ND	ND	ND	ND
20/12/2014	7.35	13.50	48.40	211.3000	4.7000	3.3000	27.6670	0.3333	0.6667	94.42%	5.58%	ND	ND	ND	ND	ND	ND
25/12/2014	7.49	16.10	47.40	191.7000	2.3000	1.7000	28.0000	1.0000	1.0000	92.88%	7.12%	5.9415	0.1154	0.1154	6.0409	0.1114	0.1114
31/12/2014	7.46	16.00	49.00	221.0000	5.0000	6.0000	32.0000	1.0000	1.0000	92.69%	7.31%	5.8859	0.0226	0.0226	5.9769	0.0275	0.0275

Appendix 3: Processed Data for Samples Collected from Pasteurised Sampling Point



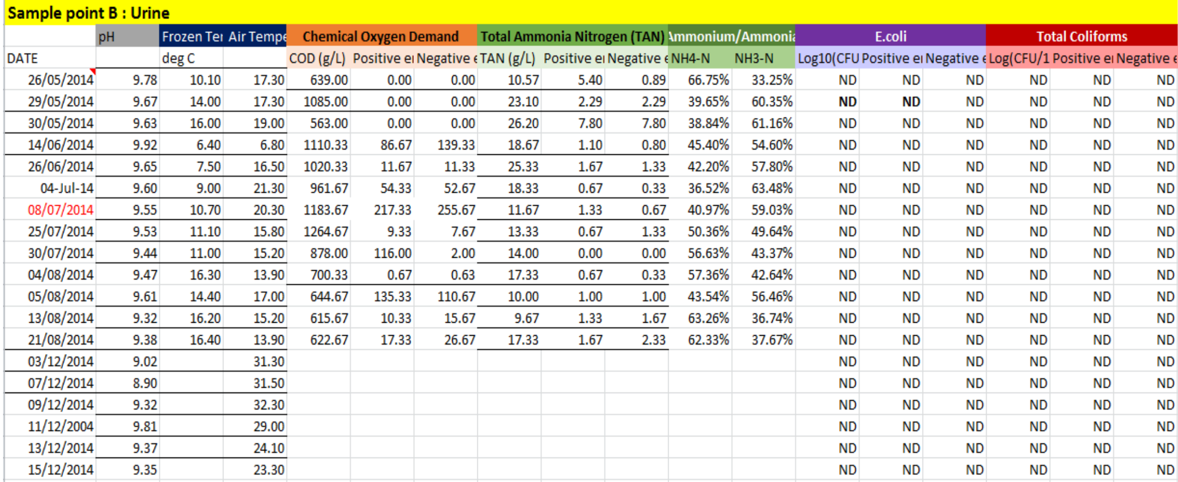
Sample point C : Pasteurised Digestate																	
	pH	Frozen T	Tempera	Chemical Oxygen Demand			al Ammonia Nitrogen (T			nmonium/Ammor		E.coli			Total Coliforms		
DATE		deg C		COD (mg/L	Positive e	Negative	TAN (mg	Positive e	Negative	NH4-N	NH3-N	Log10(CF	Positive e	Negative	Log(CFU	Positive e	Negative
26/05/2014	7.60	12.00	30.10	71.0000	0.0000	0.0000	14.5000	0.0000	0.0000	96.87%	3.13%	5.4566	0.1344	0.1344	5.6668	0.0234	0.0234
29/05/2014	6.82	12.70	35.60	115.3000	23.7000	17.3000	20.9000	0.0000	0.0000	99.23%	0.77%	5.5367	0.0315	0.0315	5.7265	0.0930	0.0930
30/05/2014	6.98	15.30	41.60	96.7000	22.3000	7.7000	13.4000	0.0000	0.0000	98.38%	1.62%	5.6805	0.0677	0.0677	5.5000	0.7000	0.7000
14-Jun-14	7.21	7.20	26.40	140.3000	7.7000	7.3000	19.4670	1.6333	0.8667	98.99%	1.01%	5.8915	0.0223	0.0223	6.1901	0.2249	0.2358
26-Jun-14	7.26	7.80	30.60	102.3000	7.7000	15.3000	13.0000	4.0000	3.0000	98.50%	1.50%	6.1450	0.3174	0.3320	6.2212	0.3103	0.3127
04-Jul-14	7.27	10.10	33.20	150.0000	15.0000	11.0000	12.6670	0.3333	0.6667	98.17%	1.83%	6.2135	0.3767	0.4745	6.2271	0.3527	0.3295
08/07/2014	8.17	9.50	31.00	107.3000	7.7000	13.3000	13.6670	1.3333	1.6667	88.69%	11.31%	6.1693	0.2931	0.2716	6.2386	0.2929	0.2892
25/07/2014	7.08	10.50	28.60	135.7000	5.3000	8.7000	14.3330	1.6667	1.3333	99.13%	0.87%	5.7548	0.0305	0.0305	5.8803	0.0542	0.0542
30/07/2014	7.25	11.60	33.60	106.7000	17.3000	14.7000	17.3330	0.6667	0.3333	98.21%	1.79%	5.4529	0.0912	0.0912	5.5886	0.0835	0.0835
04/08/2014	7.34	15.40	32.20	142.0000	6.0000	7.0000	13.0000	0	0	97.99%	2.01%	5.5292	0.1142	0.1142	5.4590	0.0973	0.0973
05/08/2014	7.01	15.80	25.00	115.0000	11.0000	6.0000	14.3000	0.6667	0.3333	99.42%	0.58%	6.1842	0.3598	0.3781	6.2772	0.3460	0.3634
13/08/2014	7.39	17.20	41.20	66.7000	14.3000	7.7000	16.3330	0.6667	1.3333	96.03%	3.97%	6.2050	0.3748	0.3242	6.3076	0.3826	0.3391
21/08/2014	7.25	20.60	43.00	82.0000	5.0000	4.0000	14.3330	0.6667	0.3333	96.75%	3.25%	6.4905	0.3960	0.3634	6.5797	0.3697	0.3570
27/09/2014	7.23	20.00	43.70	594.0000	34.0000	30.0000	19.3330	0.6667	0.3333	96.76%	3.24%	ND	ND	ND	5.5307	0.0256	0.0256
04/10/2014	7.23	17.60	41.50	609.3000	15.7000	28.3000	21.0000	0.0000	0.0000	97.16%	2.84%	ND	ND	ND	ND	ND	ND
10/10/2014	7.37	20.80	44.60	592.7000	14.3000	12.7000	21.3330	0.6667	0.3333	95.33%	4.67%	ND	ND	ND	ND	ND	ND
14/10/2014	7.34	16.60	41.20	600.7000	3.3000	5.7000	20.6670	0.3333	0.6667	96.44%	3.56%	ND	ND	ND	ND	ND	ND
20/10/2014	7.34	18.30	48.30	141.7000	34.3000	17.7000	22.3330	0.6667	0.3333	94.58%	5.42%	ND	ND	ND	ND	ND	ND
13/11/2014	7.34	18.30	43.00	141.0000	6.0000	11.0000	24.0000	1.0000	1.0000	96.03%	3.97%	ND	ND	ND	ND	ND	ND
01/12/2014	7.34	16.60	46.40	56.7000	34.3000	17.7000	30.6670	0.3333	0.6667	95.14%	4.86%	ND	ND	ND	ND	ND	ND
18/12/2014	7.49	17.00	43.90	47.0000	1.0000	1.0000	27.3330	0.6667	0.3333	94.18%	5.82%	ND	ND	ND	ND	ND	ND
20/12/2014	7.49	14.00	45.30	126.0000	9.0000	8.0000	29.0000	1.0000	1.0000	93.69%	6.31%	ND	ND	ND	ND	ND	ND
25/12/2014	7.44	15.50	42.60	83.7000	5.3000	2.7000	32.3330	1.6667	2.3333	95.17%	4.83%	ND	ND	ND	ND	ND	ND
31/12/2014	7.41	16.60	44.60	67.3000	13.7000	18.3000	33.3330	0.6667	1.3333	94.91%	5.09%	ND	ND	ND	ND	ND	ND

Appendix 4: Processed Data for Samples Collected from Lacto- Fermented Sludge



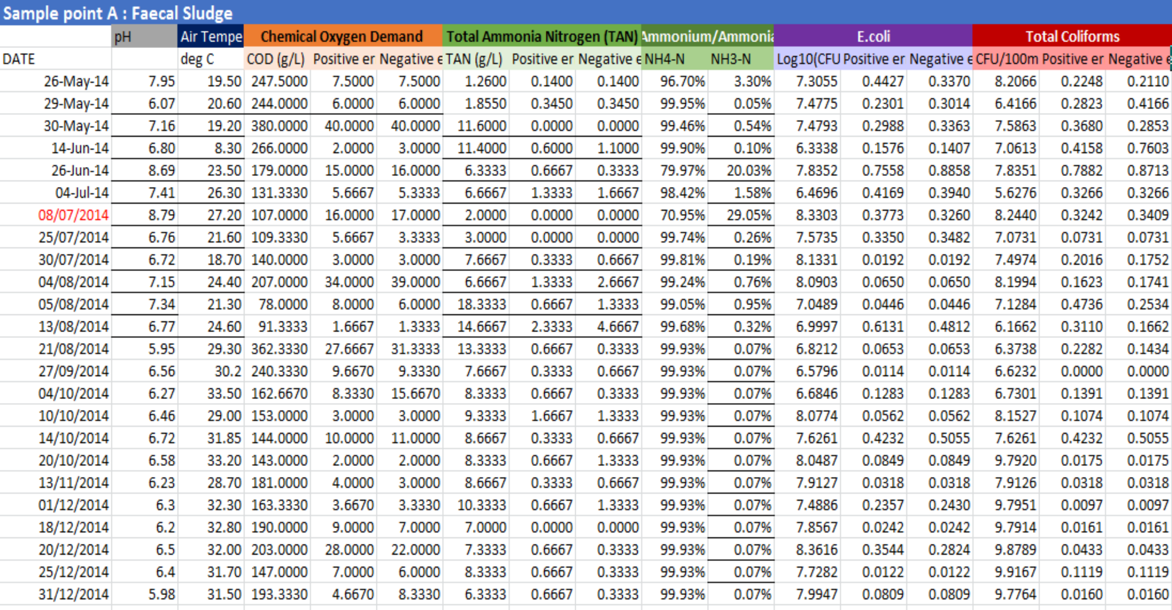
Sample point A : Lacto-fermented sludge																	
	pH	Frozen Te	Air Tempe	Chemical Oxygen Demand			Total Ammonia Nitrogen (TAN)			Ammonium/Ammonia		E.coli			Total Coliforms		
DATE		deg C	deg C	COD (g/L)	Positive e	Negative e	TAN (g/L)	Positive e	Negative e	NH4-N	NH3-N	Log10(CFU	Positive e	Negative e	CFU/100m	Positive e	Negative error
26/05/2014	6.13	11.90	18.40	82.67	10.73	6.47	2.40	1.00	1.00	99.95%	0.05%	7.1044	0.3427	0.3373	7.2428	0.4562	0.4400
29/05/2014	6.31	12.50	22.60	209.00	0.00	0.00	19.55	3.50	3.50	99.90%	0.10%	6.6238	0.2395	0.2400	6.7554	0.2532	0.2783
30/05/2014	6.10	11.00	21.40	285.00	25.00	25.00	24.11	2.39	2.39	99.94%	0.06%	5.5112	0.0799	0.0799	6.0868	0.4317	0.4534
14/06/2014	6.32	8.50	11.10	304.67	36.33	70.67	17.73	1.00	0.13	99.96%	0.04%	7.5410	0.9904	0.8689	7.6928	0.9885	0.9293
26-Jun-14	6.20	7.70	19.10	223.67	13.33	8.67	15.00	0.00	0.00	99.94%	0.06%	7.0896	0.6738	0.5321	7.5832	0.8640	0.8205
04-Jul-14	6.15	9.80	20.80	840.00	38.00	66.00	14.33	0.67	0.33	100.00%	0.00%	7.2771	0.5863	0.5940	8.4983	0.2726	0.2311
08/07/2014	6.70	10.10	19.80	974.67	41.33	50.67	8.00	1.00	1.00	100.00%	0.00%	7.3344	0.7447	0.6755	7.2743	0.5891	0.5123
25/07/2014	6.10	12.90	20.30	931.00	63.00	73.00	8.33	6.67	3.33	99.95%	0.05%	6.7593	0.3774	0.3773	7.1131	0.6029	0.6360
30/07/2014	6.44	12.80	16.10	177.60	0.33	0.67	8.00	1.00	1.00	99.92%	0.08%	8.7168	0.3167	0.3085	8.8243	0.2621	0.2411
04/08/2014	7.06	16.40	15.70	356.33	7.67	9.33	7.00	0.00	0.00	99.67%	0.33%	7.7984	1.0404	1.0138	7.8783	1.0562	1.0496
05/08/2014	7.63	12.10	13.30	442.00	59.00	40.00	7.33	6.67	3.33	98.98%	1.02%	8.5627	0.1777	0.1897	8.6531	0.1730	0.1907
13/08/2014	7.66	15.00	16.10	428.00	18.00	23.00	7.00	0.00	0.00	100.00%	0.00%	5.4169	0.0745	0.0745	5.6829	0.0495	0.0495
21/08/2014	7.67	16.40	13.40	351.33	32.67	31.33	14.33	0.67	0.33	100.00%	0.00%	5.5590	0.0538	0.0538	6.1214	0.3557	0.3361
03/12/2014	4.31		28.10									ND	ND	ND	5.8248	0.7067	0.8706
07/12/2014	4.39		28.30									ND	ND	ND	5.8101	0.8527	0.8558
09/12/2014	4.80		31.50									ND	ND	MD	6.2474	0.6717	0.8002
11/12/2014	4.90		32.70									ND	ND	ND	5.6276	0.0256	0.0256
13/12/2014	5.01		24.40									5.9783	0.7206	0.5312	6.4309	0.5136	0.3481
15/12/2014	6.30		33.90									6.1908	0.0447	0.0447	6.3872	0.1313	0.1247

Appendix 5: Processed Data for Samples Collected from Urine



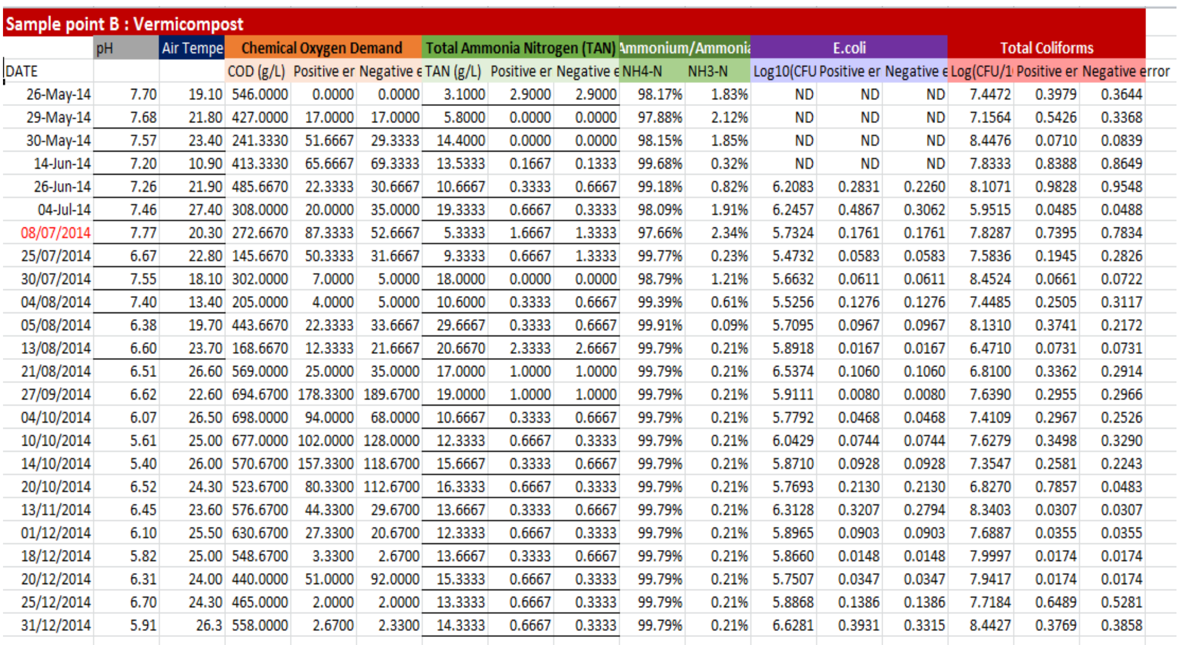
Sample point B : Urine																	
	pH	Frozen Te	Air Tempe	Chemical Oxygen Demand			Total Ammonia Nitrogen (TAN)			Ammonium/Ammonia		E.coli			Total Coliforms		
DATE		deg C		COD (g/L)	Positive e	Negative e	TAN (g/L)	Positive e	Negative e	NH4-N	NH3-N	Log10(CFU	Positive e	Negative e	Log(CFU/1	Positive e	Negative e
26/05/2014	9.78	10.10	17.30	639.00	0.00	0.00	10.57	5.40	0.89	66.75%	33.25%	ND	ND	ND	ND	ND	ND
29/05/2014	9.67	14.00	17.30	1085.00	0.00	0.00	23.10	2.29	2.29	39.65%	60.35%	**ND**	**ND**	ND	ND	ND	ND
30/05/2014	9.63	16.00	19.00	563.00	0.00	0.00	26.20	7.80	7.80	38.84%	61.16%	ND	ND	ND	ND	ND	ND
14/06/2014	9.92	6.40	6.80	1110.33	86.67	139.33	18.67	1.10	0.80	45.40%	54.60%	ND	ND	ND	ND	ND	ND
26/06/2014	9.65	7.50	16.50	1020.33	11.67	11.33	25.33	1.67	1.33	42.20%	57.80%	ND	ND	ND	ND	ND	ND
04-Jul-14	9.60	9.00	21.30	961.67	54.33	52.67	18.33	0.67	0.33	36.52%	63.48%	ND	ND	ND	ND	ND	ND
08/07/2014	9.55	10.70	20.30	1183.67	217.33	255.67	11.67	1.33	0.67	40.97%	59.03%	ND	ND	ND	ND	ND	ND
25/07/2014	9.53	11.10	15.80	1264.67	9.33	7.67	13.33	0.67	1.33	50.36%	49.64%	ND	ND	ND	ND	ND	ND
30/07/2014	9.44	11.00	15.20	878.00	116.00	2.00	14.00	0.00	0.00	56.63%	43.37%	ND	ND	ND	ND	ND	ND
04/08/2014	9.47	16.30	13.90	700.33	0.67	0.63	17.33	0.67	0.33	57.36%	42.64%	ND	ND	ND	ND	ND	ND
05/08/2014	9.61	14.40	17.00	644.67	135.33	110.67	10.00	1.00	1.00	43.54%	56.46%	ND	ND	ND	ND	ND	ND
13/08/2014	9.32	16.20	15.20	615.67	10.33	15.67	9.67	1.33	1.67	63.26%	36.74%	ND	ND	ND	ND	ND	ND
21/08/2014	9.38	16.40	13.90	622.67	17.33	26.67	17.33	1.67	2.33	62.33%	37.67%	ND	ND	ND	ND	ND	ND
03/12/2014	9.02		31.30									ND	ND	ND	ND	ND	ND
07/12/2014	8.90		31.50									ND	ND	ND	ND	ND	ND
09/12/2014	9.32		32.30									ND	ND	ND	ND	ND	ND
11/12/2004	9.81		29.00									ND	ND	ND	ND	ND	ND
13/12/2014	9.37		24.10									ND	ND	ND	ND	ND	ND
15/12/2014	9.35		23.30									ND	ND	ND	ND	ND	ND

Appendix 6: Processed Data for Samples Collected from Vermicompost Toilet Faecal Sludge



Sample point A : Faecal Sludge																
	pH	Air Tempe	Chemical Oxygen Demand			Total Ammonia Nitrogen (TAN)			Ammonium/Ammonia		E.coli			Total Coliforms		
DATE		deg C	COD (g/L)	Positive er	Negative e	TAN (g/L)	Positive er	Negative e	NH4-N	NH3-N	Log10(CFU	Positive er	Negative e	CFU/100m	Positive er	Negative e
26-May-14	7.95	19.50	247.5000	7.5000	7.5000	1.2600	0.1400	0.1400	96.70%	3.30%	7.3055	0.4427	0.3370	8.2066	0.2248	0.2110
29-May-14	6.07	20.60	244.0000	6.0000	6.0000	1.8550	0.3450	0.3450	99.95%	0.05%	7.4775	0.2301	0.3014	6.4166	0.2823	0.4166
30-May-14	7.16	19.20	380.0000	40.0000	40.0000	11.6000	0.0000	0.0000	99.46%	0.54%	7.4793	0.2988	0.3363	7.5863	0.3680	0.2853
14-Jun-14	6.80	8.30	266.0000	2.0000	3.0000	11.4000	0.6000	1.1000	99.90%	0.10%	6.3338	0.1576	0.1407	7.0613	0.4158	0.7603
26-Jun-14	8.69	23.50	179.0000	15.0000	16.0000	6.3333	0.6667	0.3333	79.97%	20.03%	7.8352	0.7558	0.8858	7.8351	0.7882	0.8713
04-Jul-14	7.41	26.30	131.3330	5.6667	5.3333	6.6667	1.3333	1.6667	98.42%	1.58%	6.4696	0.4169	0.3940	5.6276	0.3266	0.3266
08/07/2014	8.79	27.20	107.0000	16.0000	17.0000	2.0000	0.0000	0.0000	70.95%	29.05%	8.3303	0.3773	0.3260	8.2440	0.3242	0.3409
25/07/2014	6.76	21.60	109.3330	5.6667	3.3333	3.0000	0.0000	0.0000	99.74%	0.26%	7.5735	0.3350	0.3482	7.0731	0.0731	0.0731
30/07/2014	6.72	18.70	140.0000	3.0000	3.0000	7.6667	0.3333	0.6667	99.81%	0.19%	8.1331	0.0192	0.0192	7.4974	0.2016	0.1752
04/08/2014	7.15	24.40	207.0000	34.0000	39.0000	6.6667	1.3333	2.6667	99.24%	0.76%	8.0903	0.0650	0.0650	8.1994	0.1623	0.1741
05/08/2014	7.34	21.30	78.0000	8.0000	6.0000	18.3333	0.6667	1.3333	99.05%	0.95%	7.0489	0.0446	0.0446	7.1284	0.4736	0.2534
13/08/2014	6.77	24.60	91.3333	1.6667	1.3333	14.6667	2.3333	4.6667	99.68%	0.32%	6.9997	0.6131	0.4812	6.1662	0.3110	0.1662
21/08/2014	5.95	29.30	362.3330	27.6667	31.3333	13.3333	0.6667	0.3333	99.93%	0.07%	6.8212	0.0653	0.0653	6.3738	0.2282	0.1434
27/09/2014	6.56	30.2	240.3330	9.6670	9.3330	7.6667	0.3333	0.6667	99.93%	0.07%	6.5796	0.0114	0.0114	6.6232	0.0000	0.0000
04/10/2014	6.27	33.50	162.6670	8.3330	15.6670	8.3333	0.6667	0.3333	99.93%	0.07%	6.6846	0.1283	0.1283	6.7301	0.1391	0.1391
10/10/2014	6.46	29.00	153.0000	3.0000	3.0000	9.3333	1.6667	1.3333	99.93%	0.07%	8.0774	0.0562	0.0562	8.1527	0.1074	0.1074
14/10/2014	6.72	31.85	144.0000	10.0000	11.0000	8.6667	0.3333	0.6667	99.93%	0.07%	7.6261	0.4232	0.5055	7.6261	0.4232	0.5055
20/10/2014	6.58	33.20	143.0000	2.0000	2.0000	8.3333	0.6667	1.3333	99.93%	0.07%	8.0487	0.0849	0.0849	9.7920	0.0175	0.0175
13/11/2014	6.23	28.70	181.0000	4.0000	3.0000	8.6667	0.3333	0.6667	99.93%	0.07%	7.9127	0.0318	0.0318	7.9126	0.0318	0.0318
01/12/2014	6.3	32.30	163.3330	3.6670	3.3330	10.3333	0.6667	1.3333	99.93%	0.07%	7.4886	0.2357	0.2430	9.7951	0.0097	0.0097
18/12/2014	6.2	32.80	190.0000	9.0000	7.0000	7.0000	0.0000	0.0000	99.93%	0.07%	7.8567	0.0242	0.0242	9.7914	0.0161	0.0161
20/12/2014	6.5	32.00	203.0000	28.0000	22.0000	7.3333	0.6667	0.3333	99.93%	0.07%	8.3616	0.3544	0.2824	9.8789	0.0433	0.0433
25/12/2014	6.4	31.70	147.0000	7.0000	6.0000	8.3333	0.6667	0.3333	99.93%	0.07%	7.7282	0.0122	0.0122	9.9167	0.1119	0.1119
31/12/2014	5.98	31.50	193.3330	4.6670	8.3330	6.3333	0.6667	0.3333	99.93%	0.07%	7.9947	0.0809	0.0809	9.7764	0.0160	0.0160

Appendix 7: Processed Data for Samples Collected from Vermicompost



Sample point B : Vermicompost																
	pH	Air Tempe	Chemical Oxygen Demand			Total Ammonia Nitrogen (TAN)			Ammonium/Ammonia		E.coli			Total Coliforms		
DATE			COD (g/L)	Positive er	Negative e	TAN (g/L)	Positive er	Negative e	NH4-N	NH3-N	Log10(CFU	Positive er	Negative e	Log(CFU/1	Positive er	Negative error
26-May-14	7.70	19.10	546.0000	0.0000	0.0000	3.1000	2.9000	2.9000	98.17%	1.83%	ND	ND	ND	7.4472	0.3979	0.3644
29-May-14	7.68	21.80	427.0000	17.0000	17.0000	5.8000	0.0000	0.0000	97.88%	2.12%	ND	ND	ND	7.1564	0.5426	0.3368
30-May-14	7.57	23.40	241.3330	51.6667	29.3333	14.4000	0.0000	0.0000	98.15%	1.85%	ND	ND	ND	8.4476	0.0710	0.0839
14-Jun-14	7.20	10.90	413.3330	65.6667	69.3333	13.5333	0.1667	0.1333	99.68%	0.32%	ND	ND	ND	7.8333	0.8388	0.8649
26-Jun-14	7.26	21.90	485.6670	22.3333	30.6667	10.6667	0.3333	0.6667	99.18%	0.82%	6.2083	0.2831	0.2260	8.1071	0.9828	0.9548
04-Jul-14	7.46	27.40	308.0000	20.0000	35.0000	19.3333	0.6667	0.3333	98.09%	1.91%	6.2457	0.4867	0.3062	5.9515	0.0485	0.0488
08/07/2014	7.77	20.30	272.6670	87.3333	52.6667	5.3333	1.6667	1.3333	97.66%	2.34%	5.7324	0.1761	0.1761	7.8287	0.7395	0.7834
25/07/2014	6.67	22.80	145.6670	50.3333	31.6667	9.3333	0.6667	1.3333	99.77%	0.23%	5.4732	0.0583	0.0583	7.5836	0.1945	0.2826
30/07/2014	7.55	18.10	302.0000	7.0000	5.0000	18.0000	0.0000	0.0000	98.79%	1.21%	5.6632	0.0611	0.0611	8.4524	0.0661	0.0722
04/08/2014	7.40	13.40	205.0000	4.0000	5.0000	10.6000	0.3333	0.6667	99.39%	0.61%	5.5256	0.1276	0.1276	7.4485	0.2505	0.3117
05/08/2014	6.38	19.70	443.6670	22.3333	33.6667	29.6667	0.3333	0.6667	99.91%	0.09%	5.7095	0.0967	0.0967	8.1310	0.3741	0.2172
13/08/2014	6.60	23.70	168.6670	12.3333	21.6667	20.6670	2.3333	2.6667	99.79%	0.21%	5.8918	0.0167	0.0167	6.4710	0.0731	0.0731
21/08/2014	6.51	26.60	569.0000	25.0000	35.0000	17.0000	1.0000	1.0000	99.79%	0.21%	6.5374	0.1060	0.1060	6.8100	0.3362	0.2914
27/09/2014	6.62	22.60	694.6700	178.3300	189.6700	19.0000	1.0000	1.0000	99.79%	0.21%	5.9111	0.0080	0.0080	7.6390	0.2955	0.2966
04/10/2014	6.07	26.50	698.0000	94.0000	68.0000	10.6667	0.3333	0.6667	99.79%	0.21%	5.7792	0.0468	0.0468	7.4109	0.2967	0.2526
10/10/2014	5.61	25.00	677.0000	102.0000	128.0000	12.3333	0.6667	0.3333	99.79%	0.21%	6.0429	0.0744	0.0744	7.6279	0.3498	0.3290
14/10/2014	5.40	26.00	570.6700	157.3300	118.6700	15.6667	0.3333	0.6667	99.79%	0.21%	5.8710	0.0928	0.0928	7.3547	0.2581	0.2243
20/10/2014	6.52	24.30	523.6700	80.3300	112.6700	16.3333	0.6667	0.3333	99.79%	0.21%	5.7693	0.2130	0.2130	6.8270	0.7857	0.0483
13/11/2014	6.45	23.60	576.6700	44.3300	29.6700	13.6667	0.3333	0.6667	99.79%	0.21%	6.3128	0.3207	0.2794	8.3403	0.0307	0.0307
01/12/2014	6.10	25.50	630.6700	27.3300	20.6700	12.3333	0.6667	0.3333	99.79%	0.21%	5.8965	0.0903	0.0903	7.6887	0.0355	0.0355
18/12/2014	5.82	25.00	548.6700	3.3300	2.6700	13.6667	0.3333	0.6667	99.79%	0.21%	5.8660	0.0148	0.0148	7.9997	0.0174	0.0174
20/12/2014	6.31	24.00	440.0000	51.0000	92.0000	15.3333	0.6667	0.3333	99.79%	0.21%	5.7507	0.0347	0.0347	7.9417	0.0174	0.0174
25/12/2014	6.70	24.30	465.0000	2.0000	2.0000	13.3333	0.6667	0.3333	99.79%	0.21%	5.8868	0.1386	0.1386	7.7184	0.6489	0.5281
31/12/2014	5.91	26.3	558.0000	2.6700	2.3300	14.3333	0.6667	0.3333	99.79%	0.21%	6.6281	0.3931	0.3315	8.4427	0.3769	0.3858

Appendix 8: Summary on Anaerobic Digester Toilet Usage

	April	May	June	July	August	Septembe	October	November	December
Number of people Urinating	303	835	465	795	210	231	709	982	82
Number of people Defaecating	384	942	556	859	147	456	833	850	54
Sub Total	**687**	**1777**	**1021**	**1654**	**357**	**687**	**1542**	**1832**	**136**
Amount of water used by people	926.5	2280	1257	2116.2	398	902.5	1502	1241	115
General Claening Water used	98	373	270	414	220	122	650	286	85
Sub Total(litres)	**1024.5**	**2653**	**1527**	**2530.2**	**618**	**1024.5**	**2152**	**1527**	**200**

Appendix 9: Detailed specifications of Anaerobic Digester

Anaerobic Digester		
	Input volume	360L/day
	Digester Capacity	13,000L
	Hydraulic retention time in digester	36 days
	Automatic agitation Manual agitation	Yes Yes
Gas Storage System		
	Estimated Maximum biogas production	10,000L/day
	Storage capacity of one bag	1,850 L
	No. of bags	4 bags
	Total storage	7400L
Pasteurisation system		
	Length of Pasteu tube	33.5m
	Maximum depth in Pasteu tube	0.17m
	Volume in Pasteu tube	760L
	Retention time in tube at input of 360L/d	2 days
Disposal System		
	Liquid capacity of single "donut"	440L
	No. of "donuts"	3 off
	Total liquid capacity	1,320 L
	Refill cycle time at input of 350L/d	3 days
	Total soil filtration volume	3.84 m^3