MAKERERE UNIVERSITY

ENHANCING THE PERFORMANCE AND LIFE SPAN OF PIT LATRINES: PROCESSES AND IMPLICATIONS

BY

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DECLARATION

This study is original and has not been submitted for any other degree award to any other University before.

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DEDICATION

To family; past, present and future. Great men and women whose decisions in the past defined who I am today. Currently whose love, support and guidance have helped me make this possible and to those yet to come, may this motivate them to attain their goals.

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LIST OF ACRONYMS AND ABBREVIATIONS

ACH	Air Volume Changes
ANOVA	Analysis of Variance
APHA	American Public Health Association
AWW	American Water Works Association
BHC	Benzene Hexachloride
CCA	Canonical Correspondence Analysis
COD	Chemical Oxygen Demand
DDT	Dichlorodiphenyltrichloroethane
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
DO	Dissolved Oxygen
IMOs	Indigenous Microorganisms
JMP	Joint Monitoring Programme
KSMP	Kampala Sanitation Master Plan
KVIP	Kusami Ventilated Improved Pit
MOH	Ministry of Health
MUARIK	Makerere University Agricultural Research Institute, Kabanyolo
NCBI	National Centre for Biotechnology Institute
ODB	Orthodichlorobenzene
ORP	Oxygen-Reduction Potential
PCR	Polymerase Chain Reaction
PDB	Paradichlorobenzene
PRISMAS	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PWD	People with Disabilities
REC	Revised Earth Closet
RNA	Ribonucleic acid
ROEC	Reed Odourless Earth Closet
SSA	Sub-Saharan Africa
TS	Total Solids
TVS	Total Volatile Solids
UBOS	Uganda Bureau of Statistics
UNICEF	United Nations Children's Education Fund
uPVC	unPlasticized Polyvinyl Chloride
VIDP	Ventilated Improved Double Pit
VIP	Ventilated Improved Pit

WEDC	Water, Engineering and Development Centre
WHO	World Health Organisation
WRC	Water Research Commission
WSP	Water and Sanitation Program of the World Bank

ABSTRACT

Pit latrines dominate in the management of human excreta for more than half of the urban population in Sub-Saharan Africa (SSA), particularly among the low-income earners. They are adopted mainly because of their simplicity in construction, low-cost, ease in use and maintenance and are most likely to remain the technology of choice for the poor people. However, the performance of pit latrines in terms of filling, smell and insect nuisances is unsatisfactory. This has subsequently led to their abandonment and subsequent use of inappropriate methods, resulting in a high environmental and public health risk. Hence the aim of this thesis was to enhance the performance of pit latrines, so as to prolong their useful life. Studies were conducted in different slums of Kampala-Uganda, which house most of the urban poor population.

A comprehensive literature review of usage, filling, insects and odour nuisances of pit latrines in Sub-Saharan Africa was carried out. This was followed by field studies to assess the status (design, construction, operation and maintenance) of pit latrine structures, and their performance (level of pit content, smell and insect nuisances). Using multi-variate analysis of data obtained on the status of the pit latrine structures and their performance, the predictors to their performance were established. In addition, an assessment of the ambient and internal environmental conditions of pit latrines that could influence their functionality in a typical low-income urban setting was undertaken. Further, laboratory (fingerprinting for bacterial and fungal species and degradation experiments) and field studies were conducted to evaluate the potential of using indigenous microorganisms (IMOs) as a bio-stimulant to enhance pit latrine performance.

Results showed that pit latrines in the studied slums of Kampala were mainly simple/ traditional (77%), built out of brick and plastered (77%), with timber doors (89%) and corrugated iron roofing sheets (91%). In addition it was noted that there were differences and shortfalls in their construction, and usage, while their performance was found to be inadequate. The level in pit content was predicted by rain or storm water entry (β = 34.6), terrain (β = 5.3), and cleaning before or after use (β = 5.0). Smell was predicted by cleanliness (β = 97.6), stance length (β = 1.0), superstructure material (β = 0.01) and whether the latrine was private or public (β = 0.01) while presence of flies was best explained by the superstructure material (β = 70.6). Additionally, the assessment of the environmental conditions found low values of wind speed (zero to 1.8 ms^{-1}). The environment in majority of the pits (95% of pit latrines) could mainly be described as

anoxic (oxygen reduction potential (ORP) < + 50mV) with smells and flies in acid forming ORP range. A significant association (Gamma, G=0.797, *p*= 0.014) was established between the ORP and smell of only clean latrines. The IMOs in this study were found to be dominated by *Stenotrophomonas maltophilia, Bacillus sp, Chryseobacterium ureilyticum* and a number of uncultured bacterial colons. The fungal species included *Saccharomyces cerevisiae, Galactomyces geotrichum* and *Geotrichum candidum*. The results of laboratory degradation experiments and field investigations showed that while IMOs had no significant ($p>0.05$) effect on mass reduction, they significantly ($p < 0.05$), reduced ammonia concentration, smell and insect nuisance which resulted in increased user-satisfaction.

These results in this study suggest that the status of pit latrine structures and the environmental condition outside and in the pit impact on their performance. It was noted that ventilation of pit latrines within urban slums was not sufficient to exhaust odours from the superstructures. Latrine cleanliness, adequate superstructures, minimising water entry by improving the drop-hole size to minimise soiling and a change in the biological processes in the pit that could be effected by application of IMOs could address the performance of pit latrines, ultimately improving their usage in urban slums. However, this necessitates determining, developing and disseminating detailed local standards (dimensions, construction materials and number of users), as well as emphasis on supervision during construction and regular maintenance of the facilities. Sensitisation of users to minimise soiling and ensure clean latrines is also important. Lastly, additional research on IMOs application and their ecology in the pit and the enhancement of ventilated improved pit latrine technology could provide further solutions to improving the performance of pit latrines within urban slums.

CHAPTER ONE

1 Introduction

1.1 Background

Adequate sanitation involves the provision of facilities and services for the safe management of human excreta, solid waste and grey water, thereby protecting the environment and human health (Feachem et al., 1983; UNICEF & WHO, 2008). This in turn results in socio-economic development and poverty eradication (Van Minh & Nguyen-Viet, 2011; WHO & UN-WATER, 2012). Currently, access to improved sanitation is a challenge in many developing countries, especially among the urban poor population, whose solution to housing are the slums (Struyk & Giddings, 2009; UN-HABITAT, 2009). Urban slums (Figure 1.1) are heavily populated and characterised by substandard and unplanned infrastructure, inadequate basic services (i.e. water, sanitation and health), lack of secure tenure and poverty (UN-HABITAT, 2003; Isunju et al., 2011).

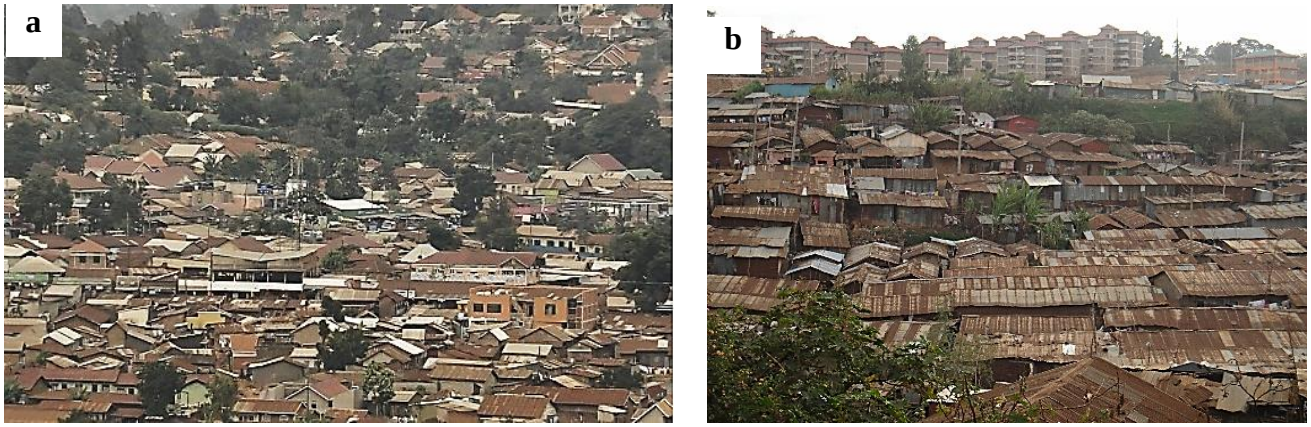

Figure 1.1 View of an urban slum (a) Kikoni slum in Kampala, Uganda (b) Kibera slum in Nairobi, Kenya (source: taken by A. Nakagiri, 2013)

Human excreta disposal in most urban slums in developing countries is met predominantly by pit latrines (Kariuki et al., 2003; Thye et al., 2011; Katukiza et al., 2012). In Sub-Saharan Africa alone, over 52.7 % of the urban population uses some form of pit latrine. Pit latrines in use within urban slums range from unimproved pit latrines that do not have a concrete slab, to improved designs like simple improved pit latrines, ventilated improved pit latrines (VIP), San Plat and a water seal / pour flush pit latrine (Buckley et al., 2008; Appiah-Effah et al., 2014; Okurut et al., 2015). Pit latrines have been mainly adopted and are used because of their simplicity in construction, low-cost and ease in use and maintenance (Franceys et al., 1992;

Pickford, 2006). They are thus most likely to remain the technology of choice for the poor people habiting in the urban slums.

In the pit latrine technology, human excreta and anal cleansing material are safely deposited in a hole dug in the ground, in a way that reduces contamination of soil, ground and surface water, minimises contact with insects or animals (Wagner & Lanoix, 1958; WHO, 1987), thereby minimising the inherent public and environmental health hazards. For their sustainability within urban area, pit latrines form the storage component, in a systems approach of managing human excreta, ahead of emptying for treatment, and safe disposal or end use (Tilley et al., 2014). If properly constructed, operated and maintained, pit latrines provide the same health benefits as the conventional sewerage system but at a low cost (Franceys et al., 1992; Black, 1998; Fang, 1999).

1.2 Problem Statement

The urban poor of Kampala City like many cities in developing countries reside in slums, where the sanitation situation is unsatisfactory, despite the wide spread use of pit latrines. Pit latrines are reportedly poorly built, heavily utilised (Figure 1.2), badly maintained, malodourous and a source of flies (KSMP, 2004; Günther et al., 2011). They thus do not meet the criteria of hygiene, safety and sustainability of sanitation systems (Jenkins et al., 2014).

Figure 1.2 Pit latrines in urban slums (a) Over flowing pit latrine in Kibera, Nairobi (b) Collapsing pit latrine in Nakulabye, Kampala, (c) Children shun pit latrines for open defecation in Bwaise, Kampala (source: taken by A. Nakagiri, 2013)

The state of poorly functioning pit latrines greatly impacts on their usage and the livelihood of the slum dwellers. For example, Tumwebaze et al. (2012) found out that smell, dirty and full latrines were attributes to user dissatisfaction. Later, Kwiringira et al. (2014) cited filthy latrines and high filling rates as barriers of latrine use and motivation for open defecation. Foul

odours from pit latrines have been reported to be a cause of a nuisance and disturbance of populations who come in contact with them, and are often associated with non-healthy, unhygienic, and dirty conditions (Rheinländer et al., 2013).

Further, the effect of inadequate sanitation facilities is estimated in the number of sanitation related diseases. Surveys and spatial analysis have shown that children in households with inadequate sanitation facilities have a higher prevalence of enteric infections than those without toilets (Berendes et al., 2017). In addition, a positive correlation was reported between malfunctioning pit latrines and children's sickness (Okurut et al., 2015). Moreover, over 6000 children die yearly due to diseases related to inadequate sanitation (Rosenquist, 2005). There is thus need improve the state of pit latrines while addressing the sanitation situation within urban slums.

1.3 Justification of the study

Access to adequate sanitation facilities leads to their usage, resulting in proper human excreta disposal. This in turn minimises the spread of faecal transmitted diseases and infections. However, the current state of pit latrines within urban slums does not ensure appropriate human excreta disposal. Studies on improving pit latrines within slums have mainly focused on their cleanliness (Tumwebaze et al., 2014; Kwiringira et al., 2014b), socio-economic issues (Isunju et al., 2011; Isunju et al., 2013; Murungi & van Dijk, 2014), pit emptying (Thye et al., 2011; Still & Foxon, 2012) and impacts on ground water pollution (Dzwairo et al., 2006; Nyenje et al., 2013). However, studies of factors causing poor performance (in terms of filling, smell and insect nuisances) of pit latrines within slum settings are scanty. These studies are vital in providing the information necessary to guide future innovations to the pit latrine technology, plus developing strategies and effective policies for improving their functioning and thus the sanitation situation especially within urban slum.

1.4 Research objectives

The overall objective of this research was to enhance the performance of the pit latrine, so as to prolong its useful life, thereby, improving the sanitation situation of communities living in urban slums. The performance issues that were addressed in this study were filling, smell and insect nuisances.

The specific objectives were to:-

i) assess the design, operation and performance of pit latrines in urban slums and processes therein.

ii) determine the key factors affecting the performance of pit latrines.

iii) determine the efficacy of application of indigenous microorganisms (IMOs) to human excreta decomposition and pit latrine use.

iv) assess the application of IMOs as a bio-solution to improve the performance of pit latrines.

1.5 Study location

This study were undertaken in different slums located in the five divisions of Kampala Capital City Authority (KCCA). These slums were Bwaise II (32º 33′ 37.2′′E, 0º 21′ 12.6′′N) in Kawempe Division, Kasubi (32º 33′ 17.8′′E, 0º 19′ 57.9′′N) and Nakulabye (32º 33′ 52′′E, 0º 19′ 42.1′′N) in Rubaga Division; Naguru-Godown(32º 36′ 14.8′′E, 0º 20′ 14.3′′N) and Kinawataka (32º 38′ 3.8′′E, 0º 20′ 3.7′′N) in Nakawa Division; Kifumbira (32º 33′ 5′′E, 0º 21′ 3′′N) in Kawempe Division; Kisenyi (32º 34′ 21.4′′E, 0º 18′ 32.2′′N) in Central Division and Namuwongo (32º 37′ 11.0′′E, 0º 18′ 10.5′′N) and Kibuye (32º 34′ 46.9′′E, 0º 17′ 38.5′′N) in Makindye Division (Fig. 1.3).

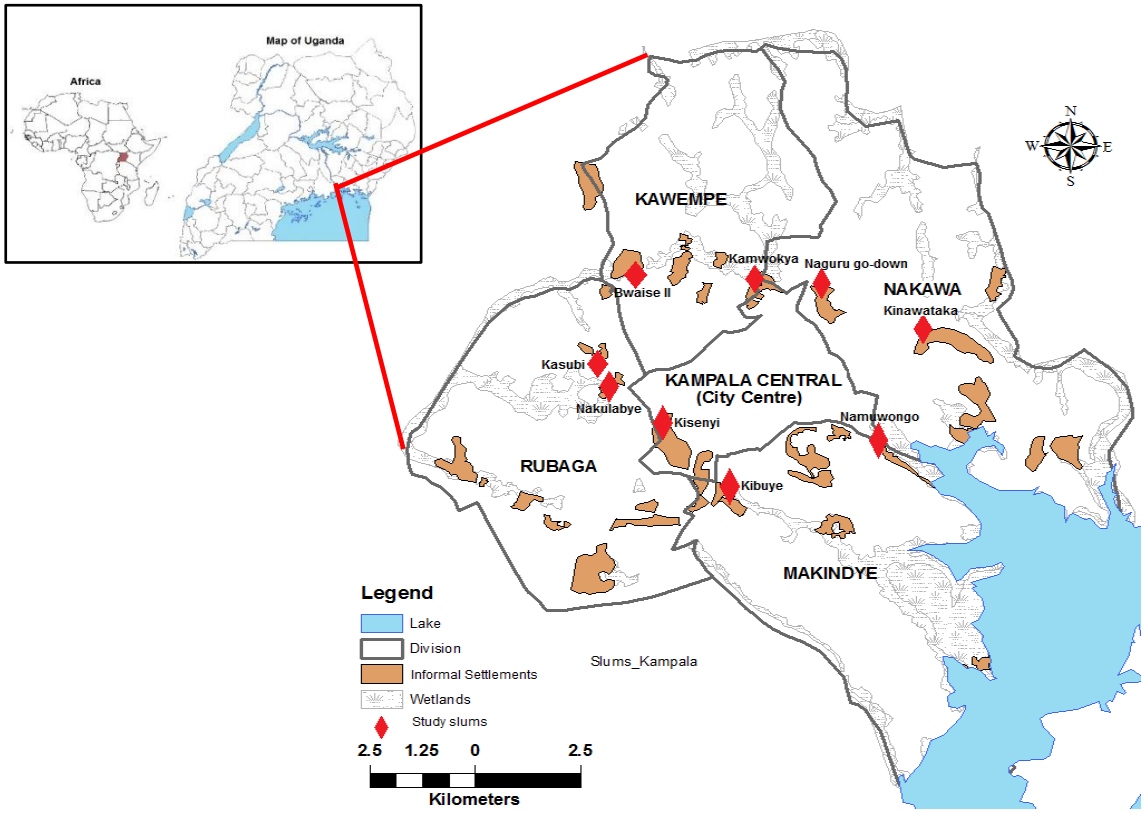

Figure 1-3 Map of Kampala Capital City showing the study area

These areas house part of the urban poor population of Kampala, are heavily populated, have substandard housing, filth and lacks basic services, which are characteristic of urban slums. Additionally, part of each slum was located in a low- lying terrain with a high ground water table (<1.5m) and always experiences floods in the rainy seasons and the other part had a low ground water table. This was necessary to capture variations resulting from differences in terrain (High and low water table) as this affected the way the pit latrines were constructed. In areas with a high water table, pit latrines were constructed and raised above the ground (KSMP, 2004; Kulabako et al., 2010).

1.6 Ethical consideration

The study was approved by both the postgraduate research committee of Makerere University College of Engineering, Design, Art and Technology (CEDAT). Clearance was also obtained from KCCA Health Department. Introductory letters issued by KCCA and the Department of Civil and Environmental Engineering (CEDAT) was presented to the house hold owners at the time of data collection (Copies have been included in the Annex). In addition, the purpose of the study, confidentiality, voluntary participation, and freedom to withdrawal were clearly explained to the participants ahead of signing a consent to their acceptance to consider their pit latrines for this study and to participate in the study.

1.7 Outline of the thesis

The thesis is organized as a series of chapters based on three published papers and two manuscripts listed below. Each chapter provides an abstract, introduction; materials and methods; results; discussion and conclusions for each study.

1. Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., & Kansiime, F. (2016). Are pit latrines in urban areas of Sub-Saharan Africa performing? A review of usage, filling, insects and odour nuisances. *BMC Public Health, 16*(1), 1-16. doi: 10.1186/s12889-016-2772-z.

2. Nakagiri, A., Kulabako, R. N., Nyenje, P. M., Tumuhairwe, J. B., Niwagaba, C. B., & Kansiime, F. (2015). Performance of pit latrines in urban poor areas: A case of Kampala, Uganda. *Habitat International, 49,* 529-537. doi: http://dx.doi.org/10.1016/j.habitatint.2015.07.005.

3. Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., & Kansiime, F. (2016). Assessing ambient and internal environmental conditions of pit latrines in urban slums of Kampala, Uganda: Effect on performance. *Journal of Water, Sanitation and Hygiene for Development, 07*(1), 92-101. doi: 10.2166/washdev.2017.085.

4. Nakagiri, A., Tumuhairwe, J. B., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., & Kansiime, F. (2016). Fingerprinting bacteria and fungi harvested in soil indigenous microorganisms (IMOs) using 16Sr-RNA and 18Sr-RNA gene sequencing for potential use in pit latrines – *manuscript*.

5. Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., & Kansiime, F. (2016). Assessing the effect of IMOs on degradation of faecal matter and improving the performance of pit latrines in urban slums - *manuscript*.

The chapters in this thesis are aimed at addressing each of the specific objectives of this study as detailed in Figure 1.4.

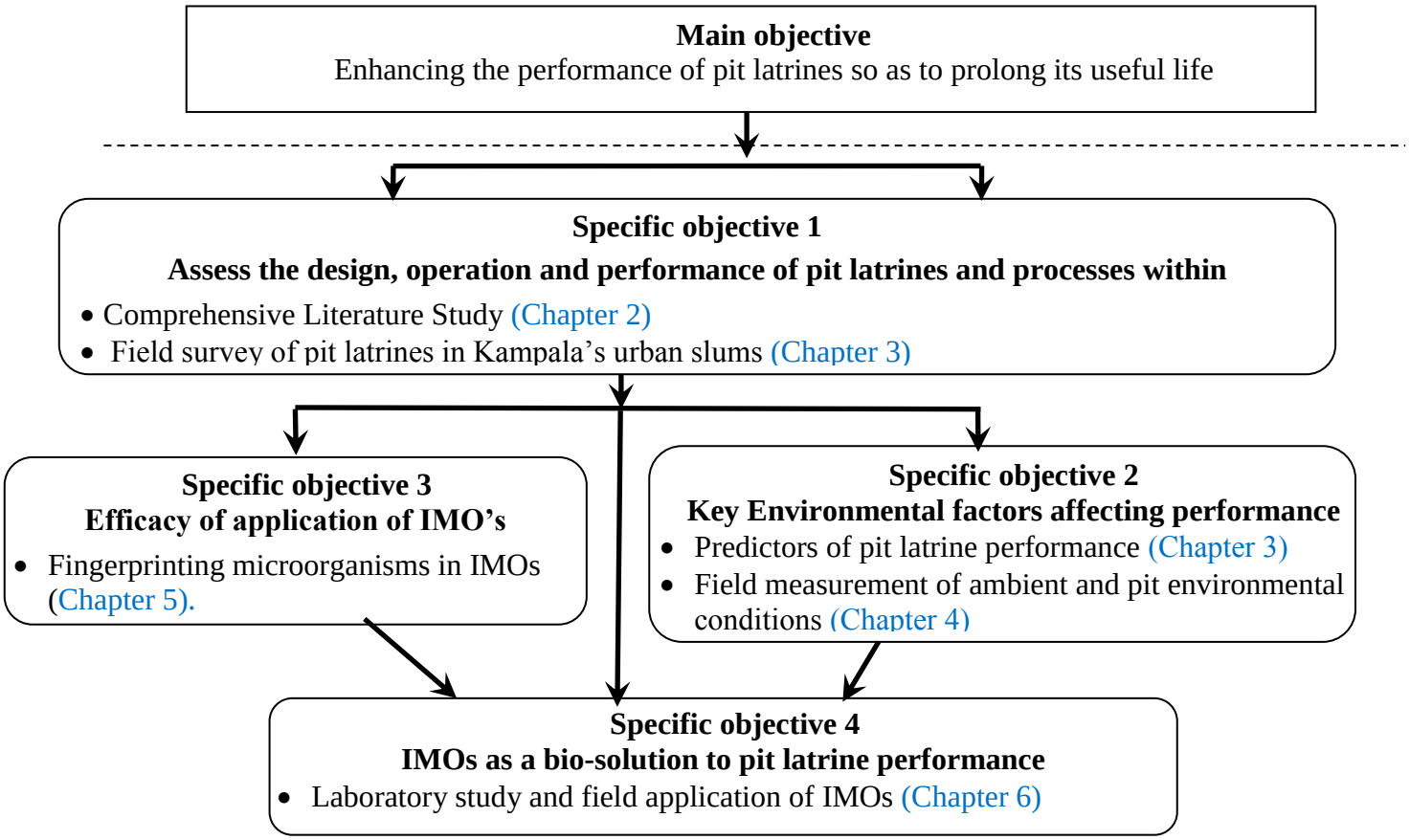

Figure 1.4 Relationship between the objectives and chapters of the thesis

In total, the thesis consists of eight chapters. Chapter 1 is the introduction to the study, which covers the study background, problem statement, research objectives and the study location. Chapter 2 is a critical review of previous and current knowledge on pit latrines focusing on usage and performance (filling, smell and insect nuisances) in urban areas of Sub-Saharan Africa (SSA) (Objective 1). Knowledge gaps and strategies / interventions to improve the

performance and sustainability of pit latrines were identified, and guided investigations in the subsequent chapters.

Chapter 3 covers a survey on the status of pit latrine structures (design, construction, operation) and maintenance within different slums of Kampala (Objective 1). In addition predictors to pit latrine performance (Objective 2) were determined through a multi-variate analysis, of data obtained on the status of the pit latrine structures and their performance in a typical urban slum. Chapter 4 assesses the ambient and internal environmental conditions of pit latrines that could influence their functionality in a typical low-income urban setting; including their implication on the performance of pit latrines (Objective 2).

In this study, IMOs were proposed as an inoculum in pit latrines. Chapter 5, thus evaluated the microorganisms collected as IMOs from different environments and established their potential at improving the performance (filling, smell and insect nuisances) of pit latrines (Objective 3). This was based on fingerprinting of 16S rRNA 18S rRNA for bacterial and fungal species. Chapter 6 presents IMOs a solution to improving pit latrine performance (Objective 4). Laboratory degradation experiments and response surface modelling, were employed to investigate the effect of IMOs on degradation of faecal matter and optimise for their use in pit latrines. Additionally, the user perceptions from three pit latrines in an urban slum of Kampala, to which IMOs were added were presented.

Chapter 7 provides a discussion that synthesises all the results in the different studies (Chapters 2, - 6) under taken in this research. Implications of the finding on the performance of pit latrines are also addressed in this section. Conclusions drawn from this study and recommendation to policy and further research are presented in chapter 8.

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CHAPTER TWO

2 Are pit latrines in urban areas of Sub-Saharan Africa performing? – A review of usage, filling, insects and odour nuisances

Abstract

A pit latrine is the most basic form of improved sanitation which is currently used by a number of people around the globe. In spite of the wide spread use, known successes and advantages associated with pit latrines, they have received little attention in form of research and development. This review focuses on the usage and performance (filling, smell and insect nuisance) of pit latrines in urban areas of sub-Saharan Africa (SSA) and proposes approaches for their improvements and sustainability. Current pit latrine usage within urban SSA was calculated from Joint Monitoring Programme (JMP) country-files of water and sanitation. The review findings indicated that more than half the urban population in SSA and especially the low-income earners are using pit latrines. However, their performance is unsatisfactory. While contributions have been made to address shortfalls related to pit latrine use in terms of science and technological innovations, further research especially in urban low-income settings is still needed. Any technology and process management innovations to pit latrines should involve scientifically guided approaches. In addition, development, dissemination and enforcement of minimum pit latrine design standards are important while the importance of hygienic latrines should also be emphasized.

This chapter is based on:

Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., & Kansiime, F. (2016). Are pit latrines in urban areas of Sub-Saharan Africa performing? A review of usage, filling, insects and odour nuisances. [journal article]. *BMC Public Health, 16*(1), 1-16. doi: 10.1186/s12889-016-2772-z

2.1 Background

Globally, providing adequate sanitation is a challenge and the situation is worse in developing countries. Improved sanitation protects the environment and improves people's health, thereby translating into socio-economic development and poverty eradication (Feachem et al., 1983; UNICEF & WHO, 2008; van Minh & Nguyen-Viet, 2011). Access to improved sanitation worldwide stands at 64%, with the lowest coverage of 41% in urban areas of Sub-Saharan Africa (SSA) (WHO & UNICEF, 2014b).

Sanitation provision in urban areas of SSA is predominantly on-site (Banerjee & Morella, 2011). A number of technologies are currently in use, each of varying affordability, suitability, adaptability and user satisfaction. These technologies include septic tanks, aqua privies, biogas latrines, composting or dehydrating toilets and pit latrines. The use of septic tanks in SSA currently stands at only 5% of the population (Strande, 2014). Challenges with the adoption and use of septic tanks are mainly high construction costs, space limitations, lack of water for flushing and blockages that result from bulk materials used for anal cleansing. The performance of aqua privies in SSA has been unsatisfactory. In Ghana, where the aqua privy was once widely used, it is now considered a failed technology at a national level because of uncontrolled odours, social /cultural issues and water shortages (Iwugo, 1981; Trawick & Parker, 2012),.

Biogas latrines have recently been installed as communal/public facilities in some areas of SSA (Jha, 2005; Schouten & Mathenge, 2010). However, their initial cost and operational skill requirements are beyond the capacity of urban-poor at a slum household level. Further, insufficient biogas to meet cooking requirements, gas leakage and the cultural issues with end-use of the slurry have hindered their adoption at household level. Replication or up-scaling composting or dehydrating toilets in SSA has registered varying levels of success. In east and southern Africa, cultural acceptance and misuse of the facilities have been cited as challenges to their use (WSP, 2005). In Ghana, failure of the Enviroloo, a type of composting toilet was caused by lack of readily available spare parts for repairing fans that were located on top of their chimney pipes (Trawick & Parker, 2012). The success and failure attributes of the different sanitation technologies used in Sub-Saharan Africa are summarised in Table 2.1.

Table 2.1 Summary of success and failure attributes of different sanitation technologies used in Sub-Saharan Africa

Sanitation technology	Attributes of success	Attributed of failure
Septic tank	• Offers a high standard of hygiene • Requires little mechanical maintenance • Permanent, emptied and reused	• High cost of installation • Shortage of space • Blockages • Water shortage
Aqua privy	• Requires less land • No pipes, less liable to blockages.	• Bad smells/odours, • Requires large volumes of water
Biogas latrine	• Provides biogas for energy, • Slurry produced is a good plant nutrient	• High installation costs • High technical skill to operate and maintain • Cultural phobia regarding slurry management
Composting/ dehydrating toilet	• Pits are re-usable, conserves space • Excreta contained, sanitized and can be recycled in agriculture	• Lack of spare parts for maintenance • High technical skill to operate and maintain. • High cost of repairs
Pit latrine	• Low cost of construction • Simple technology • Little water needed for operation • Easy to operate and maintain • Easily upgraded	• Filling up and thus need for space and money to build new ones • Bad smells/odours • Harbours insects and vermin

Pit latrines still remain widely used and are the most common basic form of improved sanitation (UNICEF & WHO, 2008). Of the 2.7 billion people using on-site sanitation facilities worldwide (Strande, 2014), an estimated 1.77 billion use some form of pit latrine as their primary means of excreta disposal (Graham & Polizzotto, 2013). Low-cost, simplicity of construction, little or no water usage, and ease in operation and maintenance, the ability to cope with bulky varied anal cleansing materials and the ease for regular improvement of the facility makes it convenient and easily taken up. The pit latrine technology currently offers a number of options ranging from simple designs like the traditional (without concrete slabs) to the simple improved, and further to more advanced Ventilated Improved Pit (VIP), Reed Odourless Earth Closet (ROEC), pour flush and borehole pit latrines. However, the use of pit latrines in urban areas of SSA has been marred by poor performance in terms of fast filling, bad smells and insect nuisances, which are associated with user dissatisfaction and a risk to disease

transmission. Yet, well-constructed, operated and maintained pit latrines isolate, store and partially treat human excreta thereby minimising human contact and their inherent public health hazards. In spite of the known successes and advantages associated with pit latrines, they have received little attention inform of research and development. The wide spread application and use of pit latrines necessitates sufficient knowledge of their performance in order to develop, design and operate them better, thereby improving the sanitation situation of the users. This chapter reviews previous and current knowledge on pit latrines usage and performance in urban areas of SSA. Knowledge gaps are identified and strategies or interventions that may improve the performance and sustainability of pit latrines are suggested. The performance elements covered in this review are pit latrine filling, smell and insect nuisances.

2.2 Methods

A comprehensive literature search according to PRISMAS guidelines (Moher et al., 2009), as shown in Figure 1, was used to find relevant documents, both published and unpublished, covering past and present knowledge on pit latrines with no date restriction (Figure 2.1). A Google (http://www.google.com/), Google scholar (https://scholar.google.com/) and Science Direct (http://www.sciencedirect.com/) was used following the keywords: "pit latrine", "pit privy", "pit latrine performance", "Pit latrine + sanitation", "Pit latrine filling smell and insects", "pit latrine filling + sub-Saharan Africa" "pit latrine smell + sub-Saharan Africa", "pit latrine + mosquitoes", "Pit latrine flies + sub-Saharan Africa", "Sanitation policy + sub-Saharan Africa".

The titles of retrieved articles were read to exclude duplication ahead of the screening process. At screening, the titles and abstracts of the documents were read to determine their eligibility of articles for full text assessment. In case of sanitation articles and reports, the complete document was obtained and scanned through to determine its eligibility. Documents selected for full text assessment were those that had information on pour/flush to pit; ventilated improved pit latrines; pit latrines with concrete slabs; traditional latrines (pit latrines with slabs not made of concrete); pit latrines without slabs/open pits (as unimproved latrines). At full text assessment, the contents of the document were critically examined to identify information on the history of pit latrines (no restriction of location), their usage. Topics that covered smell and insect nuisances (limited to SSA) and those were then considered relevant for the review. In addition, references in articles and reports guided further inquiry and review. Information from

the selected articles was extracted and the findings were used to develop this review. A figure on pit latrine and sanitation development milestones was developed from dates sited in literature. The pit latrine usage in different countries across SSA was determined based on available WHO/UNICEF survey data on estimates on the use of sanitation facilities for the different countries of SSA (WHO & UNICEF, 2014a) and the figures were then used to develop the map on pit latrines usage. The data source used for each country is indicated in the Appendix, Table A 1

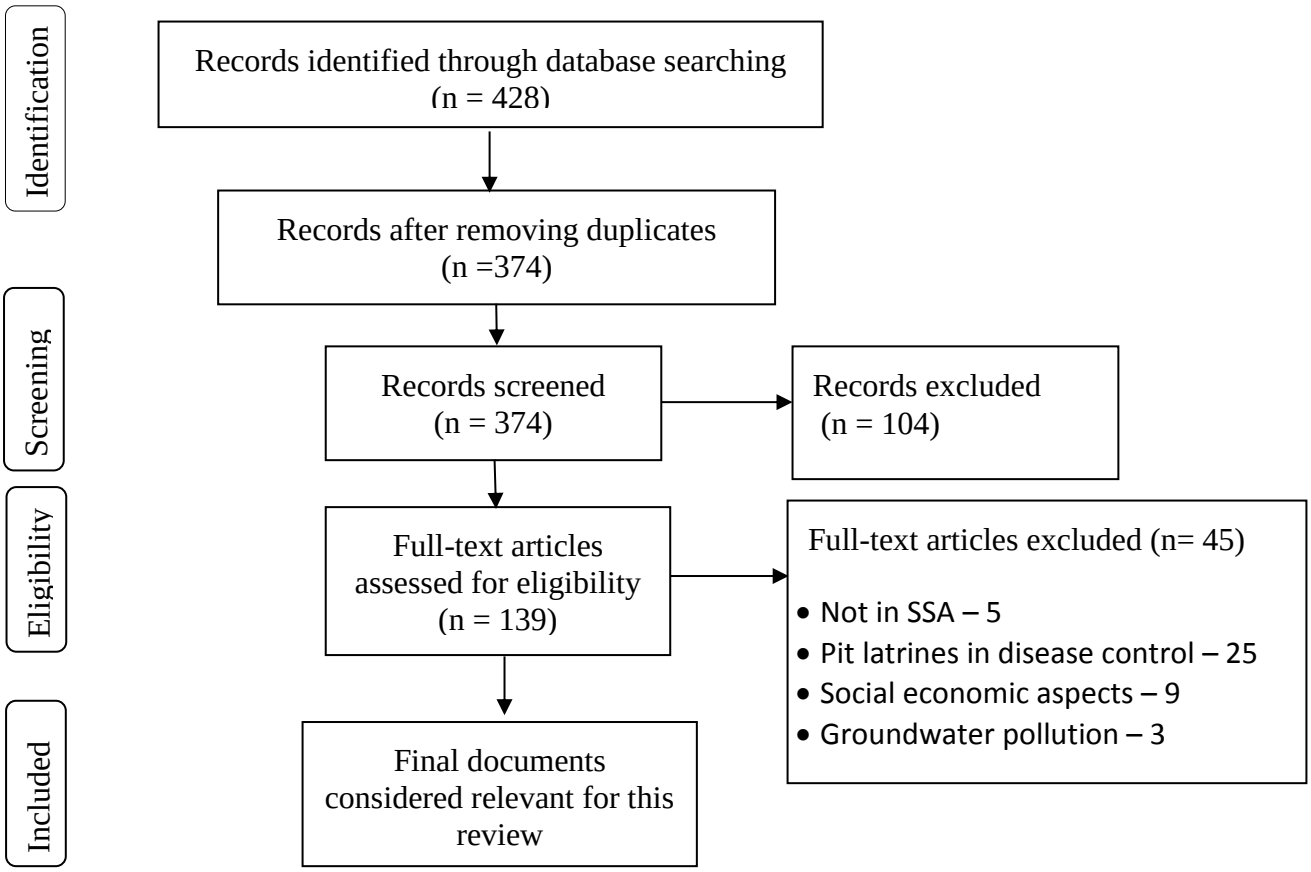

Figure 2.1 PRISMA flow diagram of the review inclusion and exclusion process

2.3 Results and Discussion

2.3.1 History of the pit latrine technology

The practice of human excreta disposal in the ground is a simple sanitation solution that has been used for thousands of years. Burying excreta in shallow holes referred to as the cat method and crude forms of pit latrines where horizontal logs were placed across the holes for support during use have been reported (Franceys et al., 1992; Pickford, 2006; Juuti et al., 2007). These

human excreta disposal solutions did not require any technical construction. Although these technologies are still used in some developing countries, and are better human excreta disposal systems than open defecation, though they are unimproved. The danger of contact with the excreta by humans, animals, and vectors of disease transmission plus soil contamination remain high in such systems.

The historical use of technical pit latrine designs dates to the early 20th century. They were developed and promoted in rural and small communities of present day developed nations to minimise indiscriminate pollution of the environment with human excreta that had resulted in high incidences of diseases. One very important World Health Organisation publication by Wagner and Lanoix (1958) in the late 1905's details technical data on pit latrines and ways of achieving successful human excreta disposal programs. The basic components of the pit latrine design are a hole dug in the ground in which excreta and anal cleansing material is deposited, a slab with a drophole that covers the pit and a superstructure for privacy (Kalbermatten et al., 1982; Cotton et al., 1995). To date, a number of design incorporations and modifications to the pit latrine have been developed, (Figure 2.2) each targeted to performance improvement, and the socio-economic status of the communities.

One such design, the borehole latrine design with small cross-sectional pit diameter (300–500 mm) evolved during the early 20th century in the Dutch East Indies. The basis of this pit latrine design is not documented. However, it was noted that borehole latrines were at times included in kits prepared for disasters as they could be quickly and easily dug (Pickford, 2006). In order to mitigate the odour and insects, a water seal by the goose neck pour flush was developed in Thailand in the 1920's. Another advanced pit latrine design aimed at addressing odour and insect problems of simple pit latrines is the Reed Odourless Earth Closet (ROEC) developed in South Africa in 1940's (Rybczynski et al., 1978)

The promotion campaign in use of a simple pit latrine in SSA dates to the 1950's – 1960's, during the heyday of the disease control campaigns. However, the pit latrine was mainly promoted for use in rural areas (Wagner & Lanoix, 1958; Black, 1996; WHO, 2003). The major health and aesthetic problems associated with pit latrines then were insects (flies and mosquitoes) and odours (Rybczynski et al., 1978). To overcome these shortfalls, the VIP, initially called the Blair Latrine, was developed in Zimbabwe in the early 1970's.

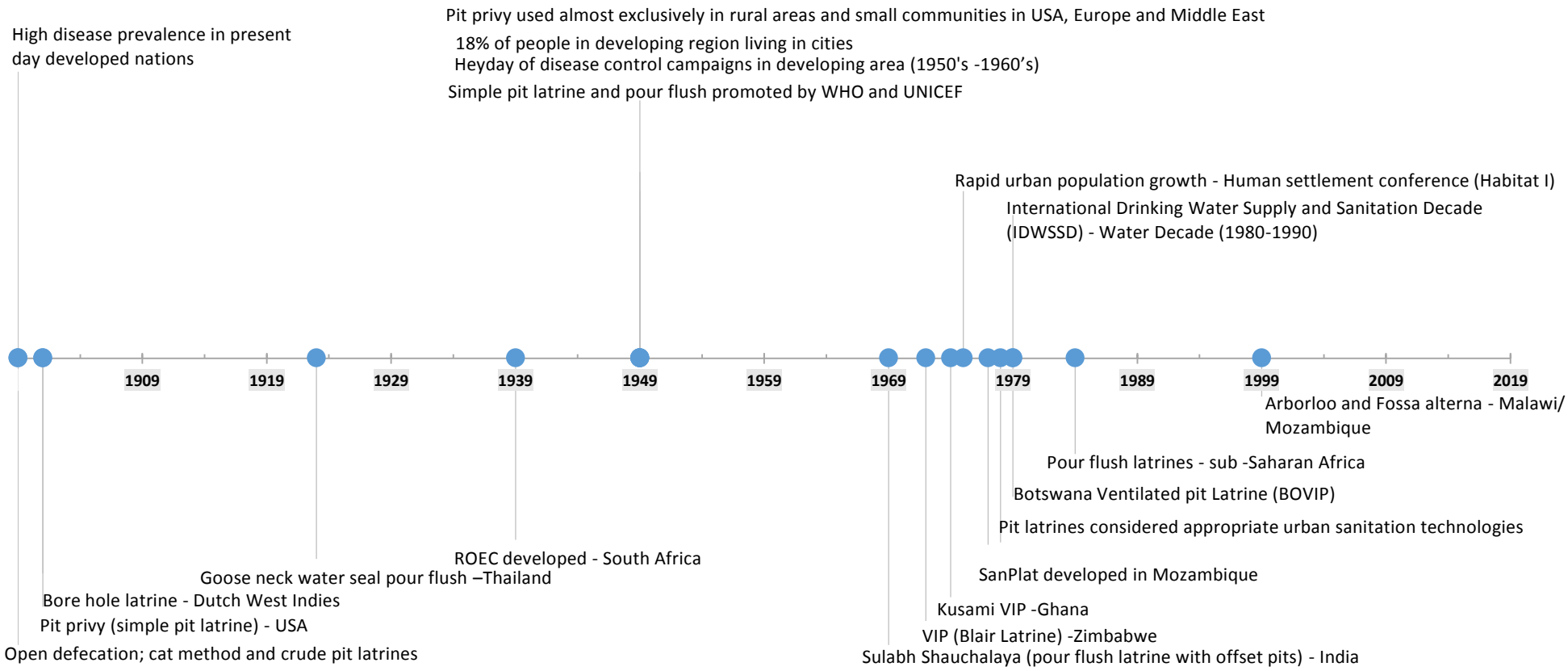

Figure 2.2 **Pit latrine and sanitation development milestones**

Modifications to the VIP made to date include the Kusami Ventilated improved pit (KVIP) in Ghana (Saywell & Hunt, 1999; Thrift, 2007) and the 'Revised Earth Closet II' (REC II), also known as the Ventilated Improved Double Pit (VIDP) latrine in Botswana (Van Nostrand & Wilson, 1983; Winblad & Kilama, 1985). In an effort to mitigate insect, odour and cost challenges of VIP latrines, another innovative design, the SanPlat was developed in Mozambique in 1979 (Solsona, 1995).

Towards the late 1970's, sanitation and health crises in developing nations were a result of rapid urban population growth and 'exploding cities'. For instance, up to 70% of new inhabitants in some African cities were residing in slums and shantytowns without amenities (Black, 1998). The World Bank, thus undertook research with emphasis directed towards low cost sanitation alternatives to sewerage. The results of the research, presented in a series of publications consider pit latrines as appropriate technologies for waste disposal in developing countries(Kalbermatten et al., 1980a; Kalbermatten et al., 1980b). Some pit latrine designs were then recommended as appropriate sanitation technologies for urban areas. Pit latrines were thereafter, accepted, adopted, promoted and used in urban areas of different countries in SSA during the Water Decade (Kalbermatten et al., 1982; Black, 1998). Currently, in the 21st century, interest in pit latrines is aimed at pit latrine filling and nutrient recovery. For example, two shallow compost pit latrines designs, the Arborloo and Fossa Alterna have been developed (Morgan, 2005, 2006). The importance of hygienic latrines has also been addressed. For example, a study by Jenkins et al. (2014) noted that beyond the Millennium Development Goal's definition of "improved" sanitation, hygienic safety and sustainability of the facilities was critical for their performance in low-income urban areas of Dar es Salaam, Tanzania. In Kampala, Uganda, it was found out that improved latrines failed to serve their purpose when misused or not properly cleaned (Günther et al., 2012; Kwiringira et al., 2014a). Other studies undertaken in urban slums of Kampala noted that understanding of the importance of using a clean toilet, the perceived disgust from using dirty toilets and user habits were essential in fostering users' cleaning intention for shared toilets. Additionally, lack of cleanliness of latrines was linked to among other things, the lack of water or a lack of responsibility to buy the water to clean latrines, especially those that were shared (Tumwebaze et al., 2012; Tumwebaze et al., 2014; Kwiringira et al., 2014b). Therefore, the availability of water and user intervention are important to assure latrine cleanliness.

2.3.2 Pit latrine usage in urban areas of SSA

Currently sanitation access for approximately 198 million (52.7 %) of the urban population in SSA is in form of a pit latrine (Appendix, Table A 1). In 2007, pit latrine use in urban areas of SSA was

at 65%, representing about 162 million people (Banerjee et al., 2008). While the percentage of pit latrine users has gone down (from 65% to 52.7%) since 2007, the actual number of people using them has risen by 36 million. This number is expected to be higher as some of the percentages used during the calculations (Appendix Table A2) are from past years. The usage of pit latrine in SSA varies notably within the different countries (Figure 2.3), and dramatically across the socio-economic spectrum, but is predominant among the low-income earners (Morella et al., 2008).

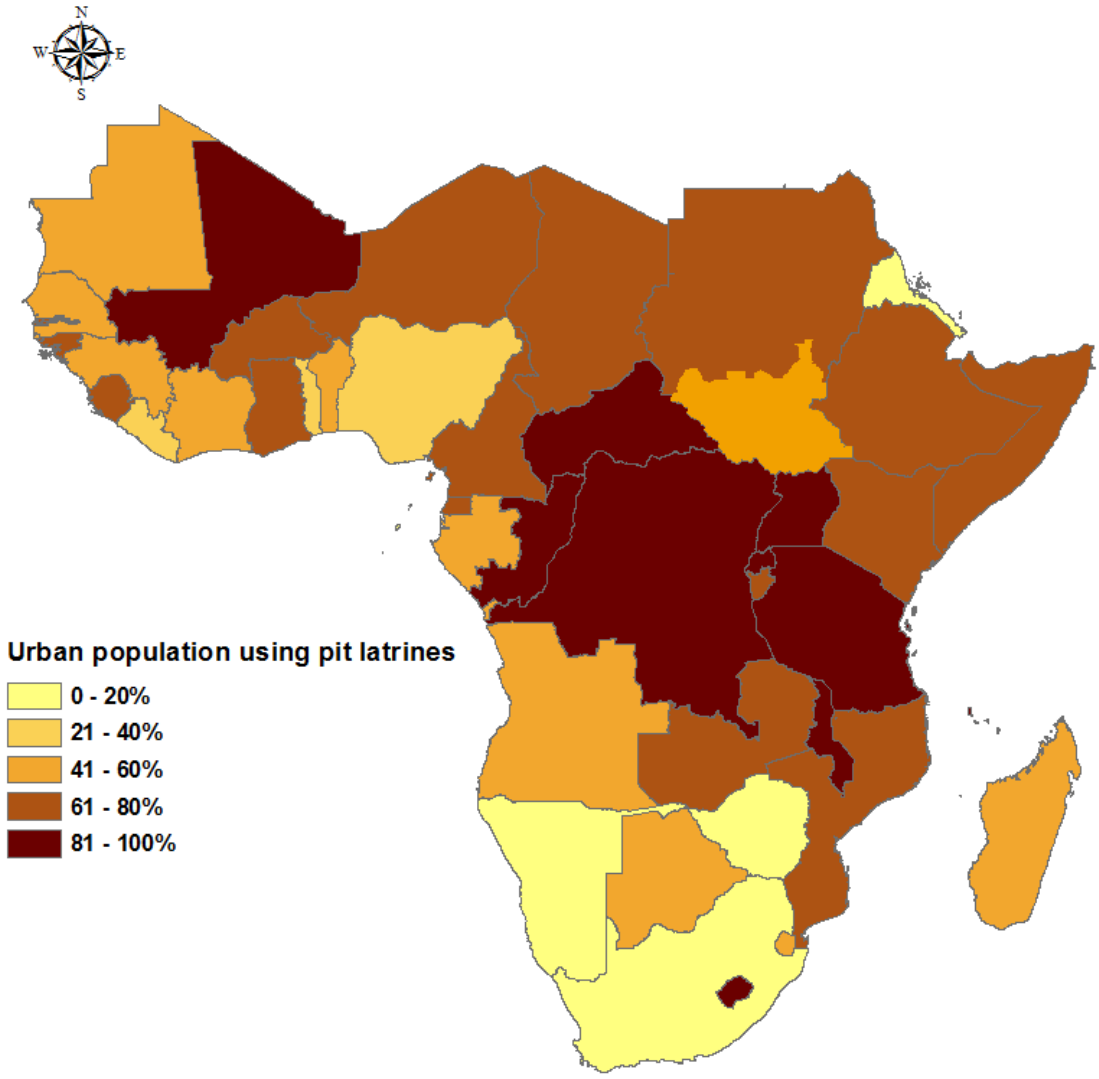

Figure 2.3 Percentage of SSA urban country populations using pit latrines (WHO & UNICEF, 2014a)

The types of pit latrines being used within the urban areas of different SSA countries also vary. Presently, access to improved pit latrines is notably high. Overall usage of improved pit latrines stands at about 63%, up from 14% noted in 2007 (Figure 2.4 A and B). A number of countries have moved from the use of traditional pit latrines to more improved types (Table A2, Appendix). The most common improved type is the simple pit latrine with a concrete slab. However, usage of VIP

and pour flush latrines still remains low. In addition, there is still usage of pit latrines without slabs in urban SSA (Figure 2.4 B). The increase in access to improved pit latrines can be explained by the high awareness and action on sanitation from 2008 onwards (UNICEF & WHO, 2008).

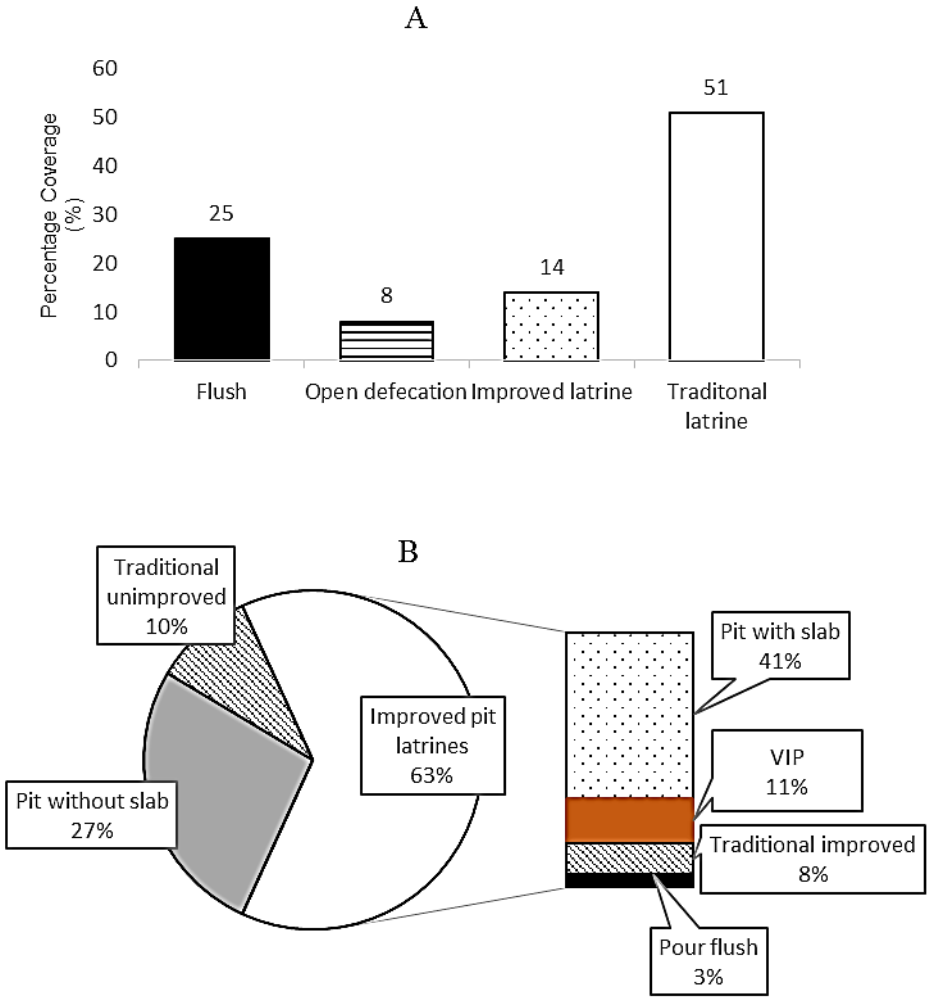

Figure 2.4 Percentage of pit latrine types in use in SSA. (A) usage in 2007 Morella et al. (2008) and (B) usage in 2015 WHO and UNICEF (2014a)

2.3.3 Sanitation policy and practice on pit latrine

One of the challenges of sanitation provision in the past was the little attention given to it and lack of clear policies to guide its provision. In the recent years, sanitation improvements have been at the forefront of most of the water and health projects (Black, 1998; WHO, 2003; UNICEF & WHO, 2008). There has been high political awareness within the international system, which has led to a number of strategies and policy reforms to address sanitation improvements. Different levels of service of pit latrines and other human excreta disposal facilities have been defined, based on the extent to which they provide improved sanitation and costs. VIP, and pit latrines with a slab are considered improved while pit latrines without slabs are considered unimproved (UNICEF &

WHO, 2008). However, while sanitation policies now exist in a number of countries in SSA, they state broadly the different sanitation technologies with no emphasis on minimum service levels of specific groups and technical details. For example, a review of policies from nine countries noted that only South Africa, Mozambique and Ghana had a VIP as their minimum sanitation standard. Additionally, most policies do not allow for funding of sanitation technologies at household level (WEDC, 2005; Potter et al., 2011). It has also been noted that sanitation service delivery is done via a multi-level process involving a number of actors (Ekane et al., 2014) of which on-site sanitation provision at household level is the responsibility of the owners. These often have limited knowledge of technical aspects on pit latrines (Kariuki et al., 2003). In addition, the type of pit latrine adopted is in most cases determined by socio- economic status of the owner. For example, sanitation improvements has been observed where government is highly involved and committed to it. One such case is Rwanda, where political will was successfully leveraged at all sanitation governance levels (Ekane et al., 2014), and improved pit latrine coverage now stands at 82.2% (WHO & UNICEF, 2014a).

2.3.4 Performance of pit latrines

There is a clear link between proper excreta disposal and improved health (Kalbermatten et al., 1982). The appropriateness of pit latrines at providing improved sanitation thus lies in its ability to safely dispose human excreta in such a way that there is minimal or no contact with humans. Furthermore, the excreta should not be accessible to insects or animals and the facility should be free from odours (Wagner & Lanoix, 1958; WHO, 1987). Research directly linking full pit latrines, their smell and insect nuisances to disease and health is limited. However, it has been reported that full and/or over flowing improved pit latrines do not meet the criteria for hygienic, safe and sustainable sanitation systems (Jenkins et al., 2014). It is not only difficult to use full or overflowing pit latrines as the content not only splashes on to the users but also the excreta poses a health risk since it is in closer contact with humans. Additionally, smell and insects nuisances of pit latrine use are the main cause of disturbance of people who come in contact with them. In the past, smell and insects significantly affected the user satisfaction, although the problem did not impact on pit latrine use (Cotton et al., 1995; Cotton & Saywell, 1998). More recently, bad smell has been frequently mentioned as a reason for dissatisfaction with shared toilets (Saywell & Shaw, 1999; Tumwebaze et al., 2012), discouraging their use and subsequent use of polyethylene bags (Kwiringira et al., 2014a). Foul smell has also been noted as a barrier for acquiring and using latrines (Rheinländer et al., 2013). Smell and insects have been associated with the hygienic nature of the pit latrine. For example, in a survey by Tumwebaze and Mosler (2014), respondents considered clean latrines as

those free from smell and insects. The subsequent sections detail pit latrine performance in terms of filling, smell and insect nuisances.

Pit latrine filling

Pit latrine filling is currently a problem associated with their performance. Notably, the first faecal sludge management seminar was held in March 2011 in Durban, South Africa and brought to light issues related to pit latrine filling (WIN-SA & WRC, 2011). One of the concerns of pit latrine filling is that a number of the pit latrines within urban areas of SSA have reached their storage capacity. For example, VIPs built in Zimbabwe from 1980 – 2000 were reported to be full or nearly full (Morgan, 2009). A study by Bakare (2014), reported that the number of pit latrines built across South Africa's municipalities were full or over flowing. In Durban, South Africa alone, 35,000 pit latrines were emptied by 2011 (Macleod, 2011). In a study undertaken in informal settlements of Kampala, Uganda, Günther et al. (2011) noted that 35% of the pit latrines had been abandoned because they had filled up while 15% of the latrines were full and still in use. Another study by Appiah-Effah et al. (2014) undertaken in the Ashanti region of Ghana reported that 31% of the latrines were found full and needed immediate de-sludging. Jenkins et al. (2014) noted that 40% of the latrines were full or nearly full in Dar es Salaam, Tanzania.

In the past, a filled pit latrine was covered and a new one sunk. Double alternating pits were also proposed for use in peri-urban areas as they sanitize and reduce the volume of human excreta prior to emptying and disposal (Winblad & Kilama, 1985). However, due to the high population density in most urban areas of SSA, digging new replacement pits and the use of alternate pits are not practical. Pit latrines can thus no longer serve as a stand-alone solution to human excreta management. A systems approach to sanitation is currently being adopted for urban settings to ensure their sustainability. In this case, the provision of access to improved sanitation facilities is considered a multi-step process, where a pit latrine is part of the chain, to be supported by the collection and transportation as well as treatment for safe end-use or disposal (Tilley et al., 2008).

Attention is currently being focused on the time it takes for the pit to fill, since it is crucial for the management and sustainability of pit latrines. The actual filling times of pit latrines as noted in literature vary (Table 2.2). The available information indicates that pit latrines are mainly filling faster than expected. This has been attributed to the rate at which sludge accumulates within the pit. Most of the studies determining sludge accumulation have been based on number of users, filling time and the size of the pit. Proposed design accumulation rates range from 40 -90 L/capita/year

(Wagner & Lanoix, 1958; Franceys et al., 1992). More recent field investigations undertaken in peri-urban of South Africa by Norris (2000) found lower rates and thus proposed 25.5 L/capita/year. In another study by Still (2002) in South Africa, sludge accumulation rates were found to range between 10 – 120.5 L/capita/year. Further studies by Still and Foxon (2012b) noted filling rates of 1 – 264 L/capita/year. Available data indicate variable pit latrine sludge accumulation/filling rates by region, even in a comparatively homogeneous environment (Table 2.3).

Table 2.2 Summary of studies on pit latrine filling time

Source	Country	Filling (Years)	Remarks
Franceys et al. (1992)	Various	15 - 25	Design recommendations for household properties
Pickford (2006)	East Africa	Over 30	Reported at a house hold level
Morgan (2009)	Zimbabwe	Over 30	Household latrine
Still and Foxon (2012a)	South Africa	20	Design recommendation
	South Africa	5 - 9	Empting time for most (85%) pit latrines. Lower and higher filling rates were also noted
Günther et al. (2011)	Uganda	5	Study in low-income areas of Kampala, Uganda (Slums)
Kulabako et al. (2010)	Uganda	< 1	Low laying areas of peri-urban settlements in Kampala
Adubofour et al. (2013)	Ghana	4.2	Average filling time
	(slums in Kusami	> 10	High income areas
	metroplis)	0.25	Low-income areas
Appiah-Effah et al. (2014)	Ghana (Ashanti region)	6 - 10	Low-income area in Ashanti region

Table 2.3 Design accumulation rates and actual excreta filling rates

Design / Place	Filling rates litres/capita/annum (l/c/a)	Reference
Design accumulation rates		
Wagner and Lanoix (1958) and Franceys et al. (1992)	40	Wet pits where degradable anal cleansing material is used
	60	Wet pits where non degradable anal cleansing material is used
	60	Dry pits where degradable anal cleansing material is used
	90	Dry pits where non degradable anal cleansing material is used
Reported pit latrine filling rates		
Wagner and Lanoix (1958) and Franceys et al. (1992)	25 (ablution water used) 35 Wet pit	West Bengal, India
Wagner and Lanoix (1958)	40 (solid cleansing material)	Philippines
Morgan et al. (1982)	20	Zimbabwe
Franceys et al. (1992)	42	USA
	47	Brazil
Bhagwan et al. (2008)	24.1(mean)	Soshongove, South Africa
	69.4 (mean)	Bester's Camp, South Africa
	18.5 (mean)	Mbila, South Africa
	27.5 (implied)	Gabarone, Dares salaam
	29 (median)	Mbazwana, South Africa
	34 (median)	Inadi, South Africa
Still and Foxon (2012a)	39 (median)	Limpopo, South Africa
	48 (median)	Mafunze, South Africa
	21 (median)	Ezimangweni, South Africa
	19 (mean)	eThekwine, South Africa

To explain the variation in sludge accumulation rates, studies have assessed different variables (Table 2.4) some of which are user related, like number of users, other material put in the pit and design related (type of pit latrine, lined or unlined), geophysical and climatic factors. Studies relating sludge accumulation rates to number of users have reported contrasting results. It is perceived that the filling rate increases with number of users. However, some field studies have reported a decrease in sludge accumulation rates with an increase in number of users (Still & Foxon, 2012b; Bakare, 2014). Additionally, Bakare (2014) based on a linear model fit to the amalgamated

data documented by Still and Foxon (2012b), showed no significant correlation (R^2 of 0.203) between sludge accumulation rate and number of users. However, it is important to note that this study was based on small increments from 5 to about 15 pit latrine users. The case could be different in urban settings where pit latrine sharing leads to higher number of users.

Relating sludge accumulation to matter other than human excreta found that the degree of abuse to which the pit is subject affects the filling rate. Throwing rubbish in a pit almost doubled its filling rate in studies undertaken in South Africa (Buckley et al., 2008; Still & Foxon, 2012b). A simple mass balance model of pit latrine filling developed and tested by Brouckaert et al. (2013) using data from VIPs in South Africa, predicted that adding non-degradable material to the pit significantly increased its filling. A study by Norris (2000) noted no effect of seasonal variations on sludge accumulation in pit latrines in South Africa. However, in Tanzania, a large temporary increase in pit content was observed in the wet periods (Todman et al., 2014). The ability of the model developed by Brouckaert et al. (2013) to simulate data collected in south-central Tanzania and a sensitivity analysis of its parameters was tested by Todman et al. (2014). The results indicated that water inflows and accumulation have an important effect on the filling rate. In Kampala (Uganda), a study relating the status of pit latrine structures to their performance noted that signs of rain or storm water entry, flooding and cleaning time were significant predictors of pit latrine filling (Nakagiri et al., 2015). This implied that water input into the pit significantly contributed to an increase in the level of pit content.

The rate of filling has also been attributed to the degradation processes occurring within the pit latrine over time. Matter starts to decompose as soon as it is deposited in the pit. Studies have depicted that the process of decomposition in pit latrines are largely anaerobic although aerobic degradation processes may occur in top layers (Wagner & Lanoix, 1958; Chaggu, 2004; Buckley et al., 2008). During decomposition, the degradable fraction of faecal matter will break down into a more stable non-odorous product. Released gases flow into the atmosphere and mineral compounds are assimilated into the ground respectively. Through this action, the volume of matter added to the pit is substantially reduced (Franceys et al., 1992; Cotton & Saywell, 1998). A possible mass - volume reduction of 50-75% (Bakare, 2014) or up to 80% (Wagner & Lanoix, 1958; Zavala et al., 2002) after well-established degradation has been reported. However, literature indicates that the uncontrolled environment within the pit may not be efficient for decomposition under either process which results in slow/incomplete breakdown of organic matter (Torondel, 2010).

Table 2.4 Summary of studies assessing sludge accumulation rates, with different variables

Source	Country	Variable of interest	Study/ experimental design	Remarks
Still and Foxon (2012b)	South Africa	Number of users	Field monitoring and measurements	A decrease in per capita filling rate with an increase in number of users.
		Rubbish content	Sorting and analysis of pit content	Throwing rubbish in a pit almost doubled its filling rate
Bakare (2014)	South Africa	Number of users	Analysis of amalgamated data documented by Still and Foxon (2012b)	No correlation (Pearson correlation coefficient of 0.203) between sludge accumulation rate and number of users.
			Field monitoring and measurements	Sludge accumulation rates decreased with increasing numbers of users.
		Degradation	Laboratory experiments on pit latrine samples	50-70% volume reduction in matter added to the VIP
		Addition of moisture	laboratory batch experiments on pit latrine samples	No evidence that an increase in moisture content of samples from VIP latrines reduced the sludge accumulation rate.
Todman et al. (2014)	Tanzania	Seasonal variation	Field monitoring and measurements	During wet periods, large temporary increases in the level (1m magnitude) of pit content was observed
		Pit latrine Modelling	Modelling pit latrine filling based on model developed by Brouckaert et al. (2013)	Water inflows and accumulation have an important effect on the filling rate
Norris (2000)	South Africa	Seasonal variation	Field monitoring and measurements	No effect of season variations on the sludge build up
Wagner and Lanoix (1958)	Various	Degradation		A possible volume reduction of up to about 80 % after well-established degradation in wet pits

Source	Country	Variable of interest	Study/ experimental design	Remarks
Buckley et al. (2008)	South Africa	Addition of moisture	Laboratory experiments on pit latrine samples	a significant increase on gas production rate was noted
		Increasing Alkalinity	Laboratory experiments on pit latrine samples	No statistically significant increases in the rate of gas production from the samples under anaerobic conditions.
		additives	Laboratory experiments on pit latrine samples	Inconclusive results
Brouckaert et al. (2013)	South Africa	Pit latrine Modelling	Developing and testing a simple mass balance model	Adding non-degradable material to the pit significantly influenced its filling
Foxon et al. (2009)	South Africa	additives	Laboratory experiments on pit latrine samples	No statistically significant effect on rate of mass loss
Taljaard et al. (2003)	South Africa	Bio additives	Laboratory studies on pit latrine samples	Use of biological product is feasible
Jere et al. (1998)	Zimbabwe	Spore forming bacteria	Pit latrine studies	Effective in reducing pit content
Kassam (2012)		Earthworm (Tiger worms)	Laboratory experiment setup	Reduction in human excreta
Banks (2014)	South Africa	Black soldier fly larvae	Laboratory studies on pit latrine samples	Potential in reduction of pit latrine content

In order to quantify the role of decomposition and stabilization on mass loss within pit latrines, laboratory batch experiments have been undertaken. Addition of moisture to samples of pit content in laboratory experiments had a significant increase on gas production rate (Buckley et al., 2008). It was thus concluded that increasing moisture content of VIP contents has the potential to increase the rate of stabilisation of buried organic material in the pit. However, in a study by Bakare (2014) no evidence was found to show that an increase in moisture content of samples from VIP latrines reduced the sludge accumulation rate. The study proposed that compaction could play an important role on the rate at which pits fill up. The effect of increasing alkalinity (addition of Sodium bicarbonate), thereby the pH buffering capacity of pit latrine samples was assessed by Buckley et al. (2008). The increase in the rate of gas production from the samples observed under anaerobic conditions was not statistically significant. It was thus concluded that alkalinity was not a limiting factor in anaerobic digestion of pit latrine contents.

Studies on inoculation with additives, containing microorganisms, enzymes or their blends, have been considered to enhance degradation of pit content. Relatedly, Taljaard et al. (2003) reported a feasibility of applying biological products for the degradation of organic matter. However, the study was inconclusive and recommended field trials to monitor contents of newly dug pits on a daily basis. A biological study into the claimed mode of action of the products, to determine the amount and type of microorganisms and enzymes present was also proposed. Earlier, Jere et al. (1998) studied the effects of spore forming non-pathogenic bacteria in reducing sludge volume in pit latrines and concluded that the bio-organic breakdown compound proved to be efficient in reducing the pit contents. However, Buckley et al. (2008) obtained no correlation in decrease of faecal matter between the used additives and the rate of change in pit matter content. The results were considered inconclusive due to the difficulty in obtaining representative measurements of any condition and lack of test control sites. Furthermore, Foxon et al. (2009) reported no statistically significant effect on the rate of mass loss from the sludge samples under either aerobic or anaerobic conditions by nine additives. It was concluded that commercial pit latrine additives did not accelerate the rate of decomposition of pit latrine contents. Subsequently, Still and Foxon (2012b) concluded that sufficient evidence was lacking to prove that pit latrine additives could cause differences in pit latrine sludge build-up.

Earth worms have also been investigated for their potential to reduce pit latrine contents with successful results (Kassam, 2012). Currently, they are the basis of the tiger toilet, a worm-based sanitation technology aimed at speeding up the decomposition of human waste (www.sanitationventures.com/). Black soldier fly larvae (BSFL), *Hermetia illucens* L., has also shown potential in reducing pit latrine sludge. Research by Banks (2014) found the characteristics of faecal sludge from different pit latrines in South Africa were within the range for BSFL development. Key factors that affected the faecal mass reduction were moisture and larvae density. However, further research is required on the applicability of these organisms in pit latrines.

Pit latrine odours and insect nuisance

The extent of the smell and insect nuisance found in literature has mainly been listed by intensities based on a pre- determined scale (Table 2.5). Only two studies listed the odour descriptions associated with particular pit latrine smell intensity (Table 2.6). Of the listed intensities, the strong, unpleasant, repugnant, foul, malodorous smell and any presence of flies are of importance in pit latrine performance. Information on the actual composition of the malodorous gases in pit latrine is limited. Methane, carbon dioxide, nitrogen, ammonia and hydrogen sulphide have for long been noted as the smell causing substances in pit latrines (Wagner & Lanoix, 1958; Mara, 1984).

Table 2.5 Pit latrine odour intensity and description scale

Source	Location	Smell description (%)	Insect nuisance (%)
Cotton et al. (1995)	Ghana and Mozambique (Simple pit latrines and VIPs respectively)	No smell (54 and 40) Slight smell (9 and 6) Strong smell (37 and 51)	None/tens (91 and 90) Hundreds (8 and 3) Thousands (1 and 7)
Kwiringira et al. (2014a)	Kampala's slums	Strong repugnant smell	
Garn et al. (2014)	Kenyan schools	Strong smell (25.6)	Many flies (10)
Nakagiri et al. (2015)	Kampala's slums	No smell, (2) Slight smell (35) Moderate smell (22) Strong smell (39) Very strong (1)	No flies (3) Few flies (80) Many flies (17)
Afful et al. (2015)	Kusumi, Ghana	Extremely annoying (69 no) Very annoying (55 no) Annoying (30 no) Some annoyance (18 no) Definitely not annoying (1 no)	

Table 2.6 Pit latrine odour intensity and description:

Source	Site	Pit latrine type	Odour intensity	odour description
Lin et al. (2013a)	Durban	VP dry pit	Weak	Sewage, phenol-like
			strong	Rotten egg, sewage, rancid
		VP wet pit	Medium	More of sewage than faecal, rotten egg
			Strong	Rotten egg, sewage, rancid
	Nairobi	VP	strong:	cheese, manure, horse, farmyard
			Strong	cheese, manure, ammonia, urine
	Kampala	VP 1	weak	farmyard, ammonia slightly urine, geosmin (earthy, moisture)
			strong	rancid, rotten onion, phenylacetic acid-like
		VP 2	medium	farmyard, ambrinol (earthy, moisture), rancid
			strong	rancid, phenolic, rotten vegetable
Chappuis et al. (2015)	Nairobi		Weak	barnyard
	Durban	VIP	Weak	Animal, faecal

However, a study by Lin et al. (2013a) using gas chromatography - mass spectrometry and olfactive analyses found many more odorants. Of the 198 volatile constituents detected (Lin et al., 2013b), isobutyric, butyric, isovaleric, 2methyl butyric, valeric, hexanoic and phenylacetic acids were responsible for the rancid, cheesy odour/smell in pit latrines. The manure, farmyard, horse-like characteristics of latrine odour were attributed to the combined effects of phenol, p-cresol, indole, skatole, and some carboxylic acids. Dimethyl sulphide, dimethyl disulphide, dimethyl trisulphide, methyl mercaptan, and hydrogen sulphide contributed to the sewage, rotten egg, and rotten vegetable odours. The sewage malodourous smell in pit latrines has been attributed to anaerobic degradation while the rancid odour was noted to be representative of latrines dominated with fresh faeces (Lin et al., 2013a). Fermenting urine resulting from enzymatic cleavage of urea by ureases has been noted to be representative of the smell found in public pit latrines (Jördening & Winter, 2005; Troccaz et al., 2013).

Unlike smell, studies characterising insects in pit latrines have been undertaken. Adult and larvae of *Chrysomya putoria, Chrysomya marginalis, Musca spp, Lucilia cuprina, Sarcophaga spp* have been reported (Curtis & Hawkins, 1982; Emerson et al., 2005; Lindsay et al., 2012). Irish et al. (2013) identified members of *Psychodidae, Culicidae, Calliphoridae, Syrphidae, Stratiomyidae, Sarcophagidae* families from pit latrines in central Tanzania. Some types of

mosquitoes especially *Culex quinquefasciatus* and species of Anopheles are known to breed in wet pits (Curtis, 1993; Satterthwaite, 1993).

Studies have linked the presence of odours and insects in pit latrines to the type and size of the superstructure, and cleanliness. Irish et al. (2013) noted that the superstructure minimises the fly nuisance in pit latrines. Absence of a roof for example is significantly associated with presence of flies. In addition more flies have been found in latrines with temporary structures. In Kampala, latrines that were not regularly cleaned were associated with bad smells (Tumwebaze et al., 2012) and caused disgust among the users (Tumwebaze et al., 2014). Another study noted that pit latrine cleanliness, stance length, superstructure material and single household use were predictors of smell. Fly presence was predicted by the superstructure material and status, plus the terrain where the pit latrines were located (Nakagiri et al., 2015). Entomological studies on pit latrines in Botswana and Tanzania (Curtis & Hawkins, 1982) linked the insect nuisance to the smell. The studies showed that insects in pit latrines were attracted by the odours as many flies and mosquitoes were caught trying to enter the vent pipe which indicated they were drawn to the smell source.

Addressing the odour and insect nuisance of pit latrines has involved simple recommendations like the concrete slab that is easily cleaned and ensuring that the pit remains dark during use, which is achieved partly by the use of hole/ seat covers (Wagner & Lanoix, 1958). The use of inorganic and organic chemicals as larvicides and disinfectants like sodium fluosilicate, borax, paradichlorobenzene (PDB), orthodichlorobenzene (ODB), aldrin, BHC and DDT has been documented (McCabe & Haines, 1957; Wagner & Lanoix, 1958; Pickford, 2006). Muscabac, a *Bacillus thuringiensis* preparation containing exotoxin, was tested and showed reasonably good control of flies in latrines in a tropical environment (Carlberg et al., 1985). Household surveys have also reported addition of oil, kerosene, ash, soil, and disinfectants to control odour and insects (Zhang et al., 1994; Nwaneri et al., 2008; Nwaneri, 2009). Laboratory and field experiments on the use of expanded and shredded waste polystyrene beads to eliminate mosquitoes in pit latrines have been very successful (Sivagnaname et al., 2005). Traps placed over the squatting plate hole have also been developed and experimented with success at controlling insects in pit latrines (Lindsay et al., 2012). Pyriproxyfen, an insect juvenile hormone, and local soap have been found to reduce flies in pit latrines (Lindsay et al., 2013).

Improvements in the design of the pit latrine have also been done to minimise the smell and fly nuisance. Incorporation of a vertical vent pipe with a fly trap and the natural effect of the sun

and wind are the principle mechanisms for the functioning of a VIP latrine. The design makes use of circulation of air from outside the latrine, through the superstructure into the pit, then up and out of the vent pipe thereby exhausting any odours emanating from the faecal material in the pit via the vent pipe. (Ryan et al., 1983; Mara, 1984). The superstructure is kept dark to prevent flies from going into the latrine. The top of VIP vent pipe is fitted with a wire mesh fly-screen that prevents any flies inside the pit from escaping via the vent pipe where they die and fall back into the pit.

Experiments on the performance of VIP latrines in Zimbabwe showed that they were effective in smell and fly control compared to identical unvented pit latrines. However, the ventilation system was not as effective at mosquito control (Morgan et al., 1982). This was because while both flies and mosquitoes were drawn to odour sources in pit latrines, (Curtis & Hawkins, 1982) the latter have a positive phototropism and fly only towards light (Wagner & Lanoix, 1958). Contrary to the studies in Zimbabwe, field investigations undertaken by Cotton and Saywell (1998) in Ghana and Mozambique that were based on a user's perceptions recorded a higher degree of odour nuisance with the use of VIPs. In a recent study undertaken on pit latrines in Kampala Uganda, VIPs did not provide superior performance (smell, flies) to the simple pit latrines. Additionally, logistic regression showed that VIPs are not likely to smell less nor have fewer flies than simple pit latrines (Nakagiri et al., 2015). This was attributed to the VIPs not meeting minimum design standards, and overcrowding in the slums that could have impeded ventilation within the VIPs to achieve odourless conditions.

In order to understand the mechanisms inducing ventilation in the VIP design, field studies were undertaken in Botswana and Zimbabwe. Morgan et al. (1982) found that the action of the wind blowing across the top of the vent pipe induced ventilation. The effect of solar heating the vent was only negligible (Mara, 1984). Additionally, satisfactory odour control in VIP latrines was achieved with a ventilation rate of 10 m^3/h and 6 superstructure air volume changes / h (ACH). Another study by Dumpert (2008) on VIP latrines in the upper west region of Ghana found out that mechanisms driving ventilation were air buoyancy forces resulting in a stack effect at times in which ambient temperatures are less than temperatures inside the pit of the latrine; and suction wind passing over the mouth of the vent pipe and when possible wind passing into the superstructure. The study further noted that, majority of the latrines (73%) achieved ventilation flow rates greater than 10 m^3/hr. However, the flow rates were not adequate enough to achieve the 6 ACH as to maintain odourless conditions. The larger volume

of the pit latrine superstructures in the study in Ghana compared to those found in Botswana and Zimbabwe was noted to contribute to the low ACH. Additionally the vent pipe sizes were found to be inadequate, while most structures were constructed with openings and entrances facing away from the wind direction.

Other design improvements to the simple pit latrine that have been noted in literature to improve the odour and smell nuisance include the SanPlat pit latrine which consists of a thin circular dome shaped slab of the pit with no reinforcement and has a removable lid cast in the squat hole to ensure it fits tightly. Contrary to the VIP latrine where air is encouraged to flow through the structure, the SanPlat prevents air in and out flows of the pit. The opening into the pit is always kept tightly closed when not in use. Thus most odours remain within the pit and are assumed to be absorbed by the pit walls (Solsona, 1995). A pit latrine modification with a specially made bowl incorporated in the ordinary concrete slab uses a water seal to control odour and insects. About 1–2 L of water is usually poured by hand into the bowl to flush faecal matter into the pit (Franceys et al., 1992).

2.4 Knowledge gaps and way forward

There is high and increasing usage of pit latrines in urban areas of SSA. However their performance in terms of filling, smell and insect nuisance is not satisfactory. Available literature shows that contributions have been made to address shortfalls related to pit latrine use. These include issues relating to the latrine cleanliness, emptying and management of faecal sludge once it is removed from the pits. However, research on the technological aspects of pit latrines in urban slums are limited. However, further research within this area is needed. Knowledge gaps that can be identified from this review include:-

1) Sludge accumulation within pit latrines is a function of a number of variables. A clear understanding of sludge accumulation within pit latrines is essential. The use of sludge accumulation rates and number of users to determine pit sizes is not sufficient. Determining the exact number of users in highly populated areas is difficult. Incidentally, pit latrines also receive additional material other than human excreta and anal cleansing material. Collecting information on the actual pit sludge accumulation rates in different settings, taking into account other materials applied in pits during their use is important. This will in turn guide prediction of sustainability and aid in better pit latrine designs.

2) Research into processes taking place within the faecal matter in pit latrines is still new and limited. While sludge accumulation has been related to moisture, alkalinity and additive inoculum, the actual contents and factors that account for the decomposition process in pit latrines cannot be conclusively stated. It has been indicated that the decomposition process is variable and the environment of the pit is uncontrolled and is affected by the design, usage and geophysical and climatic factors. Additionally, the decomposition process is responsible for the smell and insect nuisances of pit latrines. There is need to understand the content and environment within the different pit latrine types. Furthermore, an understanding of organic matter decomposition, degradation pathways and fundamental factors controlling their occurrence and their relation to filling, smell and insects is essential.
3) Microorganism inoculums, earthworms and black soldier fly larvae have been used in degradation of organic matter with varying levels of success. However, the success in their application is strongly linked to the need to have the right organism biomass and optimization of the essential environmental factors (Zhu, 2000; Juwarkar et al., 2010). In the case of pit latrines, additives have been developed without a clear understanding of the content and environmental characteristics in the pit, yet they could affect the physio-chemical and biological processes of the additives used. Additionally, the composition of pit latrine additives and their optimal operation conditions are not known.
4) The smell and insect nuisances need to be clearly quantified. Currently odour meters have been invented that can be used to give different levels of smell. A clear understanding of the composition of pit latrine smell is essential so as to help find solutions to its reduction. Such techniques have been used in the perfume industry with success. However, as there maybe limitation on adaptation of the smelling techniques from the perfume industry in the study of pit latrines, obtaining clear representative gases for smell in pit latrines could help in research for their reduction.
5) Smell and insect nuisance in pit latrines are closely associated because flies are attracted by the smell from the pits (Wagner & Lanoix, 1958), while volatile compounds from pit latrines function as pheromones to attract gravid mosquitoes to suitable breeding sites (Mboera et al., 1999; Mboera et al., 2000; Olagbemiro et al., 2004; Huang et al., 2005). Technologies for eliminating the active pheromones compounds in the gases emitted from pit latrines, will contribute to mitigating the insect nuisance.

6) Determining the appropriate superstructure sizes and construction materials for pit latrines are also essential, as these have been found to affect smell and insects within pit latrines. This will also help in developing standards for pit latrine designs.
7) Beyond technology development and process management, proper construction and maintenance of pit latrines is essential. The importance of hygienic sanitation facilities has been demonstrated, and this is largely dependent on the users. Additionally, currently urban sanitation polices lack specification of minimum technology option and service standards. Besides enforcement of sanitation policies is often lacking (Bartlett, 2008). As household owners are unaware of alternative or better functioning pit latrine designs the quality of pit latrines constructed has been greatly compromised. To improve the situation, there is need to develop, disseminate and enforce pit latrine technology specifications and service standards for different target groups and to sensitise on the need for hygienic latrines.

2.5 Summary and Conclusions

The pit latrine is a sanitation technology that has been in use for a long time and the design has evolved over time. The technology is used by majority of the people in SSA, while its use in the urban areas is currently on the rise. The current trend of usage shows adaptation of more improved designs. From this review, it can be deduced that the performance in pit latrines in terms of filling, smell and insects within urban areas is an issue that needs further investigation.

Further, advances in pit latrine technology should focus on scientifically guided approaches to enhanced and sustainable sanitation. A precursor of understanding the content, environment, decomposition process, smell/ odour and insect composition is essential in predicting and favourably altering the conditions within the pit through technological novelty or process management. In addition, development, dissemination and enforcement of minimum pit latrine design standards for target groups is important while the importance of hygienic latrines should also be emphasized.

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CHAPTER THREE

3 Performance of pit latrines in urban poor areas: A case of Kampala, Uganda

Abstract

Demand for human excreta disposal in many urban poor areas of Sub-Saharan Africa (SSA) is met, predominantly by pit latrines. Efforts towards enhancing their performance will improve the sanitation in developing countries. This study aimed at determining the status of pit latrines (design, construction, operation and maintenance) and its influence on latrine performance (filling, smell and insect nuisance). The study was conducted on 130 pit latrines in typical urban poor areas of Kampala, Uganda. Data on design, construction, usage, operation and performance of the pit latrines was collected by interviews, observations and measurements; and analysed by descriptive statistics, bi-variate analysis and logistic regression. Results showed that the level of pit content was predicted by rain or storm water entry (β = 34.6), terrain (β = 5.3), and cleaning before or after use (β = 5.0). Smell was predicted by cleanliness (β = 97.6), stance length (β = 1.0), superstructure material (β = 0.01) and whether the latrine was private or public (β = 0.01). The predictor of presence of flies was the superstructure material (β = 70.6). To improve the performance of pit latrines in urban poor areas, researchers and practitioners should develop local latrine design standards (dimensions, construction materials and number of users) and cleaning guidelines for local policy makers to implement.

This chapter is based on:

Nakagiri, A., Kulabako, R. N., Nyenje, P. M., Tumuhairwe, J. B., Niwagaba, C. B., & Kansiime, F. (2015). Performance of pit latrines in urban poor areas: A case of Kampala, Uganda. *Habitat International*, *49*, 529-537. doi: http://dx.doi.org/10.1016/j.habitatint.2015.07.005

3.1 Introduction

Access to improved sanitation in urban poor areas of developing countries is low. Urban poor areas, commonly referred to as slums, are heavily populated areas, characterized by substandard and unplanned infrastructure, poverty, and lack basic services like water and sanitation (Struyk & Giddings, 2009; UN-HABITAT, 2009). Human excreta disposal in urban slums of Sub-Saharan Africa (SSA) is predominantly by use of pit latrines (Thye et al., 2011; Katukiza et al., 2012). Pit latrines have been adopted and are used because of their low cost, simplicity of construction and ease of operation and maintenance. However, their use in urban slums is characterised by several challenges. Jenkins et al. (2014) reported that some of the pit latrines in Tanzania did not meet the criteria of hygiene, safety and sustainability of sanitation systems because they were full or overflowing. Pit latrines were found to have high numbers of *Culex quinquefasciatus* mosquitoes, *Chrysomya putoria* and *Psychodidae* fly families, in central Tanzania (Irish et al., 2013). In Kenya, the disgusting smell of latrines prevented their use by primary school pupils (Caruso et al., 2014). Smell, flies and high filling rates are problems that have been associated with pit latrine use in Kigali, Rwanda (Tsinda et al., 2013). Recently, Kwiringira et al. (2014a) reported high pit filling rates and smell as barriers for latrine usage and subsequently open defecation, in slums of Kampala Uganda. Earlier, Tumwebaze et al. (2012), faulted smell as one of the reasons for user dissatisfaction with use of their pit latrines.

Understanding the design, construction, operation and maintenance of pit latrines within urban slum contexts could help come up with strategies to improve their performance in these settings. Research has shown that the presence of a door, superstructure quality (in terms of height and construction materials for walls) as well as the slab type, affect the cleanliness of a latrine (Sonego & Mosler, 2014). Absence of a roof over the latrine and temporary superstructures as opposed to brick superstructures positively correlated with high numbers of flies (Irish et al., 2013). Relatedly, models on pit latrine filling have shown that adding non-degradable material into the pit and water inflows significantly influenced its filling (Brouckaert et al., 2013; Todman et al., 2014). Although may not be statistically related, smell has also for long been known as a proxy for dirty toilets.

The aim of this study was therefore to determine the status of pit latrine structures, in terms of design, construction, operation and maintenance and the influence of these factors on their performance (filling, smell and insects nuisances) in a typical urban slum area.

3.2 Materials and Methods

3.2.1 Study area

This was a cross-sectional study conducted in Kampala, the capital city of Uganda. Kampala has a population of 1.79 million people (UBOS, 2013), of which about 60% resides in slums (Rugadya et al., 2008). This research is part of a study being undertaken to enhance the performance of pit latrines in slums of Kampala Uganda, focusing on Lufula Zone in Bwaise II Ward/parish, Kawempe Division. To get information more representative of Kampala, other zones within Bwaise II parish and slums spread across the five divisions of Kampala, which are known to house different ethnic groups were included in the study design. The slums outside of Bwaise II were, Kasubi in Rubaga Division; Naguru-Godown and Kinawataka in Nakawa Division; Kifumbira in Kawempe Division; Kisenyi in Central Division and Namuwongo in Makindye Division (Figure 3.1).

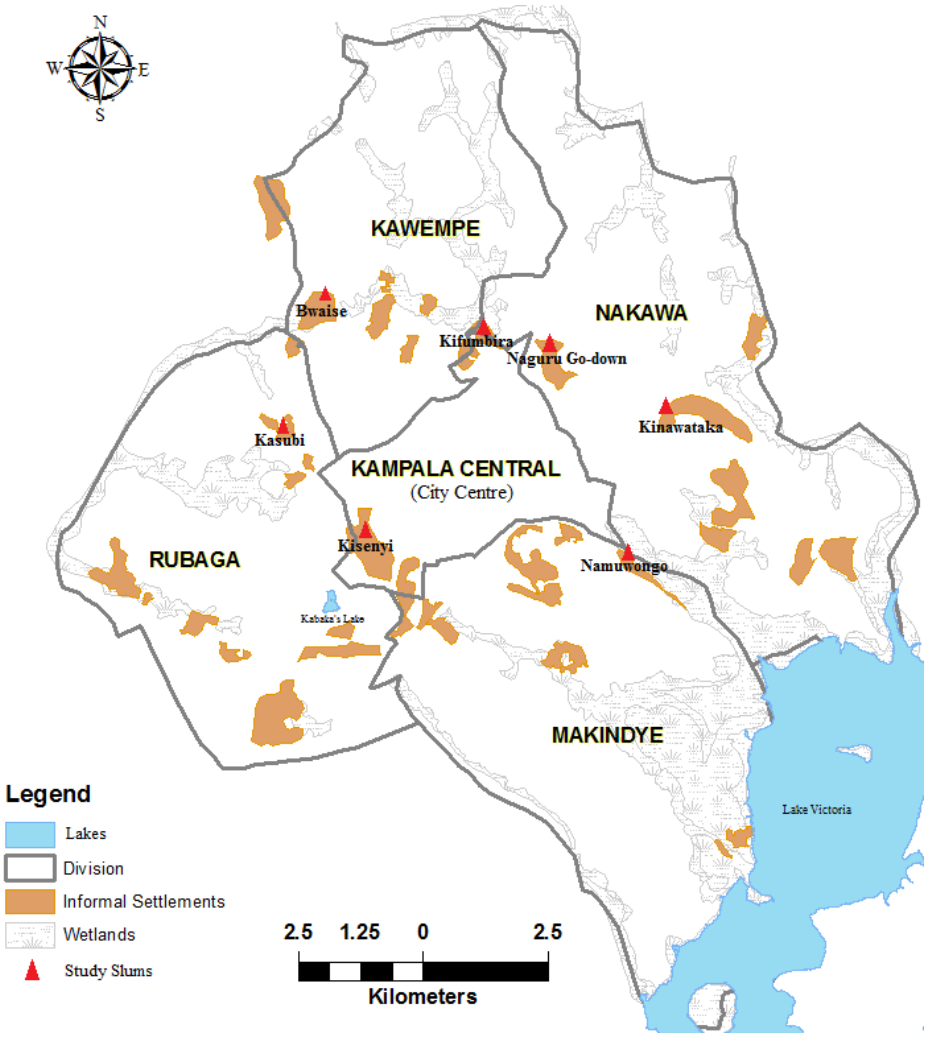

Figure 3.1 Map of Kampala Capital City showing the study slums

3.2.2 Data collection

Data were collected from traditional/simple and ventilated improved pit (VIP) latrines , which are used by over 95% of the households in slums of Kampala (Tumwebaze et al., 2012). All (38) pit latrines, were assessed within Lufula zone, Bwaise II. In addition, 44 pit latrines were randomly selected and assessed in the other zones of Bwaise II and 48 from other slums of Kampala, outside of Bwaise II. In total, therefore, 130 pit latrines were studied.

Data were obtained through field observations, measurements and user interviews. Data collected during observations and measurements of the pit latrines included facility design, stance size, materials used for construction, the structural condition of the latrine, presence of bad smell, and flies or other insects. Presence of bathroom, hand washing facilities and areas used for disposal of grey water were also noted. In addition, whether or not, the latrine had an access manhole for pit emptying was also noted. All information obtained was recorded in a pit latrine design assessment sheet. Questionnaires were used to record information obtained during the user interviews. The interview addressed the ways in which the pit latrines were operated, including public or private use, numbers and types of users, and the materials other than excreta, which were put in the pit. The details on latrine maintenance (cleaning and what is done when they are full) and user satisfaction were also noted.

3.2.3 Data analysis

The data were analysed using SPSS version 21. Descriptive statistics mainly percentages, means and standard deviations were used to describe the status of the pit latrines. Bivariate analysis (cross-tabulation and correlation) was used to establish variations within performance of pit latrines. The relationship between performance of pit latrines and their status was determined by binomial logistic regression, whereby a best fitting model was created, from which variables useful in predicting the performance factors were identified. The variables used in the regression analysis are listed in Table 3.1. All conditions for logistic regression including linearity and multicollinearity were satisfied. The difference in performance of the pit latrines between the flooded and non-flooded areas plus the different latrine design types was assessed using the ANOVA at a significance level of 95%.

Table 3.1 Variables used in the logistic regression of pit latrine performance

Study aspect	Variable name (Factor)	Scale (points)	Description	Parameter coding (1)	Parameter coding (2)	Assessment used
Design and construction	Type of pit latrine	2	Simple or traditional - basic pit with a slab and superstructure; VIP - pit latrine with a vertical vent pipe	0 = Simple or traditional 1 = VIP		Observation
	Type of slab	4	Slab material	0 = logs and mud; timber 1 = Smooth; cracked concrete slab		Observation
	Drophole cover	2	Cover on hole in the slab	0 = no cover 1 = cover		Observation
	Vent pipe	2	Vertical pipe from the pit	0 = no vent pipe 1 = vent pipe		Observation
	Superstructure walls	5	Material used for construction	1 = mud and wattle, polyethylene 0 = others	0 = other 1 = Timber, roofing sheets	Observation
	Doors	5		0 = Brick structures 0 = Timber, metallic, roofing sheets 1 = polyethylene, none	0 = others	
	Roofing	3		0 = roofing sheets 1 = polyethylene, none		
	Pit type	2	Ground level - Slab < 200mm above the ground Raised - Slab > 200mm above the ground	0 = Ground level 1 = raised		Measurement
	Nature of pit	2	Direct discharge (as shown in Figure 2 b), pit placed above and discharging into the drain. Containment pit (Figure 2a and c), stores waste until it is emptied.	0 = direct discharge 1 = containment		Observation
Structural condition	Sign of pit latrine collapse	2	Cracks in the latrine structure	0 = no cracks seen 1 = cracks seen structure		Observation
	Sign of rain or storm water entry	2	Entry of rain or storm water into the pit	0 = no rain or storm water entry 1 = rain/ storm water entry		Observation
Operation and maintenance	Private or public latrine	2	Public- facility open to everyone Private – use restricted to households it serves	0 = public 1 = private		Interviews
	How often the latrine is cleaned	3	When cleaning is done	1 = before or after use 0 = others 0 = daily	0 = others 1 = when dirty 0 = others	
Terrain	Non-flooding area Flooding area	2	Area with a low ground water table and does not flood in the rainy season. Located in a low- lying terrain with a high ground water table (<1.5m) and always experiences floods in the rainy seasons.	0 = non-flooding area 1 = flooding area		Assessment and interviews
State and performance	Level of pit content	4	Almost empty -greater than two meters below the slab; Half full- about one meter below the slab; Full - 250mm below the slab; overflowing – slab level and above.	0 = empty, half full 1 = full, overflowing		Measurement

Study aspect	Variable name (Factor)	Scale (points)	Description	Parameter coding		Assessment used
				(1)	(2)	
	Latrine cleanliness	4	Very clean - no liquid, solid on slab and walls; fairly clean - some liquid on the slab Dirty - some human excreta on the slabs and walls of the latrine: Very dirty - considerable amount of liquid and solid material on slab and wall of the latrine.	0 = clean, fairly clean 1= dirty, very dirty		Observation
	Latrine smell	5	No smell slight smell - little smell detected when within the superstructure moderate– smell detected when you are in the latrine strong smell – smell detected when outside the latrine; very strong smell – smell detected about 1 metre away from the latrine	1 = no smell, slight smell 0 = moderate, strong and very strong smell		Observation
	Latrine flies	3	No flies few flies very many	0 = no, few flies 1 = many flies		Observation
	Covariates					
Pit latrine design and construction	Latrine stance		Room on a latrine with a drophole			Observation
	Stance length		Distance from the door to the back of the latrine			Measurement
	Stance width		Distance from wall to wall			Measurement
Operation and maintenance	Households using the latrine		Households using the pit			Interviews

3.3 Results

3.3.1 Design and construction of pit latrines

The design, construction and structural condition of a pit latrine are important to ensure its proper functioning. The pit latrines in this study (Figure 3.2) were all rectangular in shape, with VIPs and simple/ traditional types at about 23% and 77%, respectively (Table 3.2). These were mainly built out of brick and plastered (77%), with timber doors (89%) and corrugated iron roofing sheets (91%) although there existed facilities with either polyethylene or mud and wattle walls (Table 3.2). The vent pipes of the VIPs were all made out of uPVC, mainly grey in colour (87%) and located within the superstructures (93%). Additionally, all vent pipes lacked fly screens. Majority of latrines were constructed using strong and durable materials which met the recommended standards.

Table 3.2 Design and construction materials of pit latrine structures

Variable (n=130)	Category	Number	Percentage (%)	Recommended standards (Wagner & Lanoix, 1958; Mara, 1984; Franceys et al., 1992)
Pit latrine type	VIP	30	23	
	Simple/ traditional	100	77	
Pit latrine shape	Rectangular	130	100	Rectangular, circular
Superstructure walls	Plastered brickwork	100	77	Bricks/ blocks, stone, sawn timber, bamboo, mud and wattle, Ferro-cement, plasticized material and galvanised/ aluminium sheets
	Brickwork - not plastered	16	12	
	Timber	7	5	
	roofing sheets	2	2	
	Polyethylene	4	3	
	Mud and wattle	1	1	
Roofing	Roofing sheets	119	91	Thatch, palm leaves, clay tiles, fibre cement, wood shingles and corrugated iron/ aluminium
	Polyethylene	6	5	
	None	5	4	
Doors	Timber	116	89	Sawn timber, metal and no door in case of spiral structures
	Metallic	1	1	
	Roofing sheets	2	2	
	Polyethylene	4	3	
	None	7	5	
Slab type	Concrete smooth finish	13	10	Reinforced concrete /brick-mortar, wood, timber and earth, fabricated slabs and plain concrete slabs – for only simple pit latrines
	Concrete cracked	111	85	
	Logs and mud	5	4	
	Timber	1	1	
Pit type	Raised pit	83	64	Raised pit (high water table, un even ground), else ground level
	Ground pit	47	36	
Nature of pit	Containment	126	97	Containment pit
	direct discharge into drains	4	3	
Vent pipe*	Vent has a fly screen	0	0	Fly screen on vent pipe
	Grey uPVC	26	87	Black colour, uPVC, brick/block and hollowed out bamboo
	Orange uPVC	4	13	

Note * n value for vent pipe = 30

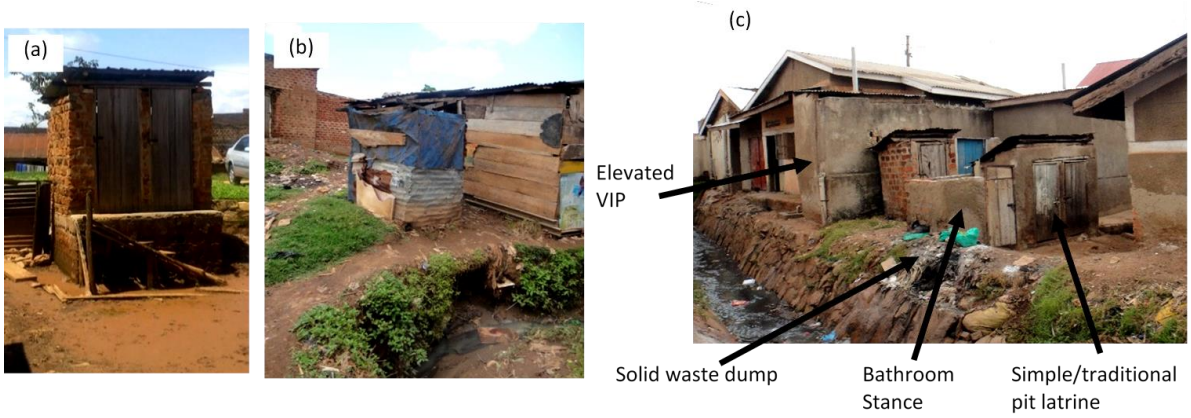

Figure 3.2 Pit latrine structures in Kampala urban slums (a) Elevated pit latrine in a flood prone area in Bwaise II Parish (b) Pit latrine constructed over and discharging directly into an open drain in Namuwongo (c) Elevated ventilated pit latrine and a simple pit latrine with an attached bathroom located in a non-flooding area

The number of stances per pit latrine ranged from 1 to 10, with a mean value of 2 (Table 3.3). The brick built structures had up to 10 stances, while timber, polyethylene, mud and wattle and roofing structures, were limited to pit latrines with 2 stances. All pit latrine superstructures were placed directly above the slab. The slabs were all squat type, majority made of concrete (95%) of which only 10% were found to be smooth (Table 3.2). The minimum drop-hole length was 180 mm and the maximum width was 150 mm (Table 3.3).

Table 3.3 Pit latrine measurements

Variable	***N***	**Min.**	**Max.**	**Mean**	**SD**	Recommended standard (Wagner & Lanoix, 1958; Mara, 1984; Franceys et al., 1992)
Stances number	130	1	10	2.4	1.5	
Stance dimensions						
Length (mm)	130	700	2060	1186	254	
Width (mm)	130	500	1800	918	206	
Height (mm)	130	1670	2200	1990	282	≥ 2000
Drophole dimensions						
Length (mm)	130	180	250	226	25	≥ 350 (to prevent soiling the drophole)
Width (mm)	130	100	150	114	22	≤ 200 (to prevent children from falling in)
Vent pipe dimensions (mm)	30	100	150	107	17	≥ 150 (for uPVC)
Height of stance above the ground for elevated latrines (mm)	81	400	2000	935	412	

Notes n= number, Min. = minimum, Max. = maximum, and SD = Standard deviation

The latrine slabs were placed directly over a single pit that was either sunk in the ground (36%) or elevated above the ground to a mean height of 935mm (Table 3.3). Raised pit latrines were found in both terrains (Table 3.4), although the number and height of pit were significantly higher ($p \leq 0.001$) in the flooding areas. Elevated pits were all constructed using plastered brick work. Access to the elevated pit latrines was by concrete steps (61%), ramp (10%) or ladders (25%) and in some cases none (4%). Some pit latrines (47%) were constructed with an attached bathroom stance (Figure 3.2), majority of which (82%) were discharging their greywater into open drains. Almost all pit latrines (98%) lacked hand washing facilities.

Table 3.4 Pit type and condition of pit latrines (n=130)

Variable	Category	Flooding area (%)	Non-flooding area (%)	Total (%)
Pit type	Raised pit	49	15	64
	Ground level pit	4	32	36
Structural condition of the pit latrine	Signs of collapse	28	11	39
	no signs of collapse	25	36	61
Storm water entry	Signs of rain/ storm water entry	30	10	40
	no signs of rain/ storm water entry	23	37	60
Total Pit latrines		53	47	100

Thirty nine percent of the pit latrines had cracks while 40% showed signs of rain or storm water entry (Figure 3.3). This indicates that some of the latrines were not structurally sound. Significant differences were noted between the structural condition of pit latrines in both terrains ($p \leq 0.001$) with more latrines having cracks and showing signs of rain or storm water entry in flooding areas. With regard to the construction materials, most of the plastered brick structures were structurally sound while more of the non-plastered ones showed signs of collapse and rain or storm water entry. All polyethylene and mud/wattle supper structures showed signs of collapse and rain or storm water entry (Figure 3.3).

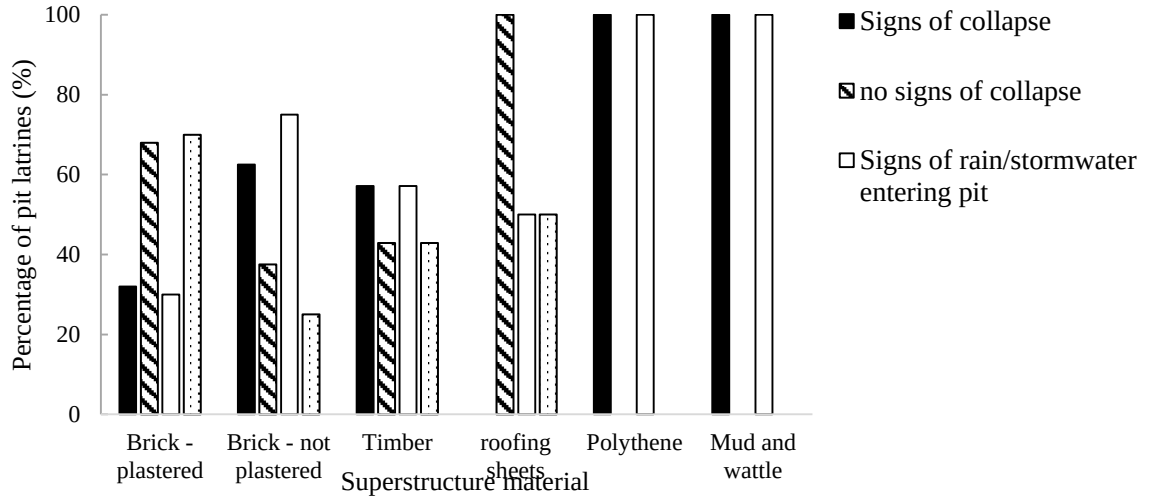

Figure 3.3 Structural condition of different pit latrine superstructures

3.3.2 Operation and maintenance of latrines

Operation and maintenance of a pit latrine is crucial for its performance. Majority of the pit latrines (85%) were operated as private to households, shared by mostly 5-8 households, although in some cases up to 20 households were found using a single latrine stance. The use of pit latrines as public facilities was at only 15%. The mean number of people per household

was 4.5. Children were present in majority of the households while only 8% of the respondents stated having elderly in their homes and 4% lived with people with disabilities (PWD). The use of pit latrines by children, elderly and PWD in this study was reported by 12% of the respondents.

Human excreta, sanitary products (baby diapers and menstrual pads), and anal cleansing material (85% of which were newspapers) were deposited in the pits. Very few respondents (4%) reportedly disposed solid waste/ rubbish in the pit latrines. The solid waste was dumped besides pit latrines as shown in Figure 2. Pit latrine cleaning was by use of water and detergents that ended up in the pit. Cleaning was mainly done (66%) before or after each use of the latrine and by every user (75%). Almost all the pit latrines (95%) contained their excreta until they were emptied. The rest discharged directly into open drains and these were all found in flooding areas. Additionally, pits in non-flooding areas were constructed as leach pits while those in flooding areas were said to be fully lined. Majority of the pit latrines had a filling time of 1 to 3 months, with longer filling time experienced in latrines located in non-flooding areas (Figure 3.4 a). Upon filling, 59% of the pit latrines were reportedly emptied, while 11% of the users supposedly dug new pits (Figure 3.4 b). Only 5% of the latrines were constructed with access manholes for emptying.

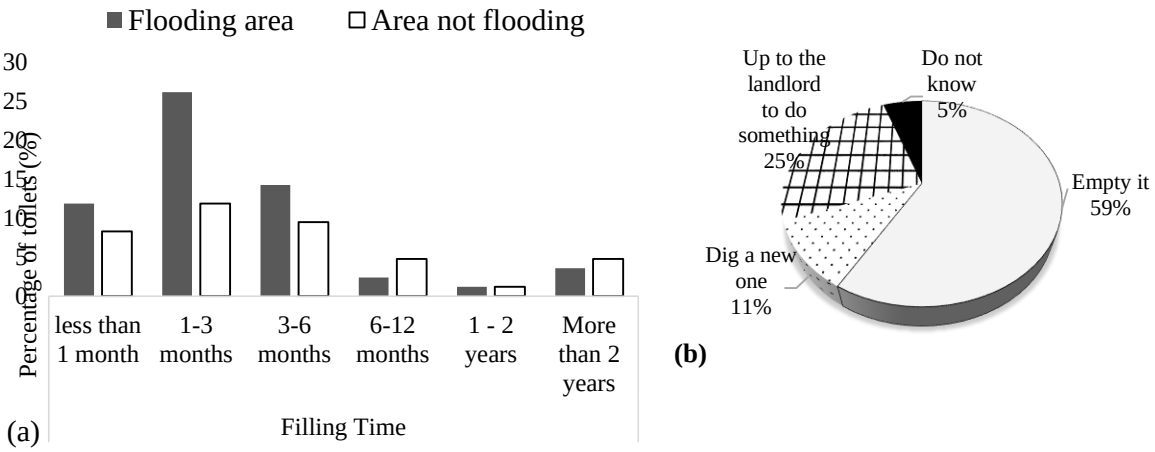

Figure 3.4 Pit latrine filling time; and (b) frequencies of action taken when the pit latrine is full

3.3.3 Performance of pit latrines

Majority of the latrines were full (51%) or over flowing (15%) (Table 3.5). A strong malodorous smell was noted in 39% of the latrines while few flies were found in majority (80%) of the latrines. Most of the latrines (43%) were dirty. Although respondents' satisfaction

with the use of their facilities was high (52%), majority of them (89%) expressed the need to improve the state and performance of the pit latrines.

Table 3.5 Performance of pit latrines

Performance variables	Pit latrines (%)
Level of pit content	
Almost Empty	7
Half full	27
Full	51
Overflowing	15
Smell of Latrine	
No smell	2
Slight smell	35
Moderate smell	22
Strong smell	39
Very strong smell	1
Fly presence	
No	3
Few	80
Many	17
Cleanliness	
Clean	18
Fairly Clean	22
Dirty	43
Very Dirty	18
User Satisfaction	
Yes	52
No	48

Comparison of means between pit latrines in different terrains (flooding to non-flooding) showed a significant differences in the level of pit content ($p = 0.036$) and smell of pit latrines ($p = 0.031$). Further, analysis was undertaken to assess the performance (smell and flies) of the different pit latrine designs (Figure 3.5). A comparison of performance variable means of the different pit latrine designs was only significant for smell. Significantly higher smell levels were noted in traditional compared to the VIP and simple pit latrines.

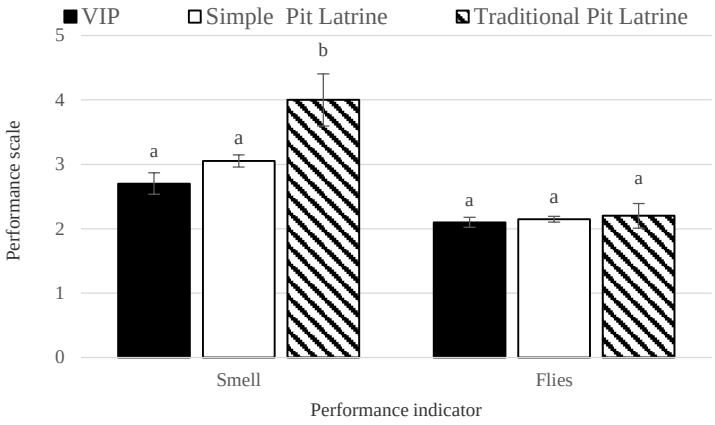

Figure 3.5 Smell and fly levels (Mean ± Standard error) in the different pit latrine designs within the slums. Means with different letters for perceived levels are significantly different (p< 0.05).

3.3.4 Relating status of the pit latrines to their performance

A logistic regression was performed to ascertain the effects of different design, construction, operation and maintenance variables on the likelihood that pit latrines were full, smelling or had flies. The results of the values of the chi-square distribution for the 3 models (level of pit content, smell and fly nuisance) were all significant at 5% level (Table 3.6). The models explained 59%, 75% and 51% (Nagelkerke R^2) of the variance in the pit content level, smell and flies in the latrines, respectively, indicating a moderately strong relationship between the predictors and performance variables.

The Wald statistics demonstrated that signs of rain/ storm-water entry (β = 34.6), flooding area (β = 5.3), and cleaning before or after use (β = 5.0) had a statistically significant relationship with the level of pit content. The odds that a pit latrine with signs of storm water entry being full are higher than those without signs. Pit latrines located in flooding areas were also more likely to be full than those in non-flooding areas, while frequently cleaned latrines (every before/after use) had a higher level of pit content.

Cleanliness was the strongest predictor (β = 97.6) of smell, implying that a dirty pit latrine was 97.6 times more likely to smell badly than a clean one. Other predictors with a notable small influence on smell were the stance length (β = 1.0), superstructure material (timber or roofing sheets, β = 0.01), latrine use by households only (β = 0.01) and cleaning before/after use (β = 0.02). The predictor of fly presence was superstructure material (β = 70.6). Timber /roofing sheet superstructures were more likely to have flies than those made of brick.

Table 3.6 Logistic regression predictors of pit latrine performance

Predictor Variables		Performance variables					
		Level of pit content		Smell of pit latrine		Fly presence	
		B(SE)	Odds Ratio	*B(SE)*	Odds Ratio	*B(SE)*	Odds Ratio
	(Constant)	-43.9		12.4		-40.9	
Design and construction	Stance length	0.0 (0.0)	1.0	0.0[b] (0.0)	1.0	0.0 (0.0)	1.0
	Superstructure (timber, roofing)	-2.0 (1.8)	0.1	-5.0[c] (2.0)	0.01	4.3[c] (2.1)	70.6
	Raised pit	-1.5 (1.2)	0.2	2.1[d] (1.1)	7.9	1.7 (1.4)	5.8
Structural condition of Pit latrine	Sign of pit latrine collapse	0.4 (0.9)	1.5	0.0 (1.1)	1.0	1.9[d] (1.1)	6.4
	Sign of rainstorm water entry	3.5[a] (1.2)	34.6	-0.7 (1.1)	0.5	-0.2 (1.1)	0.9
Operation and maintenance	Flooding area	1.7[d] (0.9)	5.3	1.2 (1.1)	3.3	-2.3[d] (1.2)	0.1
	Private to households only	-0.9 (1.7)	0.4	-4.5[c] (2.2)	0.01	20.9 (3E+04)	1E+09
	Cleaning - every after / before use	1.6[c] (0.7)	5.0	-3.7[b] (1.3)	0.02	1.3 (1.0)	3.6
State and performance of the latrine	latrine cleanliness	*NA*		4.6[a](1.1)	97.6	1.3 (1.3)	3.8
Notes:	B = regression coefficient SE = standard error R^2 = measure of goodness of model fit determined using the Cox & Snell and Nagelkerke approaches Method = the entry method	Model X^2 (15, N=108) = 61.4, p< 0.001, 81.5 (%predicted), R^2 = 0.43 (Cox & Snell), 0.59 (Nagelkerke), [a]p< 0.001, [b]p< 0.01, [c]p< 0.05, [d]p< 0.1		Model X^2 (22, N=107) = 86.8, p< 0.001, 86 (%predicted) R^2 = 0.56 (Cox & Snell), 0.75 (Nagelkerke),		Model X^2 (22, N=107) = 37.6, p = 0.02, 90.7 (%predicted) R^2 = 0.30 (Cox & Snell), 0.51 (Nagelkerke),	

Other variables that could influence the fly presence at a low significant level (p<0.1) were flooding areas and signs of collapse (Table 3.6). The type of the pit latrine; its slab; presence of a drophole cover; having a vent pipe; door and roofing material; nature of the pit; number of stances; stance width and number of households using the latrine were not significant predictors of the performance of the pit latrines. Although some of the variables were non-significant predicators, they significantly correlated with the performance of the pit latrines and can thus influence it. For instance, significant Pearson correlations were noted for containment pit (r = -0.249, p<0.001) and pit elevation (r = -0.240, p<0.001) with the level of pit content.

Binomial logistic regression analysis was carried out to predict the influence of the status of pit latrine structures on their performance in Lufula Zone in Bwaise II Ward/parish, Kawempe Division (Table 3.7). The results of the values of the chi-square distribution were significant for only the level of pit content and smell of the latrines. This indicates that the models including the predictors, significantly predict only the level of pit content and smell of the pit latrines.

Table 3.7 Logistic regression predictors of pit latrine performance in Bwaise II

	Performance indicators		
	Level of pit content	**Smell**	**Fly presence**
Model Chi-square	X^2 (1, N=38) = 12.14, p< 0.001	X^2 (2, N=38) = 24.803 p< 0.001	Initial -2log likelihood = 15.67
R^2 (Nagelkerke), %predicted	0.37 73.7	0.65 84.2	- 94.7
Significant predictors	***Variables in equation***		
	Households using the pit latrine *B(SE)* = 0.435(0.16)[b] Odds Ratio =1.55	Raised pit *B(SE)* = 3.311(1.16)[b] Odds Ratio =27.4 Latrine cleanliness *B(SE)* = 2.97(1.17)[c] Odds Ratio =19.46	Constant only
	Variables not in equation (all significant at p=0.1)		
	Flooding area Rain or storm water entry	Flooding area	Raised pit Superstructure material Cleanliness

Notes: [a]p< 0.001, [b]p< 0.01, [c]p< 0.05, [d]p< 0.1; B = regression coefficient; SE = standard error method - Forward Stepwise (Likelihood Ratio) method

Nagelkerkes R^2 indicated a moderately strong relationship between the predictors and smell (65%) and a weak relationship for level of pit content (37%). The main predictor of level of pit content was the number of households using the latrine. Other significant variables that did not contribute to the models ability to predict the level of pit content were flooding area and signs of rain or storm water entry. Smell was predicted, first by whether the pit was above the ground, followed by latrine cleanliness while fly presence had no significant predictors. Comparison of the results of slums in this study as a whole to those of Lufula zone (Table 3.6 and 3.7) indicates possibility of having different predictors of pit latrine performance between slums.

3.4 Discussion

3.4.1 Status of pit latrine structures

Majority of the pit latrines were simple or traditional, with only three VIPs in every 10 latrines. The design adapted consisted of a rectangular brick superstructure, with timber doors and roofing sheets, having single or multiple stances over a squat type slab cast directly above a single pit (Figure 1). From the technical perspective (Cotton et al., 1995) and the JMP classification of sanitation facilities (UNICEF & WHO, 2008), majority of the facilities in this study could be considered as improved pit latrines. However, there were differences and shortfalls in their construction, usage and performance.

In this study, raised pit latrines were also found in non-flooding area (Figure 3.4) perhaps due to the need for increasing the pit volume as siting new pits in slums is a challenge. The VIP latrines in this study did not meet the recommended design standards. Further, findings show that the use of polyethylene and mud/wattle structures may not be appropriate for urban slums. Majority of the pit latrines in this study were satisfactory for use by children as they had solid concrete slabs and a drophole width less than 200 mm (Mara, 1984; Franceys et al., 1992). However, access to pit latrines by PWD and the elderly was least considered in the design. Ramps were limited to only 10% of the pit latrines. This could be due to the low occupancy of PWD and the elderly within the slum households. Factors that could also have hindered proper construction of pit latrine structures are limited funds, lack of design knowledge and no enforcement to ensure good facilities (Medland et al., 2015).

The performance of pit latrines in this study was found to be inadequate. Seven in every ten pit latrines were either full or overflowing and majority of them filled in three months or less. The smelling nature of pit latrines and the presence of flies, which this study found, are consistent with findings from other studies conducted in urban slum settings (Kulabako et al., 2010; Irish et al., 2013; Jenkins et al., 2014). The use of full, smelly pit latrines that also have flies, is not only difficult but also risky to the health of the users as excreta is not properly isolated.

The use of private pit latrines shared by a number of households noted in this study is typical in slums of Kampala (Kulabako et al., 2010; Tumwebaze et al., 2012). Only about three in every ten latrines were used by the recommended four households according to the Uganda's national sanitation guidelines (MOH-Uganda, 2000) and upper limit for hygienic use of pit

latrines (Günther et al., 2012). Additionally, less were in use by two household threshold recommended by UN Habitat (UN-HABITAT, 2006). This signifies that pit latrines in urban slums of Kampala are over loaded.

Addition of waste streams other than human excreta in pit latrines was minimal in this study. Studies have reported presence of household rubbish/ garbage in pit latrine content (Buckley et al., 2008; Banks, 2014). The minimal disposal of other wastes in pit latrines could be due to the users being conscious of high filling rates of their latrines. Further, while disposal of sanitary wastes like baby diapers and menstrual pads in pit latrines was mentioned, earlier studies have shown that menstrual hygiene in slum is mainly by re-usable material owing to the expenses involved in buying pads (Kwiringira et al., 2014b). Therefore, other factors rather than external waste streams account for the poor performance of pit latrines in the studied slums.

3.4.2 Relating status of pit latrines to their performance

Logistic regression indicated a relationship between the status and performance variables of pit latrines in this study. Signs of rain or storm water entry, flooding and cleaning time were significant predictors of pit latrine filling. This is consistent with the findings in modelling pit filling by Todman et al. (2014), where it was noted that the flow and accumulation of water in the latrine has an important effect on the filling rate. Prevention of rain and storm water entry can be addressed in pit latrine construction through raising the slab to at least 150 mm above the ground and providing a roof on the latrine (Wagner & Lanoix, 1958; Franceys et al., 1992). However, in flooding areas, it will be necessary to raise the slab to a level above the highest flood level. The high level of pit content in flooding areas was probably because the pits were small and shallow. In high water table areas, lining of large volume pits is expensive while digging deep pits is hindered by the ground water table. Further, entry of groundwater into the pits cannot be ruled out. While raised pit latrines were reportedly fully lined, research has shown that the contamination of shallow aquifers in slum areas of Kampala is attributed to wastewater infiltration from pit latrines (Nyenje et al., 2013; Nyenje et al., 2014). Therefore, pit latrines are either not fully lined or they leach out liquid in surrounding soils. Additionally the cleaning before/after use by a high user number implies an increase of water input into the pit.

The predictors of smell found in this study were cleanliness, stance length, superstructure material, use by households only and cleaning after/ before use of the latrine. Studies directly relating smell to cleanliness are limited. However, smell has always been proxy for dirty toilets. Household use of latrines and cleaning before or after use could result in cleaner latrines that smell less. Sonego and Mosler (2014) found habitual cleaning behaviour to be the strongest predictor of latrine cleanliness.

The length of the latrine stance as a predictor of smell could be linked to an increase in volume/ size of the pit latrine structure, which decreases the air exchange rate. Superstructures made out of timber/ roofing sheets on the other hand, have a higher airflow rates. According to Mara (1984), at high air exchange rates, odours are less likely to accumulate within the superstructure. Additionally, the air exchange rate increases directly with ventilation rates, but decreases inversely with superstructure size. Flies presence was related to superstructure material. Superstructures made out of timber/roofing sheets have more light within the superstructure unlike brick structures. As flies are phototropic (Wagner & Lanoix, 1958; Irish et al., 2013), they will go inside the timber/ roofing sheet structures.

Interestingly, while the improved designs performed better than the traditional pit latrine, the VIP did not provide superior performance (smell, flies) to the simple pit latrine. Additionally, VIPs were not likely to smell less and have fewer flies than simple pit latrines because they were not meeting the minimum design standards (Tables 2 and 3). This finding is similar to the findings by Dumpert (2008) who noted inadequate VIP design as a hindrance to their proper functioning. The shortfalls in the VIP design could be attributed to limited knowledge of the users about pit latrine designs. Secondly, overcrowding in the slums could impede ventilation within the VIPs to achieve odourless conditions. Variations in predictors of performance were observed at individual slum level. This could be due to variations in characteristics between slums.

3.5 Conclusions

The design of pit latrines in Kampala slums was characteristically similar, but there were variations in the construction and operation/ usage and maintenance of the latrines. Further, the performance (filling, smell and insects' nuisance) of pit latrines was inadequate. Interventions to improve the performance of pit latrines should tackle their design and operation. Specific

considerations should focus on minimising water inflows into the pit, increasing the air flow rate, minimising light in the superstructures and ensuring cleanliness of the latrines. Additionally, determining and ensuring adaptation of appropriate pit latrine standards is important. The findings from this research provide important information for slum settlements, which are known to have varying characteristics and is very informative for local policy makers, practitioners, researchers and donor agencies. It provides a basis for design modifications and recommendations for pit latrine use in slums.

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CHAPTER FOUR

4 Assessing ambient and internal environmental conditions of pit latrines in urban slums of Kampala, Uganda: Effect on performance

Abstract

There is increasing interest to improve the functionality and performance of pit latrines in low-income urban areas. This study aimed at assessing the ambient and pit environmental conditions and their implications on the performance (smell and flies nuisances) of pit latrines. Forty two pit latrines were investigated in urban slums of Kampala, Uganda, through field observation and measurements of ambient and pit environmental conditions. The implications were assessed using oxygen reduction potential (ORP) and its association with smell/insect nuisances. The pit temperature (21 to 30.7 ^{o}C), pH (5.0 - 11.8) and ORP (-247 to 65.9mV) were consistently, significantly different (p<0.001) between the surface and 0.5m depth of pit content. The conditions in most (95%) pit latrines were anoxic (ORP < + 50mV), and mainly within the acid formation range (ORP -199 to -51mV). Most smelling pit latrines and flies were within the acid formation ORP range, with a significant association (Gamma, G=0.797, p=0.014) between ORP and smell in clean latrines only. The results suggest that ventilation of pit latrines within urban slums was not sufficient. Additionally, cleanliness, moisture reduction and waste stabilisation could address bad smells in pit latrines, ultimately improving their usage in urban slums.

This chapter is based on:

Nakagiri, A., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., Tumuhairwe, J. B., & Kansiime, F. (2017). Assessing ambient and internal environmental conditions of pit latrines in urban slums of Kampala, Uganda: Effect on performance. *Journal of Water, Sanitation and Hygiene for Development*. doi: 10.2166/washdev.2017.085

4.1 Introduction

The use of pit latrine in low-income areas of developing countries is high (Strande, 2014). In Sub-Saharan Africa (SSA) alone, over half of the urban population uses some form of pit latrine for human excreta disposal (Nakagiri et al., 2016). However, there is concern about their ability to provide adequate, safe, hygienic and sustainable sanitation access especially in low-income, densely populated unplanned urban areas (Jenkins et al., 2014). This is because most of them are usually full, over flowing, badly smelling, dirty, and insect infested (Nakagiri et al., 2015), which has led to user dissatisfaction and increased excreta related health risks, like open defecation, and improper pit emptying (Thye et al., 2011; Kwiringira et al., 2014). To improve the usage of pit latrines, there is a need to address these shortfalls.

Currently, there is high interest in understanding the occurrences in the pit to develop solutions to the improvement and management of pit latrines. Studies have assessed the physico-chemical, biological, and mechanical (thermal and rheological) properties of pit latrine content (faecal sludge) to give an indication of filling rates, understand and model degradation processes (Nwaneri et al., 2008; Brouckaert et al., 2013; Todman et al., 2015). Additionally, quantification and characterisation of the malodorous components of pit latrines has been carried out (Lin et al., 2013). Other investigations have focused on the efficiency of additives, (Taljaard et al., 2003; Buckley et al., 2008; Bakare et al., 2015), and developing pit emptying and faecal sludge treatment technologies (Radford & Sugden, 2014; Zuma et al., 2015). However, little attention has been paid to understanding the ambient and pit environmental conditions of latrines being used in various contexts and how these affect their performance, in terms of filling, smell and insects nuisances. Moreover, studies have shown that pit latrine functioning and contents are variable, affected by design, usage, maintenance, geophysical and climatic factors (Ryan & Mara, 1983; Bakare et al., 2012).

Information on the ambient (immediate surroundings) and pit environmental conditions could provide useful information for developing strategies to improve pit latrines. For example, ambient temperature, humidity, airflow patterns and air velocity are key factors to consider when determining ventilation and odour management in buildings (Aflaki et al., 2015) and are of special interest especially in ventilated improved pit (VIP) latrines (Ryan & Mara, 1983). During decomposition of organic matter, the environmental conditions control, ecological characteristics, microbial activity, biochemical conversions and volatilisation of gases. For

instance, the nature of degradation of the pit contents can be depicted by the environmental parameters like pH, dissolved oxygen (DO) and oxygen-reduction potential (ORP) which have for long been used in monitoring, control and management of processes in wastewater (Zipper et al., 1998; Lynggaard-Jensen, 1999). Additionally, most malodorous gases are weak acids or bases, whose volatilisation is affected by the chemical composition, pH, airflow rate and temperature at the gas- slurry surface (Blanes-Vidal et al., 2012).

The aim of this study was to assess the ambient and internal environmental conditions of pit latrines that could influence their functionality (smell, insect nuisance and thus usage) in a typical low-income urban setting. An assessment of the implication of the environmental conditions on the performance of pit latrines was also done.

4.2 Materials and Methods

4.2.1 Study area

This study was conducted in four (04) slums of Kampala, namely; Bwaise II and Kamwokya, in Kawempe Division, Kibuye I in Makindye Division and Nakulabye in Rubaga Division (Figure 4.1). The selection of the slums followed the criteria of having two types of terrains, low- lying with a high groundwater table (<1.5 m) and always flooding in the rainy seasons and the other with a low ground-water table. Pit latrines in areas with low groundwater table were sunk in the ground and unlined. Contrary, in high water table areas, pit latrines were constructed fully lined and some raised above the ground.

4.2.2 Data collection

In total, 42 simple pit and VIP latrines, located in both terrains were investigated; 15 in Bwaise II, 14 in Kibuye, 9 in Kamwokya and 4 in Nakulabye. Pit latrines selected were constructed out of brick superstructures and concrete slabs, and used by not more than four households per toilet stance. Pit latrines made of brick superstructures and concrete slabs are the most commonly used facilities within Kampala slums, which provide superior performance (reduced smells and insect nuisances) to other structures such as traditional latrines (Chapter 3) (Nakagiri et al., 2015) and are considered improved according to UNICEF and WHO (2008). Furthermore, latrines used by not more than four households (or about 20 individuals) per toilet stance ensure long-term hygienic and sustainable use (Günther et al., 2012).

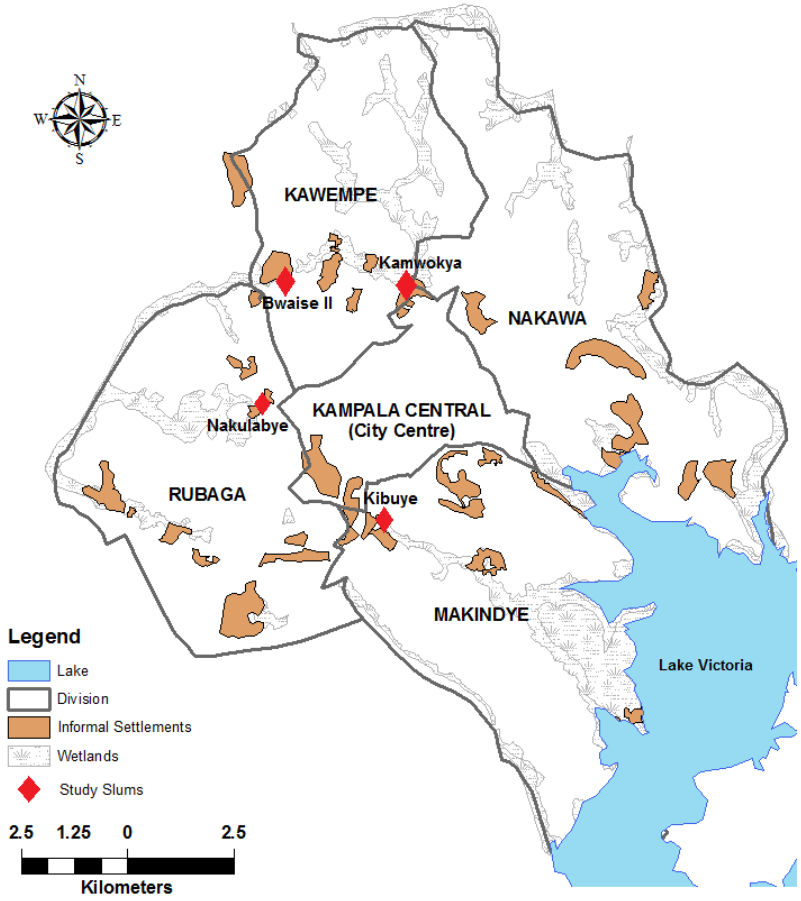

Figure 4.1 Map of Kampala Capital City showing the study areas

Information collected in this study included the ambient conditions in the slums, general characteristics of the pit latrines, and environmental conditions in the pit. Ambient conditions of temperature, wind speed and humidity in and around the pit latrines, were measured using a pocket weather meter (Kestrel 4000, USA). The general characteristics of the pit latrines included the latrine dimensions, state and odour strength. Measurements of latrine stance dimensions (length, width, height), and depth of pit content from the drophole were taken using a laser distance meter (Excelvan 60m, USA). The latrine condition, clean or dirty; smelly or not; and presence of insect or not, were noted through observation, based on a scale used in the study in Chapter 3 (Table 3.1) and (Nakagiri et al., 2015). Odour strength levels were taken from within the super structure of the pit latrine with the door shut, using a handheld odor meter (Shinyei OMX-ADM, Japan). To determine the environmental conditions in the pit, samples of the content were obtained at the surface (0 m), 0.5m, and 1m depth below the surface of the content, using a fabricated multi stage sludge sampler (Water For People, Uganda) (Figure

4.2). The pH, temperature, DO and ORP of the pit content were measured as soon as a given sample was obtained, using portable meters (Hanna HI991003, USA and Milwaukee MW600, USA). ORP was selected as it distinguishes well the biological processes, because it measures the net value of all oxidation-reduction reactions in an aqueous environment. Additionally, factors contributing to the electron activity such as pH, temperature, biological activity and chemical constituents of the system are reflected by ORP (Peddie et al., 1990). This study was carried out between September 2015 and December 2015. The information was collected during the day, between 9:00am and 3:00pm.

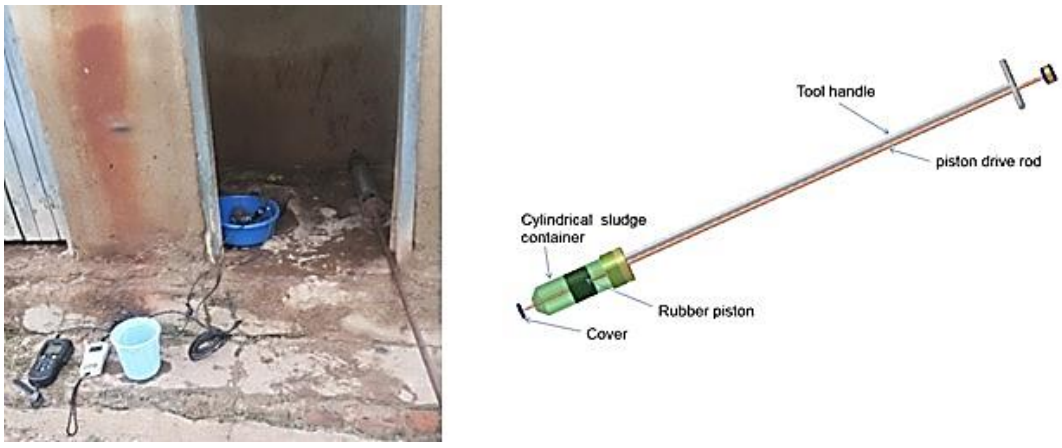

Figure 4.2 Fabricated multi stage sludge sampler used to obtain pit contents

4.2.3 Data analysis

Data analysis in this study was done using SPSS version 21. The characteristics of the ambient and environmental conditions of the pit latrines investigated were presented using descriptive statistics and box and whisker plots. Significant variations in the environmental conditions around the pit latrine and pit content variables, with respect to location, terrain and pit latrine type, were assessed using correlations, student's t-tests and analysis of variance (ANOVA).

Implications of the environmental conditions in the pit on the performance of the latrines was done in two stages. First, variation of the ORP at different depth was presented categorically using horizontal bar charts. The ORP categories (Appendix Table A.3) were adopted from literature and the redox tower, while chosen parameters were those known to impact on the performance of pit latrine (Gerardi, 2008; Madigan et al., 2015). The ranges used in this study were < -200 mV (reduction of sulphur compound, acetate fermentation and methane formation; -199 to -51 mV (acid formation); -50 to +49 mV (nitrate/ nitrite reduction) and > +50 mV

(aerobic degradation). Secondly, an association between the environmental conditions (ORP) and smell (smelly, no smell)/ insect (present, none) nuisances was assessed by cross tabulation using Goodman and Kruskais' gamma (G). This was limited to only the surface of the pit content because that is where volatilisation of malodorous compounds into the gaseous state occurs for them to be smelt while flies are drawn to the matter at the pit surface.

4.3 RESULTS

4.3.1 General characteristics of pit latrines

The study involved 45% simple and 55% ventilated improved pit latrines with mean dimensions of 1270 mm (length), 928 mm (width) and 1871 mm (height) (Table 4.1). The pit latrines were within 911 (±526) mm of filling, with 74% exhibiting a strong smell, 53% had few flies, 52% were dirty and had odour strength levels ranged from zero to 999 (odour meter limit), indicating inadequate performance (Table 4.2).

Table 4.1 Pit latrine stance dimensions, content level and odour characteristics

Variable	Category	N	Mean(± SD)	Min - Max
Stance dimensions	Stance length (mm)	42	1271(±233)	790 - 1800
	Stance width (mm)	42	928(±274)	600 - 1800
	Stance height (mm)	42	1871(±275)	800 - 2560
	Stance volume(m^3)	42	2.2(±0.75)	0.99 – 3.93
Pit content	Distance from drophole to pit content surface (mm)	42	911(±526)	0 - 2320
Odour	Odour strength level	40	484(±440)	0 - ≥999

Table 4.2 Type and performance of pit latrine structures

Variable	Category	Number	Percentage (%)
Pit latrine type	Simple	19	45
	VIP	23	55
Smell of latrine	No smell	6	14
	Slight smell	3	7
	Moderate smell	2	5
	Strong smell	23	55
	Very strong smell	8	19
Fly presence	No flies	22	53
	Few flies	16	38
	Many flies	4	9
Cleanliness	Clean	8	19
	Fairly clean	12	29
	Dirty	20	47
	Very dirty	2	5

4.3.2 Ambient conditions around and inside the pit latrine structures

The temperatures around the pit latrine structures ranged from 23.3 to 34.3 ^{o}C while the relative humidity recorded was between 38.8 and 71.4% (Figure 4.3 a and Appendix Table A.4). The wind speed varied from zero to 1.8 ms^{-1}. The range of ambient conditions inside the superstructures was 22.5 – 34.2 ^{o}C for temperature, 29.7 – 73.6% relative humidity and 0.0 to 0.6 ms^{-1} for wind speed. Analysis of variance revealed significant differences ($p \leq 0.001$) in the ambient conditions between the slums (Figure 4.3 b), while none were found with respect to pit latrine type (simple or ventilated) and terrain. This implied that variations in ambient conditions are influenced by location. There was a strong significant correlation between the ambient conditions (temperature r = 0.87, N = 42, $p \leq 0.001$ and relative humidity r = 0.74, N = 42, $p \leq 0.001$) around and inside the pit latrine structures and none with respect to wind speed. The temperature and relative humidity within the pit latrine structures increased consistently with an increase in the same conditions outside. However, wind speed outside the superstructure did not directly influence the wind speed inside the superstructure.

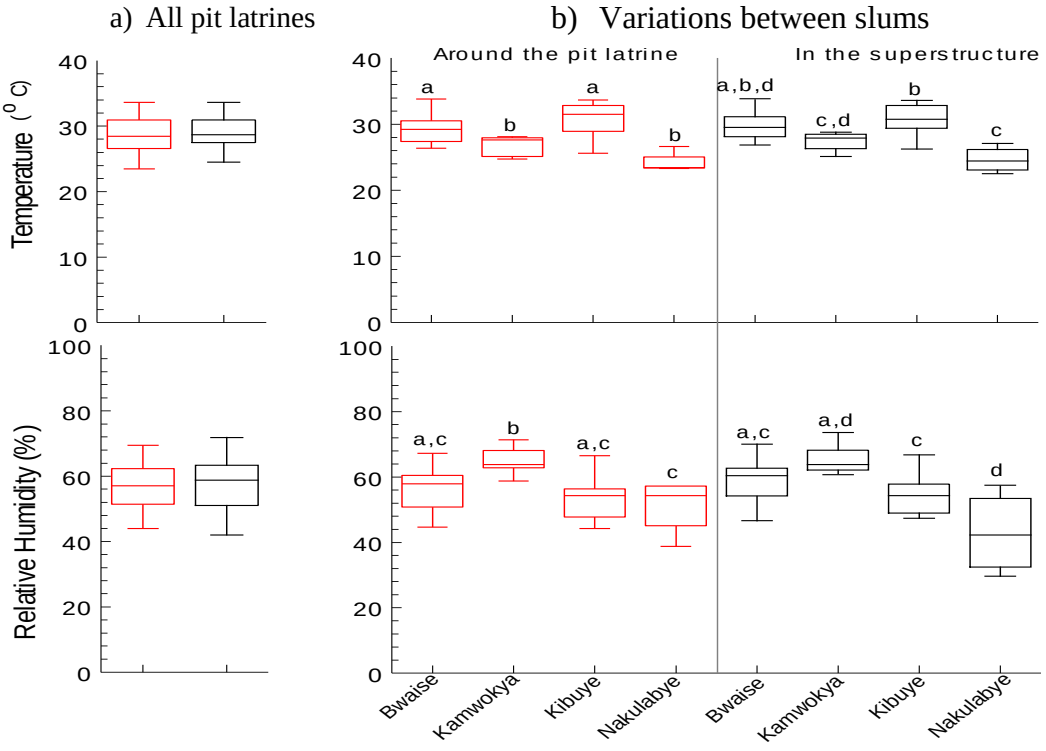

Figure 4.3 Ambient conditions around and inside pit latrine structures. Box represents 50% of the data points, whiskers represent minimum and maximum, line in box represents the median. Graphs with different letters (a, b, c and d) are significantly different from each other, $p<0.05$.

4.3.3 The environmental conditions in the pit

The environmental conditions in the latrine pit are presented in Figure 4.4 and Appendix Table A.5. The temperature inside the pit ranged from 21 ^{o}C to 30.7 ^{o}C, with mean ± standard deviation values of 26.6 ±2.3 ^{o}C (surface), 24.8 ±1.1 ^{o}C (0.5 m), and 23.78 ±0.5 ^{o}C (1 m) at the different depths. The pH of the pit content was between 5.0 and 11.8 while DO concentrations of 0 to 2.4 mg/L were recorded. The ORP ranged from -247 to 65.9mV. Analysis of variance revealed significant differences in the environmental conditions with respect to slums and none with respect to pit latrine type and terrain. This implied that variations in pit environment could be influenced by only location and not the pit latrine type or the terrain.

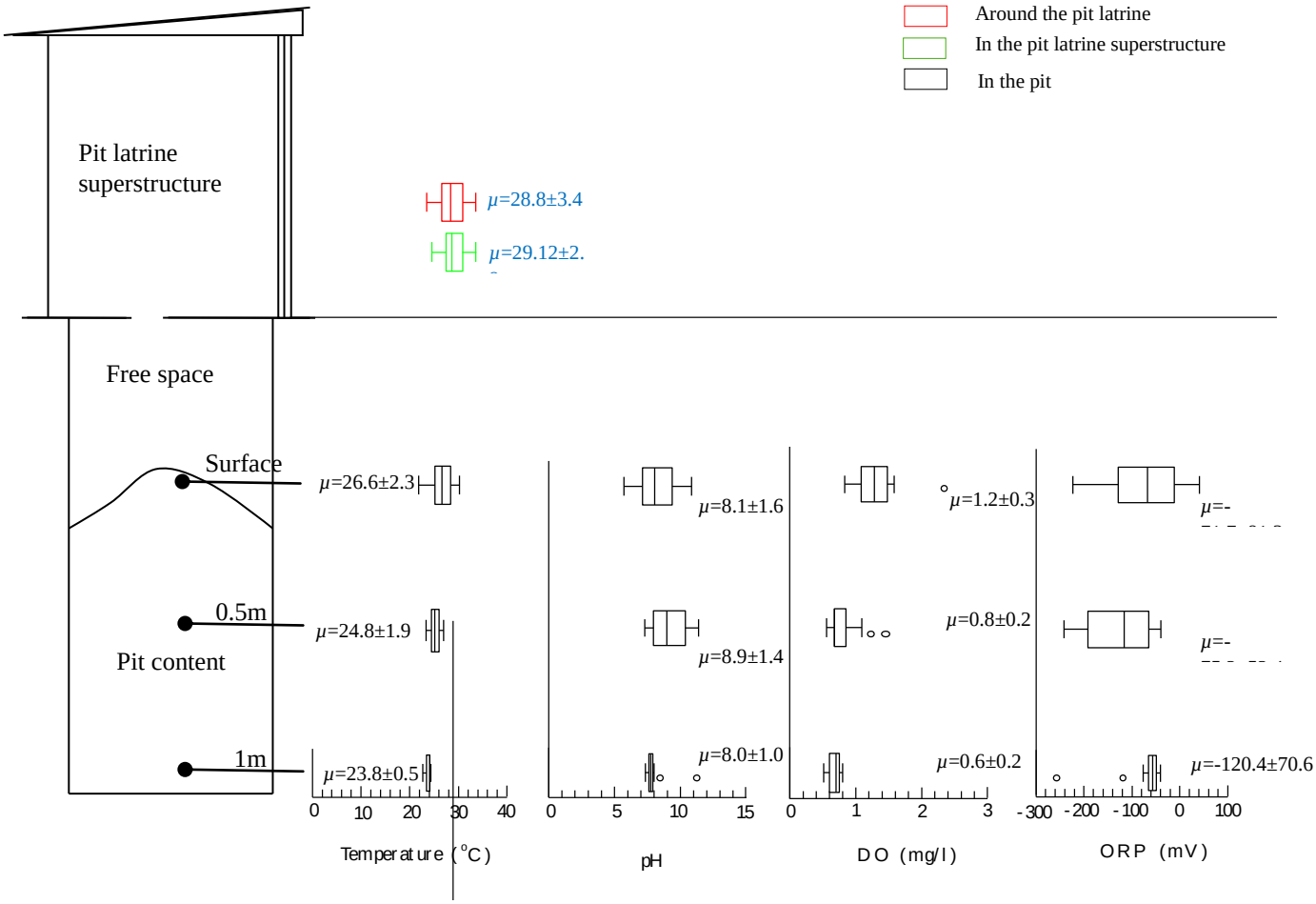

Figure 4.4 Environmental conditions in the pit. Box represents 50% of the data points, whiskers represent minimum and maximum values, lines in the box represent the median. *μ* is the mean ± standard deviation.

There was a significantly strong correlation between the ambient temperature and that at the surface of the pit content (r = 0.57, N = 42, $p \leq 0.001$; around the superstructure vs pit content

and r =0.50, N = 42, $p \leq 0.001$; in the superstructure vs pit content). The temperature at the surface of the pit content in over half of the pit latrines was consistently lower than the ambient temperature. Further, significant t-statistics (t (41) = 5.4, p< 0.001; around the superstructure vs pit content and t (41) = 6.4, p < 0001; in the superstructure vs pit content) of the ambient conditions and the pit content imply that the variation is not due to chance.

Analysis for associations in the environmental conditions at different depths revealed significant Pearson's correlation coefficients (Table 4.3). The temperature and ORP dropped with increase in depth and this was consistent in a number of the pits. A consistently significant (r =0.84, N=42, $p \leq 0.001$) increase in pH was also noted between the content at the surface and at 0.5 m and it decreased significantly at 1 m in most of the pits. In addition, a drop in DO was noted with increased depth. However, the drop was significant and consistent only between the content at the surface and that at 0.5m. Between 0.5 m and 1 m depth, the results show an overall decrease in DO. However, the correlation was not significant, implying that the decrease was not consistent across the pit latrines.

The paired t-statistics values (Table 4.3), were significant ($p \leq 0.001$) for all the pit environmental conditions at the surface and at 0.5m. This implied that the average changes in the values of each parameter was not by chance, and could thus be explained. Thereafter, between 0.5 m and 1 m the noted change was due to chance variation.

Table 4.3 T-test of different environmental variables at different locations

Parameter pairs	**Correlations**			**T-test**		
	N	**Pearson's**	**Sig.**	**t**	**df**	**Sig. (2-tailed)**
Ambient and pit content temperature						
Around latrine – Surface of pit content	42	0.57	0.000	5.4	41	0.000
In the superstructure – Surface of pit content	42	0.50	0.001	6.4	41	0.000
Pit Content						
Temperature (Surface and 0.5m)	42	0.60	0.000	6.1	41	0.000
Temperature (0.5m and 1m)	14	0.77	0.001	1.3	13	0.212
pH (Surface and 0.5m)	42	0.84	0.000	-6.0	41	0.000
pH (0.5m and 1m)	14	0.88	0.000	-1.2	13	0.252
DO (Surface and 0.5m)	27	0.55	0.003	8.4	26	0.000
DO (0.5m and 1m)	13	0.11	0.729	2.3	12	0.038
ORP (Surface and 0.5m)	41	0.85	0.000	7.2	40	0.000
ORP (0.5m and 1m)	14	0.87	0.000	1.1	13	0.263

4.3.4 Implications of the pit environmental condition on the performance of pit latrines

Categorical variations of oxygen reduction potential in the pit

The environmental conditions in the pit latrine have an implication on the nature of biological reactions, which in turn affects performance of the pit latrine. To depict the biological reactions in the pit latrines, ORP values have been presented categorically (Figure 4.5). It was noted that, 95% of the pit latrines were anoxic (ORP values less than + 50mV). Aerobic conditions (ORP values greater than + 50mV) were noted at the surface of the pit content of only 5% of the pit latrines.

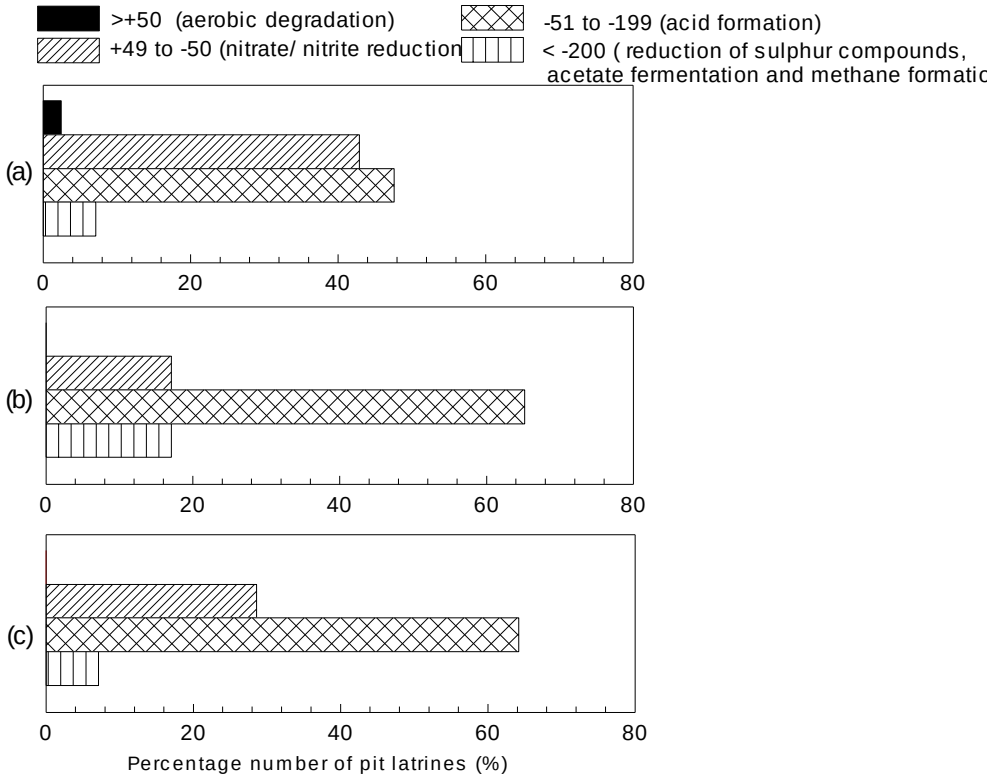

Figure 4.5 Pit latrine ORP ranges at different depth of the pit content. (a) Surface of the pit content, (b) 0.5m below the surface, and (c) 1m below the surface of the pit content

The ORP levels for samples taken from the surface of the pit content (Figure 4.5 a) in majority of the latrines (48%) were within the acid formation range (ORP values -199 mV to -51 mV), while 43% of the pit latrines were within the nitrite/ nitrate reduction level (ORP values of -50 mV to +50 mV). Reduction of sulphur compounds (ORP < -200 mV), methane formation (ORP < -240 mV) and acetate fermentation (ORP < -280 mV) conditions were in only 7% of the pit latrines. At increased depth below pit surface (Figure 4.5 b and c), acid formation (-199

mV to -51 mV) was common in majority of the pit latrines. In addition, at 0.5m, there was a decrease in the number of pit latrines in the nitrite/ nitrate reduction range (-50 mV to +50 mV) to only 17% and an increase in those in the sulphur, acetate reduction and methane formation range to 17.1%.

Relating ORP categories to smell and flies in pit latrines

Cross tabulation of ORP with smell and flies nuisances (Table 4.4 and 4.5) revealed that most smelling pit latrines (n = 17) and flies (n = 10) were found within the ORP ranges of – 199 mV to -50 mV followed by -50 mV to +50 mV (n = 12; smell and n= 8 flies). However, gamma analysis for association showed no significant correlations between ORP and smell (G = 0.483, $p = 0.115$) or flies nuisances (G = 0.081, $p = 0.767$). Results from cross tabulation analysis of only clean pit latrines (Table 4.4 and 4.5), showed that smell and flies were also found mainly within the ORP ranges of – 199 mV to -50 mV (n = 9; smell and n = 5; flies). The clean pit latrine with ORP range less than -200mV was also smelling and had flies.

Table 4.4 Cross tabulation of ORP ranges with smell

	All pit latrines			**Clean pit latrines**			**Dirty pit latrines**		
	no smell	**smell**	**Total**	**no smell**	**smell**	**Total**	**no smell**	**smell**	**Total**
>+50	0	1	1	0	0	0	0	1	1
+49 to -50	6	12	18	5	2	7	1	10	11
-51 to - 199	3	17	20	3	9	12	0	8	8
<-200	0	3	3	0	1	1	0	2	2
Total	9	33	42	8	12	20	1	21	22

Table 4.8 Cross tabulation of ORP ranges with flies nuisance

	All pit latrines			**Clean pit latrines**			**Dirty pit latrines**		
	no flies	**flies**	**Total**	**no flies**	**flies**	**Total**	**no flies**	**flies**	**Total**
>+50	0	1	1	0	0	0	0	1	1
+49 to -50	10	8	18	5	2	7	5	6	11
-51 to - 199	10	10	20	7	5	12	3	5	8
<-200	1	2	3	0	1	1	1	1	2
Total	21	21	42	12	8	20	9	13	22

Gamma analysis for associations within clean pit latrines showed a strong positive correlation between ORP and smell, which was statistically significant (G=0.797, p=0.014). However, while there was a moderate correlation between ORP and fly nuisance, it was not statistically significant (G = 0.451, p = 0.277). The results indicate that as ORP at the surface of the pit content decreases, there is likely to be smell and flies in pit latrines. However, the relationship is only statistically significant for smell among clean pit latrines. There was no significant correlations for ORP and smell (G = 0.818, p = 0.306) or fly nuisances (G = 0.70, p = 0.849) among the dirty latrines.

4.4 DISCUSSION

The study aimed at assessing the ambient and environmental conditions of pit latrines and their implication on the performance of pit latrines. General assessment of level of pit content, cleanliness, smell and flies showed that while some pit latrines are considered improved, they do not provide hygienically safe access as sanitation facilities. Moreover, earlier studies have shown that full, dirty, smelling latrines that have flies are related to user dissatisfaction and are often abandoned for open defecation (Tumwebaze et al., 2012; Kwiringira et al., 2014), posing a risk to public health. These findings are consistent with previous studies in urban slums (Kwiringira et al., 2014; Nakagiri et al., 2015; Okurut et al., 2015).

The ambient temperature (ranging 23.3 to 34.3 ^{o}C) and relative humidity (29.7 – 73.6%) around the pit latrine superstructure is typical of that of tropical climates (18 – 35 ^{o}C) (Pidwirny, 2011). However, the results of the study showed low wind speeds (0.56 ±0.46 m/s) and in some cases there was no wind movement (0 m/s). Previous studies done in Botswana and Zimbabwe found wind speeds of 2 m/s and above (Ryan & Mara, 1983). The low ambient wind speeds in this study could be attributed to obstructions from the surrounding buildings as pit latrines in urban slums are placed close to the houses due to overcrowding. While ventilation pipes are meant to increase air flow in the latrines, the results from the study showed very low air movements within the superstructures. This is attributed to shielding of the ventilation pipe on the VIP latrines by neighbouring buildings within the slum settlement, which block air movement. Secondly, air movement in VIPs is also constrained by inappropriate vent pipe sizing and location of openings (Nakagiri et al., 2015).

The environmental conditions of the contents from the pits in this study (temperature, 21 ^{o}C to 30.7 ^{o}C; pH, 5.0 to 11.8 and DO, 0 mg/L to 2.4 mg/L) varied significantly with location and

not according to pit latrine type nor terrain. This could be because classification of simple and VIP latrines is determined by presence of a vent pipe on the superstructure and not the nature of the pit. However, variations based on slums could arise from the differences in characteristic between slums. Previous studies have reported temperature of 24.2 ^{o}C – 26.2 ^{o}C and DO of 0.9 mg/L – 1.72 mg/L (Kimuli et al., 2016); temperature 25.5 ^{o}C - 33^{o}C and pH 5.2 -8.2 (Irish et al., 2013). Other studies found pH ranges of 7.31 - 9.01 (Wood, 2013), 6.4 – 6.9 (Appiah-Effah & Nyark, 2014) and from 5.3 – 7.5 (Rose et al., 2015). The higher pH within the pits in this study may be attributed to accumulation of ammonium ions from urea in the pit latrines.

There was a significant difference in the environmental conditions between the surface and that at 0.5 m depth of the pit content and not thereafter, which could be attributed to the different stages of faecal matter degradation and associated physical state, chemical and biological processes in the pit. This observation is in agreement with previous studies (Buckley et al., 2008; Bakare, 2014) which showed that degradation of matter occurred from the surface down to some section of the pit.

The ORP values have for long been used to depict different cellular activities of organic matter degradation (Koch & Oldham, 1985; Ndegwa et al., 2007). Even with the occurrence of DO in the pit content, ORP ranges show that the main form of degradation in majority (95%) of the pit latrines was anaerobic. This is contrary to assertion by Nwaneri et al. (2008) that rapid degradation of matter under aerobic conditions occurs at the surface of the pit content until the material is covered. The difference in findings could be attributed to high moisture content (about 80%) (Kimuli et al., 2016) and low air circulation indicated by the low wind speed observed in the pit latrines in this study. Among the causes of high moisture content includes cleaning the latrines before/ after use, by every user and directing the wash water into the pit and in some cases use of the facility as a bathroom (Nakagiri et al., 2015). In addition, the pit latrines are without urine diversion, thus human excreta (faeces and urine) is collected in the same pit.

Anaerobic degradation of organic matter is normally considered a two stage process involving acid formation (hydrolysis) and waste stabilisation, where microorganisms exploit any oxidation-reduction reaction resulting in formation of recalcitrant stable compounds (McCarty, 1964; Rittmann & McCarty, 2001). From the results of this study, hydrolysis may not be a limiting stage in anaerobic degradation, as majority of the latrines were in the acid formation

(-199 mV to -51 mV) range. This is further supported by the pH range (5.0 to 11.8) of the pit contents in this study and volatile organic compounds reported in other studies (Lin et al., 2013). Material stabilisation as noted in this study could have been dominated by denitrification, while optimal ORP ranges for reduction of sulphur compounds (ORP < -200mV), methane formation (ORP < -240) and acetate fermentation (ORP < -280 mV,) were not attained in most of the pit latrines. Inhibition for stabilisation (through methanogenesis) within the pits could have resulted from the high pH ranges as optimal pH ranges for methanogenic bacteria is 6.5 to 7.5 (Parkin & Owen, 1986).

Smelling clean pit latrines in this study were in the acid formation range (-199 mv to -50 mV) and ORP range less than - 200 mV. These finding are in agreement with Lin et al. (2013) who characterised a range of volatile compounds in faecal sludge from pit latrines. Further, the study showed that with a decrease in ORP in clean latrines, smell was more likely to be evident. However, flies were not significantly associated with an increase in ORP, possibly because of their phototropic nature. In addition, the dirty nature of the pit latrine could have contributed to lack of association between ORP and smell in those latrine.

4.5 Implications and Conclusion

The findings of this study suggested that improvements to the functioning of pit latrines in urban slums should consider, the ambient and internal environmental conditions and their location. The results showed that natural ventilation of pit latrines by introducing a vent pipe was not effective because of overcrowding in urban slums. This implied that improving ventilation in pit latrines in slum settings may necessitate introduction of mechanical devices to increase air flow in the structures.

Furthermore, the results showed a relationship between the environmental conditions in the pit (represented by ORP) and the performance (smell and flies nuisances). However, the association was statistically significant for only smell in clean pit latrines. This implied that changes in the biological processes in the pit could only affect the smell of the latrine and be effective when they were keep clean.

Reducing the moisture content by limiting the amount of water that gets into the pit, will improve the aerobic nature and processes of the pit content (realised by an increase in ORP), resulting in reduction in smell. This could be attained by use of urine diversion inserts in the

dropholes of the existing latrines. In addition, behaviour change could be attained though sensitisation to ensure that latrines are cleaned once a day, by mopping or directing the wash water into a separate soak away, and are not used as bathrooms. Attaining material stabilisation by use of microorganisms and/or enzymes operating within the noted environmental conditions could be sufficient. This could affect the conversion of intermittent compounds, most malodorous by nature to more stable compounds. Finally, besides pit latrine cleanliness, interventions to fly nuisances could look at entomological studies into the types of flies and their behaviour.

In conclusion, this study highlights the inadequacy in performance (smell and flies) and ventilation of pit latrines in urban slums. Addressing cleanliness, modifying the environment in the pit to reduce the moisture content through urine diversion or behaviour change, could improve the performance of pit latrine. Additionally, attaining material stabilisation and entomological studies could provide additional options for reducing smell and flies nuisances. Thus, the findings provide important information to practitioners, researches and bio additives manufacturers looking at improving the performance of pit latrines within urban slums.

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CHAPTER FIVE

5 Fingerprinting bacteria and fungi in indigenous microorganisms harvested from soil using 16S rRNA and 18S rRNA gene sequencing for potential use in pit latrines

Abstract

The use of indigenous microorganisms (IMOs) is gaining prominence in improving bio-degradation of organic compounds and reduction of foul smell, and minimising of insects' nuisance. Additionally, IMOs are being used to improve the performance (filling, smell and insects nuisances) of pit latrines. The aim of this study was to establish microbial communities in IMOs from collected from different soil environments. IMOs were collected from un-disturbed areas under bamboo trees, mango trees, open savannah grassland and home backyard waste dump sites from three different locations. Genomic DNA was directly isolated from IMOs samples, where 16S rRNA and 18S rRNA were amplified using universal primers capable of picking a broad range of organisms and sequenced for bacterial and fungal species, respectively. The soil environment had temperatures ranging from 21.0 to 29.0^{o}C, pH of 5.83 - 8.43 and soil moisture of 2 – 7. Sequencing results revealed dominance of bacterial species of *Stenotrophomonas maltophilia, Bacillus sp, Chryseobacterium ureilyticum* and a number of uncultured bacterial colons. The fungal species included *Saccharomyces cerevisiae, Galactomyces geotrichum* and *Geotrichum candidum.* Further it was deduced that the soil environmental conditions during collection of IMOs could have influenced obtainment of some microbial strains. Finally, the characteristic and functional diversity of the microorganisms found in the IMOs suggest that they could easily adapt to pit environmental conditions, degrade human faecal matter and influence insects' occurrences, thus reducing the problems related to pit latrine performance.

This chapter is based on:

Nakagiri, A., Tumuhairwe, J. B., Niwagaba, C. B., Nyenje, P. M., Kulabako, R. N., & Kansiime, F. Fingerprinting bacterial and fungi in indigenous microorganisms harvested soil using 16Sr-DNA and 18Sr-RNA gene sequencing for potential use in pit latrines – **manuscript**

5.1 Introduction

There is high usage of pit latrine by most of the urban low-income population in developing countries (Nakagiri et al., 2016). This necessitates addressing the inadequate of pit latrine performance (Nakagiri et al., 2015) so as to improve the sanitation situation, and thus the environment and public health of these areas. The challenges associated with pit latrine performance (filling, smell and insect nuisance) could be alleviated by improving the degradation processes of pit contents. There is a relationship between the degradation processes (represented by oxygen reduction potential) and the performance of pit latrines (Nakagiri et al., 2017). Moreover, studies have also indicated that problems associated with pit latrine performance are linked to slow/ incomplete breakdown of faecal matter in the pit, resulting in the build-up of intermediate volatile products, some malodorous in nature (Torondel, 2010; Still & Foxon, 2012; Lin et al., 2013). The degradation of pit latrine contents is a biochemical process where chemo-organotrophic microorganisms use their enzymes to break down and utilise the organic matter in human excreta for carbon and energy. The degradation process depends on the activity of several microorganisms although sequential activities may be done by a single member of the consortium (Rittmann & McCarty, 2001). Thus, maintaining the right microbial communities in the pit, could lead to complete degradation and stability of the excreta, which is essential for pit latrine performance. Thus since the

The environment in the pit is uncontrolled, and therefore, one way of attaining the right microbial communities could be through inoculation and bio- stimulation with effective microorganisms. During bio-stimulation, the exogenous material (amendments and nutrients or other limiting factors) and microorganisms, serve as donors of the catabolic genes that stimulate the metabolic pathways responsible for enhancing the intrinsic degradation processes of a system (Cosgrove et al., 2010; Kanissery & Sims, 2011). The use of material and microbial inoculants as bio-stimulants is popular in the field of agriculture, animal production, and waste composting and degradation of complex compounds (Calvo et al., 2014; Rushing, 2015). Single bacterial strains like; *Bacillus spp; B. thuringiensis B. sphaericus, Pseudomonas aeruginosa* and *Acidithiobacillus spp* have shown potential to improve degradation and insects control (Baumann et al., 1991; Mulligan et al., 2001). Relatedly, fungal species such as *Aspergillus niger; Trichoderma viride Penicillium spp. Trichoderma,* White-rot fungi in combination with microbial strains of *Bacillus casei, Lactobacillus buchneri* and *Candida*

rugopelliculosa have been successfully isolated, cultured and used as bio-inoculates to degrade organic matter and complex compounds in bioremediation and metal removal (Gaur et al., 1982; Malik, 2004; Wei et al., 2007).

A product of interest that could have potential in improving the performance (smell, flies and insects nuisances) of pit latrines is the Indigenous Microorganisms (IMOs). IMOs, a concept developed by Dr. Cho Han Kyu from the Janong Farming Institute, South Korea (Reddy, 2011), is a cost effective, environmentally friendly alternative to bio-fertilizers, and use in organic farming, organic-piggery and aquaculture (Kumar & Gopal, 2015). IMO cultures contain consortia of beneficial microorganisms comprising of fungi and bacteria, that are deliberately collected and cultured from soils to enhance organic matter degradation (Reddy, 2011).

The success of application of IMOs has been attributed to the functional diversity and availability of a wide consortia of microorganisms (bacteria and fungi) that can influence organic matter degradation processes. Studies have shown that the use of IMOs during composting increases the microbial communities and numbers, resulting in a better and prolonged degradation process (Hanim et al., 2012a; Bakar et al., 2015). In a related study, Anyanwu et al. (2013) noted that IMOs were effective in accelerating decomposition of farm waste and plant materials which in turn yielded high levels of macro and micro nutrients. Later, Zakarya et al. (2015) noted less decomposition time, no foul odours and leachate while composting food wastes, due to application of IMO. Sumathi et al. (2012) noted improved soil fertility resulting from an increased microbial (bacterial and fungal) population and enzyme activity when treated with IMOs. Similarly, Mbouobda et al. (2013) found improvement in the soil quality and enhanced plant (*C. esculente*) growth and yield as a result of increased microbial diversity with application of IMOs. Contrary to these findings, Zuraihah et al. (2012) reported an increase in microbial populations in soil but no increase in yield of leafy vegetables (*Brassica alboglabra, Brassica chinensis* and *Lactuca sativa*) when IMOs were applied and compared to normal compost.

In addition to improved soil fertility, enzyme reaction and microbial population in plant growth, application of IMOs has been found to reduce unpleasant odours and presence of flies in inoculated deep litter systems of swine farming (DuPonte & Fischer, 2012). In a study by

SomaSekhar et al. (2013), a decrease in foul odour and chicks faecal matter (droppings) was noted following application of IMOs. IMOs are also being applied in pit latrines to minimise filling, malodorous smell and insects nuisances. Additionally, a preliminary field application of IMOs in pit latrines in this study resulted in smell reduction with increased user satisfaction of their sanitation facilities. Previous studies have not characterised the actual microbial communities in IMOs, and yet they could vary due to the environmental conditions during the process of collection. Therefore, the aim of this study was to evaluate the abundant microbial communities in IMOs collected from different environments. Understanding the microbial communities present in an inoculum could help in establishing their degradation/ application potential, and guide their collection and enrichment for subsequent applications.

5.2 Materials and Methods

5.2.1 IMO collection sites and study design

IMOs were collected from four different environmental sites namely; (i) undisturbed area under bamboo trees, (ii) un-disturbed area under mango trees, (iii) un-disturbed open savannah grassland and (iv) home backyard waste dump. This was done to assess if the area where IMOs were collected had an effect on the consortium of microorganisms present. For each environmental site, three locations were selected where four replicates of IMOs were simultaneously collected. The sampling locations were within urban and peri- urban areas of Uganda, namely Makerere University Agricultural Research Institute Kabanyoro (MUARIK), Lungujja and Nabbingo.

At each IMO collection site and within the different locations at each site, measurements for environmental variables, namely, soil temperature, and moisture content was done at the time of IMO collection, using a TFA soil and compost thermometer (Green Wash Ltd., UK), and Bosmere K176 Moisture Meter (measurement range 1 - dry to 10 – wet) (Bosmere, UK) respectively. After *in-situ* measurements, soil samples were also collected and taken to the laboratory to determine the pH. pH of the soil was determined by adding 50 mL of deionized water to 20 g of soil in a 100 mL beaker. The beaker was then placed on a shaker and swirled for 30 minutes. It was thereafter covered and left to stand for an hour. The pH of the supernatant was then measured using a portable meter (Hach HQ30d flexi model, USA).

5.2.2 Collection and culturing of IMOs

The IMOs used in this study were collected using the cultivation method described by Reddy (2011). Four small wooden boxes (25 x 25 x 5 cm), sealed at the bottom and open at the top were half filled with steamed white rice and covered with a paper towel. Rubber bands were tied around the boxes to secure the paper towel in place. A wire mesh (30 x 30 cm) was then placed on top of each box to protect the rice from being eaten by animals. The box was then placed halfway in the ground, at different locations within the environmental site and covered with fallen leaves. A clear plastic sheet was placed at some distance above each box and anchored on all sides with small stones to prevent entry of rain. The boxes were left undisturbed for 3- 5 days. After this period, IMOs grown on rice (white mold) were collected (Figure 1) and these are referred to as IMO1.

Figure 5.1 IMO1, indigenous microorganisms (white mold) grown on rice collected from different locations

The IMO1 in the four boxes was mixed together to get a composite sample. Fifty grams of composite IMO1 was placed in falcon tubes and stored at – 80^{o}C for genomic DNA extraction. The remaining composite IMO1 was then cultured to increase their population. This was done by hand kneading equal amounts of composite IMO1 and brown sugar (1:1 ratio). The mixture was then placed in a clay pot, up to two-third of its volume. The pot was then covered with a paper towel secured in place with rubber bands and stored at room temperature (23^{o}C – 30^{o}C) for a period of 6 days to produce IMO2. Fifteen millilitres of IMO2 were placed in falcon tubes and stored at – 80^{o}c until the time for genomic DNA extraction.

5.2.3 DNA extraction, amplification and sequencing

Genomic DNA was extracted from IMO1 and IMO2 using a Powersoil DNA isolation kit, according to the manufacturer's protocol (Mo Bio Laboratories Inc., Carlsbad, CA, USA). Briefly, Cell lysis was done by adding 10g of IMO sample and 60 µL of cell lysis solution to power bead tubes and vortexed horizontally at room temperature for 10 minutes. The bead

tubes were then centrifuged for 30 minutes. Thereafter, 400 µL supernatant in the bead tubes was then transferred to a 2 mL collection tubes, to which 250 µL of an inhibitor removal reagent was added. The collection tubes were vortexed for 5 seconds and incubated at 4^{o}C for 5 minutes. The tubes were then centrifuged for one minute. This was done to precipitate non DNA organic and inorganic substances. The inhibitor removal step was repeated using a clean collection tube to which 500 µL of supernatant and 200 µL of removal reagent were added.

Seven hundred microliters of each supernatant was then obtained and placed in a clean 2 mL collection tube and 1.2 mL of a high concentrated salt solution was added and vortexed to mix. The solution was then placed in a spin filter and centrifuged to bind the DNA on a silica membrane in the filter. 500 µL of an ethanol based wash solution was then placed in the spin filters and centrifuged for 30 seconds to further clean the DNA that was bound to the silica membrane. The flow through in the collection tubes was then discarded and the spin filter centrifuged further for one minute to remove any excess wash solution. Thereafter, 100 µL of sterile elution buffer solution was then introduced at the centre of the membrane of the spin filters placed in clean 2 mL collection tubes and centrifuged to release the DNA from the silica spin filter membranes. The spin filter was discarded thereafter and the solution in the tubes was the DNA used in this study. All centrifuging was done at room temperature at 10,000xg. The DNA was purified by electrophoresis through 2% agarose gel at 100v for 1 hour. The DNA bands were excised and their quality checked on 2% agarose gel by electrophoresis stained with ethidium bromide solution (Figure 5.2).

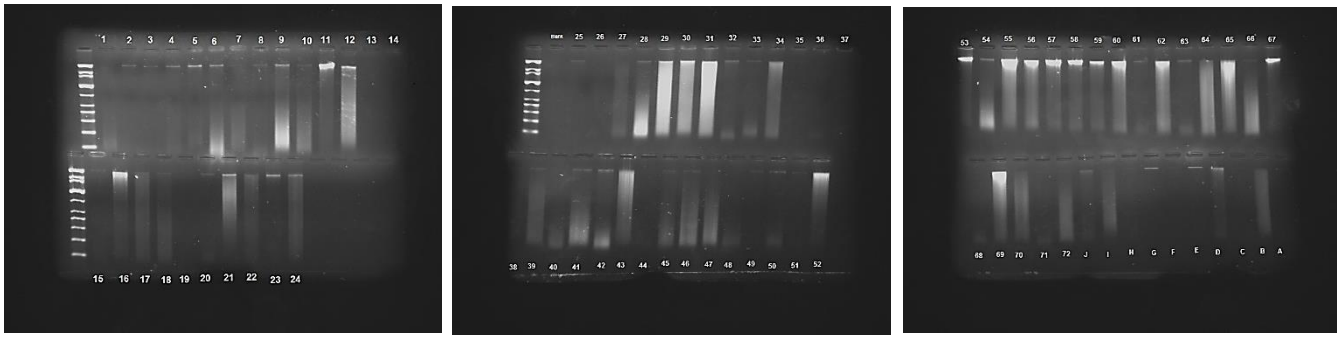

Figure 5.2 Gel images of the DNA extracted from the IMO samples. Label details are included in the appendix as Table A6

The extracted DNA was amplified using universal bacterial primers targeting the 16S rRNA gene. The primers were 27F (5' AGAGTTTGATCMTGGCTCAG- 3') and 907R (5'-CCGTCAATTCCTTTGAGTTT-3'). For fungi, ITS region of 18S rRNA was amplified using

primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Each 50 µL PCR reaction mixture contained 1 µL of 10 µM of each primer; 25 µL of One*Taq* Quick-load 2X master mix with standard buffer (New England BioLabs Inc.); 4 µL (bacterial) / 8 µL (fungi) of DNA template; and nuclease-free water up to 50 µL.

The PCR amplification was done using a Mastercycler nexus (Eppendorf, Germany) at the following amplification conditions. For bacteria, there was an initial denaturation at 94 ^{o}C for 5 minutes; followed by 30 cycles of 30 seconds denaturing at 94 ^{o}C; 30 seconds at 55 ^{o}C and annealing for 30 seconds of elongation at 72 ^{o}C; and a final elongation for extension of 7 minutes at 72 ^{o}C and stored at 4 ^{o}C infinity. For fungi, there was an initial denaturation of 5 minutes at 94 ^{o}C; followed by 25 cycles of 30 seconds of denaturing at 94 ^{o}C, 30 seconds of annealing at 58 ^{o}C and 30 seconds of elongation at 72 ^{o}C and a final extension of 7 minutes at 72 ^{o}C. The integrity of PCR products was checked using 2% agarose gel electrophoresis (Figure 5.3 and 5.4).

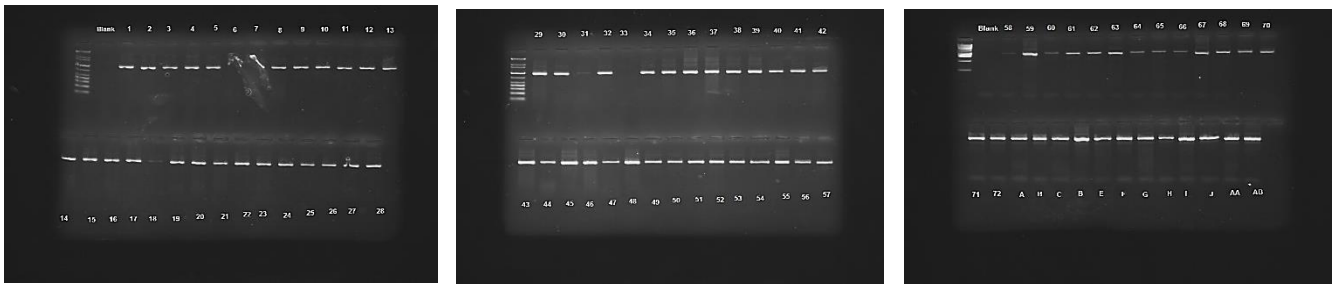

Figure 5.3 Gel images of the amplified bacterial 16S- rRNA gene fragments of PCR products from the IMO samples Label details are included in the appendix as Table A6

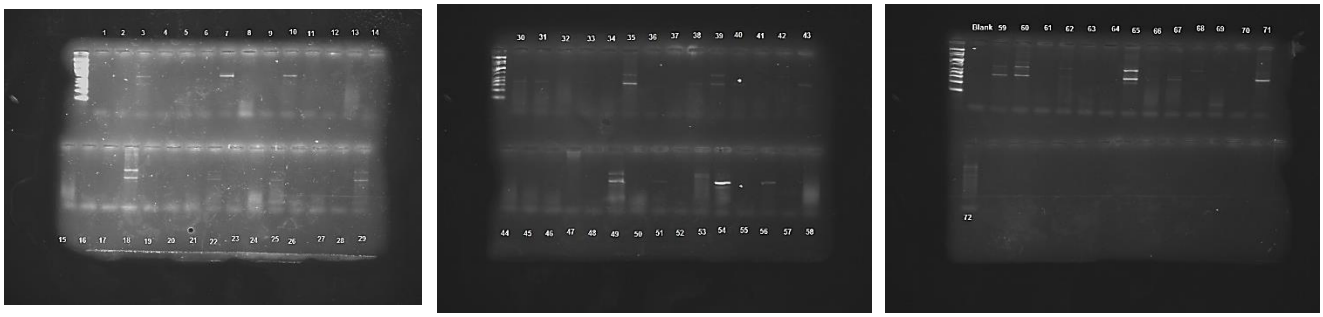

Figure 5.4 Gel images of the amplified fungal 18S- rRNA gene fragments of PCR products from the IMO samples. Label details are included in the appendix as Table A6

The PCR products were then excised and purified using a QIAquick PCR Purification Kit according to manufacturer's protocol (Qiagen, USA). Briefly, 500 µl of Buffer PB was added to 100 µl PCR sample in a collection tube and mixed. The mixed sample was then poured into a spin column placed in 2 ml collection tube, which was centrifuged thereafter for 30 seconds to bind the DNA. The flow-through in the collection tube was then discarded and the spin column placed back. 0.75 mL of Buffer PE was added to the column and centrifuged at 17,900xg for 30seconds to wash the DNA. The flow-through in the collection tube was discarded and the spin column centrifuged further for 1 minute to remove residual wash solution. The spin column was thereafter placed in a clean 1.5mL microcentrifuge tube, and 50 µL Buffer EB added to the centre of the membrane and centrifuged for 1minute to release the DNA. The purified PCR products were then sent to Macrogen Europe (The Netherlands) for library construction and sequencing.

The sequences obtained were aligned using BioEdit sequence alignment editor (Hall, 1999) and then submitted to the Basic Local Alignment Search Tool (BLASTn) search in the National Centre for Biotechnology Institute (NCBI) nucleotides sequence database. The sequences with the highest percentage matches were retrieved from the database (Appendix, Table A7).

5.2.4 Data analysis

The data for the environmental variables were analysed using SPSS version 21 and XLStat, in Microsoft excel. Descriptive statistics, which is box and whiskers, means and maximum-minimum values) were used to describe the environmental variable at each site/location. Significant differences between the environmental variables were assessed using the ANOVA at a significance level of 95%.

Phylogenetic analysis was conducted using MEGA7 (Kumar et al., 2016). This was done by first producing nucleotic alignments using Cluster W followed by phylogenetic tree constriction using neighbour-joining method (Saitou & Nei, 1987) with 1000 boot strap re-samplings.

Canonical correspondence analysis (CCA) was used to demonstrate the effect of environmental factors on the distribution patterns of the abundant microbial communities' composition and study locations. Comparison between the CCA ordination were quantified using eigen values,

while CCA coefficients were used to assess the importance of contributing environmental variables. The variations in microbial community composition as expressed by the environmental variables including locations were presented by the ordination diagram of the CCA. CCA analysis in this study was done using XLStat for Microsoft excel.

5.3 Results

5.3.1 Environmental conditions of IMO collection sites.

The soil conditions for all sites considered together were temperature ranging from 21.0 to 29.0^{o}C, pH of 5.83 - 8.43 and soil moisture of 2 – 7 (Figure 5.5).

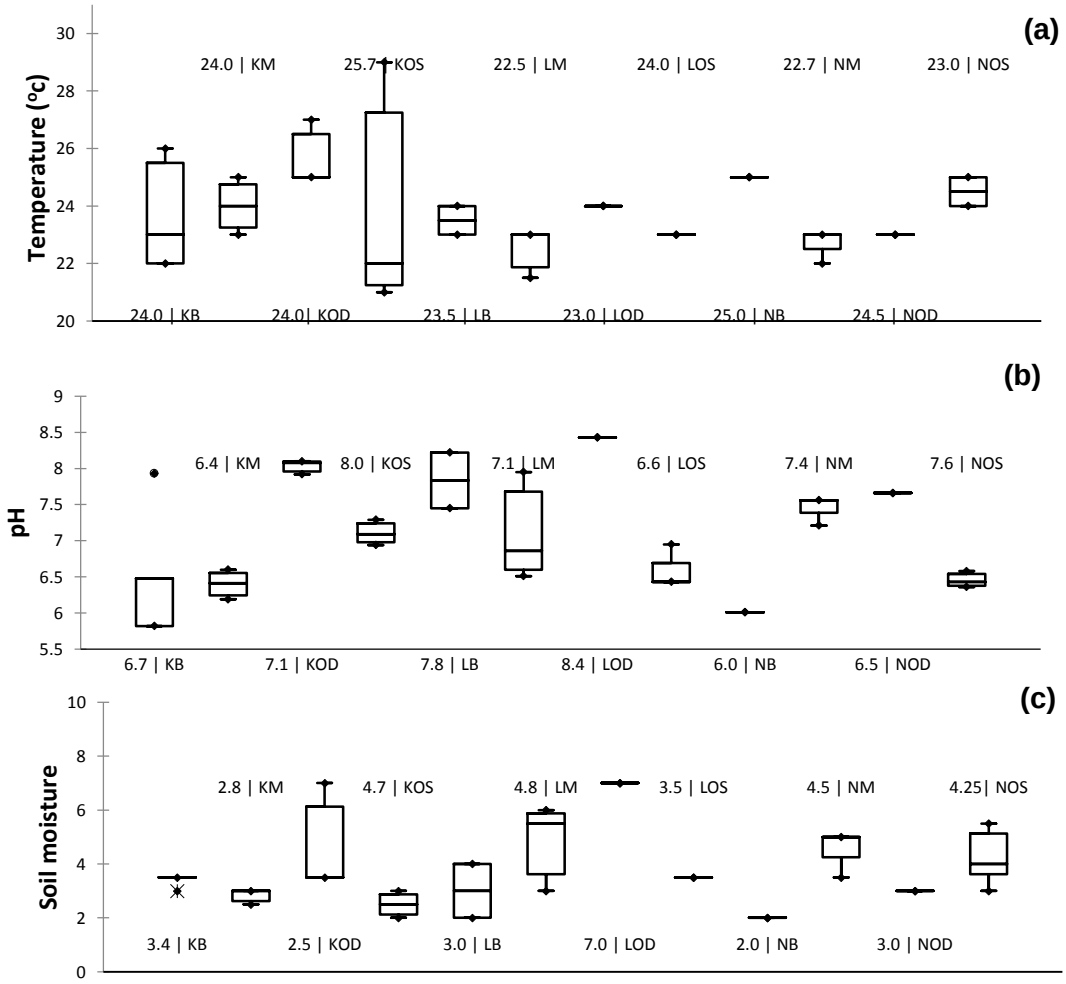

Figure 5.5 Characteristics of IMO collection locations; (a) – Temperature, (b) - pH, (c) – soil moisture. Box represents 50% of the data points, whiskers represent minimum and maximum, line in box represents the median. K stands for Kabanyolo, L –Lungujja, N –Nabbingo, B – bamboo, M –mango, OS – open space and OD- open dump

Comparison of data from the individual environmental sites depicted significant differences in the temperature ($p = 0.02$) and moisture content ($p = 0.036$) between the locations. In general, the mean of the pH for open dumps was significantly higher than that of bamboo ($p = 003$), mango ($p < 0.001$) and open space ($p < 0.001$). Additionally, a significantly higher ($p = 0.038$) soil moisture content was noted for open dump compared to bamboo areas. ANOVA results between the different niches/ locations also depicted significant differences within a particular environmental site.

At MUARIK, the pH of bamboo and open dump soils was significantly higher than that of mango ($p = 0.04$ and $p < 0.001$ respectively), while the moisture content at the open dump was significantly higher than that in the other three locations (i.e, $p < 0.001$ for bamboo; $p = 0.001$ for mango; and $p < 0.001$ in open space). In Lungujja, the pH in the open space soils was significantly lower ($p = 0.03$) than that at the open dump, while the soil moisture at the open dump was significantly higher ($p = 0.044$) than that of the bamboo soils. In Nabbingo, the pH of bamboo soils was significantly lower ($p < 0.001$) than that of the other three locations, while that at the mango and open dump sites was significantly higher ($p < 0.001$) than that of open space. The temperature of the bamboo soils was significantly higher ($p < 0.001$) than that for the mango and open dump, while that for mango and open dump were significantly lower ($p <0.001$) than that for open space. The moisture content for the bamboo soils was significantly lower than that for mango ($p = 0.005$) and open space ($p = 0.001$) while that at the open dump was significantly lower ($p = 0.026$) than that for open space.

5.3.2 Microbial community analysis of the IMO samples

The IMO1 samples collected in this study mainly had white moulds. However, black, green, purple, yellow, pink and orange microbes were also noted on the rice (Figure 5.1), which implied collection of a wide range of soil microorganisms. Sequencing the amplified bacteria 16S- rRNA gene fragments of PCR products from the IMO samples from the bamboo areas showed dominance of *Stenotrophomonas maltophilia* (Figure 5.6 and Table 5-1). Similarity to *Bacillus subtilis*, was also noted for a sample at MUARIK, *Xanthomonas retroflexus* in Nabbingo while several uncultured bacteria clones were observed in IMO1. Phylogenetic analysis of the sequences (Figure 5.6), showed that the uncultured bacterial clones could have had a close relation to the *Stenotrophomonas maltophilia* strain.

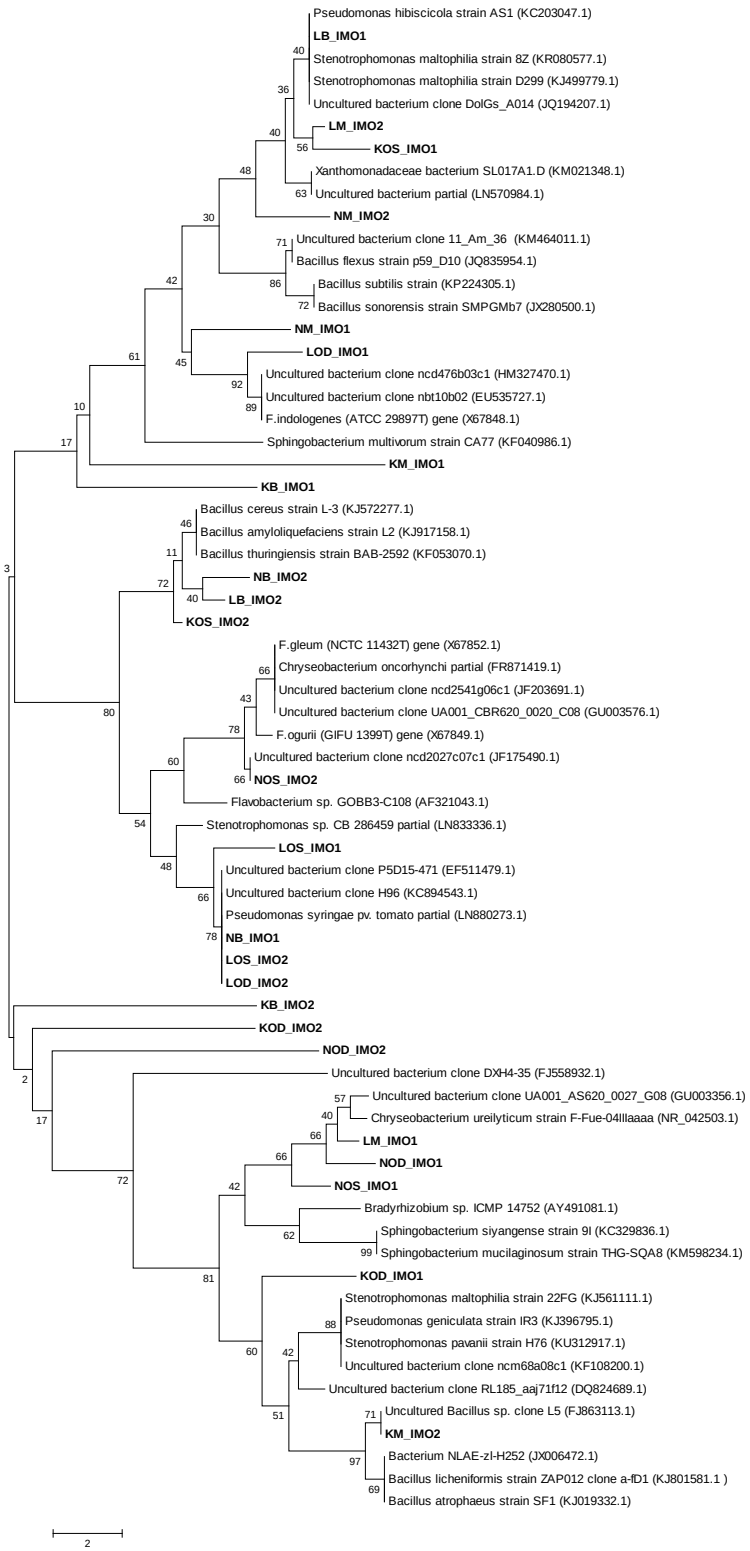

Figure 5.6 The neighbour-joining tree of partial 16S rDNA sequences from the PCR products of the IMO samples. The tree was constructed as described in the text. The Nucleotide sequences of IMO samples in this study are represented in bold. K stands for Kabanyolo, L –Lungujja, N –Nabbingo, B – bamboo, M –mango, OS – open space and OD- open dump.

Dominance of bacteria strains *Stenotrophomonas maltophilia* and *Chryseobacterium ureilyticum* was noted at the mango sites with an additional strain, *Sphingobacterium spp.* identified at the MUARIK site. Uncultured bacteria clone were identified at all the locations (Table 5.1) that could have had a close relation to *Stenotrophomonas maltophilia* and *Chryseobacterium ureilyticum* strains. The bacterial strain *Stenotrophomonas maltophilia* was found to be dominant at the MUARIK and Lungujja open space sites, while *Chryseobacterium ureilyticum* was dominant at the Nabbingo site (Table 5.1).

Table 5.1 Dominant bacterial 16S rDNA and fungi 18S rDNA gene sequences obtained from IMO sample from different niches

IMOs type	Microorganism	Species	Environmental sites			
			B	M	OS	OD
IMO1	Bacteria	*Bacillus spp.*	*			
		Chryseobacterium ureilyticum strain		*	*	*
		Flavobacterium Ogurii (GIFU 1399T) gene				*
		Pseudomonas geniculata strain				*
		Sphingobacterium spp.		*		
		Stenotrophomonas spp.	*	*	*	*
		Uncultured bacterium clone	*	*	*	*
		Xanthomonas retroflexus strain	*			
	fungi	*Galactomyces candidum* strain			*	*
		Geotrichum candidum stain				*
IMO2	Bacteria	*Bacillus spp.*	*	*	*	*
		Bacterium NLAE-zl-H252 1				*
		Chryseobacterium ureilyticum strain	*		*	*
		Pseudomonas spp.	*			
		Stenotrophomonas maltophilia strain	*	*	*	*
		Uncultured *Bacillus spp.*	*			
		Uncultured bacterium clone	*	*	*	*
	fungi	*Galactomyces spp.*	*	*		
		Geotrichum candidum stain	*	*	*	
		Saccharomyces cerevisiae strain	*	*		

Three uncultured bacteria clones were also identified that could have had close relationship to *Stenotrophomonas maltophilia* and *Chryseobacterium ureilyticum* strains (Figure 5.6). The open dump site in MUARIK was dominated by *Stenotrophomonas maltophilia* and *Pseudomonas geniculata* strains, while similarity to *Flavobacterium Ogurii* was noted at Nabbingo and *Chryseobacterium ureilyticum* strain for Lungujja.

Three uncultured bacteria clones were found at the open dumps that could be closely related to *Stenotrophomonas maltophilia* to *Flavobacterium Ogurii* and *Chryseobacterium ureilyticum*

bacterial strains. Analysis for fungal strains gave positive hits for only open space and open dump samples from Nabbingo. The results found close similarity to *Galactomyces geotrichum* and *Geotrichum candidum* strains (Figure 5.7 and Table 5.1). These results indicate a variation in dominant microbial communities between different environmental sites even with location in each site.

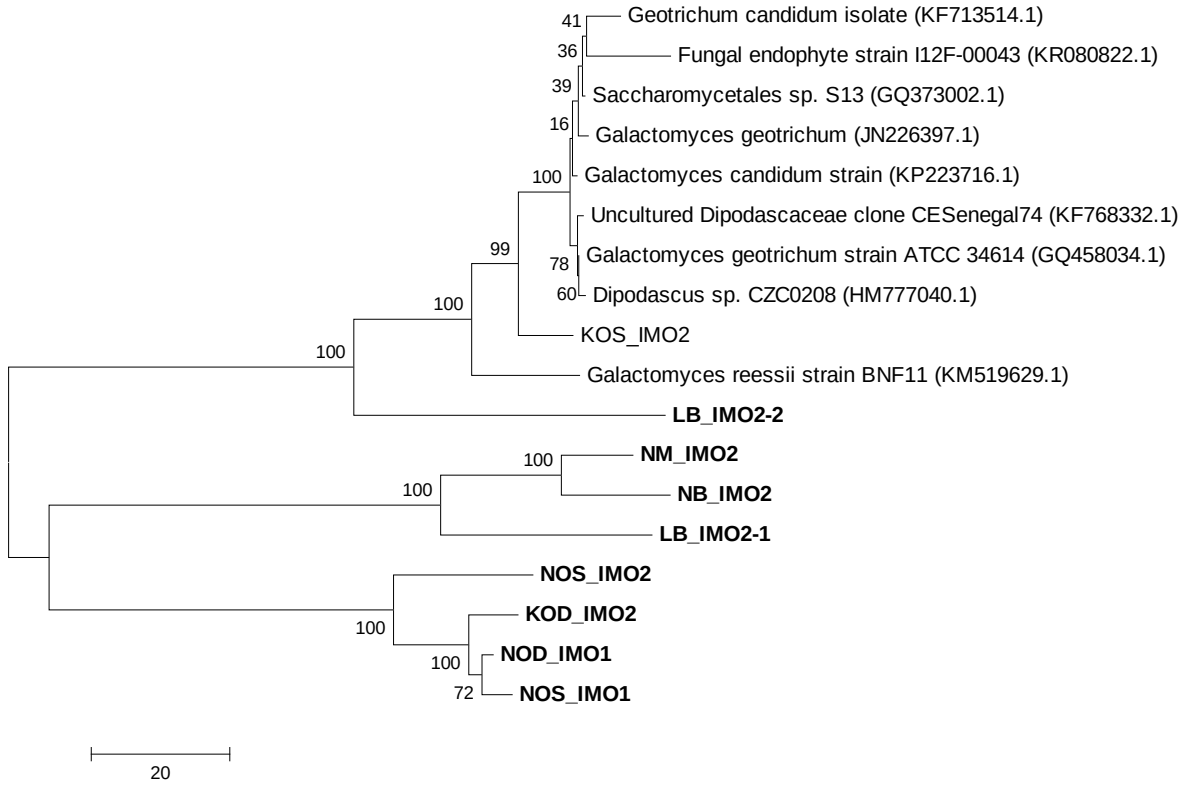

Figure 5.7 The neighbour-joining tree of partial 18S rRNA sequences from the PCR products of the IMO samples. The tree was constructed as described in the text. The Nucleotide sequences of IMO samples in this study are represented in bold. K stands for Kabanyolo, L –Lungujja, N –Nabbingo, B – bamboo, M –mango, OS – open space and OD- open dump.

Progressing to IMO type 2, *Bacillus subtilis*, *Stenotrophomonas maltophilia*, *Chryseobacterium ureilyticum*, *Pseudomonas spp.* and five uncultured bacteria clones plus fungal species *Galactomyces geotrichum* and *Geotrichum candidum* were observed (Figures 5.6 and 5.7, Table 5.1). Additionally, bacterial strains *Bacillus thuringiensis,* and *Bacillus cereus* and fungal species *Saccharomyces cerevisiae* fungal strains that were not identified in IMO1 were noted to be dominant in IMO2. This indicated some similarities in dominant

microbial communities in IMO1 and IMO2. However, the results also highlight that some microbial communities may be suppressed during preparation of IMOs.

5.3.3 The influence of environmental variables on the microbial community during IMO collection

The relationship between the environmental sites and microbial community was assessed using canonical correspondence analysis (CCA). The eigenvalues of the first two CCA axes of ordination were 0.33 and 0.29 respectively, which indicated that most of the relationship between the environmental conditions and the microbial community was mainly explained in the first axis. In total, both axes accounted for 81.8% of the total variance in the weighted averages of the species. The correlation coefficients between the ordination axes and environmental variables showed that the first axis was highly correlated to moisture content and pH, while the second axis had the highest correlation with temperature (Table 5.2).

Table 5.2 Correlation coefficients between environmental variables and the first two CCA axes

Variables	Axis 1	Axis 2
pH	-0.708	-0.603
Temperature	-0.141	0.658
Moisture content	1.029	-0.303

The main pattern of the variation in microbial community composition that was explained by the environmental variables was shown on the ordination diagram of CCA (Figure 5.8). From the diagram it can be depicted that *Stenotrophomonas maltophilia* strain, and the uncultured bacterium clones were found close to the centre were abundant in all niches and therefore unrelated to the ordination axes. This implied that the bacteria strains may not have been affected by the different environmental variables. Additionally, the presence of *Pseudomonas geniculata* and fungal *Galactomyces candidum* strains close to the centre could imply that the microbe may not have been related to the environmental variables but could be specific to sites, Kabanyoro open dump (KOD) and Nabbingo open space (NOS) respectively.

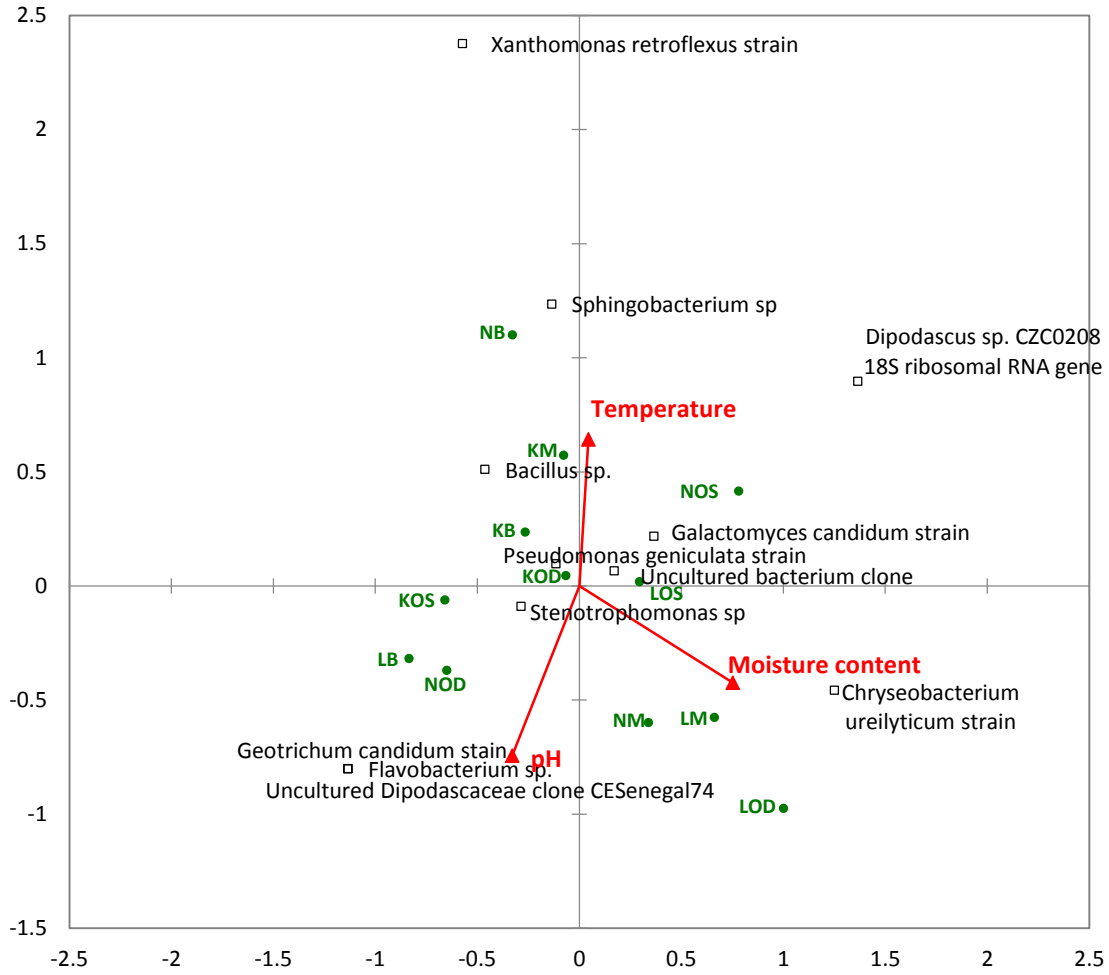

Figure 5.8 CCA ordination diagram with IMO microbial species (black open squares), Locations (green dots), and environmental variables (red lines with arrows). K stands for Kabanyolo, L –Lungujja, N –Nabbingo, B – bamboo, M –mango, OS – open space and OD- open dump.

Chryseobacterium ureilyticum strain was strongly correlated to wetter soils, a situation at the mango sites in Nabbingo and Lungujja plus Lungujja open dump, while at the Kabanyoro mango, and Nabbingo bamboo with dryer soils, the growth of *Bacillus sp.*, *Sphingobacterium sp.* and *Xanthomonas retroflexus* strain was correlated with an increase in temperature. The presence of fungal species *Geotrichum candidum and* uncultured *Dipodascaceae clone CESenegal74* as well as bacteria strain *Flavobacterium spp.* correlated with an increase in pH, as noted at Nabbingo open dump (NOD).

5.4 Discussion

5.4.1 Microbial communities in IMOs

The application of IMOs has been reported to increase the microbial community types, improve bio-degradation of organic compounds and reduces foul odours and insect intensity (DuPonte & Fischer, 2012; Hanim et al., 2012b; Kumar & Gopal, 2015). The results of this study, showed that the environmental temperature (21 – 29^{o}C) and pH (5.83 - 8.43) for IMO collection allowed for the cultivation of beneficial microorganisms as shown in earlier studies (Park & DuPonte, 2010; SomaSekhar et al., 2013). Various microorganisms were collected in this study as indicated by the variation in their colour (Figure 5.1). The most prominent bacteria strains were *Stenotrophomonas maltophilia, Bacillus subtilis, Sphingobacterium spp, Xanthomonas, retroflexus Chryseobacterium ureilyticum, Pseudomonas geniculate, Flavobacterium Ogurii,* uncultured bacterial colons while the fungi were *Galactomyces geotrichum* and *Geotrichum candidum* fungal strains. This is because the soil contains a diverse array of soil organisms including bacteria and fungi (Bollag et al., 1994; Torsvik & Øvreås, 2002). Additionally, these microorganisms are often found in the environment, on or in plants (Ryan et al., 2009; Madigan et al., 2015) and are associated with organic matter decomposition. Bacterial populations of *Bacillus sp.* and *Stenotrophomonas spp,* have been reported in decomposing sewage sludge and garbage (Ishii & Takii, 2003; YE & DU, 2005; Horisawa et al., 2008). Similarly, *Galactomyces candidum,*and *Geotrichum candidum* have been reported in composting sawdust and coffee residue (Eida et al., 2013). Furthermore, *Bacillus spp. Saccharomyces, candidum* and *Sphingobacterium* have been reported in anaerobic degradation of bio-wastes (Ritari et al., 2012), while *Bacillus sp., Pseudomonas spp.* and *Chryseobacterium spp.* species were reportedly responsible for the degradation of keratin in poultry feather waste (Charimba, 2012). It should also be noted that *Chryseobacterium spp., Saccharomyces cerevisiae, Galactomyces candidum* and *Geotrichum candidum,* oxidise glucose during fermentation of beverages and wastes (Herzog et al., 2008; Jin et al., 2017), while *Pseudomonas spp, Saccharomyces cerevisiae* and *Sphingobacterium spp.* have been isolated from biofilms of fermenting food (Jahid & Ha, 2014).

From this study, it was noted that the location may affect the dominant microbial community in IMO1. Most sites were dominated by *Stenotrophomonas maltophilia,* and many uncultured bacterial strains. Further, it was deduced from the CCA that the presence of *Stenotrophomonas*

maltophilia, and uncultured bacterial colons may not have been affected by the soil temperature, moisture or pH. This could be because some uncultured strains showed close similarity to *Stenotrophomonas maltophilia* and could thus have similar properties as the microorganism. The abundance of *Stenotrophomonas maltophilia's* in most soils has been attributed to its ability to highly colonize and survive on plant surfaces and also it's adaptably to hostile and nutrient-limited environments. Additionally, *S. maltophilia* can also effectively colonize different biotopes (Ryan et al., 2009), and could thus have easily grown on the cooked rice that was used for IMO cultivation. The presence of fungal *Galactomyces geotrichum* and *Geotrichum candidum* strains in only Nabbingo open space and dump could have been attributed to inhabitation of fungi by *Stenotrophomonas maltophilia* in other environmental sites (Jakobi et al., 1996; Graupner et al., 1997). Additionally, the pH shifts from acidic to alkaline during decomposition and alkaline pH resulting from cation salts in kitchen wastes on the dumping sites, may have favoured dominance of fungal species (Tumuhairwe et al., 2009).

Chryseobacterium ureilyticum favoured wetter soils because water is one of its natural habitat (Bernardet et al., 2015), while *Bacillus subtilis* adopt more in dryer areas and can be reproduced and stored in powder form (Singh & Deverall, 1984). Besides environmental conditions, enrichment of minor microbial populations during the preparation of IMO2 where sugar was used, could account for the dominant microorganisms identified in IMO2 and not IMO1 (Torsvik & Øvreås, 2002; Henri-Dubernet et al., 2004).

5.4.2 Implications of the results on improving the performance of pit latrines

IMOs have been applied to improve the performance of pit latrines without knowledge of the dominant microbial communities present. The cultivation temperature of IMOs in this study (21.0 to 29.0^{o}C) did not differ from that noted during the normal operation of pit latrines (21 ^{o}C to 30.7 ^{o}C) (Nakagiri et al., 2017), which implies that the IMOs could easily adapt to the environment in the pit. Additionally, the characteristic of some organisms could ensure their survival when applied to pit latrines. For example, the effective colonizing ability of *Stenotrophomonas maltophilia* could enable it to easily adapt to the pit latrine environment (Ryan et al., 2009). *Bacillus spp.* possess a network of global regulatory responses that may help them to sense changes and mount appropriate responses in gene expression and protein activity when applied to the pit environment (Msadek et al., 1998; Atkinson & Williams, 2009).

The facultative nature of strains *Pseudomonas spp.*, *Galactomyces candidum, Geotrichum candidum, Saccharomyces cerevisiae, Sphingobacterium spp.* and *Chryseobacterium spp.* shows adaptation potential in pit latrines experiencing anoxic conditions (ORP values less than + 50mV) demonstrated by ORP ranges (Nakagiri et al., 2017).

Based on the microbial diversity in the IMOs, broad metabolic properties are possible in a very complex microbial system like pit latrines that will in turn have an effect on its performance (filling smell, and insect nuisances). For example, *Bacillus spp.* and *Stenotrophomonas maltophilia,* can degrade phenolic compounds, hydrocarbons and many complex compounds (Zissi & Lyberatos, 2001). *Pseudomonas spp.* anaerobically reduce nitrate to nitrogen (Madigan et al., 2015), while the fungi, *Galactomyces candidum, Geotrichum candidum*, and *Saccharomyces cerevisiae* are fermenters that degrade matter to produce alcohol and carbon dioxide. *Sphingobacterium spp.* are important decomposers of lignin cellulose. *Bacillus spp.* can also utilise ammonium ions in organic matter during aerobic conditions. These microbial species have potential in the biodegradation of human faecal matter which contains mainly easy to degrade organic molecules of proteins, carbohydrates and fats. Additionally, the diverse enzymes released by some of the microorganisms like *Galactomyces candidum, Geotrichum candidum*, and *Saccharomyces cerevisiae* during fermentation or bioconversion could influence the volatile organic compounds and thus the smell within the latrine, while *Bacillus thuringiensis strain* is known to produce proteins that are toxic to fly larvae and mosquitoes and could help in mitigation of insects (Madigan et al., 2015).

Finally, regardless of the environmental site, collection of similar dominate microbial strains for use in pit latrines is possible. However, improving microbial population of IMOs for particular use in pit latrines necessitates consideration of the site for collection. For example, enrichment of IMOs with *Bacillus spp.* for minimising insects in pit latrines necessitates collection from drier sites, while obtaining more fungal strain implies collection from areas with high pH and less vegetation.

5.5 Conclusions

By employing fingerprinting technology, the dominant bacterial species in the IMOs were found to be *Stenotrophomonas maltophilia, Bacillus spp,* and *Chryseobacterium ureilyticum.* Additionally, a number of uncultured bacterial colons were also found in the IMOs, while

fungal species were dominated by *Saccharomyces cerevisiae*, *Galactomyces geotrichum* and *Geotrichum candidum*. The results further showed that while obtaining similar microbial strain was possible during IMOs collection, the occurrence of same strains could have been influenced by variations in the environmental conditions. Thus, enhancing the functionality of IMOs could necessitate consideration of soil environmental conditions during their collection. The functional diversity of the microorganisms found in the IMOs suggest that they could easily adapt to pit environmental conditions. Additionally, through enhanced human excreta degradation and suppression of insects' occurrences, IMOs could influence performance (filling, smell and insects) issues associated with the use of pit latrines.

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CHAPTER SIX

6 Assessing the effect of indigenous microorganisms on degradation of faecal matter to improve performance of pit latrines

Abstract

Enhancing the rate of faecal matter degradation is a major attribute to improve the performance of pit latrines and consequently the environmental and public health. This study assessed the application of indigenous microorganisms (IMOs) as an inoculum to improve the degradation of human faecal matter in pit latrines and thus their performance. IMO type 3 was used to degrade faecal matter in the laboratory under ambient temperature (23 – 28 ^{o}C). Response surface methodology was used to evaluate and optimise the effect of IMOs on degradation of faecal matter. A performance evaluation was done when IMOs were applied to pit latrine. Results showed that ammonia was an appropriate surrogate to odours in pit latrines as it significantly correlated with smell levels (r_s = 0.447, p=0.037). Treatment with IMOs showed no significant ($p>0.05$) difference in mass reduction but a significant reduction in ammonia concentration was realised. Response surfaces showed that mass reduction was significantly affected by the only faecal load (p=0.0019). Ammonia concentration significantly increased with faecal matter load (p=0.0005) but reduced with IMOs application (p=0.0007). The effect of both faecal matter loading and IMO application was significant and quadratic (p=0.0438). Field application of IMOs found a reduction in smell and flies in clean pit latrines with increased user satisfaction. Therefore, IMOs have a potential to reduce smell in pit latrines but are effective up to a faecal load of 16 kg/day and when the latrines are kept clean.

This chapter is based on:

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6.1 Introduction

Pit latrines have been designed to safely isolate, store and partially treat human excreta until they are emptied or covered and replaced, thereby minimising health hazards associated with improper excreta disposal (Franceys et al., 1992). The technology has been highly adopted and is in use by most (over 50%) of the urban population in Sub-Saharan Africa (SSA) (Nakagiri et al., 2015). However, the functionality of pit latrines, especially in urban high density, and low-income areas, has raised issues of environmental and public health concern. Pit latrine functionality viewed as high filling rates, bad smells and insect nuisances have resulted in human health risks and user dissatisfaction. Consequently, there has been increased open defecation or flying toilets (Tumwebaze et al., 2012; Jenkins et al., 2014; Kwiringira et al., 2014).

Improving the environmental and public health situation in urban low-income areas necessitates addressing the problems associated with current sanitation systems. Research has indicated that the above mentioned problems of pit latrine use are associated with the degradation process of faecal matter. For example, the slow, and insufficient decomposition or incomplete breakdown of human excreta accounts for faster filling of pits than their design life (Torondel, 2010). Additionally, smell is a result of accumulation of malodourous volatile organic compounds (Still & Foxon, 2012; Lin et al., 2013). Some of the volatile organic compounds that attract and influence the feeding and breeding behaviour of insects, have been found in pit latrines (Cosse & Baker, 1996; Davis et al., 2013; Afify & Galizia, 2014).

Enhancing *in-situ* degradation in pit latrines to obtain a more stable, non-malodorous product could address the shortfalls related to pit latrines. Currently, the use of additives constituted of microorganisms, hydrolytic enzymes, singly or in combination have been reported to enhance degradation processes in pit latrines and these products are increasingly being adopted in low-income countries (Foxon et al., 2009). Available laboratory and field studies on inoculation of commercial additives to pit latrine contents have reported inconsistent results. The use of microbial derived products and spore forming non-pathogenic bacteria were found to degrade and reduce faecal matter volumes, and thus considered to be feasible in reducing pit latrine contents (Jere et al., 1998; Taljaard et al., 2003). On the contrary, Buckley et al. (2008) obtained no correlation in decrease of faecal matter with application of additives. Relatedly, Foxon et al. (2009) and Bakare et al. (2015) found no evidence that the use of commercial pit additives

had any beneficial effect in reducing pit contents. While the effectiveness of applying commercial additives in pit latrines still shows contradictory results, the use of naturally occurring beneficial microorganisms to prevent malodorous, fly breeding and effect waste decomposition is widely used in organic/ natural farming, such as organic-piggery production systems and sustainable aquaculture (Higa & Parr, 1994; Zhou et al., 2009; Reddy, 2011; Kumar & Gopal, 2015).

IMOs are a consortium of beneficial microorganisms comprising of fungi and bacteria deliberately collected and cultured from the soil environment (Reddy, 2011). In a previous study by (Nakagiri et al., 2017 - Manuscript), the dominant fungal species in IMOs were found to be *Saccharomyces cerevisiae, Galactomyces geotrichum* and *Geotrichum candidum*, while bacteria was dominated by *Bacillus spp. Stenotrophomonas maltophilia, Chryseobacterium ureilyticum* and uncultured bacterial strains. IMOs have been promoted, and are successfully being used as a bio-stimulate to increase microbial biodiversity, population density, enzyme reaction and process stimulation in agriculture, animal production and waste composting (Reddy, 2011; Sumathi et al., 2012; Anyanwu et al., 2013; SomaSekhar et al., 2013; Bakar et al., 2015; Zakarya et al., 2015).

Currently there is interest in the application of IMOs to improve the functionality (high filling rates, bad smells and insect nuisances) of pit latrines. However, their application in pit latrines has not been scientifically explored. Yet the functional diversity of the microorganisms found in the IMOs suggests that they could easily adapt to pit environmental conditions, enhance human excreta degradation and suppression of insects' occurrences which makes them an attractive microbial inoculum for use in pit latrines (Nakagiri et al., 2017 - Manuscript). Additionally, preliminary findings from field application of IMOs in this study noted smell and insects reduction with increased user satisfaction of the facilities.

Therefore, the aim of this study was to assess the effect of application of IMOs on the degradation of human faecal matter from pit latrines as well as their effect in improving the performance of the latrine. This study was carried out in three parts. First a laboratory evaluation of the degradation of human excreta from pit latrines with IMOs was carried out. Secondly, the optimisation of IMOs for application in pit latrines carried out. Finally, IMOs were then applied to pit latrines in an urban slum, and an evaluation of their performance noted.

Two variables mass reduction and ammonia concentration were used as proxies to pit latrine filling rate and odour intensity, respectively.

6.2 Materials and Methods

6.2.1 IMOs collection and culturing

The IMOs used in this study were collected from bamboo forests by adopting the cultivation method described in (Nakagiri et al., 2017 - Manuscript). Briefly, at each sampling unit, four wooden boxes, each about 25 x 25 x 5 cm (LxWxH) were half filled with steamed white rice and covered with a paper towel, held in position with rubber bands. Each box top was covered with a wire screen to protect the rice from being eaten by animals. The boxes were then placed halfway in the ground, in different locations of the site where decomposed matter was found. A clear plastic sheet was placed some distance above each box and anchored on all sides with small stones to prevent entry of rain. The boxes were then left undisturbed for 3- 5 days. After this period, IMOs type 1 grown on rice were collected. IMO1 from four replicates were mixed together to form composite sample.

The composite IMO1 were cultured to increase microbial population by mixing an equal amount in brown sugar (1:1 ratio). The mixture was placed in a clay pot, up to two-thirds of its volume. The pot was then covered with a paper towel secured in place with rubber bands and stored at room temperature for six days to produce IMO type 2. Thereafter, IMO2 were mixed in 10L of water and slowly mixed with dry maize flour bran to a moisture content of 65% - 70%, and incubated for 7 days under shade and agitated daily to allow for cooling and ensure uniform mixing. At maturity, IMO type 3 were ready for use. IMO3 was placed in sisal bags and stored away from sunlight in an aerated area until the time for use in the experiments.

6.2.2 Determining a surrogate to odours in pit latrines

The use of compounds whose concentrations correlate well with odour offers an objective and low cost option for representing odour intensity in research especially in developing countries (Powers, 2004). N-Butanol, acetate, hydrogen sulphide and ammonia have been identified to correlate well with odours and perceptions of latrine users' and have thus been suggested as surrogates or proxies for odour intensity (Ryan et al., 1983; Powers, 2004; Obeng et al., 2016). In this study, three compounds, acetate, hydrogen sulphide and ammonia were investigated to determine an appropriate surrogate to odour in pit latrines within urban slums of Kampala.

The concentration of each odorant was determined in 37 pit latrines within the slums of Kampala. Gas samples were drawn from the pit latrine dropholes using an aspirating pump (Kitagawa AP-20, Japan) through detector tubes for ammonia (Kitagawa 105SD and 105SE, Japan), hydrogen sulphide (Kitagawa 120SB, Japan) and acetic acid (Kitagawa 216S, Japan). The concentration of the different odour components were then determined by reading the level of the stain on each tube. In addition, the users perspectives on the smell of their latrine were recorded based on a scale described in (Nakagiri et al., 2015).

Data analysis was done using SPSS version 21. Non parametric spearman's correlation coefficient was used to measure the strength of the relationship between the odour concentrations and smell perception. Following data analysis, ammonia was chosen as the appropriate surrogate to odours in pit latrines.

6.2.3 Faecal matter sample collection

The faecal matter used in the experiments was collected from four pit latrines in Bwaise II, an urban slum in Kampala, Uganda. The faecal matter was obtained from the surface of the pit content below the drophole of the pit latrines, using a fabricated multi stage sludge sampler (Semiyaga et al., 2017). The faecal matter from all the pit latrines was then sorted to remove solid waste and mixed together to get a composite sample. The composite sample was placed in a black polyethylene bag, in a cooler box with ice cubes and transported immediately to the Public Health and Environmental Engineering Laboratory at Makerere University for use in the degradation experiments.

6.2.4 Degradation of Faecal matter with IMOs

The initial step was to evaluate the degradation of faecal matter from pit latrines with IMOs. Faecal matter in this study was degraded in small scale batch reactors, made out of 1L amber coloured glass bottles, placed in an open area in the laboratory under room temperature (23 – 28^{o}C). Treatments in this experiment are presented in Table 6.1. There were four replicates arranged in a complete randomised design. The weights used were based on the current application quantities of IMOs (not standardised) in pit latrines (I kg of IMO3) and a user load of 20 people or 4 households per stance (which is a faecal load of about 5000 g/d; using a per capita loading of 250 g/p/d) the recommended user limit for hygienic use of pit latrines

(Günther et al., 2012). Prior to loading, IMO3, were mixed with the faecal matter and placed in the reactor bottles.

Table 6.1 Treatments during an experiment to test degradation of faecal matter with IMOs

Treatment	Material	Weight used (g : g)
Test	IMO3 : faecal matter	50 : 250
Control 1	Maize flour bran : faecal matter	50 : 250
Control 2	Faecal matter only	250

Each of the reactor bottles was weighed before and after loading with faecal matter or the mixture. The weight of the contents was determined as the difference between the two weights. Reduction in faecal matter during the experimental time was monitored by measuring the reactor weights. Measurements of oxidation reduction potential (ORP), pH and temperature was done at the beginning and end of the experiment using a portable meter (Hach HQ30d flexi model, USA). The material in each reactor was characterised for moisture content, total solids (TS), total volatile solids (TVS) and COD according to standard methods for the examination of water and wastewater (APHA, 2012). The TS concentration was determined gravimetrically by taking the weight of an oven dried sample at 105^{o}C till a constant weight (after 24 hours) as a fraction of wet sample volume. The TVS was determined by taking the weight difference between oven dried solids and the 2-hour muffle furnace ignited sample at 550^{o}C and expressed as a percentage of TS. COD was determined using the closed reflux colorimetric method (APHA, 2012).

Gas samples were drawn from the free space of each reactor bottle using an aspirating pump (Kitagawa AP-20, Japan) through detector tubes for ammonia (Kitagawa 105SD and 105SE, Japan). The concentration of the ammonia was then determined by reading the level of the stain on each tube. The experiments were left to run until an increase in ammonia in the reactors was observed.

Data analysis was done using SPSS version 21. Data were normalised by log transformation. Linear regression of the independent variables was used to determine the rates of mass reduction and ammonia concentration. Analysis of variance (ANOVA) was used to determine the significant effect of treatments on the parameters measured.

6.2.5 Optimisation of IMOs for application in pit latrines

The optimisation of IMOs for application in pit latrines involved response surface experiments using a central composite design (Montgomery, 2013) to develop a predictive model of the relationship between the main factors (faecal load and IMO dosage), and the responses (ammonia concentration and mass reduction) to accurately describe the degradation process of faecal matter. In addition, the optimal load for faecal matter and IMOs were determined. Design of experiments and statistical data analyses for the second phase were done using JMP 10 statistical software (SAS Institute - North Carolina, USA). The central composite design in this study was set to five centre points giving a total of 13 experimental runs in randomized order. The experiment was conducted under laboratory conditions at room temperature (23 - 28^{o}C). IMO3 was varied with faecal load so as to account for the natural degradation of faecal matter. Additionally, in the normal operation of pit latrines within most urban slums, sharing is the norm, with no defined number of users, which affects the user load while the application dosage of IMO3 is not standardised. Since the study was at laboratory scale and in 1 L bottles, the measurements were scaled down by 0.01 (Table 6.2). The factors (independent variables) including their levels as determined by design of experiments are shown in Table 6.2. Mass reduction and ammonia concentration in the free space were determined as described previously (Section 6.2.4).

Table 6.2 Factors and their levels of the experimental design

Factor	Symbol	Felid scale Levels (g)					Levels at laboratory scale (g)				
		a	**-**	**0**	**+**	**A**	**a**	**-**	**0**	**+**	**A**
IMO Application (g)	IA	250	500	1000	1500	1750	2.5	5	10	15	17.5
Faecal matter load (g)	FL	5000	10000	20000	30000	35000	50	100	200	300	350

ANOVA was carried out to establish the main factors and interaction between the process variables and the responses. The quality of the fit polynomial model was expressed by the coefficient of determination R^2, and its statistical significance was checked by the Fisher's F-test in JMP 10 statistical software. Model terms were determined based on significant, (*p*-value) at a confidence level of 95%. Three-dimensional plots and their respective contour plots were obtained based on effects of the main factors and the optimum regions were identified.

6.2.6 Field application of IMOs

IMO3 was applied to three pit latrines, according to the rate determined during laboratory optimisation, which was 1.3kg of IMO3, twice a week. Information on smell and cleanliness of the pit latrines was collected before and during application of IMOs. The concentration of ammonia was determined as described in section 6.2.2. In addition, information about the user perception on the state of their pit latrines was collected.

6.3 Results

6.3.1 Surrogate to odours in pit latrines

The concentrations of the malodorous odours components as determined by Kitagawa gas detection tubes are given in Table 6.3. Ammonia concentrations ranged from none to 50 ppm were detected in the pit latrines. Acetic acid and hydrogen sulphide were detected in only two and three strongly smelling pit latrines respectively, at respective mean levels of 0.5 ppm and 5 (+1.7) ppm. In the study, only ammonia gas was detected at all smell levels with a wider range of values noted in the strongly smelling pit latrines. Further the results showed a significant, moderately strong relationship between ammonia and smell levels ($r_s = 0.447$, $p = 0.037$).

Table 6.3 Ammonia, acetic acid and hydrogen sulphide levels in pit latrine

Smell scale		Acetic acid (ppm)	Hydrogen Sulphide (ppm)	Ammonia (ppm)
No smell	N	ND	ND	2
	Min - max			2.54 - 400
	Mean (SD)			3.25(1.06)
Slight smell	N	ND	ND	2
	Min - max			4.00 – 6.00
	Mean (SD)			5.00 (1.41)
strong smell	N	1	3	17
	Min - max	0.5	3 - 6	2.00 – 50.00
	Mean (SD)	0.5	5 (1.73)	9.07 (10.3)

Notes ND- not detected

6.3.2 Degradation of faecal matter with IMOs

The results from the degradation experiments as depicted in Figure 6.1, show a decrease in reactor weight, for all treatments during the experiments. Results from the regressed rate of mass (Table 6.4) depicted a lower mass reduction rate per day for the test treatment to which

the IMOs were added, with overlapping confidence ranges for all reactors. ANOVA depicted no significant differences (p > 0.05) between the rates of mass reduction for the different treatments.

Table 6.4 Log –linear regressed rates of mass reduction and NH_3 in the head space of the laboratory reactors

Variable	Treatment	Log – linear regressed NH_3 concentration (ppm. Reactor^{-1}.day^{-1})	
		Beta	95% confidence interval for B
Mass reduction	Control - faecal matter only	0.902	2.08 – 10.35
	control– faecal matter and water	0.906	2.164 – 10.14
	Test –	0.876	1.11 – 8.28
Ammonia	Control - faecal matter only	0.974	64.28 – 103.60
	Control - maize flour bran	-0.375*	-19.52 – 8.26
	Test –	-0.999	-9.06 - -6.649

Note: * values is not significant (p>0.05)

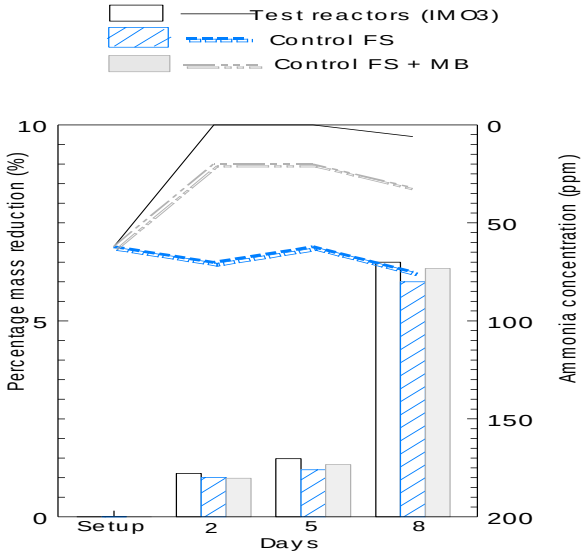

Figure 6.1 Cumulative percentage mass reduction and ammonia concentrations during degradation of faecal matter with IMOs. Line charts represent ammonia concentration (ppm) while bar charts refer to cumulative mass reduction. MB = Maize flour bran, FS = Faecal sludge

Analysis for ammonia in the head space of the reactors (Figure 6.1), showed lower concentration values for the treatment with application of IMOs than the control with only faecal matter. In the test reactors to which IMOs were applied, no ammonia was noted in the first five days of the experiment. Further, the log–linear regression results indicated an increase

in ammonia concentration in the control reactors with faecal matter, at a rate of 0.974 and a decrease in the test reactors (0.999). This indicated a 97.4% increase and 99.9% decrease in ammonia concentration per day in the head space of the control with only faecal matter and test reactors respectively. The test reactors to which IMO3 was added had significantly lower concentrations (p<0.001) of ammonia than both controls. Additionally, the control treatment where maize flour bran was added had a significantly lower ammonia concentration (p<0.001) than that with faecal matter.

The physico-chemical properties of the substrate (faecal matter and mixtures with IMOs) at the beginning and end of the degradation experiments indicated an increase in COD, TS and TVS with a decrease in moisture content obtained with addition of IMO3 (Table 6.5). Additionally, there was a decrease in both moisture content (84.3 to 72%) and pH (7.48 to 6.01) with addition of IMO3 at the beginning of the experiment. The change in physico-chemical characteristics of the contents in the reactors implied that, degradation of faecal matter occurred during the experimental time.

Table 6.5 Physico-chemical properties of substrate with application of IMO3

Parameter	Beginning	End				
	Test	Control		Test	Control	
		maize flour bran	Faecal matter		maize flour bran	Faecal matter
pH	7.94	7.6	7.48	5.84	6.01	8.1
ORP (mV)	-49.1	-48.5	-52	49.4	39.6	-77.7
Moisture content (%)	73.03	71.95	84.28	75.11	73.97	86.46
COD (mg/L)	224,700	222,220	215,300	221,000	219,460	160,900
NO_3 (mg/L)	446	464	388	486	448	408
TS (g/kg)	269.73	280.45	157.21	248.86	260.29	135.38
TVS (g/kg)	232.14	241.53	126.25	216.67	229.76	105.18
COD/TVS	0.97	0.92	1.70	1.02	0.96	1.53

6.3.3 Optimisation of IMOs for application in pit latrines

The relationship between faecal load and IMO3 dosage on ammonia concentration and mass reduction for the degradation process of faecal matter was analysed by response surface methodology. The experimental design matrix and results of each response are given in Table

6.6. The estimates of the model coefficients of both responses, calculated by least squares linear regression are shown in Table 6.7.

Table 6.6 Experimental design matrix and results of the degradation of faecal matter by IMOs

Run order	Pattern	IMO application (IA) (g)	Faecal load (FL) (g)	Percentage mass reduction	Ammonia concentration (ppm)
1	00	10	200	1.94	50
2	0A	10	350	0.99	60
3	−−	5	100	2.68	52
4	A0	17.5	200	1.39	50
5	00	10	200	1.9	65
6	++	15	300	1.62	60
7	a0	2.5	200	1.47	95
8	00	10	200	1.48	53
9	00	10	200	0.95	58
10	−+	5	300	1.02	110
11	0a	10	50	3.23	20
12	+−	15	100	1.63	40
13	00	10	200	1.47	52

Table 6.7 Analysis of variance (ANOVA) for the response surface quadratic model for faecal matter degradation

Response	Source	Sum of squares	DF	Mean square	F-Value	*p*-value (*p*>F)
Mass reduction*						
	Model	4.28	5	0.86	6.74	0.0132
	Residual (error)	0.89	7	0.13		
	Lack of fit	0.24	3	0.08	0.5	0.7021
	Pure Error	0.65	4	0.16		
	Total Error	0.89	7			
Ammonia reduction**						
	Model	5694.31	5	1138.86	19.01	0.0006
	Residual (error)	419.38	7	5.91		
	Lack of fit	274.178	3	91.3928	2.5177	0.1901
	Pure Error	145.2	4	36.3		
	Corr. Total	419.38	7			

Notes *R^2 = 0.828; Adj R^2 =0.705; ** R^2 = 0.931; Adj R^2 =0.882

The ANOVA of quadratic regression model demonstrates that the model is highly significant and accounted for 82.8% of the variability in data for mass reduction and 93.1% for ammonia concentration. Further, the adjusted correlation coefficients values (R^2 = 0.705 for mass reduction and R^2 = 0.882 for ammonia concentration) are close to the predicted R^2 values which also supports the high significance of the model. The p-values of lack of fit for both response variables were greater than 0.05, an indication that the model was adequate for the observed data at 95% confidence level. The significance of each coefficient as determined by t-values and *p*-values are listed in Table 6.8.

Table 6.8 Significance of regression coefficients (least-squares fit and parameter estimates)

Response	Model term	Parameter estimate	standard error	Computed t-value	*p*-value
Mass reduction					
	Intercept	**1.55**	**0.16**	**9.73**	**<0.0001***
	IA	-0.07	0.12	-0.55	0.6007
	FL	**-0.59**	**0.12**	**-4.82**	**0.0019***
	IA*FL	0.41	0.18	2.32	0.0540
	IA^2	-0.05	0.12	-0.43	0.6763
	FL^2	0.25	0.12	2	0.0853
Ammonia reduction					
	Intercept	**56.06**	**3.45**	**16.24**	**<0.0001***
	IA	**-15.24**	**2.65**	**-5.74**	**0.0007***
	FL	**16.24**	**2.65**	**6.12**	**0.0005***
	IA*FL	**-9.5**	**3.87**	**-2.45**	**0.0438***
	IA^2	**9.35**	**2.69**	**3.48**	**0.0103***
	FL^2	-5.09	2.69	-1.89	0.1002

Note: * values are significant (P<0.05). FL = Faecal load, IA = IMO application

The relationship between the factors and responses was further illustrated in three dimensional representation of the response surfaces and two- dimensional contour plots generated by the model. The effect of faecal matter loading and IMO3 application on mass reduction is depicted in Figures 6.2 and 6.3. The results from the surface plots (Figure 6.2a) indicate that mass reduction was highest at lower faecal matter loading and IMO3 application rates. From the regression coefficients in Table 6.8, faecal matter loading only had a significant effect on mass reduction (p=0.0019). From the contour plots (Figure 6.2b) the contours are parallel to the horizontal, indicating that the effect on mass reduction for the two parameters was independent

of each other. These results imply that regardless of IMO application, mass reduction is affected by faecal matter loading.

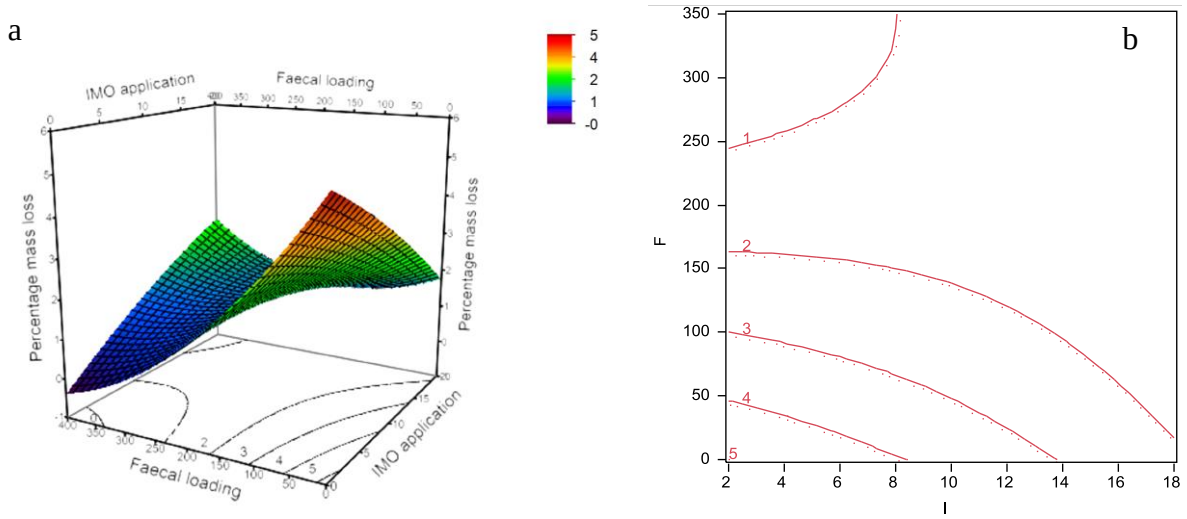

Figure 6.2 Effect of faecal matter loading and IMO3 application on mass reduction (a) Response surfaces plot, (b) and contour plot

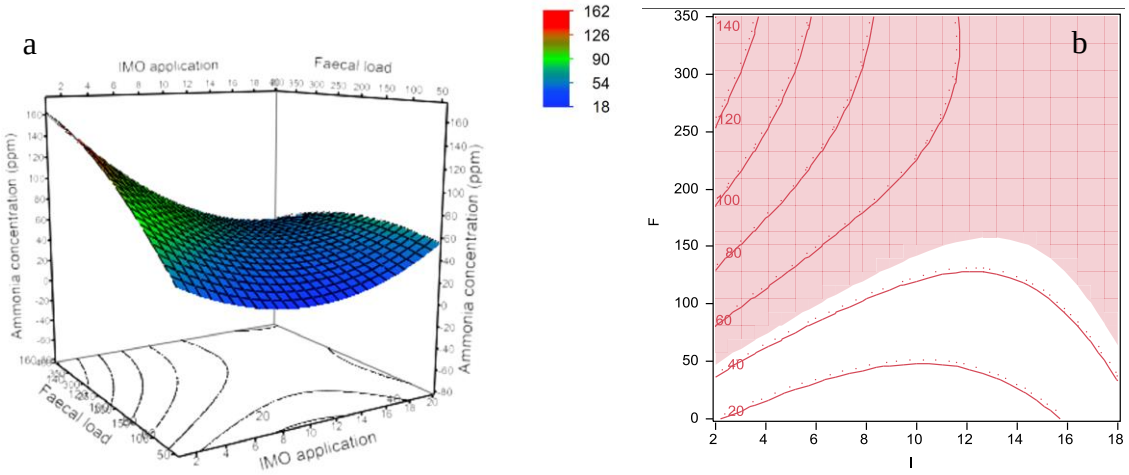

Figure 6.3 Effect of faecal matter loading and IMO3 application on ammonia concentration (a) Response surfaces plot, (b) and contour plot

Ammonia concentration was affected by both faecal matter and IMO3 loading (Table 6.8). The surface plots (Figure 6.3a) demonstrated an increase in ammonia concentration with increasing faecal matter loading. Further, the surface plots demonstrated a quadratic effect and decrease in ammonia concentration, with application of IMOs. Based on the contour plots (Figure 6.3b) the quadratic effect of ammonia concentration with IMO3 application was noted up to levels

between 40 – 60 ppm. The shaded region indicates the region in which the concentration of ammonia is pungent and irritating. To obtain levels were ammonia is not irritating, IMO3 application of 1300g is effective to a maximum faecal load of 16,000g.

The interaction between faecal matter loading and IMO application on ammonia concentrations was significant (p<0.05; Table 6.8) and this was further depicted by the inclination of the contour plots (Figure 6.3 a and b) to the horizontal. The results imply that ammonia concentration is affected by faecal loading and application of IMOs. However, the two variables, faecal loading and IMO application do not function independently of each other.

6.3.4 Field application of IMOs

The application of IMOs in this study was partly aimed at assessing their implication on reducing the smell nuisance associated with pit latrine use. An assessment of the state of the pit latrines showed variations in their performance during the study time (Table 6.9). At the start of the IMO application, Pit latrine 1 was clean with a strong smell with ammonia concentration of 3 ppm and the pit latrine also had few flies. During the time of IMO application, a decrease in smell, and ammonia concentration was noted. Furthermore, the flies were absent from the toilet. The users were happy with their facility and noted that, *"There is an improvement in our pit latrine. The smell and flies are gone"*.

Table 6.9 State of the pit latrines during application of IMOs

IMO application	Variable	Pit latrine number		
		Pit latrine 1	Pit latrine 2	Pit latrine 3
Start / 1st	Cleanliness	Clean	Clean	Dirty
	Flies	yes	None	None
	Smell	strong	Slight	Strong
	Ammonia (ppm)	3	-	2
2nd	Cleanliness	Clean	Clean	dirty
	Flies	None	None	Non
	Smell	Slight	None	Strong
	Ammonia (ppm)	3	-	10
3rd	Cleanliness	Clean	Clean	Dirty
	Flies	None	None	None
	Smell	None	none	Very strong
	Ammonia (ppm)	-	-	20

Pit latrine 2 was clean with a slight smell that was no longer noted after IMOs application. No ammonia was recorded from the pit latrine from all measurement times. Pit latrine 3 presented challenges. While it was found dirty and the importance of keeping the latrine clean stressed, the users did not maintain the latrine clean during the time of IMO application. The smell in the pit latrine remained strong. Additionally an increase in the concentration of ammonia was noted (Table 6.9). One user's perspective of the latrine was;

"The pit latrine smells and the users do not care about keeping it clean. Even when I clean it, they come to use it and some don't clean after making it dirty".

6.4 Discussion

Acetic acid (0.5 ppm) and hydrogen sulphide (5 +1.7 ppm) were detected in a few latrines. However this did not mean that acetic acid and hydrogen sulphide were not present in the pit latrines. Hydrogen sulphide concentrations as low as 0.01ppm have been reported by Obeng et al. (2016). Ammonia was detected in most pit latrines and correlated well with smell levels in this study. These findings are contrary to those by Obeng et al. (2016), where hydrogen sulphide was found to be a more reliable surrogate of the level of odour. The presence of ammonia gas could be attributed to enzymatic cleavage of urea by ureases (Jördening and Winter 2005), since urine and human faecal matter are not separated during pit latrine use. These findings suggest that ammonia could be considered a surrogate to odour levels in the pit latrines in the study setting.

The characteristics of faecal matter used in this study, are within the range reported in literature for studies on pit latrine in different locations (Chaggu, 2004; Bakare et al., 2012; Semiyaga et al., 2015). The moisture content of the faecal matter (84.3% and 90.6%) was higher than that reported by Bakare et al. (2012) (57.58 – 85.71%), suggesting wetter pits in this study. The mass reductions among the different treatments were not significant ($p>0.05$). These results are consistent with the findings in previous studies in which commercial additives were used to degrade pit latrine contents (Foxon et al., 2009; Bakare et al., 2015). This result indicates that the pit latrines, which have been in use for a long time already have sufficient population and diversity of saprophytic microorganisms and perhaps only require a favourable environment for them to perform material stabilisation and loss. Therefore, IMOs added little effect on the rate of matter reduction but could have changed the pattern manifested by increased

degradation. This is further indicated by a higher COD:TVS ratio where the IMOs were added than the control (Table 6.5), signifying more degradation had occurred (Gebauer & Eikebrokk, 2006). However, the ORP values (-77.7 to + 49.4) for all treatments indicated that the conditions could have been favourable for hydrolysis but not optimal for material stabilisation, which results in mass loss. ORP values of less than -200 are favourable for the reduction of sulphur compounds, acetate fermentation and methane formation (Madigan et al., 2015), which are the material stabilisation phases of anaerobic degradation.

Ammonia concentration was lowest in all the treatments where IMO3 were applied, while a reduction in smell was noted in the clean toilets to which IMO3 was added. IMOs are a consortium of different organisms, some of which synthesize malodorous gaseous products that are used by another group of organisms. For example, ammonia produced by ammonifying bacteria is a source of energy for some chemoheterotrophs species of IMOs which oxidse NH_3 to NO_3^- decreasing NH_3 emission. *Bacillus. spp, and Pseudomonas* utilise free fatty acids to synthesise major, saturated, branched and/ or long chain fatty acids which are not malodorous (Zhang et al., 1999; Fujita et al., 2007; Diomande et al., 2015). *Stenotrophomonas maltophilia,* can degrade phenolic compounds and hydrocarbons. Additionally, microbial groups that produce malodourous gases could have been suppressed through competition (Higa & Parr, 1994).

Furthermore, ORP (+49.6 and +39.6) and pH (5.84 and 6.01) indicated denitrification processes by denitrifying bacteria present in the IMO3 (Madigan et al., 2015). Some species of denitrifying bacterial strains such as, *Pseudomonas* and *Stenotrophomonas maltophilia* found in the IMOs are active under anaerobic conditions (Rittmann & McCarty, 2001; Mukherjee & Roy, 2016). Additionally, the decrease in ammonia could be attributed to the reduction of pH or acidification which is known to reduce ammonia emissions (Petersen et al., 2012). Further, the carrier media (maize flour bran) could have had an effect on the gas volatilisation resulting in further reduction in ammonia concentration. This is because the addition of maize flour bran reduced the moisture content which could have influenced the gases realised as volatilisation is liquid-phase controlled, which allows gas bubbles to form and escape to the atmosphere (Blanes-Vidal et al., 2012).

This study further depicted the effects of application of IMO3 using response surface methodology. From the results, only faecal matter loading had a significant effect on mass reduction (p = 0.0019). Higher faecal loads, demonstrated a decrease in mass reduction (Figure 2). In addition, faecal matter loading significantly increased the ammonia concentration (p = 0.0005). The high concentration of ammonia at higher faecal matter loads could have resulted from high quantities of nitrogen as proteins in the faecal matter, which are putrefied to ammonia under anaerobic conditions. Application of IMOs significantly reduced (p=0.0007) the ammonia emmisions. In addition, the interaction between IMO application and faecal matter loading had a significant, quadratic effect on to the reduction in ammonia. These findings are important as they signify that IMOs are effective in reducing ammonia. However, the faecal load which reflects the numbers of users is equally significant and the two act interactively. Thus, an upper limit to both faecal load and IMO application rates should be considered during ammonia and smell management in pit latrines.

The results further suggest that in the normal operation of pit latrines, at a faecal load of about 5000g/d; which corresponds to about 20 people or four households at a per capita loading of 250g/p/d, the ammonia concentration in pit latrines is expected to be below detection limits. Twenty people or four households is the recommended user limit to meet hygienic use of pit latrines (Günther et al., 2012). IMO application is effective up to an application of 1300g at a faecal load of 16,000g. This implies an application of IMOs twice a week in a pit latrine used daily by four households.

The reduction in flies noted during the field application is consistent with results where IMOs were applied in inoculated deep litter systems of swine farming (DuPonte & Fischer, 2012) and poultry farming (SomaSekhar et al., 2013). This could be attributed to the toxic insecticidal proteins released by microbial strains of *Stenotrophomonas maltophilia* and *Bacillus thuringiensis* that were found in the IMOs. Additionally, the formation of long chain fatty acids could have led to the elimination of some volatiles that act as pheromones to flies while an increase in ORP implies conditions not favourable for formation of methyl- sulphides known to attract flies (Cosse & Baker, 1996).

6.5 Conclusion

The study demonstrated the potential of application of IMOs in degradation of faecal matter for improving the performance of pit latrines by reducing ammonia and their malodourous nature. Using response surface methodology, it was noted that mass reduction decreased with increasing faecal matter loading rate. Ammonia concentration increased with faecal matter loading but reduced following the application of IMO3. Furthermore, application of IMOs in pit latrines reduced the smell and eliminated flies resulting into user satisfaction due to better performance of their facility. The recommended application rate of IMOs in a pit latrine of 20 users per stance is twice a week. In addition, to applying IMOs, it is important to maintain clean and hygienic pit latrines.

6.6 References

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CHAPTER SEVEN

7 General Discussion

7.1 Pit latrine usage in urban slums

Access to adequate sanitation is crucial for improved human health and environmental protection. Improved human excreta disposal can reduce morbidity rates and improve the livelihoods of many people by substantially minimising the severity of various diseases (Bartram & Cairncross, 2010; Mara et al., 2010). Demand for human excreta disposal in most low-income areas is mainly met by on-site facilities, predominantly pit latrines (Strande, 2014). Globally, about 1.77 billion people, use pit latrines as their primary means of sanitation (Graham & Polizzotto, 2013). Analysis of estimates on usage of sanitation facilities, found out that about 53% of the urban population of SSA use pit latrines (Chapter 2).

Within the urban poor areas, higher figures for pit latrine access than other sanitation technologies, have been noted. For example, in Kampala, Uganda, over 95% of the slum population use some form of pit latrine (Tumwebaze et al., 2012). Similarly, 87% of the respondents in low-income areas of Ashanti region, Ghana, (Appiah-Effah et al., 2014), and about 83% in those of Dar es Salaam, Tanzania were using pit latrines (Jenkins et al., 2014). The wide spread access to pit latrines has been attributed to high acceptability, simplicity and low cost of the sanitation technology (Franceys et al., 1992). Pit latrines are most likely to remain the technology of choice for the low-income people within urban slums. This is because sewerage sanitation coverage is very low (Strande, 2014), moreover sanitation provision is at self-help basis by household owners/ landlords who opt for cheaper technologies (Kariuki et al., 2003; KSMP, 2004). The importance of pit latrines within urban slums has also been recognised by practitioners who consider them collection and storage units of human excreta, ahead of emptying, and transportation for further treatment and safe disposal or reuse (Katukiza et al., 2012; Tilley et al., 2014). However, the performance (filling, smell and insects nuisances) of pit latrines in urban slums raises issues of concern (Chapter 2).

An assessment of pit latrines within slums of Kampala found out, that most did not offer hygienically safe sanitation access to their users (Chapter 3). Seven in every ten of the pit latrines were either full or overflowing. Findings from the study of environmental conditions

in pit latrines (Chapter 4) showed that pit latrines were within 911 (±526) mm of filling. These findings are similar to those reported earlier by Günther et al. (2011) and studies undertaken in other urban slums (Appiah-Effah et al., 2014; Bakare, 2014; Jenkins et al., 2014). The presence of strong smells and flies, which were found in this study (Chapter 3 and 4) are consistent with findings from other studies undertaken in urban slums (Tumwebaze et al., 2012; Irish et al., 2013; Kwiringira et al., 2014). The presence of full, smelly pit latrines having flies greatly compromises the user's ability to accessing the facilities, consequently increasing the chances of open defecation and occurrence of health issues related to improper human excreta disposal. This highlights a need to address pit latrine performance related issues in urban slums.

7.2 Key factors affecting pit latrine performance

In order to get an insight into key factors affecting pit latrine performance, an investigation into the influence of the status of pit latrine structures (design, construction, operation and maintenance) on their filling, smell and presence of insects was undertaken. The predictors of pit filling were found to be signs of rain/storm water entry, flooding areas and cleaning times. These findings implied that strategies to address pit latrine filling problems should minimise water entry into to the pit. Most pit latrines within urban slums were with superstructure that were not structurally sound (i.e had cracks) (Chapter 2) allowed entry of rain/ storm water. In areas with high water table, whereas the pits were constructed and raised above the ground, most of the bottoms were not lined, thus groundwater entry was likely. Additionally, many users cleaned and directed the wash water into the pit, which increased water entry and thus filling. Minimising the cleaning frequencies could be done by improving the pit latrine design to reduce soiling of the squatting slabs. This could be attained by improving the dropholes of the pit latrines within the slums. Keyhole shaped openings, that are above 350 mm long, have been found to be adequate to prevent soiling of the squatting plate during use of the latrines (Wagner & Lanoix, 1958; Franceys et al., 1992).

The strongest determinant of pit latrine smell was found to be its cleanliness (Chapters 3). This points out the importance of latrine cleanliness during smell management. Thus interventions aimed at improving pit latrine cleanliness within urban slums could potentially reduce their smell. Cleaning before/ after use of the facility ensured cleaner pit latrines and thus less smell. Furthermore, pit latrines used by households only were more likely to smell less that those

open to the public, which could be attributed to household latrines being presumed to be less effortless to clean (Tumwebaze et al., 2014). As pit latrine cleaning is a behavioural hygiene practice, involving users/caretakers in strategies to improve smell of pit latrines is crucial.

Other determinants of latrine smell were found to be superstructure material and stance length (Chapter 3). Timber/ roofing superstructures allowed more air flow than the block structures. However, longer stance lengths not only increased the chances of pit latrine soiling but, they also decrease the air exchange rate, and odours were thus not well exhausted but retained in the latrine (Wagner & Lanoix, 1958; Mara, 1984). The study also found that, having a vent pipe did not signify less smell in the latrines. The air flow rate in VIPs within the slums ($0 – 1.8 ms^{-1}$) (Chapter 4) were too low to maintain odourless superstructures. The inappropriate VIP design (pipe sizes/ colour, siting and cross ventilation) (Chapter 3), and overcrowding in the slums (Isunju et al., 2011) could have led to the low air flow rates.

The presence of flies in pit latrines within urban slums was related to their superstructure material, its state (signs of collapse) and the terrain (flooding area). This finding is consistent with Irish et al. (2013) who noted that absence of a roof over the latrine and temporary superstructures as opposed to brick superstructures were positively correlated with high numbers of flies. Interestingly, dirtiness did not contribute to flies presence. This could imply that factors other than presence of faecal matter contributed to the presence of flies in the pit latrines.

The degradation of human excreta has been linked not only to flies nuisances (Wagner & Lanoix, 1958), but also smell and filling problems of pit latrines (Mara, 1984; Still & Foxon, 2012). In this study, the effect of the degradation processes in the pit (represented by the ORP of the content) on the performance of the latrine was investigated (Chapter 4). The ORP of the excreta at the surface of the content in most clean, smelling latrines that had flies indicated anaerobic conditions in the acid formation range (-199 mV to -51 mV). These findings are consistent with Lin et al. (2013) who mainly attributed the rancid smell in a ventilated pit of Kampala, to carboxylic acids, including isobutyric, butyric, isovaleric, 2-methylbutyric, valeric, hexanoic, and phenylacetic acid. Flies on the other hand have been found to be attracted to mixtures of acetic acid, furfural, butanoic acid, isovaleric acid, hexanoic acid, 2-phenylethanol, p-cresol, 3-methylbutanoic acid, phenol, benzene ethanol, indole, 3-

methylindole dimethyldisulfide, dimethyltrisulfide and dimethyltetrasulfide (Cosse & Baker, 1996; Qian et al., 2013), which were noted as some of the volatile constituents of pit latrine contents (Lin et al., 2013b)

An alteration in the degradation processes in the pit could have a significant effect ($G = 0.797$, $p = 0.014$) on the latrines smell and not flies. A decrease in ORP could lead to emission of sulphur compounds like methyl sulphides, methyl mercaptan, and H_2S, which are known to give a strong, sewage, rotten egg, and rotten vegetable odour (Lin et al., 2013). Contrary to smell, the absence of a correlation between flies and the degradation process could be because flies are phototropic in nature (Wagner & Lanoix, 1958). Additionally volatiles that act as their pheromones drawing them to the pit latrines can be emitted during the different degradation processes. Modifying of the environment in the pit and thus the degradation processes could be done by bio-stimulation, which involves introduction of exogenous material and/ or microorganisms (Cosgrove et al., 2010) in the pit that could stimulate the metabolic pathways of human excreta decomposition. This was explored in Chapters 5 and 6 and discussed in the next section.

7.3 The role of Indigenous microorganisms in improving the performance of pit latrines

Microorganisms are drivers of matter degradation and thus the pit latrine performance related issues could be improved by amending the functional microbial communities in the pit. In this study, the application of indigenous microorganisms (IMOs), a bio-stimulant that has been successfully used to improve degradation of wastes (Hanim et al., 2012; Bakar et al., 2015; Zakarya et al., 2015) and reduce foul odours and flies during swine and poultry farming (DuPonte & Fischer, 2012; SomaSekhar et al., 2013) was proposed.

IMOs are a consortium of microorganisms, and in this study there were collected from the soil using cooked white rice, cultured in brown sugar and further propagated on maize flour. The IMOs in this study were cultivated from soils whose environment (21.0 ^{o}C to 29.0^{o}C temperature and 5.83 – 8.43 pH) did not differ from that of pit latrines within Kampala's slums (21^{o}C to 30.7^{o}C, temperature and 5.0 and 11.8 pH). However, the major challenge of their applicability was having the right microbes that could survive and succeed when used in pit latrines.

An evaluation of microbial communities in the IMOs (Chapter 5) showed the possibility of obtaining a consortium of microorganisms that include *Bacillus subtilis, Stenotrophomonas maltophilia, Chryseobacterium ureilyticum, Xanthomonas retroflexus, Sphingobacterium spp., Pseudomonas geniculata* strains, *F.ogurii, Bacillus thuringiensis, Bacillus cereus,* uncultured bacterial strains and fungal *Saccharomyces cerevisiae, Galactomyces geotrichum* and *Geotrichum candidum* strains. Based on their characteristics that include high colonising abilities and global regulating responses (Atkinson & Williams, 2009; Ryan et al., 2009); broad metabolic properties to degrade organic matter and complex compounds; and their wide biotechnological applicability (Ishii & Takii, 2003; Charimba, 2012; Ritari et al., 2012; Jahid & Ha, 2014; Jin et al., 2017), IMOs can easily adopt and regenerate by degrading the organic molecules (proteins, carbohydrates and fats) of human excreta in pit latrines.

Application of IMOs during degradation of human excreta from pit latrines had a significant (p = 0.0005) effect on the of malodour ammonia, which was a surrogate of smell and could thus account for the reduction in the smells observed during its application in pit latrines (Chapter 6). Moreover, the enzymes and proteins released during the degradation of excreta could be toxic to flies (Zissi & Lyberatos, 2001; Madigan et al., 2015; Mukherjee & Roy, 2016) and result in their elimination (Chapter 7). The reduced smell and elimination of flies in pit latrines following the application of IMOs led to improved performance. Even with no significant mass reduction following the application of IMOs, the decrease in moisture content (from 84.3% to 72%) that was attributed to the carrier media (maize flour bran) implies less challenges during management of faecal sludge once the pits are emptied (Semiyaga et al., 2017).

The efficiency of application of IMOs is a subject of repeated application of the inoculum so as to establish an indigenous microbial community in the pit latrine. Models for degradation of pit latrine content with IMOs (Chapter 6) demonstrated a quadratic effect in ammonia reduction to a maximum faecal load of about 16,000 g and IMO mass of 1.3 kg. This would imply that, at the recommended user limit of 20 people or 4 households per stance for attaining hygienically clean pit latrines (Günther et al., 2012), which translates to a daily faecal matter load of 5000g/d (at a per capita loading of 250g/p/d), IMOs application should be re-applied after 3days (or twice a week). However, field application also demonstrated the importance of maintaining clean pit latrines for the effectiveness of IMOs.

7.4 Implications of the findings in this study on improving the performance of pit latrines

Knowledge of performance of pit latrines within specific settings is essential for any interventions to make them appropriate. Interventions to improve sanitation conditions should not be limited to only access to sanitation facilities, but also consider their adequacy. Developing and dissemination of standards for urban slum pit latrines and their cleanliness are vital. Pit latrine filling was best explained by the condition of the superstructure and was found to be critical within flooding areas. Moreover, stance length and the type of the superstructure material seemed to have an influence on the latrines smell and flies. Higher superstructure standards seem to be adequate for urban slums. However, the use of VIP may not be appropriate due to obstruction from buildings resulting from overcrowding in the slums (Chapter 3).

The cleanliness of the pit latrines best explained their smell while latrines that were cleaned more often (before/ after use) were more likely to smell less. Additionally, household usage of pit latrines ensured their cleanliness. However, the chances of having a high pit content level in regularly cleaned latrines were noted to be high. Interventions to address the cleanliness of the pit latrines within urban slums should look at minimising soiling during their use (Chapter 3). Decreasing the super structure length, increasing the current length of pit latrine dropholes to the recommended minimum standard and providing properly located footrest could minimise soiling of pit latrines. As cleanliness is a behavioural hygiene practice, sensitising users on the need to minimise soiling and cleaning times of the pit latrines to improve their performance is important.

Improving the environment in the pit could minimise smell and flies nuisances of pit latrines (Chapter 4). In this study, it was demonstrated that IMOs had the potential to enhance the degradation of human excreta and improve the performance (smell and flies) of pit latrines (Chapter 5 and 6). However, pit latrine usage by the recommended limit of 20 people or 4 households per stance (Günther et al., 2012), their cleanliness and repeatedly applying the bio-stimulant are important during IMO application.

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CHAPTER EIGHT

8 Conclusions and Recommendations

8.1 General conclusions

The following are conclusions drawn from the research in line with the study objectives:

Specific objective 1: A review and assessment of the design, operation, performance and processes of pit latrines in urban slums

- A pit latrine is the most basic form of sanitation which is currently used by a number of people around the globe and urban areas of SSA. A review of literature found out that varying pit latrine design were in use in urban SSA with adaptation of improved than traditional pit latrines. The commonest design within urban slums was a simple pit latrine with a concrete slab.
- The performance of pit latrines in terms of filling, smell and insects was found to be inadequate. Scientifically guided studies into the performance and processes of pit latrines especially in urban slums was found to be limited.

Specific objective 2: The key factors affecting the performance of pit latrines

- The status of pit latrine structures had an effect on their performance. The level of pit content was predicted by signs of rain or storm water entry, terrain, and cleaning before or after use.
- Smell was determined by the latrines cleanliness, stance length, superstructure material and whether the latrine was private or public, while the predictor of presence of flies was the superstructure material, the state of the latrine (signs of collapse) and the terrain (high/low water table). Additionally, having a VIP did not indicate better performance (smell, insect).
- The environment in the pit was found to be mainly anoxic (ORP < + 50mV). There was a positive correlation between the processes in the pit latrine represented by ORP and their performance (smell and insects nuisance). However, the correlation was only significant ($G=0.797$, $p=0.014$) for smell in clean pit latrines. Therefore, changing the processes in the pit would only affect the smell of clean pit latrines.

Specific objective 3: The efficacy of application of indigenous microorganisms (IMOs) in human excreta decomposition and pit latrine use

- The environment in which IMOs were collected (21.0 ^{o}C to 29.0^{o}C temperature and 5.83 – 8.43 pH) did not differ from that of pit latrines within Kampala's slums (21^{o}C to 30.7^{o}C, temperature and 5.0 and 11.8 pH).
- Fingerprinting of microorganisms in IMOs found that most prominent bacteria strains were *Stenotrophomonas maltophilia, Bacillus spp., Sphingobacterium spp., Xanthomonas, retroflexus Chryseobacterium ureilyticum, Pseudomonas geniculate*, *Flavobacterium Ogurii*, and a number of uncultured bacterial colons, while the fungi was dominated by *Galactomyces geotrichum* and *Geotrichum candidum* strains. The broad microorganisms' properties indicated that they could adopt to the pit environment, degrade human excreta and minimise pit latrine performance problems.

Specific objective 4: IMOs as a solution to improving the performance of pit latrines

- Application of IMOs reduced the ammonia concentration, smell and flies in pit latrines. IMOs application was found to be effective up to 1.3 kg at a faecal load of 16 kg/day which corresponds to an application frequency of two times a week at a pit latrine user limit of 20 people per stance. Keeping pit latrines clean even with IMOs application was crucial for smell management.

8.2 Recommendations

This research has provided knowledge on the factors affecting the performance of pit latrines within urban slum areas. Additionally, a scientific basis for the use of IMOs has been provided. However, more studies in the areas indicated below that were not covered under this research could help improve the understanding of performance related issues of pit latrines and how to overcome them.

8.2.1 General recommendation

- There is need to develop appropriate pit latrine standards for use in urban slums. Determining appropriate construction material for use within the slums is also essential. While Brick superstructures were found to be adequate, polyethylene and mud/wattle structures may not be appropriate for urban slums.

- There is need to determine appropriate dimension of pit latrine superstructures as this impacts on their volume and air exchange rate that are critical for smell management. Additionally, improving the drop holes of the pit latrines to keyhole shaped openings, that are above 350 mm long would minimise soiling of the slab during use of the facility ensuring cleaner facilities and reducing water entry resulting from regular cleaning.
- IMOs are appropriate in improving the smell and flies in pit latrines. An application rate of 1.3kg twice a week is appropriate in a pit latrine used daily by 20 people per stance.
- Improving the performance of pit latrines necessitate sensitisation of the users/ owner and builder on the appropriate designs, factors that affect their performance and recommended strategies that could improve their performance.

8.2.2 Policy recommendation

- Proper performance of pit latrines within urban slums necessitates adoption of appropriate pit latrine standards. Therefore, approving, ensuring dissemination and implementation of pit latrine standards within urban slums, and proper use of the facilities is important. This necessitates a collaboration between different key players in sanitation who include; policymakers, authorities, household owners/ landlords and pit latrine users.

8.2.3 Perspectives for research

- There is need to upscale IMO application and undertake longitudinal studies assessing their efficiency in improving pit latrine performance. Additionally, employing fingerprinting techniques like Denaturing Gradient Gel Electrophoresis (DGGE) during application of IMOs will provide more insight to justify their use in pit latrines
- Research into the pit ecology, employing finger printing techniques (PCR-DGGE) and primer tailored to decomposition processes could provide a better insight into the decomposition processes, their pathways and effects on pit latrine performance. An insight into the determinants of the insect nuisances could also be made possible.
- Characterisation of insects found in pit latrines is important. The characterisation of the pit latrine environment, and available insects will help provide a clear understanding of

their pheromones behaviour and what draws them to pit latrines thereby developing more strategies to mitigate them.

- The study found that VIPs did not provide superior performance to the simple pit latrine which was attributed to inadequate designs and limitation of air movement resulting from surrounding buildings. There is need to carry out studies to improve the VIP latrine design to make it appropriate for use in overcrowded urban slums.

Appendix

Letters

MAKERERE UNIVERSITY

P. O. Box 7062 Kampala Uganda
Cables: "Makunika"

Telephone : 256 - 41 - 530686
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Email : Civil@tech.mak.ac.ug

DEPARTMENT OF CIVIL AND ENVIRONMENTAL ENGINEERING

Your Ref:

Our Ref:

16th September 2013

To Whom It May Concern
Kampala

Dear Sir/ Madam,

RE: INTRODUCING Ms. ANNE NAKAGIRI

I hereby introduce to you Ms. Anne Nakagiri, a PhD student in the Department of Civil and Environmental Engineering in the College of Engineering, Design, Art and Technology (CEDAT) at Makerere University. Ms. Anne Nakagiri is collecting Data for her PhD studies, on pit latrines in Bwaise II, Kampala. I believe that the findings of this study will be useful to Kampala Capital City Authority (KCCA) in tackling the challenges of the performance and fast filling up of pit latrines in urban slums in Kampala.

The purpose of this communication is to kindly request you to assist her. I will greatly appreciate any assistance rendered to her to enable her collect data for her studies.

Yours faithfully,

Dr. Charles B. Niwagaba
Head of Department

CIVIL AND ENVIRONMENTAL ENGINEERING DEAPRTMENT
16 SEP 2013
MAKERERE UNIVERSITY P.O.BOX 7062, KAMPALA

KCCA
KAMPALA CAPITAL CITY AUTHORITY
For a better City

OFFICE OF THE TOWN CLERK
KAWEMPE DIVISION URBAN COUNCIL
P.O BOX 7010, KAMPALA

Our ref: KDUC/KCCA/201/17
Tuesday 24th September, 2013

The LC 1 Bwaise II Parish,
Kawempe Division.

Re: **INTRODUCTION OF MS. ANNE NAKAGIRI**

The above named is a student of Makerere University, Department of Civil and Environmental Engineering, College of Engineering, Design, Art and Technology (CEDAT). She is collecting data for a PhD studies on pit latrines in Bwaise II, Kawempe Division (find attached photocopies of university introduction letter and identity card).

This letter requests you to offer her the necessary assistance to enable her accomplish the task successfully.

Yours faithfully,

KAMPALA CAPITAL CITY AUTHORITY
KAWEMPE DIVISION URBAN COUNCIL
KCCA
24 SEP 2013
TOWN CLERK'S OFFICE

Robert Katungi
For: TOWN CLERK

Tables

LIST OF TABLES

Table A. 1 Summary data on pit latrine use in urban areas of Sub-Saharan Africa

Country	Data Source	Report year	Flush Toilet (%)	Pit Latrine usage (%)	Pit latrine usage by type (%)						
					Pour flush	VIP	Pit latrine with slab (covered pit)	Traditional latrine	Pit latrine without slab (open pit)	Traditional latrines considered improved	2015 Population (Thousands)
Burundi	DHS-MIS	2012	34.3	64.6	0.0	0.0	32.9	25.6	6.0	14.6	1,305
Comoros	EIM	2004	14.9	83.7	0.0	0.0	35.2	19.2	29.2	0.0	219
Eritrea	DHS	2002	41.8	18.8	0.0	3.2	0.0	15.6	0.0	7.8	1,564
Ethiopia	DHS	2011	7.2	72.2	0.0	3.3	31.7	0.0	37.1	18.6	17,873
Kenya	MIS	2010	29.4	65.4	0.0	26.2	0.0	39.2	0.0	19.2	11,985
Madagascar	MIS	2011	16.7	49.3	0.0	0.3	3.9	0.0	39.0	19.5	8,512
Mauritius	CEN	2011	99.6	0.7	0.1	0.0	0.0	0.3	0.0	0.2	525
Rwanda	DHS	2010	5.6	92.9	0.0	3.1	79.1	0.0	10.7		2,530
Somalia	MICS	2005	22.6	69.8	41.3	1.6	12.5	0.0	14.4		4,410
South Sudan	CEN	2008	1.0	41.5	0.0	0.0	0.0	41.5	0.0	20.6	2,289
Sudan	MICS	2010	21.5	66.9	0.0	10.6	28.3	0.0	28.0		13,405
Uganda	DHS	2011	11.6	86.2	0.0	17.2	39.1	22.6	5.5	11.3	6,930
United Republic of Tanzania	LSMS	2011	27.7	88.7	20.7	6.4	35.3	26.3	0.0		14,953
Benin	DHS	2012	12.4	54.8	0.0	4.4	35.8	0.0	13.0		5,169
Burkina Faso	DHS	2010	7.0	79.6	0.0	1.8	72.8	0.0	5.0		5,352
Côte d'Ivoire	DHS-MIS	2012	44.6	47.5	0.0	0.0	31.6	0.0	15.9		11,536
Cameroon	DHS	2011	20.7	77.6	0.0	2.0	60.8	0.0	14.5		12720

Country	Data Source	Report year	Flush Toilet (%)	Pit Latrine usage (%)	Pit latrine usage by type (%)						
					Pour flush	VIP	Pit latrine with slab (covered pit)	Traditional latrine	Pit latrine without slab (open pit)	Traditional latrines considered improved	2015 Population (Thousands)
Cape Verde	CEN	2010	74.2	22.0	21.6	0.0	0.0	0.4	0.0	0.2	333
Equatorial Guinea	MICS	2000	30.3	69.7	32.7	0.0	29.0	0.4	0.0	0.2	321
Gambia	MICS	2010	33.7	65.8	0.0	5.9	51.5	0.0	8.4		1,171
Ghana	DHS	2011	29.1	60.9	0.0	40.0	10.7	0.0	9.7		14,702
Guinea	DHS	2012	45.7	53.7	0.0	3.0	37.4	0.0	13.1		4,619
Guinea-Bissau	MICS	2010	22.7	74.6	4.4	0.0	0.0	62.3	0.0	7.9	833
Liberia	DHS	2011	40.9	38.4	0.0	4.6	8.3	0.0	15.1		2,239
Mali	MICS	2010	13.2	84.3	0.0	0.8	59.5	0.0	24.0		6,100
Mauritania	MICS	2007	35.2	51.5	0.0	11.5	21.6	0.0	18.4		1,739
Niger	LSMS	2011	8.0	76.3	0.0	0.0	29.0	27.4	19.9	13.7	3,637
Nigeria	MICS	2011	50.5	37.3	0.0	2.2	25.9	0.0	9.0		95,564
Sao Tome and Principe	DHS	2009	40.6	6.6	0.0	6.6	0.0	0.0	0.0		132
Senegal	DHS	2013	43.2	54.1	0.4	11.7	34.0	8.0	0.0	4.0	6,554
Sierra Leone	OSM	2011	15.7	77.6	0.0	4.1	45.1	0.0	27.9		2,576
Togo	MICS	2010	41.4	38.8	0.0	0.7	30.9	0.0	6.2		2,866
Central African Republic	MICS	2010	1.4	91.6	0.0	1.4	9.7	0.0	18.3		1926
Chad	MICS	2010	18.5	63.1	0.0	1.0	33.3	0.0	24.7		3,019
Congo	DHS	2012	15.1	83.5	0.0	2.0	42.2	0.0	37.0	13.5	3053
Democratic Republic of the Congo	MICS	2010	12.6	83.1	0.6	0.7	0.0	21.4	60.4	11.1	25,996

Country	Data Source	Report year	Flush Toilet (%)	Pit Latrine usage (%)	Pit latrine usage by type (%)						
					Pour flush	VIP	Pit latrine with slab (covered pit)	Traditional latrine	Pit latrine without slab (open pit)	Traditional latrines considered improved	2015 Population (Thousands)
Gabon	DHS	2012	38.0	59.1	0.0	6.2	26.7	0.0	22.3		1,530
Angola	MICS	2011	42.3	45.2	0.0	2.2	36.2	0.0	6.0		14,193
Botswana	BAIS	2008	41.0	56.0	0.0	0.0	0.0	56.0	0.0	33.9	1,319
Lesotho	CMS	2012	11.1	84.0	0.0	42.9	24.0	0.0	17.1		646
Malawi	DHS	2012	13.7	82.3	0.0	7.2	24.1	0.0	51.0		2,822
Mozambique	DHS	2011	18.6	77.1	9.6	22.5	15.9	0.0	29.0		8,746
Namibia	CEN	2011	68.7	8.5	0.0	3.6	2.2	0.0	1.4		973
South Africa	IES	92.3	5.8	0.0	1.8	0.0	3.3	0.0	1.8	2.1	34,101
Swaziland	MICS	46.4	52.0	0.0	7.8	40.1	0.0	4.1	47.9		272
Zambia	CEN	31.9	67.8	0.0	2.6	0.0	65.2	0.0	2.6	49.0	6,352
Zimbabwe	DHS	88.2	9.8	0.0	3.1	3.1	0.0	1.9	6.2		6,106

Notes;

Data source DHS: Demographic and Health Survey, CEN: Census, MICS: Multiple Indicator Cluster Survey, MICS: Multiple Indicator Cluster Survey, AGVSAN: Analyse Globale de la Vulnérabilité, de la Sécurité Alimentaire et de la Nutrition, LSMS: National survey on household living conditions and agriculture, OSM Opportunities for Sanitation Marketing in Sierra Leone, BAIS Botswana Aids Impact Survey

Table A. 2 Comparison of 2015 and 2007 pit latrine coverage figures

Country	Sanitation access 2015					Sanitation access 2007			
	Flush Toilet	VIP/ pit latrine with slab/ pour flush latrines/ SANPLAT	Traditional latrine	Pit latrine without slab (open pit)	2015 Population (Thousands)	Flush Toilet	VIP/ Ventilated toilet/ SANPLAT	Traditional pit latrine	Population 2007 (Thousands)
Benin	41.4	31.6	0.0	6.2	5169	6.0	29.0	26.0	3683
Burkina Faso	7.0	74.6	0.0	5.0	5352	8.0	70.0	14.0	3297
Cameroon	20.7	62.8	0.0	14.5	12720	16.0	41.0	41.0	9499
Central African Republic	1.4	11.1	0.0	18.3	1926	2.0	6.0	86.0	1574
Chad	18.5	34.3	0.0	24.7	3019	7.0	12.0	64.0	2317
Comoros	14.9	35.2	19.2	29.2	219	8.0	35.0	56.0	176
Congo	15.1	44.2	0.0	37.0	3053	10.0	25.0	62.0	2326
Côte d'Ivoire	44.6	31.6	0.0	15.9	11536	30.0	23.0	44.0	8663
Democratic Republic of the Congo	12.6	1.3	21.4	60.4	25996	4.0	26.0	60.0	12518
Ethiopia	7.2	35.0	0.0	37.1	17873	8.0	4.0	77.0	12949
Gabon	38.0	32.9	0.0	22.3	1530	32.0	27.0	40.0	1224
Ghana	29.1	50.7	0.0	9.7	14702	23.0	39.0	27.0	11067
Guinea	45.7	40.4	0.0	13.1	4619	8.0	3.0	87.0	3382
Kenya	29.4	26.2	39.2	0.0	11985	39.0	11.0	44.0	8464
Lesotho	11.1	66.9	0.0	17.1	646	8.0	38.0	45.0	483
Madagascar	16.7	4.2	0.0	39.0	8512	7.0	67.0	6.0	5820
Malawi	13.7	31.3	0.0	51.0	2822	18.0	2.0	74.0	2089

Country	Sanitation access 2015					Sanitation access 2007			
	Flush Toilet	VIP/ pit latrine with slab/ pour flush latrines/ SANPLAT	Traditional latrine	Pit latrine without slab (open pit)	2015 Population (Thousands)	Flush Toilet	VIP/ Ventilated toilet/ SANPLAT	Traditional pit latrine	Population 2007 (Thousands)
Mali	13.2	60.3	0.0	24.0	6100	15.0	21.0	62.0	4117
Mauritania	32.2	33.1	0.0	18.4	1739	4.0	9.0	66.0	1353
Mozambique	18.6	48.0	0.0	29.0	8746	8.0	5.0	68.0	6732
Namibia	68.7	5.8	0.0	1.4	973	79.0	2.0	5.0	752
Niger	8.0	29.0	27.4	19.9	3637	5.0	55.0	21.0	2420
Nigeria	50.5	28.1	0.0	9.0	95564	28.0	5.0	58.0	69281
Rwanda	5.6	82.2	0.0	10.7	2530	6.0	48.0	43.0	1789
Senegal	43.2	46.1	8.0	0.0	6554	65.0	9.0	22.0	4944
South Africa	92.3	1.8	3.3	0.0	34101	80.0	0.0	9.0	29852
South Sudan	1.0	0.0	41.5	0.0	2289	14.0	0.0	60.0	12518
Sudan	21.5	38.9	0.0	28.0	13405				
Togo	41.4	31.6	0.0	6.2	2866	0.0	33.0	35.0	2107
Uganda	11.6	56.3	1.9	5.5	6930	11.0	9.0	78.0	4298
United Republic of Tanzania	27.7	62.4	26.3	0.0	14953	10.0	12.0	75.0	10280
Zambia	31.9	2.6	65.2	0.0	6352	47.0	2.0	47.0	4535
Zimbabwe	88.2	6.2	0.0	1.9	6106	95.0	2.0	2.0	4681

Table A. 3 Oxidation-reduction Potential (ORP) and Cellular Activity

Approximate ORP, mV	Redox Couple	Condition	Respiration
> +50	Oxygen (O_2)	Oxic	Aerobic
-50 to +50	Nitrate (NO^-_3) and nitrite (NO^-_2)	Anaerobic	Anoxic
- 170	SO_3^{2-} / H_2S	Anaerobic	Fermentation, Sulphide reduction
< -220	Sulphate (SO^{2-}_4)/ H_2S	Anaerobic	sulphate reduction
<-240	CO_2 /CH_4	Anaerobic	Fermentation, methane production
< -280	CO_2 /acetate	Anaerobic	Fermentation, mixed acid production
-280	S /H_2S	Anaerobic	Sulphur reduction

Source (Gerardi, 2008; Madigan et al., 2015)

Table A. 4 Ambient conditions around and inside the pit latrine superstructure

Location	Parameter	N	Average (SD)	Min	Max	*P* values		
						Between location	Pit latrine types	Water table level
Outside the latrine	Ambient Temp (°c)	42	28.84 (3.40)	23.3	39.70	0.000	0.540	0.253
	Relative Humidity (%)	42	56.68 (7.70)	38.80	71.40	0.000	0.524	0.652
	Wind speed (m/s)	42	0.56 (0.46)	0.00	1.80	0.357	0.683	0.103
In the superstructure	Temp (°c)	42	29.12 (2.80)	22.50	34.20	0.000	0.058	0.393
	Relative Humidity (%)	42	57.32 (9.17)	29.70	73.60	0.000	0.567	0.816
	Wind speed (m/s)	14	0.07 (0.18)	0.00	0.60	0.150	0.097	0.000

Table A. 5 Environmental conditions in the pit

Parameter	Level	N	Average (SD)	Min	Max	*P value*		
						Between location	Pit latrine types	Water table level
Temp °C	Surface	42	26.58 (2.29)	21.00	30.70	0.000	0.973	0.551
	0.5m	42	24.81 (1.09)	22.60	27.00	0.000	0.124	0.246
	1m	14	23.78 (0.55)	22.60	24.40	0.206	0.963	0.435
pH	Surface	42	8.13 (1.58)	5.00	10.94	0.000	0.852	0.041
	0.5m	42	8.94 (1.41)	6.04	11.80	0.000	0.265	0.264
	1m	14	8.03 (1.02)	7.32	11.35	0.000	0.565	0.196
Dissolved Oxygen (mg/L)	Surface	27	1.29 (0.33)	0.60	2.40	0.851	0.110	0.863
	0.5m	27	0.83 (0.23)	0.50	1.50	0.277	0.240	0.552
	1m	13	0.63 (0.21)	0.00	0.80	0.433	0.906	0.452
ORP (mV)	Surface	42	-71.69 (8.1.32)	65.90	-230.00	0.000	0.517	0.027
	0.5m	41	-120.43 (70.60)	28.50	-247.00	0.000	0.262	0.154
	1m	14	-75.24 (52.40)	-40.60	-246.00	0.000	0.482	0.186

Table A. 6 IMO samples label details

Location	IMO	niche	N	Sample label	
Kabanyolo	1	Bamboo	1	1	K111
			2	2	K112
			3	3	K113
		Mango	1	4	K121
			2	5	K122
			3	6	K123
		Open space	1	7	K131
			2	8	K132
			3	9	K133
		Open damp	1	10	K141
			2	11	K142
			3	12	K143
	2	Bamboo	1	13	K211
			2	14	K212
			3	15	K213
		Mango	1	16	K221
			2	17	K222
			3	18	K223
		Open space	1	19	K231
			2	20	K232
			3	21	K233
		Open damp	1	22	K241
			2	23	K242
			3	24	K243
Lungujja	1	Bamboo	1	25	L111
			2	26	L112
			3	27	L113
		Mango	1	28	L121
			2	29	L122
			3	30	L123
		Open space	1	31	L131
			2	32	L132
			3	33	L133
		Open damp	1	34	L141
			2	35	L142
			3	36	L143
	2	Bamboo	1	37	L211
			2	38	L212
			3	39	L213
		Mango	1	40	L221
			2	41	L222
			3	42	L223
			1	43	L231
			2	44	L232

		Open	3	45	L233
		Open damp	1	46	L241
			2	47	L242
			3	48	L243
Nabbingo	1	Bamboo	1	49	N111
			2	50	N112
			3	51	N113
		Mango	1	52	N121
			2	53	N122
			3	54	N123
		Open space	1	55	N131
			2	56	N132
			3	57	N133
		Open damp	1	58	N141
			2	59	N142
			3	60	N143
	2	Bamboo	1	61	N211
			2	62	N212
			3	63	N213
		Mango	1	64	N221
			2	65	N222
			3	66	N223
		Open space	1	67	N231
			2	68	N232
			3	69	N233
		Open damp	1	70	N241
			2	71	N242
			3	72	N243

Table A. 7 Blastn results for 16S rRNA and 18S r RNA gene sequences

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-008_A01_K111_27F	500	135	386	gb	KJ126927.1	Bacillus subtilis strain B-33 16S ribosomal RNA gene, partial sequence	1401	158	406	152	82	6e-33	197	253	78
161116-008_C01_K112_27F	905	364	815	gb	EF511479.1	Uncultured bacterium clone P5D15-471 16S ribosomal RNA gene, partial sequence	1485	373	824	204	110	3e-48	356	470	76
161116-008_E01_K113_27F	906	19	864	gb	KJ396872.1	Stenotrophomonas maltophilia strain IR95 16S ribosomal RNA gene, partial sequence	928	2	847	1291	699	0.0	797	846	94
161116-008_G01_K121_27F	909	2	892	gb	JQ308603.1	Stenotrophomonas maltophilia strain PPA N3 16S ribosomal RNA gene, partial sequence	1480	1	893	1221	661	0.0	820	897	91
161116-008_I01_K122_27F	914	12	854	gb	KF040986.1	Sphingobacterium multivorum strain CA77 16S ribosomal RNA gene, partial sequence	1436	1	834	728	394	0.0	708	856	83
161116-008_K01_K123_27F	978	21	807	gb	KJ499779.1	Stenotrophomonas maltophilia strain D299 16S ribosomal RNA gene, partial sequence	1420	8	789	468	253	1e-127	625	802	78
161116-008_M01_K131_27F	510	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_O01_K132_27F	991	25	820	gb	KJ499779.1	Stenotrophomonas maltophilia strain D299 16S ribosomal RNA gene, partial sequence	1420	3	798	706	382	0.0	679	817	83
161116-008_A03_K133_27F	972	20	903	gb	JQ194207.1	Uncultured bacterium clone DolGs_A014 16S ribosomal RNA gene, partial sequence	1366	29	906	569	308	3e-158	712	903	79
161116-008_C03_K141_27F	997	304	526	gb	JN968371.1	Pseudomonas geniculata strain BP14 16S ribosomal RNA gene, partial sequence	493	271	492	172	93	1e-38	183	226	81
161116-008_E03_K142_27F	494	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_G03_K143_27F	439	11	230	gb	KM886570.1	Stenotrophomonas sp. D6 16S ribosomal RNA gene, partial sequence	1440	7	226	385	208	5e-103	216	220	98
161116-008_I03_K211_27F	919	7	903	gb	FJ863113.1	Uncultured Bacillus sp. clone L5 16S ribosomal RNA gene, partial sequence	1257	6	902	1607	870	0.0	890	899	99
161116-008_K03_K212_27F	907	1	907	gb	JQ773351.1	Bacillus cereus strain ZK2 16S ribosomal RNA gene, partial sequence	1498	1	907	1548	838	0.0	889	912	97
161116-008_M03_K213_27F	488	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_O03_K221_27F	485	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A05_K222_27F	522	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_C05_K223_27F	924	9	906	gb	KU291379.1	Bacillus thuringiensis strain YJB4 16S ribosomal RNA gene, partial sequence	1080	8	907	1511	818	0.0	873	900	97

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-008_E05_K231_27F	978	20	876	gb	KR061403.1	Bacillus subtilis strain 2C-62 16S ribosomal RNA gene, partial sequence	1488	12	864	893	483	0.0	740	864	86
161116-008_G05_K232_27F	970	108	841	emb	FR746071.1	Stenotrophomonas sp. PG-2010-7 partial 16S rRNA gene, isolate 7	1406	83	817	370	200	3e-98	574	752	76
161116-008_I05_K233_27F	604	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_K05_K241_27F	519	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_M05_K242_27F	884	208	417	gb	JX006472.1	Bacterium NLAE-zl-H252 16S ribosomal RNA gene, partial sequence	1363	121	330	198	107	1e-46	176	211	83
161116-008_O05_K243_27F	477	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A07_L111_27F	503	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_C07_L112_27F	1012	9	935	gb	KF286281.1	Stenotrophomonas maltophilia strain JMUZJ-1 16S ribosomal RNA gene, partial sequence	1507	30	926	1375	744	0.0	873	929	94
161116-008_E07_L113_27F	970	17	896	gb	KJ499779.1	Stenotrophomonas maltophilia strain D299 16S ribosomal RNA gene, partial sequence	1420	6	881	852	461	0.0	755	894	84
161116-008_G07_L121_27F	841	17	742	gb	KM878735.1	Stenotrophomonas maltophilia strain EN1 16S ribosomal RNA gene, partial sequence	1200	7	742	791	428	0.0	645	746	86
161116-008_I07_L122_27F	917	29	888	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	27	885	667	361	0.0	709	875	81
161116-008_K07_L123_27F	961	12	879	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	14	885	1530	828	0.0	858	872	98
161116-008_M07_L131_27F	543	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_O07_L132_27F	914	10	891	gb	KF358259.1	Stenotrophomonas maltophilia strain L16 16S ribosomal RNA gene, partial sequence	1447	14	896	1053	570	0.0	788	892	88
161116-008_A09_L133_27F	903	32	902	gb	KJ499779.1	Stenotrophomonas maltophilia strain D299 16S ribosomal RNA gene, partial sequence	1420	6	879	1306	707	0.0	823	878	94
161116-008_C09_L141_27F	565	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_E09_L142_27F	918	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_G09_L143_27F	981	25	887	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	24	884	1133	613	0.0	788	871	90
161116-008_I09_L211_27F	504	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_K09_L212_27F	891	18	874	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	29	885	1317	713	0.0	814	862	94

Query				Subject						Score			Identities		
Name	**Length**	**Start**	**End**	**DB**	**AC**	**Gene**	**Length**	**Start**	**End**	**Bit**	**Raw**	**EValue**	**Match**	**Total**	**Pct.(%)**
161116-008_M09_L213_27F	983	9	910	gb	KJ496376.1	Bacillus subtilis strain DL47 16S ribosomal RNA gene, partial sequence	1507	31	932	1467	794	0.0	867	903	96
161116-008_O09_L221_27F	903	21	898	gb	KJ499779.1	Stenotrophomonas maltophilia strain D299 16S ribosomal RNA gene, partial sequence	1420	6	885	1336	723	0.0	833	885	94
161116-008_A11_L222_27F	913	19	878	gb	KJ499779.1	Stenotrophomonas maltophilia strain D299 16S ribosomal RNA gene, partial sequence	1420	6	867	1303	705	0.0	811	863	94
161116-008_C11_L223_27F	964	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_E11_L231_27F	914	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_G11_L232_27F	534	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_I11_L233_27F	920	11	893	gb	KF358259.1	Stenotrophomonas maltophilia strain L16 16S ribosomal RNA gene, partial sequence	1447	12	896	1301	704	0.0	829	889	93
161116-008_K11_L241_27F	969	23	903	gb	KJ396872.1	Stenotrophomonas maltophilia strain IR95 16S ribosomal RNA gene, partial sequence	928	1	883	1435	777	0.0	849	884	96
161116-008_M11_L242_27F	804	8	799	gb	KM464011.1	Uncultured bacterium clone 11_Am_36 16S ribosomal RNA gene, partial sequence	1494	10	807	1014	549	0.0	717	799	90
161116-008_O11_L243_27F	967	83	878	gb	JF203691.1	Uncultured bacterium clone ncd2541g06c1 16S ribosomal RNA gene, partial sequence	1345	93	885	381	206	1e-101	624	820	76
161116-008_A13_N111_27F	976	9	897	gb	KT034435.1	Stenotrophomonas sp. 282 16S ribosomal RNA gene, partial sequence	1479	6	889	1448	784	0.0	856	890	96
161116-008_C13_N112_27F	912	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_E13_N113_27F	559	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_G13_N121_27F	918	19	865	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	29	874	688	372	0.0	709	867	82
161116-008_I13_N122_27F	498	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_K13_N123_27F	807	14	791	gb	KR818084.1	Stenotrophomonas maltophilia strain S33 16S ribosomal RNA gene, partial sequence	990	10	787	1000	541	0.0	705	784	90
161116-008_M13_N131_27F	912	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_O13_N132_27F	894	65	630	gb	GU003576.1	Uncultured bacterium clone UA001_CBR620_0020_C08 16S ribosomal RNA gene, partial sequence	600	6	581	435	235	1e-117	477	589	81
161116-008_A15_N133_27F	904	16	881	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	27	885	1038	562	0.0	770	870	89

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-008_C15_N141_27F	980	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_E15_N142_27F	916	27	856	gb	HM327470.1	Uncultured bacterium clone ncd476b03c1 16S ribosomal RNA gene, partial sequence	1344	27	851	785	425	0.0	706	840	84
161116-008_G15_N143_27F	491	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_I15_N211_27F	918	44	837	gb	FJ867919.1	Bacillus cereus strain LCB13 16S ribosomal RNA gene, partial sequence	1514	75	871	316	171	4e-82	613	821	75
161116-008_K15_N212_27F	915	12	843	gb	JF772472.1	Bacillus sp. bb41(2011) 16S ribosomal RNA gene, partial sequence	1454	9	845	793	429	0.0	709	844	84
161116-008_M15_N213_27F	908	21	891	gb	KM253087.1	Pseudomonas sp. GT 2-02 16S ribosomal RNA gene, partial sequence	1421	8	880	941	509	0.0	760	881	86
161116-008_O15_N221_27F	493	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A17_N222_27F	963	330	795	gb	JN248443.1	Stenotrophomonas maltophilia strain CAB27 16S ribosomal RNA coding gene gene, partial sequence	974	45	503	272	147	9e-69	370	475	78
161116-008_C17_N223_27F	696	99	622	gb	DQ824689.1	Uncultured bacterium clone RL185_aaj71f12 16S ribosomal RNA gene, partial sequence	818	151	675	287	155	2e-73	421	544	77
161116-008_E17_N231_27F	904	18	872	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	24	880	1515	820	0.0	845	857	99
161116-008_G17_N232_27F	495	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_I17_N233_27F	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_K17_N241_27F	516	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_M17_N242_27F	906	17	879	gb	JF175490.1	Uncultured bacterium clone ncd2027c07c1 16S ribosomal RNA gene, partial sequence	1345	24	887	1561	845	0.0	859	865	99
161116-008_O17_N243_27F	522	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_E21_K111_907R	729	13	417	gb	JQ073789.1	Bacillus sp. SDT1S41 16S ribosomal RNA gene, complete sequence	1375	818	410	348	188	1e-91	343	416	82
161116-008_G21_K112_907R	928	6	822	gb	KR080577.1	Stenotrophomonas maltophilia strain 8Z 16S ribosomal RNA gene, partial sequence	1147	820	4	1227	664	0.0	769	820	94
161116-008_I21_K113_907R	923	12	873	gb	HM755568.1	Stenotrophomonas sp. C-LS-PYD2 16S ribosomal RNA gene, partial sequence	1214	864	1	1489	806	0.0	845	864	98
161116-008_K21_K121_907R	919	13	887	gb	EU539794.1	Uncultured bacterium clone nbt106f08 16S ribosomal RNA gene, partial sequence	1403	880	4	1507	816	0.0	858	878	98
161116-008_M21_K122_907R	931	224	888	gb	HM823742.1	Uncultured bacterium clone nby402b08c1 16S ribosomal RNA gene, partial sequence	1359	653	1	514	278	1e-141	545	672	81

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-008_O21_K123_907R	979	7	793	gb	KF108200.1	Uncultured bacterium clone ncm68a08c1 16S ribosomal RNA gene, partial sequence	1368	871	91	291	157	3e-74	607	816	74
161116-008_A23_K131_907R	965	188	753	gb	GU003356.1	Uncultured bacterium clone UA001_AS620_0027_G08 16S ribosomal RNA gene, partial sequence	600	585	32	409	221	7e-110	459	572	80
161116-008_C23_K132_907R	920	12	859	gb	KC849451.1	Stenotrophomonas maltophilia strain JF66 16S ribosomal RNA gene, partial sequence	1510	863	17	1399	757	0.0	821	851	96
161116-008_E23_K133_907R	986	11	808	gb	KT719879.1	Stenotrophomonas maltophilia strain MSL_3045 16S ribosomal RNA gene, partial sequence	1412	835	38	1369	741	0.0	782	801	98
161116-008_G23_K141_907R	994	25	869	gb	KJ561111.1	Stenotrophomonas maltophilia strain 22FG 16S ribosomal RNA gene, partial sequence	867	852	17	848	459	0.0	724	851	85
161116-008_I23_K142_907R	525	14	525	emb	LN890053.1	Stenotrophomonas maltophilia partial 16S rRNA gene, strain L57	1671	980	465	645	349	0.0	465	520	89
161116-008_K23_K143_907R	919	8	882	gb	KC894543.1	Uncultured bacterium clone H96 16S ribosomal RNA gene, partial sequence	1525	895	18	1602	867	0.0	875	878	99
161116-008_M23_K211_907R	923	6	894	gb	KF053070.1	Bacillus thuringiensis strain BAB-2592 16S ribosomal RNA gene, partial sequence	1533	911	22	1637	886	0.0	889	890	99
161116-008_O23_K212_907R	494	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A02_K213_907R	523	134	403	emb	LN833336.1	Stenotrophomonas sp. CB 286459 partial 16S rRNA gene, strain CB 286459	712	691	425	346	187	3e-91	245	272	90
161116-008_C02_K221_907R	681	373	429	gb	FJ558932.1	Uncultured bacterium clone DXH4-35 16S ribosomal RNA gene, partial sequence	174	173	117	78.7	42	1e-10	52	57	91
161116-008_E02_K222_907R	817	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A04_K242_907R	504	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_C04_K243_907R	834	111	834	gb	KT734803.1	Stenotrophomonas maltophilia strain Zunyi-F 16S ribosomal RNA gene, partial sequence	1412	728	4	1050	568	0.0	678	730	93
161116-008_A06_L131_907R	585	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_C06_L132_907R	911	14	849	gb	AY972181.1	Stenotrophomonas maltophilia strain P24 16S ribosomal RNA gene, partial sequence	852	15	852	1402	759	0.0	812	838	97
161116-008_A08_L213_907R	935	15	893	gb	KP224305.1	Bacillus subtilis strain RW-401 16S ribosomal RNA gene, partial sequence	1499	885	7	1574	852	0.0	870	879	99
161116-008_C08_L221_907R	901	9	874	gb	KP296212.1	Stenotrophomonas maltophilia strain Nc15MA-2 16S ribosomal RNA gene, partial sequence	1447	881	12	1531	829	0.0	857	870	99
161116-008_G08_L223_907R	908	14	838	dbj	LC066105.1	Stenotrophomonas sp. T6220-6-1b gene for 16S ribosomal RNA, partial sequence	1470	860	33	894	484	0.0	726	840	86

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-008_A10_L242_907R	909	7	891	gb	CP010577.1	Bacillus thuringiensis serovar morrisoni strain BGSC 4AA1, complete genome	5652292	228017	498424	1251	677	0.0	826	896	92
161116-008_A12_N131_907R	934	304	869	gb	EU535727.1	Uncultured bacterium clone nbt10b02 16S ribosomal RNA gene, partial sequence	1382	580	12	289	156	9e-74	448	585	77
161116-008_C12_N132_907R	922	292	866	gb	JQ433943.1	Chryseobacterium sp. VT1 16S ribosomal RNA gene, partial sequence	1457	579	4	248	134	1e-61	444	591	75
161116-008_C14_N221_907R	562	20	562	gb	KT734803.1	Stenotrophomonas maltophilia strain Zunyi-F 16S ribosomal RNA gene, partial sequence	1412	829	289	824	446	0.0	512	544	94
161116-008_A16_N242_907R	916	9	873	ref	NR_042503.1	Chryseobacterium ureilyticum strain F-Fue-04IIIaaaa 16S ribosomal RNA gene, partial sequence	1472	868	2	1561	845	0.0	861	868	99
161116-008_C16_N243_907R	861	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A18_KH1_907R	905	74	533	gb	HM335667.1	Uncultured bacterium clone ncd1062c01c1 16S ribosomal RNA gene, partial sequence	1357	787	331	93.5	50	7e-15	344	480	72
161116-008_A20_K233_ITS1	501	37	340	gb	KP223716.1	Galactomyces candidum strain feni 105 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	924	64	368	512	277	2e-141	297	306	97
161116-008_A22_L213_ITS1	514	144	360	gb	KF713514.1	Geotrichum candidum isolate L16H internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	347	133	347	294	159	1e-75	199	218	91
161116-008_C22_L223_ITS1	498	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-008_A24_N142_ITS1	485	80	335	gb	KF713514.1	Geotrichum candidum isolate L16H internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	347	92	347	351	190	5e-93	235	257	91
161116-008_C24_N143_ITS1	455	2	337	gb	KF975700.1	Geotrichum candidum strain UoS001 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	342	2	342	569	308	1e-158	331	341	97
161116-008_E24_N212_ITS1	519	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_M08_N243_ITS1	496	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-009_O08_K112_ITS4	507	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_A10_K123_ITS4	496	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_C10_K133_ITS4	502	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_E10_K222_ITS4	500	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_G10_K233_ITS4	505	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_I10_K243_ITS4	506	23	349	gb	KT175200.1	Geotrichum candidum isolate 52 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	373	329	1	590	319	1e-164	326	329	99
161116-009_K10_L121_ITS4	495	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_M10_L122_ITS4	496	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_O10_L123_ITS4	502	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_A12_L141_ITS4	509	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_C12_L211_ITS4	498	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_E12_L212_ITS4	495	54	393	gb	CP011093.1	Saccharomyces cerevisiae strain UOA_M2 chromosome 15 sequence	1057749	530752	530409	300	162	2e-77	286	346	83
161116-009_G12_L213_ITS4	503	35	336	gb	KT175200.1	Geotrichum candidum isolate 52 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	373	308	7	403	218	2e-108	277	305	91
161116-009_I12_L223_ITS4	506	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_K12_L243_ITS4	503	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_M12_N112_ITS4	496	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_O12_N121_ITS4	491	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Query				Subject						Score			Identities		
Name	Length	Start	End	DB	AC	Gene	Length	Start	End	Bit	Raw	EValue	Match	Total	Pct.(%)
161116-009_A14_N122_ITS4	626	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_C14_N131_ITS4	722	12	349	gb	KC816559.1	Galactomyces candidum strain UIMC44 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	339	1	592	320	5e-165	333	339	98
161116-009_E14_N132_ITS4	742	15	346	gb	KC816559.1	Galactomyces candidum strain UIMC44 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	336	4	597	323	1e-166	331	334	99
161116-009_G14_N142_ITS4	756	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_I14_N143_ITS4	613	8	349	gb	KC816559.1	Galactomyces candidum strain UIMC44 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	344	1	606	328	1e-169	339	344	99
161116-009_K14_N212_ITS4	709	23	707	gb	CP011093.1	Saccharomyces cerevisiae strain UOA_M2 chromosome 15 sequence	1057749	530792	530137	911	493	0.0	632	691	91
161116-009_M14_N222_ITS4	494	20	424	gb	CP011093.1	Saccharomyces cerevisiae strain UOA_M2 chromosome 15 sequence	1057749	530785	530381	577	312	8e-161	380	411	92
161116-009_O14_N231_ITS4	509	83	343	gb	KJ755081.1	Geotrichum candidum strain 147 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	362	262	3	392	212	3e-105	245	261	94
161116-009_A16_N232_ITS4	555	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_C16_N242_ITS4	540	-	-	-	-	-	-	-	-	-	-	-	-	-	-
161116-009_E16_N243_ITS4	735	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Questionnaires

PIT LATRINE DESIGN OPERATION AND PERFORMANCE BASELINE SURVEY QUESTIONNAIRE

Pit Latrine Design Operation and Performance Survey

Objective of the survey:

This survey is conducted for research purposes only to better understand the design operation and performance of pit latrines in slums in Kampala. The answers of the interviewees shall be strictly used for research purposes and kept confidential.

Parts

The survey questionnaire is divided into two parts

PART 1 : **DESIGN ASSESSMENT SHEET:**
To be filled by the one assessing the pit latrine

PART 2: HOUSEHOLD QUESTIONNAIRE:
Form A
Questions to be answered by landlord/caretaker of the pit latrine

Form B
Questions to be answered by any tenant using the pit latrine

Location:

A3 Pit Latrine No. [ ]

A0	Division:	.....
A1	Parish:	.....
A2	Zone:	.....

A4	Name of person doing the survey			
A5	Date of survey			
A6	Time of survey			

Consent of Owner/ user (signature/ tel no)				
Supervisor	Data Entrant			
Name and Signature	Date of Data Entry			

PART 1

DESIGN ASSESSMENT SHEET

To be filled by the one assessing the pit latrine

PIT LATRINE DESIGN ASSESSMENT SHEETS

Pit Latrine Measurements ***(Tick most appropriate)***							
General							
G0	Photograph no. of Latrine:- Front: Side: Back Drophole Vent Pipe Vent Access Others:	…… …… …… …… …… …… …… ……					
G1	Pit latrine type	VIP	1				
		Simple traditional Pit latrine	2				
		Others *(specify)*	3				
Superstructure							
S1	Shape of the latrine *(Draw rough sketch including dimensions of the exterior)*						
	Rectangular/ Square 1 *Sketch*	Circular 2 *Sketch*					
S2 a	How many pit latrine stances does the latrine have?	1	2	3	Others (no)		
b	What are the dimensions inside each stance	Stance	1	2	3	Others	
		Length					
		Width					
		Height					
S3a	Is the drophole covered	Yes	1				
		No	2				
b	What is the shape of the drophole *(Draw rough sketch including dimensions)* *Sketch*						
S4	What openings are there? *(Sketch and include their dimensions)*	Windows Doors					

		Ventilators	
S5 a	Does the latrine have a vent pipe?	Yes	1
		No	2
b	If so, what are the vent pipe dimensions?	Diameter……. Height above roof……..	
c	Does the vent have a fly trap?	Yes	1
		No	2
d	Describe the fly trap (photo)		
S6	Does the latrine have a roof	Yes	1
		No	2
S8 a	Does the latrine have a bathroom stance?	Yes *(IncludeDimensions)*	1
		No	2
b	If so, Where does the bathroom drain to?	Pit latrine	1
		Soak pit	2
		Drainage ditch	3
		Others (specify)	4
S9 a	Does the latrine have a hand washing facility	Yes	1
		No	2
b	Where does the hand washing facility drain to?	Pit latrine	1
		Soak pit	2
		Drainage ditch	3
		Others (specify)	4
Pit			
P1 a	Is the pit raised above the ground	Yes	1
		No	2
b	If yes, how high above the ground (mm)		
c	How is the Latrine accessed	Using Ladders	1
		Using Steps	2
		Using a rump	3
		Others	4
Construction Materials used*(give a description of the materials used in the construction of the pit latrine and their condition)*			

C1	Superstructure			
C2	Roof			
C3	Latrine Slab			
C4	Door, windows and ventilators			
C5	Vent Pipe *(include colour of pipe*			
C6	Pit *(if raised above the ground)*			
C7	Bathroom			
C8	Hand washing facility *(include detail of source of the water used)*			
Pit Latrine condition – (observation)				
Ob 1	Does the pit latrine show any signs of collapsing		Yes	1
			No	2
Ob2 a	Is the area subjected to flooding		Yes	1
			No	4
b	Are there signs of rain or storm water entering the pit		Yes	1
			No	2
Ob3	Is the Latrine full?		Almost Empty	1
			Half full	2
			Full	3
			Overflowing	4
b	Are human faeces and/or urine on the slab		Yes	1
			No	2
Ob4	How would you class your latrine		Very Clean	1
			Clean	2
			Fairly Clean	3
			Dirty	4
			Very Dirty	5
Ob5	Does the pit latrine smell?		No	1
			Slight Smell	2
			Moderate Smell	3
			Strong Smell	4
			Very Strong Smell	5
Ob6	Are there flies around the pit latrine?*(take photo)*		No	1
			Few flies	2
			Many flies	3

		Very many flies	4
Ob7a	Are there any other organisms present in and around the pit?	Yes	1
		No	2
b	If yes identify them including photos	……. ……. …… ……	
Ob7a	Is there a rubbish dumping site nearby?		
b	If yes how far is it from the pit latrine (take photo)		
Ob87	Any other remarks	…….. …… ……	

PART 2
HOUSEHOLD QUESTIONNAIRE

Questions to be answered by landlord/caretaker of the pit latrine

Socio-economic Characteristics						
SE1	What is the respondent's position?			Landlord	Caretaker	
SE2 a	What is the use of the households/ premises					
	Living only		Living and business/trade	Business only (indicate type of business)		
SE3	How long have the households/ premises been in this area?*(include number)*			Years		
				Months		
SE4	How many households use the pit latrine			(indicate number)		
SE5a	Average number of people in per household (excluding children)					
	1	2	3	4	Others (specify)	
b	Average number of children in a household			*(Indicate no.)*		
SE6a	Religion of most household owners					
	Moslem	Catholic	Protestant	Orthodox	SDA	Other (specify)….....
b	Does your religion affect the way you use the pit latrine? *(include how)*			Yes	No	
SE7a	Vulnerable people living in the households			Elderly	People with disabilities	Others (specify
b	Do children, elderly and disabled use the latrine			Yes	1	
				No(include reason)	2	Children
						Disabled
						elderly
Access and Ownership of the Latrine						
Ow1a	Is the pit latrine you use		Private (only households)		Public (used by general public)	
b	Do you have your own stance		Yes		No	
c	If yes give a reason		Landlords stance	Paid to have own stance	Each house has its own stance	
Ow2a	Who built the latrine					
	Contribution from all households		Landlord		Government	NGO
b	How much did it cost?	Pit			Superstructure	
c	Where did you get the pit latrine design?					
	Same as neighbours	From town council		Project design		Others
Ow3	How old is the latrine					
	< 1yr	1to 2yrs	2-3yrs	3-4yrs	>4yrs	
Performance of pit latrine						
Pf1	Are you satisfied with your pit latrine?			Yes		No
Pf2	What do you consider to be main problem with your Latrine?					
	Too expensive to maintain	Design		Over flows during the rainy season		Bad smell
	Insects flies and pests	Nearly full		Fills quickly	Dirty/ not easily cleaned	
	Dangerous to children	Collapses frequently		Not easily accessed		4No privacy
	Others					
Pf3		Very Clean			Dirty	

	How would you class your latrine	Clean				Very Dirty
Pf4a	If your latrine has flies how would you class them?	No flies				Many flies
		Few flies				Very many flies
b	When are the flies there	All year round	In the dry season			In the rainy season
Pf5	If your latrine smells how would you class it?					
	No smell	Slight Smell	Moderate Smell		Strong Smell	Very Strong Smell
b	When does your latrine smell most?	All year round	In the rainy season			In the dry season
Pf6	Does your pit latrine flood?	Yes			No	
b	When does it flood?	All year round	In the rainy season			In the dry season
Pf7	How often does your pit latrine get full? (months)					
	Less than 1	1-3	3-6	6-12	1yr – 2yrs	More than 2yrs (specify)
b	What do you do/ intend to do when your pit latrine gets full?(include problems encountered)					
	Empty it	Dig a new one	Up to the Landlord to do something			
	Us the neighbours	Don't know	Others (specify)			
Operation of the Pit latrine						
Pit latrine use						
Pu 1a	What is disposed in the latrine? (Y/N)					
	Faeces	Urine	Cleansing material.		Water	
	Baby Pampers	Sanitary products (like pads)			Garbage / solid waste	
Pu2a	What cleansing material is used					
	Newspapers	Pit latrine roll	Water	Rag		
	Leaves	Stones	Other (specify)			
b	Do you put the cleansing material in the pit after use?			Yes	No	
Pu3	Where do you dispose of dirty washing water			Pit latrine	If not where	
Pu4	How do you dispose of any rubbish/ garbage			In the pit latrine	If not where	
Maintenance						
M1a	Who is responsible for the pit latrine cleaning			Caretaker/ Landlord	Every user	
				Rotational among users	Others (specify)	
b	How often is the latrine cleaned					
	Daily	Once in 2 weeks	Every after/ before use		As and when dirty	
	Others (specify)				3	
M2a	What is used when cleaning the pit latrine?					
	Water	Soap / detergents (include name)		Others		
b	Where does the material above end up?		In the pit		Out of the pit	
M3a	Is anything added to the pit to reduce *(include what is used and how much)*:- Smell Flies Other pests Fill of the pit latrine others					

b	Who is responsible for adding the substance				
	Landlord	Every one	Others (specify)		
c	Do you think the substances are they effective?				
Aspiration for improved pit latrine facilities					
As1 a	What do you like most about your latrine *(include reasons why)*				
b	Do you see a need for improving your present pit latrine?			Yes	No
c	If yes what improvement would you suggest				
As2	Would you buy substances/ powder so as to apply to you latrine to prevent it from filling?			Yes	No

PIT LATRINE ENVIRONMENTAL CONDITION QUESTIONNAIRE AND DATA SHEET

Pit Latrine Environmental conditions assessment

Location:					A3 Pit Latrine No.
A0	Division:	.....			
A1	Parish:	.....			
A2	Zone:	.....			
A4	Coordinates				
A5	Date of survey				
A6	Time of survey				

Pit Latrine Measurements ***(Tick most appropriate)***					
General					
1	Photograph no. of Latrine:-	……			
2	Pit latrine type	VIP	1		
		Simple traditional Pit latrine	2		
3	Location	High water table			
		Low water table			
4	Number of users (families)				
Superstructure					
1	How many pit latrine stances does the latrine have?	1	2	3	Others (no)
2	What are the dimensions inside the stance	Stance			
		Length			
		Width			
		Height	F -		B -
3	Is the drop hole covered	Yes	1		
		No	2		
4	What are the dimensions of the drop hole	L-	W		
5	If so, what are the vent pipe dimensions?	Diameter……Height above roof……..			
6	Height of the pit above the ground				
7	Does the pit latrine show any signs of collapsing	Yes	1		
		No	2		
8	Are there signs of rain or storm water entering the pit	Yes	1		
		No	2		
9	Distance of pit content surface from the drop hole				
10	How would you class your latrine	Very Clean	1		
		Clean	2		
		Fairly Clean	3		
		Dirty	4		
		Very Dirty	5		
11	Does the pit latrine smell?	No	1		
		Slight Smell	2		
		Moderate Smell	3		
		Strong Smell	4		
		Very Strong Smell	5		
12	Are there flies around the pit latrine?*(take photo)*	No	1		

		Few flies	2
		Many flies	3
		Very many flies	4
13	Is there a rubbish dumping site nearby?		
14	If yes how far is it from the pit latrine (take photo)		
15	Any other remarks		

Measurements

Variable	**Outside the latrine**	**Inside the superstructure**	**In the pit**
Ambient temperature			
Humidity			
Airflow rate			

Odour in the superstructure

Distance	**Strength**	**Intensity**
In superstructure		
500mm from pit		
1m from pit		

VFA component	
Hydrogen sulphide component	
Ammonia	

In-situ measurements

Variable	**Samples (depth of content)**			
	Pit surface	**0.5m**	**1m**	**1.5m**
Moisture Content				
Temperature				
pH				
Dissolved oxygen				
ORP				

IMO APPLICATION QUESTIONNAIRE/ DATA SHEET

A	Pit latrine number	
B	Date of survey	
C	Time of survey	

User perspective on the performance of their pit latrine				
How would you class your latrine	Very Clean			Dirty
	Clean			Very Dirty
If your latrine has flies how would you class them?	No flies			Many flies
	Few flies			Very many flies
If your latrine smells how would you class it?				
No smell	Slight Smell	Moderate Smell	Strong Smell	Very Strong Smell

Investigator's perspective on the performance of their pit latrine					
1	How would you class your latrine				
	Very Clean	Clean	Fairly Clean	Dirty	Very Dirty
2	Does the pit latrine smell?				
	No	Slight Smell	Moderate Smell	Strong Smell	Very Strong Smell
3	Are there flies around the pit latrine?*(take photo)*				
	No	Few flies	Many flies	Very many flies	

Measurements

Before application of IMOs		
Ambient Temp	Wind speed	Humidity
Depth of pit content		
Temp	pH	ORP
Ammonia	Hydrogen Sulphide	Acetic acid