The applicability of anaerobically digested pasteurized pit latrine faecal sludge as a fertilizer to grow Radish and Garden cress

A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE (PHARMACY)

of

RHODES UNIVERSITY

by

Phindile Madikizela

April

2016

Faculty of Pharmacy

Rhodes University

Grahamstown

South Africa

Abstract

Pit latrine faecal sludge was recovered from numerous pit latrines in Hlalani Township, Grahamstown, South Africa. This material was used to prepare a fertilizer to demonstrate the value that could be captured from faecal sludge. Further anaerobic digestion, together with a co-feed demonstrated the potential of faecal sludge to produce low cost fertilizer that could be used to grow food crops. Biogas recovered from the anaerobic digester could be used to pasteurize its effluent, although effective biogas recovery and storage needs to be further addressed. Investigating the microbial community of the different depths of the pit latrine through molecular techniques showed that the fermenting bacteria family *Clostridiaceae* was the most commonly identified family throughout the different depths of the pit latrine, and that the microbial community within pit latrines was very diverse with bacterial families that are involved in nitrogen fixation, denitrification, and iron and sulphate reduction. Additionally, most of the bacterial families that dominated the seven studied pit latrines had members that were known human pathogens (*Mycobacteriaceae, Dermatophilaceae Peptostreptococcaceae, Micrococcaceae, Staphylococcaceae, Leptospiraceae*, *Listeriaceae, Bradyrhizobiaceae* and *Brucellaceae*). Effluent from a wastewater treatment works was selected as a co-feed to augment biogas production. The most successful faecal sludge and co-feed combination was shown to be the one made up of 33% and 66% pit latrine faecal sludge. 180 L of this effluent mixture generated 285 L of biogas over 45 days of anaerobic digestion (29±2°C). However, the recovered quantities were insufficient for pasteurization as 650 L of biogas was required to pasteurize 300 g of faecal sludge for 1 hour at 70±2°C. Therefore, liquid petroleum gas (LPG) was used as an alternative heating energy source. Anaerobic digestion and pasteurization rendered the faecal sludge safe for application as a fertilizer as the quality of the faecal sludge after treatment by anaerobic digestion and pasteurization was within the microbiological (*Escherichia coli, Salmonella* spp, *Enterococcus faecium* and helminth eggs) and trace element restrictions (Pb, Ni, Cr, Mo, As, Cu, Mn, Fe, Cd and Hg) of sludge application in agriculture as stipulated by the WHO and the South African Guidelines for Sludge Use in Agriculture. Radish (*Raphanus sativus* spp) and garden cress (*Lepidium sativum*) were cultivated to demonstrate the effectiveness of the anaerobically digested and pasteurized pit latrine faecal sludge as a fertilizer. Diluting the fertilizer prepared from faecal sludge did not reduce its efficacy and was comparable to the synthetic fertilizer used as a control in the growth trials in terms of the plant fresh weight, dry weight and plant height. Finally, the exposure to the current

state of pit latrines in Hlalani Township provided an incentive to develop a new tool to address sanitation service delivery skill shortage (artisans, plant operation and maintenance workers, and sanitation and hygiene facilitators) through the use of volunteers.

Contents

List of Tables

List of Figures

List of publications and presentations from this thesis

Chapter 5 of the thesis is based on the following paper:

Hoossein, S.; Tandlich, R.; Whittington-Jones, K.; Laubscher, R.; Madikizela, P.; Zuma, B., 2016. Disaster Management Policy Options to Address the Sanitation Challenges in South Africa. *Journal of Environmental Health*, 78(7), pp. E1-E7.

Acknowledgements

To God be the glory. I profoundly thank my supervisors, Dr R. Tandlich and Mr R. Laubscher for their devotion, assistance and guidance throughout this study, most importantly for allowing me to be your student. Thank you Dr Tandlich for sending me to the 3rd International Faecal Sludge Management Conference in Hanoi, Vietnam. Additionally, I also extend my gratitude to the staff and the postgraduates of the Faculty of Pharmacy and the Institute for Environmental Biotechnology, Rhodes University (EBRU) for assisting me throughout this study. Mr M. and Dr G. Mutero from the Grahamstown State Veterinary Laboratory, thank you for availing your resources and your skills when needed during this study. Mr M. Lepile, if it was not for you sir, sampling would have been quite a challenge thank you for always ensuring that our sampling expeditions were very peaceful. I would also like to thank the Makana Municipality for availing the vacuum truck for our sampling expeditions. To my family and friends who supported me throughout this study, who encouraged me to keep pushing even though at times I felt like giving up, your love and support is much appreciated. Postgraduate colleagues in the Environmental Health and Biotechnology Research Group in the Faculty of Pharmacy, salute! Tha Finally, I would like to thank Water Research Commission (WRC-Project K5/2306) and National Research Foundation (NRF) for the financial assistance during my studies.

Acronyms and abbreviations

^0C	-Degrees Celsius
AD	- Anaerobic digester
APHA	- American Public Health Association
As	- Arsenic
AWWA	-American Water Works Association
Ca	- Calcium
Cd	- Cadmium
CE	-Controlled Environment
CFU	- Colony forming unit
CH_4	- Methane
Co	- Cobalt
COD	- Chemical Oxygen Demand
Cogta	-Department of Cooperative Governance and Traditional Affairs
Cr	- Chromium
Cu	- Copper
DAFF	- Department of Agriculture, Forestry and Fisheries
DEA	- Department of Environmental Affairs
DMA	- Disaster Management Act
DMRDM	- Draft Disaster Management Regulations: Disaster Management
DNA	- Deoxyribonucleic acid
EcoSan	- Ecological Sanitation
EPWP	-Extended Public Works Programme
FC	- Faecal coliforms
Fe	-Iron
FS	- Faecal Sludge
Hg	-Mercury
ID	- Identity document
JMP	- Joint Monitoring Programme
LOI	- Loss on Ignition

LPG	- Liquid Petroleum Gas
Mg	-Magnesium
MGD	- Millennium Development Goals
MJ	- Mega Joules
Mn	- Manganese
Mo	-Molybdenum
Natech	- Natural Hazard Triggering Technological Disasters
NCOP	-National Council of Provinces
NDMETF	-National Disaster Management Education and Training
NGO	-Non Governmental Organisation
Ni	-Nickel
NPK	-Nitrogen, Phosphorus and Potassium
Pb	- Lead
PMG	-Parliamentary Monitoring Group
SAHRC	-South African Human Rights Commission
StatsSA	-Statistics South Africa
SuSana	- Sustainable Sanitation
TN	-Total nitrogen
TP	-Total phosphate
TS	-Total solids
TVS	-Total volatile solids
UN	- United Nations
UNICEF	- United Nations Children's Fund
UNW	-University of Northwest
USD	-United States of America Dollar
USEPA	-United States Environmental Protection Agency
UV	-Ultraviolet
VIP	- Ventilated Improved Pit latrine
WEF	-Water Environment Federation
WHO	-World Health Organisation

WWTW **-** Wastewater treatment Works

$ZnSO_4$ -Zinc Sulphate

Chapter 1: Introduction

1.1 Background

Sanitation refers to the principles and practices linked to the safe collection, storage and disposal or removal of human excreta (National Sanitation Task Team, 1996). Sanitation facilities include waterborne sanitation, pit latrines, ventilated improved pit latrines, urine diversion pit latrines, double vault latrines, composting toilets and many other types (National Sanitation Task Team, 1996; Buckley, et al., 2008). In developing countries, the most common sanitation facilities are pit latrines, ventilated improved pit latrines and urine diversion latrines (Feachem, et al., 1983). Ventilated improved pit latrines are the most common in peri-urban and rural areas of South Africa (Buckley, et al., 2008). Improper maintenance and operation of pit latrines causes problems such as short filling up periods and social unrest from the offensive odour (Tilley, et al., 2014). When the latrines are full, the municipality assumes the role of emptying the contents of the pit latrines and disposal in land fill sites or at wastewater treatment plants (Feachem, et al., 1983; Chinyama, et al., 2012). This service depends on the accessibility of the serviced area to the vacuum truck and availability of financial resources in the municipality but in some countries manual pit emptying is still the most common method.

The composition of human excreta depends on the age, gender, diet, health condition and the behavioural practices of individuals (Jensen, et al., 2008; Meinzinger, et al., 2009). Theoretically, 65-85% of human excreta is made up of water with 15-30% being particulate organic and inorganic matter (Feachem, et al., 1983; Buckley, et al., 2008). In faecal sludge, water originates from urine and greywater with organic and inorganic matter being made up of faeces, anal cleansing material and personal care products (Feachem, et al., 1983; Jensen, et al., 2008; Meinzinger, et al., 2009). High concentration of pathogenic microbes, such as bacteria (*Salmonella* spp. and *Vibrio Cholerae*), parasitic protozoa (*Cryptosporidium parvum,* *Giardia intestinalis*), viruses (rotavirus and hepatitis A virus) and helminths (*Ascaris lumbricoides, Trichuris trichura*) are found in faecal matter (Feachem, et al., 1983). Beneficial elements such as nitrogen, potassium and phosphorus are present in the excrement (Jensen, et al., 2008; Meinzinger, et al., 2009). Faecal matter has been used since ancient times as a soil conditioner and a fertilizer in China, Rome, Europe, Latin America and Africa (Buckley, et al., 2008; Mnkeni & Austin, 2008; Meinzinger, et al., 2009). The used treatment strategy for faecal sludge prior to use involved composting by mixing with animal manure, wood, ash, lime and other material under aerobic and anaerobic conditions (Esrey, et al., 2001; Duncker, et al., 2007; Drangert & Nawab, 2011). Depending on the composting conditions, the faecal matter must be stored up to 2 years to render it safe for application as a fertilizer (Jensen, et al., 2008; Mnkeni & Austin, 2008). Composting alters the repulsive appearance of faecal matter and significantly reduces the environmental health hazards posed by the presence of pathogens (Esrey, et al., 2001; Duncker, et al., 2007).The pathogenic microorganisms that are present in the faecal sludge are also reduced significantly to levels that allow the reuse of the faecal sludge as a compost (Esrey, et al., 2001; Duncker, et al., 2007)

The use of human excreta to grow fruit trees such as guava, banana, apricot, including crops and vegetables such as maize, barley, spinach, lettuce and tomato is documented in literature (Duncker, et al., 2007; Jensen, et al., 2008; Meinzinger, et al., 2009). Pathogenic microbes, parasitic protozoa, viruses and helminths are significantly reduced by composting (Esrey, et al., 2001; WHO, 2006; Jensen, et al., 2008; Mnkeni & Austin, 2008). The most important pathogenic microbes, such as *Escherichia coli*, *Salmonella* spp. viruses, such as rotavirus, hepatitis A, parasitic protozoa, such as *Cryptosporidium parvum, Giardia intestinalis,* and helminths (*Ascaris lumbricoides* and *Taenia linnsaginia*) have to be reduced significantly in fertilizers due to their faecal-oral route of transmission (Feachem, et al., 1983; WHO, 2006; Mnkeni & Austin, 2008). *Ascaris* eggs spp. are very persistent in the soil and can last up to a year or more (Esrey, et al., 2001; WHO, 2006). The most common route of transmission of these pathogens is oral transmission through ingestion of vegetables or food, soil/dust particles, that are contaminated by the pathogens or inhalation of spores that are suspended in the air (Feachem, et al., 1983; Mininni & Santori, 1987; Karak & Bhattacharyya, 2011). As such, contact with the fertilizer that is produced from human excrement should be avoided (Feachem, et al., 1983; WHO, 2006). Additionally, vegetables that are grown on the soil that is fertilized with human excrement should be washed thoroughly before consumption (Feachem, et al., 1983; WHO, 2006).

1.2 Problem statement

The human excreta contain macro and micronutrients which indicate the potential application of the excreta as a fertilizer (Guzha, et al., 2005; Jensen, et al., 2008). However, the high concentration of pathogenic microorganisms that are present in the human excreta pose public health and environmental risks (Feachem, et al., 1983; Jena, et al., 2016). Therefore, if human excreta is to be used as a fertilizer, these risks have to be addressed prior to its application as a fertilizer (Mininni & Santori, 1987; Mnkeni & Austin, 2008). Various treatment strategies exist to aid in mitigating the risks that are posed by the pathogens in the human excrement (Feachem, et al., 1983; Karak & Bhattacharyya, 2011). One of those strategies involves anaerobic digestion, a biological process that reduces the organic matter of the excreta while simultaneously producing biogas (Kawai, et al., 2012; Grim, et al., 2015). If recovered, this gas can be used as an energy source to fuel further treatment strategies, such as pasteurization, to render the human excreta safe for use as a fertilizer (SuSana, 2012; Kawai, et al., 2012).

1.3 The aims and objectives of the study

The aim of the study was to investigate the applicability of the anaerobic digested pasteurized faecal sludge from the pit latrines of Hlalani Township in Makana Municipality, Grahamstown, South Africa as a fertilizer. Specific objectives included the collection of pit latrine faecal sludge, the analysis of physicochemical and microbiological properties of the faecal sludge, investigate the microbial community structure at different depths of the pit latrine faecal sludge the anaerobic digestion and pasteurization of the faecal sludge, the microbiological analysis of the pasteurized effluent and the application of the pasteurized effluent as a liquid fertilizer to grow crops. Lastly, to review the South African Disaster Management Policies and Legislation to develop a new tool that could be used to address sanitation service delivery skill shortages such as sanitation and hygiene facilitators, artisans, plant operation and maintenance labourers through the use of volunteer units across the country.

1.4 Thesis organisation

Chapter 1; this chapter gives the background information about the research project. It also states the aims and objectives of the study.

Chapter 2; the chapter covers the published literature about sanitation coverage, uses of sanitation products, including some legislation that serve as a guide for the safe use of sanitation products.

Chapter 3; provides detailed methodology that was followed in order to meet the aims and objectives of the study.

Chapter 4; provides the findings of the study, the discussion of the findings in detail, key conclusions and recommendations.

Chapter 5; provides details about the development and the application of a disaster management tool that could be used to address sanitation service delivery backlogs in municipalities throughout South Africa.

References; is a list of all the cited literature which has been organised using the Harvard style of referencing.

Chapter 2: Literature review

2.1 Global sanitation coverage

According to the millennium development goals (MDG), the lack of access to sustainable sanitation was supposed to be halved by the year 2015. In 2012 approximately 2.5 billion of global population lacked access to improved sanitation with 15% practicing open defecation (UN, 2014). The World Health Organization (WHO) United Nations Children's Fund (UNICEF) Joint Monitoring Programme for water supply and sanitation (JMP) 2013 update showed that the global sanitation coverage was 64% in 2011 which was said to be far below the sanitation target of 75% set for 2015 (WHO/UNICEF, 2013).The majority of the population with no access to sanitation resided in developing countries with 90% living in rural areas (WHO/UNICEF, 2013). Eastern Asia (67%) had the highest improved sanitation coverage in 2011 with Sub-Saharan Africa (30%) showing the slowest progress (UN, 2014). Open defecation which was still prevalent in some developing countries (Nigeria and India) posed serious health and environmental risks as the contents of the faecal matter might contaminate the water bodies and the soil (UN, 2014). The new policies that focus on averting open defecation at community level were successful in some developing countries (WHO/UNICEF, 2013). Success was achieved through the influences in social norms within the society leading to enhanced sanitation coverage by employing community-led sanitation interventions (Lüthi, et al., 2011; WHO/UNICEF, 2013).

Due to the anticipated inability to meet the MDG sanitation target by 2015, a contingency plan, as a way forward post 2015, was formulated which incorporated complete elimination of open defecation by 2025 under the newly formed sustainable development goals (UN, 2014). By 2050, it was postulated that everyone should have adequate sanitation facilities at home (UN, 2014). Adequate sanitation according to the sanitation policy of South Africa is a constant system of maintenance and operation of disposal of human excreta (National sanitation task team, 1996; Hoossein, et al., 2014).The relevant obstacles hindering the improvement of sanitation service delivery were identified (WHO/UNICEF, 2013; UN, 2014). Amongst those was the shortage of investment in the sanitation sector, lack of political will and the difficulties in maintaining the services (Montgomery & Elimelich, 2007; UN, 2014). That led to a call for a strong collaboration between the relevant stakeholders to come up with strategies that are holistic to address the aforementioned issues including the cultural problems (UN, 2014; Kenny, 2013). These could potentially lead to the formulation of evidence based policies and regulations that permit effective investments and put emphasis on solutions that can be managed and maintained locally. An example of this is the community-led sanitation intervention (Vandermoortele, 2012; Kenny, 2013). This is a sustainable approach that supports communities by prioritizing their needs and promotes the use of local material or readily available materials to address their high priority problems thereby encouraging local innovation (Lüthi, et al., 2011).

Basic sanitation facilities are in demand due to rapid population growth in peri-urban and urban areas especially in developing countries (Schouten & Mathenge, 2010). Most of this population growth occurs in the slums where the sanitation infrastructures are difficult to construct due to lack of building space (Hanchett, et al., 2003). Additionally, the construction of informal settlements in uninhabitable lands also adds to the problem as construction of sanitation facilities sometimes becomes impossible due to the terrain (Mels, et al., 2009). One dominating problem is the inability of the already existing sewage collection systems to keep up with the increasing demand due to population explosions in urban areas (Chinyama, et al., 2012). The rapid population growth in urban areas is partly influenced by poverty, job opportunities and the perceived improvement of the population's living standard (Li, et al., 2012). High population densities and poverty in urban slums make provision of sanitation facilities very difficult because of accessibility in some developing countries (Schouten & Mathenge, 2010). The living conditions in the slums including the absence of sanitation facilities compel people to practice open defecation which is unhygienic thereby exposing people to health risks (Hanchett, et al., 2003). Furthermore, this practice poses environmental risks because human excreta contains pathogenic microorganism which result to health and environmental risks (Feachem, et al., 1983). In Rwanda, diarrhoea a sanitation-related disease, was responsible for 11.3 % mortality rate in children under the age of five (SuSana, 2012). Poor access to basic sanitation in Kenya, and insufficient water supply is responsible for almost 60 to 68% of the infectious diseases with cholera as the leading disease (SuSana, 2012). Most of these diseases are prevalent in rural communities where open defecation is still practiced due to the lack of basic sanitation. In August of 2000, there was an outbreak of cholera in KwaZulu Natal, which resulted to 259 deaths, thereby motivating the government to focus on addressing backlogs pertaining to water and sanitation (Nwaneri, 2009). The highlighted examples show the significance of properly managing faecal sludge as mismanagement might have severe health and environmental consequences.

2.2 Types of sanitation facilities and their management

Sanitation is not only about providing sanitation facilities but to also show the user's ways of operating and maintaining such facilities. The sanitation facilities vary from waterborne sanitation (flush toilets), pit latrines (ventilated improved pit latrine, urine diversion latrine, double vault pit latrine) to the bucket system (Bhagwan, et al., 2008; Tilley, et al., 2014). Waterborne sanitation facilities, given that they are home based and not communal facilities, do not have the same maintenance and operation problems as pit latrines (Chinyama, et al., 2012). These sanitation facilities are prevalent in developed countries with pit latrines commonly found in developing countries (Schouten & Mathenge, 2010; Chinyama, et al., 2012; Tilley, et al., 2014). Inappropriate operating or maintenance of the sanitation facilities (refer to figure 1) results in the shortened filling up periods, evolution of offensive odours, breeding zones for disease vectors and various other problems in pit latrines (Feachem, et al., 1983; Buckley, et al., 2008). Filling up becomes a major problem as intensive labour is involved in constructing a new structure (Meinzinger, et al., 2009). Secondly, space requirement for the new structure and cost of the material that is required for constructing the new structure also cause problems (Schouten & Mathenge, 2010). Approximately, R4000 ($274.63 USD) is required to construct a lined new VIP, but this cost is highly subsidised by the government of South Africa (Dunker & Matsebe, 2008). Other problems that are associated with constructing pit latrines, include possible underground water contamination, and soil contamination, more especially in instances where the latrine is not lined at the bottom (Chaggu, 2004). This could lead to the outbreak of diseases such as cholera (Rebaudet, et al., 2013).

The process of sludge stabilization in the pit latrines supposedly involves both anaerobic and aerobic digestion (Nwaneri, 2009). This can be attributed to the fact that the upper part of the sludge is exposed to oxygen. When well maintained and operated, the pit latrines may last for up to 5 years before filling up but some might last even longer than that (Still & Foxon, 2012). However there is no accurate information about how long a pit latrine in reality takes to fill up because many factors contribute (Still & Foxon, 2012). The material used for anal cleansing, personal care products, greywater and underground water including the run-off water during rainy seasons are thought to contribute to the filling up process (Strauss, et al., 2000; Still & Foxon, 2012). In South Africa, local municipalities are mandated to service the pit latrines through emptying as stipulated in the Waste Act of 2008 (DEA, 2011a). Apart from the costs that are involved in transporting the sludge to the disposal site which is normally the landfill, the sludge causes problems of system failure in wastewater treatment plants as the plants are not equipped to dealing with solid sludge (Nwaneri, 2009).

Educating people about proper operation and maintenance of sanitation facilities can have numerous benefits (Bardosh, 2015). These include prolonged filling up periods and easy management of the contents of sanitation facilities especially pit latrines (Strauss, et al., 2000; Still & Foxon, 2012). The presence of rubbish in the pits makes pit emptying challenging. In waterborne sanitation facilities, the rubbish causes unnecessary blockages in the sewerage (Montgomery & Elimelich, 2007; Buckley, et al., 2008). This is a challenge that could easily be addressed by awareness campaigns that promote proper operation and maintenance of sanitation facilities (Bardosh, 2015). Such campaigns could educate communities about the proper construction of sanitation facilities that suit their needs and advice on which locally available material to use when constructing such facilities (Srinavasan, 1990; Lüthi, et al., 2011). Secondly, communities would be educated on what goes into the pit and what should not. This could bring the sense of ownership of these facilities by either individual household owners or the entire community as the facility would have been their preferred choice (Srinavasan, 1990; Lüthi, et al., 2011; Hoossein, et al., 2014).

2.2.1 Types of sanitation facilities

Sanitation facilities are the facilities that are meant for safe collection and storage of human excrement while also protecting the dignity and the values of the end user of the sanitation facility (National sanitation task team, 1996; Buckley, et al., 2008; DEA, 2011b). These facilities have a user interface, and a collection, storage or a treatment facility (Tilley, et al., 2014). The user interface includes the type of the toilet, the pedestal, pan, urinal or any other component of the sanitation system that the user comes into contact with while defecating (Buckley, et al., 2008). The collection, storage or the treatment facility collects stores or treats the products of the user interface. Depending on the sanitation facility type, some form of conveyance of the products that have been collected, stored or treated is necessary. Conveyance or transportation however depends on the distance between the user interface, collection, storage or treatment (Tilley, et al., 2014). Sanitation facilities vary from onsite sanitation facilities to offsite sanitation facilities and both types are meant for the safe collection, storage and treatment of human excrement while upholding the dignity and the values of the sanitation facility user.

Onsite sanitation facilities are the facilities that store or treat human excrement at the site of its production (Still & Foxon, 2012). The different types of pit latrines which vary from ventilated pit latrines to urine diversion toilets and from cartridge toilets to septic tanks are examples of onsite sanitation facilities (Tilley, et al., 2014). Unlike onsite sanitation facilities, offsite sanitation facilities have a user interface that is connected to a distant collection, storage and a treatment facility (Lettinga, et al., 2001). An example of this would be a flush toilet that is connected to a sewerage system with a wastewater treatment plant being the collection, treatment and a storage facility (Lettinga, et al., 2001; Tchobanoglous, et al., 2002).

A single pit system is an example of an onsite treatment facility (Tilley, et al., 2014). The user interface of this system can either be a dry toilet or a pour flush toilet (Langergrabera & Muelleggera, 2005). A typical example of this system can be seen in Figure 1. Urine, faeces, dry cleansing material and anal cleaning water or flush water are the main inputs in the user interface (Chaggu, 2004). The products that go for collection, storage or treatment from the user interface are either the excreta or blackwater (Tilley, et al., 2014). Blackwater can be conveyed or transported to a semi-centralized treatment facility such as a settler or a planted drying bed (Tilley, et al., 2014). This configuration is convenient in densely populated urban settlements where there is insufficient space for constructing new pits when the old ones are full. Single pit systems are common in both developing and developed countries. The produced effluent from the semi-centralized treatment facility is disinfected and disposed in irrigation ponds, plant or fish ponds. The produced sludge after thickening or co-composting is either discharged to the designated landfill sites or applied in agriculture (Chinyama, et al., 2012).

In offsite sanitation facility, such as the sewerage system, the product of the user interface is either brown water (urine and faeces) or urine (Tilley, et al., 2014). The user interface itself might either be a flush toilet or a urinal. If the product of the user interface is urine, then it goes into the urine storage tank which serves to collect, store and treat the urine (Drangert, 1998). The treated urine can then be transported for disposal or application as a fertilizer (Drangert & Nawab, 2011). For the other user interfaces, there is no storage or collection but conveyance to a centralized or semi centralized treatment station by a sewer (Tilley, et al., 2014). Treatment occurs at the centralized or semi centralized treatment station which can either be an aerated pond, settler or an Imhoff tank (Chinyama, et al., 2012; Tilley, et al., 2014). The effluent after treatment can then disposed in irrigation ponds, plant ponds or fish ponds (Feachem, et al., 1983). The sludge after treatment can be used as a fertilizer, soil conditioner or disposed in designated landfill sites (Esrey, et al., 2001).

2.2.2 Management of sanitation facilities

Every system requires proper management, operation and maintenance to ensure that it is safe and sustainable. In the context of sanitation facilities, this means that the user-interface, collection, storage or treatment, conveyance should be operated and maintained properly (Grimason, et al., 2000). With dry toilets (Figure 1) in particular, the surface of the pedestal or the squat hole should be kept clean all the time to prevent malodour and the transmission of diseases (Feachem, et al., 1983). Since dry toilets are the simplest of the sanitation facilities that exist, there is no need for any repairs unless the facility has been damaged (Gibbs, 1984; Schouten & Mathenge, 2010).

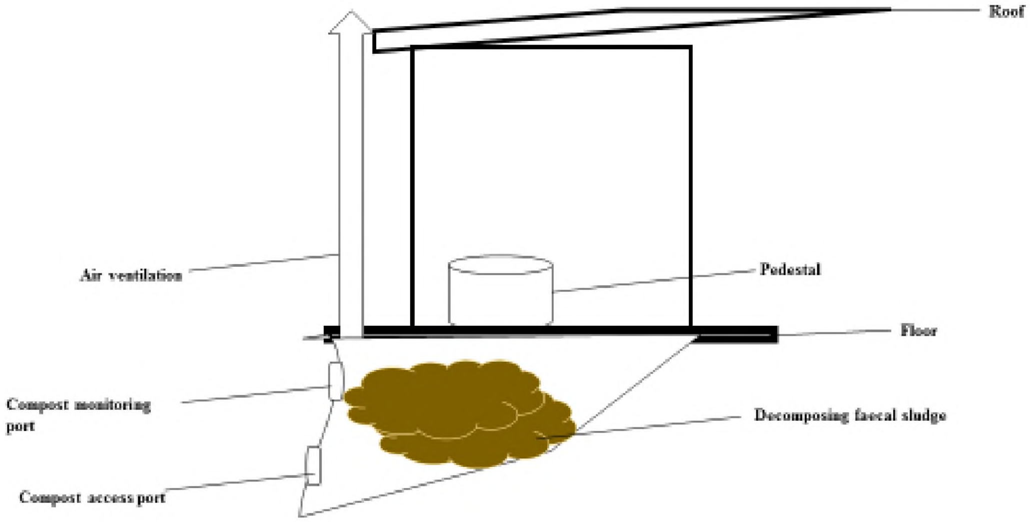


Figure 1. A schematic diagram of a typical composting dry toilet.

Unlike dry toilets, urine diversion toilets (Figure 2) are challenging to keep clean especially in water stressed areas due to water shortages and the need to separate urine from faeces (Schouten & Mathenge, 2010). Additionally, these facilities have a problem of blockage, especially if faeces are accidentally poured where in the urine collection chamber (Mnkeni & Austin, 2008; Schouten & Mathenge, 2010; Tilley, et al., 2014). Cleaning as with the dry toilets is essential to prevent malodour, disease transmission and staining. Malodour from urine can be minimized by having an odour seal which requires regular check-ups to ensure that it functions properly (Feachem, et al., 1983; Buckley, et al., 2008). Some sanitation facilities could be constructed using locally available material, a crucial aspect to consider more especially when trying to reduce maintenance costs (Grimason, et al., 2000).

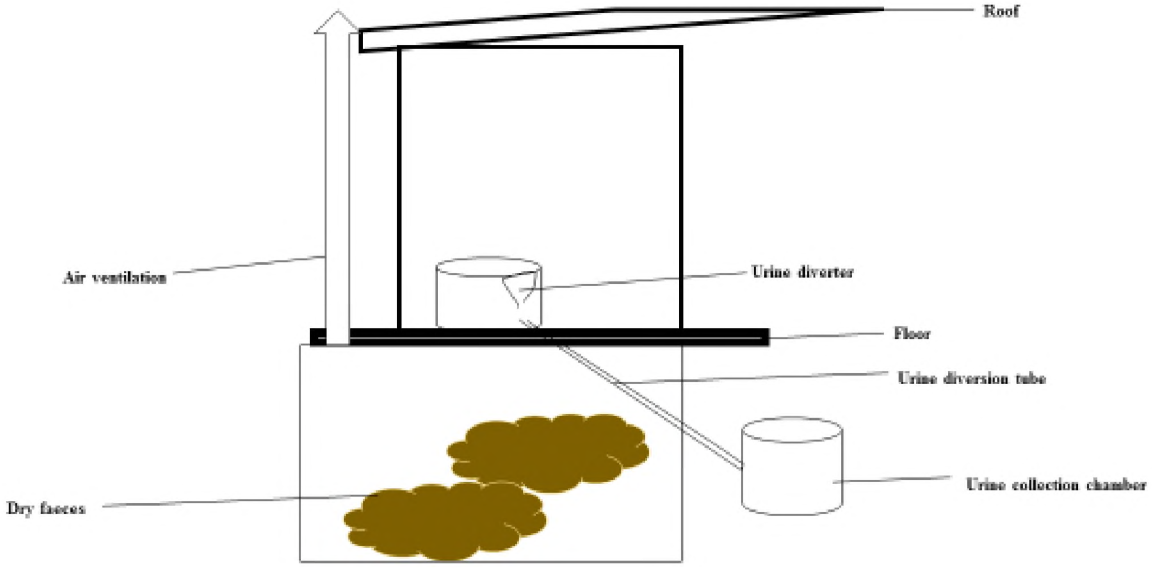


Figure 2. A schematic diagram of a urine diverting toilet.

With the flush toilets, the regular flushing cleans the bowl below the toilet seat, however scrubbing is sometimes necessary for hygiene maintenance and the prevention of stain build-up (Figure 3). The mechanical parts of the flush cistern toilet require maintenance for proper operation. Rubbish should not be flushed down the bowl as it might lead to blockages (Wilmouth, et al., 2013). As such, separate containers should be set aside for that task. Proper management, operation and maintenance of sanitation facilities not only improve the lifespan of the facility, but also improve the health of the facility users (Still & Foxon, 2012; Tilley, et al., 2014).

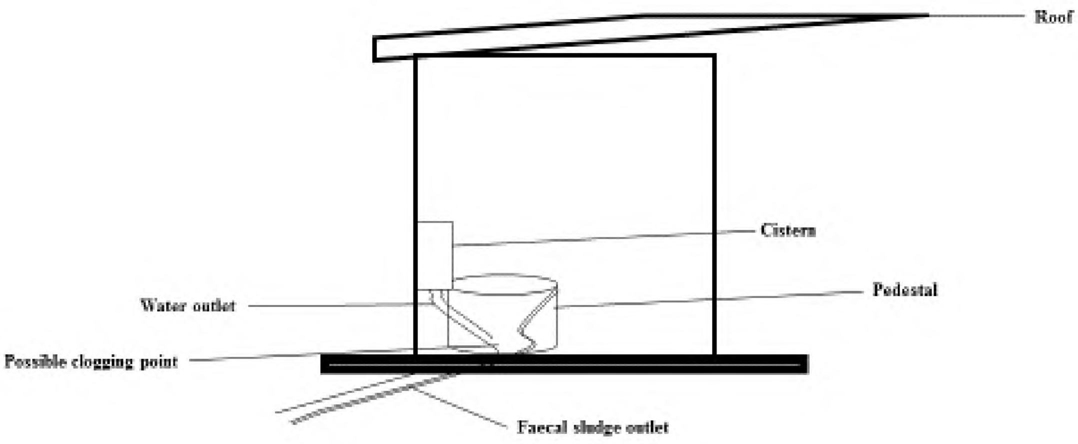


Figure 3. A schematic diagram of a typical flush toilet.

2.3 Products of sanitation facilities and their characterization

2.3.1 Faeces

In a year, each person produces 30 to 180 kg of human excrement (Feachem, et al., 1983). There are variations on exactly how much each person produces because faeces production is influenced by water and dietary uptake (Feachem, et al., 1983; Gao, et al., 2002; Vinnerås, 2002). People from developed countries are known to produce more faeces than the people from developing worlds due mostly to dietary differences. According to Dranget (1998), a person who consumes 250 kg of cereal produces excreta that contains all the nutrients that are necessary for cereal biomass production (Drangert, 1998). Various records showing the use of human excreta as a valuable resource in agriculture exist (Palmquist & Jönsson, 2004; Guzha, et al., 2005; Jensen, et al., 2008). This is mainly due to the presence of macronutrients (nitrogen, phosphorus, potassium) and micronutrients (iron, manganese, copper and zinc) which are essential for the germination of crops (Strauss, 1998; Strauss, et al., 2000). Faeces contain high concentrations of microorganisms with some being pathogenic. Thus about 70 to 80% wet weight of human faeces is water with the remaining portion being organic matter and microbes (Feachem, et al., 1983; Schouw, et al., 2002). However, survival of pathogens is shortened when the faecal sludge is composted due to high temperatures resulting from the composting process (Johansson, et al., 2001; Jonsson, et al., 2004). The heavy metals in human excreta are present in very low concentrations and as such, some authors consider them to be insignificant, especially in cases where faeces is to be used as a soil conditioner or a compost (Mininni & Santori, 1987; Jonsson, et al., 2004; Jensen, et al., 2008). As an example, trace elements that can be found in faecal sludge include As (12 mg/kg), Cd (13 mg/kg), Cr (731 mg/kg), Cu (3720 mg/kg), Hg (0.12 mg/kg), Ni (466 mg/kg), Pb (69 mg/kg) and Zn (13913 mg/kg) (Tervahanta, et al., 2014). But these concentrations might vary depending on the diet or the disposal of heavy metal containing waste inside the pit.

2.3.2 Urine

Urine has various nutrients which make it a valuable liquid fertilizer but the nutrients are available to plants in chemical forms that are accessible to plants (Jonsson, et al., 2004). The urine is made up of approximately 80-90% nitrogen, 50-65% phosphorus and 50-80% of potassium (Johansson, et al., 2001). The rate of urine production per person varies but it is estimated that each person produces up to 440 litres of urine per year (Mnkeni & Austin, 2008; Karak & Bhattacharyya, 2011; Still & Foxon, 2012). In less developed countries where the food consumed is less digestible, the total amount of nutrients that are available is low. Approximately 70-90% of nitrogen that is present in urine is urea with the remainder being ammonium ions (Lentner, et al., 1981). In nature, urine has few pathogens, and therefore its use as a fertilizer poses minimal environmental risk of disease transmission except in cases where there is possible contamination by faeces or schistosomiasis (Feachem, et al., 1983;

Schönning, et al., 2002; Ecosan Club (ed), 2010). Micropollutants in the form of steroidal hormones and pharmaceuticals can also be found in urine, but their removal can be accomplished through natural adsorption or absorption treatment technologies (Palmquist & Jönsson, 2004; Ecosan Club (ed), 2010).

2.3.4 Understanding the production of faecal sludge

Faecal sludge is a mixture of excreta and anal cleansing material with or without greywater that has been partially treated or raw and is found in onsite sanitation facilities (Strande, 2014). Dietary habits significantly affect the quantity of faecal excrement production (Lentner, et al., 1981; Feachem, et al., 1983; Vinnerås, 2002; Vinnerås, et al., 2006). Higher quantities of faecal matter are produced by people who consume food that is not processed and high in fibrous content (Lentner, et al., 1981; Feachem, et al., 1983). On the contrary, those that consume meaty meals and processed food produce less quantities of faecal matter (Lentner, et al., 1981; Feachem, et al., 1983). Factors such as the consumption of liquid, physical activity, diet and climate influence the volume of daily urine excretion (Lentner, et al., 1981; Feachem, et al., 1983; Vinnerås, et al., 2006). Daily accumulation of faecal sludge is influenced by the behaviour of people, frequency of toilet usage and the disposal of solid waste including the ingress of water and soil in pit latrines (Still & Foxon, 2012; Murungi & Pieter van Dijk, 2014). Thus, the understanding of the rate at which faecal sludge is produced at on-site sanitation facilities requires data that include all the factors discussed above (Still & Foxon, 2012). Where emptying is practiced, faecal sludge production can be estimated by looking at how much faecal excrement is brought to the disposal site (Klingel, et al., 2002). However, there are problems with this approach due to the fact that most on-site sanitation facility emptiers cannot remove all the contents of these facilities (Still & Foxon, 2012). Additionally, vacuum trucks often encounter problems of vacuum pump blockages due to the solid waste that is discarded into these facilities (Klingel, et al., 2002; Bhagwan, et al., 2008; Still & Foxon, 2012). Various estimates of the faecal sludge production rate are found in literature. According to Francey *et al.* a person produces 1 L of excreta per day with 10 to 40% of the wet weight being faeces (Franceys, et al., 1992). A study conducted by Norris in Soshanguve, in Gauteng, estimated 24 L per capita per annum to be the sludge accumulation rate in pit latrines (Norris, 2000). The reported mean filling rate for pit latrine was reportedly 69.4, 18.5, 29, 34,39, 19, 48 L per capita per annum for Besters Camp, Mbila, Mbazwana, Inadi, Limpopo, Ethekwini and Mafunze respectively in South Africa (Still & Foxon, 2012). For Besters Camp, the proper mean for the faecal sludge accumulation rate in pit latrines was found when the filling rate was calculated using the original pit volume from the construction time (Still & Foxon, 2012). This was mainly due to the wide range of faecal sludge accumulation rate during the monitoring period of the study (November 1993-December 1995) including the negative values of faecal sludge accumulation rate per capita per year.

Understanding the various factors that influence faecal sludge accumulation inside the pits is what is currently being used to estimate the sludge accumulation rate (Still, 2002; Klingel, et al., 2002; Still & Foxon, 2012). These factors vary from geophysical, biological to even social factors. The geophysical factors include the substrate and the soil type where the latrine is constructed, seasonal temperatures and rainfall. Anaerobic and aerobic digestion is the biological process that occurs within the pit with the social factors including number of facility users, the design of the facility, anal cleansing material used and the addition of solid waste in the pit. Some authors suggest that gathering faecal sludge accumulation rate through surveys, interviews, number of users of facilities, type of the facility, including the socio-economic level of the population could address the issue of faecal sludge accumulation (Klingel, et al., 2002; Still & Foxon, 2012; Kone & Chowdhry, 2012; Murungi & Pieter van Dijk, 2014).

The most common method for estimating the faecal sludge accumulation rate in pit latrines involves the use of surveys to collect information, such as age of the pit latrine, number of users per day, number of visits, socio-economic level of the household and the frequency of emptying (Still, 2002; Klingel, et al., 2002; Still & Foxon, 2012). Additionally, the pit volume is also used when estimating the accumulation rate, including the user behaviour, as that can help in estimating how much household waste is disposed of in the pit. The pit latrine faecal sludge accumulation rates that were reported by Still and Fox were estimated based on the pit volume and number of users (Still & Foxon, 2012). The author could not find any reported scientific data about the estimates of pit latrine faecal sludge accumulation from the volume of the faecal sludge collected from pit latrines by vacuum trucks or manual pit emptiers.

2.4 Valorisation of the products of sanitation facilities

On average, citizens of the developed countries produce more faecal sludge than in developing countries (Lentner, et al., 1981; Feachem, et al., 1983; Vinnerås, et al., 2006). The main contributor to this is the lifestyle and the diet choices. The products of sanitation facilities vary from faecal sludge to the various materials that are thrown into the facility (Feachem, et al., 1983; Buckley, et al., 2008; Still & Foxon, 2012). For waterborne sanitation or the sewerage system, anal wipes are always the component of the blackwater. This, however, depends on the region as most people often discard material like sanitary pads, condoms, tablets and various other things down the toilet (Schouw, et al., 2002; Still & Foxon, 2012; Guzha, et al., 2005; Bhagwan, et al., 2008; Bardosh, 2015). This is a practice not only done in developing regions but in developed regions too. As such, the composition of the contents of sanitation facilities wastes varies substantially depending on their location, the cultural practices of the region and the level of literacy of the users (Feachem, et al., 1983; Schouw, et al., 2002; Still & Foxon, 2012).

The most essential and the most relevant sanitation products are urine, faeces and faecal sludge. This is due to its physicochemical properties which make it a very useful resource for agricultural application (Esrey, et al., 2001; Jensen, et al., 2008; Meinzinger, et al., 2009). Sanitation facilities such as urine diversion toilets separate the faecal excrement from the urine (Vinnerås, 2002). Two separate products are acquired from such facilities. In other facilities however, urine and faecal excrement are mixed, hence the product is faecal sludge (Buckley, et al., 2008). These products might be mixed with water depending on the cultural practices of the users of sanitation facilities, and the type of facility (Vinnerås, 2002). The physicochemical properties of these products have made it possible for the products of sanitation facilities to be applied as valuable resources in agriculture, and in construction as bricks or other building material (Wang, 1997; Mnkeni & Austin, 2008). Due to the nature and the composition of these products, proper measures have to be taken to minimize any detrimental unintended consequences of the use of faecal excrement (Feachem, et al., 1983).

Urine is used as a fertilizer due to the presence of nitrogen (2.5-4.3 kg), phosphorus (0.4 kg) and potassium (0.9 kg) which are essential plant nutrient s (Drangert, 1998; Vinnerås, et al., 2006; Karak & Bhattacharyya, 2011). The values in brackets present the plant nutrients that are excreted through urine by each person per year. Before use, urine is fermented by storage for 6 months to eliminate any pathogens present and then diluted, but untreated urine is used as a fungicide (Duncker, et al., 2007; Ecosan Club (ed), 2010). When using urine as a fertilizer, it is recommended that the plants should be not fertilized closer to the harvesting times (Guzha, et al., 2005; Ecosan Club (ed), 2010). Urine alone might not contain heavy metals, however, it might contain pharmaceutical end products and non-metabolized drugs such as ciprofloxacin and tetracyclines which are eliminated through urine (Ecosan Club (ed), 2010; Decrey, et al., 2011). These include pharmaceuticals such as ibuprofen, estrone, β-sitosterol, diclofenac, carbamazepine and many more which are excreted either unchanged or metabolized (Tettenborn, et al., 2007; Vinnerås, et al., 2008). Considering the presence of the necessary macronutrients for plant grown in urine, urine makes a good fertilizer. As such, high yields in vegetables such as cucumber, tomatoes, lettuce, cabbage, carrots and turnips grown in soils fertilized with urine were obtained in Latin America (Esrey, et al., 2001).

Organic matter (88-97% of dry weight), phosphorus (3-5.4 % of dry weight), potassium (1-2.5% dry weight) and nitrogen (5-7%) are the major components of human faeces, thus making it a good soil conditioner (Feachem, et al., 1983). The nutrient availability for plants is low in faeces when compared to urine because the majority of the nutrients that are present in faeces exist in organically bound state (Feachem, et al., 1983; Esrey, et al., 2001; Duncker, et al., 2007). The presence of pathogenic microbes, such as eggs of *Ascaris* spp., viruses (rotavirus and hepatitis A virus) and bacteria (*Salmonella* spp and *Vibrio* cholera), in high concentrations in faeces requires proper treatment of the faecal sludge before application as a soil conditioner (Feachem, et al., 1983; Esrey, et al., 2001). In Walkerton, Ontario, in 2000, there was an outbreak of *E. coli* O157:H7 and *Campylobacter* that affected 5000 residents resulting in 7 deaths and 27 cases of haemolytic uremic syndrome (Choffnes & Mack, 2009). This outbreak occurred after 6 days of heavy rainfalls which led to cow manure contaminated runoff water from the surrounding farms contaminating a shallow well. The outbreak of cholera and typhoid fever after the 2004 Indian Ocean tsunami also serves as an example of the detrimental consequences of not managing faecal sludge (Leslie, 2005). The outbreak was the result of the consumption of water from unprotected wells (Leslie, 2005).

Treatment is a combination of two stage processes with the primary treatment aimed at reducing the weight and volume of the sludge (Duncker, et al., 2007). The secondary treatment is based on preparing faeces for application as a soil conditioner (Duncker, et al., 2007). Due to the repulsive appearance of the human faeces in some cultures, it is usually mixed with other organic wastes to reduce the repulsiveness, but in countries such as India and China, human faeces is used in agriculture without any treatment (Esrey, et al., 2001; Duncker, et al., 2007; Drangert & Nawab, 2011). There are no recorded disease outbreaks because of this practice from these countries. However, the composition of the human excreta does allude to the danger that this practice exposes to the environment and the people that consume the crops fertilized with the unsanitary sludge.

The rapid population growth creates social pressure as the demand for food also increases (Guzha, et al., 2005). Soil nutrients are declining without replenishing due to excessive farming (Guzha, et al., 2005). To compensate for that, commercial farmers often opt for chemical fertilizers. These fertilizers, although highly effective, are costly and contain compounds that can have negative environmental effects such as loss of fresh water due to salinity and heavy metal content accumulation, soil destruction and loss of biodiversity (Duncker, et al., 2007). The perception of human excreta by people for agricultural purposes is a significant determinant for its success. Before use, the excreta can either be composted or fermented inside or outside the pit depending on the toilet type and the accepted minimum composting period prior use is 6 months according WHO (WHO, 2006; Jensen, et al., 2008). This period of time is enough to eliminate the *Ascaris* eggs even though some helminths eggs are known to survive for up to a year (Jensen, et al., 2008). However various factors contribute to the die off of helminths and these include high pH (above 10), moisture content (below 20 %) and temperature (above 70°C) (Feachem, et al., 1983).

Inadequate composting time was identified as a problem for farmers that practice seasonal farming as they only get a minimum of 3-4 months for composting an excreta (Jensen, et al., 2008). Heavy metals such as cadmium, lead and chromium are toxic to human beings as well as plants, with high levels of zinc, lead and nickel known to be very phytotoxic (Mininni & Santori, 1987). Absorption of some heavy metals by crops through their roots can result in heavy metal poisoning to people that consume crops that are fertilized with faecal sludge that is contaminated by heavy metals (Mininni & Santori, 1987; Ecosan Club (ed), 2010). Consequently, these could potentially lead to health hazards as some of these metals are known to interfere with vital enzymatic activities once introduced into the blood stream (Mininni & Santori, 1987; Ecosan Club (ed), 2010; Decrey, et al., 2011). Cadmium blocks oxidative phosphorylase activity thereby affecting the activity of the enzyme and its interaction with nucleic acids (Mininni & Santori, 1987; Martin & Griswold, 2009; Singh, et al., 2011). In the liver and kidneys, it accumulates resulting in functional and histological damage (Mininni & Santori, 1987; Martin & Griswold, 2009). Lead is a known carcinogen and forms complexes with nucleic acids thereby impairing their synthesis (Martin & Griswold, 2009).

In African countries such as Zimbabwe, Tanzania, Ethiopia, Botswana, Mali, Uganda, Nigeria, Burkina Faso and other countries, human excreta have been used to grow crops (Duncker, et al., 2007; SuSana, 2012). The crops fertilized with human excreta vary from vegetables to trees.

The most prevalent use is the application of human excreta in growing fruit trees such as guava, banana, avocado, mango and many other fruit trees (Esrey, et al., 2001). Based on the available literature, only a few commercial farmers exploit the benefits of human excreta as a fertilizer (Duncker, et al., 2007; Meinzinger, et al., 2009; Cofie, et al., 2010; SuSana, 2012; Mor, et al., 2013). The perception of human excreta by society is dependent on the cultural and traditional ties, which influence its acceptability for use as a fertilizer in agriculture (Esrey, et al., 2001; Ecosan Club (ed), 2010).

In South Africa and based on anecdotal evidence, human excreta have been used in various applications which vary from medicinal, traditional and agricultural purposes (Duncker, et al., 2007). However, there is little quantitative data available in scientific literature about the extent of this practice. The emergence of the current information is linked to the recent introduction of ecological sanitation to various provinces within the country (Duncker, et al., 2007). In one example, human excreta is collected and taken to a communal composting facility in Northern Cape where inorganic material and particulate matter such as stones are removed and the excreta is then mixed with animal manure (Duncker, et al., 2007). The mixing of faecal matter with animal manure or plant matter is a global trend and the reason behind that is to make the compost appealing and to also seed the faecal sludge with the bacterial consortia to enhance stabilization (Esrey, et al., 2001; Mnkeni & Austin, 2008; Jensen, et al., 2008; Cofie, et al., 2010; Drangert & Nawab, 2011; Karak & Bhattacharyya, 2011).

In a study conducted in Pakistan, farmers demonstrated a sound knowledge of the benefits of human excreta (Drangert & Nawab, 2011). Although they claimed not to use excreta for crop production, they allowed the disposal of human excreta or defecation in the fields (Drangert &

Nawab, 2011). Farmers allowed the human excreta to dry and introduced a partly decomposed substance to the field (Drangert & Nawab, 2011). A study conducted in a small community in Ghana found that there were no cultural ties linked to the use of human excreta in agriculture (Cofie, et al., 2010). In fact people were attracted to the bigger size of the crops grown in faecal matter (Cofie, et al., 2010). The only available information about the conventional processing of faecal matter prior use in Africa is composting. Composting in this respect is done only alter the physical appearance of the faecal matter with less emphasis being put on ensuring that the compost does not pose any environmental or health risks (Cofie, et al., 2010; Drangert & Nawab, 2011; Karak & Bhattacharyya, 2011). Neglecting the importance of proper treatment of faecal matter or failure to disseminate information pertaining to the proper handling and treatment of faecal matter might endanger many lives (Esrey, et al., 2001; Hayman, et al., 2013).

2.4.1 Anaerobic digestion

The first ever anaerobic digester was built in India in 1897 by Matunga Leper Asylum and used human waste for gas generation to supply energy in the form of light (Chawla, 1986; Khanal, 2008). In 1937 a microbiologist by the name of Desai constructed a first ever anaerobic digester that could produce biogas from manure (McCarty, 1982). In China, there are over 25 million households that use biogas that is produced through anaerobic digestion of organic waste (Chen, et al., 2010). The large and expanding animal husbandry sector in Vietnam shows a high potential for biogas generation (Nguyen, 2011). More than 20000 digesters were installed by small scale farmers in Vietnam, but integration of the digesters to farming still remains a challenge because of the limited use of the resultant effluent (Nguyen, 2011). Poor design, lack of skills that are required for maintenance and inadequate capacity are the major obstacles of biogas digesters in Sri Lanka (de Alwis, 2012). In developing countries, the use of biogas as a fuel source could potentially reduce deforestation, indoor pollution and also reduce the amount of time that women take when gathering firewood for cooking (Sagar & Kartha, 2007).

Anaerobic digestion is a biological process that stabilizes organic matter with simultaneous production of biogas and sludge that can be used as a soil amendment because of its nutrient rich content (Gunnerson & Stuckey, 1986; Chen, et al., 2008). The produced biogas consists mainly of methane (60%>), carbon dioxide (29%) and a small percentage of other gases such as hydrogen sulphide (Osorio & Torres, 2009). The major component of the sludge is organic matter (88-97% of dry weight), phosphorus (3-5.4 % of dry weight), potassium (1-2.5% dry weight) and nitrogen (5-7%) (Feachem, et al., 1983; Mnkeni & Austin, 2008; Cofie, et al., 2010). Because of such properties, the produced sludge has a value and can be used as a fertilizer. Faecal sludge contains pathogens such as *E.coli* (0157:H7), *Salmonella* spp, *Ascaris* eggs, *Giardia*, *Hepatitis* virus and therefore treatment is necessary prior its application as a fertilizer or soil amender (Feachem, et al., 1983).

In rural China, India, Africa, Nepal and Latin America small scale anaerobic digesters are used to treat animal and food waste (Müller, 2007). Large anaerobic digesters are used worldwide to treat municipal waste in wastewater treatment plants. The implementation of small scale anaerobic digesters in rural communities has a positive impact on the livelihood of community dwellers and individual households (Arthur, et al., 2011; Garfía, et al., 2012). The positive social and economic benefits come in the form of reduced indoor air pollution from cooking fires, energy recovery through biogas that can be applied as a cooking fuel, empowerment of women by eliminating the time used to collect fire wood from the forest, a significant reduction in the amount of biosolids produced and the nutrient rich effluent and organic matter which could be used as a fertilizer or soil amender (Arthur, et al., 2011; Garfía, et al., 2012). In rural communities where solid biomass and heating fuels are used for cooking, anaerobic digesters serve as a valuable technology (Arthur, et al., 2011; Garfía, et al., 2012).

Anaerobic digestion is an efficient process for the treatment of organic matter because of energy recovery in the form of biogas which can be used in many applications involving cooking and transport (Cantrell & Ducey, 2008). Furthermore, the process promotes and permits nutrient recycling through the application of the effluent residue in crop production. Anaerobic digestion works best when provided with a proper feedstock (Gunnerson & Stuckey, 1986). Typically, the carbon to nitrogen ratio has to be between the ranges of 20 to 30:1 for optimum operation (Ward & Hobbs, 2008). The suggested optimum pH range for anaerobic digestion is between 6.8 and 7.8 (Ward & Hobbs, 2008). This is a relatively wide range but the final pH value varies with the substrate and the digestion technique. As a biological process, anaerobic digestion is carried out at specified temperatures depending on the type of anaerobic microorganisms that are present in the reactor (Gunnerson & Stuckey, 1986; Ward & Hobbs, 2008).

The optimum temperature range for anaerobic microorganisms ranges from psychrophilic (less than 20℃), mesophilic (20℃- 45℃), thermophilic (above 45℃ but less than 60℃) and hyper-thermophilic (above 60℃). Mesophilic and the thermophilic temperatures are the most commonly used for the digestion of organic matter (Gunnerson & Stuckey, 1986; Ward & Hobbs, 2008). This is mainly due to the elevated metabolic rates of microorganisms that function at these temperatures and the possibility of die off of pathogens. The reduced ammonia content and the reduced formation of long chain fatty acids in mesophilic temperatures is ideal for anaerobic digestion as the two are known to inhibit anaerobic digestion (Gunnerson & Stuckey, 1986; Khanal, 2008). In sludges whose moisture content is above 90% methane production increases but the pH should range between 6.6 and 7.8 (Gunnerson & Stuckey, 1986). Where methane production is low, its yield could be improved by pre-composting the sludge to enhance the biodegradation of organic matter or thermally treating the sludge (Gunnerson & Stuckey, 1986; Khanal, 2008).

In an attempt to improve methane production in anaerobic digestion, some researchers go for extracellular commercial enzymes such as proteinases, peptidases, carbohydrates and lipases to enhance the breakdown of organic matter (Valladão, et al., 2007; Romano, et al., 2009). The inclusion of these enzymes, however, has huge implications on the process cost of anaerobic digestion (Coultry, et al., 2013). The presence of refractory compounds or inhibitory soluble molecules such as polychlorinated biphenyls and chlorinated benzene can, however, result in reduced anaerobic biodegradation of organic matter (Chen, et al., 2008; Kawai, et al., 2012). The high molecular weight compounds could potentially result in reduced biodegradability of organic matter and bio-toxicity (Yang & Speece, 1986). On the contrary, organic matter can split into smaller molecules which are prone to biological degradation at high temperatures and pressures (Gunnerson & Stuckey, 1986; Khanal, 2008).

In community healthcare centres, hospitals, schools, boarding schools or prisons, small scale digesters are implemented to treat human waste (Chawla, 1986; Khanal, 2008; Appels, et al., 2008). The performance of anaerobic digesters is affected by hydraulic retention time, solid retention time, organic loading rate, mixing, pH, alkalinity, temperature and the reactor configuration (Appels, et al., 2008). The hydraulic retention time dictates the contact time period that is required for the removal of particular constituents by the biomass in the reactor (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008).Solids retention time is the mass of the settleable solids including the microbial consortium within the reactor divided by the mass of solids that get washed out of the system each day. This is important because if it is too low, microorganisms will be washed out and if it is too long the system will become nutrient deficient and fail (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). The effectiveness of microorganisms that have optimal growth conditions within the reactor are dictated by the solid retention time and this also alters the microbial ecology within the system (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). The quantity of volatile solids added to the reactor daily per reactor volume is the organic loading rate. The two methanogenesis processes (acetotrophic methanogenosis and hydrogenotrophic methanogenosis) are inhibited by the organic loading rate in anaerobic digestion (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). During the process start up, the produced organic acids, if in high concentrations, can negatively affect methane production (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). Since the efficiency of the process is regulated by microorganisms, temperature is an important component for optimal operation of the digester (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). Hydrolysis is a rate limiting step in anaerobic digestion, therefore prior physicochemical treatment will improve organic matter solubilisation (Appels, et al., 2008).

The processes that occur in anaerobic digestion include hydrolysis (fermentation), acidogenesis, acetogenesis and methanogenosis (Gunnerson & Stuckey, 1986; Müller, 2007; Khanal, 2008). During fermentation, fermentative bacteria break macromolecules down through the excretion of extracellular enzymes (Appels, et al., 2008). Proteins, polysaccharides and phospholipids are the macromolecules that are broken down into soluble organic compounds in a process called hydrolysis (Gunnerson & Stuckey, 1986; Müller, 2007; Khanal, 2008). The energy output from hydrolysis is used to produce organic acids, hydrogen and carbon dioxide through the process called acidogenesis (Müller, 2007; Khanal, 2008). The stoichiometric equation is presented in equation 1. Propionic acid (CH_3CH_2COOH) and butyric acid ($CH_3CH_2CH_2COOH$) are the organic acids that are formed.

$$C_5H_9O_3N + nH_2O \rightarrow CH_3CH_2COOH + nCO_2 + nH_2 + NH_3 \qquad [1]$$

These are reduced by acetogenic microbes forming acetic acids (CH_3COOH), hydrogen and water in a process called acetogenesis (Gunnerson & Stuckey, 1986; Müller, 2007; Khanal, 2008). The stoichiometric equation is presented below.

$$CH_3CH_2COOH + nH_2O \rightarrow CH_3COOH + CO_2 + nH_2 \qquad [2]$$

For acetogenesis to be thermodynamically favourable and the forward reaction to occur within the system, the partial pressure of hydrogen has to be 10^{-3} atm (Gunnerson & Stuckey, 1986; Müller, 2007; Khanal, 2008). Hydrogen scavenging by methanogenic archaea lowers the partial pressure and keeps a thermodynamically favourably process of acetogenesis (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). Two processes catalyse methane production via methanogenesis. One process utilizes hydrogen and carbon dioxide to form methane through hydrogenotrophic methanogenesis (Appels, et al., 2008). The stoichiometric equation for the reaction is presented in equation 3. In the other process, acetate is formed from the conversion of hydrogen and carbon dioxide through homoacetogenesis (Khanal, 2008; Appels, et al., 2008). Thereafter, acetotrophic methanogens convert acetate into methane and carbon dioxide (stoichiometric equation 4).

$$CO_2 + nH_2 \rightarrow CH_4 + nH_2O \qquad [3]$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \qquad [4]$$

In summary, that is how biogas is produced through anaerobic digestion (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). Various designs of anaerobic digesters that are specifically designed to treat faecal sludge exist globally (Appels, et al., 2008). However, the quality of the sludge produced by such digesters might not be sufficient to either meet the regulations for the effluent disposal in landfill sites or agricultural reuse regulations (Gunnerson & Stuckey, 1986; Müller, 2007; Appels, et al., 2008; Khanal, 2008). As such, additional treatments such as the use of planted or unplanted drying beds, waste stabilization ponds, aerated ponds and pasteurization could be used.

2.4.2 Pasteurization

A process of increasing the temperature of the sludge sufficiently enough to reduce the concentration of pathogenic microorganisms to concentrations that do not pose health or environmental risks, is pasteurization (Ciochetti & Metcalf, 1984; Saitoh & El-Ghetany, 1999). Instead of a complete die off of pathogens, pasteurization significantly reduces the colony count to levels that do not pose any environmental or health risks, especially if the sludge is to be reused in agriculture (Coultry, et al., 2013; Grim, et al., 2015). This process is achieved either through a batch or a flow through process (Ciochetti & Metcalf, 1984; Saitoh & El-Ghetany, 1999). Faecal sludge containing vessels are heated up using liquid fuels, wood or sunlight as energy sources in batch pasteurization. The flow through process involves passing of the sludge through a heated pipe or a duct, emerging as a pasteurized sludge (Grim, et al., 2015). It is less expensive to construct the batch processes, but the high capital cost might make them to be less attractive (Coultry, et al., 2013). Both batch and flow through processes are extremely efficient in their function, however, the flow through process is the commonly used procedure as it is mostly applied not only in wastewater treatment plants, but in the dairy industry as well (Ciochetti & Metcalf, 1984; Saitoh & El-Ghetany, 1999; Grim, et al., 2015). The operational costs of the pasteurizer, however, can dictate the preferred option as the process requires extensive heating (Grim, et al., 2015).

The principal aim of pasteurization is the reduction of pathogens to concentrations that do not pose environmental or public health risks. Generally, pathogens can survive the elevated temperatures but only for a definite time period. Their survival is mostly influenced by pasteurisation temperature and the type of the pathogen (Sahlstrom, 2003). Complete treatment of composting sludge at the temperature range of 60-70^{0}C for 3 days was achieved by Wiley and Westerberg (Wiley & Westerberg, 1969). Inactivation of hepatitis MS -1 and MS-2 strains with exposures of 56 and 98^{0}C for 30 and 1 minute respectively have been obtained (Ziemba & Peccia, 2011). *Giardia lamblia* inactivation was achieved at exposure of 70 and 64^{0}C for 10 and 5 minutes (Ongerth, et al., 1989; Aukerman & Monzingo, 1989). The infective capacity of thermotolerant *Cryptosporidium* oocysts was reduced after being exposed to elevated temperatures for a defined time period (Harp, et al., 1996; Hogland & Stenstrom, 1999). From the aforementioned examples, it is evident that time of exposure and temperature play a significant role in the inactivation of pathogens by pasteurization. In fact, with increasing temperatures, exposure time decreases significantly. However, the pathogen type also matters as higher temperatures and prolonged exposures might be necessary for resilient pathogens such as helminth ova (Wiley & Westerberg, 1969; Feachem, et al., 1983). Complete inactivation of *Salmonella* and the loss of viability in *Ascaris* ova after 20 minute exposure at 70^{0}C were observed by Strauch and Berg (Strauch & Berg, 1980).

To effectively eliminate pathogens, pasteurization of bio-waste for an hour at 70^0C is the most appropriate strategy (Bohm, et al., 1999; Bendixen, 1999). Standard pasteurization procedures, however, fail to eliminate bacterial endospores that are present in sewage sludge, thus their presence needs to be investigated before the sludge application in agriculture (Mitscherlich & Marth, 1984; Bagge, et al., 2005). To eliminate endospores, two pasteurization rounds might be necessary to effectively reduce the endospores, but that increases operational costs substantially (Foster & Johnstone, 1989). The idea behind the two runs is that the primary pasteurization should induce the vegetative cells from the spores to germinate and grow (Ward, et al., 1981; Shin & Kang, 2003; Cuba, et al., 2003; Cuba, et al., 2003). With the secondary pasteurization procedure, the heat-labile newly germinated bacteria are eliminated (Ward, et al., 1981; Shin & Kang, 2003; Cuba, et al., 2003; Cuba, et al., 2003). However, the incubation time period between the pasteurization steps should be minimal to prevent endospore formation (Ward, et al., 1981; Shin & Kang, 2003; Cuba, et al., 2003; Cuba, et al., 2003).

In reality, endospore forming bacteria are present in the soil, and therefore, the health risks that are imposed by the endospore forming pathogenic bacteria presence in the sludge that is to be used for agricultural practices needs investigation (Mitscherlich & Marth, 1984; Foster & Johnstone, 1989). Regrowth of pathogens in the sludge from partially or highly disinfected sludge also needs investigation (Yeager & Ward, 1981). In composting treatments, regrowth of pathogens has been observed where additives, such as rice hulls have been used because this material is rich in soluble sugars which acts as an additional nutrient source (Gantzer, et al., 2001). Hot weather or rainy periods are associated with regrowth of enteropathogenic microorganisms in sludges that are applied to soils (Hagedorn, 1980; Gibbs, et al., 1997).

Pasteurization is effective in reducing or eliminating pathogens, however, it fails to address the issue of endospores as stated above. As such, irrespective of the sludge sanitization process, one needs to have a proper understanding of basic microbiological principles when handling biological waste to prevent recontamination and regrowth. One problem with pasteurization is the capital cost requirement as the process has high energy requirement and thus requires a heat exchanger (Bagge, et al., 2005; Grim, et al., 2015). This therefore, shows the importance of a combination of treatment processes using anaerobic digestion to generate energy rich biogas for the downstream pasteurization. When treating the sludge, one has to consider the fact that the microorganisms might protect themselves from thermophilic treatment by embedding into the sludge (Sahlstrom, 2003). This is mainly due to the fact that the die off of microorganisms in pasteurization or thermophilic treatment is influenced by operational factors and sludge characteristics, especially physical heat transfer in huge volumes of sludge solids (Sahlstrom, 2003).

2.5 Environmental and health risks associated with mismanaging sanitation facilities and their products

The contents of the faecal sludge are influenced by age, gender, health status, diet and the daily operation of the latrine (Still & Foxon, 2012). Urine and faeces contain nutrients such as potassium, phosphorus, calcium and nitrogen which are essential for plant growth (Feachem, et al., 1983). When the sludge is disposed in wastewater treatment plants, these essential nutrients are lost. Pit latrine faecal sludge reportedly contains 3400-5000 mg/l of total kjedhal nitrogen, 450-500 mg/l of total phosphorus, 2000-9000 mg/l of NH_4-N, elements that are useful as nutrients for the growth of crops (Semiyaga, et al., 2015). The presence of the nutrients that are required for plant growth shows that the sludge has economic value. Both urine and human faeces have been used as compost since ancient times (Jensen, et al., 2008). Before use, however, treatment is necessary to completely eliminate pathogenic microorganism and some toxic compounds which might have adverse environmental and health effects (Jensen, et al., 2008; Semiyaga, et al., 2015). The protection of human and environmental health is the main objectives of the treatment of faecal sludge, hence regulatory legislation for endues, discharge or disposal is of paramount importance.

If not used properly, the pit latrines fill up very quickly (Chaggu, 2004; Buckley, et al., 2008). The quick filling up is not desirable in that it causes problems in areas where there is not enough space for the construction of new pit latrines (Buckley, et al., 2008; Still & Foxon, 2012). Secondly, the location of some pit latrines makes it very difficult to access the material where emptying is possible (Buckley, et al., 2008; Still & Foxon, 2012). As such, instead of using mechanized methods, some households are obliged to hire manual pit emptiers (Strauss, et al., 2000; Chinyama, et al., 2012). Most of these emptiers are not qualified to do such jobs and are not educated on safe ways of disposing human excrement, and as such are exposed to dangerous pathogens such as *Listeria* spp, *Staphylococcus* spp, *Mycobacteria* spp and *Brucella* spp (Strauss, et al., 2000; Still & Foxon, 2012). As a result, the contents of the pit latrine end up in an open land, rivers or drainage systems (Strauss, et al., 2000; Still & Foxon, 2012). This act could potentially result in outbreak of diseases such as brucellosis, listeriosis, cholera, as the human excrement might end up in water bodies or come into contact with people (Feachem, et al., 1983; Al-Jessim, et al., 2015). The pollution of the environment by pathogens could have dire consequences in farming communities resulting in a decrease in food security (Feachem, et al., 1983; Palmquist & Jönsson, 2004; Montgomery & Elimelich, 2007).

Faecal sludge from pit latrines or any other onsite sanitation facilities can be a useful resource (Meinzinger, et al., 2009; Cofie, et al., 2010). However, if proper measures are not taken to control the unsafe use of faecal sludge, there can be detrimental consequences (Hagedorn, 1980; Mininni & Santori, 1987; Gibbs, et al., 1997). As such, it is important that the standard guidelines are set for the safe use of faecal sludge (Jonsson, et al., 2004). These guidelines should not only address the issues that are related to the safe use of faecal sludge but incorporate the proper use of on-site sanitation facilities (Jensen, et al., 2008; Chinyama, et al., 2012; Hayman, et al., 2013). In turn this could potentially result in a reduction in the amount of rubbish that is often thrown into the pit latrines (Strauss, et al., 2000; Buckley, et al., 2008; Still & Foxon, 2012). The reduction in the amount of rubbish that is inside the pit could have a positive impact on the quality of faecal sludge and on the emptying of pit latrines. Improved quality of faecal sludge could potentially reduce the costs that are sometimes incurred when treating faecal sludge, especially when the mechanized treatment methods are used (Kone & Chowdhry, 2012; Murungi & Pieter van Dijk, 2014).

The reduction in the amount of rubbish that is often thrown in the pit latrine could possibly help in reducing the costs of pit emptying and significantly reduce the volume of water that is used pit emptying (Kone & Chowdhry, 2012; Murungi & Pieter van Dijk, 2014). Rubbish reduction could have a positive impact because most pit emptiers base their prices on the amount of rubbish that is present in the pit latrine (Kone & Chowdhry, 2012; Murungi & Pieter van Dijk, 2014). In areas where the municipality offers the emptying service for free, the reduction in the rubbish could reduce the maintenance costs on the vacuum truck as the rubbish often damages the extraction pipe (Kone & Chowdhry, 2012; Murungi & Pieter van Dijk, 2014).

2.6 Policies and regulations of using the products of sanitation facilities

The United Nations Environmental Program has set guidelines for waste management strategies. These serve as a guideline for countries that have waste management strategies and countries that want to modify their policies so that they can meet up with the waste management problems that they might be facing (Hayman, et al., 2013). The policies already implemented in certain countries, especially the ones which are quite efficient in managing waste, are catered for within the guidelines. Reduction, recycling and recovering of waste as a form of sustainable development, is proposed (Hayman, et al., 2013). Economic, environmental and social factors that affect waste management are comprehensively elucidated in the guidelines (Hayman, et al., 2013). Successive stages beginning from product design until it becomes a waste are well explained (Hayman, et al., 2013). However, some wastes such as human excreta do not follow the product development stages but require management at the end. Products of economic value can be generated from human excreta and these include methane, soil conditioner and a fertilizer. It is of paramount importance that the countries set policies that address waste management problems that are of high priority within its boundaries instead of simply following the international guidelines as some of these guidelines might not apply to the problems being experienced (Hayman, et al., 2013).

In South Africa, all the waste generated within the boundaries of the country is managed under the Waste Act of 2008 (DEA, 2013). This act promotes the reduction, recycling and recovering of the waste with the disposal in landfill sites regarded as the last resolution in cases where the waste can neither be recovered, recycled, nor reused (DEA, 2013). The human refuse is manageable on site as its source is usually within the household (Esrey, et al., 2001; DEA, 2013). The type of management depends on the population density in a particular location, including the terrain, as the commonly used refuse management facilities are pit latrines (DEA, 2011b). In this respect, the household owners are fully responsible for managing their waste. When the latrines are full, emptying is necessary in cases where new structures cannot be constructed or the householders cannot afford the new structure (DEA, 2011a).

According to the national policy for basic refuse removal services under the Waste Act of 2008, the municipality has to assume the role of emptying the pits as that role falls under the scope of basic refuse removal services (DEA, 2011a). Thus, the fate of the collected refuse depends on the municipality and the presence of the resources needed to manage it dictate where it is disposed of. This service is available to everyone who is within the jurisdiction of the municipality, regardless of the economic situation as stipulated by the national policy (DEA, 2011a). Thus, the national policy for basic refuse removal service mainly focuses on ensuring that the refuse from the households within specific municipality boundaries is removed.

The information pertaining to the reuse and recycling of any waste material is within the scope of the Waste Act of 2008. The rules and regulations, however, differ based on the waste source and type (DEA, 2013). This is due to the existing classification system which requires that certain waste be handled, treated and disposed in specified ways. There is no specific information about the handling and treatment of human excreta within the act. From the complexity of constituents of the human excreta and the information that is available in the literature, there is a gap of knowledge that still needs to be filled. This refers to the information pertaining to the safe handling and use of the human excreta, especially when it is to be recycled and used as a fertilizer in South African context. The WHO guidelines for safe use of wastewater, excreta and greywater do address the safety measures that have to be taken when handling human excreta for use in agriculture (WHO, 2006) .

The major concern of the current literature seems to be around the reduction of potential contaminants, such as pathogenic microorganism and helminths. There is little information about the presence of the pharmaceutical by-products in the human excreta (Ecosan Club (ed), 2010; Decrey, et al., 2011). There does seem to be an agreement amongst researchers who have investigated the longevity of pharmaceutical by-products in the soil that they do not last. As such, it is assumed that their impact is not that significant. However, a study by Drewes *et al.,* (2002), found that carbamazepine and primidone were not reduced after 6 years of passage through a soil aquifer treatment system (Drewes, et al., 2002). Neglecting these by-products might pose environmental and health risks, more especially when human excreta is used either as a fertilizer or soil conditioner (Ecosan Club (ed), 2010). This is mainly due to the shortage of information pertaining to the adsorption of these by products or compounds by plants.

Based on the available information about the concentration of pharmaceutical by-products in either human faeces or urine, authors conclude that these concentrations are negligible and unlikely to cause harm (Feachem, et al., 1983; Drangert, 1998; Palmquist & Jönsson, 2004; Karak & Bhattacharyya, 2011). However, in cases where animal fertilizer is to be used, the concentration of these products is known to be very high, and therefore caution should be taken, more especially with animals that are given drugs (Feachem, et al., 1983). The dissemination of the information pertaining to the potential value of human excreta without proper policies to regulate its use might in fact lead to problems (Hayman, et al., 2013). Some of these problems might include outbreak of diseases, contamination of soil and water bodies which might consequently result in environmental and health hazards, including high food insecurity (Feachem, et al., 1983; Sahlstrom, 2003). Currently, it is within the interest of a licensed waste manager to ensure safe reuse and disposal of a particular waste product, since failure to do so attracts severe penalties from the state (DEA, 2011b). Although the rules and regulations were set, detailed information about human refuse management and reuse is lacking. The dissemination of the relevant information could possibly ensure safe implementation of the policies and legislation that regulate the use of human excreta.

2.7 Sanitation service delivery in South Africa

Access to basic water and sanitation services is a constitutional right in South Africa with the National government's set minimum sanitation standards (National sanitation task team, 1996). These standards are very flexible in that they allow the local authorities to make independent decisions about the suitable sanitation facilities based on the available information about the area that requires servicing (Meiklejohn & Cotzee, 2003). Additionally, the authorities can decide on the best suitable strategy to recover the costs that are involved in providing, maintaining and operating the sanitation facilities. The unpredictable urbanization which has resulted in the establishment of informal settlements has caused major problems in terms of service delivery planning (Fox, 2012; Gauri, 2013). This is not only a problem in South Africa but a global problem that is being experienced in both developed and developing countries (Brockerhoff, 2000; Fox, 2012; Gauri, 2013; Cobbinah, et al., 2015).

In South Africa, however, the shortage of expertise in fields such as financial management, project management and engineering within the local municipalities has caused numerous service delivery backlogs (Godfrey, 2008; Tempelhoff, 2009). Additionally, the prevalence of corruption across all the spectrums of governing bodies makes effective service delivery by the municipalities challenging (Tomlinson, 2011). Municipalities according to the Municipal Systems Act (Act 32 of 2000) are mandated to include local communities in the integrated development planning and the implementation process (The National Treasury, 2015). This does not happen, however, due to the distorted line of accountability within the governing structures of the municipalities and the dissatisfaction of communities with service delivery (Nzimakwe, 2009).

In 2012, South Africa achieved the MGD goal of halving the number of people without access to improved sanitation (Statistics South Africa, 2015). However, several challenges emerged in addressing sanitation service backlogs, including sustaining adequate sanitation services (Statistics South Africa, 2015). These encompassed upgrading and expanding of the bulk infrastructure capacity, improving already existing structures and ensuring that the newly built facilities are of good quality (Department of Water and Sanitation, 2015a). Additionally, proper maintenance and reticulation, cost recovery to support sanitation service delivery, community involvement as the recipients of the facilities, responsibility adoption of the facilities, effective oversight and proper regulation and management at all of sanitation services at all government levels, are part of the challenges that are faced by the sanitation sector (Department of Water and Sanitation, 2015a). The expansion of formal and informal settlements in urban areas because of rural-urban migration, the influx of foreign nationals and population growth, amplify these challenges (Fox, 2012; Cobbinah, et al., 2015).

The sector is affected by the lack of clarity about the institutional, policy and regulatory frameworks and poor coordination between the key stakeholders that are involved in various aspects of sanitation (Department of Water and Sanitation, 2015a). The government made plans to address the aforementioned issues through the establishment of the Department of Water and Sanitation in 2014 whose main role is to formulate and implement the policies that govern the water and sanitation sector, including the predominance of the responsibilities for water services that are provided by the local government (Department of Water and Sanitation , 2015b).

As stated above, there is a variety of backlogs in the sanitation sector of South Africa and they need to be addressed. Before addressing them, however, the availability of the relevant information pertaining to the sources of sanitation service backlogs is important. Information regarding to sanitation service backlogs can be found on the Water Services National Information system, Statistics South Africa (STAS SA) data and the Department of Water Services Reference Framework Planning data set. This source contains information pertaining to census data about calculated yearly modifications of population growth and service delivery, people's opinions about services rendered at household level and also the status of the infrastructure for water services (South African Human Rights Commission, 2012). In Cape Town, the implementation of sanitation service delivery systems is mostly hindered by the unsuitable settlement areas, especially the informal settlements (Water Services Department of Cape Town, 2006). A study conducted by Mels *et al.,* 2009, showed that about approximately 85% of the informal settlements were constructed on areas that were prone to flooding (Mels, et al., 2009). Additionally, 70% of these settlements were in areas that were partially accessible for servicing by the municipality.

When looking at the living conditions of people in informal settlements, it is easy to assume that people are unhappy more, especially given the social unrests related to service delivery issues in South Africa (Nembambula, 2014). A study that was conducted in Soweto found that the informal settlement dwellers were much more satisfied with their quality of life there and little concerned about other basic services, such as schools and health care services (Westaway, 2006). In the context of the study, these basic services were grouped under environmental quality. What was established through the findings was that people were more satisfied with the houses that they lived in, regardless of the environmental conditions (Westaway, 2006). Evidently, these people moved to the informal settlements to be with their families or for work (Moller, 2001). In light of the aforementioned factor, the service delivery protests are possibly incited by other factors post relocation to the informal settlements (Nembambula, 2014). Based on the SHARC report, sewages spills, un-serviced pit latrines, poorly constructed pit latrines and the rape incidences associated with abandoned or dysfunctional pit latrines were amongst the highlighted problems that were closely associated with service delivery protests across the country (SAHRC, 2014). Consequently, water borne sanitation systems were a preferred sanitation type over the standard VIP latrines.

In the South African context, it is clear that sanitation service delivery is an ongoing problem. With the introduction of ventilated improved pit latrines and the known challenges that they pose after filling up, it is evident that alternative methods of addressing the pit emptying issues need to be addressed. The most common method of addressing this issue is through the use of vacuum trucks by the municipalities, a process which is costly and requires a lot of water (Still, 2002; Still & Foxon, 2012). Eventually, the contents of the contents of the pit latrine either end up in wastewater treatment plants or designated landfill sites. Wastewater treatment plants are not designed to treat faecal sludge with high solid content, and therefore, treating faecal sludge might result in operational problems that in turn could lead to system failures (Tilley, et al., 2014). Additionally, the costs that are involved in extracting and transporting the pit latrine faecal sludge to either landfills or wastewater treatment plants are high and considering the fertilizer value of faecal sludge, cost effective methods to reduce the prices involved transporting and treating faecal sludge need to be investigated (Bhagwan, et al., 2008; Kone & Chowdhry, 2012). This study investigated mesophilic anaerobic digestion of the pit latrine faecal sludge with the aim of recovering biogas and applying the recovered biogas to pasteurize the anaerobically digested pit latrine sludge through pasteurization. Part of the study involved investigating the microbial consortia of the pit latrine faecal sludge as a way of assessing the risks that re posed by the presence of pathogens to faecal sludge handers and to also investigate the presence of the necessary microbial consortia which required for anaerobic digestion.

Chapter 3: Materials and methods

3.1 Reagents and consumables

The following consumables and chemicals were purchased from Merck (Pty) Ltd. (Johannesburg/Cape Town, South Africa): $C_{10}H_{14}O_8$ (EDTA); $CuSO_4.5H_2O$; NH_4NO_3; $ZnSO_4.7H_2O$; KNO_3; H_3BO_4; COD solution A ,(Catalogue No.114679) and COD solution B, (Catalogue No. 114680, test range 500-10000 mg/l); PO_4^{3-} test kit (Catalogue No. 1.14729.0001, test range 0.010-5.00 mg/l); NH_4–N test kit (Catalogue No. 1.14559.0001, test range 0.010-150.0 mg/l); NO_3^- test kit (Catalogue No. 1.14542.0001, test range 0.4-110.7 mg/l); Cl^- test kit (Catalogue No. 1.14897.0001, test range 2.5-250 mg/l); K test kit (Catalogue No. 1.14562.0001, test range 5.0 -50.0 mg/l); Tetrathionate Broth Base (Catalogue No. HGC31.500); XLD agar for microbiology (Catalogue No. 1.55287.00500) and TWEEN 80. The following consumables and chemicals were purchased from Sigma-Aldrich (Johannesburg, South Africa): $FeSO_4.7H_2O$; $MnCl_2.4H_2O$; Ca $(NO_3)_2.4H_2O$; KOH; Hi-Crome m-TEC agar (Catalogue No.90924-500g) and Hi-Crome *Enterococcus faecium* agar (Catalogue No.90919-54.2G). The following material and equipment were used: ZR Fecal DNA MiniPrep kit (Catalogue No. D6010); Merck Thermoreactor TR300 (Merck, Johannesburg, South Africa); Membrane filter (MicroSep Cellul-sic white-grid-sterile, 0.45 micrometer, 47 mm, 2001PK Catalogue No- E04WG04751); Beckman Coulter Avanti J-E centrifuge (Beckman Coulter, United States of America); Merck Nova 60 spectrophotometer (Merk, South Africa); Shimadzu UV-1201 spectrophotometer (Shimadzu, Japan); UWE OFW-B60 platform scale (UWE Scales, Johannesburg; South Africa); Heidolph Type RZR1 overhead stirrer (Heidolph, Germany) ; Atlas CF -200A gas ring and Liquid petroleum gas (LPG) (BUCO Hardware, Grahamstown, South Africa).

3.2 Sample collection and processing

Hlalani Township in Grahamstown, Eastern Cape, South Africa was chosen as a potential sampling site since the households of the township still used ventilated improved pit latrine (VIP) as sanitation facilities (Figure 4). The selection criterion for the potential sampling VIP was that the contents had to be easily accessible, through the extraction port or that the pedestal could be moved, and also contain less rubbish, for the ease of extraction without clogging due to rags, plastics and braids. Permission to use Hlalani Township was granted by the Makana Municipality and the sampling site visits were facilitated by a facilitator, an employee of Khulumani Water for Dignity Project, a project under the Khulumani Support Group. Basically, the aim of sampling was to recover material from VIP for analysis and to recover material for anaerobic digestion purposes.

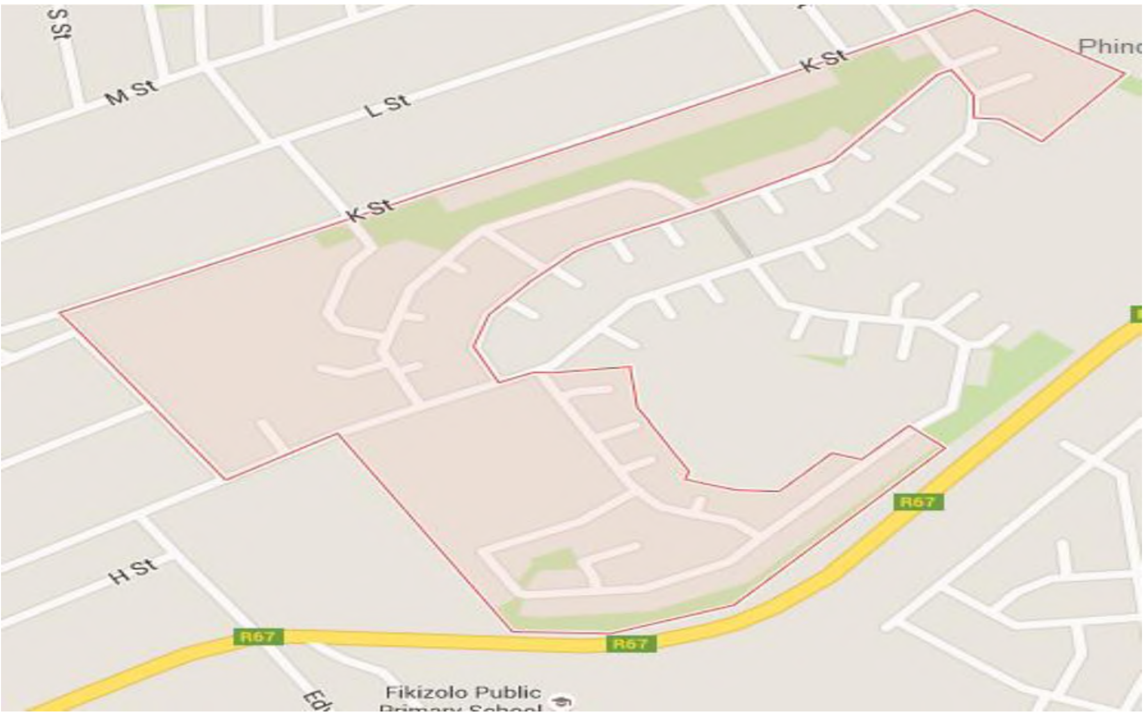


Figure 4. The map of Hlalani Township in Grahamstown (circled area) where the samples were acquired (image adapted from Google maps on 29 August 2014).

Initially, a 2 metre long hollow steel (2 mm thick) corer with an external diameter of 80mm was used (Figure 5 a). The distal end of the corer had welded to it an auger of the same diameter. The proximal end had a metal T piece handle to allow the corer to be rotated. The corer was used to manually extract the contents of the pit latrine and two people were required for the extraction process. The corer was inserted through the defecation hole of the overlying concrete plinth of the pit latrine and into the faecal sludge. While driving the corer into the sludge, it was constantly rotated until the bottom of the pit was reached. Once the bottom of the pit was reached, the sampler was carefully retrieved to prevent the collected material from sliding out of the corer. The collected pit latrine faecal sludge was transferred from the corer into 25 L plastic bucket and transported to the laboratory.

At the laboratory, the collected pit latrine faecal sludge was passed through a coarse nylon mesh (typically used in grocery stores to pack vegetables, approximately 1 cm) to remove extraneous materials (condoms, plastic bags, hair braids, cloth, etc.). Thereafter, the filtered material was transferred into a tall 100 L Perspex digester (Figure 9) for anaerobic digestion (details of the digestion and sampling for physicochemical analysis are reported in the anaerobic digestion in section 3.3. This faecal sludge recovery method was not repeated for the following sampling expeditions as it was very laborious and only permitted a recovery of limited amount of the pit latrine faecal sludge. With the redesign and fabrication of larger 200 L anaerobic digesters (Figure 10), larger amounts of faecal sludge needed to be recovered. For this purpose, a vacuum truck from the Makana Municipality was used to collect the faecal sludge from multiple pit latrines within Hlalani Township. In order to sample the different strata of pit contents, a newly designed core sampler was fabricated for a subsequent experiment. This allowed the microbiological community of the pit latrine to be sampled at different depths within the pit (Figure 5 b).

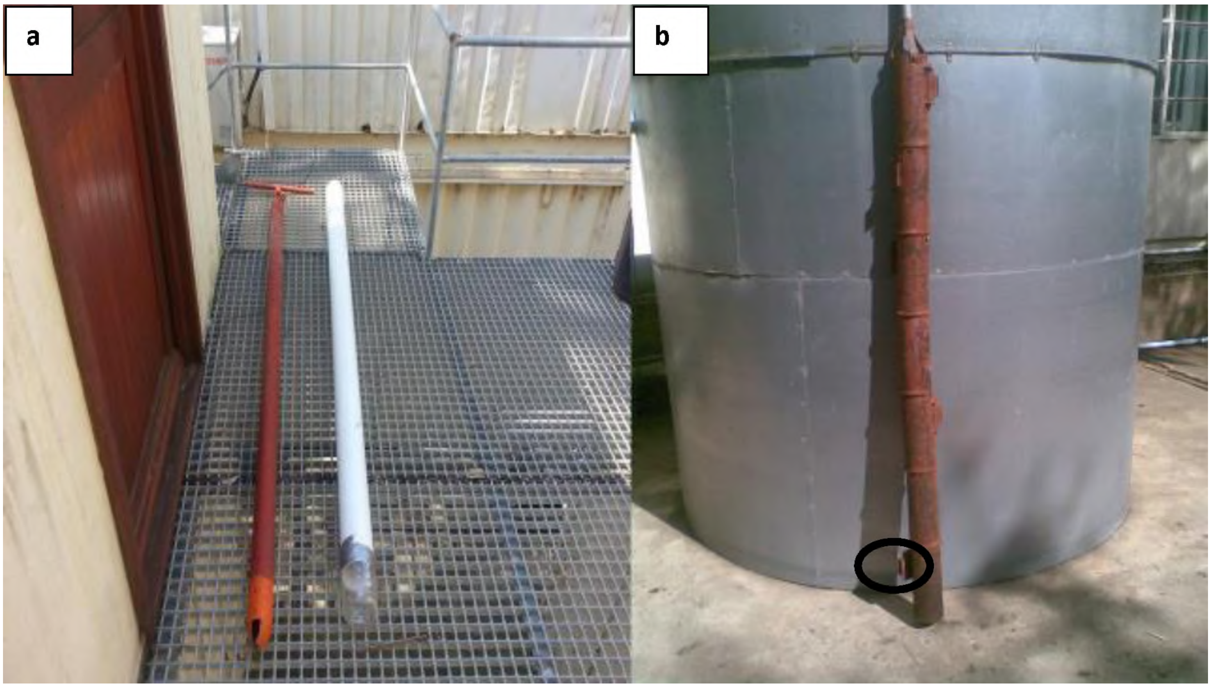


Figure 5 .The hollow sampler that was used to collect the pit latrine faecal sludge on the first sampling expedition with the plastic cover sleeve next to it. 4b the newly designed segmented sampler that was used to sample at different depths of the pit latrine. The circled part indicates the position of the opening flaps on the sampler.

The new sampler consisted of seven interlocking cylindrical segments. Each segment was 250 mm in length and consisted of a chamber with an opening on the side. The opening could be sealed with a hinged lid. When the corer was inserted into the pit faecal sludge, it was rotated clockwise causing the lids to lie flush against the cylinder sides. After the maximum depth of the pit was reached, the corer was rotated anticlockwise a number of times. Sills on the lids caused the lids to open as the corer was rotated anti-clockwise in the sludge. These lids now acted as scoops channelling sludge into the segment chambers. After a number of rotations to ensure enough material had been collected, the corer was rotated clockwise again to cause the lids to lie flat against the cylinders. As the corer was retrieved from the sludge, it was rotated clockwise to ensure that lids remained flat against the cylinder sides to prevent ingress of material from different strata. Each cylinder was detached by unbolting it from its neighbouring cylinder. Samples collected in the cylinders' chambers were decanted into sterile colourless zip bags (UV exposer for 15 minutes) at the site of collection. At the laboratory, the collected samples were transferred to 40 ml sterile plastic screw cap urine jars and stored at -20^{0}C until used for later metagenomics studies.

3.3 Anaerobic digestion of the pit latrine faecal sludge

In the first anaerobic digestion experiment, approximately, 20L of the pit latrine faecal sludge that was collected using the steel corer and screened through a coarse nylon mesh bag (mesh size ~ 1 cm), was anaerobically digested in a tall Perspex digester that had an electric stirrer attached to the top part of the digester (Figure 6). For biogas collection, an inner tube of a car tyre (white arrow, Figure 6) was fitted to the digester. The faecal sludge was mixed with the electric stirrer prior anaerobic digestion. There was no inoculum added to seed the microbial community in the reactor because of the assumption that the microbial community responsible for anaerobic digestion was present in the pit latrine faecal sludge. The anaerobic digester was run at 29 ± 2℃ for 60 days to improve biogas recovery in a controlled environment room (CE).

Approximately, 500ml of the anaerobically digested pit latrine faecal sludge was collected using Schott bottles and stored at 8℃. Physicochemical properties (COD, ammonium, chloride, phosphate and nitrate) of the effluent were determined within 24 hours of sampling (the experimental details are explained in the physicochemical analysis section 3.6). For biogas collection, the inner car tyre tube was weighed before and after anaerobic digestion. The mass of the car tyre did not change after anaerobic digestion. Before anaerobic digestion, the inner car tyre tube was weighed and the mass was recorded as 0.85 kg (the minimum weight of the scale, 0.10 kg) and no increase in the inner car tyre tube was recorded. As such, it was concluded that biogas collection was unsuccessful possibly due to the excessive headspace compared to the quantity of the sludge within the digester. Poor gas collection and the energy demand associated with using the electric stirrer made this design impractical and resulted in the change in the anaerobic digester design (Figure 7).

For the next set of experiments, pit latrine faecal sludge from multiple pit latrines was collected with a vacuum truck and used as feedstock for the newly designed anaerobic digesters. In order to promote anaerobic digestion, effluent from the Belmont Valley Wastewater Treatment Works (WWTW) in Grahamstown was added as a co-feed. Different proportions of the pit latrine faecal sludge and the effluent from the WWTW were mixed. The faecal sludge was transported to the Institute for Environmental Biotechnology Rhodes University where it was transferred to a series of 200L plastic drums for further processing. The rubbish that was present in the pit latrine faecal sludge was manually separated from the sludge before being used as a feedstock for the following anaerobic digestion experiment. Three 200 L modified plastic drums were used as anaerobic digesters. Two digesters had added to them as a co-feed, the effluent from an anaerobic digester at Belmont Valley WWTW. The co-feed material was collected and stored in a separate 200 L plastic drum before being mixed with the pit latrine faecal sludge. The proportions that were mixed were 1:2 pit latrine faecal sludge and WWTW anaerobic effluent (AD) sludge, 2:1 pit latrine faecal sludge and WWTW AD sludge and 100% pit latrine faecal sludge which served as the control.

Each digester had the following treatments, 2:1 pit latrine faecal sludge to WWTW AD effluent, 1:2 pit latrine faecal sludge to WWTW AD effluent and 100 % pit latrine faecal sludge. Each digester was filled to 180 L leaving 20 L headspace for gas collection. This experiment was run for 45 days, as this was considered sufficient time for biogas recovery (Ward & Hobbs, 2008). Approximately 100 ml of the mixture was collected from a drainage valve at the bottom of each 200 L anaerobic digester using autoclaved Schott bottles (250 ml). The physicochemical properties (dry weight, ash content, pH, COD, ammonium, nitrate, phosphate, chloride, potassium and trace metals) of the different mixtures were determined. Details about the experimental procedure are explained in detail under the physicochemical properties of the pit latrine faecal sludge section 3.6. A third anaerobic digestion experiment was conducted after the completion of this digestion experiment. The experimental set up was identical to the one outlined above. To improve gas production in the third anaerobic digestion, each digester was inoculated with 2 kg of bovine paunch manure obtained from an abattoir near Grahamstown, to provide the microbial consortium required for anaerobic digestion. After each digestion, a sizeable portion of the effluent was collected from the drainage valve using sterile Schott bottles and stored at 8°C for 18 hour prior to pasteurization.

Figure 6. The Perspex digester used for anaerobically digesting pit latrine faecal sludge. An inner tube of a car tyre (white arrow) was attached to the top of the digester collected biogas

Figure 7 a. The second type of anaerobic digesters with the attached inner car tyre tube . Figure 10b. The crank handle with the stirrer attached to the lid of the anaerobic digester. This was to permit stirring of the anaerobic digester contents.

3.5 Pasteurization of the pit latrine faecal sludge prior use

The surface of the laboratory work bench was chemically sterilized with 70% ethanol followed by a commercial bleach (Jeyes Fluid, Pick ‘n Pay; Grahamstown, South Africa). In a sterilized pre-weighed 5 L tin container, 3 kg of the effluent was weighed using an UWE OFW-B60 platform scale (Figure 8 b). Liquid petroleum gas (LPG) was used as a heating source because biogas had not been collected in sufficient quantities to provide the heating energy for the pasteurization process. Based on the COD value of the digested pit latrine faecal sludge, the theoretical quantity of biogas that could be recovered from anaerobic digestion was 49 L whereas 663.4 L of biogas would have been required for pasteurization (the calculations used to estimate the total of biogas that was required for pasteurization are explained below). The theoretical biogas yields were estimated from COD data using the following formula (Angelidaki & Sanders, 2004);

$$CH_4\ yield\ at\ STP = \frac{0.35(L)}{g\ COD}$$

Before and after pasteurization, the weight of the LPG cylinder was determined to ascertain the amount of LPG gas used for heating (Figure 8 b). The amount of LPG gas used was found to be 331 g to pasteurize 3000 g of the material for 1 hour. The calorific value of LPG is 46.1 MJ/kg, thus a total of 13.83 MJ was used for pasteurizing pit latrine faecal sludge equating to 4.18 MJ of LPG consumed per 1 kg of the pit latrine faecal sludge that was pasteurized. To estimate how much biogas would have been required to provide the same heat energy, the calorific value was used which is estimated at 23 MJ/Nm^3 (lower heating value). For every 1 kg of pit latrine faecal sludge, an estimated value of 5.5 m^3 would have been required if the process was self-sufficient. A 5L tin container was used as the pasteurization vessel. The tin vessel was weighed and effluent added, after which the tin vessel and effluent contents were weighed and weight recorded. Prior to pasteurization, the tine vessels and its contents were placed on an Atlas CF -200A gas ring that was connected to the LPG cylinder, (Figure 8 a). A thermometer mounted on a retort stand and inserted into the effluent was used to measure the temperature throughout pasteurization process. Mixing of the sludge was provided by a Heidolph Type RZR1 overhead stirrer which was used to maintain constant distribution of heat in the effluent during pasteurization (Figure 7 a). Once the temperature of the effluent reached 70 ±2℃, it was maintained at that point by continuously turning the heat source on and off for an hour. Using autoclaved Schott bottles, 50 ml of the samples were collected before and after pasteurization and stored at 8℃ for 18 hours and then physicochemical and microbiological properties were determined. A further pasteurization efficiency study was set up using fresh material from the WWTW anaerobic digester to ensure the presence of living faecal bacteria in the pasteurization material. Effluent from an anaerobic digester at Belmont Valley Wastewater plant was used for this trial. Fifty ml samples of the effluent undergoing pasteurization were collected at defined time intervals (0.5; 10; 15; 30; 60& 120 minutes) and stored at 8 ℃ for 18 hours before analysis. Pasteurization efficiency was assessed by monitoring indicator microorganisms (*E. coli, Salmonella* spp. and *Enterococcus feacium*) before, during and after the pasteurization process at the defined time intervals specified above.

As an alternative to pasteurization, alkalinisation of the pit latrine faecal sludge with fly ash was investigated. The faecal sludge and 5 L tin vessel was weighed out as outlined above. An overhead stirrer was employed to mix the faecal sludge and fly ash. In this experiment, fly ash was added to the faecal sludge until a final pH of 12 was reached. The mixture was allowed to stand for 2 hours. Then 50 ml samples of the mixture were collected at defined time intervals (0.5; 10; 15; 30; 60& 120 minutes) and stored at 8℃ for 18 hours before being analysed.

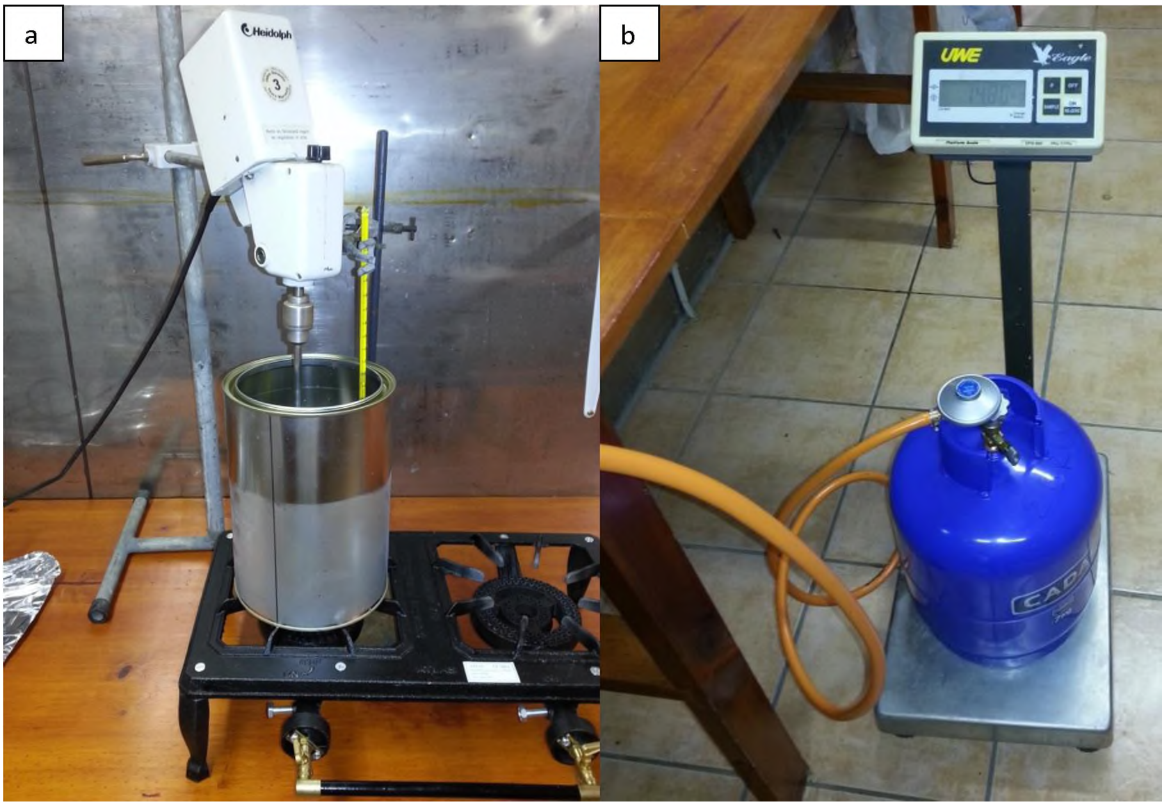


Figure 8a. The pasteurization tin placed on a gas plate that is connected to an LPG cylinder. The overhead stirrer and a thermometer are immersed on the effluent. 8b, the LPG cylinder on a scale used to determine the amount of gas used.

3.6 Physicochemical analysis of the pit latrine faecal sludge

3.6.1 The dry weight and the ash content of the pit latrine faecal sludge

The dry weights of the pit latrine faecal sludge were determined by weighing out 2.00 g of the wet samples in pre-weighed crucibles using a Pioneer PA2102 analytical balance with the accuracy of 0.01 g (Ohaus Corporation, United States of America) (EPA, 2001). The crucibles were then placed in an oven for 24 hours at 105°C after which the crucibles with the dried samples were placed in a desiccator and allowed to cool down. Dry weights were determined using the following equation;

$$Dry\ weight\ (\%\ of\ wet\ weight) = \frac{W2}{W1} \times 100 \qquad [1]$$

Where *W1* represents the wet weight of the sample before drying at 105°C and *W2* is the mass after drying at 105°C.

Thereafter, the loss on ignition (LOI) was determined by incinerating the samples at 550°C for 2 hours in a muffle furnace (Carbolite-ELF 11/6). The samples were then transferred to a desiccator and allowed to cool down after which the crucibles and combusted residues were weighed to determine the ash content (loss on ignition). The ash content was calculated using the following equation;

$$LOI\ (\%) = \frac{W2-W3}{W2} \times 100 \qquad [2]$$

In the equation, *W3* is the mass after ignition at 550°C (EPA, 2001). The other items in equation 1 have the same meaning as the ones in equation 1

3.6.2 The pH of the pit latrine faecal sludge

The pH of the pit latrine faecal sludge was determined before anaerobic digestion and after pasteurization using a calibrated Hanna Model HI 8314 pH meter (Hanna Instruments; United States of America). The pH was measured directly from the sample by dipping the pH probe into the faecal sludge. Then the pH reading was allowed to stabilize and then the pH was recorded. All the recorded pH values were discrete real time values without replication and there was no aqueous medium added because the sludge was liquid (indicated by moisture content of greater than 90%, see Table 3 in Section 4.1.1. The physiochemical and biological properties of the pit latrine faecal sludge) (APHA,AWWA and WEF, 1998) .

3.6.3 The chemical oxygen demand of the pit latrine faecal sludge

The chemical oxygen demand (COD) was determined using COD test kits from Merck (APHA,AWWA and WEF, 1998). A low range COD test (COD solution A, Catalogue No.112677 and COD solution B, Catalogue No. 112678; 25-1500 mg/l) was used for the COD test of the first digestion but was later changed to the high range test kit (COD solution A, Catalogue No.114679 and COD solution B, Catalogue No. 114680; test range 500-5000 mg/l). The chemical reaction is equation 3;

$$2\, KC_8H_5O_4 + 10\, K_2Cr_2O_7 + 41\, H_2SO_4 \rightarrow\ 16\, CO_2 + 46\, H_2O + 10\, Cr_2(SO_4)_3 + 11K_2SO_4 \qquad [3]$$

For the low range COD test kit, 3 ml of the sample was pipetted into the digestion tube. The remaining steps of the protocol are explained below. In the high range COD, 1 ml of the faecal sludge was pipetted into the COD digestion tubes. 0.3 ml of COD solution A was pipetted into the test tube followed by mixing. After that 2.85 ml of COD solution B was carefully pipetted into the mixture. The mixture was mixed thoroughly by vortexing for one minute (MT19 Deluxe Vortex mixer, Chiltern Scientific, London, UK). Then the COD digestion tubes were placed into a preheated electrical heating block (Merck Thermoreactor TR300; Merck; Johannesburg, South Africa); and digested for 120 minutes at 148°C. The tubes were removed from the thermoreactor and allowed to cool to room temperatures for 10 minutes. After that the absorbance was spectrophotometrically measured at 610 nm using Shimadzu UV-1201 spectrophotometer. Distilled water was used as a blank to and any interference by the reagents were rectified by subtracting the blank from the actual sample reading. Potassium hydrogen phthalate (KHP) was used to prepare the standards for the COD calibration curve. The calibration curve was constructed by multiplying the concentration of potassium hydrogen phthalate (KHP) by 1.17 to get the mg/L of COD. Figure 9 shows the calibration curve used to calculate the COD values.

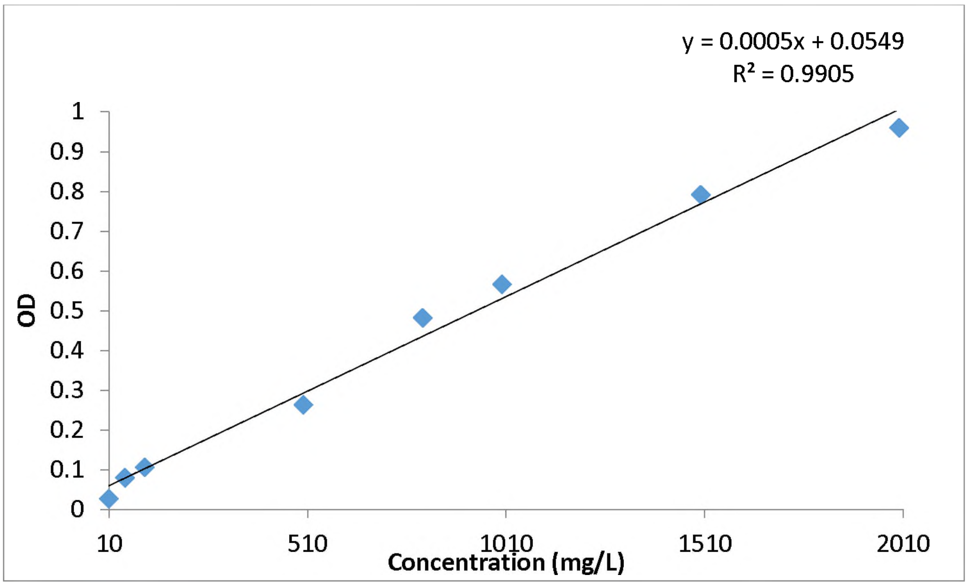


Figure 9. The standard curves of COD. The linear equation was used to determine the concentration of COD from the absorbance (OD).

3.6.4 The ammonium content of the pit latrine faecal sludge

The concentration of ammonium nitrogen in the sludge was determined using ammonium nitrogen test kits from Merck (Catalogue No. 1.14559.0001) (APHA,AWWA and WEF, 1998). The chemical reaction is presented below; ammonium nitrate (Catalogue No 221244-500G, Sigma Aldrich, South Africa) was used when preparing the calibration curve.

$$NH_3 + OCl^- + 2\ C_6H_5OH \rightarrow OC_6H_8NC_6H_6O^- \qquad [4]$$

Following the filtration step, 5.0 ml of the sample was pipetted into a test tube into which 0.60 ml of ammonium nitrogen reagent 1 was pipetted. The sample was mixed and 1 level blue microspoon of ammonium nitrogen reagent 2 was added. The test tube was thoroughly mixed by vortexing until the reagent dissolved completely. The mixture was allowed to stand for 5 minutes and then 4 drops of ammonium nitrogen reagent 3 was added. The reaction mixture was allowed to stand for a further 5 minutes before the absorbance was measured at 690 nm using a Shimadzu UV-1201 spectrophotometer. Distilled water was used as a blank to and any interference by the reagents were rectified by subtracting the blank from the actual sample reading. To prepare the standard for the calibration curve, ammonium nitrate was used. The calibration curve is represented below;

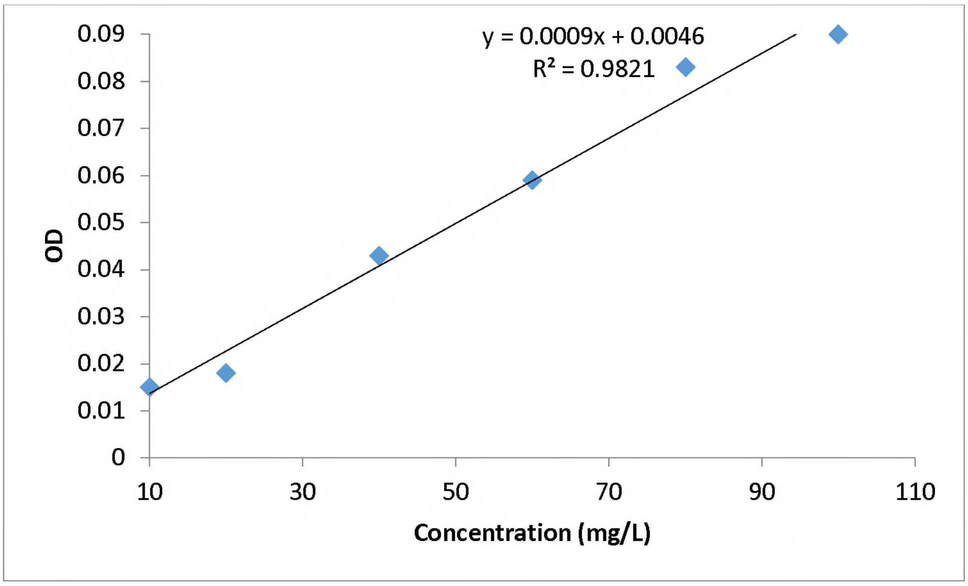


Figure 10. The standard curve for ammonium. The linear equation was used to calculate the concentration of ammonium from the absorbance.

3.6.5 The nitrate content of the pit latrine faecal sludge

The nitrate test kit from Merck (Catalogue No. 1.14542.0001) was used to determine the concentration of nitrate in the pit latrine faecal sludge (APHA,AWWA and WEF, 1998). The chemical reaction is presented in equation 5;

$$R - NH_2 + NO_2 \rightarrow R - N_2 + R_1 - NH_2 \rightarrow R - N = N - R_1 - NH_2$$

[5]

Following the filtration step, 4.0 ml of the pre-treated faecal sludge was pipetted into a test tube, following which 0.5 ml of nitrate reagent 1 was added. Without mixing, 0.5 ml of nitrate reagent 2 was added after which the mixture was mixed by vortexing. The reaction mixture was allowed to stand for 10 minutes and then the absorbance was measured at 520 nm using a Shimadzu UV-1201 spectrophotometer. Distilled water was used as a blank to and any interference by the reagents were rectified by subtracting the blank from the actual sample reading. Below is the standard curve for nitrate. Potassium nitrate (Catalogue No P8394-500G, Sigma Aldrich, South Africa) was used to make a calibration curve.

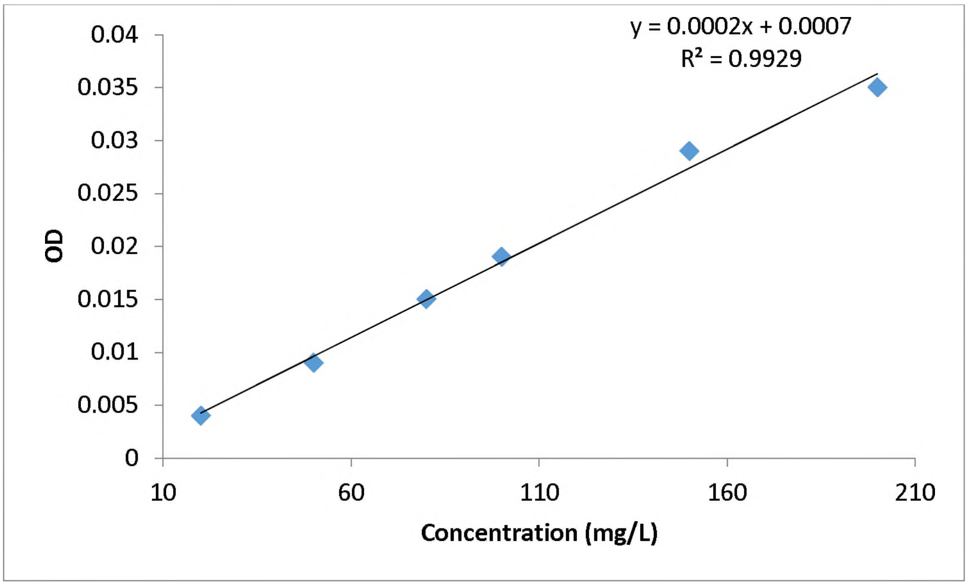


Figure 11. The standard curve for nitrate. The linear equation was used to determine the concentration of nitrate from the absorbance.

3.6.6 The phosphate content of the pit latrine faecal sludge

The phosphate concentration in the pit latrine faecal sludge was determined using a phosphate test kit from Merck (Catalogue No. 1.14729.0001) (APHA,AWWA and WEF, 1998). Presented below is the chemical reaction for the phosphate test;

$$H_3PO_4 + 6\ Mo(VI) \rightarrow 12\ MoPA + 9\ H^+ \qquad [6]$$

Following the filtration step, 5.0 ml of pre-treated faecal sludge sample was pipetted into a test tube, following which 5 drops of phosphate reagent 1 were added into the sample and the sample was mixed. Afterwards, 1 level blue microspoon of phosphate reagent 2 was added followed by mixing through vortexing until the reagent dissolved. The reaction mixture was allowed to stand for 5 minutes and after which the absorbance was measured at 650 nm using a Shimadzu UV-1201 spectrophotometer. Distilled water was used as a blank to and any interference by the reagents were rectified by subtracting the blank from the actual sample reading. The standard curve for phosphate is presented below; dipotassium monohydrogen phosphate (Catalogue No, P3786-500G, Sigma Aldrich, South Africa) was used to make the calibration curve.

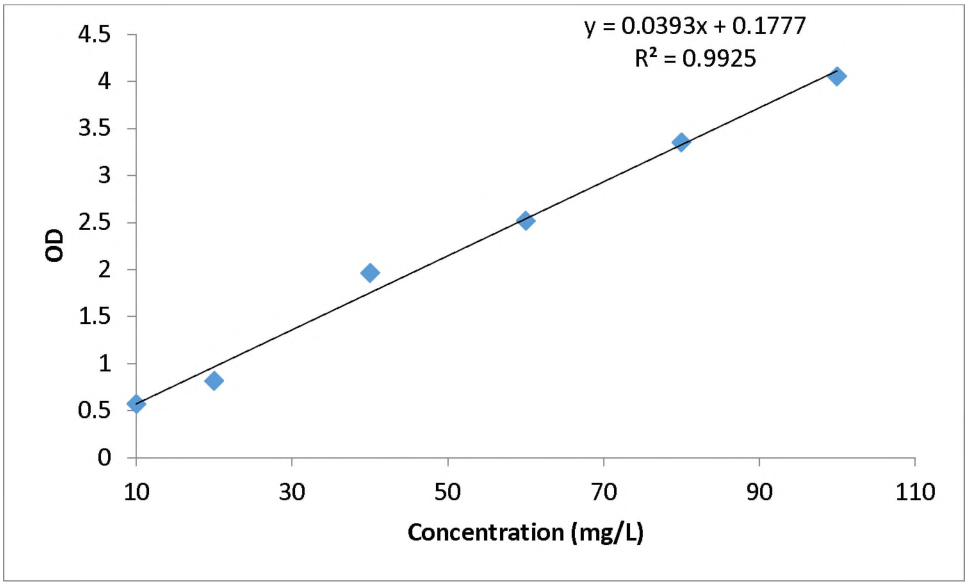


Figure 12. The standard curve for phosphate. The linear equation was used to determine the concentration of phosphate from the absorbance.

3.6.7 The chloride content of the pit latrine faecal sludge

The concentration of chloride was determined using the Merck test kit for chloride testing (Catalogue No. 1.14897.0001) (APHA,AWWA and WEF, 1998). The chemical reaction of chloride ions with the reagents is presented below; calcium chloride (Catalogue No 1016-500G, Sigma Aldrich, South Africa) was used when preparing the calibration curve and the chemical reaction is presented in equation 7.

$$Hg(SCN)_2 + 2Cl^- \rightarrow HgCl_2 + 2SCN^- \rightarrow 3SCN^- + Fe^{3+} \rightarrow Fe(SCN)_3 \quad [7]$$

Following the filtration step, 5.0 ml of a pre-treated pit latrine faecal sludge sample was pipetted into a test tube, following which 2.5 ml of the chloride reagent 1 was pipetted into the same test tube and mixed. After that, 0.50 ml of chloride reagent 2 was pipetted into the test tube followed by mixing. The mixture was allowed to stand for 1 minute, after which absorbance was measured at 450 nm using a Shimadzu UV-1201 spectrophotometer. Distilled water was used as a blank to and any interference by the reagents were rectified by subtracting the blank from the actual sample reading. To prepare a standard, calcium chloride was used. Presented below is the standard curve for chloride;

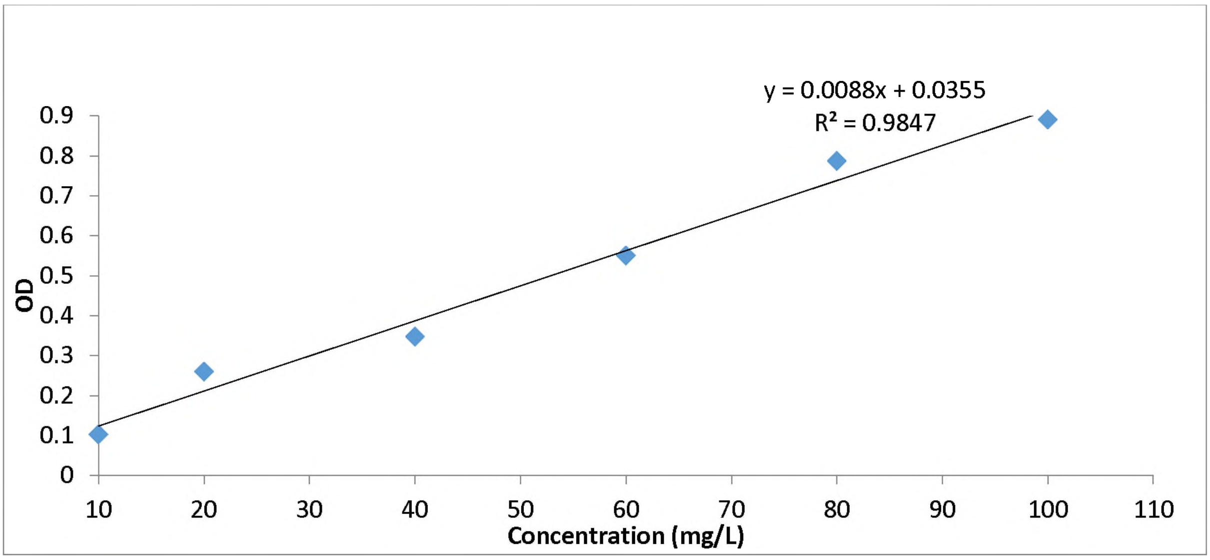


Figure 13. The standard curve for chloride. The linear equation was used to determine the concentration of chloride from the absorbance.

3.6.8 The potassium content of the pit latrine faecal sludge

The concentration of potassium in the pit latrine faecal sludge was determined using the Merck potassium test kit (Catalogue No. 1.14562.0001) (APHA,AWWA and WEF, 1998). The chemical reaction that occurs during the potassium test is presented in equation 8;

$$NaB(C_6H_5)_4 + K^+ \rightarrow KB(C_6H_5)_4 + Na^+ \qquad [8]$$

2.0 ml of the pre-treated faecal sludge sample was pipetted into a reaction cell and the cell was closed and its contents mixed. Then 6.0 drops of potassium reagent 1 were added into the reaction cell followed by mixing. After that, 1.0 level blue microspoon of potassium reagent 2 was added and the contents were mixed until the reagent dissolved completely. The reaction cell was allowed to stand for 5 minutes and after which the concentration of potassium was determined using a Spectroquant NOVA 60 from Merck (Johannesburg, South Africa).

3.6.9 The trace element content of the pit latrine faecal sludge

For metal analysis, autoclaved faecal sludge samples were sent to Bemlabs (16 Van der Berg Crescent, Grant's Center, 7137). The selected trace elements for analysis were arsenic (As), lead (Pb), chromium (Cr), manganese (Mn), magnesium (Mg), mercury (Hg), iron (Fe), copper (Cu), calcium (Ca), molybdenum (Mo), nickel (Ni) and cadmium (Cd). Approximately, 40 ml of the faecal sludge samples were transferred into urine jars (40 ml, Cleansafe; South Africa), placed in the cooler box and then couriered to Bemlabs. At Bemlabs, the samples were analysed with inductively coupled optical emission spectroscopy (ICP-OES) with a detection limit of 0.001 mg/l. The method that was used for analysis by the lab was USEPA Method 3146 (nitric acid digestion) (APHA,AWWA and WEF, 1998).The method involves complete digestion of the sample in an acidic solution. A gram (dry weight) of the faecal sludge was digested with nitric acid and hydrogen peroxide (1:1) and refluxed at 95 ± 5°Cfor two hours. The digested faecal sludge was allowed to cool down and then diluted to 100 ml with chromatogram grade water. The sample was then analysed using ICP-OES.

3.7 Biological analysis of the pit latrine faecal sludge

3.7.9 Extraction of microorganisms from the faecal sludge

Five grams of each of the samples was aseptically transferred to pre-weighed autoclaved 100 ml Erlenmeyer flasks. Then 50 ml of 9% (w/v) sterile saline solution (9g NaCl dissolved in 1L milliQ water) was added. The suspensions were agitated by vortexing for 2 minutes.

3.7.2 Spread plating of *E. coli, Enterococcus faecium and Salmonella* spp.

The serial dilution of the above suspension were prepared by aseptically pipetting 500 µl of the suspension into 4.5 ml of the sterile saline solution (1 in 10 dilution) and mixed by vortexing for 1 minute. From the 1 in 10 dilution, 500 µl was pipetted into 4.5 ml of the sterile saline solution (1 in 100 dilution). The serial dilutions were repeated until the dilution of 1 in 10^6 was reached. A triplicate series of pre-prepared (prepared according to the manufacturer's instructions) Hi-Crome m-TEC agar (Sigma, Catalogue No.90924-500G) and Hi-Crome *Enterococcus faecium* (Sigma, Catalogue No.90919-54.2G) agar was aseptically inoculated with 100 µl of each of the prepared dilutions and spread over the agar surface using a sterile glass rod. The inoculated agar plates were incubated at 44.5±0.2 and 35℃ for 24 hours respectively. *E.coli* colonies appeared pink on the Hi-Crome m-TEC agar and *Enterococcus faecium* appeared black on the Hi-Crome *Enterococcus faecium agar*. The number of colonies formed in each selective medium plate were enumerated and recorded as colony forming units (CFU/ g of dry weight). The following equations were used;

$$Multiplication\ factor = \frac{W1}{W2} \qquad [9]$$

$$\frac{CFU}{g\ of\ wet\ weight} = CFU\ \times\ dilution\ factor \qquad [10]$$

$$\frac{CFU}{g\ of\ dry\ weight} = \frac{CFU}{g\ of\ wet\ weight} \times multiplication\ factor \qquad [11]$$

In order to grow *Salmonella* spp and to suppress the growth of other bacteria, Tetrathionate Enrichment Broth (Merck, Catalogue No. 110863) was used to prepare the serial dilution of the suspension as outlined above. Using a triplicate series of pre-prepared XLD agar (Merck, Catalogue No. 1.05287.00500) plates, 100 µl of each dilution was spread plated as outlined above. The inoculated plates were incubated at 35℃ for 24 hours. *Salmonella* spp. colonies appeared pink red with black centres and were enumerated and recorded as CFU/g of dry weight.

3.7.3 Helminth enumeration from the pit latrine faecal sludge

For the enumeration of Helminths, two methods were used. Two methods were the sucrose flotation method (Reinecke, 1983; Margaret, 1970) and the zinc flotation (Moodley, et al., 2008). The experimental procedures are outlined below:

3.7.4 Zinc flotation method

The sample to be analysed was thoroughly mixed by stirring with a glass rod. Fifteen millilitre portions of the sample were transferred into separate test tubes and 14 ml of 0.1% ammonium bicarbonate solution was added and mixed by vortexing for 5 minutes. The mixture was filtered through a 150 µm nylon mesh and the filtrate collected in a glass beaker. The filtrate was poured into a centrifuge tube and centrifuged at 1 389g (3 000 rpm) for 3 minutes on a JA-20 rotor in a Beckman-Coulter Avanti J-E Model centrifuge. The supernatant was discarded and pellet re-suspended in 12 ml of $ZnSO_4$ (specific gravity of 1.3; 600 g of $ZnSO_4$ in 800 ml of milli-Q water) and mixed by vortexing. After that, the test tubes were centrifuged at 617 g (2 000 rpm) for 3 minutes. Supernatant was filtered through a 20 µm nylon mesh and the retentate washed into test tubes with distilled water. The test tubes were centrifuged at 964g (2 500 rpm) for 3 minutes and the supernatant discarded. The pellet was centrifuged again at 964 g (2 500 rpm) for 3 minutes after which 60 µl of the supernatant was removed and analysed under the microscope (x10/x40 magnification). Any eggs visible under the microscope were counted and reported as eggs/g of wet sludge. The results were calculated as follows;

$$\frac{Helminth\ ova}{wet\ weight\ (g)} = \frac{viable\ ova\ in\ wet\ sample \times \frac{100}{moisture\ content\ (\%)}}{wet\ weight\ (g)} \qquad [12]$$

3.7.5 Sucrose flotation method

One gram of the faecal matter was weighed out and transferred to a 100 ml Schott bottle (Glassworld, Johannesburg; South Africa). To this was added 59 ml of a 40 % sucrose (Catalogue No S7903-250G, Sigma Aldrich, South Africa) solution and mixed thoroughly by shaking by hand. Four to five drops of amyl alcohol was added to reduce the bubbles that form in the emulsion. After further mixing by shaking, the mixture was allowed to settle for 5 minutes. The supernatant was pipetted into a Macmaster slide and filled. The slides were allowed to stand for 2 minutes to allow the helminth eggs to float to the surface. The Macmaster slides were viewed under a light microscope at 10x magnification. All the eggs that were in the rectangle on the lower surface of the cover slide of each chamber (12×8.5×1.5 mm) were counted. Each chamber represented a 0.15 ml aliquot of the emulsion with the eggs.

3.7.6 Plant growth studies

Radish and garden cress seeds were used to examine the fertilizer potential of the pit latrine faecal sludge. As growth substrates, vermiculite and a mixture of perlite and coco-peat were used to minimize the effect of soil heterogeneity in terms of its nutrient content. Initially a 1:1:1 NPK fertilizer was used as a positive control but was later changed to a Hoagland's solution, a chemically defined solution that is known to supply all the nutrients that plants require for growth (Hoagland & Arnon, 1950; Hojjat, 2014). Tap water was used as a control. The experimental design was a randomized block setting (the pots of each treatment were placed on the same tray and trays were randomly switched every three days). Approximately 300 g of vermiculite or perlite and coco-peat mixture (500 g: 1000 g) was weighed and placed in 5 separate plastic containers. In each plastic container, 500 ml of the different concentrations of the fertilizer treatment (0.1 %, 1%, 10% and 100% pasteurized pit latrine faecal sludge made up to IL with milliQ-water in Schott bottles) were added. These containers were left overnight to allow the absorption of the treatment solutions by the growth substrate. Fifty grams (6 replicates) of the growth substrate was weighed from each plastic container into a series of plastic flower pots.

A plastic mesh was placed at the base of each pot to prevent the loss of the growth substrate through perforations at the bottom of the flower pots. In each flower pot, four 2 mm holes were punched into the surface of the growth substrate and into which the seeds were sowed. The experiment was conducted in a controlled environment room (12 hours of light and 12 hours of darkness) at 26- 27℃ for 35 days. Light was supplied by an overhead light source consisted of 15W fluorescent bulbs (Eveready Cool Daylight) and two types of fluorescent strip lights (Osram L36W/33-640 Cool White and Osram L36W/77 Fluora). Irradiance ranged from 70 to 90 $\mu mol/m^2/sec$. The grown growth was kept moist by irrigating with 10 ml of tap water every third day. After 7 days, the germination percentage was investigated so as to view the phytotoxicity of the fertilizer treatments. Germination percentage was determined after 7 days using the following equation;

$$Germination\ \% = \frac{Totalgerminatedseeds}{Numberoftheseedssowed} \times\ 100\% \qquad [13]$$

After 35 days, the experiment was terminated and the yield parameters (total number of plants that remained viable when the experiment was terminated, plant height (cm), number of leaves, root length (cm), fresh weight (g), dry weight (g) and moisture content percentage) were investigated. A 30 cm ruler was used to determine the plant height and the root length. For the fresh and dry weight, each plant was weighed separately and oven dried at 65℃ for 72 hours. After that, the plants were reweighed and the dry weight and the moisture content was determined (refer to equation 3). The measured yield parameters were analysed using single Anova from Microsoft excel 2013.

3.8 The metagenomics studies

The study was conducted to investigate the microbial community that is found at the different depths in the pit latrine. The metagenomics study is based on the diversity of a single gene that codes for the 16S subunit of prokaryote ribosome (Thomas, et al., 2012). Briefly, the deoxyribonucleic acid (DNA) is extracted from a particular sample. The extracted DNA is amplified to increase the concentration of the extracted DNA. Thereafter the extracted DNA is taken for sequencing where the amplified DNA fragments are translated into the identity of the microorganisms that gave the template of the original fragments for the 16S subunit.

3.9.1 DNA extraction

Faecal sludge pit latrine DNA collected using the segmented core sampler and stored at -20°C was extracted using the Zymo Research Faecal DNA MiniPrep kit (Catalogue No.D6010). The samples were thawed before extraction. Approximately 0.15 g of the thawed pit latrine faecal sludge was weighed into the ZR BashingBead lysis tube and 750 µl of a lysis solution was added into the tube. The ZR BashingBead lysis tube was then vortexed at maximum speed for 5 minutes and thereafter centrifuged at 10 000 x g (9 708 rpm) for 1 minute using an Eppendorf Model 5810 centrifuge (Eppendorf F45-30-11 rotor). After that 400 µl of the supernatant was pipetted into a Zymo-Spin IV filter that was placed in a collection tube. This tube was then centrifuged at 7 000 x g (8 122 rpm) for 1 minute. 1 200 µl of faecal binding DNA binding buffer and 6 µl of beta-mecaptoethanol (0.5%, v/v) was added to the filtrate in the collection tube. After that, 800 µl the mixture was transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10 000 x g for 1 minute.

The filtrate was discarded and the previous step was repeated. 200 µl of the DNA pre-wash buffer was added to the Zymo-Spin IIC column in a collection tube and the tube was centrifuged at 10 000 x g for 1 minute. This was followed by the addition of 500 µl of faecal DNA wash buffer to the Zymo-Spin IIC column and centrifugation at 10 000 x g for 1 minute. After that, the Zymo-Spin IIC column was transferred to a clean 1.5 ml microcentrifuge tube. Furthermore 100 µl of the DNA elution buffer was added directly into the column matrix and the tube was centrifuged at 10 000 x g for 30 seconds. The eluted DNA was then pipetted into a prepared Zymo-Spin IV-HRC Spin Filter in a clean 1.5 ml microcentrifuge tube. The tube was centrifuged at 8 000 x g (8 683 rpm) for 1 minute. The filtered DNA was stored in a fridge at -20°C until analysis. The concentration and the purity of the DNA from each sample was analysed using a NanoDrop UV/Vis spectrophotometer (2 µl sample; wavelength; 260 & 280 nm). The samples were sent to Inqaba Biotechnical Industries (Pty) Ltd (525 Walker St, Pretoria, 0002) for metagenomics analysis. The metagenomics analysis at Inqaba Biotechnical Industries was conducted using the Roche Genome Sequencer 454 FLX system where approximately 5 µg of a pure and concentrated DNA is run on the sequencer. The sequencer generates sequence reads of approximately 200-300 bases which can then be analysed using either BLASTX or MEGAN applications to search against sequence database for homologs.

Chapter 4: Results and discussion

4.1 Physicochemical and biological properties of the pit latrine faecal sludge

A study was initiated to determine the fertilizer value of anaerobically digested and pasteurized pit latrine faecal sludge. A part of the study included characterizing pit latrine faecal sludge before anaerobic digestion, biogas recovery during anaerobic digestion, pasteurization of the effluent, and characterizing the anaerobically digested and pasteurized faecal sludge. The other part included the application of the pasteurized pit latrine faecal sludge as a fertilizer. The current sanitation delivery backlogs such as poor operation and maintenance of pit latrines in Hlalani Township led to the development of a tool that could be used to address the backlogs through the use of volunteers. For the ease of reading, the order of the results follows the aforementioned order.

The results obtained from characterizing the pit latrine faecal sludge before anaerobic digestion and after anaerobic digestion and pasteurization, including the microbial community structure of the different depths of pit latrines are presented in this section. Physicochemical properties of the sludge were not determined after anaerobic digestion because digestion was mainly focused on biogas recovery. After this section, the results from the plant growth studies are presented, followed by a detailed information about the development of a tool that could be used to address sanitation delivery challenges in municipalities across the country. The physicochemical properties of the pit latrine faecal sludge before the first anaerobic digestion experiment are shown in Tables 1. Table 2 shows the microbiological properties of the pit latrine faecal sludge before anaerobic digestion and after pasteurization.

Table 1. The physicochemical properties of the pit latrine faecal sludge before the first anaerobic digestion experiment.

Parameter	Measurements (n=3)
Dry weight % of wet weight (g/g)	92.8 ± 0.3
Moisture content (%) (g/g of dry weight)	7.2 ± 0.3
LOI % (g/g of dry weight)	96.1 ± 0.2
COD (mg/l)	1 406 ± 0.4
PO_4^{3-} (mg/l)	48.1 ± 3
NH_4^+ (mg/l)	10.9 ± 5
NO_3^- (mg/l)	26 ± 2
Cl^- (mg/l)	2 293 ± 95

The observed results for the moisture content were out of the range that was reported by Bakare and colleagues who reported moisture content (%) range of 60 to 80% in VIP's as shown by the high dry weight %, an indication that the pit was well drained (Table 1) (Bakare, et al., 2012). The COD concentration reported in this pit latrine was lower than the range (4 613-9 118 mg/l) that was reported by Khanto and Banjerdikij for pit latrine faecal sludge, suggesting a substantial degree of faecal sludge stabilization in the pit latrine (Table 1) (Khanto & Banjerdikij, 2013). Kuai and colleagues reported a COD concentration of 1831 ± 1621 mg/l in faecal sludge (Kuai, et al., 2000). PO_4^{3-}, NH_4^+ and NO_3^- give the faecal sludge the fertilizer value for agricultural application (Guzha, et al., 2005; Jensen, et al., 2008). The concentration of NH_4^+ (10.9 ± 5 mg/l) obtained from the pit latrine faecal sludge was lower than the range (2 000-9 000 mg/l) that was reported by Semiyaga and colleagues for pit latrine faecal sludge but was within the reported range (30-70 mg/l) of NH_4^+ in raw sewage (Semiyaga, et al., 2015). The observed difference in NH_4^+ concentrations might be attributed to several factors, which include storage period, the method used for extraction, pH and temperature which determine the form of nitrogen that dominates at a particular time (Karak & Bhattacharyya, 2011; Semiyaga, et al., 2015). Kouawa and colleagues reported a concentration of 84.4 ± 194.0 mg/l and 51.9 ± 61.9 mg/l for NO_3^- and PO_4^{3-} for faecal sludge (Kouawa, et al., 2015). The concentrations reported for the studied pit latrine however were lower possible due to the assimilation of this compounds by the microorganisms or leaching for NO_3.$^-$

The biogas could not be recovered from the pit latrine faecal sludge that was in the Perspex digester. This can be attributed to the quantity of the sludge in relation to the volume of the reactor itself which was too small (see figure 5). Ideally, in an anaerobic digester, a particular percentage of the total volume of the bioreactor should be reserved as a headspace where biogas can accumulate, generating enough pressure that can then force the gas to diffuse into the gas collection chamber (Nguyen, 2011). Improving biogas collection through the use of a co-feed is recommended in literature to inoculate the microbiological consortium responsible for the breakdown of biodegradable matter under anaerobic conditions (Valladão, et al., 2007; Romano, et al., 2009). In this particular study, a co-feed was not used because of the assumption that the necessary microorganisms were already present in sufficient concentrations in the faecal sludge. Based on the experience from this study however, it was then decided that a co-feed be included to improve biogas production as that formed a crucial part on the success of the subsequent studies and also the 10% headspace which was implemented in the 200 L drum reactors. The chosen indicator pathogenic microorganisms could not be detected after anaerobic digestion and pasteurization at 70℃ for an hour. Thus *E.coli* and *Salmonella* spp. appeared to be completely eliminated from the effluent (Table 2).

Table 2. The microbiological properties of the pit latrine faecal sludge before anaerobic digestion and pasteurization.

Bacteria	Before anaerobic digestion	After pasteurisation
***Salmonella* (cfu/ g of dry weight)**	0	0
***E.coli*(cfu/ g of dry weight)**	0	0

E.coli and *Salmonella* spp. were not detected in the faecal sludge before anaerobic digestion in this particular study. However, *E.coli* concentration of $4.07 \pm 2.04 \times 10^8$cfu/g in faecal sludge has been reported by Cofie and colleagues (Cofie, et al., 2009). The presence of *Salmonella* spp. in the faecal sludge depends mainly on the health of the pit latrine users, thus its detection could serve as an indicator of a possible infection in the pit latrine users (Gibbs, et al., 1997; Esrey, et al., 2001).

In this study, 331 g (649 L) of LPG gas was used to pasteurize 3 000 g of the pit latrine sludge which equated to 15.3 MJ of the energy used. This was because of poor biogas recovery during anaerobic digestion. The amount of the gas used was determined by determining the mass of the LPG gas container before and after pasteurization and then the difference was the mass of the gas used. To calculate energy consumption, the mass of the LPG gas was converted to MJ by multiplying the mass of LPG gas used with a conversion value of 25 MJ and then dividing with 0.51 kg (a known mass of LPG gas). To pasteurize the same mass of the sludge using biogas, 663.4 L of biogas would have been required. The theoretical biogas yields were estimated from COD data using the following formula (Angelidaki & Sanders, 2004);

$$CH_4\ yield\ at\ STP = \frac{0.35(L)}{g\ COD} \qquad [14]$$

According to the regulations for fertilizer use in South Africa, faecal sludge must have 0 *Ascaris* ova/10 g of dry sludge, 0 *Salmonella* spp./10 g of dry sludge and less than 1 000 faecal coliforms/ 10 g of dry sludge (DAFF, 2012). The WHO guidelines for excreta use in agriculture clearly state that the concentration of *E.coli* in sludges to be applied in agriculture should be less than 1 000 per gram of total solids (WHO, 2006). Based on the WHO guidelines for excreta use and the regulations for fertilizer use in agriculture, it was then concluded that the faecal sludge could be applied as a fertilizer. Regardless of the quality of the faecal sludge, thorough washing of vegetables grown in soils that are fertilized with faecal sludge is necessary prior consumption (Cofie & Adamtey, 2009). For root crops and fruit vegetables peeling reduces pathogens but cooking achieves a complete reduction of pathogens (Duncker, et al., 2007).

4.1.1 Physicochemical properties of the faecal sludge before anaerobic digestion

To address the issue of biogas production, the pit latrine faecal sludge was seeded with the effluent from the anaerobic digester of the Belmont Valley WWTW at different ratios. The physicochemical and microbiological properties of the different ratios of pit latrine faecal sludge before anaerobic digestion are presented in Table 3. The physicochemical properties of the effluent from the Belmont Valley WWTW were not quantified, because the material was used solely as a co-feed in the reactors and as such its influence on the general characteristics of the faecal sludge was deemed negligible. Therefore, the physicochemical properties of the 33% and 66% FS reflect the product of the combination of the effluent and the pit latrine sludge. The physicochemical properties of the original pit latrine faecal sludge are presented as 100% FS.

Table 3. The physicochemical properties of the pit latrine faecal sludge mixed with the effluent from the anaerobic digester of Belmont Valley WWTW at different ratios [2:1 pit latrine faecal sludge to effluent (66% FS), 1:2 pit latrine faecal sludge to effluent (33% FS) and 100 % pit latrine faecal sludge (100% FS)] before anaerobic digestion.

Parameter	33% FS	66% FS	100% FS
Dry weight % of wet weight (g/g)	1.30 ± 0.30	2.79 ± 0.61	2.77 ± 0.30
Moisture content (%) of dry weight (g/g)	98.7 ± 0.3	97.2 ± 0.6	97.2 ± 0.3
LOI (%)of dry weight (g/g)	61.1 ± 10.1	77.1 ± 5.0	64.4 ± 0.1
COD (mg/l)	46 317 ± 12 872	43 580 ± 10 763	35 780 ± 3 935
PO_4^{3-} (mg/l)	103 ± 6	133 ± 23	137 ± 15
NH_4^+ (mg/l)	8 537 ± 3 575	7 600 ± 990	10 080 ± 439
NO_3^- (mg/l)	15 600 ± 3 524	24 277 ± 7 588	24 017 ± 6 985
K (mg/l)	670 ± 375	1 267 ± 35	553 ± 12
Cl^- (mg/l)	297 ± 91	350 ± 46	393 ± 86
pH	7.50	7.57	7.00
Pb (mg/kg)*	0.23	0.49	0.14
Ni (mg/kg)*	0.26	0.33	0.23
Cr (mg/kg)*	0.46	11.1	3.9
Mo (mg/kg)*	0.02	5.0	0.02
As (mg/kg)*	0.09	0.1	0.03
Cu (mg/kg)*	1.45	50.20	1.97
Mn (mg/kg)*	3.48	10.70	4.29
Fe (mg/kg)*	128	267	72
Ca (mg/kg)*	289	541	310
Cd (mg/kg)*	0.01	0.01	0
Hg (mg/kg)*	0	0	0
Mg (mg/kg)*	90	128	80
***E.coli* (cfu/g of dry weight)**	$7.5x10^2$	$3.7x10^3$	$6.6x10^2$
***Salmonella* spp. (cfu/g of dry weight)**	$7.5x10^2$	$3.7x10^2$	$3.3x10^2$
Total helminths/ g of dry weight	0	0	1

All treatments analysis were done in triplicate except the ones indicated by * where n =1.

The dry weight (1.30 ± 0.30 - 2.79 ± 0.61 % of wet weight in g/g) of the pit latrine faecal sludge was low throughout the different digesters with high moisture content (97.2 ± 0.6 to 98.7 ± 0.3 % of wet weight in g/g), possible due to the addition of water by the vacuum truck operators during pit emptying (Table 3). Moisture content range of 60 to 80% is reported by Bakare and colleagues in faecal sludge (Bakare, et al., 2012). At this stage, high moisture content was desirable as it promotes anaerobic conditions. The COD concentration in all the digesters was within the COD concentration range of 20 000 and 50 000 mg/l was reported by Heinss and colleagues for pit latrine faecal sludge (Table 3) (Heinss, et al., 1998). LOI of 65 ± 21% for faecal sludge was reported by Kouawa and colleagues, and was similar to the LOI of each digester in Table 3 (Kouawa, et al., 2015). COD and LOI are the presentation of the organic matter of the faecal sludge and therefore suggested that the faecal sludge could be digested further to recover biogas.

The reported concentrations for PO_4^{3-}, NH_4^+ and NO_3^- were higher than the concentration of PO_4^{3-} (51.9 ± 21 mg/l), NO_3^- (84.4 ± 194 mg/l) and NH_4^+ (67 ± 27 mg/l) that were reported by Kouawa *et al.* and Kuai *et al* for faecal sludge, possible due to the fact that the sludge used for this study was from multiple pit latrines and therefore, the degree of faecal sludge stabilization might have varied significantly resulting in high concentrations (Kuai, et al., 2000; Kouawa, et al., 2015). However, the concentration of NH_4^+ reported in this study is within the range (2 000-9 000 mg/l) that was reported by Semiyaga *et al* (2015) for pit latrine faecal sludge (Semiyaga, et al., 2015). K and Cl^- concentrations in faecal sludge ranged from 553 ± 12 to 1 267 ± 35 mg/l and from 297 ± 91 to 393 ± 86 mg/l respectively.

Bacterial growth is stimulated by K, Ca, Mg and NH_4^+ therefore, the presence of these nutrients in faecal sludge is important in ensuring the survival of microbial consortia responsible for breaking down organic matter in anaerobic digesters and the production of biogas (Daisy & Kamaraj, 2011). However, the concentration range of K^+ (2 500- 4500 mg/l), Ca $^{++}$ (2 500-

4500 mg/l) and Mg^{++} (1 000- 1 500 mg/l) is known to have an inhibitory effect on bacterial growth during anaerobic digestion (Daisy & Kamaraj, 2011). The concentrations of K, Ca and Mg that were found in this study were below these concentration ranges and as such could not have had any negative effect on the growth of the anaerobic bacteria (Table 3). Biological processes are regulated by pH amongst other factors, and such serves as an important parameter in anaerobic digestion. From the study the pH ranged between 7.0 and 7.5 and was within the desirable range of 6.5 and 7.8 for anaerobic digestion (Appels, et al., 2008).

The concentrations of the trace elements reported in this study are lower that the concentrations of Cu (97.50 ± 6.05 mg/kg), Mn (195.33 ± 1.69 mg/kg), Fe (938.4 ± 25.39 mg/kg), Pb (156.67 ± 34.0 mg/kg) and traces of Cd and Hg that were reported in faecal sludges from Ghana (Cofie & Adamtey, 2009). That can be attributed to the fact that Grahamstown is not an industrial area and therefore heavy metals might be only be from household waste or from food sources. Lower concentrations of Fe (4 462 µg/g), Mn (189 µg/g), Pb (189 µg/g) and Cu (85 µg/g) present in faecal sludge have been reported by Nikiema and colleagues (Nikiema, et al., 2013). Heavy metal concentrations of 10 mg/kg, 414 mg/kg, 576 mg/kg, 4 mg/kg, 9 mg/kg, 296 mg/kg and 318 mg/kg for Cd, Cr, Cu, Hg, Mo, Ni and Pb respectively, have been reported in South African sewage sludges (Snyman, et al., 2004). In pit latrine faecal sludges, the concentration of heavy metals is expected to be lower because their source is the disposal of household waste such as batteries and chemicals in the pit with a minor contribution from food consumed. Low concentrations of these trace elements suggested that they will not have a negative effect on the anaerobic digestion process as higher concentrations of elements such as Mn (greater than 1 000 mg/l) have been reported to have an inhibitory effect on anaerobic bacteria (Daisy & Kamaraj, 2011; Feachem, et al., 1983).

Substantial concentrations of pathogenic bacteria *E.coli* and *Salmonella* spp. were detected in the faecal sludge including a single helminth egg (Table 3). However, higher concentrations of *E.coli* ($4.07\pm2.04\text{x}10^{8}$cfu/g), and helminths eggs (30 000- 40 000 eggs/ g of total solids) have been reported in literature (Cofie, et al., 2009; Semiyaga, et al., 2015). Yadav and colleagues reported *Salmonella* spp., and viable helminths ova of $5.1\text{x}10^{4} \pm 1.6\text{x}10^{2}$ MPN/g dry weight and 12.5 ± 3.5 ova/4g of dry weight respectively in faecal sludge (Yadav, et al., 2012). At this stage, the detected microorganisms were not of concern because the sludge was yet to undergo the anaerobic digestion.

Approximately 48 hours after the second anaerobic digestion experiment was initiated, the inner car tyre tube (18 L) that was attached to the reactor with 33% FS mixed with the effluent from the Belmont Valley WWTW started expanding until it filled up. That was an indication of biogas production within the reactor. The tubes on other reactors, however, remained deflated. An estimation made from the volume of the tyre suggested that 18 L (see equation 15) of gas would have been produced. The following equation was used to calculate the volume of the tires;

$$V(m^3) = 2\pi^2 \times Rr^2 \qquad [15]$$

In this equation, V is the volume of the tire, R is the centre of the torus (average of internal diameter and external diameter of the tire) and r is the thickness of the tire divided by 2.To show that there was flammable gas inside the tube, the tube was detached from the reactor and connected to a Fisher burner (Fisher Scientific, USA) and the gas was ignited. The produced gas, however, was still insufficient as only a single tyre was filled up until gas production stopped. Consequently, for pasteurization LPG gas was used. So, 330 g (647.1 L) of LGP gas was used to pasteurize 3210 g of the effluent. This equated to 15.2 MJ of energy required for pasteurization. If biogas was used, 661.4 L of biogas would have been used to pasteurize the same material. Poor gas collection was mainly attributed to the gas leakage from the manifold accommodating the crank handle despite the use of silicone (polydimethylsiloxane) sealant to reinforce the sealing (refer to Figure 7b). Hydrogen sulphide gas could be smelt from the crank handle. Gases that are likely to permeate the silicone include CO_2, N_2, O_2 and CH_4 with the diffusivity of 11, 15, 16 and 13 cm^2/s respectively (Zhang, 2006). Thus, the type of sealant used was not gas tight and therefore a new sealant, with lower gas permeability properties, was introduced in the third anaerobic digestion experiment.

To address the aforementioned problems, the sealant was changed to an acrylic glue (poly (methyl methacrylate), and an acrylic paint was used to reinforce the sealing and to provide a thickened, low gas permeable layer to minimize gas leakage in the third anaerobic digestion experiment (Alcolin Contractors Silicone, Cape Town, South Africa) (Dooerra & Lehmkul, 2008; Ali, et al., 2015). Additionally, PTFE Teflon plumbing tape was used to improve the sealing between the reactors and their lids. Lastly, the reactors were seeded with cow paunch manure to enrich the anaerobic microbial community and to enhance biogas production in the reactors. The physicochemical and microbiological properties of the mixtures of the pit latrine faecal sludge, the effluent from an anaerobic digester from Belmont Valley WWTW and the cow paunch manure before anaerobic digestion are presented in Table 4.

Table 4. The physicochemical properties of the different proportions of pit latrine faecal sludge and the effluent from the WWTW mixed with cow paunch before the third anaerobic digestion experiment.

Parameter	33% FS	66% FS	100% FS
Dry weight % of wet weight (g/g)	2.12 ± 0.60	1.64 ± 0.60	0.82 ± 0.60
Moisture content (%) of dry weight (g/g)	97.9 ± 0.6	98.4 ± 0.6	99.2 ± 0.6
LOI (%)of dry weight (g/g)	57 ± 14	33 ± 29	0
COD (mg/l)	3 639 ± 87	3 216 ± 70	2 993 ± 66
PO_4^{3-} (mg/l)	82 ± 1	95 ± 7	99 ± 3
NH_4^+ (mg/l)	21 067 ± 5 041	18 673 ± 5 065	19 187 ± 1 123
NO_3^- (mg/l)	5 943 ± 178	5 533 ± 806	5 443 ± 525
K (mg/l)	313 ± 6	340 ± 10	427 ± 21
Cl^- (mg/l)	583 ± 11	590 ± 6	712 ± 22
pH	7.32	7.51	7.79
Pb (mg/kg)*	0	0	0
Ni (mg/kg)*	5.51	2.03	0.849
Cr (mg/kg)*	1.22	1.04	0.377
Mo (mg/kg)*	2.69	0.430	0.425
As (mg/kg)*	0	0	0.236
Cu (mg/kg)*	0.122	0.184	0.283
Mn (mg/kg)*	15.90	6.14	1.42
Fe (mg/kg)*	18.6	20.1	45.4
Ca (mg/kg)*	10 784	4 542	2 384
Cd (mg/kg)*	0	0	0
Hg (mg/kg)*	0.245	0.123	0.094
Mg (mg/l)*	3189.7	948.5	348.6
***E.coli* (cfu/g of dry weight)**	$1.4x10^5$	$1.3x10^5$	$1.5x10^4$
***Salmonella* spp. (cfu/g of dry weight)**	0	0	0
***E.faecium* (cfu/g of dry weight)**	$1.6x10^4$	$2.3x10^3$	$1.6x10^3$
Total helminths/ g of dry weight	0	0	0

All treatments analyses were done in triplicate except the ones indicated by * where n =1.

The reported moisture content in this study was higher than the one reported by Bakare and colleagues (2012) because of the sampling method (Table 4). The method used by these researchers involved the use of a gulper, a manually operated pump for pit emptying, emptying without any addition of water, whereas in this study, sampling involved addition of large quantities of water by the vacuum truck operators. LOI% range of 45 to 60% has been reported in literature, thus the LOI% reported in this study was similar to the one found in literature (Semiyaga, et al., 2015). The COD concentration reported in this study was lower than the COD concentration range of 4 613 to 9 118 mg/l that was reported by Khanto and Banjerdkij for faecal sludge (Khanto & Banjerdikij, 2013). High organic content of the sludge as indicated by the LOI and COD suggested possible biogas recovery during anaerobic digestion.

PO_4^{3-} and NO_3^- concentrations in faecal sludge were reported to be 51.9 ± 61.9 mg/l and 84.4 ± 194 mg/l respectively (Kouawa, et al., 2015). These concentrations were lower than the ones reported in this study, possible because the faecal sludge used was collected from multiple pit latrines and therefore mixing of the fresh and stabilized faeces could have affected the concentration (Table 4). The concentration of NH_4^+ in pit latrine faecal sludge was high and concentration range of 2 000 to 9 000 mg/l for NH_4^+ has been reported in literature including NH_4^+ concentration of 67 ± 27 mg/l (Semiyaga, et al., 2015; Kuai, et al., 2000). NH_4^+ is produced by fermentative bacteria during the anaerobic breakdown of organic matter and used up by other microorganisms as a nitrogen source and therefore, high concentrations might suggests elevated microbial activity within the pits (Wagner, et al., 2012). Yadav and colleagues reported a pH of 7.70 in faecal sludge which is similar to the one reported in this study, suggesting that the faecal sludge could be anaerobically digested (Yadav, et al., 2012).

Pb and Cd were not detected from the faecal sludge and As was only detected in the 100% FS as 0.236 mg/kg (Table 4). The concentration of the trace elements in faecal sludge were lower than the concentrations that were reported by Tervahauta and colleagues for As (12 mg/kg), Cd (13 mg/kg), Cr (731 mg/kg), Cu (3 720 mg/ kg), Hg (0.12 mg/kg), Ni (466 mg/kg) and Pb (69 mg kg) (Tervahauta, et al., 2014). A possible explanation for the low concentration of trace elements in faecal sludge might be the leaching of these elements to the surrounding soils or that the pit latrine users did not dispose household waste such as batteries in the pit (Martin & Griswold, 2009; Bakare, et al., 2012). Cofie and Adamtey reported concentrations of 195.33 ± 1.69 mg/kg and 938.4 ± 25.33 mg/kg for Mn and Fe while Nikiema and colleagues reported the concentrations of Fe and Mn to be 4 462 µg/g and 189 µg/g with Pb and Cu concentrations as 189 µg/g and 85 µg/g respectively (Cofie & Adamtey, 2009).

The concentrations reported in this study were lower suggesting minimal contamination of pit latrine faecal sludge with trace elements. *Salmonella* spp. and helminths eggs were not detected in the faecal sludge. *E.coli* and *E. faecium* ranged between 1.5×10^4 and 1.4×10^5 and 1.6×10^3 and 1.6×10^4 cfu/g of dry weight (Table 4). Higher concentrations of this microorganism have been reported in faecal sludge (Cofie, et al., 2009; Yadav, et al., 2012; Semiyaga, et al., 2015).During anaerobic digestion period, the pH, COD and NH_4^+ were monitored to check if the conditions within the reactor were conducive for anaerobic digestion. In the 33% FS, the COD concentration ranged between 3 600 ± 87 mg/l and 1 300 ± 12 mg/l with the level of significance (p=0.05) of 1.01×10^{-7} (Figure 8).

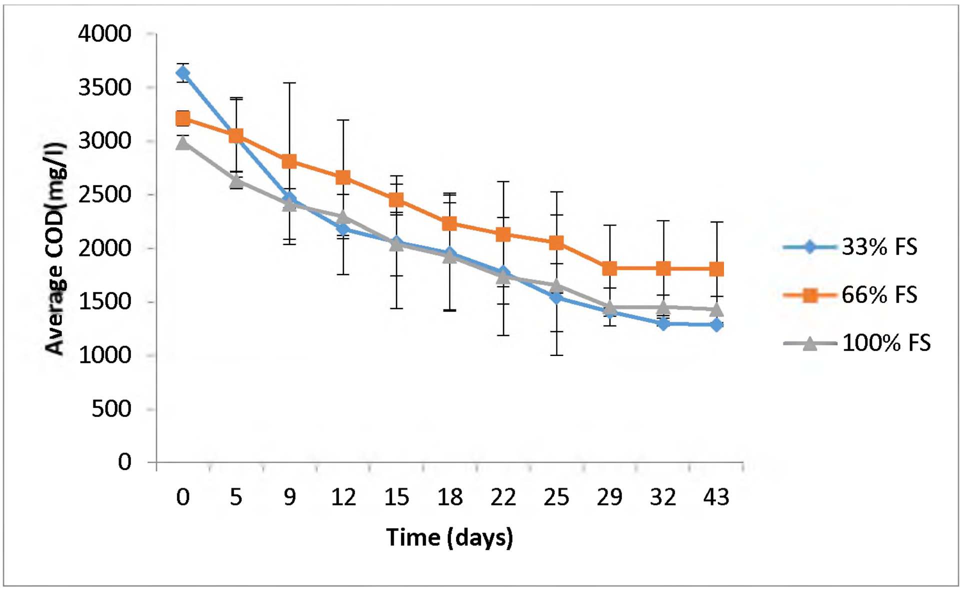


Figure 14. The removal of COD during anaerobic digestion shown against the time.

The maximum concentration of COD in the 66% FS was 3 200 ± 70 mg/l with the minimum concentration of 1 800 ± 440 mg/l.

The level of significance (p=0.05) of the decrease in COD concentration was 0.0037. From the 100% FS, the COD concentration ranged between 1 400 ±200 mg/l and 3 000 ± 70 mg/l with the level of significance (p=0.05) of $8x10^{-5}$. Thus there was a significant decline in COD in all the reactors suggesting biodegradation of organic matter. On the contrary, fluctuations were observed in the concentration of NH_4^+ from all the reactors (Figure 9). During anaerobic digestion NH_4^+ is produced from the biodegradation of amino acids by fermentative microorganisms, and is used by other microorganisms as a nitrogen source (Wagner, et al., 2012). Therefore, the observed fluctuations might be due to the overlap between NH_4^+ uptake by microorganisms and its production.

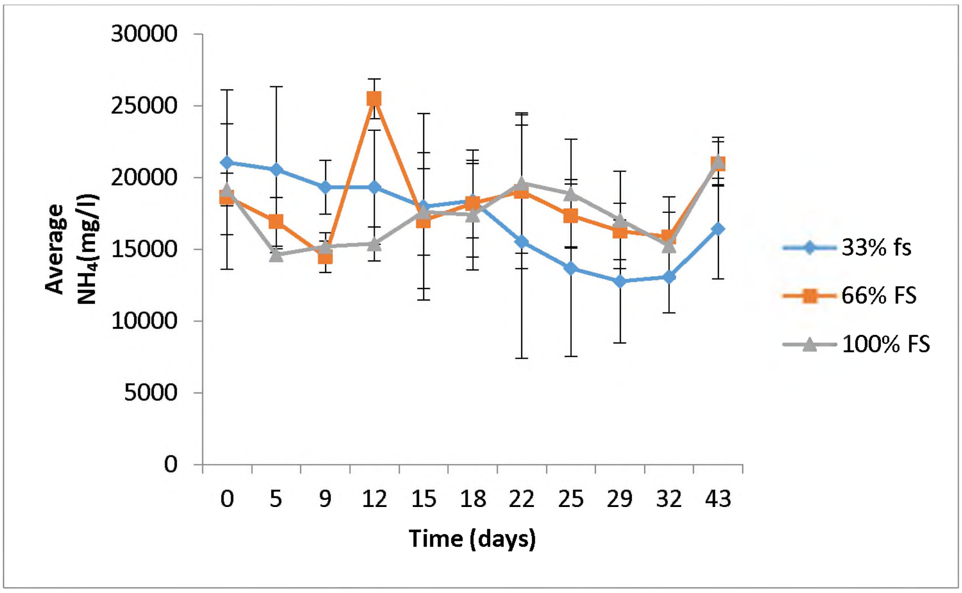


Figure 15. The fluctuation of NH_4^+ during anaerobic digestion shown against time.

In the 33% FS, the concentration of NH_4^+ ranged between 12 800 ± 4 300 mg/l and 21 000 ± 5 000 mg/l while it ranged between 14 500 ± 1 100 mg/l and 25 500 ± 1 400 mg/l in the 66% FS with the level of significance (p=0.05) of 0.402 and 0.033 respectively. Concerning the 100% FS, the NH_4^+ concentration ranged between 14 600 ± 400 mg/l and 21 200 ± 1 600 mg/l with a level of significance (p=0.05) of 0.127. From monitoring the reactors, it was concluded that the conditions within all the reactors were conducive for anaerobic digestion. The pH in the 33% FS, 66% FS and 100% FS ranged between 7.13 - 7.51, 7.30 – 7.62 and 7.23- 7.79 respectively (Figure 10). The pH range was within the accepted pH range (6.6 - 7.8) for anaerobic digestion in all the reactors (Gunnerson & Stuckey, 1986; Nguyen, 2011).

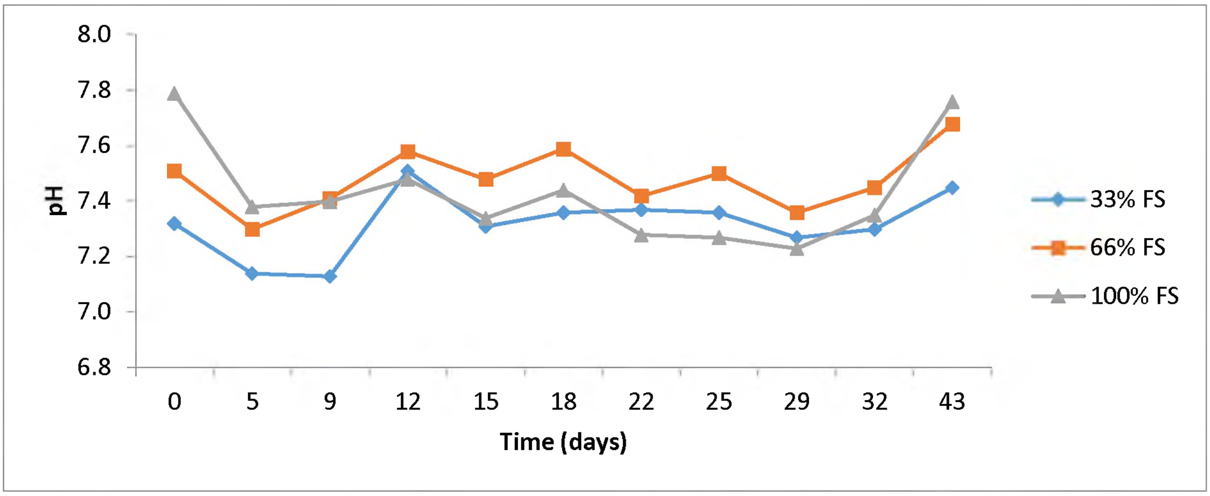


Figure 16. The pH of the material within the different reactors during anaerobic digestion shown against time. Each pH was measured (n=1) at discrete time intervals not continuously.

The 66% FS digester on the third anaerobic digestion experiment filled up 4 inner car tyre tubes, one inner tractor tyre tube and partial filling (half of the total volume) of one more inner tractor tyre tube with biogas. In total, the 4 tyres produced a total volume of 72 L of biogas (see equation 15). The volume of the inner tractor tyre tube was 142 L, thus a full tyre tube produced a total volume of 142 L of biogas. The half-filled inner tractor tyre produced an estimated total volume of 71 L gas. In total, the two tractor tyre tubes produced 213 L of biogas. Poor gas recovery was observed from the other reactors. The possible source for poor gas recovery could have been a leak from the manifold that supported the crank handle which was attached to the stirrer due to poor sealing. Apart from changing the sealant from the silicone glue to the acrylic glue, the crank handle was also only used to mix the reactor contents prior to sampling to minimize gas leakage. The rubber material used in the inner car tyre tube appeared to be permeable to biogas. This observation suggested that gas retention properties of inner tubes are poor with respect to biogas and an alternative collection vessel needs to be investigated using either a canister, a tedlar film (polyvinyl fluoride) or flexfoil opaque flexible material with 4 ply construction). Because insufficient quantities of biogas were produced, LPG gas was used for pasteurization throughout the study. The different sludges characterized in this section were then pasteurized to render them safe for application as a fertilizer. Accordingly, the next section presents physicochemical properties of the anaerobically digested and pasteurized faecal sludge.

4.1.2 Physicochemical properties of the faecal sludge after pasteurization

For feacal sludge to be applied in agricultural land, it should have less than 1000 cfu/ 10 g dry sludge of *E.*coli and 0 cfu/10 g of dry feacal sludge *Salmonella* spp (DAFF, 2012). Pasteurization efficiency study appreaded to suggest a complete elimination of *E.coli* and *Salmonella* spp. in less than 20 minutes of exposure to 70℃ of heat (Figure 17 a and b).This was desirable in that there was a certainty in the die off of potential pathogenic microorgansism during pasteruization. To test wether there was regrowth, samples were taken an hour later after pasteurization after the sludge had cooled down. There were no signs of regrowth of either *E.coli* or *Salmonella* spp.

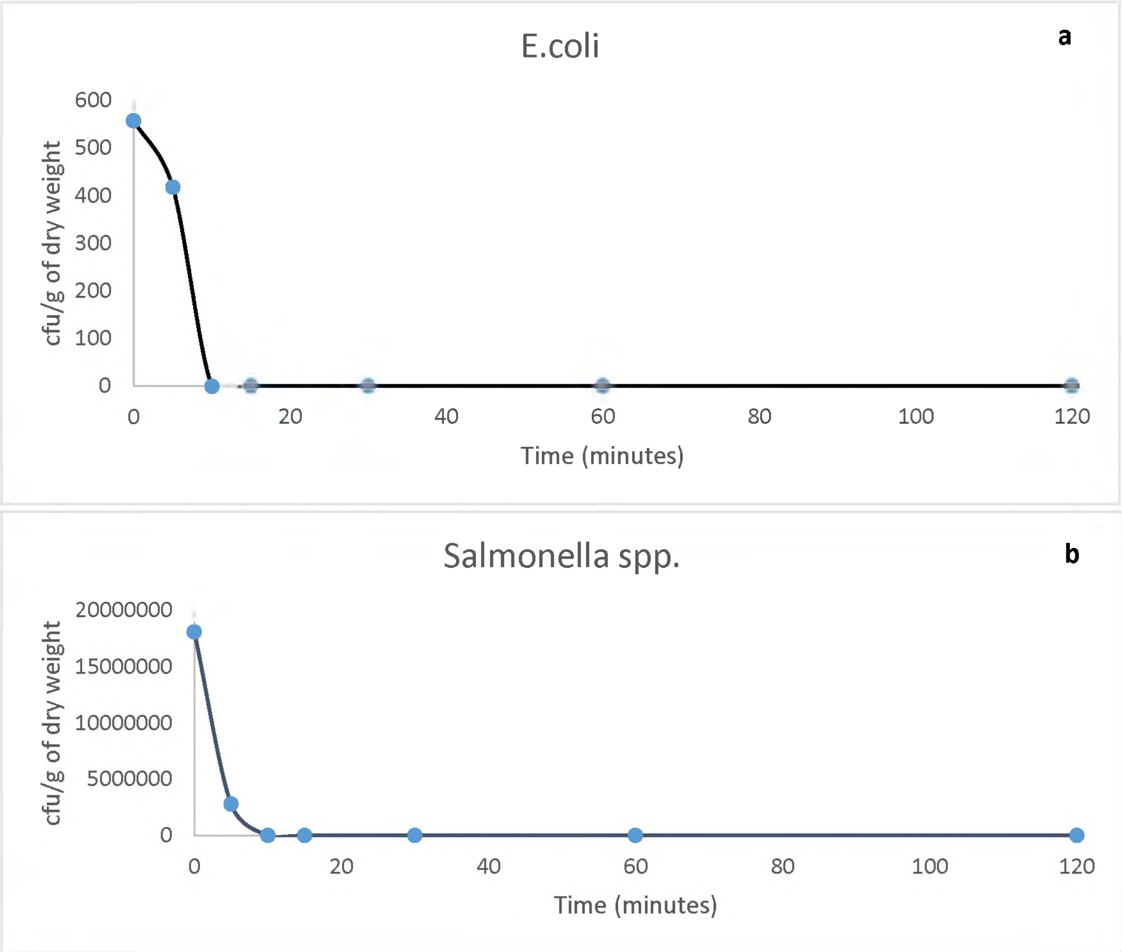


Figure 17 (a-b). The die off of *E.coli* and *Samonella* spp. after heat exposure at 70^{0}C shown against time.

The physicochemical and microbiological properties of the faecal sludge from the second anaerobic digestion experiment mixed with the effluent from Belmont Valley WWTW post anaerobic digestion and pasteurization are shown in Table 5. After pasteurization, the dry weight % was similar throughout the different digesters with moisture content greater than 90% (Table 5). High moisture content supports bacterial growth and as such might lead to the recontamination of the pasteurized faecal sludge if it is not stored in closed, sterile vessels (Gantzer, et al., 2001; Gibbs, et al., 1997). Based on the reported COD concentration, the faecal sludge was still of high strength as COD concentration was greater than 20 000 mg/l (Heinss, et al., 1998). The high COD concentration in the faecal sludge suggested that the treated faecal sludge still contained a substantial quantity of organic matter or contained recalcitrant organic material such as humics which could have been degraded further post pasteurization.

Table 5. The physicochemical and microbiological properties of the pit latrine faecal sludge mixed with the effluent from the WWTW after pasteurization. The pasteurized material was from the second anaerobic digestion experiment.

Parameter	33% FS	66% FS	100% FS	Fertilizer limit*
Dry weight % of wet weight (g/g)	3.97 ± 0.50	2.95 ± 0.02	3.95 ± 0.80	n.a
Moisture content (%) of dry weight (g/g)	96.0 ± 0.5	97.1 ± 0.02	96.1 ± 0.8	n.a
LOI (%)of dry weight (g/g)	66.7 ± 5.0	55.6 ± 19.0	70.9 ± 14.0	n.a
COD (mg/l)	22 747 ± 5 231	26 577 ± 4 907	27 113 ± 5 189	n.a
PO_4^{3-} (mg/l)	67.5 ± 4.0	63.1 ± 2.0	82.2±7.0	n.a
NH_4^+ (mg/l)	172 373 ± 19 804	217 717 ± 38 059	174 883 ± 32 434	n.a
NO_3^- (mg/l)	6 290 ± 500	5 807 ± 228	5 960 ± 1 032	n.a
K (mg/l)	347 ± 12	350 ± 60	460 ± 165	n.a
Cl^- (mg/l)	520 ± 27	490 ± 80	970 ± 121	n.a
pH	7.71	7.80	7.77	n.a
Pb (mg/kg)	0.65	0.57	1.82	400
Ni (mg/kg)	0.42	0.41	1.16	200
Cr (mg/kg)	0.95	12.4	48.5	1 750
Mo (mg/kg)	0.06	0.03	0.13	25
As (mg/kg)	0.23	0.15	0.32	15
Cu (mg/kg)	3.86	6.72	10.30	750
Mn (mg/kg)	11.9	11.8	19.5	n.a
Fe (mg/kg)	312	294	493	n.a
Ca (mg/kg)	858	590	911	n.a
Cd (mg/kg)	0.02	0.01	0.03	20
Hg (mg/kg)	0	0	0	10
Mg (mg/kg)	249	155	190	n.a
***E.coli* (cfu/g of dry weight)**	0	0	0	<1 000
***Salmonella* spp. (cfu/g of dry weight)**	0	0	0	0
Total helminths/ g of dry weight	0	0	0	0

Fertilizer limits*- regulatory limits of sewage sludge fertilizers in South Africa (DAFF, 2012). n.a, not available

The concentration of NH_4^+ ranged between 172 300 ± 19 800 and 217 700 ± 38 100 mg/l while NO_3^- concentrations ranged from 5 800 ± 230 to 6 300 ± 500. Plants rapidly assimilate inorganic nitrogen (NH_4^+; NO_3^-), however, inorganic nitrogen can easily be lost through leaching in soils (Mininni & Santori, 1987). The pH of the pasteurized faecal sludge ranged between 7.71 and 7.80. Most nutrients around this pH are available for plant uptake and thus the sludge could be applied as a fertilizer (Jadhao, et al., 1999). For faecal sludge to be used as a fertilizer, Cd, Co, Cr, Cu, Hg, Mo, Ni, Pb and As concentration (all reported in mg/kg) should not exceed 20, 100, 1750, 750, 10, 25, 200, 400 and 15 respectively (DAFF, 2012). The concentration of trace elements was in pasteurized pit larine faecal sludge was within the accepted limits for faecal sludge use in agriculture (Table 5).

The pathogenic microorganisms, *E.coli, Salmonella* spp, *E.faecium*, appeared to be completely eliminated in the faecal sludge after pasteurization. Thus, these pathogens were within the accepted range for faecal sludge reuse in agricultural soils of South Africa (Table 5) (DAFF, 2012). Helminth eggs were also not detected from the sludge post pasteurization either due to the die off or because the pasteurized faecal sludge was too dilute as suggested by the high moisture content. Ideally, there should be 0 *Ascaris* ova/10 g of dry sludge for it to be used as a fertilizer in South Africa and therefore, these results seem to suggest that the pasteurized faecal sludge was within the accepted limit for microbiological classification, and therefore could be applied in agriculture (Table 5).

The physicochemical and microbiological properties of the anaerobically digested mixture of pit latrine faecal sludge and the effluent from the Belmont Valley WWTW with cow paunch manure presented in Table 4 was pasteurized and the results are presented in Table 6. The dry weight of the pasteurized faecal sludge was below 3% with moisture content that was greater than 95%. High moisture content increases chances of sludge recontamination post pasteurization and therefore a proper storage container is necessary to prevent recontamination (Feachem, et al., 1983). The COD concentration of faecal sludge from each digester was comparable after pasteurization, and was similar to COD concentration (1 831 ± 1 621 mg/l) that was reported by Kuai and colleagues (Kuai, et al., 2000).

Table 6. The physicochemical and microbiological properties of the mixture of pit latrine faecal sludge and the effluent from the WWTW with cow paunch manure after pasteurization. The material used for pasteurization was an effluent from the third anaerobic digestion experiment.

Parameter	33% FS	66% FS	100% FS	Fertilizer limits
Dry weight % of wet weight (g/g)	1.16 ± 0.80	2.32 ± 0.80	2.32 ± 0.60	n.a
Moisture content (%) of dry weight (g/g)	98.8 ± 0.8	97.7 ± 0.8	97.7 ± 0.6	n.a
LOI (%)of dry weight (g/g)	50.0 ± 5.0	64.4 ± 4.0	0	n.a
COD (mg/l)	1 294.5 ± 8.1	1 812.3 ± 438.0	1 439.3 ± 121.1	n.a
PO_4^{3-} (mg/l)	111.8±20.0	73.3 ± 4.0	100.3 ± 3.0	n.a
NH_4^+ (mg/l)	231 733.3 ± 11 196.0	171 633 ± 35 228	182 700 ±23 157	n.a
NO_3^- (mg/l)	8 312.3 ± 2 600.0	6 590.7 ± 819.0	7 776.7 ± 850.1	n.a
K (mg/l)	850 ± 171	686.7 ± 127.2	770 ± 160	n.a
Cl^- (mg/l)	528.7 ± 12.3	605.3 ± 29.1	705.3 ± 15.1	n.a
pH	7.45	7.68	7.76	n.a
Pb (mg/kg)	0	0	0.0862	400
Ni (mg/kg)	1.77	0.250	3.36	200
Cr (mg/kg)	0.129	0.293	0.172	1 750
Mo (mg/kg)	1.47	0.142	2.85	25
As (mg/kg)	0.467	0.0056	1.72	15
Cu (mg/kg)	4.27	1.06	8.62	750
Mn (mg/kg)	0.862	0.129	1.72	n.a
Fe (mg/kg)	72.2	5.23	143.5	n.a
Ca (mg/kg)	757.3	100.0	1478.3	n.a

Cd (mg/kg)	0	0	0	20
Hg (mg/kg)	0.0862	0.0086	0.172	10
Mg (mg/kg)	199.8	49.8	408.6	n.a
***E.coli* (cfu/g of dry weight)**	0	0	0	<1000
***Salmonella* spp. (cfu/g of dry weight)**	0	0	0	0
***E.faecium* (cfu/g of dry weight)**	0	0	0	n.a
Total helminths/ g of dry weight	0	0	0	0

The pH of the pasteurized faecal sludge was neutral (Table 6). pH plays an important role in the availability of nutrients such as phosphorous to plants. For an example, $H_2PO_4^-$ is the most dominant form of soluble phosphorus at the pH range of 5 and 7 and thus its absorption by plants is most favourably around within the reported pH range (Mininni & Santori, 1987). In order for faecal sludge to be applied in agriculture, the South African regulations state that the concentrations of As, Cd, Cr, Cu, Pb, Hg and Ni should be below 40 mg/kg, 40 mg/kg 120 mg/kg 1500 mg/kg, 300 mg/kg, 15 mg/kg and 420 mg/kg respectively (Snyman, et al., 2004). Trace metals in faecal sludge were low and thus within the accepted limits for faecal sludge application in agriculture (Table 6). Microbiological classification limits *Ascaris* ova to 0 ova/100 g of dry sludge, *Salmonella* spp. to 0 spp./10 g of dry sludge and less than 1000 faecal coliforms/ 10 g of dry sludge for sludge application in agriculture (DAFF, 2012). *E.coli, Salmonella* spp., *E. faecium* and helminth eggs were not detected from the faecal sludge after pasteurization, suggesting that the chosen treatment strategy might have been effective in eliminating the studied microorganisms. Helminths eggs were of concern although they were also not detected in the original faecal sludge because the sludge was too dilute and as such that could have made it difficult to detect them.

To conclude, the physicochemical properties of the faecal sludge that give it value as a fertilizer were retained throughout although minor increases were observed in some nutrients post pasteurization. Additionally, the pathogenic microorganisms which could expose humans, animals and environment to health risks were removed successfully by the combination of anaerobic digestion and pasteurization. After the treatments, the sludge was safe for application as a fertilizer to grow crops and was within the required limits for sludge application in agriculture as per the South African Guidelines for sludge use in agriculture. The following section presents and discusses the plant growth studies that were conducted using the anaerobically digested and pasteurized pit latrine faecal sludge that is characterized above.

4.1.3 Microbial community structure at different depths of the pit latrine faecal sludge

4.1.3.1 Background

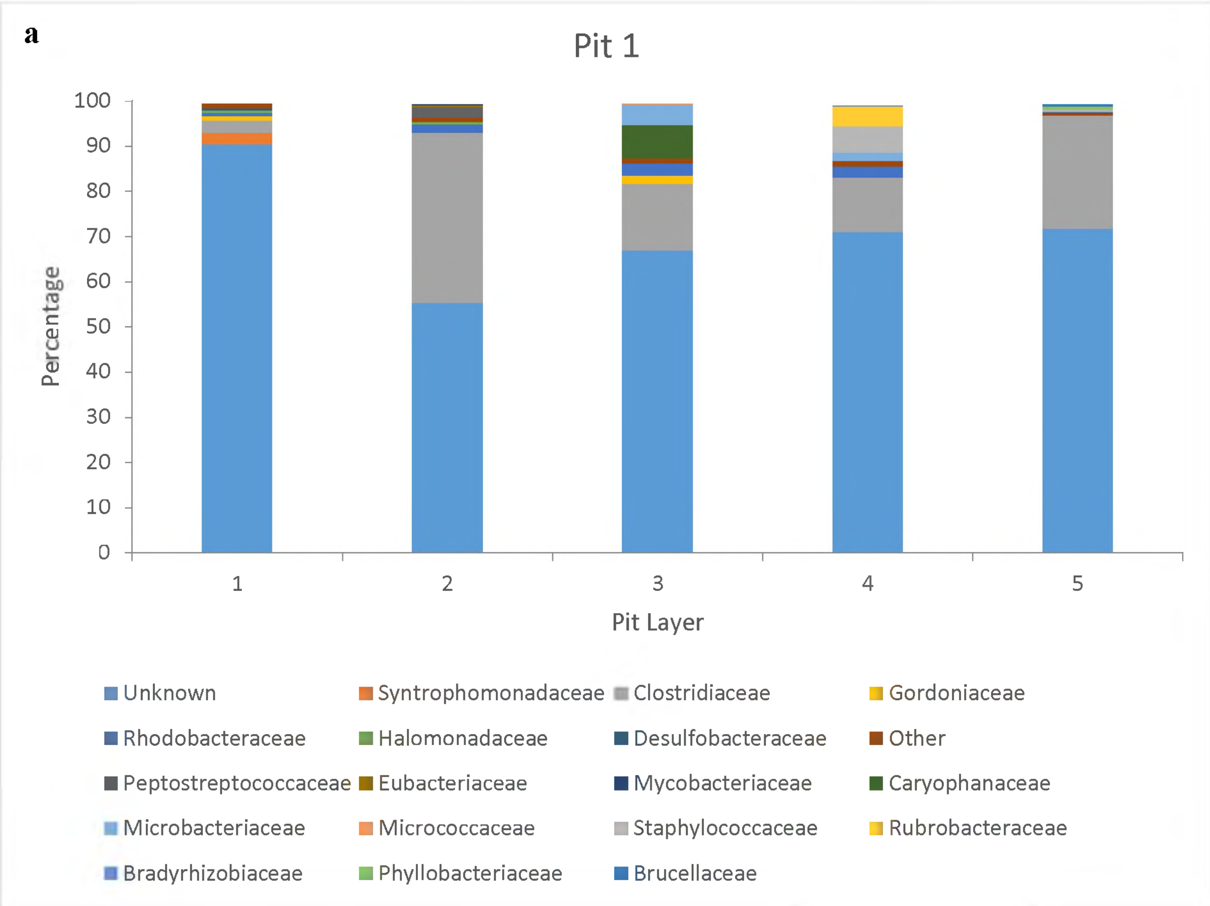
Anaerobic digestion (AD) is carried out by a consortia of microorganisms which include bacteria and archaea. Most of these microorganisms occur naturally in the environment and therefore play an important role in the stabilization of faecal sludge in pit latrines. Apart from the evidence of stabilization through the decrease in organic matter in various depths of pit latrine faecal sludge, there is no scientific evidence to substantiate the involvement of microorganisms in the stabilization process. A lot of research has been conducted on the microbial consortia responsible for AD in wastewater treatment plants mostly because of their significant role in wastewater treatment (Appels, et al., 2008; Dooerra & Lehmkul, 2008; Garcia-Pena, et al., 2011). It has been reported that stabilization of faecal sludge in pit latrines occurs through aerobic and anaerobic processes.

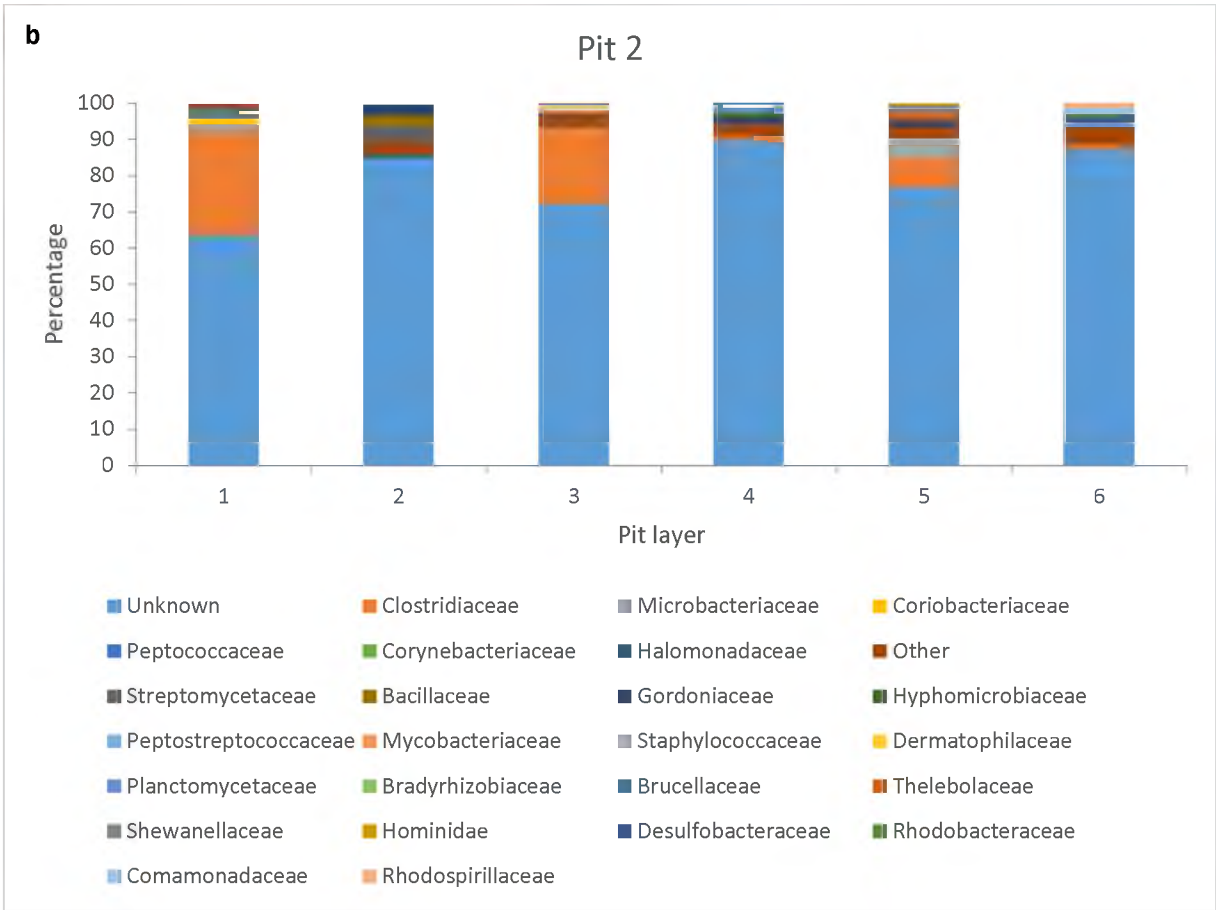
In anaerobic digesters, it was reported that members from the bacterial orders *Clostridiales*, *Flavobacteriales*, *Pseudomonadates* and *Syntrophobacteriales* were the dominating orders thus suggesting that members within these orders play an important role in anaerobic digestion (Maspolin, et al., 2015). One of the benefits of anaerobic digestion is biogas recovery. In an AD experiment conducted in this research to produce biogas for pasteurization, pit latrine faecal sludge on its own could not be shown to produce biogas. This led to a conclusion that the microbial consortia responsible for biogas production were insufficient or absent. Therefore part of this study was to determine the presence of the relevant microbial consortia in pit latrine faecal sludge through metagenomic analysis of the DNA.

During AD, organic matter is biodegraded into less biodegradable organics in a series of biological steps leading to the production with methane and carbon dioxide forming the bulk the gas. The steps that are involved in breakdown of organic matter under anaerobic conditions include hydrolysis, acidogenesis, acetogenesis and methanogenesis. The dominating bacterial community at a phylum level during hydrolysis, acidogenesis and acetogenesis include *Bacteriodetes, Proteobacteria* and *Firmicutes* which are responsible for generating volatile fatty acids for methanogens to produce biogas (Tang, et al., 2005; Cardinali-Rezende, et al., 2012). Under the kingdom Archaea, the phylum *Euryarcheota,* which produces methane from acetate, molecular hydrogen, and carbon dioxide and methyl compounds, dominates the archaeal community of methanogens during methanogenesis (Nelson, et al., 2011; Cardinali-Rezende, et al., 2012). Thus the bacterial and archaeal communities play a significant role in the degradation of organic matter, and as such should be expected to be present in the pit latrine faecal sludge. It is an indisputable fact that faecal sludge contains substantial quantities of pathogens that might pose health and environmental risks (Feachem, et al., 1983). Their detection in the faecal sludge with respect to agricultural application of pit latrine faecal sludge is imperative in taking preventative measures of the likely disease outbreaks and also in choosing the appropriate protective gear for sanitation service workers.

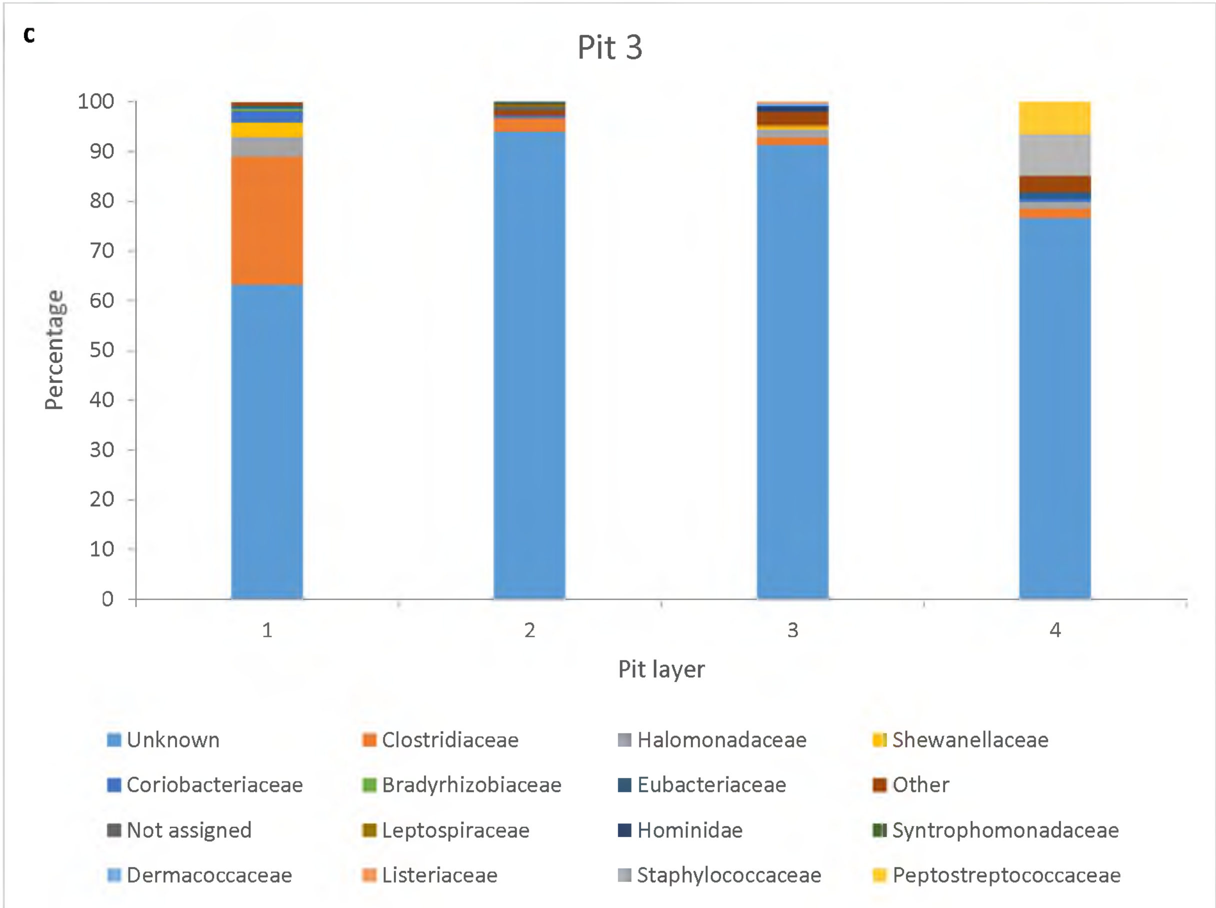
4.1.3.2 Results and discussion

In this study, faecal sludge samples were taken to extract DNA for estimating the microbial community of the different strata of pit latrine faecal sludge through 16S rRNA analysis, however, the community structure was not confirmed through direct observation by the use of bacterial culturing methods. Thus, the results presented in this study were obtained through the application of a molecular technique. The universal primers 27F (5' AGAGTTTGATCMTGGCTCAG 3') and 518R (5' ATTACCGCGGCTGCTGG 3') were used for amplifying the 16S subunit of the ribosomal RNA (rRNA). Sequencing data showed that more than 99% of the analysed 16S rRNA genes were of bacterial origin with minor genes from the kingdom Viruses, Archaea, Plantae, Animalia, Protozoa and Chromista. For the ease of interpreting and analysing the data, the results from the family level of classification are presented. Additionally, the top 7 families were taken from each of different faecal sludge samples and other families were presented as other. The presented top 7 results are in that category because they represented the highest percentage of the families that were found to be present in different faecal sludge samples. Each depth in the figures is presented as layers as follows, 0-25 cm (pit layer 1), 25 - 50 cm (pit layer 2), 50 - 75 cm (pit layer 3), 75 - 100 cm (pit layer 4), 100 - 125 cm (pit layer 5) and 125 - 150 cm (pit layer 6). The relative abundance of the microbial community sequences at different depths of pit latrine faecal sludge at the family level of classification is presented in Figure18. Majority (> 70%) of the analysed DNA sequences throughout the different samples were unknown, suggesting that the sequences had never been identified before. Some of the sequences were unassigned, meaning that they are known but have not yet been classified.

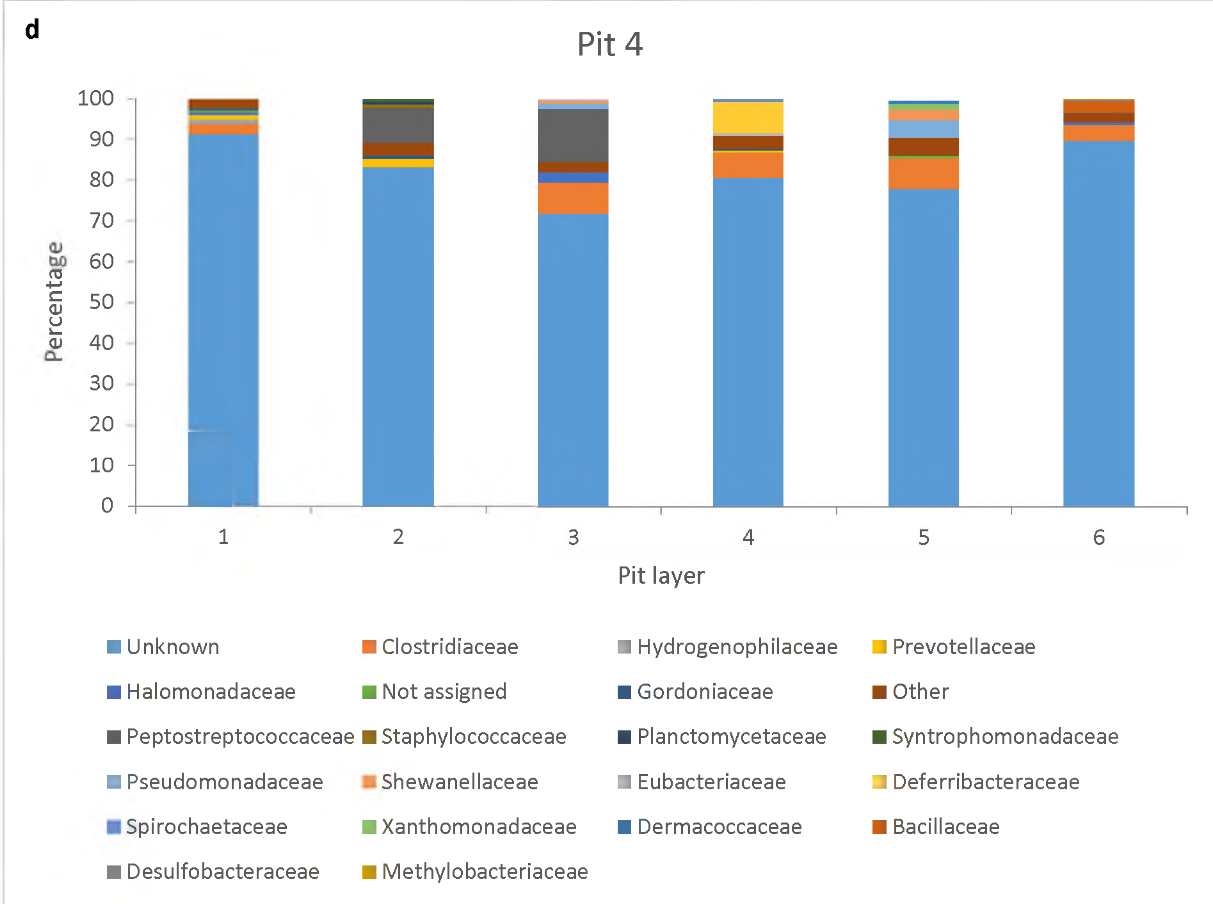


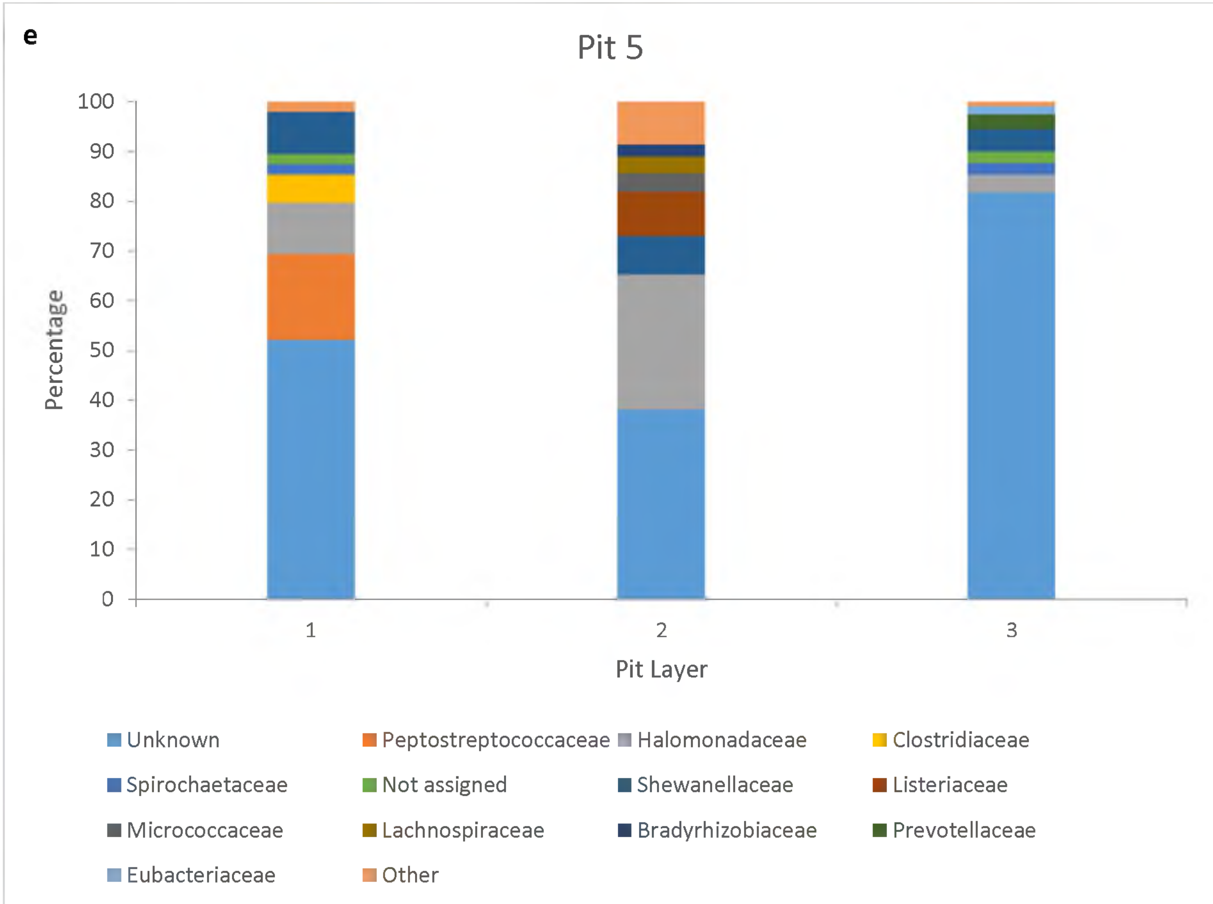


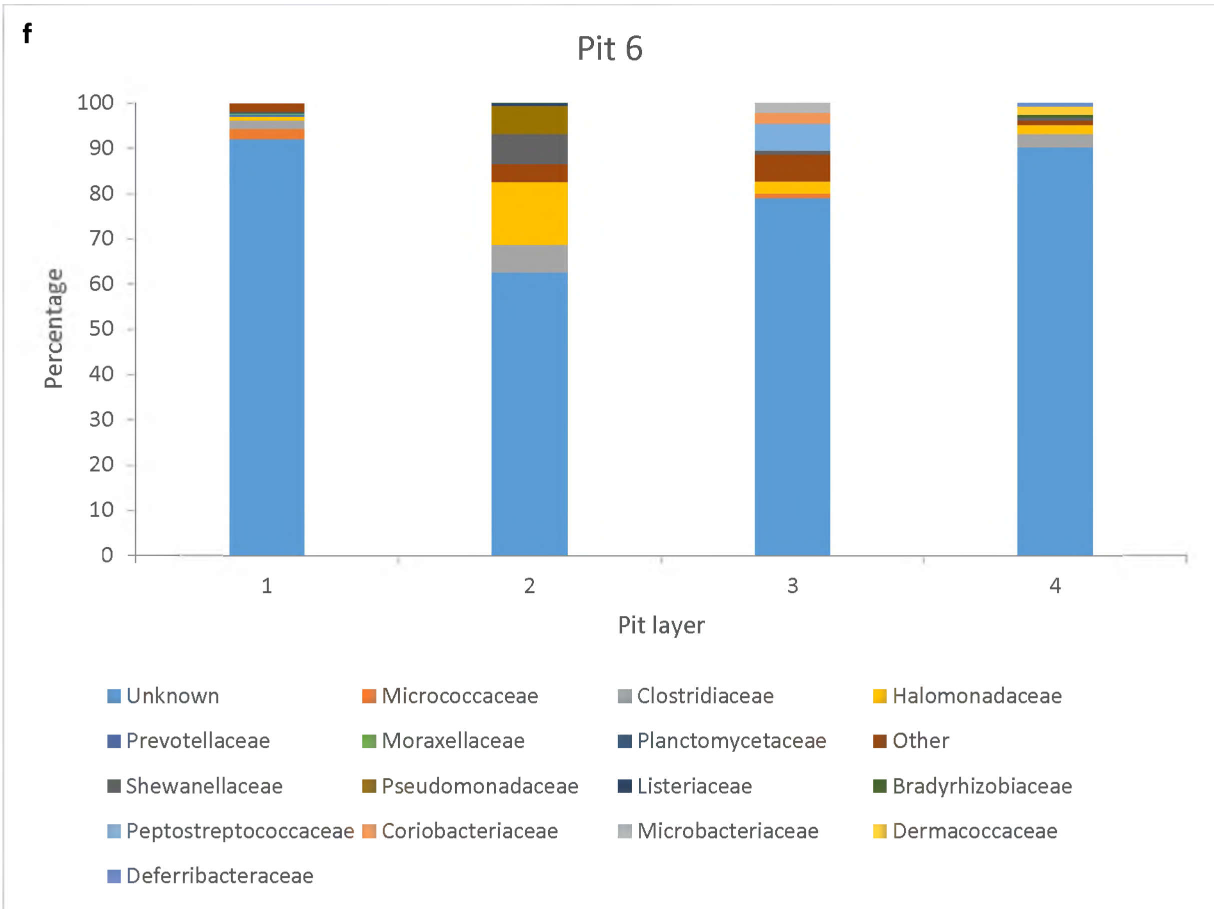
b
Pit 2
Percentage
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
Pit layer
Unknown
Clostridiaceae
Microbacteriaceae
Coriobacteriaceae
Peptococcaceae
Corynebacteriaceae
Halomonadaceae
Other
Streptomycetaceae
Bacillaceae
Gordoniaceae
Hyphomicrobiaceae
Peptostreptococcaceae
Mycobacteriaceae
Staphylococcaceae
Dermatophilaceae
Planctomycetaceae
Bradyrhizobiaceae
Brucellaceae
Thelebolaceae
Shewanellaceae
Hominidae
Desulfobacteraceae
Rhodobacteraceae
Comamonadaceae
Rhodospirillaceae



c
Pit 3
Percentage
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Pit layer
Unknown
Clostridiaceae
Halomonadaceae
Shewanellaceae
Coriobacteriaceae
Bradyrhizobiaceae
Eubacteriaceae
Other
Not assigned
Leptospiraceae
Hominidae
Syntrophomonadaceae
Dermacoccaceae
Listeriaceae
Staphylococcaceae
Peptostreptococcaceae


d
Pit 4
100
90
80
70
60
50
40
30
20
10
0
Percentage
1
2
3
4
5
6
Pit layer
Unknown
Clostridiaceae
Hydrogenophilaceae
Prevotellaceae
Halomonadaceae
Not assigned
Gordoniaceae
Other
Peptostreptococcaceae
Staphylococcaceae
Planctomycetaceae
Syntrophomonadaceae
Pseudomonadaceae
Shewanellaceae
Eubacteriaceae
Deferribacteraceae
Spirochaetaceae
Xanthomonadaceae
Dermacoccaceae
Bacillaceae
Desulfobacteraceae
Methylobacteriaceae


e
Pit 5
100
90
80
70
60
50
40
30
20
10
0
Percentage
1
2
3
Pit Layer
Unknown
Peptostreptococcaceae
Halomonadaceae
Clostridiaceae
Spirochaetaceae
Not assigned
Shewanellaceae
Listeriaceae
Micrococcaceae
Lachnospiraceae
Bradyrhizobiaceae
Prevotellaceae
Eubacteriaceae
Other


f
Pit 6
Percentage
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
Pit layer
Unknown
Micrococcaceae
Clostridiaceae
Halomonadaceae
Prevotellaceae
Moraxellaceae
Planctomycetaceae
Other
Shewanellaceae
Pseudomonadaceae
Listeriaceae
Bradyrhizobiaceae
Peptostreptococcaceae
Coriobacteriaceae
Microbacteriaceae
Dermacoccaceae
Deferribacteraceae

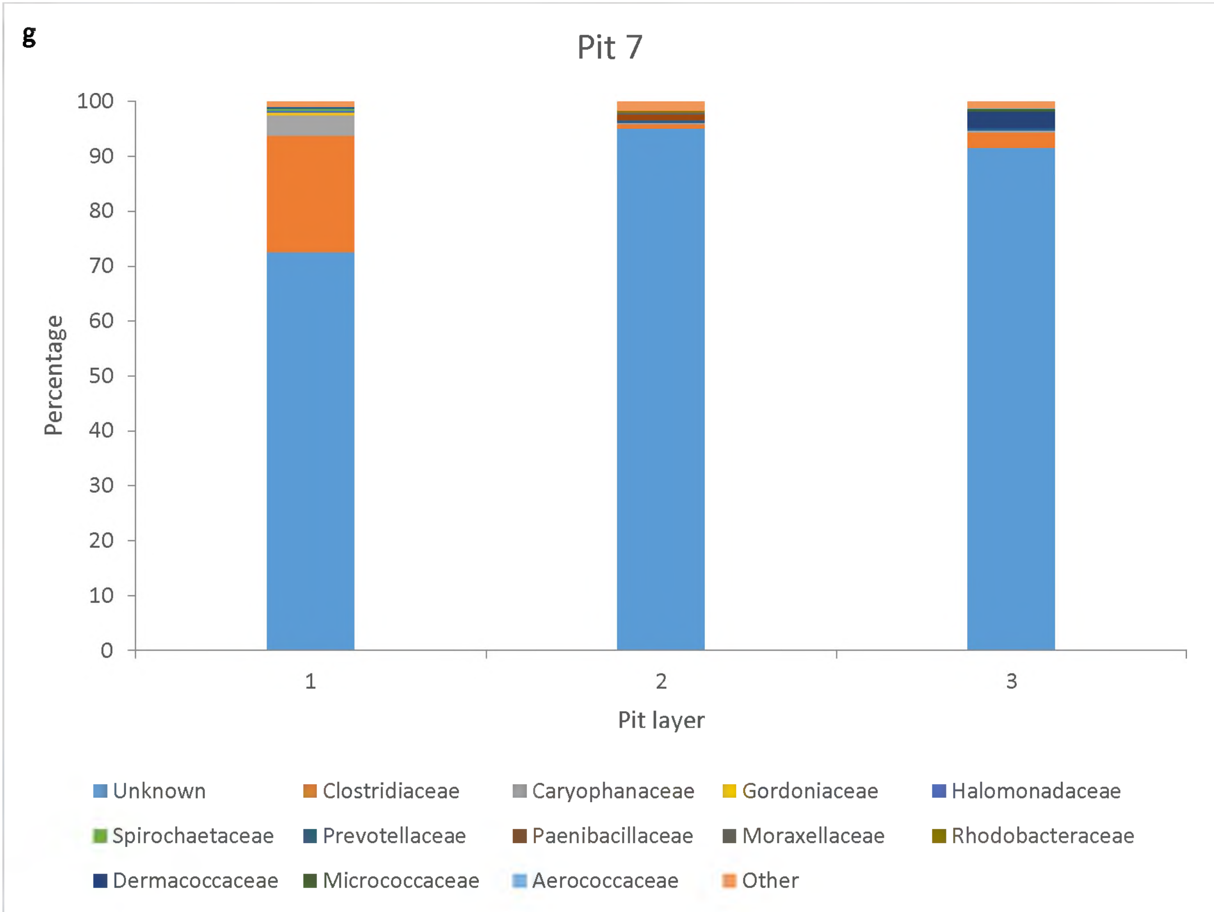

g
Pit 7
Percentage
100
90
80
70
60
50
40
30
20
10
0
1
2
3
Pit layer
Unknown
Clostridiaceae
Caryophanaceae
Gordoniaceae
Halomonadaceae
Spirochaetaceae
Prevotellaceae
Paenibacillaceae
Moraxellaceae
Rhodobacteraceae
Dermacoccaceae
Micrococcaceae
Aerococcaceae
Other

Figure 18 a-g.Relative abundance of microbial community at the different strata of pit latrine faecal sludge presented at the family level of classification.

The breakdown of organic matter requires cooperation between a consortia of microorganisms and these include fermentative, syntrophic, acedogenic, acetogenic and methanogenic bacteria. The relative abundance of the microbial community at the different strata of pit latrine faecal sludge at the family level of classification is presented in Figures 18a-g. In the bottom layer (reported as pit layer 1 in Figure 18) of pit 1, the most commonly identified families were *Syntrophomonadaceae* (2.64%), *Clostridiaceae* (2.61%), *Gordoniaceae* (1.01%), *Rhodobacteraceae* (0.74%), *Halomonadaceae* (0.56%) and *Desulfobacteraceae* (0.47%) respectively (Figure 18a). The percentages in brackets represent the read count of the sequences on the sequencer, thus a number of hits a particular sequence obtained. A major portion of the microbial community was unknown (90.35%) and this was consistent throughout different layers of the pit latrine. The most frequently identified bacterial families in the faecal sludge from the top layer (Pit layer 5) of the pit were *Clostridiaceae* (25.00%), *Mycobacteriaceae* (0.14%), *Microbacteriaceae* (0.35%), *Staphylococcaceae* (0.18%), *Phyllobacteriaceae* (0.74%) and *Brucellaceae* (0.53%). As with the bottom layer, a major portion of the bacteria was unknown (71.74%). The other most frequently identified bacterial families in layer 2 were *Peptostreptococcaceae* (2.13%), *Halomonadaceae* (0.62%), *Eubacteriaceae* (0.47%) and *Mycobacteriaceae* (0.27%). In layer 3, *Caryophanaceae* (7.39%), *Microbacteriaceae* (4.48%), *Gordoniaceae* (1.77%) and *Micrococcaceae* (0.29%) were the commonly identified bacterial families while in the 4th layer *Staphylococcaceae* (5.74%), *Rubrobacteraceae* (4.36%), *Microbacteriaceae* (1.89%) and *Bradyrhizobiaceae* (0.33%) were the most commonly identified families.

The family *Clostridiaceae* plays an important role in the anaerobic hydrolysis and fermentation of organic matter and therefore its prevalence throughout the different layers of the pit latrine serves as an indication of the role of its members in faecal sludge stabilization. (Rigueiro, et al., 2015) *Syntrophomonadaceae* inhabits environments where methanogens are present and members of this family are responsible for degrading propionate and butyrate under anaerobic conditions and were only detected at the bottom layer of the pit latrine suggesting that methanogens might have been present (Sobieraj & Boone, 2006; Rigueiro, et al., 2015). The *Gordoniaceae* family members are gram positive aerobic bacteria that occur-naturally in the environment (Goodfellow & Maldonado, 2006). They are capable of degrading various substrates including rubber and the pathogenicity to humans is closely associated with the ability to degrade rubber (Gupta, et al., 2010). This bacterial family was found in most depths of the pit latrine faecal suggesting a possible die of because members of this family are aerobic and there is no record of their isolation in anaerobic environments (Linos, et al., 2002).

Members of the *Rhodobacteraceae* family are aquatic gram negative bacteria that are mainly aerobic but have been isolated in anaerobic environments. In the environment, members of this family are actively involved in denitrification (Rajesabapath, et al., 2015). *Rhodobacteraceae* family members have been isolated in sludges from wastewater treatment plants (Jena, et al., 2016). The detection of this bacterial family in the pit latrine faecal sludge highlights its significance in the environment, more specifically in denitrification. The *Halomonadaceae* family members are gram negative strictly aerobic bacteria that are mostly found in the marine habitats and are also suspected human pathogens (de la Haba, et al., 2014; Leon, et al., 2014). Members of this family have been isolated from sewage sludge although their role in the wastewater treatment is not fully explained (Arahal, et al., 2007). Some members of the *Halomonadaceae* family have been shown to germinate under anaerobic conditions using nitrate, nitrite and furamate as an electron acceptor (Mata, et al., 2002). Based on the aforementioned factors, it can thus be suggested that these microorganisms thrived in the pit latrine faecal sludge because of the presence of nitrogen as high concentrations of nitrate were observed in pit latrine faecal sludges that were analysed in Hlalani (see table 4 in section 4.1). *Desulfobacteraceae* constitute a family of gram negative strict anaerobic bacteria that are responsible for the reduction of sulphate to sulphide under anaerobic conditions (Kuever, 2014). Members of this family have been isolated from various habitats including brackish water. Some members of the families of *Clostridiaceae, Mycobacteriaceae, Peptostreptococcaceae, Micrococcaceae, Staphylococcaceae, Bradyrhizobiaceae* and *Brucellaceae* are known human pathogens, as such, their detection in the faecal sludge from this particular pit latrine indicates a possibility of infected pit users (Lory, 2014; Slobodkin, 2014; Lorenzo, et al., 2012; de Souza, et al., 2014; Kampfer, et al., 2014). However, it should also be noted that these bacteria are also soil bacteria as such could have seeped from the surrounding soil.

The faecal sludge from the bottom layer of pit latrine 2 was dominated by the family of *Clostridiaceae* (28.56%), *Microbacteriaceae* (1.78%), *Coriobacteriaceae* (1.71%), *Peptococcaceae* (1.21%), *Corynebacteriaceae* (1.00%), and *Halomonadaceae* (0.44%) respectively (Figure 18b). In layer 2, the families *Streptomycetaceae* (3.86%), *Bacillaceae* (3.10%), *Gordoniaceae* (2.41%), *Halomonadaceae* (0.97%), *Hyphomicrobiaceae* (0.76 %), *Peptostreptococcaceae* (0.40%). Apart from the bacterial family of *Clostridiaceae* which was prevalent in all the layers, the bacterial families *Mycobacteriaceae* (0.83%), *Staphylococcaceae* (0.81%), *Dermatophilaceae* (0.66%), *Gordoniaceae* (0.58%) and *Planctomycetaceae* (0.51%) were found in layer 3. *Clostridiaceae, Planctomycetaceae* and *Gordoniaceae* families were common in both layers 3 and 4 except the families

Hyphomicrobiaceae (1.26%), *Bradyrhizobiaceae* (0.91%) and *Brucellaceae* (0.56%). In addition to *Clostridiaceae, Microbacteriaceae* and *Gordoniaceae* bacterial families which appeared to be prevalent in all the layers, the bacterial families *Thelebolaceae* (2.20%), *Shewanellaceae* (2.03%) and the eukaryotic family *Hominidae* (0.53%) were also found in layer 5 (pit latrine faecal sludge from the depth of 100-1250 cm from the bottom of the pit). The families *Desulfobacteraceae* (1.41%), *Rhodobacteraceae* (1.34%), *Comamonadaceae* (1.30%) and *Rhodospirillaceae* (1.25%) were some of the dominant bacterial families in layer 6. Members of the families *Hyphomicrobiaceae, Comamonadaceae*, *Planctomycetaceae* and *Rhodobacteraceae* are known denitrifiers while *Bradyrhizobiaceae* family members are known to be involved in nitrogen fixation and have been isolated in activated sludge (Saia, et al., 2016; de Souza, et al., 2014). Members of the family *Clostridiaceae*, *Bacillaceae, Peptococcaceae* and *Gordoniaceae* actively degrade organic matter and the different bacterial families suggests that different bacterial families are necessary in degrading faecal sludge due to the its complex nature.

The eukaryotic family *Hominidae* DNA that was identified in layer 5 and its presence in this particular pit latrine is unknown and might possibly serve as an indication of an error in the specificity of the primers used because members of this family are apes. The detection of this eukaryotic family partially highlights the limitations of metagenomics studies more specifically, the use of universal primers in characterizing microbial communities. Cross reactivity of universal primers with human DNA during the amplification of the bacterial 16S rRNA gene of clinical specimens was reported by Kommedal and colleagues, a phenomenon that might be attributed to the mismatches due to the low ratio of the target sequence to the non-target including the variations on the primer binding site (Kommedal, et al., 2012). Thus, the detection of the eukaryotic family *Hominidae* could be due to the aforementioned reasons since universal primers were used in this study. The identification of the bacterial family members such as *Clostridiaceae, Corynebacteriaceae, Mycobacteriaceae, Peptostreptococcaceae, Micrococcaceae, Gordoniaceae, Dermatophilaceae, Staphylococcaceae, Bradyrhizobiaceae* and *Brucellaceae* with members that are known to be human pathogens shows the potential danger posed by faecal sludge to the surrounding environment (Lory, 2014; Slobodkin, 2014; Lorenzo, et al., 2012; de Souza, et al., 2014; Kampfer, et al., 2014). Moreover, it also indicates that unsafe handling of human excreta could possibly lead to various infections and that the list of microorganisms that have been for a long time used in identifying the threats posed by application of faecal sludge in agriculture might need some amendments. In light of the use of faecal sludge in agriculture, more stringent measures need to be taken in order to prevent future outbreaks of diseases. Such outbreaks may be due to pathogenic microorganisms that have not been detected been neglected in faecal sludge due to difficulty in isolating them using the standard microbiological culturing methods.

In a different pit latrine, *Halomonadaceae* (4.09%), *Shewanellaceae* (2.76%), *Coriobacteriaceae* (2.40%), *Bradyrhizobiaceae* (0.49%) and *Eubacteriaceae* (0.44%) families were dominant in the bottom layer with *Clostridiaceae* being the most dominant family in all the layers of the pit latrine faecal sludge (Figure 18c). *Clostridiaceae* belongs to a group of fermenting bacteria, and therefore, its prevalence throughout the different layers of the pit latrine could be linked to their role in degrading organic matter while the *Bradyrhizobiaceae* family is known to be involved in nitrogen fixation in the environment. (Maspolin, et al., 2015; de Souza, et al., 2014). The bacterial family *Coriobacteriaceae* (0.46%), *Leptospiraceae* (0.45%), *Syntrophomonadaceae* (0.11%) including the eukaryotic family *Hominidae* (0.30%) dominated the layer 2 of the pit latrine. Members of the bacterial family *Syntrophomonadaceae* syntrophic bacteria which are found in environments where there is methane production and are actively involved in the degradation of propionate and butyrate (Sobieraj & Boone, 2006; Rigueiro, et al., 2015). Some of the sequences detected in layer 2 were not assigned to any family, which means that they are known and that there is insufficient information to assign them to particular families.

The bacterial families *Halomonadaceae* (1.67%), *Shewanellaceae* (0.62%), *Dermacoccaceae* (0.49%), *Listeriaceae* (0.39%) and eukaryotic family *Hominidae* (1.21%) were commonly identified in Layer 3. *Staphylococcaceae* (8.40%), *Peptostreptococcaceae* (6.49%), *Halomonadaceae* (1.33%) and *Eubacteriaceae* (1.20%) were the most common families in the top layer (layer 4). Although bacterial family *Clostridiaceae*, the known fermenting bacterial family was detected from the faecal sludge including nitrogen fixing bacterial family *Bradyrhizobiaceae,* families that are potential human pathogens such as *Staphylococcaceae, Dermacoccaceae, Peptostreptococcaceae, Halomonadaceae, Leptospiraceae* and *Listeriaceae* were also detected and were more common through the pit latrine strata and were more diverse in the top layer. The prevalence of the potential human pathogens especially in the faecal sludge from the top of the pit latrine where there is fresh faeces might suggest possible infections in the pit latrine users. Since the bacteria were not cultured, it is not known if they were viable or whether they were truly pathogenic strains and therefore the extent of the danger they pose cannot be ascertained.

The family *Clostridiaceae* was detected in most layers of pit latrine 4 but was the most frequent family in layers 1, 5 and 6. In layer 1 the bacterial families *Hydrogenophilaceae* (1.11%), *Prevotellaceae* (1.10%), *Halomonadaceae* (0.64%) and *Gordoniaceae* (0.44%) were commonly identified, including a portion of unassigned bacterial families (0.56%) (Figure

18d). Members of the family *Clostridiaceae* and *Gordoniaceae* have an important role in stabilizing the organic matter under anaerobic conditions and as such their dominance might be related to waste stabilization, regardless of some of the members of the two families being human pathogens (Goodfellow & Maldonado, 2006; Gupta, et al., 2010; Rigueiro, et al., 2015). It should however be noted that a major portion of the analysed DNA was unknown and that the observation consisted throughout the reported results.

The family *Prevotellaceae* was also detected in layers 2 (2.04%) and 4 (0.50%) including family *Halomonadaceae* as 2.64% and 0.65% in layers 3 and 6 respectively. The families *Peptostreptococcaceae* (8.75%), *Staphylococcaceae* (0.71%), *Gordoniaceae* (0.70%), *Planctomycetaceae* (0.64%), *Syntrophomonadaceae* (0.62%) were identified in layer 2. *Peptostreptococcaceae* bacterial family was also reported in layers 3 and 6 as 12.98% and 0.24% respectively and also the family *Gordoniaceae* in layer 4 (0.52%). Some of the common bacterial families in layer 3 included *Pseudomonadaceae* (1.38%), *Shewanellaceae* (0.52%) and *Eubacteriaceae* (0.48%). The bacterial families *Pseudomonadaceae* and *Shewanellaceae* were also detected in layer 5 as 4.22% and 2.60% respectively. In layer 4 *Deferribacteraceae* (7.70%), *Eubacteriaceae* (0.71%), and *Spirochaetacea*e (0.62%), were some of the commonly identified bacterial families. The denitrifying bacteria family *Hydrogenophilaceae, Planctomycetaceae, Prevotellaceae* and *Pseudomonadaceae* were common in this particular pit latrine and their diversity might indicate that nitrate was bound to different substrates (Saia, et al., 2016). Apart from the bacterial families *Pseudomonadaceae* and *Shewanellaceae* in layer 5, *Xanthomonadaceae* (1.46%) and *Dermacoccaceae* (0.69%) were also common in the layer including some unassigned bacterial families (0.64%). In addition to the previously identified bacterial families in layer 6, the families *Bacillaceae* (2.73%), *Desulfobacteraceae* (0.27%) and *Methylobacteriaceae* (0.24%), were commonly identified bacterial families in the 6th layer.

The iron reducing bacteria family *Deferribacteraceae* including the sulphate reducing family *Desulfobacteraceae* were also detected although they were not that common (Garcia-Pena, et al., 2011; Maspolin, et al., 2015; Saia, et al., 2016). Bacterial families, such as *Spirochaetacea*e, *Xanthomonadaceae, Methylobacteriaceae, Dermacoccaceae* and *Pseudomonadaceae* that are known to have pathogenic members were also prevalent indicating the diversity of putative pathogens present in the faecal sludge (Cutino-Jimenez, et al., 2010; Lorenzo, et al., 2012; Cardinali-Rezende, et al., 2012; Jena, et al., 2016).

Pit latrine 5 was one of the pit latrines with the lowest depth as can be seen in Figure 18e, where only a maximum of 3 layers were sampled. The common bacterial families from this pit were the *Peptostreptococcaceae* (17.22%), *Halomonadaceae* (10.30%), *Clostridiaceae* (5.75%), *Spirochaetaceae* (2.03%), *Shewanellaceae* (1.89%) and some unassigned families (2.02%). The family *Halomonadaceae* was also common in layer 2 (27.23%) and 3 (3.55%). Other commonly identified bacterial families in layer 2 included *Listeriaceae* (8.87%), *Shewanellaceae* (8.68%), *Micrococcaceae* (3.79%), *Lachnospiraceae* (3.30%) and *Bradyrhizobiaceae* (2.47%). In the top layer, the common bacterial families included *Prevotellaceae* (3.03%), *Spirochaetaceae* (2.33%), *Eubacteriaceae* (1.62%) and *Shewanellaceae* (0.93%). Apart from the known fermenting bacterial family *Clostridiaceae* and the nitrogen fixing bacteria family *Bradyrhizobiaceae* the majority of the bacterial families that were identified in this pit latrine were the families with members that are known to be pathogenic (Rigueiro, et al., 2015; Nelson, et al., 2011; Slobodkin, 2014; Jena, et al., 2016). However, it cannot be confirmed whether the strains of the identified bacterial members were pathogenic or not as the resolution of this study was low and additional steps of isolating and culturing of the pure strains followed by the biochemical tests to confirm their pathogenicity were not undertaken.

The family *Micrococcaceae* (2.28%), *Clostridiaceae* (1.87%), *Halomonadaceae* (0.92%), *Prevotellaceae* (0.40%), *Moraxellaceae* (0.29%) and *Planctomycetaceae* (0.25%) were common in the bottom layer of pit latrine 6 (Figure 18f). *Micrococcaceae* bacterial family was also commonly identified in layer 3 (0.96%) and *Clostridiaceae* family in layer 2 (6.13%) and 4 (2.97%). The family *Halomonadaceae* was also common in layers 2 (13.86%), 3 (2.75%) and 4 (1.93%) respectively. Other frequent families in layer 2 included *Shewanellaceae* (6.67%), *Pseudomonadaceae* (6.13%), *Listeriaceae* (0.73%) and *Bradyrhizobiaceae* (0.70%). The family *Shewanellaceae* was also one of the commonly identified bacterial families in layers 3 (0.86%) and 4 (0.61%).

Fermenting (*Clostridiaceae and Coriobacteriaceae*), nitrogen fixing (*Bradyrhizobiaceae*), denitrifying (*Planctomycetaceae, Prevotellaceae, Pseudomonadaceae*) and iron reducing (*Deferribacteraceae*) bacteria were widely distributed throughout the various depths of the pit latrine and have been isolated in activated sludge from wastewater treatment plants (Garcia-Pena, et al., 2011; Cardinali-Rezende, et al., 2012; Lorenzo, et al., 2012; Nelson, et al., 2011). *Peptostreptococcaceae* (5.93%), *Coriobacteriaceae* (2.48%) and *Microbacteriaceae* (2.13%) were the commonly identified bacterial families in layer 3. The other common bacterial families in layer 4 were *Dermacoccaceae* (1.90%) and *Deferribacteraceae* (0.75%). The pathogenic bacteria containing families (*Micrococcaceae, Listeriaceae, Pseudomonadaceae*, *Dermacoccaceae* and *Moraxellaceae*), however, were also part of the most frequently identified bacterial families throughout the pit latrine. Although known opportunistic pathogens exist within the family *Pseudomonadaceae* the role of the members of this family as denitrifying bacteria in wastewater treatment plants is widely known and therefore, it is not clear if the strains that were identified in this study were pathogenic or just actively involved with denitrification (Saia, et al., 2016).

The prevalence of the *Clostridiaceae* family was observed throughout layers 1 (21.26%), 2 (0.81%) and 3 (2.77%), followed by the bacterial family *Caryophanaceae* (3.66%), *Gordoniaceae* (0.52%), *Halomonadaceae* (0.36%), *Spirochaetaceae* (0.36%) and *Prevotellaceae* (0.27%) respectively in layer 1 (Figure 18g). The family *Caryophanaceae* was also common in the layers 2 (0.26%) and 3 (0.42%) as well as *Prevotellaceae* family in both layers 2 (0.38%) and 3. (0.40%). The other commonly identified bacterial families included *Paenibacillaceae* (1.06%), *Moraxellaceae* (0.44%) and *Rhodobacteraceae* (0.34%) in layer 2 while *Dermacoccaceae* (3.01%), *Micrococcaceae* (0.50%) and *Aerococcaceae* (0.16%) bacterial families were common in layer 3 as with the other pit latrines, it is evident that the bacterial family of fermenters *Clostridiaceae* plays an important role in the pit latrine faecal sludge stabilization as it was prevalent throughout the different depths of the pit latrine including the family *Gordoniaceae* although it was mostly common in the bottom layers of the pit latrine in various pit latrines (Nelson, et al., 2011). The bacterial family members that are involved in denitrification such as *Rhodobacteraceae* and *Prevotellaceae* were also common throughout the various pit latrines suggesting that they too form part of the microbial consortia that is actively involved in stabilizing faecal sludge (Maspolin, et al., 2015). Potential pathogenic bacteria families were also very common throughout the pit latrine faecal sludge and seemed to be mostly found in the shallow depth of the pit latrine.

In conclusion, the microbial consortia found in pit latrines were very diverse throughout the different depths of the pit latrine and amongst pit latrines. Evident, however, was the common occurrence of the family *Clostridiaceae* as a dominating fermenting bacteria throughout, which seemed to suggest that this bacterial family plays a key role in faecal sludge stabilization. Various bacteria families ranging from nitrogen fixing, denitrifying to iron reducing bacteria were identified in the sludge and their presence varied between pit latrines. Common occurrence of bacterial families that are known to have members that are human pathogens was also observed. Since the bacteria were not cultured, it is not known if the detected bacterial family members contained pathogenic strains or not and therefore, future work involving the culturing of such microorganisms from faecal sludge might be necessary to simplify differentiating between the actual pathogens and the bacteria which might be involved in stabilizing faecal sludge. Major portion of the unknown bacterial families suggests that more work is needed to update the existing information about the microbial community in faecal sludge as that could help in understanding the link between the different types of sanitation facilities and the outbreak of sanitation related infectious diseases. Such information could help in control disease outbreaks during natural disasters such as floods or earthquakes where people are often relocated to refugee camps that sometimes do not have adequate sanitation facilities.

4.2 : Plant growth studies

4.2.1 The growth response of radish to the fertilizer treatment

A series of plant growth studies was conducted to assess the fertilizer value of anaerobically digested and pasteurized pit latrine faecal sludge. Radish and garden cress were selected as suitable candidates for this plant growth trials based on their rapid growth and the differences in measureable plant characteristics (tuber, roots, leaves, etc). For the ease of reading, the study outlines and discusses the results of the radish first and then garden cress. Radish (*Raphanus sativus spp.*) is a tuber forming edible crop plant that is widely used as a root vegetable and is a good source of vitamins A and C, carbohydrates and proteins (Jilani, et al., 2010). The results for the fertilizer testing using radish as a test plant are shown in Table 7. Only two (1% and 10% adjustment by volume) out of the four (0.1%, 1%, 10%, 100 %) tested treatments showed evidence of growth. There was also no evidence for growth in either the positive or the negative control. Germination % in the 1% and 10% faecal sludge treatments was 56 %. Poor germination was attributed to the sowing depth. Changing the sowing depth from 3 cm to 1 cm gave a 100 % germination in all the treatments as outlined in the plant growth trial section (Figure 19).

Table 7. The average physiological parameters of radish after it had been grown on a growth substrate (vermiculite) that had been fertilized with anaerobically digested and pasteurized pit latrine faecal sludge. Parameters are expressed as mean ± standard deviation (SD)

Physiological parameters (n=2)	Treatment					
	0.1%	1%	10%	100 %	(NPK) control	(Water) control
Fresh plant mass (g)	0	0.27 ± 0.17	1.46 ± 0.79	0	0	0
Fresh leaf mass (g)	0	0.075 ± 0.007	0.20 ± 0.049	0	0	0
Fresh root mass (g)	0	0.20 ± 0.15	1.26 ± 0.83	0	0	0
Dry plant mass (g)	0	0.045 ± 0.021	0.19 ± 0.028	0	0	0
Dry leaf mass (g)	0	0.01 ± 0	0.035 ± 0.007	0	0	0
Dry root mass (g)	0	0.035 ± 0.021	0.155 ±0.035	0	0	0
Number of leaves (average)	0	2.0 ± 0	2.0 ± 0	0	0	0
Root length (mm)	0	32.5 ± 31.82	40 ± 0	0	0	0
Root diameter (mm)	0	4.00 ± 4.24	9.0 ± 4.24	0	0	0
Germination %	0	56	56	0	0	0
Total yield %*	0	40%	40%	0	0	0

* Total yield % is calculated as the total number of plants harvested divided by the total number of seeds that germinated x 100.

There was no significant difference (p= 0.05) between the fresh plant weight (p= 0.17), root length (p= 0.77), leaf weight (p=0.07), root fresh weight (p= 0.22), and the root dry weight (p= 0.05) of radish grown in a growth substrate fertilized with 1% and 10% fertilizer strength treatments. The treatments did have an effect on the plant dry weight (p= 0.02) and leaf dry weight (p=0.03) respectively. Thus, the different fertilizer strengths only had an influence on the dry weight of the plant and the leaves. Since radish is a tuber forming plant, there were no tubers observed in all the plants except long thick roots (results not shown). Thus the root diameter presented is not the diameter of the tuber but that of the thickened root which suggested that the tuber might have been on its early development stage since fully developed tubers are harvested after 34 days post seedling germination. From literature, the tuber development is delayed by low light intensity (95 to 100 µmol. m^2 s^{-1}) (Rostika-Rick & Manning, 1993). As such plants tend to increase their leaf size to capture more light to compensate for reduction in light energy input (Rostika-Rick & Manning, 1993). Regarding the nutrient requirement for radish, it is reported that radish can utilize low to moderate nutrients and that the mass of the tuber increases with an increase in nitrogen content of the fertilizer (Rostika-Rick & Manning, 1993).

According to Chohura and Kolota, 50 kg N per hectare (ha), 20 kg P_2O_5 per ha, 50 kg K_2O per ha is required for high yields of radish (Chohura & Kolota, 2011). Based on the recommended dose of nitrogen for radish by Chohura and Kolota (2011), the radish used in this study could have absorbed most of the nitrogen during its early growth stages, or it could have either leached out during the watering of the growth substrate (Brintha & Seran, 2009). Another important macronutrient for plant growth and development is phosphate. The deficiency of this macronutrient stunts plant growth resulting to poor yields (Sadia, et al., 2013). From Table 1, it can be seen that the concentration of phosphate in the fertilizer was 48.1 ± 2.79 mg/l, thus the nutrient supply might not have been sufficient to sustain radish development to its marketable size.

For tuber formation, high quantities of potassium are necessary (Inam, et al., 2011). Jadhao and colleagues reported that applying 40 kg/ ha of potassium in soils is sufficient for producing radish with tubers of marketable size (Jadhao, et al., 1999). Because of the poor yield which resulted in a significant reduction in the number of the plants that could be analysed, the study was repeated. In a follow up study, a combination of pit latrine faecal sludge (FS) and the effluent from Belmont Valley Wastewater Works was used (33% FS, 66% FS and 100% FS). This was mainly because gas production was one of the objectives of the study, so the effluent was added to improve biogas production.

Figure 19. The germination of radish after the sowing depth had been changed to 1 cm.

This study was conducted using radish and the fertilizer used were the same as stated above. However, the composition of the fertilizers was different. The mixtures used were composed of 33%, 66% and 100% FS made up with WWTW AD effluent. The results of the germination % and the total yield % of radish from the study are presented in Table 8. If germination % is below 60%, the fertilizing material is considered to be toxic to the seeds of a particular crop (Murrilo, et al., 1995). However, seed germination might also be affected by the moisture content and the inherent characteristics of the seeds such as vitality and seed dormancy (Pervez, et al., 2004; Baloch, et al., 2014; Buss & Masek, 2014).

Table 8. Germination and yield percentage of radish grown on a growth substrate (perlite and cocopeat) that was fertilized with anaerobically digested and pasteurized pit latrine faecal sludge mixed with the effluent from the Belmont Valley WWT.

Sludge mixture	Fertilizer strength	Parameter	
		Germination %	Total yield %
33% FS	0.1%	63	33
	1%	63	27
	10%	46	18
	100%	42	10
66% FS	0.1%	42	20
	1%	50	25
	10%	50	8
	100%	29	14
100% FS	0.1%	38	33
	1%	75	39
	10%	38	22
	100%	58	7
Control	Hoagland Solution	75	16

Poor germination was observed throughout the different fertilizer strengths which were prepared from the various mixtures of pit latrine faecal sludge (FS) and the effluent from the wastewater works. The germination % was above 60% for 0.1% and 1% strengths fertilizer treatments from the 33% FS and 100% FS. For the 10% and 100% fertilizer strengths, germination % was below 60% suggesting that these concentrations might have been toxic to radish seeds. These findings contradict the findings of Mor and colleagues who reported that low concentrations of landfill leachate from municipal solid waste lowered germination % in bread wheat seeds (Mor, et al., 2013). Groundnut seeds showed a positive germination % when treated with the 25% sewage water which was the highest concentration with the COD value of 348 mg/l (Girisha & Raju, 2008). The yield% was higher in the 0.1 and 1% fertilizer treatments throughout the study. Thus, the germination and the yield % suggest-that the 0.1% and the 1% fertilizer strengths were the most tolerable treatments for radish and also that fertilizer strengths above that negatively affect the two parameters for radish. The different fertilizer strengths did not have a significant impact on the fresh weight of radish ($p = 0.26$) (Table 9). In this study, the average fresh weight of radish ranged between 0.03±0 and 2.12 ±0 g between the different fertilizer strengths. In literature, radish with the fresh mass of 400 g has been reported and was higher than the one reported in this study suggesting that supplementary nutrients might be necessary to improve the full development of radish to marketable sizes (Baloch, et al., 2014).

Table 9. The response of the fresh weight (g) of radish to different the fertilizer strengths as treatment.

	Plant fresh weight (g)												
	33% FS				**66% FS**				**100% FS**				
Treatment	0.1%	1%	10%	100%	0.1%	1%	10%	100%	0.1%	1%	10%	100%	**C**
Min	0.30	0.50	0.19	2.12	0.33	0.52	0.17	0.03	0.40	0.90	0.38	1.70	0.40
Max	0.39	0.83	0.23	2.12	0.38	0.83	0.17	0.03	0.52	1.99	0.66	1.70	0.46
Median	0.38	0.62	0.21	2.12	0.36	0.69	0.17	0.03	0.43	1.50	0.52	1.70	0.46
Average	0.37	0.64	0.21	2.12	0.36	0.68	0.17	0.03	0.45	1.42	0.52	1.70	0.44
SD	0.04	0.14	0.03	0	0.04	0.16	0	0	0.06	0.35	0.20	0	0.03
N	5	4	2	1	2	3	1	1	3	7	2	1	3

SD = standard deviation, C is a control (Hoagland solution)

The reason for the difference can be attributed to the fact that most studies use the recommended nutrient requirements for radish (Pervez, et al., 2004; Brintha & Seran, 2009; Baloch, et al., 2014). This study, however, focused on assessing the fertilizer value of pit latrine faecal sludge without the addition of supplementary nutrients to account for the nutrient imbalances.

The number of leaves, the plant height, root length and diameter influence the fresh and the dry mass of radish. As expected, the average dry weight of radish was small and ranged between 0.03 and 0.14 g between the different fertilizer strengths (Table 10). The difference between the dry mass of radish from the different fertilizers strengths was not significant (p = 0.56).

Table 10. The response of the dry weight of radish (g) to different fertilizer strengths.

	Plant dry weight (g)												
	33% FS				**66% FS**				**100% FS**				
Treatment	0.1%	1%	10%	100%	0.1%	1%	10%	100%	0.1%	1%	10%	100%	**C**
Min	0.01	0.03	0.03	0.14	0.04	0.09	0.02	0.02	0.01	0.08	0.07	0.13	0.01
Max	0.05	0.09	0.13	0.14	0.05	0.10	0.02	0.02	0.06	0.25	0.20	0.13	0.06
Median	0.02	0.07	0.08	0.14	0.05	0.10	0.02	0.02	0.03	0.15	0.14	0.13	0.03
Average	0.03	0.06	0.08	0.14	0.05	0.10	0.02	0.02	0.03	0.15	0.14	0.13	0.03
SD	0.02	0.03	0.07	0	0.01	0.01	0	0	0.03	0.06	0.09	0	0.03
N	5	4	2	1	2	3	1	1	3	7	2	1	3

Sousa and Figueiredo (2015) reported a maximum dry weight of 2.1 g per radish plant. A dry weight of 1.2 g per radish plant has been reported in the literature (Chan, et al., 2007). Thus the dry weight of the radish reported in this study was lower than that reported in literature. That can be attributed to the fact that the reported radish had developed tubers in literature, whereas in this study radish did not have tubers. Plant height is an indicator for vegetative growth. Average radish height from this study ranged between 3.0 and 42.6 mm (Table 11). Statistically, the difference between the radish height from the different fertilizer strengths was not significant (p=0.57).

Table 11.The response of the height of radish (mm) to different fertilizer strengths.

	Plant height (mm)												
	33% FS				**66% FS**				**100% FS**				
Treatment	0.1%	1%	10%	100%	0.1%	1%	10%	100%	0.1%	1%	10%	100%	**C**
Min	27.0	27.0	10.5	95.0	12.5	20.0	3.5	3.0	9.0	34.0	9.0	9.0	11.0
Max	30.0	36.0	11.2	95.0	16.5	24.0	3.5	3.0	16.0	48.0	11.2	9.0	15.0
Median	29.0	30.6	10.9	95.0	14.5	20.0	3.5	3.0	16.0	45.0	10.1	9.0	14.0
Average	28.6	31.1	10.9	95.0	14.5	21.3	3.5	3.0	13.7	42.6	10.1	9.0	13.3
SD	1.5	3.7	0.5	0	2.8	2.3	0	0	4.0	5.9	1.6	0	2.1
N	5	4	2	1	2	3	1	1	3	7	2	1	3

From literature, radish can grow up to 83 cm in height (Pervez, et al., 2004; Jilani, et al., 2010; Baloch, et al., 2014). A maximum radish height of 30 cm was reported by Sousa and Figueiredo when using sewage sludge biochar as a fertilizer. (Sousa & Figueiredo, 2015). The radish height reported in this study was lower than the one reported in literature. This could be attributed to nutrient imbalances during germination, possibly nitrogen deficiency as it plays an important role in cell division during the plant development, as such its deficiency can retard plant growth (Inam, et al., 2011; Baloch, et al., 2014). In general, fast growing plants require a balanced nutrient supply as imbalances could stunt growth, thus affect the normal development of the plant (Inam, et al., 2011). Plant roots are important for nutrient and water uptake by plants. Nutrient absorption by roots is influenced mostly by the nutrient concentration at the surface of the root (Marcelis & Hooijdonk, 1999). Average root lengths of radish reported in this study ranged between 2.5 and 60 mm (Table 12). Statistical analysis showed that the difference in the roots from the different fertilizer strengths was not significant (p = 0.46).

Table 12. The response of the length of the roots (mm) of radish to the different fertilizer strengths.

	Root length (mm)												
	33% FS				66% FS				100% FS				
Treatment	0.1%	1%	10%	100%	0.1%	1%	10%	100%	0.1%	1%	10%	100%	**C**
Min	6.0	6.0	3.5	60.0	2.5	5.0	5.0	2.5	5.0	10.0	3.8	7.0	5.0
Max	8.0	13.0	4.5	60.0	5.5	9.0	5.0	2.5	11.0	25.0	5.0	7.0	13.0
Median	7.0	9.0	4.0	60.0	4.0	7.0	5.0	2.5	5.0	13.0	4.4	7.0	9.0
Average	6.8	9.3	4.0	60.0	4.0	7.0	5.0	2.5	7.0	15.1	4.4	7.0	9.0
SD	0.8	3.0	0.7	0	2.1	2.0	0	0	3.5	5.2	0.8	0	4.0
N	5	4	2	1	2	3	1	1	3	7	2	1	3

The literature shows that increasing concentration of nitrogen increase the root length of radish, however, that was not the case in this study (Marcelis & Hooijdonk, 1999; Brintha & Seran, 2009; Jilani, et al., 2010; Baloch, et al., 2014). Maximum root length of 84.4 cm for radish is recorded in literature with the minimum root length being 11.03 cm (Pervez, et al., 2004; Jilani, et al., 2010). In another study, the root length of radish grown in soil fertilized with farm yard manure, gamma irradiated sewage sludge and non-irradiated sewage sludge reported as 23.97, 25.53 and 23.85 cm respectively (Rathood, et al., 2011). The reported root length in this study however was below the values that were reported in literature. This could be due to the fact that this study used a soilless growth substrate whereas soil or peat is used in other studies, therefore, any growth occurring solely relied on the fertilizer supplying the plant with the necessary nutrients (Chohura & Kolota, 2011; Baloch, et al., 2014). The leaves of a plant play an important role in photosynthesis, transpiration and food storage. As such they play a significant role in the development of the plant. The average number of leaves of radish that grew per fertilizer treatment ranged from 1.0 to 10.3 and the difference was not significant ($p = 0.06$) (Table 13).

Table 13. The response of the leaves of radish (average) to the different fertilizer strengths.

	Number of leaves												
	33% FS				**66% FS**				**100% FS**				
Treatment	0.1%	1%	10%	100%	0.1%	1%	10%	100%	0.1%	1%	10%	100%	**C**
Min	1.0	5.0	3.0	7.0	5.0	3.0	3.0	1.0	6.0	1.0	5.0	6.0	3.0
Max	11.0	16.0	4.0	7.0	7.0	12.0	3.0	1.0	9.0	7.0	9.0	6.0	6.0
Median	10.0	10.0	3.5	7.0	6.0	9.0	3.0	1.0	8.0	3.0	7.0	6.0	5.0
Average	8.0	10.3	3.5	7.0	6.0	8.0	3.0	1.0	7.7	3.7	7.0	6.0	4.7
SD	4.8	5.5	0.7	0	1.4	4.6	0	0	1.5	2.1	2.8	0	1.5
N	5	4	2	1	2	3	1	1	3	7	2	1	3

6.4 leaves per radish plant were reported by Sousa and Figueiredo when using sewage sludge biochar as a fertilizer (Sousa & Figueiredo, 2015). In a different study, the highest recorded number of radish leaves was found to be 19 leaves per plant with the minimum number being 9 (Jilani, et al., 2010; Baloch, et al., 2014). The results reported in the current study, however, are more similar to the ones reported by Sousa and Figueiredo (2015). Based on the obtained results for the measured physiological parameters for radish, it was concluded that there is a possibility to use the anaerobically digested and pasteurized pit latrine faecal sludge as a fertilizer given that the fertilizer is diluted properly. The composition of pit latrine faecal sludge varies between pit latrines depending on the user behaviour and the surrounding terrain (Bakare, et al., 2012). As such, the observed growth response of radish to the fertilizer only represents the response of the nutrients that were present in the used pit latrine faecal sludge. The following section presents plant growth studies conducted using garden cress, a leafy vegetable.

4.2.2 The growth response of garden cress to the fertilizer treatment

Garden cress, a plant scientifically known as *Lepidium sativum* is an edible herbaceous plant that is mostly consumed in salads. This crop is mainly cultivated for its seeds but the leaves and the roots also have economic value (Souri, et al., 2004; Mohammed Ali, 2013). Its ability to grow fast amongst other factors is what motivated its use in this study .The results for the physiological parameters of garden cress are shown in Table 14. In all the treatments, the germination was above 90% after 7 days.

Table 14. The average physiological parameters of garden cress after it had been grown on a growth substrate (perlite and cocopeat) that had been fertilized with an autoclaved anaerobically digested and pasteurized pit latrine faecal sludge.

Physiological parameters	0.1%	1%	10%	100%	Control
Fresh plant mass (g)	0.039 ± 0.021	0.030 ± 0.015	0.039 ± 0.015	0.071 ± 0.027	0.19 ±0.070
Dry plant mass (g)	0.0056 ± 0.003	0.0052 ± 0.002	0.0067 ±0.002	0.010 ± 0.004	0.017 ± 0.006
Number of leaves (average)	4.3 ± 1.0	4.2 ± 1.1	4.2 ± 0.9	5.9 ±1.2	6.4 ± 0.9
Plant height (mm)	101.2±42	97.3 ± 39	127.9 ± 47	116.7 ± 57	151.2 ± 43
Root length (mm)	71.8 ± 38	60.9 ± 30	87.5 ± 41	60.4 ±36	87.6 ± 35
Germination %	92	92	100	92	100
Total yield %	100	100	96	100	100
N	22	22	23	22	24

Similar results were reported by Zuokaite and Scupakas when using sewage sludge as a fertilizer for garden cress and spinach (Zuokaite & Scupakas, 2007). Germination % that is below 60% serves as an indication that the fertilizing material is phytotoxic to the crop (Murrilo, et al., 1995). As a fast growing plant, garden cress is known to be very sensitive to phytotoxic substances (Zucconi, et al., 1985). As such, it is a suitable plant to test for the phytotoxicity of the fertilizer (Buss & Masek, 2014). Phytotoxicty manifests itself through delayed germination and inhibition of root development or the damage of the root system (Pare, et al., 1997). Sometimes burning, chlorosis, necrosis yellowing and stunting on developing crops can also be an indication of phytotoxicity (de las Heres, et al., 2005). The total yield after harvesting was above 95% in all the treatments showing a better response of garden cress to the fertilizer than the radish.

The physiological parameters measured for garden cress, such as the plant fresh mass (g), dry mass (g), number of leaves, plant height (mm) and root length (mm) showed a varied response to the effect of the different strengths of the fertilizer. After harvest, the fresh weight, dry weight, plant height, root length and the number of leaves per treatment ranged (min- max) between 0.030 ± 0.015 – 0.071 ± 0.027 g, 0.0052 ± 0.002 – 0.010 ± 0.004 g, 97.3 ± 39 – 127.9 ± 47 mm, 60.4 ± 36 – 87.5 ± 41 mm and 4.2 ± 0.9 – 5.9 ± 1.2 respectively for garden cress. Different fertilizer strengths had a significant effect (p <0.05) on the fresh weight, dry weight, plant height, the number of leaves per treatment with the exception of the root length (p >0.05). Increasing the dosage of sewage sludge in soil has been reported to positively influence the physiological parameters of crops (Atiyeh, et al., 2001; Chan, et al., 2007; Girisha & Raju, 2008). The 10 and 100% fertilizer strength showed better performance in terms of the measured physiological parameters. Rong and colleagues reported that 4%and 10% concentration of sewage sludge improved the physiological parameters of water spinach (Rong, et al., 2015). Subsequently this study was repeated only using the 10% and the 100% fertilizer strength.

Although the fertilizer strengths were the same, the different fertilizer strengths were prepared from the different mixtures of faecal sludge namely, 33% FS, 66% FS and 100% FS.

Germination % was above 70% in all the different fertilizer strengths including the control (Table 15). These results are similar to the ones reported in literature for garden cress germination in sewage sludge (Zuokaite & Scupakas, 2007; Buss & Masek, 2014). The total yield % was above 85% in all the different fertilizer treatments and a 100% yield % was obtained from the control. These results suggest that the fertilizer was able to maintain the required nutrients throughout the plant growth period.

Table 15. Germination and yield percentage of garden cress grown on a growth substrate (vermiculite) that was fertilized with anaerobically digested and pasteurized pit latrine faecal sludge mixed with the effluent from the Belmont Valley WWTW.

Sludge mixture	**Fertilizer strength**	**Parameter**	
		Germination %	**Total yield %**
33% FS	10%	100	96
	100%	75	100
66% FS	10%	79	90
	100%	83	90
100% FS	10%	96	87
	100%	79	87
Control	Hoagland Solution	91	100

There was an increase in average fresh weight of garden cress with increasing fertilizer strengths (Table 16). The minimum fresh weight was 0.41 ± 0.1g with the maximum being 0.81 ± 0.2 g and was obtained from the 100% fertilizer strength prepared from the 100% FS. These results agree with the findings of Girisha and Raju (2008) who reported a positive response of ground nut with increasing sewage concentrations. Statistical analysis, however, showed that the difference between the fresh weights of garden cress treated with different fertilizer strengths was not significant (p=0.42).

Table 16. The response of the fresh weight of garden cress to different fertilizer strengths. Data presented as average ± SD.

Parameter	**33% FS**		**66% FS**		**100% FS**		
Treatment	10%	100%	10%	100%	10%	100%	C
Fresh weight (g)	0.58 ±0.2	0.68±0.3	0.41±0.1	0.68±0.3	0.67±0.2	0.81±0.2	0.83±0.2
Dry weight (g)	0.17±0.07	0.07±0.04	0.07±0.03	0.16±0.05	0.11±0.06	0.12±0.07	0.16±0.04
N	4	4	4	4	4	4	4

The dry weight of garden cress ranged between 0.07±0.03 g and 0.17±0.07 g and the maximum weight was obtained from the 10% fertilizer strength of 33% FS. There was an insignificant difference in the dry weights of garden cress (p=0.72). Similar concentrations were reported to increase the dry weight of water spinach (Rong, et al., 2015). The response of garden cress height to the different fertilizer treatments is presented in Table 17. Garden cress height ranged between 15.3±8.5 mm and 57.0 ±35.6 mm in the respective fertilizer strengths. The maximum height was reached with the garden cress grown in the 100% fertilizer strength of the 100% FS.). Statistical analysis indicated that the different fertilizer treatments did not have a significant effect on the height of the plant (p=0.65). Garden cress can grow up to 50 cm in height but garden cress with the height of 118 cm is also recorded in literature (Diwakar, et al., 2008; Kumari & Patel, 2013). Increases in the concentration of nitrogen have been shown to improve the height of garden cress (Kumari & Patel, 2013).

Table 17. The response of the height of garden cress (mm) to different fertilizer strengths.

	Plant height (mm)						
	33% FS		**66% FS**		**100% FS**		
Treatment	10%	100%	10%	100%	10%	100%	C
Min	4.0	4.0	3.0	7.5	4.0	15.0	6.0
Max	261.0	45.0	37.0	100.0	68.0	135.0	103.0
Median	15.0	15.0	15.0	20.5	15.0	56.5	17.0
Average	30.5	19.8	15.3	25.8	19.9	57.0	26.1
SD	52.2	14.8	8.5	21.5	16.6	35.6	25.9
N	23	18	17	18	20	17	22

Roots are important for nutrient and water absorption by plant. Their damage either by high salt content, heavy metals or insects can have a negative impact on the nutrient uptake of the plant (Januskaitiene, 2008). Response of garden cress roots to different fertilizer treatments is presented in Table 18. The maximum root length (43.2 ±22.4 mm) was reached with the 10% fertilizer treatment from the 33% FS, and the minimum root length (23.8 ± 18.4 mm) observed with the 10% fertilizer treatment from the 100% FS. It is reported that increasing the concentrations of the sludge positively affects the roots of crops (de las Heres, et al., 2005). However, damage in roots because of heavy metals present in the sewage sludge has been reported (Moreno, et al., 1998; Mor, et al., 2013; Buss & Masek, 2014). The difference between the root length of garden cress grown in growth substrates fertilized with different fertilizer strengths was not significant ($p = 0.31$).

Table 18. The response of the roots (mm) of garden cress to the different fertilizer strengths.

	Root length (mm)						
	33% FS		**66% FS**		**100% FS**		
Treatment	10%	100%	10%	100%	10%	100%	C
Min	5.0	5.0	7.0	0.5	6.0	5.0	5.0
Max	95.0	69.0	78.0	90.0	72.0	90.0	160.0
Median	41.0	35.0	29.0	22.0	15.5	25.0	31.5
Average	43.2	35.9	31.7	31.7	23.8	35.2	40.3
SD	22.4	21.6	18.4	23.0	18.4	25.1	41.5
n	23	18	17	18	20	17	22

Plant leaves play a significant role in the life cycle of plants more especially during the vegetative stage. Any damage to the leaves could have negative impact on photosynthesis, transpiration and food storage thereby affecting the yield (Mondal, et al., 2013). In garden cress, leaves are very important because they are the edible component of the plant. The lowest average number of garden cress leaves was 4.5± 1.9 leaves per treatment and the maximum number was 9.1±4.1 leaves (Table 18). Atiyeh and colleagues reported an increase in the number of leaves of tomato with increasing concentrations of pig manure vermicompost (Atiyeh, et al., 2001). Some authors even suggest that the response of crop physiological parameters to fertilizer treatments is influenced by the fertilizer type and the time of application (Moreno, et al., 1998; de las Heres, et al., 2005; Kumari & Patel, 2013; Mondal, et al., 2013; Rong, et al., 2015). Statistical analysis showed that the different fertilizer treatments had an effect on the leaves of garden cress ($p = 6.5 \times 10^{-5}$).

Table 19. The response of the leaves of garden cress to the different fertilizer strengths.

Number of leaves							
	33% FS		**66% FS**		**100% FS**		
Treatment	10%	100%	10%	100%	10%	100%	**C**
Min	2.0	4.0	3.0	2.0	2.0	2.0	2.0
Max	9.0	15.0	12.0	11.0	15.0	10.0	11.0
Median	4.0	9.0	7.0	4.5	6.0	7.0	7.0
Average	4.5	9.1	6.3	5.3	6.6	6.3	6.4
SD	1.9	4.1	2.3	2.7	3.0	2.6	2.1
n	23	18	17	18	20	17	22

The results for the physiological parameters of garden cress suggest that the fertilizer was able to meet the nutrient requirements for garden cress. This is further confirmed by the similar performance of the used fertilizer to the control, a Hoagland solution known to provide all the nutrients that plants require for plant growth. The 10% and the 100% produced the best physiological parameters which seemed to suggest that the two fertilizer strengths were appropriate for growing garden cress. The pasteurised effluent was suitable as a fertiliser, and the lower dilution strength would be the most economical.

Conclusion

The plant growth studies showed that the anaerobically digested and pasteurized pit latrine faecal sludge has value as a fertilizer. Diluting the fertilizer to different fertilizer strengths did not have a significant effect on the measured physiological parameters of radish. However, a positive response to the different fertilizer strengths was observed with garden cress when the fertilizer source was a single pit latrine but that changed when the source of the fertilizer was from various pit latrines. The response of the test plants to different fertilizer strengths was mainly due to the overall physiochemical properties of the fertilizer. Germination yield suggested partial toxicity of the sludge but the seed behaviour could have also contributed to poor germination. For future studies, the uptake of the nutrients by the plants could be conducted including the bioavailability of the nutrients for plant absorption. Additionally, soil could be used as a growth substrate instead of a soilless growth substrate as this could help in identifying the nutrients that are most likely to be depleted first by plants. This could also help in addressing issues of nutrient imbalance in the sludge when considering wide scale use in subsistence farming.

Chapter 5: The development of a tool to address the sanitation service delivery backlogs in municipalities throughout South Africa.

5.1 Introduction

By 2015, it was projected that the global population would reach 9.6 billion with a 6.3 billion rise occurring in the urban population globally (UN, 2012; UN, 2011). Over the 2011-2050 periods, most of the urban population growth was expected to occur in Africa (UN, 2011). In South Africa, the population is estimated to be 52.98 million with 24 %, 19.7 % and 11.4 % of the people living in the Gauteng Province, KwaZulu-Natal and the Western Cape Province respectively (StatSA, 2013). With unprecedented population increases in urban and peri urban areas, people are likely to inhabit land sites that are prone to natural disasters (low lying areas), and the mostly likely natural disaster might be the outbreak of diseases due to poor sanitation coverage and unhygienic practices in densely populated areas (Beall, et al., 2002; Cobbinah, et al., 2015). One of the major problems with poor sanitation coverage is the shortage of skilled personnel such as artisans, plumbers, sanitation and hygiene awareness officers which are key in addressing sanitation service delivery backlogs in South Africa, and disaster management could be applied in addressing these backlogs (SAHRC, 2014). The economic hubs of the country are based in these provinces where major metropolitan centres, such as Johannesburg, Durban, Richard's Bay, Pretoria and Cape Town are based. Out of the nine provinces, Gauteng experienced the largest population growth between 2002 and 2013 (StatSA, 2013). Similar migration patterns are likely to be observed in KwaZulu-Natal and the Western Cape.

The influx of people into the informal settlements and backyard shacks is the indirect consequence of the economic in-migration and the rapid urbanization (Beall, et al., 2002; NDMETF, 2013). As low-income areas, these communities are more susceptible to disasters than high income areas (Tandlich, et al., 2013). The shortage of financial and material resources coupled with poor nutrition, poor service delivery in areas such as sanitation, water and health are the contributing factors to the susceptibility of these areas to disasters and these are exacerbated by climate change (O'Brien, et al., 2006). The location of informal settlements is the cause for the elevated susceptibility of the settlements population to disasters and the high population density exposes the inhabitants to unhygienic conditions (Zuma, et al., 2012; Lamond & Kinyanjui, 2012). Exposure to such conditions can result in increased chances of an outbreak of sanitation related diseases such as cholera which can reach epidemic proportions and eventually become disasters (Lamond & Kinyanjui, 2012).

The disaster management cycle is shown in Figure 20. It illustrates the way in which the government, businesses, and the civil society plans for and alleviate the effect of the disaster during and instantly after the disaster and also the steps that are followed for recovery post the disaster. A complete disaster management cycle includes mitigation, preparedness, response and recovery (Coetzee & Van Niekerk, 2012). Mitigation involves the elimination or the reduction of the disaster occurrence or the reduction of the impact of inevitable disasters. Measures that are taken to mitigate disasters include building codes, zoning, land use management, safety codes, preventative health care and public education (NDMF, 2005).

In sanitation, this would mean ensuring that the sanitation facilities are well operated and maintained including the promotion of sanitation hygiene through well thought out regulations and guidelines. These could minimize the outbreak of sanitation related diseases, such as cholera, diarrhoea and the typhoid fever. Knowledge of the type of sanitation facilities in a particular region would be useful in investigating the possible disasters such as the disease outbreak and therefore the relevant stakeholders that might be required in case of a natural disaster. Prior knowledge of the presence of potential pathogens in communities can better prepare management for responding to possible outbreaks of these diseases. This is especially so when the point sources have been identified, such as pit latrines. For mitigation to be effective such information is crucial as it can aid in formulating the necessary countermeasures. The ability to respond to an emergency situation through programs that reinforce the technical and the managerial capacity of the government, organisations and communities shows preparedness (Coetzee & Van Niekerk, 2012). Necessary measures for preparedness include preparedness plans, emergency communication systems, emergency personnel or contact lists, warning systems, emergency training and resource inventories (NDMF, 2005). When the necessary measures of preparedness have been taken into account, then disaster response and recovering of the affected area is less challenging (NDMF, 2005).

Figure 20. The disaster management cycle.

Sanitation can be linked to hygiene and diarrhoeal epidemics or disasters. In the South African context, sanitation has a disaster management aspect that is linked to natech events (technological disaster triggered by natural disasters) including the secondary effects such as in the case of floods (Ozunu, et al., 2011; Luyt, et al., 2011). Sanitation service delivery lags behind the rate of urbanization and the needs of informal settlement inhabitants in South Africa. An example is the Moqhaka Local Municipality in the Free State Province of South Africa, where around 1620 waterborne sewage facilities/toilets were built by the municipality as means of eradicating the bucket system between 2001 and 2010, but only 24.7 % of the toilets were enclosed with the top structure (SAHRC, 2010). Similar sanitation service delivery problems were also reported from the City of Cape Town (IOL News, 2011). The lack of the superstructure of the toilet compromises the dignity of the user. As such, the users of such facilities are compelled by circumstances to look for alternatives of defecating and open defecation is a viable alternative, which results in low hygiene levels in informal settlements in South Africa. Poor hygiene and sanitation service delivery is linked to the prevalence of HIV/AIDS infections within the population (Luyt, et al., 2011). Therefore, urgent means to curb this problem are required because of the impact that they might have in natural disasters or epidemics.

The national government of South Africa has given attention to the problems that are linked to sanitation service delivery and the resulting impacts since 2001 (Hoossein, et al., 2014). The evidence for that include the testing of various strategies to address the sanitation service delivery backlogs through handing over the responsibility to the National Department of Human Settlements in 2009, and also the South African Department of Water and Sanitation which came into existence in 2014 (Hoossein, et al., 2014; SA Presidency, 2014). The number of the relevant stakeholders participating in sanitation projects was maximized as a form of cooperative intervention in the planning stage (Hoossein, et al., 2014). To speed up targeted and localized sanitation service delivery, funds were availed to local government and significant improvements were made (Hoossein, et al., 2014; SAHRC, 2010). Thus the cooperative interventions are most likely to remain with the Department of Water and Sanitation.

Shortage of sanitation related skills at the local government level, the lack of buy in from the community and the users of sanitation facilities according to research are the major contributors to the factors that hinder sustainability of sanitation provision in South Africa (Hoossein, et al., 2014; Whittington-Jones, et al., 2011). Acceptance of ventilated improved pit (VIP) latrines as sanitation facilities by communities is mostly influenced by how community members perceive the facility including the potential problems that the facility itself might bring to the community (Buckley, et al., 2008; Bardosh, 2015). In Hlalani Township in Grahamstown, most households have VIP's as sanitation facilities, most of which are not maintained or not serviced properly, a problem that stems from ignorance about the proper maintenance and operation of this facilities. Pit latrines have a filling up problem which serves as part of maintaining the facility and the municipality assumes that role as it is mandated to do so as stipulated in the Water Services Act (DEA, 2011a). When the local government fails to fulfil its legislative obligations, it is necessary that the provincial administration intervenes (RSA, 1996).The intervention of the provincial administration at Makana Municipality, in which Hlalani Township is located, to address issues pertaining to institutional challenges such as vacant key positions, lack of capacity and the skills audit is an example of failure by the local government to fulfil its legislative obligations (PMG, 2015). In Mpumalanga, the provincial government had to intervene at the Thaba Chweu Municipality in 2005 after the audits reports showed the prevalence of corruption in the municipality, which led to the failure of the municipality to deliver services including sanitation to communities (PMG, 2005). Successful interventions by the provincial government including the relevant stakeholders were reported for Polokwane Municipality (PMG, 2008). These interventions resulted in improved water service delivery by the municipality between 2001 and 2007, although backlogs still remained in house provision and electricity supply.

The implementation of the provincial government administration takes times as the intervention depends on the political will and might at times not be suitable, because of the unregulated population expansion in urban areas, especially in the metropolitan areas of South Africa (Zuma, et al., 2012). Thus, other approaches are necessary to address sanitation service delivery backlogs which are mainly due to the shortage of skilled personnel such as artisans, plumbers, operation and maintenance officers and sanitation and hygiene awareness officers. Addressing the shortage of these skills could help in minimizing some of the challenges that are linked with operation and maintenance of sanitation facilities such as VIP's including the possible outbreak of sanitation related diseases due to poor sanitation hygiene. All the functions of government operations are included in the disaster management systems regarding the immediate action required to address urgent circumstances. Hlalani Township, under Makana Municipality in Grahamstown, provides a good perspective of a typical formal settlement with sanitation challenges. Informal settlements are usually worse off with fewer sanitation services available. While innovative uses of pit latrine faecal sludge may provide a partial solution to on-site sanitation issues, considerable health risks remain and need to be addressed, or at least mitigated. This chapter investigated policy research into the legal framework and conceivable tools in disaster management that could be used in addressing sanitation service delivery backlogs such as operation and maintenance of VIP's in Makana Municipality, Grahamstown and throughout South Africa. The obtained results were then applied in formulating and proposing implementation strategies that could address the shortage of skills that are required in operation and maintenance of sanitation facilities including the promotion of sanitation hygiene and awareness to minimize future sanitation related disease outbreaks.

5.2 Research method

The South African government documents, data gathered during the sampling site scouting, online resources provided by the Parliamentary Monitoring Group (PMG) and SCOPUS database were used to search for the information. The website of the City of Cape Town Disaster Management, Johannesburg Metropolitan Municipality, Disaster Management Institute of Southern Africa, National Disaster Management Centre, Department of Cooperative Governance and Traditional Affairs, Social Justice Coalition and Community-led sanitation projects from NGOs and WHO were used to gather information and to also provide the base for the conducted analysis.

5.3 Results of the policy analysis

In South Africa each municipality is mandated to perform a specific set of disaster management functions (DMA, 2002). As such, whenever there is a shortage of either human or technical capacity, policy tools should be available to address such shortages. The conducted policy search identified the Draft Disaster Management Regulations: Disaster Management (DMRDM) as the relevant tool that could be used by local government to address capacity shortages (DMRDM, 2005). Chapter 2 of the policy facilitates the addressing of sanitation service delivery gaps through the establishment of a volunteer unit within a particular metropolitan or a district municipality whose duty it is to perform the functions that are linked to disaster management (DMRDM, 2005). Therefore, the regulations from Chapter 2 of the policy articulate the basis of action.

According to regulation 4e of Chapter 2, the role of the established volunteer unit is to assist municipalities with the management of communities during disasters by operating as emergency personnel that renders specialized assistance to disaster survivors, help in distributing relief material during disasters and also be involved in activities that help in reducing disasters and its impact in disaster prone communities (DMRDM, 2005). Technical assistance which includes water supply, roads and bridge construction, electricity supply, solid waste and wastewater services and emergency vehicle and equipment repairs could also be provided by the established volunteer unit as articulated in regulation 4j of Chapter 2. These two regulations are relevant in that improper maintenance of sanitation infrastructure results in the contamination of the environment with faecal matter and pathogens (Bakare, et al., 2012). Consequently, the health of the community and the environment could be compromised. Regulation 4j applies in this respect because sanitation involves the management of domestic wastewater (DMRDM, 2005).

During the site visits for sampling at Hlalani Township in Grahamstown, it was observed that the some VIP's were not well maintained by the owners. Most pit latrines were filled with rags, stones, corn cobs, sanitary pads and various other materials that were not supposed to be inside the pit. In most pit latrines, the emptying port at the back could not be opened, either due to the handle being broken, or the access port was completely covered with layers of soil or building material, for instance bricks, corrugated iron, etc. Consequently, the contents of the pit latrine could not be accessed by the pit emptiers. In some pit latrines, however, it was possible to remove the toilet pedestal to access the contents of the pit latrine. The pit latrines inspected or sampled in this study were found to be unlined, with no constructed walls or floor in the pit.

Additionally, the pit latrines were very close to the houses indicating that the guidelines about the distance between the pit latrine and the house were not followed by the contractors, either due to ignorance or due to the lack of clear guidance from the municipality (National Sanitation

Task Team, 1996; Buckley, et al., 2008). Some pit latrine users complained about the leaking of the pit contents into their houses during heavy rains (Figure 21). This could have detrimental health consequences to the residents, more especially infants who play barefoot on the ground (Figure 22).

Figure 21. An image of the support concrete slab for the superstructure of the pit latrine showing an area where the sludge leaches during rainy days or seasons. b. the leaching of the faecal sludge from the pit latrine

Figure 22. Sewerage leakage in one of the streets of Hlalani Township, Grahamstown.

On the basis of the needs analysis results, regulation 4n in Chapter 2 of the policy applies to sanitation because it permits either the metropolitan or district municipality to broaden the duties of the volunteer unit (DMRDM, 2005). According to the Constitution of the Republic of South Africa, a metropolitan municipality is a municipality with the exclusive municipal and executive legislative authority in a given area, while the district municipality can exercise the same authority in more than one municipality (RSA, 1996). Local municipalities share the executive and legislative authority with the district municipality whose jurisdictions it belongs. The needs analysis is an audit which enables the municipality to identify its disaster management capabilities through comparing these to the active disaster management stakeholders within its jurisdiction as stated in regulation 2a of Chapter 2 (DMRDM, 2005). That way, the scope of the functions of a volunteer unit and its size is ascertained based on the annual needs analysis as per regulation 2 b of Chapter 2 (DMRDM, 2005). Most important is that the needs analyses should be conducted on an annual basis, to gather information about the areas that are not covered by the functions and activities of the volunteer units. Operation and maintenance of sanitation facilities such as VIPs including sanitation hygiene and awareness campaigns can thus be included that way. Poor maintenance and operation of VIP's in Hlalani Township, Grahamstown could be addressed by deploying volunteers that could aid in demonstrating to the users how VIP's are operated and maintained. All the disaster management activities in a particular metropolitan or district municipality are overseen by a Municipal Disaster Management Centre, a local government entity (DMA, 2002). Chapter 2 regulation 3 sub-regulation 1a,b and Appendices A and B of the regulations allows the staff of the Municipal Disaster Management Centre to appoint members of the volunteer units (DMRDM, 2005). The Municipal Disaster Management Centre is a centre where the integrated and coordinated approach to disaster risk management is promoted and maintained by adhering to the disaster management cycle and making amendments where necessary. Instructions pertaining to the maintenance of the list of the currently and recently employed volunteers that are employed by a given municipality are described comprehensively in regulations 5 and 14 of Chapter 2.

The need for training volunteer disaster management staff is determined by the Head of Municipal Disaster Management Centre to ensure that the duties of volunteer units are performed and executed properly according to regulation 7 sub-regulation 1 of Chapter 2. Regulation 7 sub-regulation 2a-b stipulates that the financial resources of the municipality should be used for such resources. A registered training provider should be used and all the records of the attended courses should be kept as articulated in regulation 7 sub-regulation 2c-e, 3,4 and regulation 8 of Chapter 2. Registered training providers should include universities such as University of Northwest, the University of Free State and colleges across South Africa that already offer accredited courses in disaster management, water and sanitation including volunteer capacity building short courses (UNW, 2009). Volunteer based organisations such as the Red Cross South Africa do offer courses such as first aid training (basic to level 3), cardio-pulmonary resuscitation, occupational health and safety, counselling and disaster management (Red Cross, 2015). That way, volunteers can get quality education and training which can improve the effectiveness of the volunteer unit. Furthermore, an annual review of the number of volunteers required by a particular municipality should be done (DMRDM, 2005). Regulation 10 sub-regulation 1 and 2 states that the Head of Municipal Disaster Management Centre should formalize the conditions of service for the volunteers and that the volunteers should adhere to, such as stipulated by regulation 10 sub-regulation 4, or the service is terminated according to regulation 10, sub-regulation 5 of Chapter 2. In Grahamstown, key organisations that could aid the municipality in recruiting volunteers include Khulumani

Support Group and Ikamva youth because of their active engagement with the youth of the Grahamstown community. The Khulumani Support Group is already actively involved in raising sanitation awareness in communities across South Africa through the use of volunteers, as such, might have access to suitable volunteers.

Protective clothing must be worn at all times by volunteers that are on duty including official IDs that are provided by the municipality as outlined in regulations 12,13 and regulation 10 sub-regulation 3 of Chapter 2. For VIP emptying volunteers, protective clothing should include an overall with a hat, an apron, a pair of gloves (latex gloves and heavy duty gloves), dust mask, protective glasses and gum boots to minimize contact with faecal sludge which might result in unnecessary infections. Regulation 11 of Chapter 2 gives guidelines on how municipalities can appoint emergency volunteers across jurisdictional boundaries (DMRDM, 2005). As an example, the 2008 cholera outbreak in Zimbabwe was also experienced in Musina in the Limpopo province with reported deaths of two truck drivers in Durban and Johannesburg who had driven through Zimbabwe (COGTA, 2012). The cause of the outbreak was traced beak to the contamination of drinking water with human faeces due to poor maintenance of sanitation facilities which led to the overflow of sewage into the water bodies. This was a challenge as it was a cross-border epidemic and also led to the Limpopo River being contaminated with faeces. Effective measures were taken by the Zimbabwean and South African authorities to deal with the outbreak through the cross-border supply of proper medication and skilled personnel.

The outline about the command structure of the volunteer unit, and the overheads that the volunteers can be reimbursed for from the municipality's budget can be found in regulations

15 and 16 of Chapter 2 (DMRDM, 2005). The Minister of Cooperative Governance and Traditional Affairs is mandated to draft the guidelines for establishing the command structure of the volunteer unit according to Section 58 of the Disaster Management Act no. 57/2002 (Kilian, 2009). The Occupational Injuries and Diseases Act No. 130/1993 entitles the volunteers to basic insurance as stated in regulation 19 of Chapter 2. Chapter 3 specifies that each municipality should have a database containing information about the existing prevention and mitigation measures, relevant hazards, communities at risk, suppliers of important goods and services and all the relevant contact details (DMRDM, 2005).

The relevant stakeholders could be assigned to close the municipal sanitation capacity gaps based on the flexibility of the policy. Engaging non-governmental stakeholders such as NGOs could aid in facilitating the transfer of sanitation skills that are lacking in local communities to the sanitation facilities end-users. The absence of a sense of ownership of the existing sanitation facilities could be exposed because of the lack of community participation in the planning of the sanitation projects and the maintenance of sanitation facilities. The concerns that are raised by the disaster management stakeholders during a comprehensive consultative process include the time consuming legislative process that slows down practical implementation (Kilian, 2009; NCOP, 2010). The concerns raised by the stakeholders include unpreparedness of the volunteers to perform duties that the municipal staff members are paid for (Kilian, 2009). There are also additional concerns about the proper insurance coverage of every volunteer prior registering with National Disaster Management Centre. Finally, there were matters of concern about the ability of the volunteers to apply for the vacancies in disaster management based on the experience they acquired or the qualifications they obtained while volunteering (Kilian, 2009).

The Draft Disaster Management Volunteer Guidelines were gazetted in South Africa in 2010 after a consultative and a legislative process between various stakeholders and government (DMVR, 2010). The guidelines became an extension of the policy by providing structural mechanisms for the policy implementation after the comments from the consultative and legislative processes were implemented (DMVR, 2010). Initially, the Head of the Municipal Disaster Management Centre was appointed as the leader of the volunteer unit in section 3 subsection 1 of the guidelines. Approval for the transfer of the role to another municipal official was also given to the Head of the Municipal Disaster Management Centre in section 3 subsection 2a of the guidelines. A components leader according to section 1 of the guidelines is defined as a member of the volunteer unit who oversees particular activities of the volunteering unit such as sanitation as stated in section 3 subsections 3 and 4 of the guidelines (DMVR, 2010).

The duties of a particular volunteer unit are specified in section 4 of the guidelines (DMVR, 2010). Section 4g is relevant to sanitation in that it is linked to community and environmental health related activities (DMVR, 2010). Section 4i is also relevant to sanitation because it identifies technical activities which include wastewater and solid waste services, water supply, roads and bridge construction, emergency vehicle and equipment repairs and electricity supply (DMVR, 2010). The requirements for a volunteer who is a member of a unit are specified in section 5 and the detailed information about volunteer recruitment can be found in section 6 (DMVR, 2010). Section 9 and 10 specify that appropriate training is required for a volunteer unit to carry out its duties effectively with section 11 providing the procedures that have to be followed (DMVR, 2010). As can be seen, there were only minor changes from the draft of the previous policy (DMRDM, 2005).

Clarity on vague information and specifications can be obtained from guidelines hence their importance. The specific description about the protective clothing that volunteers need to wear while on duty is articulated in section 12 subsection 4 (DMVR, 2010). Section 13 deals with compliance and the quality of the equipment that is provided to the volunteers (DMVR, 2010). Annexure A (2) of the updated policy outlines the details of the code of conduct of the volunteers with Annexure A (3) highlighting the inclusion of consent forms in cases where there are non-adults in a particular volunteer unit (DMVR, 2010). Additionally, all volunteers are mandated to fill in a health questionnaire before resuming their activities as indicated in Annexure B of the updated policy and the clothing specifications and official IDs are included in Annexure C and D (DMVR, 2010). With the detailed information articulated in both policy and guidelines as a tool, sanitation service delivery backlogs could be addressed. The following section describes this in detail.

5.4 Discussion

The analysed policy clearly indicates that the tool it provides could be used by the local government to address the sanitation backlogs. Synergy between entrepreneurs who derive value from faecal sludge through the production of a sanitised fertilizer, and participants in the proposed volunteer units, should also be explored. Various stakeholders could be included in the structure of the volunteer unit. Given the capacity of the municipal disaster management centre, the stakeholders could include NGOs, tertiary institutions, business community and community members. Substantial solutions to address sanitation service backlogs could be acquired from NGOs as NGOs mostly develop low cost solutions to complex problems and also have a wide range of skills and knowledge (Kar & Chambers, 2008; Lüthi, et al., 2011). This is mostly due to their access to international donors which provide them with a platform to broaden the spectrum of their knowledge base and skills to levels which far exceed that of stakeholders that are within the municipal jurisdiction (Esrey, et al., 2001; Hanchett, et al., 2003; Kar & Chambers, 2008; Lüthi, et al., 2011). The necessary material and consumables including the expertise for construction and maintenance of sanitation facilities could be provided by the business community. That way, the logistics of sanitation provision through the volunteer unit system would be simplified.

Community-led sanitation interventions are a good example for transferring the skills necessary for addressing sanitation service delivery, and they have been successful in improving sanitation coverage through the promotion of sanitation hygiene practices in countries such as India, Bangladesh and Kenya (Kar & Chambers, 2008; Lüthi, et al., 2011).The purpose of involving the entire community is so that each member can contribute to the decision making. Such interventions are meant to raise awareness and to change sanitation behaviour and perception within communities. As such, participation of stakeholders from within and outside the affected communities is the key to the success of the interventions. These interventions follow a sequence of events which include pre-triggering, triggering, post-triggering and scaling up and going beyond community-led intervention (Kar & Chambers, 2008; Lüthi, et al., 2011). During the pre-triggering stage, a particular community is selected and introductions are made followed by building relationships (Kar & Chambers, 2008; Lüthi, et al., 2011). In the triggering step, the community members conduct an analysis of the sanitation profile and identify the urgent issues that need to be addressed (Kar & Chambers, 2008; Lüthi, et al., 2011). This is the stage at which a particular community can then decide on the appropriate sanitation facility type, thus between VIP's, composting toilets or the sewage sanitation system. Most members of the Hlalani community appeared to prefer the sewage sanitation system over the pit latrines for reasons that were linked mostly to pit emptying difficulties and the odour. When all of the above mentioned has been done, the community members then plan on how to proceed in addressing the identified sanitation related issues in the post-triggering stage (Kar & Chambers, 2008; Lüthi, et al., 2011). Scaling up involves training of the community members and providing them with the necessary skills that would aid in addressing the identified issues (Kar & Chambers, 2008; Lüthi, et al., 2011).

For an individual facilitating such interventions, the behaviour and attitude towards the community is the key to either success or failure of the intervention (Kar & Chambers, 2008). An important aspect is allowing the community members to identify their sanitation shortfalls and where improvements can be made without imposing any knowledge on the members (Lüthi, et al., 2011). An example of this would be to state that a particular community needs bucket sanitation system without considering how the members feel about it. Challenges do immerge in communities that are socially divided where there is low cohesion, weak tradition of joint action, and areas that are mostly populated by tenants where there is lack of ownership (Kar & Chambers, 2008). Cultural, religious values and beliefs also contribute to some of the challenges that need overcoming in communities where intervention is necessary (Kar & Chambers, 2008; Lüthi, et al., 2011). Heavily subsidised sanitation hardware also makes it difficult for communities to come up with their innovative challenges to address sanitation shortfalls (Kar & Chambers, 2008; Lüthi, et al., 2011). As a facilitator, clearly stating who you are and your purpose of visiting the community is a good foundation for a successful relationship. Do clarify that you are not there to change the current hygiene behaviour and that your visit is a learning expedition to understand the reason behind the current hygiene behaviour (Kar & Chambers, 2008; Lüthi, et al., 2011). This is important as it allows the community members to discuss freely without holding back any information that might be vital for the success of the intervention.

When there are meetings, collective decision making should be encouraged within the community without the influence of an outsider even when a monetary incentive is being provided (Kar & Chambers, 2008; Lüthi, et al., 2011). That way any critical decision made will be owned by the entire community and not particular individuals with hidden agendas, as that might lead to divisions within the communities. The role of the volunteer in this regard is to facilitate the meeting and not to interfere with the affairs of the community and to only provide guidance when asked to do so. Sometimes influential individuals that are affiliated with a particular political party that is active within the community might emerge and might view the intervention as a threat of competition (Kar & Chambers, 2008; Lüthi, et al., 2011). Such individuals ought to be treated with care and be kept busy with serious discussions whenever there are decision making meetings as they might have a negative influence on the community members (Kar & Chambers, 2008; Lüthi, et al., 2011). Successful community-led sanitation interventions in India, Bangladesh, Kenya, Ethiopia, Cambodia, Indonesia and Pakistan are recorded in the literature (Kar & Chambers, 2008; Lüthi, et al., 2011). Sanitation coverage after these interventions varied from the use of pit latrines, composting toilets and VIP's depending on the priorities of the affected areas. The experience from these interventions can be applied to speed up basic sanitation coverage in developing countries where there are limited financial resources and could provide sustainable solutions to the ongoing sanitation challenges.

The members of a particular community know and understand their preferred type of sanitation facility and thus serve as the best source of information and thus information could be used by a facilitator as a guide in helping communities to make informed decisions. As such, the sanitation project implementation involving community members would simplify the delivery of sanitation service delivery, including the maintenance of sanitation facilities. Additionally, new sanitation skills that otherwise would not have been known before the establishment of a volunteer unit could emerge from the interaction between the community members and the different stakeholders and municipal officials. The acquired sanitation skills could encourage the opening of new business ventures that could address the local problems through manufacturing of sanitation materials and consumables using locally available raw material(s). The business ventures could include fertilizer and biogas production for commercial purposes and subsistence farming. That way, poverty including the health vulnerability of the poor South African population, would decrease significantly due to the financial independence brought about by economic activities , such as those linked to sanitation services provided by members within that community. Parametric insurance could also stimulate economic activities (Arnold, 2008). These could be accomplished through the emergence of different industries that could help in providing disaster relief services to affected communities or regions. These activities could be in the form of acquiring the raw material necessary for building of new sanitation facilities, including the material necessary for repairing the collapsing structures and servicing them. During the planning process for sanitation service delivery or provision, the individuals or organisations that could offer such services could be involved.

The workforce that has been trained through the Expanded Public Works Programme (EPWP) could be recruited as volunteers as they might have the skills (operational, maintenance, construction skills) that are lacking (Ethekwini, 2006). EPWP is a nationwide programme that was launched in 2009. Its purpose is to provide the necessary skills that would in turn allow members of poor communities to be employable (EPWP, 2013). The skills of trained personnel such as artisans, plumbers, operation and maintenance officers including sanitation hygiene and awareness officers that are required for effective sanitation service delivery could be incorporated into the Disaster Management Volunteer Units. The principle of community-based and participatory approach to disaster management should be applied to volunteers as the model has been shown to be inclusive of all stakeholders.

The notion of informal and non-formal learning in the draft of educational framework for disaster management is coherent with the aforementioned strategies (NDMETF, 2013). The standard for learning mechanisms in disaster management is set by the National Disaster Management Education and Training Framework (NDMETF, 2013). Throughout South Africa there are variations in the uptake and establishment of volunteer units. A volunteer unit workshop was set up by the eThekwini metropolitan municipality, but as far as the author knows, there is no established volunteer unit (Durban, 2011). Progress has been made in the City of Johannesburg and the City of Cape Town regarding the establishment of the volunteer units (Cape Town, 2014; Johannesburg, 2014). However, the list of activities and functions that are included in the volunteer unit of the City of Cape Town exclude sanitation (Cape Town, 2014). In light of the continued sanitation service delivery protests that are directed towards the City of Cape Town, it is possible that sanitation might eventually be included in the list of functions and activities of the established volunteer unit, because of the growing impatience of the communities such as Khayelitsha. This would include the knowledge about the dangers that are associated with improper handling of human excrement (CapeTown2, 2014).

5.5 Conclusion

In conclusion, the combination of draft DMRDM and the Draft Guidelines for the Volunteers in disaster management can be used to forge a tool or draft an efficient policy to include volunteering as a means of addressing the shortage of skills in the sanitation sector at the local government level in South Africa. The key skilled personnel that are necessary for effective sanitation service delivery include artisans, plumbers, and operation and maintenance officers including sanitation awareness officers which could be included in the disaster management volunteer unit activities. Implementation of informal and non-formal learning across South Africa's volunteering units within municipalities should be consideration to cover a broad spectrum of skills necessary to address sanitation service delivery backlogs. New business ventures could also emerge from addressing sanitation service delivery backlogs which could aid in maintaining and sustaining various sanitation systems. Community-led sanitation interventions could also be used to address sanitation service delivery and the volunteers would play a crucial role as the facilitators of the intervention.

References

Al-Jessim, N; Ansari, M.I; Harb, M & Hong, P-Y., 2015. Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: Is the treated wastewater safe to reuse for agricultural irrigation? *Water Research,* Volume 73, pp. 277-290

Ali, U., Juhanni, K. & Karim, A. B. N., 2015. A review of the properties and applications of poly (methyl methacrylate) (PMMA). *Polymer Review,* 55(4), pp. 678-705.

Angelidaki, I. & Sanders, W., 2004. Assesment of the anaerobic biodegradability of macropollutants. *Reviews in Environmental Science and Bio/Technology,* Volume 3, pp. 117-129.

APHA,AWWA and WEF, 1998. *American Public Health Association, American Water Works Association and Water Environment Federation.Standard Methods for the Examination of Water and Wastewater.* 20 ed. Washington, DC: WEF.

Appels, L., Baeyens, J., Degre`ve, J. & Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. *Progress in Energy and Combustion Science,* Volume 34, p. 755–781.

Arahal, D.R., Vreelad, R.H., Litchfield, C.D., Mormile, M.R., Tindal, B.J., Oren, A., Bejar, V., Quesada, A & Ventosa, A., 2007. Recommended minimal standards for describing new taxa of Halomonadaceae. *International Journal of Systematic and Evolutionary Microbiology,* Volume 57, pp. 2436-2446.

Arnold, M., 2008. *The role of risk transfer and insurance in disaster risk reduction and climate change adaption,* Stockholm: Commission on Climate Change and Development.

Arthur, R., Baidoo, M. B. & Antwi, E., 2011. Biogas as a potential renewable energy source: A Ghanaian case study. *Renewable Energy,* Volume 36, pp. 1510-1516.

Asare, I., Kranjac-Berisavljevic, G. & Cofie, O., 2003. Faecal sludge application for agriculture in Tamale, Ghana. *Urban Agriiculture Magazine,* Volume 10, pp. 32-33.

Atiyeh, R., Edwards, C., Subler, S. & Metzger, J., 2001. Pig manure vermicompost as a component of horticultural bedding plant medium; effects on physiological properties and plant growth. *Bioresource Technology,* Volume 78, pp. 11-20.

Aukerman, R. & Monzingo, D., 1989. Water treatment to inactivate Giardia. *The Journal of Science and Technology for Forest Products and Processes,* Volume 87, pp. 18-21.

Bagge, E., Sahlstrom, L. & Albihn, A., 2005. The effect of hygienic treatment on the microbial flora of biowaste at biogas plants. *Water Research Journal,* Volume 39, p. 4879–4886.

Bakare, B., Foxon, K., Brouckaert, C. & Buckely, C., 2012. Variations in VIP latrine sludge contents. *Water SA,* 38(479-485).

Baloch, P. Uddin, R; Nizamanai, F. K; Solangi, A.H; Siddiqui, A, 2014. Effect of nitrogen, phosphorus and potassium on growth and yield characteristics of Radish. *Journal of Agriculture and Environmental Science,* 14(6), pp. 565-569.

Bardosh, K., 2015. Achieving ‘‘Total Sanitation” in Rural African Geographies: Poverty,Participation and Pit Latrines in Eastern Zambia. *Geoforum,* Volume 66, p. 53–63.

Beall, J., Crankshaw, O. & Parnell, S., 2002. *A matter of timing: migration and housing access in metropolitan Johanesburg.* [Online]

Available at: http://www.cssr.uct.ac.za/sites/cssr.uct.ac.za/files/pubs/wp15.pdf
[Accessed 25 November 2015].

Bendixen, H. J., 1999. *Hygienic safety—results of scientific investigations in Denmark.* Hohenheim, Proceedings of the IEA Bioenergy Workshop.

Bhagwan, J. N., Still, D., Buckley, C. & Foxon, K., 2008. Challenges with up-scaling dry sanitation technologies. *Water Science and Technology,* 1(58), pp. 21-27.

Bhagwan, J., Still, D. & Buckley, C. F. K., 2008. When last did we look down the pits. *WISA Paper.*

Bohm, R., Martens, W. & Philipp, W., 1999. *Regulation in Germany and results of investigations concerning hygienic safety of processing biowastes in biogas plants.* Hohenheim, Proceedings of the IEA Bioenergy workshop.

Brintha, I. & Seran, T. H., 2009. Effect of paired row planting of Radish intercropped with vegetable amaranthus on yield components of radish in sandy regosol. *Journal of Agricultural Science,* 4(1), pp. 19-28.

Brockerhoff, M. P., 2000. An urbanising world. *Population Bulletin,* 55(3), pp. 3–43.

Buckley, C. A. Foxon, K. M; Brouckaert, C. J; Rodda, N; Nwaneri, C; Balbon, E; Couderc, A; Magagna, D, 2008. *Scientific support for the design and operation of ventilated improved pit latrines and the efficieny of pit latrines and additives,* Pretoria: Water Research Commission.

Buss, W. & Masek, O., 2014. Mobile organic compounds in biochar-a potential source of contamination-phytotoxic effecs on cress seed germination. *Journal of Environmental Management,* Volume 173, pp. 111-119.

Cantrell, K. B. & Ducey, T., 2008. Livestock waste-to-bioenergy generation opportunities. *Bioresource Technology,* 99(17), pp. 7941-7953.

Cape Town, 2014. *Disaster Risk Management: Volunteer unit.* [Online]
Available at: http://www.capetown.gov.za/en/DRM/Pages/Volunteerunit.aspx
[Accessed 2 December 2015].

CapeTown2, 2014. *Disaster Risk Management.* [Online]
Available at: https://www.capetown.gov.za/en/DRM/Pages/HumanDiseaseOutbreak.aspx
[Accessed 19 January 2016].

Cardinali-Rezende, J. Colturato, L.F.D.B; Colturato, T.D.B; Chartone-Souza, E; Nascimento, A.M.A; Sanz, J.L, 2012. Prokaryotic diversity and dynamics in a full-scale municipal solid waste anaerobic reactor from start-up to steady-state conditions. *Bioresource Technology,* Volume 119, pp. 373–383.

Chaggu, E. J., 2004. *Sustainable environment protection using modified pit-latriens- PhD thesis,* The Netherlands: Wagenigen University.

Chan, K., Van Zwieten, I., Downie, A. & Joseph, S., 2007. Agronomic values of green biochar as a soil amendement. *Australian Journal of Soil Research,* Volume 45, pp. 629-634.

Chawla, O., 1986. *Advances in biogas technology.* New Delhi: Publications and Information Division, Indian Council of Agricultural Research.

Chen, Y., Cheng, J.& Creamer, K., 2008. Inhibition of anaerobic digestion process: A review. *Bioresource Technology,* Volume 99, pp. 4044–4064.

Chen, Y., Yang, G., Sweeney, S. & Feng, Y., 2010. Household biogas use in rural China: a study of opportunities and constraints. *Renewable and Sustainable Energy Reviews,* 14(1), pp. 545–549.

Chinyama, A., Chipato, P. T. & Mangore, E., 2012. Sustainable sanitation systems for low income urban areas-a case of the city Bulawayo, Zimbabwe. *Physics and Chemistry of the Earth,* Volume 50, pp. 233-238.

Choffnes, E. R. & Mack, A., 2009. *Global Issues in Water, Sanitation, and Health: Workshop Summary.* Washington: National Academy Press.

Chohura, P. & Kolota, E., 2011. The effect of nitrogen fertilization on radish yielding. *Scientiarum Polonorum Acta, Hortorum Cultus,* 10(1), pp. 23-30.

Ciochetti, D. & Metcalf, R., 1984. Pasteurization of naturally contaminated water with solar energy. *Applied and Environmental Microbiology,* Volume 47, pp. 223-228.

Cobbinah, P. B., Erdiaw-Kwasie, M. O. & Amoateng, P., 2015. Africa's urbanisation: Implications for sustainable development. *Cities,* Volume 47, pp. 62–72.

Cofie, O. & Adamtey, N., 2009. *Nutrient recovery from human excreat for urban and peri-urban agriculture,* Addis Ababa: Contribution to SuSanA Food and Security Work Group meeting during the WEDC International Conference.

Cofie, O., Adesti, A., Nkansa-Boudu, F. & Awuah, E., 2010. Farmers perception and economic benefits of excreta use in Southern Ghana. *Resources, Conservation and Recycling,* Volume 55, pp. 161-166.

Cofie, O., Kone, D., Rokenberger, S, Moser, D, Zurbrueg, C., 2009. Co-composting of faecal sludge and organic solid waste for agriculture: process dynamics. *Water Research,* Volume 43, pp. 4665-4675.

COGTA, 2012. *http://www.cogta.gov.za/sites/cogtapub/AnnualReport/.* [Online]
Available at:
http://www.cogta.gov.za/sites/cogtapub/AnnualReport/NDMC%20ANNUAL%20REPORT%202008-2009%20FINAL%2814%20May%202012%29.pdf?Mobile=1&Source=%2Fsites%2Fcogtapub%2F_layouts%2F15%2Fmobile%2Fview.aspx%3FList%3Df5e587b1-5b6f-4ea1-ab4e-1fccf4fb4d41%26View%3De93aa81
[Accessed 9 January 2015].

Coultry, J., Walsh, E. & McDonnell, K., 2013. Energy and economic implications of anaerobic digestion pasteurisation regulations in Ireland. *Energy,* Volume 60, pp. 125-128.

Coetzee, C & Van Niekerk, D., 2012, Tracking the evolution of the disaster management cycle: A general system theory approach, Jàmbá: *Journal of Disaster Risk Studies,* 4(1), pp. 1-9.

Cuba, V., Pospisil, M. & Mucka, V., 2003. Electron beam/biological processing of anaerobic and aerobic sludge. *Czechoslovak Journal of Physics,* Volume 53, p. 369–374.

Cuba, V. Pospisil, M; Mucka, V; Jenicek, P; Silber, R; Doha´nyos, M; Za´branska, J., 2003. Impact of accelerated electrons on activating process and foaming potential of sludge. *Radiation Physics and Chemistry,* Volume 67, p. 545–548.

Cutino-Jimenez, A. Martins-Pinheiro, M; Lima, W.C; Martin-Tornet, A; Morales, O.G & Meuck, C.F.M., 2010. Evolution and placement of Xanthomonadaceae based on conserved protein signiture sequences. *Molecular Phylogenetics and Evolution,* Volume 54, pp. 524-534.

DAFF, 2012. *Fertilizers, Farm Feeds, Agricultural remedies and stock remedies ACT, 1947 (ACT No. 36 OF 1947),* Pretoria: Department of Agriculture, Forestry and Fisheries.

Daisy, A. & Kamaraj, S., 2011. The impact and treatment of night soil in anaerobic digesters: a review. *Journal of Microbial Biochemistry and Technology,* Volume 3, pp. 43-50.

de Alwis, A. A. P., 2012. A Tool for Sustainability: A Case for Biogas in Sri Lanka. *Journal of Tropical Forestry and Environment,* 2(1), pp. 1-9.

de la Haba, R., Arahal, D.R., Sanchez-Porro, C. & Ventosa, A., 2014. The Family Halomonadaceae. In: E. Rosenberg, De Long, E.F; Lory, S; Stackebrandt, E & Thompson, F. eds. *The prokaryotes.* Berlin Heidelberg: Springer Berlin Heidelberg, pp. 325-360.

de las Heres, J., Manes, P. & Labrador, J., 2005. Effects of several applications of digested sewage sudge in soil and plants. *Journal of Environmental Science and Health, Part A; Toxic/Hazardous Substances and Environmental Engineering,* 40(2), pp. 437-451.

de Souza, J., Alves, L., Varani, A. & Lemos, E., 2014. The Family Bradyrhizobiaceae. In: E. Rosenberg, DeLong, E.F; Lory, S; Stackebrandt, E &Thompson, F. eds. *The prokaryotes.* Berlin Heidelberg: Springer Berlin Heidelberg, pp. 35-154.

DEA, 2011. *Government Gazette No 34385 :National policy for the provision of basic refuse removal services for indigent households,* Pretoria: DEA.

DEA, 2013. *Government Gazette, No 37088: National environmental management: waste act 2008,* Pretoria: DEA.

DEA-2, 2011. *A user friendly guide to the national environmental management: waste act,* Pretoria: DEA.

Decrey, L. Udert, K, M; Tilley, E; Pecson, B,M & Kohn, T., 2011. Fate of the pathogen indicators phage X147 and Ascariss suum eggs during the production of struvite fertilizer from source-seperated urine. *Water Research,* Volume 45, pp. 4960-4972.

Department of Water and Sanitation 2, 2015. *Department of Water and Sanitation webpage.* [Online]
Available at: https://www.dwa.gov.za/about.aspx
[Accessed 10 August 2015].

Department of Water and Sanitation, 2015. *Inputs and data for the MDG 7, 2015 report.,* Pretoria: Department of Water and Sanitation.

Diwakar, B., Dutta, P., Lokesh, B. & Naidu, K., 2008. Bioavailability and metabolism of n-3 fatty acid rich garden cress seeds oil in albino rats. *Prostaglandins,Leukotrienes and Essential Fatty Acids,* Volume 78, pp. 123-130.

DMA, 2002. *Disaster Management Act no. 57 of 2002.* [Online]
Available at: http://www.info.gov.za/view/DownloadFileAction?id=68094
[Accessed 28 November 2015].

DMRDM, 2005. *The Disaster Management Regulation.* [Online]
Available at: http://www.gov.za/sites/www.gov.za/files/27991b.pdf
[Accessed 30 November 2015].

DMVR, 2010. *South African Government Gazette Notice R1215. In Government Gazette 33882,* Pretoria: Department of Cooperative Governance and Traditional Affairs.

Dooerra, B. & Lehmkul, N., 2008. *Methane digesters,* Florida: Echo Technical Note.

Drangert, J., 1998. Fighting the urine blindness to provide more sanitation options.. *Water South Africa,* 24(2).

Drangert, J. & Nawab, B., 2011. A cultural-spatial analysis of excreting, recirculation of human excreta and health: the case of North West Frontier province, Pakistan. *Health and Place,* Volume 17, pp. 57-66.

Drewes, J., Heberer, T. & Reddersen, K., 2002. Fate of pharmaceuticals during indirect potable water reuse.. *Water Science and Technology,* 46(3), pp. 73-80.

Duncker, L. C., Matsebe, G. & Moilwa, N., 2007. *The social/cultural acceptability of using human excreta (faeces and urine) for food production in rural settlements in South Africa,* Pretoria: Water Research Commission.

Dunker, L. & Matsebe, G., 2008. *Prejudices and attitudes toward reuse of nutrients from urine diversion toilets in SA.* Accra, 33rd WEDC International Conference.

Durban, 2011. *Disaster Management Workshop.* [Online]
Available at: http:/www.durban.gov.za/Resource Centre/Press Releases/Pages/Disaster Management-Workshop.aspx
[Accessed 2 12 2015].

Ecosan Club (ed), 2010. Use of urine. *Sustainable Sanitation Practice,* Volume 3, pp. 1-33.

EPA, 2001. *Method 1684; Total, Fixed, and Volatile Solidsin Water, Solids, and Biosolids,* Washington, DC: Water Resource Center.

EPWP, 2013. *Extended Public Works Programme.* [Online]
Available at: http://www.epwp.gov.za
[Accessed 26 December 2015].

Esrey, S., Anderson, I., Hillers, A. & Sawyer, R., 2001. *Closing the loop; ecological sanitation for food security.* New York: Publication of Water Resources No 18.

Ethekwini, 2006. *EThekwini Municipality Expanded Public Works Programme (EPWP) Policy Framework..* [Online]
Available at: http://www.durban.gov.za/Documents/CityServices/Engineering/2 Final EPWP Policy Framework june 07.pdf
[Accessed 30 November 2015].

Fayer, R., 1994. Effect of temperature on infectivity of Cryptosporidium parvum oocysts in water. *Applied and Environmental Microbiology,* Volume 60, pp. 2732-2735.

Feachem, R. G., Bradley, D. J., Garelick, H. & Mara, D. D., 1983. *Sanitation and disease-health aspects of excreta and wastewater management.* Washington: John Willey and Sons.

Foster, S. & Johnstone, K., 1989. The trigger mechanism of bacterial spore germination . In: I. Smith, Slepecky, R & Setlow, P, eds. *Regulation of Proharyotic Development.* s.l.:American Society for Microbiology, pp. 89-108.

Fox, S., 2012. Urbanisation as a global historical process: Theory and evidence from sub-Saharan Africa. *Population and Development Review,* 38(2), p. 285–310.

Franceys, R., Pickford, J. & Reed, R., 1992. *A guide to the development of on-site sanitation.* Geneva: WHO.

Gantzer, C. Gaspard, P; Galvez, L; Huyard, A; Dumouthier, N & Schwartzbrod, J., 2001. Monitoring of bacterial and parasitological contamination during various treatment of sludge. *Water Research,* Volume 35, pp. 3763–3770.

Gao, X. Z. Shen, T; Zheng, Y; Sun, X; Huang, S; Ren, Q; Zhang, X; Tian, Y; Luan, G., 2002. *Practical manure handbook.* Beijing: Chinese Agriculture Publishing House.

Garcia-Pena, E. Parameswaran, P; Kang, D.W; Canul-Chan, M & Krajmalnik-Brown, R., 2011. Anaerobic digestion and co-digestion process of vegetable and fruit residues:Process and microbial ecology. *Bioresource Technology,* Volume 102, pp. 9447-9457.

Garfía, M., Ferrer-Martíb, L., Velob, E. & Ferrera, E., 2012. Evaluating benefits of low-cost household digesters for rural Andean communities. *Renewable and Sustainable Energy Reviews,* Volume 16, pp. 575– 581.

Gauri, V., 2013. Redressing grievances and complaints regarding basic service delivery. *World Development,* Volume 21, pp. 109–119.

Gibbs, K., 1984. Privacy and the pit privy: a technology or a technique. *Waterlines,* 1(3), pp. 19-21.

Gibbs, R. A., Hu, C. J., Ho, G. E. & Unkovich, I., 1997. Regrowth of faecal coliforms and salmonellae in stored biosolids and soil amended with biosolids. *Water Science and Technology,* Volume 35, pp. 269–275.

Girisha, S. T. & Raju, N., 2008. Effect of sewage water on seed germination and vigour index of different varieties of groundnut (Arachis hypogaea L). *Journal of Environmental Biology,* Volume 29, pp. 937-939.

Godfrey, L., 2008. Facilitating the improved management of waste in South Africa through a national waste information system. *Waste Management,* Volume 28, pp. 1660–1671.

Goodfellow, M. & Maldonado, L., 2006. The Families Dietziaceae, Gordoniaceae, Nocardiaceae and Tsukamurellaceae. In: M. Dworkin, Falkow, S; Rosenburg, E; Schleifer, K; Stackebrandt, E l. eds. *The prokaryotes.* New York: Springer , pp. 843-888.

Grimason, A. Davison, K; Tembo, K. C; Jabu, G. C & Jackson, M. H., 2000. Problems associated with the use of pit latrines in Blantyre, Republic of Malawi. *The journal of the Royal Society for the Promotion of Health,* 3(120), pp. 175-182.

Grim, J., Malmros, P., Schnürer, A. & Nordberg, A., 2015. Comparison of pasteurization and integrated thermophilic sanitation at a full-scale biogas plant-heat demand and biogas production. *Energy,* Volume 79, pp. 419-427.

Gunnerson, C. G. & Stuckey, C. D., 1986. *Anaerobic Digestion - Principles and Practices for Biogas systems,* Washington DC: The World Bank & UNDP.

Gupta, M., Prasand, D., Khara, H. & Alcid, D., 2010. A rubber-degrading organism growing from a human body. *International Journal of Infectious Diseases,* 14(1), pp. 75–76.

Guzha, E., Nhapi, I. & Rockstrom, J., 2005. An assessment of the effect of human faeces and urine on maize production and water productivity. *Physics and Chemistry of the Earth,* Volume 30, pp. 840-845.

Hagedorn, C., 1980. *Potential health hazard associated with the disposal of sewage sludge on agricultural soils in western Oregon.* Corvallis: Water Research Institute, Oregon State University.

Hanchett, S., Akhter, S; Khan, M,M; Mezulianik, S & Blagbrough, V., 2003. Water, sanitation and hygeine in Bangladeshi slums: an evaluation of the WaterAid-Bangladesh urban programme. *Environment and Urbanization,* 2(2), pp. 43-56.

Harp, J., Fayer, R., Pesch, B. & Jackson, G., 1996. Effect of pasteurization on infectivity of Cryptosporidium parvum oocysts in water and milk.. *Applied and Environmental Microbiology,* Volume 19, pp. 2866-2868.

Hayman, M., Turner, B. & Carpintero, A., 2013. *Guidelines for national waste management strategies: moving from challenges to opportunities,* s.l.: United Nations Environment Program.

Heinss, U., Larmie, S. A. & Strauss, M., 1998. *Solids Separation and Pond Systems for the Treatment of Faecal Sludges in the Tropics. Lessons learnt and recommendations for preliminary design,* Accra: SANDEC Report No. 5/98. Second Edition. Swiss Federal Institute for Environmental Science ad Technology (EAWAG) and Water Research Institute (WRI).

Hoagland, D. R. & Arnon, D., 1950. Investigation of the possible in hydroponics growing grass pea (Lathymus sativum L.). *International Journal of Agriculture and Crop Science,* Volume 347, pp. 1-32.

Hogland, C. & Stenstrom, T., 1999. Survival of Cryptosporidium parvum oocysts in source seperated human urine. *Canadian Journal of Microbiology,* Volume 45, pp. 740-746.

Hojjat, S. S., 2014. Investiigation of the possible in hydroponics growing of grass pea (Lathymus sativum L.). *International Journal of Agriculture and Crop Science,* 7(15), pp. 1485-1487.

Hoossein, S., Whittington-Jones, K. & Tandlich, R., 2014. Sanitation policy and prevention of environmental contamination in South Africa. *Environmental Engineering and Management Journal,* 13(6), pp. 1335-1340.

Inam, A., Sahay, S. & Mohammad, F., 2011. Studies on potassium content in two root crops under nitrogen ferilization. *International Journal of Environmental Sceinces,* 2(2), pp. 1030-1038.

IOL News, 2011. *Cape loses open-toilet battle.* [Online]
Available at: http://www.iol.co.za/news/crime-courts/cape-loses-open toilet-battle-1.1062794#.UmkrCHBmD4Y
[Accessed 24 October 2014].

Jadhao, B., Kulwal, L. & Mahakal, K., 1999. Effect of nitrogen, phosphorus and potassium on growth and seed yield of radish. *Journal of Vegetation Science,* 26(1), pp. 95-96.

Januskaitiene, I., 2008. The fertilization impact on garden cress resistance to substrate acidity and heavy metal cadmium. *Sodininkyste IR Darzininkyste,* 27(4), pp. 213-220.

Jena, J., Kumar, R; Saifuddin, M; Dixit, A; Das, T, 2016. Anoxic–aerobic SBR system for nitrate, phosphate and COD removal from high-strength wastewater and diversity study of microbial communities. *Biochemical Engineering Journal,* Volume 105, pp. 80-89.

Jensen, P. K. Phuc, P, D; Knudsen, L, G; Dalsgaard, A; Konradsen, F., 2008. The use of human excreta in agriculture-a Vietnamese example. *International Journal of Hygeine and Environmental Health,* Volume 211, pp. 432-439.

Jilani, M., Burki, T. & Waseem, K., 2010. Effect of nirogen on growth and yield of radish. *Journal of Agricultural Resource,* 48(2), pp. 219-225.

Johannesburg, 2014. *Emergency Management Services: Disaster Management Volunteers.* [Online]
Available at: http:/www.joburg.org.za/index.php?option=com content&limitstart=7
[Accessed 2 December 2015].

Johansson, M., Jonsson, H; Hoglund, C; Richert Stintzing, A; L, Rodhe,C., 2001. *Urine separation—closing the nutrient cycle,* Stockholm: Stockholm Water Company.

Jonsson, H., Stintzing, A., Vinneras, B. & Salomon, E., 2004. *Guidelines on use of urine and faeces in crop production,* Sweden: EcoSanRes.

Kampfer, P., Wohlgemuth, S. & Scholz, H., 2014. The Family Brucellaceae. In: E. Rosenberg, DeLong, E.F; S, Lory; Stackebrandt, E; Thompson, F. eds. *The prokaryotes.* Berlin Heidelberg: Springer Berlin Heidelberg, pp. 155-178.

Karak, T. & Bhattacharyya, P., 2011. Human urine as a source of alternate natural fertilizer in agriculture: a flight of fancy or an achievable reality. *Resources, Conservation and Recycling,* Volume 55, pp. 400-408.

Kar, K. & Chambers, R., 2008. *Handbook on community-led total sanitation.* London: Insitute of Development Studies at the university of Sussex/Plan-UK.

Kawai, M; Kishi, M; Hamersly, M.R; Nagao, N; Hermana, J; & Toda, T.,2012. Biodegradability and methane productivity during anaerobic -co-digestion of refractory leachate. *International Biodeterioration and Biodegradation,* Volume 72, pp. 46-51.

Kenny, C., 2013. *http://www.cgdev.org.* [Online]
Available at: http://www.cgdev.org/sites/default/files/un-declaration-post-2015-development-agenda.pdf
[Accessed 19 February 2015].

Khanal, S., 2008. *Anaerobic biotechnology for bioenergy production: principles and applications..* Ames: Wiley-Blackwell.

Khanto, A. & Banjerdikij, P., 2013. Methane femantation of night soil and food waste. *Journal of Clean Energy Technologies,* 1(3), pp. 234-237.

Kilian, G., 2009. *Presentation to the portfolio committee: cooperative governance and traditional affairs on disaster management volunteer regulations. Meeting minutes of the Portfolio Committe Cooperative Governance and Traditional Affairs on Disaster Management Volunteer,* Cape Town: Parliament of South Africa.

Klingel, F., Montangero, A., Koné, D. & Strauss, M., 2002. *Fecal Sludge Management in Developing Countries.* Duebendorf: EAWAG and SANDEC.

Kommadal, O., Simmon, K., Karaca, D., Langelad, N & Wiker, H.G., 2012. Dual priming oligonucleotides for broad range amplification of the bacterial 16S rRNA gene directly from human clinical specimens. *Journal of Clinical Microbiology,* 50(4), pp. 1289 - 1294.

Kone, D. & Chowdhry, S., 2012. *Business analysis of feacal sludge management: emptying and transportation services in Africa and Asia..* Seattle: Bill & Melinda Gates Foundation.

Koné, D. & Strauss, M., 2004. *Low-costOptions for Treating Faecal Sludges (FS) in Developing Countries – Challenges and Performance..* Avignon, Paper presented to the 9th International IWA Specialist group conference on wetlands systems for water pollution control; and to the 6th International IWA Specialist Group Conference on Waste Stabilisation Ponds.

Kouawa, K., Wanko, A; Beck, C; Mose, R; Maiga, A.H., 2015. Feasibility study of faecal sludge treatment by constructed wetlands in Sahelian context: Experiments with Oryza longistaminata and sporobolus pyramidalis species in Ouagadougou. *Ecological Engineering,* Volume 84, pp. 390-397.

Krugman, S., Giles, J. P. & Hammond, J., 1970. Hepatitis virus: effect of heat on the infectivity and antigenicity of the MS-1 and MS-2 strains. *International Journal of Infectious Diseases,* Volume 122, pp. 432-436.

Kuai, L., Doulani, F. & Verstraete, W., 2000. Sludge treatment and reuse as a soil conditioner for smal rural communities. *Bioresource Technology,* Volume 73, pp. 213-219.

Kuever, J., 2014. The Family Desulfobacteraceae. In: E. Rosenberg, et al. eds. *The prokaryotes.* Berlin Heidelberg: Springer Berlin Heidelberg, pp. 45-73.

Kumari, I. & Patel, R., 2013. Effect of irrigation and nitrogen on yield of cress. *Crop Resource,* 46(1,2,3), pp. 231-233.

Lamond, E. & Kinyanjui, J., 2012. *OXFAM: Cholera outbreak guidelines: preparedness, prevention and control.* [Online]
Available at: http://www.unicef.org/cholera/Annexes/Supporting Resources/ Annex 6B/ OXFAM Cholera guidelines.pdf
[Accessed 16 November 2015].

Langergrabera, G. & Muelleggera, E., 2005. Ecological Sanitation—a way to solve global sanitation problems?. *Environment International,* Volume 31, p. 433– 444.

Lentner, C., Lentner, C. & Wink, A., 1981. *Units of Measurement, Body Fluids, Composition of the Body, Nutrition.Geigy Scientific tables.* Basel: Ciba-Geigy.

Leon, M. et al., 2014. Larsenia salina gen. nov., sp. nov., a new member of the family Halomonadaceae based on multilocus sequence analysis. *Systematic and Applied Microbiology,* 37(7), pp. 480–487.

Leslie, B., 2005. *Tsunami-related diseases.* [Online]
Available at: http://academic.evergreen.edu/g/grossmaz/LESLIEBJ/
[Accessed 05 May 2015].

Lettinga, G., Lens, P. & Zeeman, G., 2001. Environmental protection technologies for sustainable development.. In: P. Lens, G. Zeeman & G. Lettinga, eds. *Decentralized sanitation and reuse—concepts, systems and implementation..* London: IWA Publishing, pp. 3– 10.

Li, J., Lui, Q. & Sang, Y., 2012. International symposium on safety science and engineering in China, 2012 (ISSSE-2012): several issues about urbanisation and urban safety. *Procedia Engineering,* Volume 43, pp. 615-621.

Linos, A; Berekaa, M. M., Steinbuchel, A., Kim, K. K., Sproer, C., Kroppenstedt, R.M., 2002. Gordonia westfalica sp. nov., a novel rubber degrading actinomycete. *International Journal of Systematic and Evolutionary Microbiology,* Volume 52, pp. 1133-1139.

Lorenzo, J., Fontan, M.C.G; Gomez, M; Fonseca, S; Franco, I; Carballo, J., 2012. Study of the Micrococcaceae and Staphylococcaceae throughout the Manufacture of Dry-Cured Lacón (a Spanish Meat Product) Made without additives. *Journal of Food Research,* 1(1), pp. 200-211.

Lory, S., 2014. The Family Mycobacteriaceae. In: E. Rosenberg, DeLong, E.F; Lory, S; Stackebrandt, E; Thompson, F. eds. *The Prokaryotes.* Berlin Heidelberg: Springer Berlin Heidelberg, pp. 571-575.

Lüthi, C., Morel, A., Tilley, E. & Ulrich, L., 2011. *Community-Led Urban Environmental Sanitation : CLUES.* WSSCC, UN-HABITAT: Eawag(Sandec).

Luyt, C. D., Muller, W. J., Wilhelmi, B. S. & Tandlich, R., 2011. *Health implications of flood disaster management in South Africa.* Bucharest, Published in peer-reviewed proceedings of the 18th Annual Conference of the International Emergency Management of Society, pp.376-385.

Marcelis, L. & Hooijdonk, J., 1999. Effectt of salinity on growth, water use and nutrient use in Radish. *Plant and Soil,* Volume 215, pp. 57-64.

Margaret, W., 1970. *Veterinary Clinical Parasitology.* 4th ed. Ames: The Iowa State University Press.

Martin, S. & Griswold, W., 2009. Human Health Effects of Heavy Metals. *Environmental Science and Technology Briefs for Citizens,* Volume 15, pp. 1-6.

Maspolin, Y., Zhou, Y; Guo, C; Xiao, K; Ng, W.J., 2015. Comparison of single-stage and two-phase anaerobic sludge digestion systems-perfomance and microbial community dynamics. *Chemosphere,* Volume 140, pp. 54-62.

Mata, J.A., Martinez-Canovas, J., Quesada, E., Bejar, V., 2002. A detailed phenotypic characterization of the type strain of Halomonas species. *Systematic and Applied Microbiology,* Volume 25, pp. 360-375.

McCarty, P. L., 1982. *One hundred years of anaerobic treatment.* Amsterdam, Proceedings of the second international symposium on anaerobic digestion. Elsevier Biomedical.

Meiklejohn, C. & Cotzee, M., 2003. *Integrated Development Planning (in the best of Hologram 2001-203): issues and practices in South African Local Government.,* Cape town: Nolwazi.

Meinzinger, F., Moldenburg, M. & Otterpohl, R., 2009. No waste, but a resource: alternative approaches to urban sanitation in Ethopia. *Desalination,* Volume 248, pp. 322-329.

Mels, A., Castellano, D; Braadbaart, O; Veenstra, S; Dijkstra, I; Meulman, B; Singels, A; Wilsenach, J.A., 2009. Sanitation services for the informal settlements of Cape Town, South Africa. *Desalination,* Volume 248, pp. 330–337.

Miller, R. O., 1998. High temperature oxidation: dry ashing. In: Y. Kalra, ed. *Handbook of reference methods for plant analysis.* Suite: CRC Press, pp. 53-56.

Mininni, G. & Santori, M., 1987. Problems and perspectives of sludge utilization in Agriculture.. *Agriculture, Ecosystems and Environment,* Volume 18, pp. 291-311.

Mitscherlich, E. & Marth, E. H., 1984. *Microbial survival in the environment.* Berlin: Springer.

Mnkeni, P. & Austin, L. M., 2008. Fertilizer value of human manure from pilot urine diversion toilets. *Water SA,* 35(1), pp. 133-138.

Mohammed Ali, R., 2013. Preparation and characterization of protein isolate and biodiesel from garden cress. *European Journal of Chemistry,* 4(2), pp. 85-91.

Moller, V., 2001. Monitoring quality of life in cities: the Durban case. *Development Southern Africa,* Volume 18, pp. 217–238.

Moller, V. & Jackson, A., 1997. Perceptions of service delivery and happiness. *Development Southern Africa,* Volume 14, pp. 169–184.

Mondal, N., Datta, J. & Banerjee, A., 2013. Biochemical response of mungbean under the influence of reduced dose of chemical fertilizer and different time and method of application of biofertilizer. *Journal of Agricultural Technology ,* 9(3), pp. 643-658 .

Montgomery, M. & Elimelich, M., 2007. Water and sanitation in developing countries: including health in the equation.. *Environmental Sciecne and Technology,* 1(1), pp. 17-24.

Moodley, P., Archer, C., Hawkswoth, D. & Leibach, L., 2008. *Standard methods for the recovery and enumeration of helminth ova in waastewater, sludge, compost and urine diversion waste in South Africa,* Pretoria: Water Research Commission.

Moreno, J., Garcia, C. & Hernadez, T., 1998. Changes inorganic matter and enzymatic activity of an agricultural soil ammended with metal-contaminated sewage sludge compost. *Communications in Soil Science and Plant Analysis,* 29(15-16), pp. 2247-2262.

Mor, S., Kaur, K. & Khawal, R., 2013. Growth behavior studies of bread wheat plant exposed to municipal landfill leachates. *Journal of Environmental Biology,* Volume 34, pp. 1083-1087.

Müller, C., 2007. *Anaerobic Digestion of Biodegradable Solid Waste in Low and Middle-Income Countries (Eawag),* Dübendorf: Switzerland: Department of Water and Sanitation in Developing Countries (Sandec).

Murrilo, J. M., Cabrera, F., Lopez, R. I. & Mardin-Olmedo, P., 1995. Testing low-quality urban composts for agriculture: germination and seedling perfomance of plants. *Agriculture, Ecosystems and Environment,* Volume 54, pp. 127-135.

Murungi, C. & Pieter van Dijk, M., 2014. Emptying, Transportation and Disposal of feacal sludge in informal settlements of Kampala Uganda: The economics of sanitation. *Habitat International,* Volume 42, pp. 69-75.

Nathan, M. V. & Sun, Y., 2006. *Methods for plant analysis: a guide for conducting plant analysis in Missouri..* s.l.:University of Missouri Soil and Plant Testing Laboratory, Division of Plant Sciences, University of Missouri-Columbia.

National Sanitation Task Team, 1996. *National sanitation policy,* Pretoria: Department of water and environmental affairs.

NCOP, 2010. *Local Government Turnaround Strategy, Funding Model for Ward Commitee Members, Participation of Municipal Staff Members as Candidates for National, Provincial and Municipal Elections Draft; DMVDR,* Cape Town: National Council of Provinces of South Africa, Parliament of South Africa.

NDMETF, 2013. *Chapter 1; Introduction: South Africa's disaster risk management context. National Disaster Management Education and Training Framework-DRAFT 3.5,* Pretoria: National Disaster Mnanagement Centre.

NDMETF, 2013. *Chapter 13: Glossary. National Disaster Management Education and Training Framework-DRAFT 3.5,* Pretoria: National Disaster Management Centre.

NDMF, 2005. *https://www.capetown.gov.za/en/DRM/Documents/.* [Online]
Available at:
https://www.capetown.gov.za/en/DRM/Documents/SA_National_Disaster_Man_Framework_2005.pdf
[Accessed 8 January 2015].

Nembambula, P., 2014. Violent Service Delivery Protests in the Governance of Public Participation in a Democratic South Africa. *Mediterranean Journal of Social Sciences,* 5(9), pp.148-151

Nelson, M., Morrison, M. & Yu,Z., 2011. A meta-analysis of the microbial diversity observed in anaerobic digesters. *Bioresource Technology,* Volume 102, pp. 3730–3739.

Nguyen, V., 2011. Small-scale anaerobic digesters in Vietnam - development and challenges. *Journal of Vietnamese Environment,* 1(1), pp. 12-18.

Norris, J.A. 2000. *Sludge build up in septic tanks, biological digesters and pit latrines in South Africa. Research Report No. 544/1/100*, Pretoria: Water Research Commission

Nikiema, J., Cofie, O., Impraim, R. & Adamtey, N., 2013. Processing of fecal sludge to fertilizer pellets using a low-cost technology in Ghana. *Environment and Pollution,* Volume 24, pp. 70-87.

Nwaneri, C. F., 2009. *Physico-chemical characterisitics and biodegradability off the contents of ventilated improved pit latrines in eThekwini municipality; Masters thesis,* s.l.: Univeristy of KwaZulu Natal.

Nzimakwe, T., 2009. Water and sanitation provision through public-private partnership: challenges for municipalities. *Africanus,* 39(1), pp. 52-65.

O'Brien, G., O'Keefe, P., Rose, J. & Wisner, B., 2006. Climatechange and disaster management. *Disaster,* 30(1), pp. 64-80.

Ongerth, J. E., Johnson, R. L; Macdonald, S. C; Frost, F & Stibbs, H. H., 1989. Backcountry water treatment to prevent giardiasis. *American Journal of Public Health,* Volume 79, pp. 1633-1638.

Osorio, F. & Torres, J., 2009. Biogas purification from anaerobic digestion in a wastewater treatment palnt for biofuel production. *Renewable Energy,* Volume 34, pp. 2164-2171.

Ozunu, A., Senzaconi, F; Botezan, C; Stefanescu, L; Nour, E & Balcu, C., 2011. Investigation on natural hazards which trigger technological disasters in Romania. *Natural Hazards and Earth Systems Sceicne,* 11(5), pp. 1319-1325.

Palmquist, H. & Jönsson, H., 2004. *Urine, faeces, greywater, greywater and biodegradable solid waste as potential fertilizers. In: Ecosan – closing the loop. Proceedings of the 2nd International Symposium on Ecological Sanitation.* Lübeck, Incorporating the 1st IWA Specialist Group Conference on Sustainable Sanitation.

Pare, T., Gregorich, E. & Dinel, H., 1997. Effects of stockpiled and composted manure on germination and initial growth of cress (Lepedium Sativum). *Biological Agriculture and Horticulture: An International Journal for Sustainable Production Systems,* 14(1), pp. 1-11.

Pervez, M., Ayub, C.M; Saleem, B.A; Virk, N.A; Mahmood, N., 2004. Effect of nitrogen levels and spacing on growth and yield of Radish. *International Journal of Agriculture and Biology,* 6(3), pp. 504-506.

PMG, 2005. *Thaba Chweu Municipality Intervention: Briefing; Moqhaka, Phumelela & Elundini Intervention Committee Reports.* [Online]
Available at: https://pmg.org.za/committee-meeting/4909/
[Accessed 20 December 2015].

PMG, 2008. *Polokwane Local Municipality interventions: Municipality, National Treasury & Provincial Government reports.* [Online]
Available at: https://pmg.org.za/committee-meeting/9465/
[Accessed 5 January 2016].

PMG, 2015. *Parliamentary Monitoring Group;SouthAfrica.* [Online]
Available at: https://pmg.org.za/committee-meeting/21414/
[Accessed 23 December 2015].

Rajesabapath, R. Mohades, C; Yoon, J; Daslager, S.G; Lui, Q; Khieu, T; Son, C.K; Li, W; Colaco, A., 2015. Nioella nitratireducens gen. nov., sp. nov., a novel member of the family Rhodobacteraceae isolated from Azorean Island. *Antonie van Leeuwenhoek,* Volume 107, pp. 589–595.

Rathood, P., Patel, J. & Jhala, P., 2011. Potential of gamma irradiated sewage sludge as a fertilizer in radish; evaluating heavy metal accumulation in sandy loam soil. *Communications in Soil Sceince and Plant Analysis,* 42(3), pp. 263-282.

Rebaudet, S; Sudre, B; Faucher, B & Piarroux, R., 2013. Environmental Determinants of Cholera Outbreaks in Inland Africa: A Systematic Review of Main Transmission Foci and Propagation Routes. *Journal of Infectious Diseases,* 208(1), pp. S46-S54

Red Cross, S., 2015. *Training courses.* [Online]
Available at: http://www.redcross.org.za/index.php/training/training
[Accessed 8 January 2015].

Reinecke, R., 1983. *Veterinary helminthology.* 1st ed. Pretoria: Butterworths.

Rigueiro, L., Lema, J. & Carballa, M., 2015. Key microbial communities steering the functioning of anaerobic digesters during hydraulic and organic overloading shocks. *Bioresource Technology,* Volume 197, pp. 208–216.

Romano, R., Zhang, R., Teter, S. & McGarvey, J., 2009. The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. *Bioresource Technology,* 20(100), pp. 4564–4571.

Rong, W., Aipig, T. & Ashraf, M., 2015. The effects of applying sewage sludge into Jiangxi red soil on the growth of vegetables and the migration and enrichment of Cu and Zn. *Saudi Journal of Biological Sciences,* p. http://dx.doi.org/10.1016/j.sjbs.2015.10.028.

Rostika-Rick, R. & Manning, W., 1993. Radish: A model for studying plant resonses to air pollutants and other environmental stresses. *Environmental Pollution,* Volume 82, pp. 107-138.

RSA, 1996. *Constitution of South Africa.* [Online]
Available at: http://www.info.gov.za/documents/constitution/1996/a108-96.pdf
[Accessed 30 November 2015].

SA Presidency, 2014. *President Jacob Zuma announces members of the National Executive.* [Online]
Available at: http://www.thepresidency.gov.za/pebble.asp?relid=17453
[Accessed 23 November 2015].

Sadia, A., Mahasen, M; Shahrin, S; Roni, M.Z.K; Jamal, A.F.M., 2013. Phosphorus levels on growth and yield of Turnip. *Bangladesh Research Publications Journal,* 8(1), pp. 29-33.

Sagar, A. D. & Kartha, S., 2007. Bioenergy and Sustainable Development. *Annual Review of Environment and Resources,* 32(1), pp. 131-167.

Sahlstrom, L., 2003. A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology,* Volume 87, pp. 161–166.

SAHRC, 2010. *Report on the submission of Gareth Van Onselen (complainant) versus the Moqhaka Local Municipality. File Ref No: FS/2010/0231,* Pretoria: South African Human Rights Commission.

SAHRC, 2014. http://www.sahrc.org.za/home/21/files/. Online]
Available at: http://www.sahrc.org.za/home/21/files/FINAL%204th%20Proof%204%20March%20-%20Water%20%20Sanitation%20low%20res%20%282%29.pdf
[Accessed 3April 2016].

Saia, F., Souza, T.S.O; R.T.D, Duarte; Pozzi, E; Fonseca, D; Foresti, E., 2016. Microbial community in a pilot-scale bioreactor promoting anaerobic digestion and sulfur-driven denitrification for domestic sewage treatment. *Bioprocess and Biosystems Engineering,* Volume 39, pp. 341-352.

Saitoh, T. & El-Ghetany, H. H., 1999. Solar water-sterilization system with thermally controlled flow. *Applied Energy,* Volume 64, pp. 387-399.

Schönning, C., Leeming, R. & Stenström, T., 2002. Faecal contamination of source separated human urine based on the content of faecal sterols.. *Water Ressource,* Volume 36, pp. 1965–1972.

Schouten, M. A. & Mathenge, R. W., 2010. Communal sanitation alternatives for slums: a case study of Kiberia, Kenya. *Physics and Chemistry of the Earth,* Volume 35, pp. 815-822.

Schouw, N., Danteravanichb, S., Mosbaeka, H. & Tjella, J., 2002. Composition of human excreta a case study from Southern Thailand. *The Science of the Total Environment,* Volume 286, pp. 155-166.

Semiyaga, S; Okure, M.A.E; Niwagaba, C.B; Katukiza, A.Y & Kanssime, F, 2015. Decentralized options for faecal sludge management in urban slums of sub-Saharan Africa: A review of technologies, practices and uses. *Resources, Conservation and Recycling,* Volume 104, pp. 109-119.

Shin, K. S. & Kang, H., 2003. Electron beam pretreatment of sewage sludge before anaerobic digestion. *Applied Biochemistry and Biotechnology,* Volume 109, pp. 227–239.

Singh, R., Gautam, N., Mishra, A. & Gupta, R., 2011. Heavy metals and living systems: An overview. *Indian Journal of Pharmacology,* 43(3), pp. 246–253.

Slobodkin, A., 2014. The Family Peptostreptococcaceae. In: E. Rosenberg, DeLong, E.F; Lory, S; E, Stackebrandt; Thompson, F. eds. *The prokaryotes.* Berlin Heidelberg: Springer Berlin Heidelberg, pp. 291-302.

Snyman, H., Herselman, J. & Kasselman, G., 2004. *Metal conntent of South African Sewage Sludges.* Cape town, Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference.

Sobieraj, M. & Boone, D., 2006. Syntrophomonadaceae. In: M. Dworkin, Falkow, S; Rosenberg, E; Schleifer, K & Stackerbrandt, E. eds. *The prokaryotes.* New York: Springer USA, pp. 1041-1049.

Souri, E., Amin, G., Farsam, H. & Andaji, S., 2004. The antioxidant activity of some commonly used vegetables in Iranian diet. *Fitoterapia,* 75(6), pp. 585-588.

Sousa, A. & Figueiredo, C., 2015. Sewage sludge biochar effects on soil fertility and growth of radish. *Biological Agricuture and Horticulture,* Volume DOI: 10.1080/01448765.2015.1093545, pp. 1-12.

South African Human Rights Commission, 2012. *South African Human Rights Commission Webpage.* [Online]
Available at:
http://www.sahrc.org.za/home/21/files/Quality%20of%20sanitation%20Main%20report%20April%202012%20final%20Aug%202012.pdf
[Accessed 10 October 2015].

Srinavasan, L., 1990. *Tools for community participation. A manual for training trainers in participatory techniques.* New York: United Nations Development Programme.

Statistics South Africa, 2015. *Statistics South Africa.* [Online]
Available at:
http://www.statssa.gov.za/MDG/Executive_Summary_MDG_Country_Report_Final_30Sep2015.pdf
[Accessed 15 October 2015].

StatSA, 2013. *Mid-year population estimates 2013. Statistical Release P0302.* [Online]
Available at: http://beta2.statssa.gov.za/publications/P0302/P03022013.pdf
[Accessed 16 November 2015].

Still, D. A., 2002. *After the Pit Latrine is Full... What Then? Effective Options for Pit Latrine Management.* Durban, WISA Biennial Conference, May 2002.

Still, D. & Foxon, K., 2012. *How fast do pit latrines fill up? a scientific understanding of sludge build up and accumulation in pit latrines,* Pretoria: Water Research Commision.

Strande, L., 2014. The global situation. In: L. Strande, M. Ronteltap & D. Brdjanovic, eds. *Faecal Sludge Management: Systems approach for Implementation and Operation.* London: IWA Publishing, pp. 1-14.

Strauch, D. & Berg, T., 1980. Microbioligcal studies on the disinfection of sludge. 2 studies on pasteurisation plants. *GWF Water /Sewage (GWF-Wasser/Abwasser),* Volume 121, pp. 184–187.

Strauss, M., 1998. *Faecal Sludge Treatment. Proceedings of Workshop on Sustainable Sanitation.* Cali, Univalle.

Strauss, M., Heinss, U. & Montangero, A., 2000. *On-Site Sanitation: When the Pits are Full – Planning for Resource Protection in Faecal Sludge Management.* Bad Elster, IWA Publishing House and WHO Water Series.

SuSan, 2012. *Compilation of 25 case studies on sustainable sanitation projects from Africa,* Eschborn: Sustainable Sanitation Alliance.

Tandlich, R., Chirenda, T. & Srinivas, C., 2013. Gender aspects of disaster management in South Africa. *Journal of Disaster Risk Studies,* 5(4), pp. 84.

Tang, Y., Shigematsu, T., Morimura, S. & Kida, K., 2005. Microbial community analysis of mesophilic anaerobic protein degradation process using bovine serum albumin (BSA)-fed continuous cultivation. *Journal of Bioscience and Bioengineering ,* Volume 99, pp. 150–164.

Tchobanoglous, G., Burton, F. & Stensel, H., 2002. *Wastewater Engineering: Treatment and Reuse.* 4th ed. New York: McGraw-Hill Science/Engineering.

Tempelhoff, J., 2009. Civil society and sanitation hydropolitics: A case study of South Africa's Vaal River Barrage. *Physics and Chemistry of the Earth,* Volume 34, pp. 164–175.

Tervahauta, I., Rani, S; Leal, L.H; Buisman, C.J.N & Zeeman, G., 2014. Blackwater sludge reuse in agriculture: are heavy metals a problem?. *Journal of Hazardous Materials,* Volume 274, pp. 229-236.

Tettenborn, F., Behrendt, J. & Otterpohl, R., 2007. *Resource recovery and removal of pharmaceutical residues. Treatment of separate collected urine within the EU-funded SCST-project,* Hamburg: Institute of Wastewater Management and Water Protection, Hamburg University of Technology.

The National Treasury, 2015. *http://mfma.treasury.gov.za/Legislation/.* [Online]
Available at: http://mfma.treasury.gov.za/MFMA/Legislation/Local%20Government%20-%20Municipal%20Systems%20Act/Local%20Government%20-%20Municipal%20Systems%20Act,%20No.%2032%20of%202000.pdf
[Accessed 28 June 2015].

Thomas, T., Gilbert, J. & Meyer, F., 2012. Metagenomics-a giude from sampling to data analysis. *Microbial Informatics and Experimentation,* 2(3), pp. 1-12.

Tietjen, C., 1975. *From biodung to biogas-a historical review of the European experience.* Ann Arbor, Proceedings of the 1975 Cornell Agricultural Waste Management Conference.

Tilley, E., Lüthi, C; Morel, A; Zurbrügg, C; Schertenleib, R., 2014. *Compendium of Sanitation Systems and Technologies.* 2nd ed. Dübendorf: WA; EAWAG.

Tomlinson, M. R., 2011. Managing the risk in housing delivery: Local government in South Africa. *Habitat International,* Volume 35, pp. 419-425.

UN, 2011. *World Urbanization Prospects. The 2011 Revision: Department of Economic and Social Affairs, Population Division.* New York: United Nations.

UN, 2012. *World Urbanization Prospects. The 2012 Revision: Department of Economic and Social Affairs, Population Division.* New York: United Nations.

UN, 2014. *The mellinium development goals report 2014,* New York: United Nations.

UNW, 2009. *African Centre for Disaster Studies.* [Online]
Available at: http://acds.co.za/index.php?page=short-courses
[Accessed 18 January 2016].

Valladão, A., Freire, D. & Cammarota, M., 2007. Enzymatic pre-hydrolysis applied to the anaerobic treatment of effluents from poultry slaughterhouses.. *International Biodeterioration & Biodegradation,* 4(60), pp. 219–225.

Vandermoortele, J., 2012. *http://www.un.org.* [Online]
Available at: http://www.un.org/millenniumgoals/pdf/jan_vandemoortele_Aug.pdf
[Accessed 19 February 2015].

Vinnerås, B., 2002. *Possibilities for sustainable nutrient recycling by faecal separation combined with urine diversion.* Uppsala: Agraria 353, Acta Universitatis Agriculturae Sueciae, Swedish University of Agricultural Sciences.

Vinnerås, B., Clemens, J. & Winker, M., 2008. *Non-metallic contaminants in domestic waste, wastewater and manures: constraints to agricultural use.* United Kingdom, pp.1-31, The International Fertiliser Society, Proceedings.

Vinnerås, B., Palmquist, H; Balmér, P; Weglin, J; Jensen, A; Andersson, Å; Jönsson, H., 2006. The characteristics of household wastewater and biodegradable waste - a proposal for new Swedish norms.. *Urban Water,* Volume 3, pp. 3-11.

Wagner, A.O., Hohlbrugger, P., Lins, P., Illmer, P. 2012. Effect of different nitrogen sources on the biogas production- a lab scale investigation. *Microbiological Research*, Volume 167, pp. 630-636.

Wang, M., 1997. Land application of sewage sludge in China. *The Science of the Total Environment,* Volume 197, pp. 149-160.

Ward, A. J. & Hobbs, P. J., 2008. Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology,* 99(17), pp. 7928-7940.

Ward, R., Krugman, S; Giles, J.P; Jacobs, A. M; Bodansky, O., 1958. Infectious hepatitis: studies of its natural history and prevention.. *The New England Journal of Medicine,* Volume 258, pp. 407-416.

Ward, R. L., Yeager, J. G. & Ashley, C. S., 1981. Response of bacteria in wastewater sludge to moisture loss by evaporation and effect of moisture content on bacterial inactivation by ionizing radiation.. *Applied Environmental Microbiology,* Volume 41, pp. 1123–1127.

Water Services Department of Cape Town, 2006. *Servicing Informal Settlements (SIS): Draft Situation Report on the Provision of Services,* Cape Town: Water Services Department of Cape Town.

Westaway, M. S., 2006. A longitudinal investigation of satisfaction with personal and environmental quality of life in an informal South African housing settlement, Doornkop, Soweto. *Habitat International,* Volume 30, p. 175–189.

Whittington-Jones, K., Tandlich, R; Zuma, B, M; Hoossein, S; Villet, M. H., 2011. Perfomance of the pilot-scale mulch tower system in treatment of greywater from a low-costhousing development in the Buffalo City, South Africa. *International Water Technology Journal,* 1(2), p. 7.

WHO/UNICEF, 2013. *progress on sanitation and drinking water 2013 update,* Geneva: WHO Press.

WHO, 2006. *Guidelines for the safe use of wastewater,excreta and greywater,* Geneva: WHO Press.

Wiley, B. & Westerberg, S. C., 1969. Survival of human pathogens in composted sewage. *Journal of Applied Microbiology,* Volume 18, pp. 994-1001.

Wilmouth, R., Truyens, C; Buckley, C; Turnberg, W; Daniell, W., 2013. *Menstrual hygiene management in communal sanitation facilities: recommendations to eThekwini municipality.* Nakuru, 36th Water, Engineering and Development Centre International Conference.

Yadav, K., Tare, V. & Ahammed, M., 2012. Integrated composting-vermicomposting process for stabilization of feacal slurry. *Ecological Engineering,* Volume 47, pp. 24-29.

Yang, J. & Speece, R., 1986. The effects of chloroform toxicity on methane fermentation. *Water Resource,* Volume 20, pp. 1273–1279.

Yeager, J. G. & Ward, R. L., 1981. Effects of moisture content on long-term survival and regrowth of bacteria in wastewater sludge. *Applied and Environmental Microbiology,* Volume 41, p. 1117–1122.

Zhang, H., 2006. *The permeability characteristics of siliconce rubber.* Dallas, Global Advances in Materials and Process Engineering, proceedings, Coatings and Sealants.

Ziemba, C. & Peccia, J., 2011. Net energy production associated with pathogrn inactivation during mesophilic and thermophilic anaerobic digestion of sewage sludge. *Water Research,* Volume 45, pp. 4758-4768.

Zucconi, F., Monico, A., Forte, M. & De Bartold, M., 1985. Phytotoxins during stabilization of organic matter . In: J. K. Gasser, ed. *Composting of Agricultural and Other Wastes.* London: Elsevier Applied Science, pp. 73-86.

Zuma, B., Luyt, C., Chirenda, T. & Tandlich, R., 2012. *Flood disaster management in South Afica: legislative framework and current challenges..* Konu, Published in the peer-reviewed proceedings from the 2012 International Conference on Applied Life Sciences, pp. 127-132.

Zuokaite, E. & Scupakas, D., 2007. Utilization of sewage sludge from acid casein production for soil fertilization. *Journal of Environmental Engineering and Landscape Management,* 15(3), pp. 166-172.