To assess the impact of black soldier fly (*Hermetia illucens*) larvae on faecal reduction in pit latrines

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“A beginning is the time for taking the most delicate care that the balances are correct”

Frank Herbert

Dune

1965

For Sarah

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Abstract

On-site sanitation solutions are an economically feasible method of improving sanitation, and for reducing the burden of diarrhoeal diseases, in low- and middle-income countries. However, suitable faecal sludge management (FSM) solutions are severely lacking in these countries. Black solider fly larvae (BSFL) efficiently reduce food-waste and animal manure, and produce valuable prepupae, high in protein and fat, supporting investigation into a novel BSFL FSM method. The aim of this study was to determine the feasibility of using BSFL as a FSM method, by evaluating their faecal matter reduction (FMR), and prepupal production capacity, when reared on FS under different conditions.

Black soldier fly larvae were found to develop successfully on fresh human faeces, effectively reducing waste and converting it to prepupal biomass. A survey of pit latrines in South Africa found physical and chemical characteristics of faecal sludge (FS) similar to previous studies in countries requiring novel FSM methods, with characteristics falling within a range suitable for BSFL development. Key rearing parameters, moisture content, feeding rate, and larval density, significantly influence FMR and prepupal production of BSFL reared on “top layer” homogenised FS. Black soldier fly larvae were found to effectively reduce FS from a variety of depths, each with a range of physical and chemical characteristics, and produce prepupae with nutritious values comparable to previous research, excepting crude fats. The study also demonstrated that reported cleaning chemicals in FS do not affect BSFL mortality at manufacturer recommended, or user reported concentrations.

It is proposed that the use of a novel BSFL FSM method is an economically feasible method of improving sanitation in low- and middle-income countries, and may help reduce the burden of diarrhoeal diseases.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

AA	Amino Acids
BSF	Black soldier fly
BSFL	Black soldier fly larvae
CF	Crude fibre
CFUs	Colony forming units
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CP	Crude protein
DALY	Disability-Adjusted Life Year
EE	Crude fat (ether extract)
EU	European union
FMR	Faecal matter reduction
FS	Faecal sludge
FSM	Faecal sludge management
GE	Gross energy
GLM	Generalized linear model
HSW	Hygiene, sanitation, and water
ICP-MS	Inductively coupled plasma – mass spectrometry
LD_{20}	Lethal dose 20%
LD_{50}	Lethal dose 50%
MOW	Municipal organic waste
NH_4^+	Ammonium
PO_4	Total phosphate

PPE	Personal protective equipment
sCOD	Soluble chemical oxygen demand
tCOD	Total chemical oxygen demand
TS	Total solids
TVS	Total volatile solids
VFAs	Volatile fatty acids
VIP	Ventilation improved pit latrine

Chapter 1) Introduction

To conduct the following literature review, Ovid and Google Scholar were used to identify peer-reviewed studies, grey literature, and organisational reports. The following keywords were used: human excreta, human faeces, human feces, diarrhoea, diarrhoeal, diarrhea, diarrheal, global disease burden, sanitation, on-site sanitation, off-site sanitation, faecal sludge, fecel sludge, pit latrine, pit latrine emptying, faecal sludge management, fecel sludge management, waste management, black soldier fly, BSF, BSFL, *Hermetia illucens*, pathogen reduction, prepupae, prepupal value, insects as animal feed, myiasis, organic fertiliser, organic fertilizer, heavy metals, endocrine disrupting chemicals, and nitrification. The search was conducted between May 2011 and July 2014.

1.1. Human excreta

Vast quantities of human excreta are produced every day around the world, and its safe disposal is essential to prevent disease transmission. It is suggested that the total amount of excreta produced is approximately 1 litre person^{-1} day^{-1} (Shaw 1962, Pradt 1971). However, the quantity depends on a number of factors, including diet, climate, occupation and water consumption, and varies widely (Franceys *et al.* 1992). Without detailed data, a reasonable estimate is that individuals consuming a high-protein diet in temperate climates can produce on average 120 grams of faeces, and 1.2 L of urine person^{-1} day^{-1}, while individuals consuming a high fibre vegetarian diet in tropical climates can produce on average 400 g of faeces, and 1 L of urine person^{-1} day^{-1} (Franceys *et al.* 1992).

There have been few studies quantifying the composition of human faeces. However, the studies which have been comprehensively reviewed by Chaggu (2004) and Buckley *et al.* (2008), and are synthesised in Table 1-1. Approximately 84% of dried human faeces is organic material (Lopez Zavala *et al.* 2002), consisting of 55% is bacteria and 17% residual dietary fibre (Stephen *et al.* 1980). Bacteria are around 80% water, consequently, 75% of wet human faeces

are bacteria (Stephen *et al.* 1980). Many species of bacteria present in excreta are anaerobic or harmless, however there are also many species which can cause disease (Mara *et al.* 1999).

Table 1-1 Summary of physical and chemical characteristics of human excreta: Sources, Buckley *et al.* (2008), Chaggu (2004), and Jensen *et al.* (1976)

Characteristic	Unit	Faeces	Urine
Total solids	%	15 – 34	0.5 – 7
Moisture content	%	66 – 85	93 – 99.5
Total volatile solids	% dry mass	79 – 84	-
pH	-	5.50 – 7.50	7.08 – 9.00
Total chemical oxygen demand (COD)	g kg^{-1} dry mass	253 – 1450	12.79
Soluble COD	g kg^{-1} dry mass	-	11.33
Total nitrogen (N)	% dry mass	5 – 7	15 – 19
Total phosphorus (P)	% dry mass	0.7 – 2.5	1.1 – 2.2
Total potassium (K)	% dry mass	0.8 – 2.1	3.0 – 4.5

1.1.1. Diseases associated with excreta

It is estimated that human excreta contains over 50 different pathogenic organisms, including bacteria, viruses, protozoa, and soil- and water-transmitted helminths (Table 1-2) (Mara *et al.* 1999). Approximately 1.4 million deaths are caused by diarrhoeal diseases which could be prevented if suitable hygiene, sanitation and water practices (HSW) were followed (Clasen *et al.* 2014). Systematic reviews of HSW interventions suggest that the risk of diarrhoea can be reduced approximately 36% by improving sanitation alone (Esrey *et al.* 1991, Cairncross *et al.* 2010). Diarrhoeal diseases account for the majority of preventable deaths, while the number of deaths caused by helminthiasis and schistosomiasis are relatively low. While diarrhoeal diseases are still the main cause of lost Disability-Adjusted Life Years (DALYs), 52 million annually, helminthiasis and schistosomiasis cause a larger percentage of DALYs than deaths, 2.9 million and 1.7 million respectively. Similarly, trachoma, the commonest infectious cause of blindness caused by *Chlamydia trachomatis*, results in approximately 1.3 million DALYs lost annually, with limited evidence for premature mortality (Burton *et al.* 2009). Therefore, it is important to consider the disability burden of excreta-related pathogens as-well as the mortality burden.

Table 1-2 Selection of excreta-related pathogenic organisms, including number of annual infections and mortality

Category	Organism	Infections (thousands)	Mortality (thousands)	Source
Bacteria	*Escherichia coli*	200,000	210	(Lozano *et al.* 2012)
	Salmonella typhi	93,800	155	(Majowicz *et al.* 2010)
	Shigella spp.	90,000	123	(Lozano *et al.* 2012)
	Vibrio cholera	4,000	58	(Lozano *et al.* 2012)
	Chlamydia trachomatis	40,000	0	(Burton *et al.* 2009)
Viruses	Rotavirus	100,000	251	(Lozano *et al.* 2012)
	Norovirus	260,000	200	(Debbink *et al.* 2012)
Protozoa	*Cryptosporidium* sp.	58,000	100	(Lozano *et al.* 2012)
	Entamoeba histolytica	50,000	56	(Lozano *et al.* 2012)
	Giardia lamblia	280,000	< 1	(Esch *et al.* 2013)
Soil/water transmitted helminths	*Ascaris lumbercoides*	819,000	3	(Pullan *et al.* 2014)
	Trichuris trichiura	464,600	< 1	(Pullan *et al.* 2014)
	Hookworm	438,900	< 1	(Pullan *et al.* 2014)
	Schistosoma spp.	238,000	12	(Vos *et al.* 2013)

It is also important to consider how frequent diarrhoeal infections and intestinal parasites are an important cause of malnutrition (Bartram *et al.* 2010). Protein-energy malnutrition is estimated to cause approximately 71,000 deaths a year in children under 5 years old. While up to 790,000 deaths, in children under 5, are caused by the consequences of malnutrition, such as impaired immune systems leading to an increase in susceptibility to other diseases (Prüss-Üstün *et al.* 2008). It has been suggested that the interaction between malnutrition and the immune system could interfere with rotavirus vaccinations (Linhares *et al.* 2002). However, recent trials suggest that rotavirus vaccines can still be beneficial in malnourished populations (Perez-Schael *et al.* 2007, Maier *et al.* 2013). This is important considering rotavirus vaccines

have significantly reduced the cases of severe childhood diarrhoea in low- and middle-income countries (Tate *et al.* 2010). Additionally, trials are currently underway to evaluate potentially effective norovirus vaccines (Debbink *et al.* 2014).

The diseases discussed, and consequences of infections, are preventable by safely separating humans from excreta. Pathogenic organisms in excreta are transmitted through a number of different routes, with safe excreta disposal acting as a primary barrier, and personal and domestic hygiene providing a secondary barrier (Figure 1-1) (Mara *et al.* 1999).

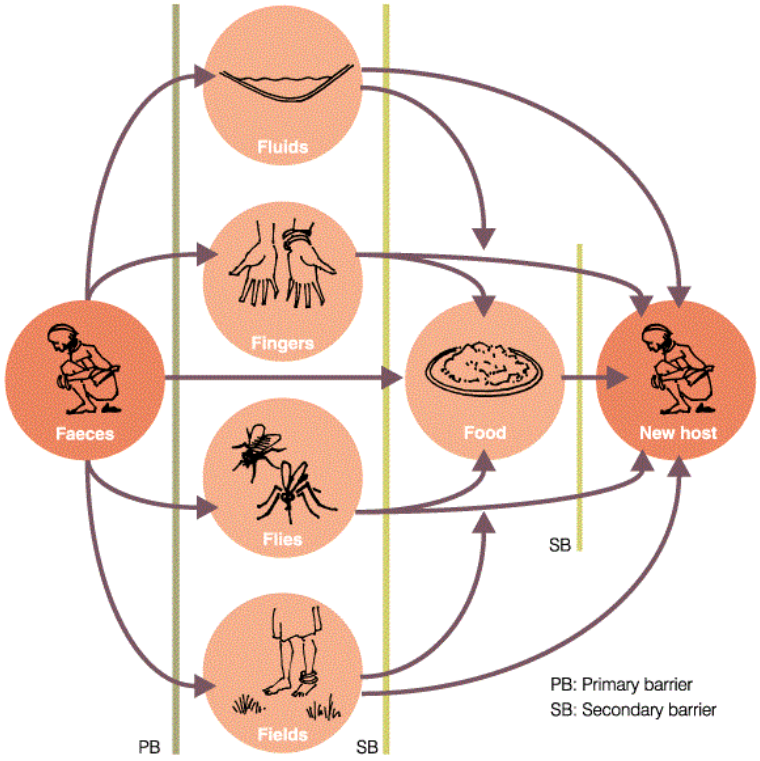

Figure 1-1 Transmission routes of pathogenic organisms found in excreta, where safe excreta disposal acts as a primary barrier (PB), and personal and domestic hygiene as a secondary barrier (SB), to pathogen transmission; © WELL 2005

The faecal-oral transmission route is where pathogens from excreta pass from one host to the oral cavity of another, including when excreta contaminates water sources used for drinking, washing, and food washing. Soil-transmitted infections occur when pathogens contaminate soil, either due to open defecation or use of untreated excreta as fertiliser (Esrey *et al.* 1991).

The pathogens are then ingested through the faecal-oral route, with contaminated soil remaining on hands or fingers due to improper hand-washing practices, or fruits and vegetables which have not been cooked, washed or peeled correctly. Hookworm infections are acquired primarily by walking barefoot on contaminated soil where larvae mature and penetrate human skin (Hotez *et al.* 1995). Pathogens, including *E. coli*, *Cryptosporidium*, *E. histolytica*, *Giardia lamblia*, *A. lumbricoides*, *T. trichiura*, hookworms, and *Taenia* sp., can also be transmitted by mechanical vectors, such as non-biting synanthropic flies (Graczyk *et al.* 2005, Adenusi *et al.* 2013), cockroaches (Majewska 1986, Fotedar *et al.* 1991, Foil *et al.* 2000), and rats (Fayer *et al.* 2000). Mechanical transmission occurs when excreta, and pathogens, are ingested by the vector or adhere to the vectors exterior. The pathogens are then transported to human foods or surfaces, where they are subsequently ingested by a new host.

Improving access to sanitation can have a significant effect on health (Esrey *et al.* 1991, Fewtrell *et al.* 2005) by blocking the transmission routes of excreta related pathogens (Figure 1-1). With approximately 2.5 billion people in low- and middle-income regions of the world having no access to improved sanitation (UNICEF/WHO 2014), the goal of providing hygienic, affordable and manageable sanitation solutions is more important than ever.

1.2. Sanitation

Sanitation generally refers to the safe storage and disposal of human excreta as a way to reduce transmission of pathogens (UNICEF/WHO 2014). Unimproved sanitation includes open defecation, pit latrines without slabs, hanging latrines, bucket latrines, or improved facilities shared between two or more households, and is used by approximately 2.5 billion people worldwide (UNICEF/WHO 2014). While improved sanitation includes pit latrines with slabs, ventilated improved pit (VIP) latrines, flush/pour flush to piped sewer systems, septic tanks, or pit latrines, and composting toilets, and are used by over 4.5 billion people (UNICEF/WHO 2014). Improved sanitation can be split between off-site and on-site technologies.

Off-site sanitation is where excreta are transported from dwellings to another location for treatment and disposal, and can be classified as decentralised and centralised (WHO/UNEP 2006). Decentralised systems involve small bore sewers linking two or more households to communal treatment systems, while centralised systems are large piped sewage systems serving one or more communities (Franceys *et al.* 1992). The goal is to treat the excreta to reduce the solids content and pathogens present in the material. There are a number of obstacles associated with the implementation of off-site sanitation. The prohibitive cost, and high water demand, make implementing a conventional sewage system in low- and middle-income countries often unfeasible (WHO/UNEP 2006). The costs include: building wastewater treatment plants, which are scarce in many low- and middle-income countries, connecting households with a sewage network, which is impractical in highly populated and dense urban areas, supplying households with potable water required for flushing, which is prohibitive in water-poor countries as households can use up to 40% of their potable water for excreta disposal, and the cost of maintaining and operating the whole system, for example, where excreta is treated aerobically, a constant supply of oxygen is required, leading to high maintenance costs. These caveats mean that on-site sanitation is more technically and economically viable as an option to improve sanitation in low- and middle-income countries (UNICEF/WHO 2014), therefore will be the focus of this thesis.

1.2.1. On-site sanitation

On-site sanitation is where excreta are stored and contained within a dwelling, or its surrounding area (WHO/UNEP 2006). There are around 1.7 billion people, in low- and middle-income countries around the world who use the most basic form of improved sanitation, the pit latrine (UNICEF/WHO 2014). These latrines consist of a single pit to store excreta, covered by a slab with a drop hole, separating humans from their excreta, and a superstructure. There are a number of ways to improve pit latrines with slabs, including: hygienic, self-draining concrete slabs with foot rests, tight-fitting drop hole lids to reduce smells and insects, raising

slabs at least 15cm to prevent flooding, lining pits with stones, bricks, concrete rings, or oil drums to prevent pit collapse, improving the superstructure, alternating between two pits, allowing decomposition of faecal sludge (FS), which, once stored for a period of 12 – 18 months, can be excavated and used as organic fertiliser, or adding ventilation pipes, which reduces flies and foul odours emanating from the pits (Franceys *et al.* 1992). In principle, all the improvements result in a system where FS is stored in a pit where it undergoes biodegradation. Flush/pour flush latrines are also used, utilising a water seal to prevent fly and mosquito breeding, and foul odours. However, these latrines often have septic tanks which are expensive. Due to this high cost, the majority of users in low- and middle-income countries use pit latrines, on which this thesis will focus on.

The main disadvantage of a pit latrine is that it will fill up over time and require emptying, discussed in detail below. In rural areas space is available to dig a new pit, but in densely populated urban areas, where the lack of space limits households to a single vault, and areas with a high water table where only shallow vaults can be dug (Patinet 2010), FS must be collected and treated off-site. Additionally, areas with high water tables are subject to pathogen infiltration and nitrate pollution of groundwater {Graham, 2013 #1389}. The frequency of pit latrine emptying depends on pit latrine fill-rate, which varies depending on FS composition and biodegradation through microbial action.

1.2.2. Faecal sludge composition and decomposition

The contents of pits latrines contain more than human excreta decomposing into FS. Water used for anal cleansing is often added to latrines (Buckley *et al.* 2008), while solid anal cleansing materials are also disposed of in the latrine, including: toilet paper, newspaper, corn cobs, cement bags, and stones (Franceys *et al.* 1992). Household refuse is also found disposed of in pit latrines (Buckley *et al.* 2008), and it has been reported that grey water from cleaning

and showering is often added to pits (Couderc *et al.* 2008). The addition of these materials influences the composition of FS, therefore its decomposition.

There have been few studies quantifying the composition of pit latrine FS. Table 1-3 shows the physical and chemical characteristics that give an approximation of biodegradability of FS. There is a wide range of values in the characteristics, indicating differences in FS biodegradability.

Table 1-3 Summary of physical and chemical characteristics of pit latrine faecal sludge (FS). Sources: Lopez Zavala, 2002; Palmquist, 2005; Buckley, 2008; Irish, 2013

Characteristic	Unit	Range
Total Solids	%	6 – 80
Total Volatile Solids	% dry mass	1 – 91
pH	-	5.25 – 8.94
tCOD	g kg^{-1} dry mass	30 - 2000
sCOD	g kg^{-1} dry mass	1 - 750

Two studies into pit latrine fill rates conducted in South Africa on 16 VIP latrines (Bakare 2014), and 50 non-improved latrines each in Tanzania and Vietnam (Torondel, LSHTM, unpublished data), suggest that there are huge variations in FS biodegradability between different pit latrines, and within different layers inside latrines. Some latrines show trends of increasing TS, and decreasing TVS and COD, from top to bottom of the latrine, whereas other latrines show no significant changes in TS, TVS, and COD, between the top and bottom layers of the latrine. Total solids can range between 6 – 80% in the top layer of latrines, to 40 – 80% past 1 metre deep, and COD content ranges from 30 – 2000 g kg^{-1} dry mass in the top layer, to 20 – 300g kg^{-1} dry mass in lower layers (Torondel, LSHTM, unpublished data).

The biodegradation process of FS is caused by aerobic and anaerobic digestion within pit latrines, and can be separated into four theoretical layers (Buckley *et al.* 2008). In the top layer, readily biodegradable components in fresh excreta are rapidly digested by aerobic micro-organisms when deposited on the surface of the pit. The second layer is the top aerobic section of the pit, where aerobic biodegradation of hydrolysable organic material occurs,

limited by the aerobic hydrolysis of complex organic material to simpler compounds. Once covered, the remaining biodegradable material is slowly degraded by anaerobic micro-organisms into water, carbon dioxide (CO_2), methane (CH_4), ammonium (NH_4^+), phosphates, organic acids, new anaerobic micro-organisms, and non-biodegradable organic material. After successful biodegradation, the final layer of FS remaining is largely non-degradable (Buckley *et al.* 2008). This model of biodegradation is relevant, but not complete. Questions have been raised over the low abundance of aerobic micro-organisms in fresh excreta, and how they could be wholly responsible for the rapid biodegradation observed on the surface of pit latrines (Buckley *et al.* 2008). The results from the aforementioned studies (Bakare 2014, Torondel, LSHTM, unpublished data) partially agree with the theoretical model. However, the results imply that pit latrines can be divided into at least two categories based on biodegradation of organic material: 1) latrines demonstrating changes in TS, TVS, and COD with depth indicated organic material in FS undergoes biodegradation over time, resulting in lower layers of pits being more stable than higher layers with newer FS, 2) latrines with no changes in TS, TVS, and COD with depth indicate there are factors preventing the successful biodegradation of FS, or that the biodegradation process has "stalled". Latrines which fall into the first category correspond to the four layer model (Buckley *et al.* 2008). However it is unknown what causes the second category of latrines to "stall", with research currently underway (Torondel, LSHTM, unpublished data),

Regardless of the exact microbial process responsible for biodegradation, it is known that a number of abiotic factors affect decomposition within pits. Temperature affects microbial growth and biological reactions, influencing enzyme catalysed reactions and substrate diffusion into cells (Chaggu 2004, Grady *et al.* 2011). While pH stability is important to the micro-organisms in FS, with a reported range of 6.5 – 8.0 appropriate (Bhagwan *et al.* 2008), and a pH close to, or below, 6.0 inhibits and kills methanogenic bacteria (Bitton 2011). A minimum moisture content (MC) of FS is necessary to enable diffusions of substrates into, and

waste products away from micro-organisms (Martin *et al.* 2003), and an excess of water, caused by rainfall, anal cleansing practice, or grey water, could result in soluble substrates leaching from the pit, slowing down the biodegradation process (Bhagwan *et al.* 2008). The location of a pit affects biodegradation processes, as the porosity of different soils alter the leaching of moisture and soluble components into and out of the pit, while flooding of pits can be caused by high water tables and increased rainfall in wet-seasons (Chaggu 2004, Bhagwan *et al.* 2008). Also pit latrine depth and side wall surface area significantly impact temperature, pH and moisture content of FS, influencing biodegradation as described previously (Bhagwan *et al.* 2008). These factors all influence the biodegradation of FS, which ultimately affects the fill-up rate of pit latrines.

1.2.3. Pit latrine filling

The fill-up rate of pit latrines varies greatly (Still 2002, Bakare 2014, Torondel, LSHTM, unpublished data), and is determined by FS composition, user behaviours, and factors which affect biodegradation, as described above. Variations in fill-up rates results in pit latrines being emptied at different frequencies. Average vault emptying frequencies of pit latrines in Dar es Salaam, Tanzania, varied according to how the vaults were lined: unlined 8.2 years, partially lined 6.5 years, fully lined 8.5 years, and drums/tyre lined 4.7 years (Jenkins *et al.* 2013). Additionally, the average fill-up rate increased with each additional pit latrine emptying. Solid waste accumulated in vaults and reduced the volume of FS which could be stored before the vault requiring emptying again (Jenkins *et al.* 2013). A separate study found that the frequency of pit emptying varied between countries worldwide, with vault emptying frequencies between once a year in Senegal, to once every 3 to 5 years in Cambodia and Vietnam. The most common emptying frequency across all countries studied was once every two years (Chowdhry *et al.* 2012).

When latrines fill up users must have the vault emptied or resort to sharing latrines or open defecation. Sharing latrines leads to an increase in vault fill-up rates, and open defecation negates the benefits of improved sanitation, leading to the spread of disease (Esrey *et al.* 1991). An increase in odours emanating from full vaults, as well as in the number of flies and mosquitoes, has been reported (Biran 2010b, Biran 2011). Pit emptying is accomplished by either mechanical or manual means, as discussed below.

1.2.4. Pit latrine emptying

Vault emptying can be conducted either mechanically, or manually. A report which investigated faecal sludge management (FSM) provisions of 30 cities, in five countries in Africa (Burkina Faso, Ethiopia, Nigeria, Kenya, and Senegal), and five countries in Asia (Bangladesh, Cambodia, India, Malaysia, and Vietnam), found that the majority of households, 63.4%, used mechanical emptying, while 34.3% using manual emptying services, and 1.4% using a combination of both (Chowdhry *et al.* 2012). However, the method of pit latrine emptying differs depending on location. For example, in Addis Ababa, Ethiopia, 100% of households reported using mechanical emptying services. This was because of heavily subsidised government provided emptying services, costing less than $5, five times less than private operators. However, in Kisumu, Kenya, manual emptying accounted for approximately 75% of services, costing $30, while mechanical emptying cost on average $52 (Chowdhry *et al.* 2012). Table 1-4 demonstrates how the cost of emptying can vary between $5 and $300, depending on method, and distance travelled by the emptying service (Still 2002) (Table 1-4).

There are many issues associated with pit latrine emptying. The act of emptying vaults poses a public health risk to emptiers and the community as exposure to pathogens through improper disposal also increases the chance of pathogen transmission by mechanical vectors (Esrey *et al.* 1991). Emptying and transportation of FS can also be inconvenient for the latrine owners, as it

may result in a contaminated households and cause bad smells in surrounding areas (Biran 2010a, Biran 2010b).

Table 1-4 Methods and cost of pit latrine emptying in three African countries. Source: Still, 2002

Emptying Method	Location	Cost [a] (Range) for $2m^3$
Manual excavation of old pit with fully decomposed contents	Standard rates for Pit Excavation in Soil	$7 – $14
Manual scooping/flushing with hand tools	South Africa	$5 – $11
Cart mounted, 200 litre vacuum tank indirectly couple to hand pump	Dar es Salaam, Tanzania	$8
Self-propelled 500 litre tank with motorised pump	Nairobi, Kenya	$18
Trailer mounted, tractor hauled 2000 litre tank	Durban, South Africa	$20 – $60
Urban Vacuum tanker, 5,000 – 15,000 litre truck mounted tank	South Africa	$20 – $100
Rural Vacuum tanker, 5,000 – 20,000 litre truck mounted tank	South Africa	$0.7 – $1.5 per kilometre e.g. 200km = $140 – $300

[a] costs calculated using present day exchange rates

A problem that occurs in cities and unplanned urban settlements is that the vacuum trucks are often too big to reach the latrines that need to be emptied (Boesch *et al.* 1985). In these situations, manual emptying must be conducted. Over 90% of manual emptying is reported to be conducted by a hired individual or company (Chowdhry *et al.* 2012). Individuals who manually empty pit latrines are often some of the poorest in their communities, unable to afford basic protective gear. This lack of equipment exposes them to diseases due to direct contact with excreta, broken glass and other discarded waste, resulting in many health problems. Additionally, the nature of the work has a negative social image, and stigmatises the workers, often required to work at night where they can be harassed by criminals (WSP 2005). Manual emptying is often done in areas which are inaccessible to mechanical methods, although there are new technologies which may get around this problem. For example, the Gulper is a manually operated pump for emptying the contents of wet pit latrines. The Gulper

consists of a two metre long PVC pipe and stainless steel valves (WaterAid 2011). Operated by one or two people the pipe is lowered into a wet pit latrine and through a pumping action lifts the pit contents up the pipe. This is then directed into a container. The small size of the equipment makes it ideal for use in densely populated cities and unplanned urban settlement. The Gulper has been trialled in a number of countries, including Tanzania (WaterAid 2011), and Uganda (Stalker, Engineers Without Borders, personal communication). Currently the Gulper is being used by 12 different entrepreneurs around Kampala, Uganda, who charge approximately $12 per 200 litre barrel of FS disposed of (Stalker, Engineers Without Borders, personal communication).

1.2.5. Faecal sludge management

Once FS has been emptied from pit latrines, it must be transported and treated. Treatment of FS can be achieved by a number of methods, including official dumping grounds, using wetlands, stabilisation ponds, mixing into wastewater treatment plants, or dedicated FSM plants (Chowdhry *et al.* 2012). However, the distance to FSM facilities, and cost of legally dumping FS, can lead to pit latrine emptying services illegally dumping FS into the environment, causing major environmental and hygiene problems in many cities (Helmer *et al.* 1997, Kariuki *et al.* 2003, WHO/UNEP 2006). It is vital to note that suitable FSM solutions are uncommon in low- and middle-income countries. Although precise data are scarce, it has been reported that only 30% of cities in countries described previously have dedicated FSM solutions (Chowdhry *et al.* 2012). In Addis Ababa, where two dedicated FSM plants are located, their combined capacity is only 66% of the total FS collected (Chowdhry *et al.* 2012). Additionally, in Bangladesh, only 1% of all FS generated throughout the country is treated, with the remaining 99% dumped into surface water (Rahman 2009).

The hygiene and environmental problems caused by the lack of suitable FSM solutions in low- and middle-income countries has stimulated innovation of alternative FSM methods. Examples

of organisations and new technologies being investigated as affordable and accessible FSM methods include Sanergy, Loowatt, and the Tiger Toilet.

Sanergy aims to improve sanitation in Kenyan urban slums by designing and implementing a scalable and sustainably viable sanitation infrastructure in the slums of Nairobi (Sanergy 2013). This is achieved by building low-cost, user friendly, and hygienic communal sanitation centres, and distributing them through franchising to local entrepreneurs. These entrepreneurs operate the sanitation centres, charging an affordable price for use, while ensuring facilities are kept clean and well maintained. The facilities are designed so excreta are stored in sealed cartridges, ensuring foul odours and filth flies are not a problem, and making it hygienic for the surrounding community and collection staff. Excreta are collected daily by properly equipped Sanergy staff. Full cartridges are gathered using wheelbarrows, handcarts and/or trucks, from facilities which could be in hard to reach areas. The excreta are then delivered to a centralised facility which converts it into useful by-products, such as organic fertiliser and renewable energy using biogas digesters. The excreta is converted into fertiliser by co-composting with sawdust, eliminating pathogens to WHO standards, and then sold to Kenyan farmers. To date Sanergy has opened 405 sanitation facilities, servicing 16,000 daily users, creating 462 jobs, and has removed and treated almost 2,500 tons of excreta (Sanergy 2013). Sanergy have developed an end-to-end approach of excreta storage, collection, transport and treatment, utilising simple technologies and a robust support structure for franchise operators. However, the development of new technologies is also being investigated.

Loowatt has developed a waterless, mechanical sealing unit to contain human excreta within a biodegradable film while removing odours, and stores the excreta in a cartridge beneath the toilet. The unit can be fitted to a range of toilets or sanitation facilities, and can be easily emptied daily or weekly, depending on usage. The collected excreta is then processed using biogas digesters, where anaerobic micro-organisms decompose the excreta, releasing biogas

composed of CH_4, CO_2, and trace gases (NNFCC 2009). Biogas can be used as a clean, renewable energy source, and can increase the value of owning a toilet with the system. The residue that remains can also be used as an organic fertiliser, although requires secondary treatment to ensure sufficient pathogen removal (NNFCC 2009). Pathogen removal can be achieved through thermophilic composting, pasteurisation, or vermicomposting, which uses detritivorous worms to compost residue. Currently Loowatt is running a pilot system in Antananarivo, Madagascar, with 6 toilets servicing around 250 people daily. In 2013, a $1 million grant was awarded by the Bill and Melinda Gates Foundation to develop the products, systems and implementation strategies of the commodity-generating waterless toilet system (BMGF 2013).

The use of detritivorous organisms to process human excreta is a prospective solution that is also being investigated. The tiger worm, *Eisenia fetida*, has been shown as a potential source of vermifiltration technology (Yadav *et al.* 2010, Kassam 2012, Furlong *et al.* 2014) for low- and middle-income countries. The "Tiger Toilet" is linked to a normal pour-flush pedestal. When flushed, the excreta are deposited onto a bed of worms living in a matrix of woodchips or similar material. The solid excreta is consumed by the worms, and the liquids are filtered through a drainage layer. The worms significantly reduced FS by up to 96%, compared to 38% in controls without worms (Furlong *et al.* 2014), and reduce the number of faecal coliform colony forming units (CFU's) by up to 97.7% (Kassam 2012). The liquid effluent was found to be of higher quality than that found in septic tanks and other vermifiltration systems (Furlong *et al.* 2014). In 2013, a "Stage 1: Proof of Concept Development Innovation Ventures" grant was awarded by USAID to Bear Valley Ventures to test the Tiger Toilet in 10 households in each of India, Myanmar, and Uganda. The project aims to determine the performance, and user acceptance, of the Tiger Toilet in rural communities, displaced person camps, and peri-urban areas. It is hoped that the Tiger Toilet will become a leapfrog technology, offering lower maintenance and better performance than a pit latrine, for a price below that of septic tanks.

An alternative detritivorous species which is being investigated is the larvae of the black soldier fly (BSFL), *Hermetia illucens* (L.). The BSFL consume large quantities of organic matter in a short period of time, reducing pathogens and converting excreta into organic fertiliser. The prepupae are also a valuable resource, as they are high in protein and fats. These qualities indicate that BSFL could be a valuable novel FSM method which can remove dangerous FS from the environment, while creating products which could potentially generate a profit.

1.3. Black soldier fly biology

1.3.1. Life cycle

The black soldier fly (BSF) is a Dipteran fly of the Stratiomyidae family, found around the world from 49°N to 42°S (Üstüner *et al.* 2003, Roháček *et al.* 2013). There have been limited studies which investigated their behaviour in the wild, but it has been shown that the adult males congregate in small numbers near secluded bushes (Copello 1926) and display lekking behaviour (Tomberlin *et al.* 2001) in order to find a mate. Once mated the females lay egg clutches of 600 to 900 eggs close to a larval food source (Booth *et al.* 1984, Tomberlin *et al.* 2002). The eggs hatch within 102-105 hours (Booth *et al.* 1984) and the newly hatched larvae crawl or fall into the food source. The larvae feed, accumulating enough resources to develop through 6 larval instars, before they pupate. The pupa undergoes complete metamorphosis, holometabolism, before eclosing as an adult (Figure 1-2).

1.3.2. Adult behaviour

The BSF adults are neither a nuisance species, nor a mechanical vector for disease. The adult females oviposit in crevices around the edges of larval food sources (Copello 1926), but neither females nor males feed as they survive on fat stores from their larval stage (Furman *et al.* 1959).

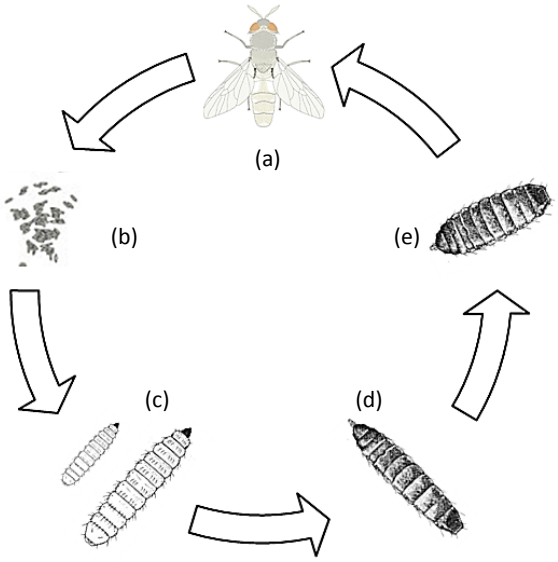

Figure 1-2 Life cycle of *Hermetia illucens*; (a) Adult (b) Eggs (c) 1st – 5th larval instars (d) 6th larval instar (prepupa) (e) Pupa

These behaviours result in no mechanical pathogen transmission, which can arise from using alternative detritivorous species, such as the house fly, *Musca domestica* (Pieterse *et al.* 2014). Nevertheless, the fact that there have been rare cases of accidental myiasis caused by people consuming ripe, unwashed fruit (Calderón-Arguedas *et al.* 2005, Gonzalez *et al.* 2009) should not be overlooked. There has also been a single incident of furuncular cutaneous myiasis recorded after an American woman spent 3 weeks in Uganda, teaching on a sugar plantation, and 5 days in Kenya at a tourist lodge (Adler *et al.* 1995). From the case report it appears that the woman involved had a skin condition rendering her uniquely susceptible to larvae that crawled onto her clothing. Given the worldwide distribution (Leclercq 1997) of BSF, and the limited number of cases described, it can be proposed that myiasis caused by BSFL presents negligible risk to humans.

Adult BSF have limited interactions with humans (Furman *et al.* 1959), therefore, little is known about their natural behaviour. However, there is sufficient knowledge to allow colonies of BSF to be reared artificially. Establishing a colony is difficult and requires a number of factors to be correct to allow successful adult mating and larval development. The adults mate

on the wing (Booth *et al.* 1984), and when enclosed require a sufficiently large cage volume to result in successful mating. Successful mating has been reported in artificial colonies enclosed in cages sized 76cm x 114cm x 137cm (w x d x h) and larger (Tingle *et al.* 1975, Tomberlin *et al.* 2002). Time of day and light intensity are significantly correlated with mating (Tomberlin *et al.* 2002), with sunlight promoting the most successful mating. However a quartz-iodine artificial light source produces a 61% mating rate compared to a sunlight control (Zhang *et al.* 2010), allowing indoor rearing of BSF. The adults lay their eggs in crevices near a larval food source, with significantly more eggs laid in dry cardboard oviposition sites than wet cardboard with approximately 52% water content (Booth *et al.* 1984). Time of day, relative humidity (RH), and temperature are positively correlated with oviposition, with more egg clutches laid later in the day when temperatures are at or above 26°C, and 80% of egg clutches laid at RH over 60% (Tomberlin *et al.* 2002). It was suggested that this behaviour ensures eggs are laid under optimal conditions for survival, where desiccation of eggs can occur due to low RH (Tomberlin *et al.* 2002). Furthermore, temperature and RH have an important influence on the rearing of BSF. When larvae were reared at 27 – 30°C, up to 97% emerged as adults. However, at 36°C, only 0.1% emerged as adults, even though 73.4% developed into prepupae (Tomberlin *et al.* 2009). Increased relative humidity also increases egg hatching and adult emergence, while development time decreases with rising relative humidity (Holmes *et al.* 2012).

1.3.3. Immature stages

The larvae are detritivores, obtaining nutrients from decomposing plant and animal matter, and faeces. Black soldier fly larvae have been known to feed on human and animal cadavers (Dunn 1916, Tomberlin *et al.* 2005), decaying vegetables (Malloch 1917), animal manure (Tingle *et al.* 1975, Booram *et al.* 1977, Newton *et al.* 2005), palm kernel meal (Hem *et al.* 2008), municipal organic waste (MOW) (Diener *et al.* 2011a) fresh human faeces (Lalander *et al.* 2013, Banks *et al.* 2014) and pit latrine FS (Bradley 1930, Fletcher *et al.* 1956). The ability of BSFL to utilise such varying organic matter as food sources could be due to their gut

microbiota. In one study gut bacterial communities in BSFL were shown to be dependent on their diet, with food-waste fed BSFL containing more complexity of bacterial species than BSFL fed on cooked rice or calf-forage (Jeon *et al.* 2011). However, 36 bacterial strains were shared between the BSFL fed on the three different diets and were unlike intestinal micro-flora found in other insects (Jeon *et al.* 2011). This indicates that BSFL have a unique composition of gut microbiota, which allows them to utilise a range of food-sources.

The larvae can reach maturity in 2-4 weeks depending on temperature and food availability, but can survive for up to 10 months in a state of quiescence (May 1961, Myers *et al.* 2008). It has also been proposed that the larvae may feed on the remains of their conspecifics in cases of extreme food deprivation (May 1961). Black soldier fly larvae are able to extend their life cycles under environmentally stressful conditions, making them suitable for FSM. The final larval instar is known as the prepupal stage (Figure 1-2d).

The prepupal stage is indicated by a change in colour and behaviour. The larvae turn from white to a dark brown colour. Prepupae crawl out of their feeding material to find a dry dark area to pupate and can crawl up to 100m to find a suitable site (Schremmer 1986). Prepupae will climb slopes of 40°, making them easy to collect (Sheppard *et al.* 1994). This behaviour facilitates prepupal harvesting, suggested due to their intrinsic value, discussed in detail later. However, BSFL have been observed during laboratory experiments performed in the United Kingdom, and South Africa, to climb up vertical surfaces if there is sufficient moisture to maintain surface tension (Banks, LSHTM, personal observations). This behaviour could also be due to BSFL seeking a more suitable food source with lower moisture content.

1.4. Black soldier fly applications

1.4.1. Manure management

Several researchers have shown that BSFL are effective at reducing animal manure and MOW. For example, Newton *et al.* (2005) fed 169kg of fresh swine manure to BSFL. The larvae were

fed in a basin with a 35° ramp that directed prepupae into guttering at the top to facilitate harvesting. A total population of 45,000 larvae were added to the basin. The larvae converted 68kg dry weight of manure into 41.6kg dry weight residue, and 26.2kg of prepupae. Newton also recorded BSFL reducing 55kg of fresh manure, dry matter, to 24kg of residue, dry matter, within 14 days. The manure was reduced by 56%, with the residue having no objectionable odour (Newton *et al.* 2005). The reduction in odour is supported by preliminary semiochemical research in a laboratory experiment, which showed a large reduction in volatiles produced by fresh human faeces after being fed on by BSFL (Banks 2010).

Sheppard *et al.* (1994) fed BSFL on chicken manure in basins with a migration slope of 40°. Approximately 5.2 tonnes of fresh chicken manure, from 460 hens, was reduced to approximately 2.6 tonnes residue, yielding 242kg of prepupae, with a mean weight of 0.22g. The BSFL reduced the manure by up to 50%, while at the same time eliminating house fly breeding (Sheppard *et al.* 1994).

Another study, aimed to evaluate the feasibility of using BSFL as a method of MOW treatment in low- and middle-income countries, fed BSFL on MOW in “larveros”, with migration ramps of 28° (Diener *et al.* 2011a). The larvae were fed different quantities of MOW, 1.5 or 4.6 kg each day, with the new food either mixed in or placed on the surface. It was found that prepupal migration varied throughout the 55 days of experiment, with daily fluctuations in prepupal production. In the treatments, where fresh MOW was manually mixed into already digested residue containing BSFL, there were lower mean prepupal weights and prepupal harvests than when the fresh food was applied to the surface. The surface fed, high feed amount produced largest mean weight of prepupae, 0.22 g, and a mean prepupal harvest of 286 g m^{-2} day^{-1}. The relative wet weight reduction varied from 46.2% to 75.8% (Diener *et al.* 2011a). Trials conducted by Banks *et al.* (2014) reported that BSFL fed 100mg $larvae^{-1}$ day^{-1} of fresh human

faeces every three days, resulted in mean prepupal wet weights of 0.30g, with a wet weight faecal matter reduction (FMR) of 46%.

All of these experiments produced similar feed conversion rates (FCR) (Table 1-5), with the exception of BSFL fed on fresh human faeces. The FCR is a measure of an animal's efficiency in converting feed mass into increased body mass (USAID 2011), and is calculated using the following equation:

$$FCR = \frac{Feed\ added\ (faecal\ sludge)}{Total\ prepupal\ biomass}$$

Bioconversion rate is also used to quantify BSFL production efficiency, using the following equation:

$$Bioconversion\ rate = \frac{Total\ prepupal\ biomass}{Feed\ added\ (faecal\ sludge)} \times 100$$

The BSFL fed on fresh human faeces resulted in a higher bioconversion rate than when fed on other faecal material.

Table 1-5 Biomass yield and faecal matter reduction (FMR) of different pilot scale experiments

Feed Source	Total Amount of feed	Residue	FMR	Prepupal yield	Bioconversion (%)	FCR
Swine manure (Newton *et al.* 2005)	68 kg DW	42 kg DW	~39% DW	~2.7 kg DW	3.97	9.6
Chicken Manure (Sheppard *et al.* 1994)	5,240 kg WW	~2,620 WW	~50% WW	196 kg WW	3.74	13.4
MOW (Diener *et al.* 2011a)	151 kg DW	48 kg DW	68% DW	17.8 kg DW	11.78	5.8†
Fresh human faeces (Banks *et al.* 2014)	~480g WW	~260g WW	~46% WW	108g WW	22.3	2.0

MOW: municipal organic waste, DW: dry weight, WW: wet weight, FCR: feed conversion ratio, † value different to published paper (Diener, Upsala, personal communication)

In another experiment, it was demonstrated that BSFL can develop on pure FS taken from septic tanks and dewatered to 63% moisture content (Diener *et al.* 2011b). The BSFL reduced the FS by 54.7%. However, the mean dry prepupal weight was 0.02 g, far lower than found in other studies (Sheppard *et al.* 1994, Newton *et al.* 2005, Diener *et al.* 2009, Diener *et al.* 2011a, Banks *et al.* 2014).

1.4.2. Prepupae value

Another advantage of BSFL is the potential value of the prepupae. Prepupae contain high protein and fat concentrations, 42-45% and 31-35% respectively (Sheppard *et al.* 1994, Newton *et al.* 2005). Research has shown how the prepupae can act as a replacement for conventional protein and fat sources in a number of animal feeds, including cockerels (Hale 1973), swine (Newton *et al.* 1977), catfish and tilapia (Bondari *et al.* 1987, Hem *et al.* 2008), and rainbow trout (St-Hilaire *et al.* 2007b). It is also possible to fractionate the prepupae into their constituent parts, using the proteins for animal feed and converting the fats into biodiesel (Li *et al.* 2011a, Li *et al.* 2011b, Zheng *et al.* 2012a, Zheng *et al.* 2012b). It is also possible to utilise the chitin from the larval cuticles into chelating agents (Kumar 2000). Chelating agents form multiple bonds with a single central metal atom, forming a chelate complex, stopping the metal reacting with other elements and producing precipitates (Muller 1994). Chelating agents can be used in producing fertilisers (Ashmead 1993), and other industrial or medical applications. It is even possible to use the residue remaining after feeding, comprised of the larval excreta and undigested material. This has been found to be of similar chemical composition to commercial fertiliser. Also, when used as a fertiliser in growth trials using Chinese cabbages, it resulted in no significant difference in cabbage mass and chemical composition compared with commercial fertilisers (Choi *et al.* 2009).

1.4.2.1. Alternative species as animal feed

The use of insect protein in animal feed is increasingly becoming an issue of worldwide importance. This is due to the rising cost of animal feed protein, animal feed insecurity, environmental pressures, population growth, and increasing demand for protein amongst the middle class (FAO 2013). Black soldier flies are just one of a number of different insect species that have been suggested as a resource to be used as animal feed (Table 1-6). These species include: the common house fly, *M. domestica*, silkworms, *Bombyx mori*, *Samia cynthia ricini*, *Antheraea assamensis*, *An. mylitta* and *An. paphia*, mealworms, *Tenebrio molitor*, *T. obscurus*, and several species from the order Orthoptera, including locusts, grasshoppers, and crickets (FAO 2013). Table 1-6 summarises the nutritional values from a selection of these different species, as well as BSF prepupae. The protein content of prepupae is lower than other species listed. However, the fat content is higher than *M. domestica* and Orthoptera species, with content and fatty acid composition depending on diet (St-Hilaire *et al.* 2007a). The prepupae are rich in calcium and iron compared to other species, although phosphorus, potassium, magnesium, manganese, sodium, zinc, and copper concentrations are lower than *M. domestica* pupal meal. The amino acid profile of BSF prepupae is rich in lysine, and comparable to *M. domestica* (Calvert *et al.* 1969).

1.4.3. Pathogen reduction

The ability of the larvae to reduce the pathogen loads in manure has been shown in a number of studies. The BSFL reduce *E. coli* O157:H7 and *Salmonella enterica* serotype *Enteritidis* (ME18) in chicken manure (Erickson *et al.* 2004). After three days of larval feeding on chicken manure, there was a 3 $\log_{10}$ (CFU g^{-1}) inactivation of *E. coli* compared to a control. On days two and four of the experiment, there were significant reductions of *S. enterica* in manure treated with BSFL, 4.9 $\log_{10}$ (CFU g^{-1}) and 1.41 $\log_{10}$ (CFU g^{-1}) respectively. However, after two days of feeding on *Salmonella*-treated chicken faeces, the BSFL became contaminated with the pathogen, with the *Salmonella* population 1-$\log_{10}$ higher than in the manure. Between days

four and six there was no further decrease in *Salmonella* populations in both manure and BSFL, but during this time larval mass decreased suggesting a reduction in feeding. The reduction in larval mass suggests that the inactivation of pathogens is linked to feeding and larval growth. A later study found that BSFL significantly reduced *E. coli* populations in dairy manure (Liu *et al.* 2008). It was also found that the amount of manure added significantly influenced the larval weight gain and the ability to reduce *E. coli*. A second experiment found that BSFL were able to significantly reduce bacterial growth at 23, 27 and 31°C, with greatest suppression at 27°C (Liu *et al.* 2008). There is also evidence that BSFL reduce the concentration of *Salmonella* spp. in human faeces by 6 $\log_{10}$ (CFU g^{-1}) in eight days (Lalander *et al.* 2013), a significantly accelerated reduction compared to less than 2 $\log_{10}$ (CFU g^{-1}) in controls. These results indicate that using BSFL to treat human FS can remove zoonotic pathogens, decreasing the risk of transmission to animals and humans should the residues be used further in agriculture. Unfortunately, there was no significantly accelerated reduction of *Enterococcus* spp., bacteriophage ΦX174, and *Ascaris suum* ova. This suggests that an additional treatment of residue would be required if it was to be used in food crop production. It was also suggested that, before using the prepupae in animal feed, additional processing would be required to destroy any pathogens that may remain in the prepupal gut (Lalander *et al.* 2013).

1.4.4. Control of *Musca domestica*

It has been suggested that a dense population of BSFL will inhibit the development of *M. domestica* larvae. For example, in chicken manure treated with BSFL, *M. domestica* larvae were unable to develop, possibly due to competition for food (Furman *et al.* 1959). It was found that in pit latrines already containing BSFL, there were very low levels of *M. domestica* (Kilpatrick *et al.* 1959). Kilpatrick also found that, when pits were treated with the insecticide dieldrin, there was a subsequent increase in *M. domestica*, which he attributed to insecticide resistance of *M. domestica* but BSFL's susceptibility to dieldrin.

Table 1-6 Nutritional values of insect species proposed as alternative sources of animal feed, source: FAO's Animal Feed Resources Information System www.feedipedia.org/content/feeds?category=17919

		Main analysis						Minerals								
		Dry matter	Crude protein	Crude fibre	Ether extract	Gross energy	Ash	Calcium	Phos-phorus	Potas-sium	Sodium	Magne-sium	Manga-nese	Zinc	Copper	Iron
	Unit	% as fed	% DM	% DM	% DM	MJ/kg DM	% DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM	mg/kg DM	mg/kg DM	mg/kg DM	mg/kg DM
Black soldier fly prepupae (dried)	Mean	91.3	42.1	7	26	22.1	20.6	75.6	9	6.9	1.3	3.9	246	108	6	1370
	SD	1.1	1		8.3		6	17.1	4							
Musca domestica pupal meal (dried)	Mean	92.1	70.8	15.7	15.5	24.3	7.7	5.2	17.2	12.5	5.7	8.2	416	363	38	258
	SD		5.3		1		2.1									
Musca domestica larval meal (dried)	Mean	92.4	50.4	5.7	18.9	22.9	10.1	4.7	16	5.7	5.2	3.4	91	119	27	995
	SD	1	5.3	2.4	5.6	1.4	3.3	1.7	5.5	3.5	2.4	4	114	118	6	440
Silkworm pupal meal (dried)	Mean	91.4	60.7	3.9	25.7	25.8	5.8	3.8	6			3.7	18	224	15	326
	SD	4.4	7	1.1	9		2.4	3	2.4			2.5		126	12	67
Mealworm (*Tenebrio molitor*) meal (fresh)	Mean	42.2	52.8		36.1	26.8	3.1	2.7	7.8	8.9	0.9	2.3	9	116	16	57
	SD	6.3	4.2		4.1	0.4	0.9	1.9	3.7			0.4	4	24	1	32
Locust or Grasshopper Meal (dried)	Mean	91.7	57.3	8.5	8.5	21.8	6.6	1.3	1.1	1.1	3.2	1.5		10		13
	SD	2.3	11.8	4.1	3.1	2	2.5									
House Cricket (*Acheta domesticus)* (fresh)	Mean	28.4	63.3		17.3		5.6	10.1	7.9			1.2	40	215	15	116
	SD	4.5	5.7		6.3		2.4	5.3					10	60	7	58
Mormon Cricket (*Anabrus simplex*) (dried)	Mean	94.1	59.8	8.2	13.3	23	6.5	2	10.4	13.5	9.7	1.2	68	268	25	164
	SD	0.5	4.1	1.1	5		1.9									

Black soldier fly larvae were also shown to reduce oviposition of *M. domestica* on chicken faeces by 94-100% (Sheppard 1983). In contrast, observations from large scale breeders noticed no reduction in filth fly quantities (Drew, personal communication).

The mechanisms causing any reduction in larval survival or oviposition of *M. domestica* due to BSFL have not been fully elucidated. Kilpatrick found that the presence of BSFL combined with water in the pit maintained a semi-liquid or liquid medium that was unsuitable for the development of *M. domestica* larvae (Kilpatrick *et al.* 1959). There is also evidence to suggest that an allomone, a chemical released by BSFL larvae resulting in interspecific chemical communication with the *M. domestica* gravid females, is present that reduces oviposition (Bradley *et al.* 1984). Egg laying behaviour of gravid females of *M. domestica* is known to be affected by semiochemical (behaviour modifying chemical) cues released by fungal competitors on animal faeces (Lam *et al.* 2010). It is also possible that a combination of both chemical and physical factors could work together to repel *M. domestica*.

1.4.5. Current and potential BSFL applications

The method of how to use BSFL for FSM in different situations must be considered. There are a number of different techniques suggested, including decentralised BSFL treatment plants, in-situ BSFL, and a BSFL toilet.

Decentralised BSFL FSM plants would be located in communities which are lacking suitable FSM solutions, and could be suitable for urban, peri-urban, and potentially rural locations, depending on population density. The plants would receive a constant stream of FS from surrounding pit latrines, either collected manually or mechanically, in order to continuously rear BSFL. This would yield a steady supply of prepupae and residue that would be properly sterilised to ensure safety. Health benefits would be provided by the provision of a properly regulated, hygienic, pit emptying service, while economic benefits, in the form of employment, and income from sale of products, would benefit the communities covered by the plant,

similar to the Sanergy model. There is currently a pilot BSFL FSM plant running in South Africa operated by the BioCycle (BioCycle 2014). The BioCycle is a joint venture between Bear Valley Ventures and Agriprotein Technologies that aims to develop commercially viable and scalable methods of bioconversion of human FS into valuable products using BSFL. The pilot plant is currently processing 150kg of FS per week, covering approximately 25 bucket latrines and 125 users. The Biocycle are also conducting pathogen trials on prepupae and residue, to determine health and safety issues related to the use of the products (Lewis, The BioCycle, personal communication).

Further evidence to support the decentralised processing plant model is provided by a business feasibility study conducted by the HAAS Business School, University of California, Berkeley, in 2011 (Appendix A). Data were gathered from internet resources, research reports, publications, and field interviews in Dar es Salaam, Tanzania. The study used a range of assumptions based on data collected, and produced three business models: Model 1 – Crude Oil and BSFL feed, Model 2 – Biodiesel and BSFL feed, and Model 3 – BSFL feed only. The results showed that a decentralised BSFL FSM treatment plant is feasible under a number of scenarios. Model 1 is only feasible in the best case scenario, where highest revenues and lowest costs are assumed, resulting in fixed investment breakeven and setup time to be 1.82 years. In Model 2, the best case scenario is 1.23 years, while the worst case scenario, assuming lowest revenue and highest costs, would take 13.51 years to breakeven and setup. However, Model 3 is predicted to be unfeasible, never achieving a fixed investment breakeven. This suggests that the sale of prepupal lipids, as crude oil or biodiesel, is integral to the business model. The model is designed to be flexible. The cost, revenue, and BSFL production data can be amended depending on localised information. Also, the model does not account for the sale of residue, as an organic fertiliser, which could be a major revenue source. Considering these details, the model provides strong supporting evidence for decentralised BSFL FSM solutions.

In-situ BSFL treatment could be suitable in rural areas, where the cost of collecting and transporting FS to a decentralised BSFL treatment plant would be uneconomical. Faecal sludge could be managed by introducing BSFL into pit latrine vaults, subsequently harvesting the prepupae from the vault. The primary aim of an in-situ treatment solution would be to reduce the FS inside of a pit latrine. This could extend the life of the pit latrine, by reducing the vault fill rate, subsequently reducing the frequency of vault emptying. Overall, this would help extend the sanitation of the household, and the surrounding community. A secondary benefit is that prepupae could be sold to official companies to process safely into a protein source for animal feed. It is suggested that to maintain the FSM benefits of the BSFL, larvae could be regularly added to the vault or adult BSF could be encouraged to lay eggs within the vault. The use of BSFL in-situ may be recommended for use in older latrines, where the technology can be retrofitted with a prepupal harvesting system. Harvesting system currently being investigated following an InnoCentive Challenge includes the "Kone" and "Daisy Chain" (SV 2011). The Kone was designed by Swedish water and waste engineering consultants, and is comprised of a rubber cone, made from recycled tires, that is placed into a latrine. Prepupae migrate up the slopes of the cone into a removable collection pot which is emptied periodically. The Daisy Chain was developed following a BSF Toilet Design Workshop, and is a flexible lightweight circular tube which sits on top of the FS in a pit latrine. Prepupae migrate into the tube through multiple access points, and the tube is pulled up and emptied via the drop hole (SV 2011).

Finally, it is possible to design a new toilet based around BSFL. A BSFL toilet would comprise of a BSFL FS treatment unit, which directed migrating prepupae for easy harvesting. Using the FMR capabilities of the BSFL, FS would be reduced to residue, resulting in a slow fill rate, and reduced emptying and maintenance. This would be most suitable in crisis situations, where a long lasting and robust solution is required, due to high usage. The BSFL toilet could help prevent disease but reducing FS, but also potentially prevent pathogen transmission via

controlling *M. domestica* populations which would normally breed in pit latrines. A conceivable concern with a BSFL toilet is how to maintain the BSFL population. The toilet could be seeded with early stage BSFL at regular intervals, or alternatively, a specially designed adult cage could be built onto the superstructure. However, it is believed that these problems are not impossible to solve with sufficient research and ingenuity.

1.4.6. Potential pitfalls

A potentially major drawback of using BSFL to consume FS is the bioaccumulation of hazardous compounds. Bioaccumulation is the build-up of a substance by an organism at a greater rate than which it is lost. This is important when the substance being absorbed is toxic, such as pesticides, organic chemicals or heavy metals (IUPAC 1993). Heavy metal bioaccumulation must be taken into consideration when dealing with BSFL due to biomagnification. Biomagnification is when there is an increase in concentration of a substance from one trophic level to another (Walker 1987). It has been found that the prepupae accumulate cadmium within their body, and lead and zinc were found in the larval exuviate: the shed skin of the larvae (Diener 2010). The heavy metals had no significant effect on prepupal weight, development time, sex ratio and adult bilateral symmetry. However, a later study demonstrated the influence of heavy zinc contamination caused reduced adult egg laying and increased mortality of young BSFL (Diener *et al.* 2011a).

Even though heavy metals such as zinc, cadmium and lead do not affect the development of individual BSF (Diener 2010), the problem of biomagnification occurs when the prepupae are used for animal feed, such as when larvae are fed directly to chickens. Even though the larvae only contain a trace amount of a heavy metal, a large quantity of larvae eaten will cause a magnifying effect of the heavy metal. This is magnified again when the chicken is eaten by humans.

1.5. Rationale

Improved sanitation is fundamental to enhancing social and economic development (Mara *et al.* 2010). Improved on-site sanitation is low-cost, and the only feasible solution for low- and middle-income countries. However, issues arise with on-site sanitation, when pit latrines become full and require emptying. The lack of FS treatment and disposal sites means there is a necessity for the investigation into novel FSM technologies. Using BSFL as a technology to manage pit latrine FS has been suggested due to their FMR efficiency and prepupal value (Newton *et al.* 2005, Diener *et al.* 2011a). It is suggested that BSFL could be an environmental, scalable, and suitable technology alternative FSM solution, which could reduce indiscriminate dumping, disease, and provide an income for entrepreneurs.

However, there are significant gaps in research that must be filled before BSFL can be utilised as a FSM solution. Although previous research into BSFL behaviour has shown that they can digest fresh human faeces (Banks 2010, Lalander *et al.* 2013), there is little data on their ability to develop on, and digest, pit latrine FS. How effective BSFL are at FMR and prepupal biomass production while feeding on fresh faeces and pit latrine FS under different feeding conditions must be determined. Variations in the physical characteristics, and chemical components of different layers of pit latrine FS, and their effects on BSFL efficiency must be identified, specifically in relation to FMR and prepupal yield. Also, it is necessary to determine whether heavy metal contaminants found in FS bioaccumulate in prepupae. Finally, it is important to understand the effect of commonly used cleaning chemicals, which could be present in FS, on the mortality of BSFL. The PhD presented here aims to assess the full potential of BSFL in this role.

1.6. Objectives

The overall aim of this PhD is to determine whether BSFL are a viable faecal sludge management solution. The specific objectives are:

1) To determine the growth rates and FMR of black soldier fly larvae on fresh human faeces, verifying whether BSFL have the potential as a method of faecal sludge management
2) To determine the physical and chemical characteristics of pit latrine faecal sludge in a country where BSFL technology could provide a solution to faecal sludge management issues, while establishing whether the characteristics fall within the range of a suitable food source for BSFL
3) To determine the effect of key rearing parameters: faecal sludge moisture content, feeding rate, and larval density, on black soldier fly larvae FMR efficiency, and prepupal biomass production when reared on pit latrine faecal sludge, establishing an optimum FMR model for future trials
4) To determine the FMR efficiency, and prepupal production, of black soldier fly larvae reared on different layers of pit latrine faecal sludge with variations in physical and chemical composition, ascertaining the economic practicality of using BSFL as a faecal sludge management technology, and determining bioaccumulation of heavy metals in prepupae
5) To conduct a preliminary investigation into the effect of common non-excreta additives in pit latrine faecal sludge on black soldier fly larvae mortality, determining whether commonly used chemicals could potentially negatively affect BSFL faecal sludge management technologies

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Chapter 2) Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation

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2.1. Abstract

OBJECTIVES: To determine the efficacy of black soldier fly larvae (BSFL) (*Hermetia illucens*) at converting fresh human faeces into larval biomass under different feeding regimes, and to determine the viability of BSFL as a means of human faecal sludge management (FSM).

METHODS: Black soldier fly larvae were fed fresh human faeces. The frequency of feeding, number of larvae and feeding ratio were altered to determine their effects on larval growth, prepupal weight, faecal matter reduction (FMR), bioconversion and feed conversion rate (FCR).

RESULTS: The larvae that were fed a single mass of faeces developed into significantly larger larvae and prepupae than those fed incrementally every 2 days. However, the development into prepupae took longer. The highest FMR was found in the group containing the most larvae, with no difference between feeding regimes. At an estimated 90% pupation rate, the highest bioconversion (16–22%) and lowest, most efficient FCR (2.0–3.3) occurred in groups that contained 10 and 100 larvae, when fed both single-feed and incremental feeding regimes.

CONCLUSION: The prepupal weight, bioconversion and FCR results surpass those from previous studies into BSFL management of swine, chicken manure and municipal organic waste (MOW). This suggests that the use of BSFL could provide a solution to the health problems associated with poor sanitation and inadequate FSM in developing countries.

2.2. Introduction

Providing hygienic, affordable and manageable sanitation is vital to the improvement in public health in both developed and developing countries. Two and a half billion people in low- and middle income regions have no access to improved sanitation (UNICEF/WHO 2014). With 44% of these people practicing open defecation, there are serious risks to public health that can lead to an increase in disease spread (Esrey *et al.* 1991). Strong evidence suggests that improved sanitation has a significant effect on health in developing regions (Esrey *et al.* 1991).

On-site improved sanitation includes pit latrines with slabs, ventilated improved pit latrines (VIP), pour-flush pit latrines and composting toilets (UNICEF/WHO 2014). 1.7 billion people in low- and middle-income communities around the world use these forms of improved sanitation (UNICEF/WHO 2014). However, it has been reported in Vietnam (Biran 2010a) and Tanzania (Biran 2010b) that the biggest problem faced by pit latrine owners is the disposal of pit latrine faecal sludge (FS). Adequate pit latrine emptying services are not available in many areas in developing countries and can be expensive (Still 2002). The emptying process can also be inconvenient for the latrine owner and cause bad smells in the surrounding area (Biran 2010a, Biran 2010b). Digging a new pit is an alternative, but too expensive for many. Also, it may be impossible in areas which lack space, such as emergency camps and unplanned settlements (Patinet 2010).

Effective faecal sludge management (FSM) is vital to prevent adverse health and environmental effects (WHO/UNEP 2006). The method of FSM must be considered, particularly in low-income countries with insufficient piped sewerage systems. It is possible to remove pathogens while transporting FS to wastewater treatment plants, but in practice, unregulated services and the prohibitive cost, lack of infrastructure and resources render this method of FSM in developing countries unfeasible (Helmer *et al.* 1997, Kariuki *et al.* 2003, WHO/UNEP 2006). Composting can be used to remove pathogens in FS if maintained correctly

(USEPA 2003). But pathogens are not always inactivated throughout the entire compost mass (Droffner *et al.* 1995, Hutchison *et al.* 2005). Biogas systems combine FS with animal waste, agricultural waste and water (NNFCC 2009), but only remove some of the pathogenic organisms. With a large increase in the number of pit latrines being built in developing countries, more consideration needs to be given to improving methods of pit emptying and suitable FSM.

One prospective solution for FSM is the larvae of *Hermetia illucens* (L.), commonly known as the black soldier fly larvae (BSFL). The adult flies are neither a nuisance species nor a mechanical vector of disease, as they do not need to feed, surviving on fat stores from their larval stage (Furman *et al.* 1959). As the females oviposit around the edges of larval food sources (Copello 1926), they do not transmit pathogens from FS to human food unlike filth flies such as *Musca domestica*. Although there have been rare cases of accidental myiasis caused by the consumption of ripe, unwashed fruit (Calderón-Arguedas *et al.* 2005, Gonzalez *et al.* 2009), but given their worldwide distribution (Leclercq 1969), such cases represent negligent risks to humans. Unlike the adults, the larvae are detritivores feeding on human cadavers (Dunn 1916), decaying vegetables (Malloch 1917), pit latrine FS (Bradley 1930) and animal manure (Tingle *et al.* 1975, Booram *et al.* 1977, Newton *et al.* 2005). The final larval stage (prepupal) is indicated by a change in colour from white to dark brown (May 1961). The prepupae crawl out of the feeding material to pupate, climbing slopes of 40° when dry, making them easy to direct for harvesting (Sheppard *et al.* 1994). The prepupal stage contains high protein and fat levels, 42–45% and 31–35%, respectively (Hale 1973, Newton *et al.* 1977). These nutritional qualities give the prepupae value, as they can be converted into beneficial end products (Sheppard *et al.* 1994). They can provide a suitable replacement for conventional fat and protein sources and can be fed to animals such as cockerels (Hale 1973), pigs (Newton *et al.* 1977), catfish and tilapia (Bondari *et al.* 1987), and rainbow trout (St-Hilaire *et al.* 2007). The prepupae can also be fractionated into their component parts, protein separated for

animal feeds and fats converted into biodiesel (Li *et al.* 2011a, Li *et al.* 2011b). BSFL are also known to reduce oviposition of the disease-spreading house fly, *M. domestica* (Sheppard 1983). The quantities of organic material consumed by BSFL can significantly reduce swine, chicken and cattle manure in the animal husbandry industry (Sheppard *et al.* 1994, Newton *et al.* 2005). BSFL can also reduce *Escherichia coli* and *Salmonella enterica* pathogen loads in chicken and cattle manure (Erickson *et al.* 2004, Liu *et al.* 2008), and human faeces (Lalander *et al.* 2013).

Although there has been much research focusing on the use of BSFL to manage swine, chicken and cattle manure (Sheppard *et al.* 1994, Newton *et al.* 2005), as well as municipal organic waste (MOW) (Diener *et al.* 2009, Diener *et al.* 2011), few studies investigated their consumption of FS (Dang 2010, Lalander *et al.* 2013). This study aims to determine the efficiency of BSFL at consuming fresh human faeces, under different feeding conditions and feeding rates. Efficiency is determined by calculating faecal matter reduction (FMR), bioconversion and feed conversion rates (FCR). The results will help optimise the way in which BSFL are fed human faeces, increasing FMR and prepupal biomass generation. The value of the various components of the prepupae could provide a source of income, while the economic benefits through selling BSF products could be an incentive to communities, entrepreneurs, non-government organisations and governments to improve FSM.

2.3. Methods

2.3.1. Black soldier fly larvae

The experiments were carried out at the London School of Hygiene and Tropical Medicine, UK. The BSFL used in the study were 18 days old, extra small Phoenix Worms™ (ISR, Georgia, USA). The larvae were kept in an inert material provided by the supplier that prevented the larvae from gaining weight. The BSFL were stored at 20 °C in an insectary at LSHTM until they were needed for the experiments.

2.3.2. Faecal sample collection

Ethical approval for sampling human tissue was obtained from the LSHTM Ethics Committee (Appendix B), and all experiments complied with current laws. Volunteers for the study were recruited from university staff and the general public. The project was explained to all volunteers by the author who conducted the experiment, and the volunteers signed an informed consent form. A collection kit, consisting of a sealable faecal collection pot, a pair of purple nitrile gloves, a ziplock bag and a large padded envelope, was given to volunteers at least 1 day prior to the sample being produced. The volunteers produced a faecal sample, ensuring no urine was mixed into the sample, and then contacted the author on the same day for collection. Samples were collected every 2 days from different volunteers throughout the experiment and stored in a refrigerator at 5°C for a maximum of 48 h, until required.

2.3.3. Experimental design

The experiment used two feeding regimes: Feeding Regime 1 (FR-1) and Feeding Regime 2 (FR-2). In FR-1, the larvae were provided with fresh faeces every 2 days (incremental feeding) for 12 days. In FR-2, the larvae were only provided with one sample of faeces at the beginning of the experiment (lump amount feeding). The quantity of faeces added was calculated according to an optimal feeding ratio of 100 mg/food (faeces) larva/day, as determined by a study that fed BSFL on MOW (Diener *et al.* 2009, Diener *et al.* 2011), or an excess feeding ratio of 1000 mg/faeces larva/day. The feeding regimes were divided into three groups (A, B and C), which differed in larval density (1, 10 or 100 larvae per treatment) and feeding ratio (Table 2-1). Equal quantities of faeces, without larvae, served as controls for all groups within both feeding regimes.

Table 2-1 Description of *Hermetia illucens* Feeding Regime 1 (incremental feeding), and Feeding Regime 2 (lump sum feeding) used in the experiment, includes group allocation, feeding ratio, number of larvae, quantity of faeces added, feeding occasions, total feed added and number of treatment and control replicates

	Incremental feeding (FR-1)			Lump sum feeding (FR-2)		
Group	A	B	C	A	B	C
Feeding ratio (mg/larva/day)	1000	100	100	1000	100	100
Number of larvae (*n*)	1	10	100	1	10	100
Quantity feed added (g)	2	2	20	12	12	120
Feeding occasions (*n*)	6 (every 2 days)			1 (at beginning)		
Total feed added (g)	12	12	120	12	12	120
Replicates with BSFL (*n*)	40	40	6	40	40	6
Replicates without BSFL (*n*)	10	10	3	10	10	3

2.3.4. Faecal and larval weights (Day 0)

Sterilised 50-ml falcon tubes (VWR International Ltd, Leicestershire, UK) were weighed and labelled for Groups A and B. Larger containers for Group C, autoclaved 324- ml glass jars (Jam Jar Shop, Telford, UK), were weighed and labelled to include Feeding Regime, Group and replicate number. Prior to distribution, the samples of human faeces were combined and mixed thoroughly in a large bowl to remove variation between samples. The mixed faeces was weighed (Oertling RB153, Birmingham, UK) and divided between treatment and control replicates. Larvae were counted and weighed on an analytical balance (Oertling NA114, Birmingham, UK) in groups for each treatment before adding to the faecal sample. Parafilm (Bemis Flexible Packaging, Oshkosh, USA) was stretched over the top of the containers to prevent the larvae from escaping and then perforated with a needle to allow the larvae to breathe. Treatment and control replicates were stored at 27 °C and 67% relative humidity (Diener *et al.* 2009, Diener *et al.* 2011) for 2 days in an incubator (GenLab, Widnes, England).

2.3.5. Faecal and larval weights (Day 2–12)

New falcon tubes and glass jars were weighed and labelled using the FR-1 nomenclature. Fresh faecal samples were mixed in a large bowl to remove variation between samples. The faeces

were divided between the new treatment and control replicates, and the containers and faeces were weighed.

All FR-1 and FR-2 replicates were taken out of the incubator. The larvae were removed from the FR-1 treatment replicates, weighed and then placed in a new FR-1 treatment replicate, with a fresh faecal sample and the same identification number. Sample of larvae was removed from the FR-2 treatment replicates, counted, weighed and returned to their treatment replicates. The FR-2 control replicates were weighed. Parafilm was replaced over the top of the new FR-1 and original FR-2 treatment and control replicates and perforated with a needle. All treatment and control replicates were returned to an incubator at 27 °C and 67% relative humidity.

It was not necessary to wash the larvae before weighing, as it was shown in a preliminary experiment that washing did not significantly alter larval weight (Banks 2010). This process was repeated every 2 days for the 12 days of the experiment. Once the experiment ended, the larvae were removed from the treatment replicates. Once larvae developed into prepupae, indicated by a change in colour from white to dark brown (May 1961), they were removed from their treatments and weighed. The prepupae were then freeze-dried (Edwards Modulyo, West Technology, Bristol, England) until a constant weight was reached.

2.3.6. Statistical analysis

The data were analysed using Intercooled Stata 12.0 for Windows (StataCorp LP, TX, USA). Data were visualised using box-plot graphs, tested for normality using the Shapiro–Wilk test and, if necessary, and log-transformed. Percentage data were arcsine transformed, depending on range (Parsad 2005).

Analysis of variance (ANOVA) was used to determine significant differences between feeding regimes, and between groups within the same feeding regime. The variables comprised total feed added, total residue, FMR, larval mean weight, prepupal mean weight, percentage

pupation and prepupal yield. The bioconversion and FCR were calculated for actual yield and an estimated 90% harvest.

2.4. Results

2.4.1. Larval development

Mean larval weight data were normal (Shapiro–Wilk $P > 0.05$) after the removal of outlying data points that were the result of a single larva that failed to thrive (E. Pieterse, personal communication). There were significant differences between mean larval weights in all three groups of the different feeding regimes (Figure 2-1).

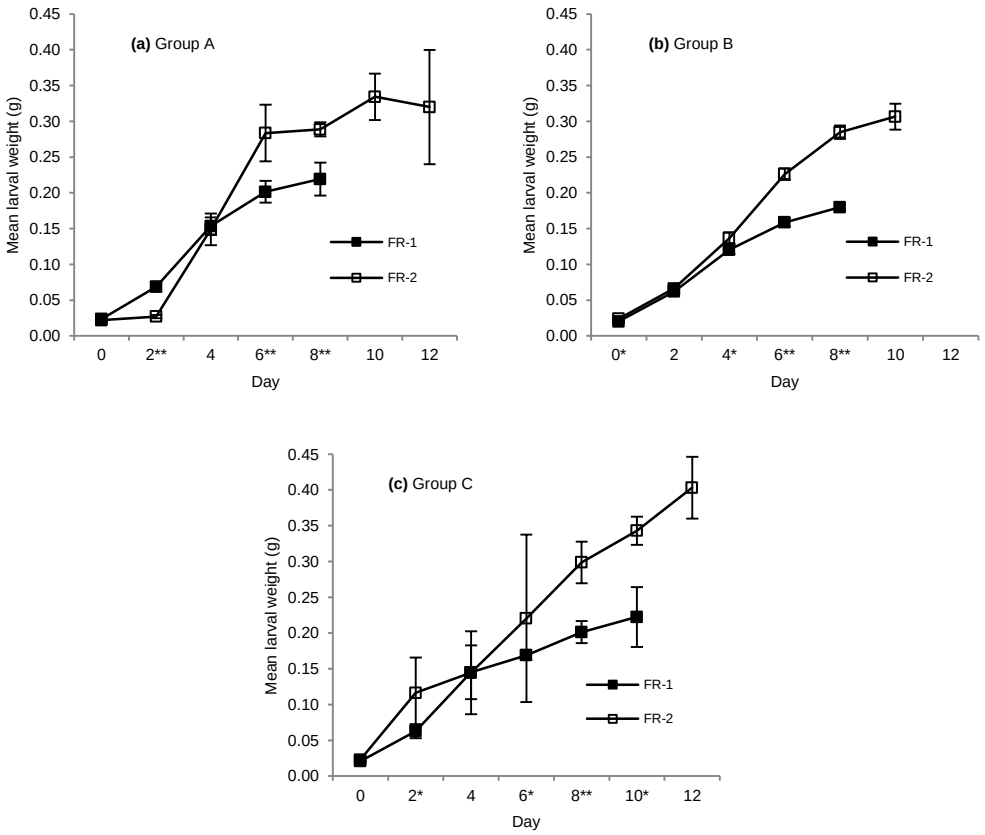

Figure 2-1 *Hermetia illucens* larval wet weight in grams (arithmetic mean ± 95% CI), over 12 days, for two different feeding regimes, FR-1 (filled squares), were fed fresh faeces every 2 days, and FR-2 (empty squares) were fed a large lump sum of faeces at the start of the experiment. Panel (a) contains Group A data from replicates (n = 40) of a single larva fed 1000 mg/faeces larvae/day. Panel (b) contains Group B data from replicates (n = 40) of 10 larvae fed 100 mg/faeces larvae/day. Panel (c) contains Group C data from replicates (n = 6) of 100 larvae fed 100 mg/faeces larvae/day. Day numbers followed by a * indicate significant difference (P ≤ 0.05), and ** indicates highly significant difference (P ≤ 0.0001).

In Groups A and B, by day 6, the larvae in Feeding Regime 2 (FR-2) were highly significantly larger than in Feeding Regime 1 (FR-1) (Group A – $P < 0.0001$, $F = 23.69$, df = 1, 45; Group B – $P < 0.0001$, $F = 117.04$, df = 1, 48). In Group C, the larvae in FR-2 were significantly larger ($P = 0.0254$, $F = 8.00$, df = 1, 7) by day 6, and highly significantly larger ($P < 0.0001$, $F = 98.15$, df = 1, 7) by day 8. In Groups A and B, the majority of the FR-1 larvae had developed into prepupae by day 8. In Group A, it took further 4 days for the larvae in FR-2 to reach the same stage. In Group B, it took further 2 days for the larvae in FR-2 to reach the same stage. In Group C, the majority of prepupae in FR-1 had developed by day 10. Only 8.2% (Table 2-3) of the larvae in FR-2 reached the prepupal stage by day 12.

2.4.2. Faecal matter reduction

Faecal matter reduction data were between 0 and 100%. The data were arcsine-transformed before an ANOVA was performed. All treatment groups had significantly higher FMR ($P < 0.0001$) than control groups, in both feeding regimes. The lowest treatment FMR (Table 2-2) was found in Group A, FR-2 (25.2% ± 0.80 SE). This was significantly lower ($P < 0.0001$, F = 26.15, df = 1, 78) than FR-1 (33.4% ± 1.44 SE). In Group B, there was a significant difference between the feeding regimes ($P = 0.0032$, $F = 9.24$, df = 1, 78), with FR-1 having higher FMR than FR-2, 49.7% ± 1.03 SE and 45.8% ± 0.73 SE, respectively. The highest FMR (FR-1 = 54.2% ± 0.86 SE, FR-2 = 54.6% ± 2.20 SE) was found in Group C, with no significant difference ($P = 0.8633$, $F = 0.03$, df = 1, 10) between the feeding regimes.

2.4.3. Prepupal yield, bioconversion and feed conversion rate

Pupation data were between 0 and 100% and therefore arcsine-transformed before an ANOVA was performed. The prepupal weight was significantly higher in FR-2 for all groups than in FR-1 (Table 2-3). Group A, FR-2 had the largest mean prepupal weight of 0.3151 g. All groups showed high levels of pupation, with the exception of Group C, FR-2. Prepupal mean weight was significantly affected by Feeding Regime ($P < 0.0001$, $F = 68.03$, df = 1,158) and Group ($P <$

0.0001, F = 13.60, df = 2,158), but not by an interaction between Feeding Regime and Group (P = 0.0646, F = 2.79, df = 2,158).

Table 2-2 Total and arithmetic mean (±SE) wet weight of feed added and residue remaining, and geometric mean (±SE) percentage faecal matter reduction (FMR), by wet weight. P values indicate statistical differences in FMR between groups in different feeding regimes, significant effects are in bold

Group	Feeding regime	Feed Added (Wet Weight)		Residue (wet weight)		FMR (wet weight)	
		Total (g)	Mean (g)	Total (g)	Mean (g)	Mean (%)	*P*
A	FR-1	390.3	9.8 ± 0.23	260.2	6.5 ± 0.20	33.4 ± 1.44	<0.0001
	FR-2	481.5	12.0 ± 0.04	360.3	9.0 ± 0.10	25.2 ± 0.80	
B	FR-1	436.5	10.9 ± 0.08	219.8	5.5 ± 0.12	49.7 ± 1.03	0.0032
	FR-2	482.5	12.1 ± 0.04	261.4	6.5 ± 0.09	45.8 ± 0.73	
C	FR-1	658.1	109.7 ± 1.43	301.1	50.2 ± 0.81	54.2 ± 0.86	0.86
	FR-2	720.5	120.1 ± 0.08	327.1	54.5 ± 2.67	54.6 ± 2.20	

When bioconversion and FCR were calculated (Table 2-4) for actual prepupal yield, Group B, FR-2, had the highest bioconversion (22.9%) and most efficient FCR value of 2.0. The lowest bioconversion and least efficient FCR was in Group C, FR-2 (1.6% and 33.9, respectively), followed by Group A, FR-1 (2.2% and 15.2, respectively). When prepupal total weight was estimated using a 90% yield, Group B, FR-2 maintained the highest bioconversion (22.3%) and most efficient FCR value (2.0), however, Group C, FR-2 had improved bioconversion to 18.1% and FCR efficiency to 3.0. The lowest bioconversion (2.1%) and least efficient FCR (15.6) were found in Group A, FR-1.

Table 2-3 *Hermetia illucens* prepupal geometric mean (±SE) wet weight, and percentage of larvae reaching prepupal stage. *P* values indicate statistical differences in prepupal weight and pupation between groups in different feeding regimes, significant effects are in bold

Group	Feeding regime	Prepupal Wet Weight		Pupation	
		Mean (g)	*P*	Percentage (%)	*P*
A	FR-1	0.2258 ± 0.0078	<0.0001	92.5	0.4624
	FR-2	0.3151 ± 0.0124		87.5	
B	FR-1	0.1936 ± 0.0026	<0.0001	82.8	0.0001
	FR-2	0.2986 ± 0.0039		92.5	
C	FR-1	0.1998 ± 0.0034	0.0023	85.0	<0.0001
	FR-2	0.2410 ± 0.0098		8.2	

Table 2-4 Bioconversion and feed conversion rate (FCR) of *Hermetia illucens* converting human faeces into prepupal biomass, for actual prepupal yield, and estimated 90% yield. The most efficient bioconversion and FCR for actual and estimated yield are in bold

Group	Feeding regime	Actual prepupal yield				
		Prepupal weight (g)	Feed added (g)	Bioconversion (%)	Feed consumed (g)	FCR
A	FR-1	8.5	390	2.2	130.1	15.2
	FR-2	11.3	482	2.3	121.2	10.7
B	FR-1	65.3	437	14.9	216.7	3.3
	FR-2	110.7	483	**22.9**	221.1	**2.0**
C	FR-1	104.8	658	15.9	357.0	3.4
	FR-2	11.6	721	1.6	393.4	33.9

Group	Feeding regime	Estimated 90% yield				
		Prepupal weight (g)	Feed added (g)	Bioconversion (%)	Feed consumed (g)	FCR
A	FR-1	8.3	390	2.1	130.1	15.6
	FR-2	11.6	482	2.4	121.2	10.4
B	FR-1	69.9	437	16.0	216.7	3.1
	FR-2	107.9	483	**22.3**	221.1	**2.0**
C	FR-1	107.9	658	16.4	357.0	3.3
	FR-2	130.7	721	18.1	393.4	3.0

2.5. Discussion

The BSFL fed fresh faeces every 2 days developed into smaller prepupae (Table 2-3) faster than the larvae fed once at the beginning of the experiment (Figure 2-1). Based on the slower development and larger prepupae of the larvae fed once, it is theorised that there was a nutritional imbalance in the lump amount diet that led to an increase in consumption to compensate for deficient nutrients (Raubenheimer *et al.* 1997, Bennett 2000, Wright *et al.* 2003). Both proteins and carbohydrates are critical in the development of insect larvae (Bennett 2000, Nijhout 2003, Lee *et al.* 2004, Simpson *et al.* 2006). However, there are few data regarding the protein and carbohydrate content of fresh and ageing faeces. If pit latrine material is used as a proxy, with the top layer being fresh material, and lower layers aged material, the protein content of the material drops rapidly within the first 20 cm (J. H. J. Ensink & B. Torondel, LSHTM, unpublished data). The increase in development time and larval size supports the hypothesis that reduced protein content in the lump sum diet causes a nutritional

imbalance that leads to compensatory feeding. If the ageing material is losing nutritional value over time, and the feeding rate of insect larvae is highest in the later instars, it is likely that the older larvae will need to consume more low nutrition feed than larvae fed fresh, nutritionally balanced feed.

Growth rate plasticity (Metcalfe *et al.* 2001, Tu *et al.* 2003, Wright *et al.* 2003, Dmitriew *et al.* 2005, Dmitriew 2011) means that larvae are capable of successfully developing on a range of resources that may be transient in nature. Insect herbivores are known to increase their consumption of plant tissue when feeding on low-quality plants (Kondoh *et al.* 2001), which increases developmental time and leads to higher vulnerability to natural predators. A slow-growth, high-mortality hypothesis has been proposed in Lepidoptera (Benrey *et al.* 1997, Fordyce *et al.* 2003, Medina *et al.* 2005, Cornellisen *et al.* 2006) and Coleoptera (Häggström *et al.* 1995). Growth rate plasticity indicates that BSFL could be capable of consuming pit material with a range of nutritional contents and still be capable of developing into valuable prepupae.

2.5.1. Faecal matter reduction, prepupal yield and feed conversion rates

The results from this study were calculated using wet weight, meaning results can only be compared to studies that calculate wet weight FMR. It can be seen that FMR levels in Groups B and C are comparable (Table 2-5) to those found when BSFL feed on chicken manure (Sheppard *et al.* 1994).

It is possible that dry weight FMR could compare to that found with BSFL feeding on MOW (Diener *et al.* 2011), however, those data were not collected in this experiment. The FMR in Group A was far lower than Groups B and C. However, this is to be expected with only one larva present for each replicate. The percentage pupation ranged from 82.8 to 92.5% (Table 2-3), excluding Group C, Feeding Regime 2. The low figures of pupation in this group (8.2%) could be due to competition between the larvae combined with reducing quality of feed.

Table 2-5 Effect of different feed sources on *Hermetia illucens* mean (±SE) prepupal weight, faecal matter reduction (FMR) capacity, prepupal yield, bioconversion and feed conversion rate (FCR). The most efficient bioconversion and FCR results are in bold. Other data are from previous studies using swine manure (Newton *et al.* 2005), chicken manure (Sheppard *et al.* 1994) and municipal organic waste (MOW) (Diener *et al.* 2011)

Feed Source		Mean prepupal weight (g)	Feed added	Residue	Feed consumed	FMR (%)	Prepupal yield	Bioconversion (%)	FCR
Swine manure*		N/A	68 kg	42 kg	26 kg	~39	~2.7 kg	3.97	9.6
Chicken manure†		0.220 ± N/A	5,240 kg	~2,620 kg	2620 kg	~50	196 kg	3.74	13.4
MOW*		0.220 ± 0.008	151 kg	48 kg	103 kg	68	17.8 kg	11.78	5.8±
Human faeces‡									
Group	Feeding Regime								
A	FR-1	0.2258 ± 0.0078	390g	260g	130g	33	8g	2.1	15.6
	FR-2	0.3151 ± 0.0124	482g	360g	121g	25	12g	2.4	10.4
B	FR-1	0.1936 ± 0.0026	437g	220g	217g	50	70g	16.0	3.1
	FR-2	0.2986 ± 0.0039	483g	261g	221g	46	108g	22.3	2.0
C	FR-1	0.1998 ± 0.0034	658g	301g	357g	54	108g	16.4	3.3
	FR-2	0.2410 ± 0.0098	721g	327g	393g	55	131g	18.1	3.0

*** Dry weight,† Wet weight, ‡ Estimated 90% prepupal yield, ± value different to published paper (Diener, Upsala, personal communication)**

However, a higher rate would have been recorded if the experiment had lasted longer. Therefore, a 90% yield of prepupae was calculated to compare the FCR against previous research (Table 2-5). The bioconversion and FCR of the single prepupae in Group A were comparable to the rates found in previous studies (Sheppard *et al.* 1994, Newton *et al.* 2005, Diener *et al.* 2011). However, Groups B and C have higher bioconversion rates and lower FCR values than reported in previous studies. The high bioconversion rates show that BSFL are effective at reducing human faeces, and a low FCR indicates that the larvae feeding on the lower feeding ratio of 100 mg/larva/day are more efficient at converting fresh human faeces into biomass than swine manure, chicken manure and MOW.

Based on a yield of 90% prepupae, the high FMR and effective FCR results support the use of BSFL in FSM. The prepupae can be collected for their protein and fat, taking advantage of their self-harvesting behaviour of crawling out of their feeding medium. This behaviour removes issues that arise from separating them from remaining residue. However, it is unlikely that all of the prepupae will self-harvest, suggesting alternative methods of prepupal collection must be investigated for large-scale FSM. Also, with further research, the FMR could be optimised, resulting in less remaining residue. Additionally, urine was not present in the faeces provided by volunteers, and the moisture content of the faeces was not increased artificially. Therefore, the samples were not representative of fresh faeces found in standard non-urine diverting latrines. The presence of urine may affect the FMR efficacy of BSFL when converting pit latrine faecal sludge into biomass. Furthermore, it is important to consider how the presence of urine could alter BSFL faecal sludge reduction efficacy.

In summary, the study has demonstrated that BSFL feeding on fresh human faeces can develop successfully. The largest prepupae are produced when given a large quantity of feed, resulting in prepupae of a higher mass than previous studies. The larvae are effective at FMR and converting the human faeces into a valuable biomass. These results support the use of BSFL in FSM. However, a number of issues still need to be addressed. It has been shown that BSFL are capable of consuming fresh human faeces on a small scale, but up scaling of this experiment is needed to test whether BSFL are capable of developing into prepupae at high densities. To help develop the technology for use in developing countries, more research needs to be conducted on the ability of BSFL to consume pit latrine FS. Previous research shows how BSFL development time varies depending on diet, feeding rate, temperature and humidity (Tomberlin *et al.* 2002, Diener *et al.* 2009, Tomberlin *et al.* 2009, Diener *et al.* 2011, Holmes *et al.* 2012). Therefore, further research is needed to assess the growth rate plasticity of BSFL on low-quality diets like pit latrine material. The BSFL should be tested using material from different latrine types, with different physical and chemical characteristics recorded to

determine effects on FMR and prepupal yield. Also, considering that the prepupal biomass could be used to feed animals that are part of the human food chain, it is important to assess the potential risks regarding bioaccumulation of heavy metals and contamination by pathogens.

Ultimately, BSFL have the potential to improve sanitation in developing countries by providing a way to process dangerous FS, with the benefit of having the prepupae produced have a value that could provide a source of income for communities or local entrepreneurs, while the remaining residue, if safe, may be used as a fertiliser or soil conditioner.

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Chapter 3) Pit latrine faecal sludge characteristics, and suitability for black soldier fly rearing

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3.1. Abstract

OBJECTIVES: To determine the physical and chemical properties of faecal sludge (FS) collected from stratified layers in pit latrines in South Africa, and to see whether they fall within the range suitable for rearing black solider fly larvae (BSFL) for use in a novel faecal sludge management (FSM) method.

METHODS: Faecal sludge was collected from 25 latrines, in 3 communities (urban, peri-urban, and rural) around South Africa. Samples were collected from four different layers, 0 – 20 cm, 21 – 50cm, 51 – 100cm, and 101 – 150cm. Samples were analysed for physical and chemical parameters and heavy metals using standard methods.

RESULTS: Total solids (TS) and pH of FS from peri-urban latrines was significantly lower than urban and rural latrines ($P < 0.05$). Faecal sludge from peri-urban latrines had significantly higher total chemical oxygen demand ($P = 0.019$), total phosphate ($P = 0.018$), volatile fatty acids ($P = 0.002$), and carbohydrates ($P = 0.033$), than rural latrines. The FS collected from all communities had similar physical and chemical characteristics to FS analysed in Tanzania, and previously in South Africa.

CONCLUSION: The study shows there is differences in FS characteristics of latrines in different communities, urban, peri-urban, and rural. It is suggested that differences in FS characteristics, and biodegradation of FS are due to different latrine design. The characteristics fall within the range suitable for rearing BSFL. Black soldier fly larvae FSM could be used to improve sanitation in communities where pit latrines contain FS with similar properties to those found in this study.

3.2. Introduction

An estimated 2.5 billion people world-wide lack access to an improved form of sanitation (UNICEF/WHO 2014). The high cost associated with piped sewage systems and wastewater treatment plants in low- and middle-income countries means that the most appropriate solution is on-site sanitation (WHO/UNEP 2006). On-site sanitation solutions have the major drawback that they will eventually fill-up and will thus require emptying, transport and treatment of faecal sludge (FS). The lack of suitable faecal sludge management (FSM) methods in low- and middle-income countries (Chowdhry *et al.* 2012) may result in illegal dumping of FS which causes major environmental and hygiene problems in many cities (Helmer *et al.* 1997, Kariuki *et al.* 2003, WHO/UNEP 2006).

There is a great need for alternative FSM solutions to help improve sanitation in communities which face problems with FS disposal. One such alternative is the use of black solider fly larvae (BSFL), which have been shown to effectively reduce fresh human faeces (Lalander *et al.* 2013, Banks *et al.* 2014), and yield a potentially valuable product in the form of high protein and fat rich prepupae (Hale 1973, Newton *et al.* 1977). It is known that the physical, biological, and chemical characteristics of the larval diet affects the development, and subsequent value, of BSFL and similar insect species (Fatchurochim *et al.* 1989, Bennett 2000, Nijhout 2003, Lee *et al.* 2004, Simpson *et al.* 2006, Chaudhury *et al.* 2009, Diener 2010, Diener *et al.* 2011, Popa *et al.* 2012). Therefore, before determining how effective BSFL are as a FSM method, it is vital to determine what physical and chemical characteristics are present in FS, and whether they are suitable for the rearing of BSFL.

However, there have been few studies quantifying the composition of pit latrine FS, as discussed by Chaggu (2004) and Buckley *et al.* (2008), and synthesised in Table 3-1.

Table 3-1 Summary of physical and chemical characteristics of pit latrine faecal sludge (FS). Sources: Lopez Zavala, 2002; Palmquist, 2005; Buckley, 2008; Irish, 2013

Characteristic	Unit	Range
Total solids	%	6 – 80
Moisture content	%	66 – 85
Total Volatile Solids	% dry mass	1 – 91
pH	-	5.25 – 8.94
Total COD	g kg^{-1} dry mass	30 - 2000
Soluble COD	g kg^{-1} dry mass	1 - 750
Total Nitrogen (N)	% dry mass	5 – 7
Total Phosphorus (P)	% dry mass	0.7 – 2.5
Total Potassium (K)	% dry mass	0.8 – 2.1

Two studies examining pit latrine fill rates were conducted in South Africa on 16 VIP latrines (Bakare 2014), and in Tanzania and Vietnam using 50 non-improved latrines in each country (Torondel, LSHTM, unpublished data). In both studies, there were huge variations in FS physical and chemical characteristics between different pit latrines, and between different layers within each latrine. Some latrines showed trends of increasing total solids (TS), and decreasing total volatile solids (TVS) and chemical oxygen demand (COD), from top to bottom of the latrine, whereas others showed no significant changes in TS, TVS, and COD between the top and bottom layers of the latrine. Total solids ranged from between 6 – 80% in the top layer of latrines, to 40 – 80% past 1 metre deep, and COD content ranged from 30 – 2000 g kg^{-1} dry mass in the top layer, to 20 – 300g kg^{-1} dry mass in lower layers (Torondel, LSHTM, unpublished data).

In order to determine whether pit latrine FS is a suitable rearing material for BSFL, and how it affects BSFL faecal matter reduction (FMR) efficiency, development and survival, it is important to understand what range of FS physical and chemical characteristics occur in pit latrines in a country which could benefit from BSFL FSM. To determine this, FS will be excavated from selected pit latrines, the physical and chemical characteristics ascertained, and the characteristics assessed to determine how suitable they are as BSFL rearing material.

Additionally, household surveys will be conducted to gather information on user behaviour and pit latrine design.

3.3. Methods

3.3.1. Study area

South Africa was chosen as the location to conduct the experiments for a number of reasons. Over 4.7 million latrines are used throughout the country, including approximately 2 million ventilation improved pit (VIP) latrines, 2.6 million pit latrines without ventilation improved pipes, and 79,000 bucket toilets (StatsSA 2012). The number of pit latrines in South Africa, and the issue of FSM already faced by municipal governments (DWAF 2005), suggests that an alternative FSM solution would benefit the country's sanitation provisions.

The survey was conducted in the Western and Eastern Cape Provinces, South Africa. In the Western Cape, the survey was conducted in an urban informal settlement (33.9804 S, 18.5792 E), located near Cape Town International Airport. The area was selected due to the high number of "self-built" pit latrines, which possessed easily accessible vaults. In the Eastern Cape, the survey was conducted in a rural community (32.2735 S, 28.2002 E) where household owners constructed their own pit latrines, and in a peri-urban community (32.1255 S, 28.2789 E), a rural village, where the local municipal government provided each household with a urine diversion, double vaulted VIP latrines to replace self-built, non-improved pit latrines.

3.3.2. Data collection

Between the 29th of April and 16th of May 2013, 29 households in the urban settlement, with self-built pit latrines, agreed to participate in the study (Appendix C). Household information was collected using a questionnaire (Appendix D), and a visual inspection of the pit latrines was conducted. The top mound and 20 cm layer of FS was removed from 17 pit latrines which fulfilled the emptying inclusion criteria, described below. Between the 6th and 8th of June 2013, 20 randomly selected households in the rural and peri-urban settlements were surveyed, and a

visual inspection of the pit latrines was conducted. Four layers of FS (0 – 20cm, 21 – 50cm, 51 – 100cm, and 201 – 150cm) were removed from 3 pit latrines in each community that fulfilled the emptying inclusion criteria. Latrines selected for emptying were suitable if they fulfilled the following inclusion criteria: 1) no chemicals were added to the vault, 2) the FS was sufficiently solid (MC approximately 50 – 90%), as determined manually by digging into the top layer, 3) the pit latrines superstructure allowed access to the vault and 4) the FS was readily accessible.

3.3.3. Sample collection and analysis

Faecal sludge was collected by removing latrine superstructures to gain access to vaults. Entire layers were excavated using a spade to prevent mixing between layers, and sealed inside containers (Addis Roughtote, 68 litre). The FS was immediately stored and refrigerated (4°C) after collection. On arrival at the local laboratory it was frozen at -20°C for 48 hours to kill any fly larvae present. Once defrosted, items of household waste were removed and the FS was homogenised using a drill with a paint mixer bit. Representative samples of homogenised FS were taken for analysis to determine physical characteristics and chemical contents.

All FS samples were analysed for total solids (TS), using Official Method 934.01 (AOAC 2002), and pH was measured with a hand-held pH meter (PHH-5012, Omega, UK). A subsample of residue remaining from the TS analysis was used to determine ash/total volatile solids (TVS) of peri-urban and rural FS, using Official Method 942.05 (AOAC 2002). Total protein and carbohydrate values of peri-urban and rural FS was measured using the Lowry assay method (Lowry *et al.* 1951), and phenol-sulphuric acid technique (Masuko *et al.* 2005), respectively.

One gram of peri-urban and rural homogenised FS was diluted in 40 millilitres of double distilled H_2O, and the following chemical analysis performed. Total (tCOD) and soluble (sCOD) chemical oxygen demand was determined with Aqualytic COD VARIO kits (tCOD #420721, sCOD #420720, Aqualytic, Germany), using the dichromate method. Ammonia (NH_4^+) was determined with Aqualytic Ammonia VARIO kits (#535650, Aqualytic, Germany), using the

salicylate method. Total phosphate (PO_4) was determined using Hach Lange kits (LCK350, Hach-Lange, USA) and the phosphormolybdenum blue method. Volatile fatty acids (VFA's) were determined using Hach Lange kits (LCK365, Hach-Lange, USA) and the esterification method. For sCOD, NH_4^+, and VFA analysis, samples were passed through a 0.45 µm filter. Aqualytic and Hach Lange reagent kits were digested, when required by methods, with a heatblock (HT200S, Hach-Lange, USA), and analysed with a spectrophotometer (DR3900, Hach-Lange, USA. Heavy metal analysis was conducted by microwave digestion of 0.5g dry weight of FS (US EPA method 3015), and trace elements determined by inductively coupled plasma – mass spectrometry (ICP-MS) (US EPA method 200.8, 6020A). Specific elements determined were: Aluminium (Al), Antimony (Sb), Arsenic (As), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Lead (Pb), Manganese (Mn), Mercury (Hg), Molybdenum (Mo), Nickel (Ni), Selenium (Se), Tin (Sn), Vanadium (V), and Zinc (Zn).

3.3.4. Data analysis

Using Stata 13 (Statacorp, Texas, USA), data were tested for normality visually using qnorm and pnorm functions, and histograms, and statistically using the Shapiro-Wilk, Shapiro-Francia, and Skewness-Kurtosis tests. Linear regression was used to analyse whether physical and chemical characteristics varied between communities and layer. Trends in physical and chemical characteristics were determined by linear regressions followed by F-tests for normally distributed data, and non-parametric trend tests for non-normally distributed data.

3.3.5. Ethical clearance

Ethical approval for this study was granted by LSHTM Observational/Interventions Research Ethics Committee (#5972, amendment #A394) (Appendix B). All study participants provided written, informed consent (Appendix C) after having the study explained to them, and before filling in the questionnaire (Appendix D).

3.4. Results

3.4.1. Physical and chemical characteristics

3.4.1.1. Differences between communities

Table 3-2 contains a summary of the physical and chemical characteristics of FS collected from urban, peri-urban and rural communities. Total solids and pH were compared between all three communities. Total solids varied significantly between communities (F = 5.58; df = 2, 40; P = 0.007). Peri-urban TS was significantly lower than urban ($\overline{\Delta}$ TS increase = 6.4%; 95% CI 1.9 – 10.9; P = 0.006) and rural ($\overline{\Delta}$ TS increase = 7.4%; 95% CI 2.5 – 12.4; P = 0.004). Faecal sludge pH also varied significantly between communities (F = 5.35; df = 2, 42; P = 0.009). Peri-urban pH was significantly lower than urban ($\overline{\Delta}$ pH increase = 0.24; 95% CI 0.06 – 0.42; P = 0.01) and rural ($\overline{\Delta}$ pH increase = 0.31; 95% CI 0.10 – 0.51; P = 0.004).

Table 3-2 Arithmetic mean, and 95% confidence interval, of faecal sludge collected from pit latrines in urban (n = 21), peri-urban (n = 3), and rural (n = 3) communities in South Africa. Means followed by a different letter are significantly different (F tests; $P < 0.05$)

		Urban		Peri-urban		Rural	
Characteristic	Unit	Mean	95% CI	Mean	95% CI	Mean	95% CI
Total solids	%	32 [a]	(29 – 35)	26 [b]	(24 – 27)	33 [a]	(28 – 38)
pH	-	7.30 [a]	(7.22 – 7.39)	7.06 [b]	(6.99 – 7.13)	7.37 [a]	(7.12 – 7.62)
tCOD	g kg^{-1}			1012 [a]	(726 – 1297)	642 [b]	(496 – 787)
sCOD	g kg^{-1}			117 [a]	(51 – 183)	75 [a]	(32 – 117)
Protein	g kg^{-1}			95 [a]	(66 – 724)	76 [a]	(56 – 96)
Total phosphate	g kg^{-1}			24 [a]	(21 – 28)	19 [b]	(16 – 22)
Ammonium	mg kg^{-1}			133 [a]	(83 – 183)	93 [a]	(45 – 141)
Volatile fatty acids	mg kg^{-1}			19 [a]	(14 – 24)	10 [b]	(7 – 12)
Carbohydrates	g kg^{-1}			822 [a]	(666 – 979)	641 [b]	(561 – 721)

There was a significant difference in specific chemical characteristics of FS collected from peri-urban and rural communities, including: tCOD (F = 6.44, df = 1, 22; P = 0.019), total phosphate (F = 6.48; df = 1, 22; P = 0.018), volatile fatty acids (F = 12.15; df = 1, 22; P = 0.002), and carbohydrates (F = 5.14; df = 1, 22; P = 0.033). The results indicate that FS collected from

double vaulted VIP latrines in a peri-urban community has higher concentrations of specific characteristics.

3.4.1.2. Differences in depth

There was a significant difference in tCOD concentrations of FS extracted from peri-urban pit latrines (F = 8.86; df = 3, 8; P = 0.0064): FS from 21 – 50cm (1393g kg^{-1}; 95% CI 1055 – 1731) had significantly higher tCOD than 101 – 150cm ($\overline{\Delta}$ tCOD decrease = 1000; 95% CI 523 – 1478; P = 0.001), but not 0 – 20cm ($\overline{\Delta}$ tCOD decrease = 180; 95% CI -297 – 658; P = 0.41) or 51 – 100cm ($\overline{\Delta}$ tCOD decrease = 346; 95% CI -131 – 824; P = 0.134). Also in peri-urban pit latrines, there was a significant difference in sCOD concentrations of FS (F = 7.03; df = 3, 8; P = 0.012), FS from 21 – 50cm (253 g kg^{-1}; 95% CI 168 – 338) had significantly higher sCOD than 0 – 20cm ($\overline{\Delta}$ sCOD decrease = 203; 95% CI 83 – 323; P = 0.005), 50 – 100cm ($\overline{\Delta}$ sCOD decrease = 132; 95% CI 12 – 252; P = 0.035), and 100 – 150cm ($\overline{\Delta}$ sCOD decrease = -211; 95% CI 91 – 331; P = 0.004). The total phosphate concentrations of FS differed significantly within rural pit latrines (F = 4.46; df = 3, 8; P = 0.04): FS from 21 – 50cm (24g kg^{-1}; 95% CI 20 – 28) had significantly higher total phosphates than 101 – 150cm ($\overline{\Delta}$ PO_4 decrease = 9; 95% CI 3 - 15; P = 0.007), but not 0 – 20cm ($\overline{\Delta}$ PO_4 decrease = 4; 95% CI -2 – 11; P = 0.13) or 51 – 100cm ($\overline{\Delta}$ PO_4 decrease = 6; 95% CI -0.2 – 12; P = 0.06).

3.4.1.3. Heavy metals

There were significant differences in several heavy metal concentrations between peri-urban and rural FS (Table 3-3).

There was a significant difference in Co concentrations of FS from peri-urban pit latrines (F = 6.30; df = 3, 8; P = 0.017), FS from 101 – 150cm (12mg kg^{-1}; 95% CI 8 – 15) had significantly higher Co than 0 – 20cm ($\overline{\Delta}$ Co decrease = 7.9mg kg^{-1}; 95% CI 2.9 – 12.9; P = 0.007), 21 – 50cm ($\overline{\Delta}$ Co decrease = 8mg kg^{-1}; 95% CI 3.1 – 13.0; P = 0.006), and 51 – 100cm ($\overline{\Delta}$ Co decrease = 6.9mg kg^{-1}; 95% CI 1.9 – 11.8; P = 0.013).

Table 3-3 Arithmetic mean concentration of heavy metals, including 95% CI, of faecal sludge from peri-urban (n = 3) and rural (n = 3) pit latrines, analysed using standard methods (US EPA method 200.8, 6020A). Means followed by a different letter are significantly different (*F* tests; *P* < 0.05)

	Peri-urban		Rural		*Regression model*		
Element	Mean (mg kg^{-1})	95% CI	Mean (mg kg^{-1})	95% CI	*F*	df	*P*
V	9 [a]	(2 – 17)	67 [b]	(46 – 89)	31.56	1, 22	< 0.0001
Co	6 [a]	(4 – 9)	21 [b]	(17 – 25)	46.08	1, 21	< 0.0001
Ni	49 [a]	(30 – 68)	88 [b]	(63 – 113)	7.58	1, 22	0.012
Cu	72 [a]	(57 – 86)	70 [a]	(61 – 79)	0.04	1, 20	0.85
As	0.9 [a]	(0.3 – 1.5)	2.2 [b]	(1.7 – 2.8)	13.83	1, 22	0.0012
Se	1.7 [a]	(1.2 – 2.1)	2.3 [b]	(1.9 – 2.8)	5.72	1, 22	0.026
Mo	4.3 [a]	(3.3 – 5.3)	5.0 [a]	(4 – 6)	1.24	1, 21	0.28
Cd	0.3 [a]	(0.25 – 0.4)	0.3 [a]	(0.2 – 0.4)	0.14	1, 18	0.71
Sn	2.6 [a]	(2.2 – 3.0)	4.2 [b]	(3.5 – 4.9)	18.26	1, 21	0.0003
Sb	0.2 [a]	(0.1 – 0.3)	0.2 [a]	(0.1 – 0.3)	0.52	1, 21	0.48
Hg	0.04 [a]	(0.02 – 0.05)	0.04 [a]	(0.03 – 0.05)	0.18	1, 21	0.67
Pb	5.1 [a]	(2.5 – 7.7)	13 [b]	(8 – 18)	9.59	1, 19	0.006
Al	4766 [a]	(1701 - 7831)	13328 [b]	(9939 - 16717)	17.31	1, 21	0.0004
Cr	103 [a]	(62 - 145)	222 [b]	(169 – 274)	15.24	1, 22	0.0008
Fe	4326 [a]	(1114 - 7539)	22213 [b]	(16233 – 28193)	35.91	1, 21	< 0.0001
Mn	366 [a]	(276 - 456)	692 [b]	(548 – 836)	19.93	1, 20	0.0002
Zn	816 [a]	(514 - 1118)	897 [a]	(645 – 1149)	0.20	1, 21	0.66

Additionally, there were significant differences in Mn concentrations of FS from peri-urban pit latrines (F = 9.25; df = 3, 8; P = 0.006): FS from 101 – 150cm (568mg kg^{-1}; 95% CI 463 - 673) had significantly higher Mn than 0 – 20cm ($\overline{\Delta}$ Mo decrease = 310mg kg^{-1}; 95% CI 162 - 458; P = 0.001), 21 – 50cm ($\overline{\Delta}$ Mn decrease = 252mg kg^{-1}; 95% CI 104 - 400; P = 0.004), and 51 – 100cm ($\overline{\Delta}$ Mn decrease = 248mg kg^{-1}; 95% CI 100 – 396; P = 0.005).

The results demonstrate that the majority of FS from rural pit latrines has higher heavy metal concentrations than peri-urban pit latrines. Additionally, only copper and manganese concentrations changed significantly between layers, and only in peri-urban pit latrines.

3.4.2. Household survey

In the urban and rural communities surveyed, all latrines surveyed had a single, unlined vault, and varied in design, construction material, age and structural integrity. In the peri-urban community surveyed, all latrines had the same design, with two concrete lined vaults, ventilation pipes, and urine diversion fitted. Table 3-4 shows a summary of responses from all households surveyed. There were significant differences in type of latrine, age of pit, number of users, age of users, garbage disposal, cleaning chemicals, top layer consistency, and reported depth of vault (χ^2 test; $P < 0.05$).

Table 3-4 Questionnaire responses from urban (n = 29), peri-urban (n = 20) and rural (n = 20) pit latrine owning households. Significant differences in responses between communities was tested using χ^2 test ($P < 0.05$)

		Community				
		Urban	Peri-urban	Rural	Chi^2 Test	
			%		χ^2	*P*
Type of Latrine	Family	34	100	100	36.16	<0.001
	Communal	66	0	0		
Age of pit (Years)	≤1	59	0	0	63.2	<0.001
	>1 to ≤5	38	5	55		
	≥5	3	55	45		
	Unknown	0	40	0		
Number of daily users	≤5	28	75	70	14.24	0.007
	>5 to ≤15	69	25	30		
	≥15	3	0	0		
Age of users	≤5	11	12	18	29.24	<0.001
	>5 to ≤15	13	20	35		
	≥15	76	68	46		
Distance to sludge?	≤50cm	38	45	75	8.92	0.063
	>50cm to ≤150cm	55	55	25		
	≥150cm	7	0	0		
Garbage disposed of in vault?	Yes	17	0	0	7.44	0.024
	No	83	100	100		
Cleaning chemicals?	Yes	41	0	10	14.38	0.001
	No	59	100	90		
Pit performance additives?	Yes	3	0	0	1.4	0.497
	No	97	100	100		
Solid/liquid top layer?	Solid	100	75	100	13.21	0.001
	Liquid	0	25	0		
Reported depth of vault	≤100cm	0	10		32.04	<0.001
	>100 to ≤150cm	0	15			
	>150 cm	14	25			
	Unknown	86	50			

None of the latrines surveyed had been emptied, with urban and rural households reporting that once vaults had filled, a new vault was dug, the superstructure moved, and the old vault filled in with soil. No household reported separating urine, even in the peri-urban community where latrines were designed for urine diversion. It was also observed that none of the urine diversion pipes were connected. There were anecdotal reports of male members of households often urinating outdoors. All households used newspaper or toilet-paper for anal cleansing, and disposed of the paper in the vault. No households reported using FS for agricultural use.

Only 17% of households in the urban community reported disposing of other waste in the vault, such as diapers, food-scraps, and washing water. However, all of the latrines that were emptied contained non-faecal waste, including: building materials (wood, stone, metal), clothes, condoms, diapers, food packaging, food-scraps, and paint tins. There was evidence of similar materials in pits which were not emptied. No peri-urban or rural households reported disposing of other waste in the vaults, however all latrines that were emptied had items similar to the urban community, and there was evidence of materials in pits that were not emptied.

3.4.2.1. Chemical use

In the urban community, 41% of the households reported using cleaning chemicals to maintain the latrines, including: pine antiseptic, bleach, Dettol®, Jeyes Fluid®, and Madubula®. Cleaning frequency ranged between twice weekly to once a month. Only a single household (3%) reported using pit-additives to increase decomposition in the vault. However, the household was unable to identify which product was used. In the peri-urban community, two households (10%) reported using chlorine once a month to clean their pit latrines. In the rural community, there was no reported use of cleaning chemicals, and no pit-additives were used to increase decomposition in the peri-urban or rural communities. None of the households which reported

using cleaning chemicals, or pit-additives, had FS excavated, in accordance to emptying inclusion criteria.

3.4.2.2. Emptied latrines

There were no significant differences in the type of latrines, age of the latrines, number of daily users, and reported garbage disposal practices for latrines that were emptied and latrines that were not emptied (Table 3-5). This indicates that households where FS was collected were representative of their communities.

Table 3-5 Linear regression analysis comparing household survey responses between emptied and non-emptied latrines

		Regression model		
Variable	Community	*F*	df	*P*
Type of Latrine	Urban	2.20	1, 27	0.15
	Urban	0.36	1, 27	0.55
Age of pit (Years)	Peri-urban	0.06	1, 10	0.81
	Rural	0.02	1, 18	0.90
	Urban	0.64	1, 27	0.43
Number of daily users	Peri-urban	1.39	1, 18	0.25
	Rural	1.23	1, 18	0.28
Garbage disposed of in vault?	Urban	< 0.01	1, 27	0.95

3.5. Discussion

The study found differences in physical and chemical characteristics of FS collected from urban, peri-urban, and rural pit latrines. Peri-urban FS had significantly lower TS and pH from urban and rural latrines, but higher tCOD, total phosphate, volatile fatty acids, and carbohydrates than rural latrines. Also, 11 out of 17 heavy metal concentrations were higher in rural latrines than peri-urban.

3.5.1. Physical and chemical characteristics

Table 3-6 shows how all physical and chemical characteristics of FS, from both peri-urban and rural communities sampled, are comparable to fresh human faeces undergoing aerobic biodegradation in laboratory experiments (Lopez Zavala *et al.* 2002, Chaggu 2004), blackwater

collected from Swedish households (Palmquist *et al.* 2005), pit latrine FS collected from the top 15cm layer (Irish *et al.* 2013), and pit latrine FS from throughout latrines (Bakare 2014).

Table 3-6 Arithmetic mean, and 95% CI, of physical and chemical characteristics of faecal sludge collected from peri-urban and rural latrines, compared to previous studies

		This study				
		Peri-urban		Rural		Previous studies†
Characteristic	Unit	Mean	95% CI	Mean	95% CI	Range
Total Solids	%	26	(24 – 27)	33	(28 – 38)	6 – 80
ph	pH	7.06	(6.99 – 7.13)	7.37	(7.12 – 7.62)	5.25 – 8.94
tCOD	g kg^{-1}	1012	(726 – 1297)	642	(496 – 787)	30 – 2000
sCOD	g kg^{-1}	117	(51 – 183)	75	(32 – 117)	1 – 750
Protein	g kg^{-1}	95	(66 – 724)	76	(56 – 96)	9 – 674
Total Phosphate	g kg^{-1}	24	(21 – 28)	19	(16 – 22)	1.0 – 88
Ammonium	mg kg^{-1}	133	(83 – 183)	93	(45 – 141)	0.1 – 38
Volatile fatty acids	mg kg^{-1}	19	(14 – 24)	10	(7 – 12)	0.6 – 577
Carbohydrates	g kg^{-1}	822	(666 – 979)	641	(561 – 721)	0 – 1000

† (Lopez Zavala *et al.* 2002, Chaggu 2004, Palmquist *et al.* 2005, Buckley *et al.* 2008, Irish *et al.* 2013)

The results also indicate that there were significant differences in TS, pH, tCOD, total phosphate, volatile fatty acids, and carbohydrates, between peri-urban and rural latrines. The characteristics are known to affect aerobic and anaerobic micro-organisms, varying biodegradation of FS (Martin *et al.* 2003, Chaggu 2004, Bhagwan *et al.* 2008), and differences are likely to be due to the design of latrine. The rural latrines were unlined, non-improved pit latrines, whereas the peri-urban latrines were double-vaulted, VIP latrines, with vault walls concrete lined, but no lining on the bottom. The results indicate that biodegradation of FS is slower in the peri-urban latrines, resulting in FS with higher organic material. This is important when considering the implementation of BSFL FSM, as an increase in organic material could result in higher FMR efficiency and prepupal production. It is recommended that future work investigates the influence variations in FS physical and chemical characteristics have on the rearing of BSFL (see Chapter 4) and Chapter 5).

3.5.1.1. Heavy metals

The FS collected from rural pit latrines had higher heavy metal concentrations compared to peri-urban latrines. This is likely caused by the increased biodegradation of FS in rural pits, resulting in a decrease in organic material. The inorganic nature of the heavy metals means that they are not reduced by biodegradation, therefore a higher proportion of heavy metals are present in further biodegraded FS. It is also possible that the higher concentrations are due to contamination of the FS due to large quantities of garbage found in all pit latrines, including metallic building materials and paint tins.

The FS collected had lower mean concentrations of Cadmium, Copper, Nickel, Lead, and Mercury than European Union (EU) standards for admissible heavy metal concentrations in sludge which can be used in agriculture (EU 1986) although Zinc concentrations were higher (Table 3-7).

Table 3-7 Mean concentration (mg kg^{-1}), and range, of heavy metals determined in the current study, and European Union (EU) standards for admissible heavy metal concentrations in sludge which can be used in agriculture

	This Study		EU Standards
Element	Mean	Range	Range
Cadmium	0.31	0.14 – 2.69	1 – 3
Copper	70	32.9 – 169.4	50 – 140
Nickel	68	22.3 – 170	30 – 75
Lead	9.2	2.1 – 74	50 – 300
Zinc	855	181.2 – 2995	150 – 300
Mercury	0.04	0.01 – 0.29	1 – 1.5

These results suggest that the specific heavy metal concentrations are too high in FS for direct use in agriculture, although a more extensive study is recommended as only a small number of latrines were analysed in the present study. It is important to determine what concentrations of heavy metals are present in the residue and in prepupae, and to determine whether prepupae bioaccumulate heavy metals, as previously shown (Diener 2010) (see Chapter 5).

3.5.1.2. Chemical use

The use of chemicals by household owners varied between communities. This is likely due to the availability of commercial chemicals in urban areas, and the lack of availability in rural areas. It was found that two households in the peri-urban community used chlorine as a cleaning chemical. In South Africa, chlorine is sold for swimming pool maintenance, and is not recommended for any other purpose. Although only a single household reported using pit latrine additives to speed up digestion, it should be noted that Drakenstein local municipality, Western Cape, routinely add bio-additives to long-drop pit latrines and conservancy tanks that they service (Kowalewski, Drakenstein Municipality, personal communication).

The use of cleaning chemicals and additives is important when considering the effect on BSFL development during FSM. The results presented here indicate that urban communities are more likely to use cleaning chemicals. Depending on how BSFL FSM is implemented, the presence of chemicals in FS could increase BSFL mortality. This subject will be investigated in future studies (see Chapter 6). However, further work is recommended to determine how widespread cleaning chemicals and additive use is, in areas where BSFL FSM is proposed.

3.5.2. BSFL suitability

The TS, and moisture content, in FS analysed falls within a range that has been shown to be suitable for BSFL development (Fatchurochim *et al.* 1989), although BSFL have been reported in semi-liquefied pit latrines (Copello 1926, Furman *et al.* 1959, Axtell *et al.* 1970, Booth *et al.* 1984). It has been previously shown that BSFL can develop on organic leachate with a pH as low as 4.0, rising to 9.0 in just seven days (Popa *et al.* 2012). This implies that the pH of the FS analysed in this study is suitable for BSFL development. The total COD found in the FS is far higher than organic leachates, which have shown successful development of BSFL (Popa *et al.* 2012). The ammonia content of the FS analysed in this study was higher than a previous study which showed BSFL developing successfully on human faeces (Lalander *et al.* 2013). Currently

there are no data on BSFL development associated with protein, carbohydrates or total phosphate levels, however BSFL have been identified in FS with comparable concentrations to FS analysed in the current study (Irish *et al.* 2013).

Before this study was conducted, it was unknown whether pit latrine FS had physical and chemical characteristics suitable for successful BSFL development. The results presented indicate that FS, from peri-urban and rural latrines, is suitable for rearing BSFL. If BSFL can be successfully reared (see Chapter 5), because of the similarities between FS in this study and previous studies, it can be inferred that BSFL FSM could be implemented in low- and middle-income.

3.6. Conclusion

The study has shown that there are differences in FS characteristics of latrines between urban, peri-urban and rural communities. It is suggested that differences in FS characteristics and biodegradation of FS are due to different latrine design and management. The results also indicate that the FS characteristics found in the present study is comparable to previous research. The results also suggest that the range of characteristics found in FS is suitable for BSFL development. These results can be extrapolated to suggest that similar variation in characteristics will be in FS from similarly designed pit latrines and environments found globally. If correct, then the use of BSFL as a FSM technology could benefit billions of people worldwide. It is important to identify how key rearing parameters affect BSFL FMR and prepupal production when reared on FS, what affect the physical and chemical properties of FS identified in this study have on BSFL, and their efficiency at FMR and prepupal production, and also what influence chemical additives have on BSFL development. These three issues will be addressed in the following Chapters.

3.7. Acknowledgements

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Chapter 4) The effect of varying faecal sludge moisture content, feeding rate, and larval density on faecal matter reduction by black soldier fly larvae

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4.1. Abstract

OBJECTIVES: The aim of this study was to determine how key parameters affect the faecal matter reduction (FMR), and prepupal production, of black soldier fly larvae (BSFL) developing on homogenised top layer pit latrine faecal sludge (FS), in relation to how the parameters will affect BSFL faecal sludge management (FSM).

METHODS: A 2 x 3 x 3 x 3 factorial design tested the following factors: presence of BSFL (BSFL absent, BSFL present), FS moisture content (MC 65%, 75%, 85%), feeding rate (FR 50mg larvae^{-1} day^{-1}, 100mg larvae^{-1} day^{-1}, 200mg larvae^{-1} day^{-1}), and larval density (LD 400, 800, 1200).

RESULTS: All factors significantly influenced wet weight FMR ($P < 0.0001$): presence of BSFL, FR, MC, and LD ranked in order of most- to least-influence (*F*-test value). Optimum FMR (57.5%; 95% CI 54.0 – 61.1) was in the presence of BSFL, MC 75%, FR 50mg larvae^{-1} day^{-1}, and LD 400. Prepupal production measurements were significantly influenced by the following factors: mean prepupal dry weight – MC, FR, and LD ($P < 0.0001$), pupation – MC ($P < 0.0001$) and FR ($P = 0.035$), growth rate – FR ($P < 0.0001$), and bioconversion – MC ($P < 0.0001$) and FR ($P = 0.0056$). Optimum prepupal production was with factors MC 85%, FR 200mg larvae^{-1} day^{-1}, and LD 1200.

CONCLUSION: The results indicate that the key parameters analysed will influence BSFL FSM efficiency in different interventions: in-situ, decentralised treatment plants, and BSFL toilets. The moisture content of FS in an in-situ intervention must be within a suitable range for successful FSM. In decentralised FSM plants, parameters can be adjusted to adapt to different aims, either FMR or prepupal production. For a BSFL toilet, anal cleansing behaviour and toilet design will be influenced by MC. The study has provided further evidence that the use of BSFL at managing FS is a viable alternative to current FSM practices, and could provide an additional tool in helping improve sanitation worldwide.

4.2. Introduction

There are currently 2.5 billion people in the world who have no access to an improved form of sanitation (UNICEF/WHO 2014). It is known that access to sanitation has a significant effect on health (Esrey *et al.* 1991, Fewtrell *et al.* 2005) by preventing disease (Mara *et al.* 1999), and that the appropriate solution in low- and middle-income countries is on-site sanitation (WHO/UNEP 2006). Issues arise with all forms of on-site sanitation when they fill-up and require emptying, transport and treatment of faecal sludge (FS) (Helmer *et al.* 1997, Kariuki *et al.* 2003, WHO/UNEP 2006, UNICEF/WHO 2014). The transportation of FS to treatment plants using manual or mechanical emptying services is a solution (Chowdhry *et al.* 2012). However the distance to faecal sludge management (FSM) facilities, and the cost of legally disposing of FS, can lead to indiscriminate dumping, causing major environmental and hygiene problems (Kariuki *et al.* 2003). Therefore it is important to consider alternative methods of on-site FSM.

The larvae of the black soldier fly (BSFL), *Hermetia illucens* (L.), have been proposed as an alternative method of FSM. This worldwide distributed (Leclercq 1997), non-disease spreading, non-nuisance species (Copello 1926, Furman *et al.* 1959) has been demonstrated to be suitable for managing animal manure (Tingle *et al.* 1975, Booram *et al.* 1977, Newton *et al.* 2005) and municipal organic waste (MOW) (Diener *et al.* 2011). Research has also shown how BSFL can develop successfully on fresh human faeces (Lalander *et al.* 2013, Banks *et al.* 2014), and in pit latrine FS (Bradley 1930, Fletcher *et al.* 1956, Irish *et al.* 2013). The BSFL have an intrinsic value, the final larval stage, known as prepupae, contain high protein and fat levels (Hale 1973, Newton *et al.* 1977). The prepupae can be used to replace conventional protein and fat sources in animal feeds (Hale 1973, Newton *et al.* 1977, Bondari *et al.* 1987, St-Hilaire *et al.* , Hem *et al.* 2008), or the fat can be fractionated to produce biodiesel (Li *et al.* 2011a, Li *et al.* 2011b, Zheng *et al.* 2012a, Zheng *et al.* 2012b). It is known that variations in key environmental and biological parameters, such as feed moisture content (MC) (Fatchurochim *et al.* 1989), and BSFL feeding rate (FR) (Diener *et al.* 2009) have been shown to affect the faecal matter

reduction (FMR) and prepupal production efficiency of BSFL. Chicken manure with MC within the range of 40 – 60% resulted in higher prepupal production than < 40% and > 70% (Fatchurochim *et al.* 1989). While feeding rates of 50 and 100 mg larvae^{-1} day^{-1} resulted in increased FMR, but lower prepupal production, compared to a high feeding rate of 200mg larvae^{-1} day^{-1}. Considering that FMR and prepupal production are vital to the effectiveness of BSFL as a FSM method, it is important to investigate how the key parameters affect BSFL reared on FS.

The necessity for alternative FSM technologies is vital when improving sanitation worldwide. So far, research has shown that BSFL can develop successfully on fresh human faeces (Lalander *et al.* 2013, Banks *et al.* 2014), and the FMR and prepupal production efficiency of BSFL is affected by key parameters, including feed moisture content (Fatchurochim *et al.* 1989), and feeding rate (Diener *et al.* 2009). Therefore, it is important to determine how these key parameters affect the FMR, and prepupal production, of BSFL developing on pit latrine FS. Understanding and optimising these key parameters is vital when considering how a human FSM system utilising BSFL would operate, specifically in how effectively FS is reduced and prepupal yield is produced. The present study aims to determine the efficiency of BSFL in reducing FS at varying MC, FR, and larval quantities, hereto after referred to as larval density (LD). This will be accomplished by feeding BSFL under different conditions, and measuring FMR and prepupal production.

4.3. Methods

4.3.1. Study area

Faecal sludge was collected from pit latrines in the KTC informal settlement, Cape Town, South Africa (see previous Chapter 3). Non-faecal waste, including diapers, building materials and clothes, were removed. The FS was passed through a continuous ribbon mixer into a large plastic container until it was evenly homogenised, and where smaller non-faecal waste was

then removed. Non-faecal waste was removed to accurately measure FMR, as items would not have been consumed by the BSFL. The samples were homogenised to remove any variation in FS characteristics. Samples were taken of the homogenised FS and MC analysed using standard methods (APHA 2012). Four kilograms of homogenised FS were placed into 5 L containers, divided into three groups and labelled, 65%, 75% and 85% MC. Using the MC of the homogenised FS, a calculated measure of water was added to the containers to rehydrate the FS to the required moisture content, with samples taken for TS analysis before being stored at 4°C until needed.

Feeding experiments were conducted at Mariendahl Experimental Farm in Stellenbosch, South Africa. The experimental containers were stored in a controlled environment at approximately 27°C, 70% relative humidity (RH), with a 12:12 day/night light cycle. Faecal sludge was added at the start of the experiment, and then every 7 days after as described in the experimental design section below.

4.3.2. Black soldier fly larvae

The BSFL used in the experiments were collected from a colony at the Mariendahl Experimental Farm, maintained by AgriProtein Technologies and the University of Stellenbosch, South Africa. Adult black soldier flies (BSF) were maintained at approximately 27°C, 70% RH, with natural sunlight provided to encourage mating (Tomberlin *et al.* 2002). Egg clutches were collected from the colony laid in the previous 24hrs, and placed on chicken layer mash (14% protein, 25% fat; Laymax 75/85, Pioneer Foods Ltd, South Africa), which was kept moistened to prevent dehydration. The larvae and layer mash, which served as a food source for hatched larvae, were stored for six days at approximately 27°C, 70% RH. The 6 day old larvae were separated from the layer mash by placing the larvae and layer mash onto a sheet of shade cloth which served as a sieve for the larvae to crawl through, separating them from the feed. The larvae were then placed on non-insecticide treated netting (1.36mm gauge)

which served as a sieve to clean off small pieces of feed. One hundred larvae were counted and weighed. This was repeated three times and the mean individual weight calculated. The mean individual weight was used to measure approximately 400, 800 and 1200 BSFL for different replicate tests to be used in the experiment.

4.3.3. Experimental design

The experiment consisted of a 2 x 3 x 3 x 3 factorial design testing the following factors: presence of BSFL (BSFL absent = 0, BSFL present = 1), FS moisture content (MC 65% = A, 75% = B, 85% = C), feeding rate (FR 50mg larvae^{-1} day^{-1} = A, 100mg larvae^{-1} day^{-1} = B, 200mg larvae^{-1} day^{-1} = C) and larval density (LD 400 = A, 800 = B, 1200 = C). Faecal sludge MC levels were selected based on previous research demonstrating how MC affects BSFL survival, development time, and dry adult weight (Fatchurochim *et al.* 1989). Three FR regimes were chosen due to their significant effects on BSFL development time, FMR, and prepupal dry weight (Diener *et al.* 2009). Using FR and proposed LDs, the quantity of FS added per week was calculated (Table 4-1). The estimated total mass of FS influenced the size of experimental containers required, and due to limitations in experimental space, the most appropriate LDs were selected. For the factor variations where BSFL are absent, the quantity of FS added were based on the same FR and LD calculations described above, emulating the same conditions as with the presence of BSFL.

Table 4-1 Mass of pit material, in grams, to be added to treatment and control replicates per day and per week, depending on BSFL feeding rate and larval density

Feeding Rate (mg larvae^{-1} day^{-1})	50			100			200		
Larval Density (# of larvae)	400	800	1200	400	800	1200	400	800	1200
Pit material per Day (g)	20	40	60	40	80	120	80	160	240
Pit material per week (g)	140	280	420	280	560	840	560	1120	1680

There were a total of 54 variations of factor levels, with four replicates each, 27 variations without BSFL, and 27 variations with BSFL (Table 4-2). Variations of factor levels, MC, FR, and LD, were paired by the presence of BSFL factor. For logistical reasons, fourteen randomly

chosen paired groups were set up on one day (batch 1). The following day the remaining thirteen paired groups were tested (batch 2). Each paired group was randomly allocated a set of shelves in the experimental room. The position of each replicate (4 without BSFL, 4 with BSFL) within the paired group was randomly allocated and rotated every three days according to a Latin-square design (Appendix E).

Table 4-2 2 x 3 x 3 x 3 Factorial design showing the 54 variations of factors; presence of BSFL, moisture content (MC), feeding rate (FR), and larval density (LD). Each variation is made up of four factor levels, in the order BSFL, MC, FR, LD, e.g. 0-AAA = without BSFL, 65% MC, 50 FR, 400 LD; 1-BBB = with BSFL, 75% MC, 100 FR, 800 LD; 0-CCC = without BSFL, 85% MC, 200 FR, 1200 LD

		Factor					
		Presence of BSFL					
		0			1		
FR	LD	Moisture Content (MC)			Moisture Content (MC)		
Level		A	B	C	A	B	C
	A	0-AAA	0-BAA	0-CAA	1-AAA	1-BAA	1-CAA
A	B	0-AAB	0-BAB	0-CAB	1-AAB	1-BAB	1-CAB
	C	0-AAC	0-BAC	0-CAC	1-AAC	1-BAC	1-CAC
	A	0-ABA	0-BBA	0-CBA	1-ABA	1-BBA	1-CBA
B	B	0-ABB	0-BBB	0-CBB	1-ABB	1-BBB	1-CBB
	C	0-ABC	0-BBC	0-CBC	1-ABC	1-BBC	1-CBC
	A	0-ACA	0-BCA	0-CCA	1-ACA	1-BCA	1-CCA
C	B	0-ACB	0-BCB	0-CCB	1-ACB	1-BCB	1-CCB
	C	0-ACC	0-BCC	0-CCC	1-ACC	1-BCC	1-CCC

Due to the interaction between FR and LD, there were seven different masses of FS added each week (Table 4-1). Different sized, adjustable containers were used for different variations of factors in order to standardise the feeding depth. The feeding depth was maintained at 5cm or below to ensure BSFL were not crushed under the weight of the FS (Banks, LSHTM, personal observation). Four different sized plastic containers were fitted with temporary plastic divisions, held in place with a waterproof, temporary adhesive (Prestik, Bostick). This division was moved each week when FS was added to maintain depth. Containers were labelled with the factor variation and replicate number, e.g. Label: 0-AAA-1 represented - without BSFL, 65% MC, 50 FR, 400 LD, replicate 1, Label: 1-CCC-4 represented with BSFL, 85% MC, 200 FR, 1200 LD, replicate 4 (Table 4-2).

4.3.3.1. Faecal matter reduction and prepupal production

Labelled containers were cleaned, dried, and weighed. The appropriate quantity of FS was added to a container and weighed. The appropriate numbers of six day old BSFL were added to factor variations designated as "with BSFL". Each container was then held in a larger "crawl-off" container, and placed at a randomly allocated position in the environmentally controlled experimental room.

Every three days the weight of each replicate was recorded. For the replicates with BSFL, 10 BSFL were selected at random, weighed individually, and returned to the replicate. When returned to the experimental room, the replicates were moved to the next randomly allocated position to reduce environmental bias that could occur from container position. Every seven days after placement, experimental containers were weighed, and a fresh quantity of FS was added dependent on the factor variation (Table 4-1). To maintain a depth of 5cm, the temporary partition in the container was moved. This process of weighing and adding FS continued until 50% of the larvae began to develop into prepupae, identified by a change in colour from white to dark brown. Prepupae were collected from the "crawl-off" containers, counted and weighed. The 50% prepupal endpoint was chosen for each replicate with BSFL, and its paired replicate without BSFL, because it had been used in previous studies (Diener *et al.* 2009), and indicated that the FS was suitable for BSFL development. At the endpoint, all remaining BSFL and prepupae were removed from the treatment.

The remaining FS residue and prepupae were weighed, and representative samples collected for total solids (TS) analysis. Total solids were determined using Official Method 934.01 (AOAC 2002). A subsample left from the TS analysis was used to determine ash/total volatile solids (TVS) using Official Method 942.05 (AOAC 2002)

4.3.4. Data analysis

Data were entered into Excel 2013 (Microsoft, Washington, USA), and analysed in Stata 13.1 (Statacorp, Texas, USA). Faecal matter reduction was calculated for both wet and dry weight FS and residue data, while bioconversion was calculated using dry weight FS and dry weight prepupal data. The outcome variables, FMR, prepupal dry weight, pupation, growth rate, and bioconversion data were tested for normal distribution visually using qnorm and pnorm functions, and histograms, and statistically using the Shapiro-Wilk, Shapiro-Francia, and Skewness-Kurtosis tests. Non-normally distributed data were transformed where possible to result in a normal distribution. Normally distributed data were analysed using univariate and multivariate linear regression. Linear regression analysis was used to determine whether factors tested, BSFL, MC, FR, LD, and interactions between them, significantly affected the outcome variables.

4.3.5. Ethical clearance

Ethical approval for this study was granted by LSHTM Observational/Interventions Research Ethics Committee (#5972, amendment #A394) (Appendix B).

4.4. Results

4.4.1. Faecal matter reduction

4.4.1.1. Wet weight faecal matter reduction

Table 4-3 displays the arithmetic mean wet weight FMR for all dependant factors, and regression models indicate that all factors significantly affected FMR. The presence of BSFL resulted in significantly higher FMR than when BSFL were absent ($\overline{\Delta}$ FMR = 7.6%; 95% CI 5.3 – 10.0; $P < 0.001$) (Table 4-3). Moisture content had a significant influence on FMR, with 75% MC having higher FMR than 65% ($\overline{\Delta}$ FMR = 6.6%; 95% CI 3.7 – 9.6; $P < 0.001$) and 85% ($\overline{\Delta}$ FMR = 6.0%; 95% CI 3.1 – 9.0; $P < 0.001$) (Table 4-3). Feeding rate had a significant influence on FMR, with 50mg larvae^{-1} day^{-1} having a moderately significant higher FMR than 100mg larvae^{-1} day^{-1}

($\overline{\Delta}$ FMR = 3.1%; 95% CI 0.2 – 6.0; P = 0.034), and significantly higher than 200mg larvae^{-1} day^{-1} ($\overline{\Delta}$ FMR = 8.9%; 95% CI 6.0 – 11.7; P < 0.001) (Table 4-3). Larval density had a significant influence on FMR, with 400 larvae causing significantly higher FMR than 1200 larvae ($\overline{\Delta}$ FMR = 6.7%; 95% CI 3.8 – 9.7; P < 0.001), but not significantly higher than 800 larvae ($\overline{\Delta}$ FMR = 2.1%; 95% CI -0.9 – 5.1; P = 0.17) (Table 4-3).

Table 4-3 Arithmetic mean wet weight faecal matter reduction (FMR), including 95% CI's, for investigated factors and levels. Means followed by a different letter indicates a significant difference between levels within the factor (*F*-test; $P < 0.05$)

		Wet weight FMR (%)		Overall Regression Model		
Factor	Level	Mean	95% CI	*F*	df	*P*
BSFL	without	29.3[a]	(27.6 – 31.1)	42.01	1, 212	< 0.0001
	with	38.2[b]	(36.5 – 39.8)			
Moisture Content (%)	65	32.0[a]	(30.0 – 34.0)	11.90	2, 211	< 0.0001
	75	38.6[b]	(36.3 – 41.0)			
	85	32.5[a]	(30.5 – 34.7)			
Feeding Rate (mg larvae^{-1} day^{-1})	50	38.4[a]	(36.2 – 40.5)	18.94	2, 211	< 0.0001
	100	35.3[b]	(33.5 – 37.0)			
	200	29.5[c]	(27.2 – 31.8)			
Larval Density (number of larvae)	400	37.3[a]	(34.9 – 39.8)	10.45	2, 211	< 0.0001
	800	35.3[a]	(33.7 – 36.8)			
	1200	30.6[b]	(28.3 – 32.9)			

There were significant differences between the FMR effects of FR at different LDs (Figure 4-1), 50mg larvae^{-1} day^{-1} and 400 larvae caused significantly higher FMR than all other combinations ($\overline{\Delta}$ FMR = 4.7 – 23.3%; 95% CI 0.5 – 27.5; P < 0.05). A two-way linear regression indicates there was a significant interaction between FR and LD (F = 17.85; df = 8,205; P < 0. 001). There were significant differences in FMR effects when MC, FR and LD interacted, 75% MC, 50mg larvae^{-1} day^{-1} and 400 larvae caused significantly higher FMR than all other combinations ($\overline{\Delta}$ FMR = 10.2 – 38.1%; 95% CI 4.5 – 43.6; P < 0.001). The three-way linear regression indicates that there was a significant interaction between moisture content, feeding rate and larval density (F = 10.24; df = 8,187; P < 0.001). However, the F-values of the two models indicate that the association

between FR and LD is stronger than MC, FR, and LD. This difference indicates that the interaction between feeding rate and larval density was mainly responsible for increased FMR.

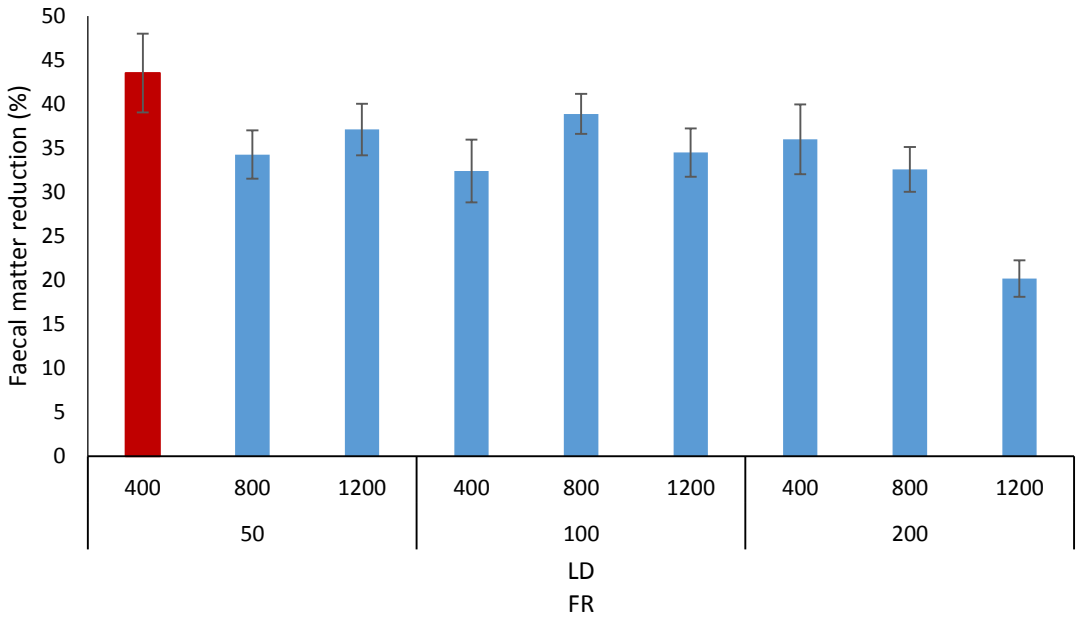

Figure 4-1 Arithmetic mean of percentage wet weight faecal matter reduction (FMR), including 95% CI's, for significantly interacting factors, FR (bottom row of horizontal axis) and LD (top row of horizontal axis). Column highlighted in red has significantly higher mean wet weight reduction than all other factor combinations (*F*-test; $P < 0.05$)

The results of a multivariate regression model indicate that the highest wet weight FMR was found in the presence of BSFL, MC = 75%, FR = 50mg larvae^{-1} day^{-1}, LD = 400 (1-BAA) ($\bar{x}$ FMR = 57.5%; 95% CI 54.0 – 61.1; $P < 0.001$). However, there was no significant interactions between all four factors ($F = 1.14$; df = 8,160; $P = 0.34$; see Appendix F) for regression table, and detailed interactions). None of the interactions between BSFL, MC, FR, and LD listed in Table 4-4 were found to be significant.

4.4.1.2. Dry weight faecal matter reduction

Dry weight FMR data were calculated using the FS wet weight and TS, and residue wet weight and TS. However, errors occurred in the procedure to obtain residue TS. These errors resulted in replicates where residue dry weight was higher than the FS dry weight, resulting in a net

mass gain. A net gain in mass is impossible, as no other solids were added to the replicates. Although only 9.2% of all calculations returned erroneous results, it was decided to exclude dry weight FMR data from analysis as the source of the error could not be identified.

Table 4-4 Linear regression analysis of wet weight faecal matter reduction (FMR) (%); Non-significant interactions between factors BSFL, MC, FR, and LD; (F-test; P > 0.01)

	Wet weight FMR		
Factor Interaction	*F*	df	*P*
BSFL * MC	0.23	2, 208	0.79
BSFL * FR	1.07	2, 208	0.34
BSFL * LD	1.85	2, 208	0.16
MC * FR	0.57	2, 208	0.68
MC * LD	2.00	2, 208	0.10
BSFL * MC * FR	0.20	4, 196	0.94
BSFL * MC * LD	0.61	4, 196	0.66
BSFL * FR * LD	0.24	4, 196	0.92

4.4.2. Prepupal production

All data were found to be normally distributed, except for prepupal dry weight, which was cube-root transformed to result in a normal distribution. All prepupal production data were calculated from factor variations where BSFL were present. Table 4-5 displays the mean prepupal dry weight, pupation rate, growth rate, and bioconversion rate of the three dependent factors, showing that 85% MC resulted in the heaviest prepupae, highest growth rate and bioconversion, but lowest pupation, the highest FR resulted in the heaviest prepupae, highest pupation, and growth rate, but lowest bioconversion, and the highest LD resulted in the heaviest prepupae, highest pupation, second highest growth rate, but lowest bioconversion.

Mean prepupal dry weight was significantly affected by: MC (F = 24.97; df = 2, 1067; P < 0.001), FR (F =887.26; df = 2, 1067; P < 0.001), and LD (F = 10.08; df = 2, 1067; P < 0.001). Pupation rate and growth rate were significantly influenced by: MC (F = 13.89; df = 2,1067; P < 0.001 and F = 3.44; df = 2,104; P = 0.036; respectively), FR (F = 3.48; df = 2,1067; P = 0.035 and F = 85.02; df = 2,104; P < 0.001; respectively), but not LD (F = 0.21; df = 2,1067; P = 0.81 and F =

1.23; df = 2,104; *P* = 0.30; respectively). Dry weight bioconversion also follows a similar trend by being significantly influenced by: MC (*F* = 40.22; df = 2,104; *P* < 0.0001), FR (*F* = 5.45; df = 2,104; *P* = 0.0056), but not LD (*F* = 0.38; df = 2,104; *P* = 0. 69).

Table 4-5 Back transformed cube root mean of prepupal dry weight, arithmetic mean pupation, growth rate and bioconversion, including 95% CI's, for investigated factors and levels. Means followed by a different letter indicate a significant difference between levels within the factor; data analysed using linear regression model (*F*-test; *P* < 0.05)

		Mean prepupal dry weight (g)		Pupation (%)		Growth rate (mg day^{-1})		Bioconversion (dry weight)	
Factor	Level	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Moisture Content (%)	65	0.0495^{a}	(0. 0480 – 0.0514)	57.8^{a}	(53.3 – 62.4)	8.4^{a}	(7.7 – 9.1)	3.5^{a}	(3.1 – 3.8)
	75	0.0485^{a}	(0.0466 – 0.0504)	56.8^{a}	(51.5 – 62.1)	7.5^{b}	(6.6 – 8.3)	4.9^{b}	(4.4 – 5.3)
	85	0.0572^{b}	(0.0553 – 0.0590)	43.8^{b}	(40.6 – 47.0)	8.8^{a}	(8.1 – 9.5)	6.4^{c}	(5.8 – 7.0)
Feeding Rate (mg larvae^{-1} day^{-1})	50	0.0345^{a}	(0.0334 – 0.0357)	47.7^{a}	(43.0 – 52.3)	6.0^{a}	(5.6 – 6.4)	5.4^{a}	(4.7 – 6.0)
	100	0.0507^{b}	(0.0495 – 0.0519)	55.1^{b}	(50.9 – 59.2)	8.4^{b}	(7.9 – 8.9)	5.3^{a}	(4.7 – 5.9)
	200	0.0696^{c}	(0.0684 – 0.0708)	55.3^{b}	(49.8 – 60.7)	10.2^{c}	(9.7 – 10.8)	4.1^{b}	(3.6 – 4.7)
Larval Density (number of larvae)	400	0.0482^{a}	(0.0463 – 0.0501)	53.1^{a}	(49.1 – 57.1)	7.8^{a}	(7.1 – 8.4)	5.1^{a}	(4.4 – 5.9)
	800	0.0534^{b}	(0.0515 – 0.0553)	51.4^{a}	(56.5 – 56.3)	8.6^{a}	(7.8 – 9.4)	4.9^{a}	(4.2 – 5.6)
	1200	0.0537^{b}	(0.0518 – 0.0556)	53.4^{a}	(47.9 – 58.9)	8.3^{a}	(7.5 – 9.1)	4.7^{a}	(4.3 – 5.1)

Table 4-6 presents the linear regression results of mean prepupal dry weight, pupation, growth rate and bioconversion, for the investigated factors. The MC factor significantly affected prepupal dry weight, pupation, and bioconversion, FR affected mean prepupal dry weight, pupation, growth rate, and bioconversion, while LD only significantly affected prepupal dry weight. This indicates that FR and MC have the most effect on prepupal production. It was observed that in FS with a moisture content of 85%, the BSFL took longer to "settle" in the food. A lot of surface activity was witnessed, and when the BSFL were initially placed on the FS a higher number of larvae crawled out than in the two lower moisture contents.

Table 4-6 Linear regression analysis of mean prepupal dry weight, pupation, growth rate and bioconversion, for investigated factors and levels, including; estimated model coefficients, 95% CI's, and significant differences (*t*-test; $P < 0.05$) between levels of factors, and overall regression model displaying significant effect of factor on variable (*F*-test; $P < 0.05$)

			Estimated Model Coefficients			Overall Regression Model		
	Factor	Level	Unstandardised Coefficients	95% CI	*P*	*F*	df	*P*
Mean prepupal dry weight	Moisture Content (%)	65	-0.008	(-0.0019 – -0.0019)	<0.001	25.05	2,1067	< 0.0001
		75	-0.009	(-0.0019 – -0.0019)	<0.001			
		85	0.0572	(0.055 – 0.059)	-			
	Feeding Rate (mg larvae^{-1} day^{-1})	50	-0.0351	(-0.0012 – 0.0012)	<0.001	886.13	2,1067	< 0.0001
		100	-0.0189	(-0.0012 – 0.0012)	<0.001			
		200	0.0696	0.068 – 0.071)	-			
	Larval Density (number of larvae)	400	-0.0055	(-0.0019 – 0.0019)	< 0.001	10.11	2,1067	< 0.0001
		800	-0.0003	(-0.0019 – 0.0019)	-			
		1200	0.0537	(0.052 – 0.056)	0.46			
Pupation	Moisture Content (%)	65	57.8	(53.7 – 62.0)	-	13.89	2,100	< 0.0001
		75	-1.0	(-7.1 – 5.0)	0.74			
		85	-14.1	(-19.9 – -8.2)	< 0.001			
	Feeding Rate (mg larvae^{-1} day^{-1})	50	-7.6	(-14.2 – -1.0)	0.025	3.48	2,100	= 0.035
		100	-0.23	(-6.8 – 6.4)	0.95			
		200	55.3	(50.5 – 60.1)	-			
	Larval Density (number of larvae)	400	-0.3	(-7.2 – 6.5)	0.93	0.21	2,100	= 0.81
		800	-2.0	(-8.6 – 4.6)	0.55			
		1200	53.4	(48.7 – 58.1)	-			
Growth rate	Moisture Content (%)	65	-0.4	(-1.4 – 0.6)	0.44	3.44	2,104	0.36
		75	-1.3	(-2.3 – -0.3)	0.012			
		85	8.8	(8.1 – 9.5)	-			
	Feeding Rate (mg larvae^{-1} day^{-1})	50	-4.3	(-4.9 – -3.6)	< 0.001	85.02	2,104	< 0.0001
		100	-1.9	(-2.5 – -1.2)	< 0.001			
		200	10.2	(9.8 – 10.7)	-			
	Larval Density (number of larvae)	400	-0.8	(-1.9 – 0.2)	0.12	1.23	2,104	0.30
		800	8.6	(7.9 – 9.3)	-			
		1200	-0.3	(-1.4 – 0.7)	0.55			
Bioconversion	Moisture Content (%)	65	-3.0	(-3.6 – -2.3)	< 0.001	40.22	2,104	< 0.0001
		75	-1.6	(-2.2 – 0.9)	< 0.001			
		85	6.4	(6.0 – 6.9)	-			
	Feeding Rate (mg larvae^{-1} day^{-1})	50	5.4	(4.8 – 6.0)	-	5.45	2,104	0.0056
		100	-0.1	(-1.0 – 0.7)	0.79			
		200	-1.3	(-2.1 – -0.4)	0.004			
	Larval Density (number of larvae)	400	5.1	(4.5 – 5.7)	-	0.38	2,104	0.69
		800	-0.2	(-1.1 – 0.7)	0.69			
		1200	-0.4	(-1.3 – 0.5)	0.39			

4.5. Discussion

This study has determined that the presence of BSFL has a significant effect on FMR compared to their absence. It has also verified that a low FR, 75% MC, and low LD result in the highest

FMR when BSFL are present, however, high FR, 85% MC, and high LD result in the largest prepupae and highest growth rate.

4.5.1. Faecal matter reduction

The presence of BSFL had the strongest association with FMR of human FS. These results are consistent with previous studies of BSFL feeding on fresh human faeces, where the presence of BSFL resulted in significantly higher FMR compared to interventions without BSFL (Lalander *et al.* 2013, Banks *et al.* 2014). The presence of BSFL in the most effective variation of factor levels, 75% MC, 50mg larvae day FR, 400 LD, resulted in an 8.7% higher FMR than replicates without BSFL. This is far lower than previously reported by Lalander *et al.* (2013), with the presence of BSFL resulting in a net 30% higher FMR, than the absence of BSFL. This difference is most likely due to the containers with FS in the Lalander *et al.* (2013) study being covered with perforated aluminium foil, reducing dehydration of the control material. The Lalander *et al.* (2013) study demonstrated that the reduction in total solids of FS was 43% higher in the presence of BSFL than in the absence. Additionally, dehydration and the biodegradation of FS by bacteria will occur in both the presence and absence of BSFL. However due to different bacterial communities of FS used in the two studies, the speed of biodegradation could vary. Unfortunately, it was not possible to calculate the total solid reduction of FS in the present study because of an anomaly in the data, as discussed below.

The most effective FMR rate with BSFL present, approximately 58%, is comparable to BSFL FMR of chicken manure, approximately 50% (Sheppard *et al.* 1994), but higher than the reduction of chicken manure and cow manure by common houseflies, *Musca domestica,* approximately 30%, and 25%, respectively (Morgan *et al.* 1975). This indicates that the use of BSFL to manage human FS could effectively reduce the mass of the FS by half, resulting in easier residue management, post-processing. It has been suggested that with suitable treatment, residue could be a safe and effective fertiliser for crops suitable for human

consumption (Lalander *et al.* 2013). Future work is required to investigate what value residue may contain, and what processes are required to reduce the risk of disease transmission to humans and animals.

The moisture content of FS had a significant effect on FMR, with 75% moisture content resulting in the highest FMR. This could be due to the addition of moisture in order to raise the MC of the FS from 65% to 75%. However, even more water was added to raise the MC from 65% to 85%, and the mean FMR was lower than in the 75% MC interventions. This suggests that that human FS with an MC of 75% is optimum for rearing BSFL. These results are in contrast to a previous study which investigated the moisture content of chicken manure and how it affected BSFL production (Fatchurochim *et al.* 1989) that suggested that prepupal production was greatest for chicken manure with a moisture content from 40 – 60%, significantly higher than BSFL reared on chicken manure with a moisture content of with 70 – 80%. However, the study using chicken manure did not investigate FMR. The results presented in the current study indicate that optimum FMR of FS occurs when the starting sludge contains 75% moisture. Also, the results in the current study imply that successful FMR can occur at all moisture contents tested, although with varied efficiency. Further research must be completed to determine the upper and lower tolerances of BSFL in relation to MC of food, with evidence that BSFL can successfully develop in chicken manure with an MC of approximately 55 – 80% (Sheppard 1983). The results of future studies should determine whether FS collected from pit latrines, which can range from 20 – 94% (Torondel, LSHTM, unpublished data), is suitable without any treatment, or whether extra steps, such as drying or addition of water, must occur. In the present study, feeding rate was significantly associated with FMR, where lower feeding rates resulted in higher FMR. This finding was consistent with previous research (Diener *et al.* 2009, Banks *et al.* 2014), and was anticipated, as the quantity of food that BSFL can consume is limited. With higher feeding rates, there is a larger proportion of residue left over which has already passed through the gut of the BSFL, or not eaten at all, compare to

lower feeding rates (Diener *et al.* 2009). This means that the feeding rates used in any proposed BSFL FSM system are a vital component, determining the quantity of FS that can be processed, and also how much residue remains. The post-processing of the residue must be taken into account also. As mentioned previously, residue has the potential of having value as a fertiliser. However, further investigation should be conducted into residue that remains post-BSFL treatment, and whether it contains sufficient nutrients to be fed back to BSFL, to extract as much value from the FS as possible.

The larval density had a significant association with FMR where, surprisingly, lower larval densities produced higher FMR than the highest. These results could be due to increased intraspecific competition for resources, such as food and space. The effect of larval competition has been observed in the Mediterranean fruit fly (Dukas *et al.* 2001), with larval growth and pupal mass showing larger variance when egg clutches were laid on successive days than on the same day, implying a competitive advantage for older larvae over younger larvae. The BSF egg clutches used in the present study were gathered on the same day, and in preliminary research it was found that egg hatching occurred between 84 and 108hrs post-collection (Banks, LSHTM, unpublished data). This variance in egg hatching could result in a competitive advantage for older larvae. With a higher larval density, the number of older larvae would increase, amplifying the effect of larval competition. These results suggest that FMR efficiency will reduce as the number of BSFL and quantity of FS increases. However, this suggestion conflicts with previous research that demonstrated efficient BSFL management of chicken manure on a scale 5000 times larger than the experiments conducted in this study (Sheppard *et al.* 1994). Therefore, it is important for future work to investigate how variations in early-stage BSFL, caused by egg collection and larval rearing practices, could affect FMR, and the scalability of BSFL FSM. This research is especially important when considering large scale, decentralised BSFL FSM sites, due to the necessity of standardised methods that will ensure reliable and robust FS treatment.

Reporting dry weight FMR is more accurate than wet weight FMR, because it excludes the varying water concentrations in the FS and residue. However, due to an anomaly where residue dry weight was higher than FS dry weight, numerous dry weight FMR values were negative, indicating there had been a net increase in dry matter. The technique used to determine dry weight of residue and FS is an internationally recognised method (APHA 2012), and has been used extensively for similar applications (Newton *et al.* 2005, Diener *et al.* 2009, Diener *et al.* 2011, Lalander *et al.* 2013, Banks *et al.* 2014). The authors investigated whether anomalies, such as found in this experiment, had been previously reported, but with no success. The protocol was followed correctly, with container weights subtracted where appropriate, and the calculations used in the analysis were checked by a number of collaborators, however it was not possible to identify the source of the error. This leads to the conclusion that an error occurred while recording data or determining the dry weight of FS and residue. Due to the anomaly in the data, the wet weight FMR data must be presented in this study, resulting in less accurate indications of FMR.

4.5.2. Prepupal production

The FS moisture content of 85% resulted in significantly higher prepupal dry weight, pupation rate and bioconversion compared to lower MCs. Previous research found that significantly higher BSFL production, characterised by survival to adulthood, days to emergence, and dry adult weight, occurred in manure moisture levels of 40 – 60%, compared to manure with a moisture content of 70 - 90% (Fatchurochim *et al.* 1989). However, the study also discussed how its results conflicted with previous research that demonstrated the abundance of BSFL in semi-liquefied FS (Copello 1926, Furman *et al.* 1959, Booth *et al.* 1984). Diet moisture content is also known to significantly affect larval survival and size of emerging adults in *M. domestica*, *Muscina stabulans*, *Fannia fenoralis*, *F. canicularis*, and *Ophyra aenescens* (Fatchurochim *et al.* 1989), while low-water reared *Manduca sexta* (Sphingidae: Lepidoptera) larvae initially grew

slowly, but resulted in pupae and adult sizes comparable to controls with 30% higher moisture content.

In FS with 85% moisture content, the BSFL were more active, constantly moving around the surface of the FS resulting in dehydration. This allowed the BSFL to burrow into the FS in a behaviour which was similar to the two lower moisture content levels. By the end of the experiment the moisture content of the residue was still suitable for BSFL feeding. This is different to the 65% moisture content FS, where in some factor variations, the residues moisture content had reduced to as low as approximately 10%, hypothetically too dry for BSFL feeding. The changes in the FS over time explain why 85% moisture content results in the highest mean prepupal dry weight, growth rate, and bioconversion. Although not ideal for the BSFL when first placed on the FS, their natural behaviour altered their environment to make it more suitable. Due to the initially high moisture content, the FS maintained moisture content more appropriate for sustained feeding. The BSFL could move through the FS faster than the more viscous FS with lower moisture contents. Also, the increased moisture in the FS would have made it easier for the BSFL to feed, as their macerating mouthparts could scrape at solids softened by the moisture (Schremmer 1986), once more, allowing the BSFL to feed more effectively for longer. However, the 85% moisture content did result in a lower pupation rate, possibly due to slower initial development. This is important to consider, as the higher prepupal dry weight, growth rate and bioconversion could have an increased benefit to prepupal production than the additional time required to reach higher pupation levels.

The highest feeding rate resulting in the highest mean prepupal dry weight, pupation, and growth rate, confirming previous results from studies performed using chicken feed and dairy manure as larval food sources (Myers *et al.* 2008, Diener *et al.* 2009). The results published (Diener *et al.* 2009) show prepupal dry weights comparable to results in the present study for all three feeding rates examined, as well as increased growth rate (Table 4-5). While Myers *et*

al. (2008) reported highest survival to prepupal stage in a feeding rate range of 133 – 180mg larvae^{-1} day^{-1}. However, this increase in prepupal production is counteracted by a decrease in FMR efficiency, also shown by Diener *et al.* (2009). The increased quantity of FS available to the BSFL results in an increase in weight and growth rate. However, as there is more food available, more residue remains, either not eaten, or already passed through the gut of the larvae. The higher quantity of residue leads to lower bioconversions, regardless of an increase in prepupal biomass.

It is important to note that an increase in larval density results in an increase in prepupal dry weight, while having no significant effect on pupation, growth rate or bioconversion. These results suggest that it is possible to scale up the number of BSFL in a FSM system, without negatively affecting the mean prepupal dry weight, pupation, growth rate or bioconversion. All of these measurements are vital when considering prepupal production.

The present study has shown that the factor feeding rate has the most significant effect on BSFL reduction of FS and prepupal production. This factor should be considered the most important when applying the results to a real-world situation. The moisture content of FS used in a BSFL FSM system will vary depending on its source, however the additional steps required to ensure standardised optimum FMR efficiency are easily accomplished. The results produced by this study in regard to larval density and FMR are puzzling, and need to be further investigated. After checking the coding of data was correct, the results still suggested that more BSFL added resulted in lower FMR. As discussed above, this is contrary to previous research, although these differences could be caused by the differences in rearing diets and must be investigated further. Further research into the scalability of BSFL FSM is vital to for the future implementation of it as a technology, as discussed in the next section.

4.5.3. Implications

The study presented here has shown that key rearing parameters have a significant influence on BSFL reared on FS, resulting in changes in FMR and prepupal production. These parameters are important when considering the method of how to use BSFL for FSM in different situations. There are a number of different techniques suggested, including: decentralised BSFL treatment plants, in-situ BSFL, and a BSFL toilet.

In a decentralised FSM plant, the moisture content, feeding rate and larval density are vital to successful and efficient operation. Previous research shows the moisture content of pit latrine FS from different depths of latrines can vary between 24% - 79% (see Chapter 1). However, this variation is irrelevant in this context because collected FS would become homogenised, by combining FS from different pit latrines. However, it would be possible to incorporate a pre-BSFL treatment stage which could alter moisture content to the desired level. This could be achieved by drying FS which is too wet on drying beds, or adding water to FS with low moisture content. Using this technique, the FS moisture content could be adjusted to result in more effective FMR, or prepupal production, depending on what is required. The feeding rate used in a FSM plant will depend heavily on what the desired outcome of the plant is. If a treatment plant's primary aim is to reduce the mass of FS to a safer residue, then low feeding rates will result in more efficient FMR. However this will also result in low prepupal production. Conversely, if prepupal production is the primary aim of a BSFL treatment plant, and FMR secondary, then a higher feeding rate would be beneficial. It is also possible to apply a feeding rate which results in a balance between FMR and prepupal production. The larval density results presented in the present study indicate that FMR is reduced at higher larval densities, however this is contrary to previous studies which utilised BSFL to efficiently manage tons of chicken manure (Sheppard *et al.* 1994). The results suggest that prepupal production is not affected by larval density, and that the scale of BSFL FSM can increase without adversely affecting prepupal production. This is important when the primary aim of a BSFL treatment

plant is the production of prepupae as a protein source of animal feed. The study presented here suggests that the purpose of a decentralised BSFL treatment plant can be tailored by adjusting the key parameters investigated.

When considering using an in-situ BSFL treatment method, the key parameters presented in this study must be considered, particularly the moisture content of FS in a pit latrine, before implementation. The moisture content must fall within a specific range if BSFL are to be used. Additionally, the water table, and seasonality must be considered before implementation. A rising water table, due to seasonal rainfall, could cause flooding of the pit latrines. This would raise the moisture content of the FS to inappropriate levels. This study has demonstrated that FS moisture contents of 65 – 85% are suitable for BSFL. However, it is vital to conduct more comprehensive research using a wider range of FS moisture contents, considering the contrasting results to previous work (Fatchurochim *et al.* 1989). The feeding rate of BSFL in-situ would be difficult to determine. However, the results presented indicate that lower feeding rates result in increased FMR. This is important when the main aim of an in-situ treatment solution is to improve FMR, and increase the life span of a pit latrine. Considering this, an approximate feeding rate can be determined by controlling the larval density in the latrine. A suitable quantity of BSFL could be seeded into the latrine regularly, with the quantity established by calculating how much excreta is added to the latrine under user conditions, taking into account the number of users and frequency of use. It may be possible to increase the FMR, by the addition of more BSFL, potentially having the BSFL reduce the FS which is already in the vault, and not just the fresh excreta added. However, the physical and chemical characteristics of FS have been found to alter as depth increases in some latrines (see previous Chapter 3), which could affect how BSFL develop, in-situ and in a decentralised BSFL treatment plant.

Finally, the design of a specialised BSFL toilet will be heavily influenced by moisture content, feeding rate and larval density. Fresh human faeces can range between 75 – 85% moisture content in healthy people (Chaggu 2004, Buckley *et al.* 2008). However, the use of water for anal cleansing would increase the moisture content of the FS, resulting in the need for a draining bed to promote BSFL feeding. It could be possible to incorporate the BSFL into a sawdust matrix/feeding bed as found in the Tiger Toilet (Furlong *et al.* 2014). As discussed above, the quantity of fresh excreta which is added to the BSFL toilet, and larval density, will determine what feeding rate occurs. A lower feeding rate will result in higher FMR, but lower prepupal production. This is important when considering where a BSFL toilet is implemented, as discussed previously (see previous Chapter 3). However, a significant amount of research and engineering must be undertaken before a BSFL toilet is an achievable FSM technology.

4.6. Conclusion

The study presented here has determined that key parameters can affect FMR and prepupal production of BSFL when reared on pit latrine FS. Due to the variation in how BSFL can be utilised to manage FS, the results indicate that the key parameters can be adjusted depending on what the primary aim is, either FMR or prepupal production. However, it is vital for future research to concentrate on a number of areas which are integral to BSFL FSM, including: how variations in physical and chemical characteristics of FS affect the development of BSFL, value of prepupae reared on FS, value of residue, suitability of prepupae and residue for subsequent use, in relation to harmful contamination by pathogens or heavy metals. Importantly, the study presented here has provided further evidence that the use of BSFL in the management of FS is a viable alternative to current FSM practices, suggesting that a BSFL FSM technology could provide an additional tool in helping improve sanitation worldwide.

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Chapter 5) Feeding efficiency of black soldier fly larvae on different layers and mixtures of pit latrine faecal sludge, and the chemical and physical characteristics that affect faecal matter reduction

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5.1. Abstract

OBJECTIVES: To determine faecal matter reduction (FMR) and prepupal production efficiency of black soldier fly larvae (BSFL) feeding on pit latrine faecal sludge (FS), and to assess whether different physical and chemical characteristics from different layers of pit latrines influences prepupal production. In addition the nutritional value of prepupae and residue post-BSFL development will be determined.

METHODS: Factors tested were presence of BSFL (BSFL absent, BSFL present) and FS "layer" (6 latrines, 5 layers per latrine: 0 – 20 cm, 21 – 50cm, 51 – 100cm, 101 – 150cm, and homogenised layer). Approximately 400 BSFL were fed 50mg larvae^{-1} day^{-1} until 50% developed into prepupae. Prepupae, FS, and residue samples were analysed for physical and chemical characteristics (total solids, total volatile solids, pH, total chemical oxygen demand, soluble COD, NH_4^+, protein, carbohydrates, VFAs, heavy metals), and nutritional value using standard methods.

RESULTS: Wet weight FMR was significantly influenced by the presence of BSFL (P = 0.013) and layer (P < 0.0001). Optimum wet weight FMR occurred in FS from 0 – 20 cm (65%, 95% CI 58.1 – 71.8). Layer and physical and chemical characteristics did not influence prepupal production.

CONCLUSION: The results show that BSFL are efficient at reducing FS from a variety of depths with a range of physical and chemical characteristics. The prepupae produced have a nutritional value comparable to previous research, except for crude fat which was lower than previous studies. The study shows under an optimum situation, a decentralised BSFL treatment plant has a strong business model. However, further investigation is required to determine potential problems in pathogen transmission or heavy metal bioaccumulation, and appropriate treatment, and value, of residue and prepupae. Solutions suggested in this study could help improve sanitation for billions of people around the world.

5.2. Introduction

Providing improved sanitation for the 2.5 billion people (UNICEF/WHO 2014) worldwide who currently have no access, is vital to the improvement of public health (Esrey *et al.* 1991, Fewtrell *et al.* 2005). However, the implementation of large scale piped sewage systems, and off-site treatment plants in low- and middle- income countries can be prohibited by a lack in suitable infrastructure and high costs (WHO/UNEP 2006). Therefore, a more suitable solution is to provide on-site sanitation. However, problems arise with the emptying, transportation, and treatment of faecal sludge (FS) that collects in pit latrine vaults (Helmer *et al.* 1997, Kariuki *et al.* 2003, WHO/UNEP 2006, UNICEF/WHO 2014). Furthermore, the distance and cost of legally disposing FS at faecal sludge management (FSM) facilities can lead to indiscriminate dumping, which causes major environmental and hygiene problems (Kariuki *et al.* 2003). Therefore it is important to consider alternative methods of on-site FSM.

An alternative method of on-site FSM is the larvae of the black soldier fly (BSFL), *Hermetia illucens* (L.). The detritivorous larvae of this species have been proven to be effective at managing animal manure (Tingle *et al.* 1975, Booram *et al.* 1977, Newton *et al.* 2005) and municipal organic waste (MOW) (Diener *et al.* 2011). Previous research has shown that BSFL can effectively reduce fresh human faeces (Lalander *et al.* 2013, Banks *et al.* 2014), and the top layer of FS from selected South African pit latrines (see previous Chapter 4). The BSFL can develop successfully on FS into the final larval stage, known as prepupae. Prepupae reared on animal manure have high protein and fat levels, 43-45% and 31-35% respectively (Hale 1973, Newton *et al.* 1977). These characteristics give the prepupae an intrinsic value, as they can be used as a replacement for conventional protein and fat sources in animal feed (Hale 1973, Newton *et al.* 1977, Bondari *et al.* 1987, St-Hilaire *et al.* , Hem *et al.* 2008). The fat can also be fractionated to produce biodiesel (Li *et al.* 2011a, Li *et al.* 2011b, Zheng *et al.* 2012a, Zheng *et al.* 2012b).

The nutritional value and faecal matter reduction (FMR) efficiency of the prepupae depends on what they feed on as larvae. A number of biological, chemical, and physical characteristics are known to affect the development of BSFL, and similar insects, including: total solids (TS) (Fatchurochim *et al.* 1989), feeding rate (Diener *et al.* 2009, Banks *et al.* 2014), larval density (see previous Chapter 4), pH (Chaudhury *et al.* 2009), chemical oxygen demand (COD), ammonium (NH_4^+) (Popa *et al.* 2012), protein (Bennett 2000, Simpson *et al.* 2006), carbohydrates (Nijhout 2003, Lee *et al.* 2004) and heavy metals (Diener 2010, Diener *et al.* 2011). It is known that the physical and chemical characteristics of pit latrine FS vary between pits (Lopez Zavala *et al.* 2002, Chaggu 2004, Palmquist *et al.* 2005, Buckley *et al.* 2008, Irish *et al.* 2013). Variation in pit latrine FS has been investigated in South Africa (Bakare 2014), Tanzania and Vietnam (Torondel, LSHTM, unpublished data). The studies suggest that there are huge variations between different pit latrines, and between different layers within and between latrines. Some latrines show trends of increasing % total solids, and decreasing COD, NH_4^+, and proteins, from top to bottom of the latrine, whereas other latrines show no significant changes from top to bottom layers. Total solids can range between 6 – 80% in the top layer of latrines, to 40 – 80% past 1 metre deep, with COD concentrations ranging from 30 – 2000 g kg^{-1} dry mass in the top layer, to 20 – 300g kg^{-1} dry mass in lower layers (Torondel, LSHTM, unpublished data). This variation in FS is important, as the physical and chemical properties alter its potential usefulness as a resource, as discussed below. Additionally, heavy metal bioaccumulation must be taken into consideration when dealing with BSFL due to biomagnification. Biomagnification is when there is an increase in concentration of a substance from one trophic level to another (Walker 1987). It has been previously found that cadmium bioaccumulates in prepupae (Diener 2010), and even though heavy metals such as zinc, cadmium and lead do not affect the development of individual BSF (Diener 2010), the problem of biomagnification occurs when the prepupae are used for animal feed, such as when larvae are fed directly to chickens.

The demand for an alternative method of FSM for low- and middle-income countries is necessary to help improve sanitation. Previous research has determined that the physical and chemical characteristics of pit latrine FS, collected from communities which would benefit from an alternative FSM technology, fall within a range suitable for BSFL development (see Chapter 3). The use of BSFL to manage FS has been shown to be possible, with the correct key rearing parameters (see Chapter 4). However, further investigation must be conducted into how the physical and chemical characteristics of FS affect the development of BSFL, and their FMR efficiency, and prepupal production. The present study aims to determine the FMR efficiency and prepupal production of BSFL feeding on pit latrine FS from different layers of pit latrines. Faecal sludge physical and chemical characteristics will be identified, and their influence on BSFL FMR efficiency and prepupal production correlated. The study aims to quantify nutritional value of prepupae produced on FS, and the macro- and micro-nutrient value of FS residue post-BSFL development, and identify if bioaccumulation of heavy metals in prepupae is a problem. Additionally, the study will determine whether a BSFL FSM is economically feasible using business feasibility models (see Appendix A).

5.3. Methods

5.3.1. Study site

Faecal sludge for the experiments was collected from the communities Mnyamanzane (32.2735 S, 28.2002 E), and Sheshegu (32.1255 S, 28.2789 E), Eastern Cape Province, South Africa. Research was conducted at Mariendahl Experimental Farm (33.8506 S, 18.8262 E), Stellenbosch, South Africa. The experimental containers were stored in a controlled environment at approximately 27°C, 70% relative humidity, and a 12:12 day/night light cycle. Faecal sludge collection procedure was detailed in a previous study (see Chapter 3).

5.3.2. Black soldier flies

The BSFL used in the experiments were collected from a colony at the Mariendahl Experimental Farm, maintained by AgriProtein Technologies and the University of Stellenbosch, South Africa. The method of BSF egg collection and early-stage larval rearing is described in a previous study (see Chapter 4). The mean individual weight was used to measure approximately 400 BSFL for each replicate to be used in the following experiment.

5.3.3. Faecal sludge

Faecal sludge was collected by removing latrine superstructures to gain access to vaults. Entire layers (0 – 20cm, 21 – 50cm, 51 – 100cm, and 101 – 150cm) were excavated using a spade to prevent mixing between layers, and sealed inside containers (Addis Roughtote, 68 litre). The FS was immediately stored and refrigerated (4°C) after collection. On arrival at the local laboratory it was frozen at -20°C for 48 hours to kill any fly larvae present. Once defrosted, non-faecal waste, including: diapers, building materials, clothes, condoms, and food packaging, were removed from each sample, and the FS was homogenised using a drill with a paint mixer bit. Representative samples of homogenised FS were taken for analysis to determine physical characteristics and chemical contents. Four layers of FS were collected from six pit latrines. A 5th layer was produced, containing a sample of FS from each of the four layers. This "combined" layer imitated the mixture of a whole vault of FS, with all four layers mixed together and is representative of how FS could be presented to BSFL in a decentralised FSM system. All 30 layers (5 layers per latrine, and 6 latrines) had their identities blinded for the experiment by a third party to reduce observational bias. Samples were taken from each layer for physical and chemical analysis as described in a previous study (see Chapter 3). The FS was stored at 4°C until needed, and warmed to room temperature before use.

5.3.4. Experimental design

”Presence of BSFL” and “layer” were the two factors tested in this experiment. The quantity of FS added to each sample (140g week^{-1}) was calculated using a feeding rate of 50mg larvae^{-1} day^{-1} and a larval density of 400 larvae. These amounts were chosen because they caused the most efficient FMR in a previous study (see Chapter 4). All 30 layers were set up on a single day. Each layer was paired by the presence of BSFL factor (with or without BSFL), and there were four replicates per treatment. Each paired group was randomly allocated a set of shelves in the experimental room. The position of each replicate (4 without BSFL, 4 with BSFL) within the paired group was randomly allocated and rotated every three days.

5.3.4.1. Faecal matter reduction and prepupal production

One litre plastic containers were cleaned, dried, labelled, and weighed. Faecal sludge was added to all replicates and the weight recorded. Six day old BSFL were added to the replicates designated as “with BSFL”. Each 1 L container was then placed in a larger “crawl-off” container to prevent BSFL escape. All replicates were then placed in the environmentally controlled experimental room.

Every three days the weight of each replicate was recorded. For the replicates with BSFL, 10 BSFL were selected at random, weighed individually, and returned to the replicate. When returned to the experimental room, the replicates were moved to the next randomly allocated position to reduce environmental bias that could occur from different microclimatic conditions associated with the different container positions. Every seven days after placement, experimental containers were weighed, and a fresh quantity of FS was added dependent on the factor variation. After the first seven days, a square of fine netting was placed over the top of all 1 L containers, and held in place by a lid with a large hole cut in it. This was to prevent further oviposition of invasive dipteran species, as invasive larvae had been found unexpectedly in a number of replicates without BSFL.

The process of weighing, and addition of FS, continued until the BSFL began to develop into prepupae, indicated by a change in colour from white to dark-brown. Prepupae were collected from the "crawl-off" containers, counted and weighed. Once approximately 50% of the larvae had developed into prepupae (see Chapter 4), remaining BSFL and prepupae were removed from replicate, and both the replicate and its paired replicate without BSFL were weighed and residue samples collected for subsequent analyses.

5.3.5. Material analysis

For FS, residue, and prepupae, total solids (TS) were determined using Official Method 934.01 (AOAC 2002), as described in a previous study (see Chapter 3). For FS and residue samples, pH, total (tCOD) and soluble (sCOD) chemical oxygen demand, ammonia (NH_4^+), total protein, total carbohydrate, and volatile fatty acids (VFA's), and heavy metals were determined using methods described previously (see Chapter 3).

Crude protein (CP) of prepupae was determined by measuring total nitrogen (N) content by block digestion method using copper catalyst and steam distillation into boric acid, Official Method 4.2.07 (AOAC 2002), and calculated using the following equation:

$$CP = N\ x\ 6.25$$

Crude fibre (CF) of prepupae was determined using the ceramic fibre filter method, Official Method 962.09 (AOAC 2002): two 1 g samples were placed into a crucible (WW_{CF}), then into a Fibertec/Dosifiber extrusion apparatus, boiling H_2SO_4 was added and left to cook for thirty minutes, samples were washed three times with dH_2O, then 0.313M NaOH added and left to cook for a further 30 minutes, the samples were washed three times more with dH_2O, then dried for 24 hours at 100°C (DW_{CF}), and combusted in a muffle filter for 6 hours at 500°C (ash_{CF}), crude fibre content was calculated using the following equation:

$$CF = \left(\frac{DW_{FC} - ash_{CF}}{WW_{CF}}\right) \times 100$$

Crude fats (EE) of prepupae were determined using the diethyl ether reagent method, Official Method 920.39 (AOAC 2002): two 2 g samples were placed in a soxhlet fat beaker (WW_{EE}), 50 ml of diethyl ether was added, and samples placed in a Tecator Soxtex System HT1043 Extraction Unit, samples were dried for 2 hours at 100°C (DW_{EE}), and CF was calculated using the following equation:

$$EE = \left(\frac{DW_{EE}}{WW_{EE}}\right) \times 100$$

Gross energy (GE) of prepupae was measured using a CP 200 isothermal bomb calorimeter (IKA, Germany): two 0.5g samples were pelletized, then placed in the bomb which was filled with pure oxygen up to 3000kPa, the bomb was placed in the isothermal bomb calorimeter and GE was directly measured in MJ kg^{-1}, and standardised with benzoic acid.

Amino acids (AA), excluding tryptophan and cysteine, determined using hydrolysis, high performance liquid chromatography (HPLC), and a fluorescence detector (Cunico *et al.* 1986): a 0.1g sample was placed into a hydrolysis tube, and 6ml of 6N HCl and 15% phenol solution added, samples were placed under a vacuum, and N added under pressure, samples were sealed off with a blue flame and left to hydrolyse for 24 hours at 110°C, samples underwent a pre-column derivatisation of AAs, and were separated using HPLC, finally AAs were detected using a fluorescence detector.

To determine whether heavy metals accumulate in prepupae, a bioaccumulation factor (BAF) will be calculated using the following equation (Walker 1990):

$$BAF = \frac{heavy\ metal\ concentration\ in\ prepupae}{heavy\ metal\ concentration\ in\ faecal\ sludge}$$

5.3.6. Data analysis

Data were entered into Excel 2013 (Microsoft, Washington, USA), and analysed using Stata 13 (Statacorp, Texas, USA). When described in subsequent results, faecal sludge (FS) refers to the

excavated pit latrine material which was utilised throughout the experiment, while residue is defined as the remaining mass of biodegraded FS at the end of the experiment. Layer, presence of BSFL, and change in FS and residue physical and chemical characteristics: tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, and heavy metals, were investigated for association with the outcome variables, FMR and prepupal data: total solids, ash, dry weight, bioconversion, CP, CF, EE, GE, and AAs. Faecal matter reduction, prepupal dry weight and bioconversion data were calculated using methods previously described (see Chapter 4).

Data were tested for normality visually using qnorm and pnorm functions, and histograms, and statistically using the Shapiro-Wilk, Shapiro-Francia, and Skewness-Kurtosis tests. Data which were non-normally distributed were transformed, but still found to be non-normally distributed, therefore were analysed using the non-parametric a Mann-Whitney test. Normally distributed data were analysed using univariate and multivariate linear regression. Univariate analyses defined significant variables to be retained for the multivariate analysis (*F*-test; $P < 0.1$). In the multivariate analysis, non-significant variables (*F*-test; $P > 0.05$), and interactions between significant variables (*F*-test; $P > 0.01$), were dropped in a backwards stepwise analysis until all variables were significant. Data were un-blinded after analysis.

5.3.7. Ethical clearance

Ethical approval for this study was granted by LSHTM Observational/Interventions Research Ethics Committee (#5972, amendment #A394) (Appendix B).

5.4. Results

5.4.1. Faecal matter reduction

5.4.1.1. Wet weight faecal matter reduction

Wet weight FMR (Figure 5-1) was significantly affected by the presence of BSFL ($F = 6.60$; df = 1, 58; $P = 0.013$), and layer depth ($F = 14.72$; df = 4, 55; $P < 0.0001$). The highest wet weight FMR occurred in FS from 0 – 20cm deep, in the presence of BSFL (65%, 95% CI 58.1 – 71.8),

significantly higher than the 101 – 150cm layer ($\overline{\Delta}$ wet weight reduction = 26.3; 95% CI 16.5 – 36.1; $P < 0.001$), and the combined layer ($\overline{\Delta}$ wet weight reduction = 13.2; 95% CI 3.5 – 13.2; $P = 0.01$). However FMR was not significantly higher than 21 – 50cm deep ($\overline{\Delta}$ wet weight reduction = 0.2%; 95% CI -9.6 – 9.9; $P = 0.97$), or 51 – 100cm deep ($\overline{\Delta}$ wet weight reduction = 8.1%; 95% CI -1.7 – 17.8; $P = 0.1$).

The wet weight FMR was significantly higher in the presence of BSFL in FS from 0 – 20cm deep ($\overline{\Delta}$ wet weight reduction = 8.7; 95% CI 3.5 – 13.9; $P = 0.004$), and moderately significantly higher than 21 – 50cm deep ($\overline{\Delta}$ wet weight reduction = 9.0; 95% CI 0.1 – 17.9; $P = 0.048$). However, there was no significant difference when BSFL were present or when absent in FS from 51 – 100cm deep ($\overline{\Delta}$ wet weight reduction = 8.2; 95% CI 5.8 – 22.1; $P = 0.22$), 101 – 150cm deep ($\overline{\Delta}$ wet weight reduction = 3.6; 95% CI -8.0 – 15.2; $P = 0.5$), and the combined layer ($\overline{\Delta}$ wet weight reduction = 10.3; 95% CI -1.4 – 22.0; $P = 0.078$).

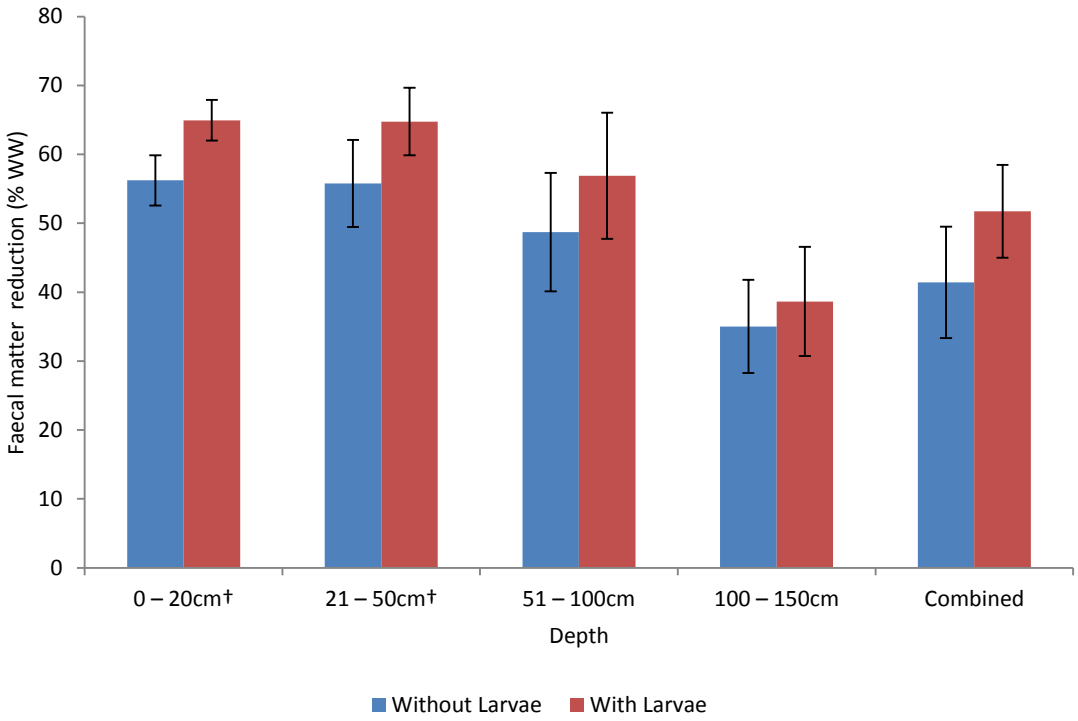

Figure 5-1 Arithmetic mean wet weight faecal matter reduction (FMR), including 95% CIs, of faecal sludge in the presence and absence of BSFL; depths followed by a † indicate a significant difference in FMR between BSFL factor (*F*-test; $P < 0.05$)

Interactions between change in pH, tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, heavy metals, and presence of BSFL and layer did not have a significant effect on FMR (*F*-test; $P > 0.05$, see Appendix G).

5.4.1.2. Invasive species

The presence of invasive species of filth fly larvae was found in 86% of interventions without BSFL, however there was no significant difference in FMR ($\overline{\Delta}$ wet weight reduction = 0.7%; 95% CI -13.5 – 12.1; $F = 0.01$; df = 1, 28; $P = 0.91$). No invasive larvae were discovered in interventions with BSFL.

5.4.1.3. Dry weight faecal matter reduction

Dry weight FMR data were calculated using the FS wet weight and TS, and residue wet weight and TS. However, errors occurred at some point during the experiment. These errors resulted in replicates where residue dry weight was higher than the FS dry weight, a net mass gain. A net gain in mass is impossible, as no other solids were added to the replicates, and 23.3% of calculations returned erroneous results. Therefore, it was decided to exclude dry weight FMR data from analysis, as the source of the error could not be discovered.

5.4.2. Prepupal production

The prepupal dry weight (Figure 5-2) was not significantly affected by the depth of FS ($F = 1.47$; df = 4, 21; $P = 0.25$). However, the prepupal dry weight of BSFL fed on FS from 21 – 50cm deep, (0.0133g; 95% CI 0.0102 – 0.0164), was significantly higher than 101 – 150cm deep ($\overline{\Delta}$ prepupal weight reduction = 0.0069; 95% CI 0.0007 – 0.013; $P = 0.03$). Dry weight bioconversions (Figure 5-3) were not significantly affected by layer ($F = 2.55$; df = 4, 25; $P = 0.065$). The highest bioconversion was in FS from 51 – 100cm (6.5%; 95% CI 3.9 – 9.1), significantly higher than FS from 101 – 150cm ($\overline{\Delta}$ bioconversion reduction = 4.8; 95% CI 1.1 – 8.5; $P = 0.012$). However, there was no significant difference between the bioconversion rates in depths 0 – 20cm, 21 – 50cm, 51 – 100cm, and the combined layer FS (*F*-test; $P > 0.05$). Changes in pH, tCOD, sCOD,

NH_4^+, protein, carbohydrates, VFAs, and heavy metals do not have a significant effect on prepupal dry weight or bioconversion (*F*-test; $P > 0.05$, see Appendix G).

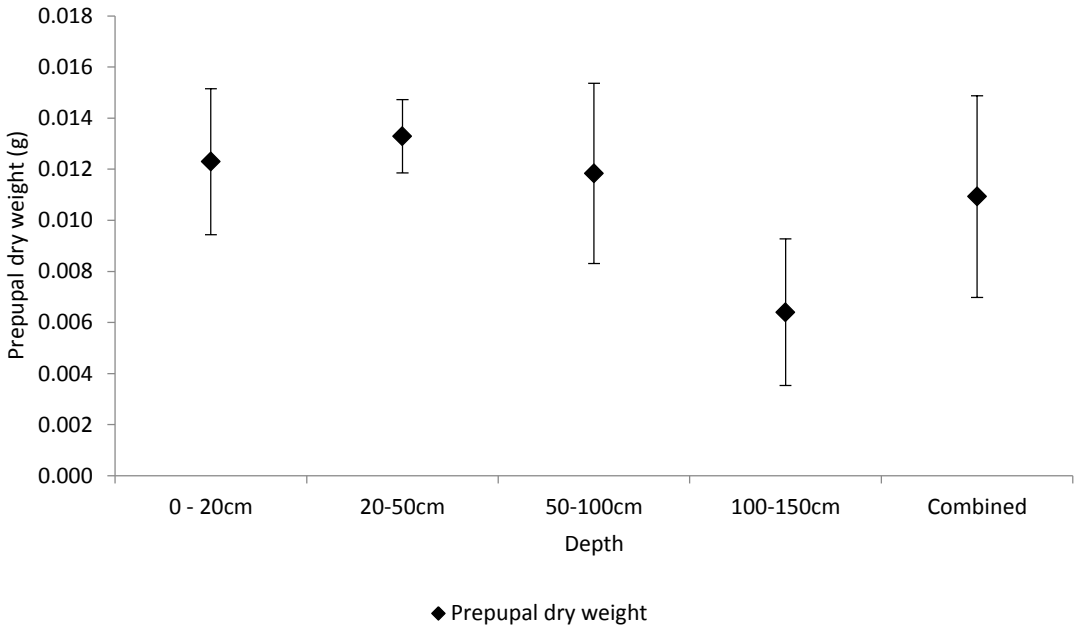

Figure 5-2 Arithmetic mean prepupal dry weight, including 95% confidence intervals, of BSFL reared on pit latrine faecal sludge from different depths

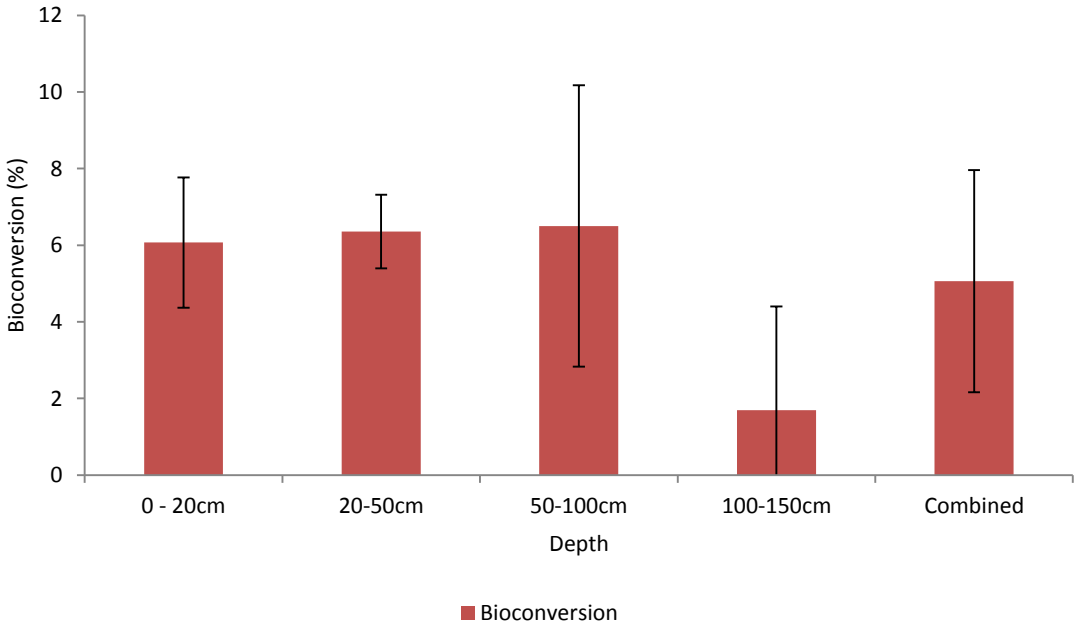

Figure 5-3 Arithmetic mean dry weight bioconversion, including 95% confidence intervals, of faecal sludge from different depths

The pupation data (Table 5-1) collected during the experiment were not normally distributed. The data were excluded from analysis, as over 100% of the BSFL placed reached prepupal stage in every depth, except 101 – 150cm, where mortality was 100% in FS from two latrines.

Table 5-1 Mean percentage of BSFL reaching prepupal stage, including range, on different depths of faecal sludge

Depth	Mean pupation (%)	Range
0 – 20cm	134	(118 - 151)
21 – 50cm	124	(111 - 138)
51 – 100cm	139	(132 - 151)
101 – 150cm	68	(0 - 159)
Combined	129	(114 - 151)

5.4.2.1. Prepupal nutritional composition

"Layer" significantly affected crude protein content of prepupae (F = 6.72; df = 4, 24; P = 0.0009). Prepupae which developed on FS from 21 – 50cm deep had the highest crude protein (35.3%; 95% CI 32.3 – 38.3), significantly higher than 101 – 150cm deep ($\tilde{x}$ crude protein reduction = 9.4; 95% CI 4.9 – 13.8; P < 0.001), and the combined layer of FS ($\overline{\Delta}$ crude protein reduction = 7.6; 95% CI 3.3 – 11.8; P = 0.001). There was no significant difference between crude protein content of prepupae reared on FS from 0 – 20cm ($\overline{\Delta}$ crude protein reduction = 2.3; 95% CI -6.5 – 1.9; P = 0.28), and 51 – 100cm deep ($\overline{\Delta}$ crude protein reduction = 2.9; 95% CI -7.1 – 1.4; P = 0.18). "Layer" also had a significant effect on ash content of prepupae (F = 3.68; df = 4, 24; P = 0.019). Highest ash content (48% of TS; 95% CI 40.6 – 55.5) was found in prepupae reared on 101 – 150cm deep FS, significantly higher than 0 – 20cm FS ($\overline{\Delta}$ ash reduction = 13.6; 95% CI 4.5 – 22.8; P = 0.005), and 21 – 50cm ($\overline{\Delta}$ ash reduction = 11.6; 95% CI 2.4 – 20.7; P = 0.016). There was no significant difference in ash content of prepupae reared on FS from 51 – 100cm ($\overline{\Delta}$ ash reduction = 5.8; 95% CI -3.4 – 14.9; P = 0.20), and the combined layer of FS ($\overline{\Delta}$ ash reduction = 4.2; 95% CI -5.0 – 13.4; P = 0.35).

Changes in pH, tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, and heavy metals did not have a significant effect on ash and crude protein contents of prepupae (*F*-test; $P > 0.05$, see Appendix G). Table 5-2 shows the proximate analysis of prepupae reared on FS, with data presented combined from all five layers of FS tested. Depth of FS and changes in pH, tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, and heavy metals do not have a significant effect on total solids, crude fat, crude fibre and gross energy (*F*-test; $P > 0.05$, see Appendix G).

Table 5-2 Proximate analysis, including 95% CI, of prepupae reared on faecal sludge

Parameter	Unit	Mean	95% CI
Total solids	%	24.4	(22.6 - 26.1)
Ash	% TS	41.4	(37.7 - 45.2)
Crude protein	% TS	31.9	(29.5 - 34.2)
Crude fat	% TS	3.4	(2.7 - 4.1)
Crude fibre	% TS	8.0	(7.1 - 9.0)
Gross energy	MJ kg^{-1}	8.2	(7.3 - 9.1)

Table 5-3 Mean amino acid content, including 95% CI, of prepupae reared on faecal sludge; amino acids followed by a * are essential amino acids

Amino Acid	Mean (% total)	95% CI
Histidine*	2.5	(2.3 – 2.6)
Serine	4.9	(4.7 – 5.1)
Arginine	4.0	(3.8 – 4.1)
Glycine	10.5	(10.0 – 10.9)
Aspartic acid	13.2	(12.2 – 14.3)
Glutamic acid	11.4	(11.1 – 11.8)
Threonine*	4.5	(4.5 – 4.6)
Alanine	7.1	(6.9 – 7.3)
Proline	5.9	(5.8 – 6.1)
Cysteine	0.4	(0.3 – 0.4)
Lysine*	4.9	(4.7 – 5.1)
Tyrosine	5.4	(5.2 – 5.6)
Methionine*	1.6	(1.4 – 1.7)
Valine*	6.8	(6.7 – 6.9)
Isoleucine*	4.6	(4.6 – 4.7)
Leucine*	7.6	(7.5 – 7.8)
Phenylalanine*	4.7	(4.6 – 4.9)

Table 5-2 presents the AA contents of prepupae reared on FS, where data presented are combined from all five layers of FS tested. Depth of FS and changes in pH, tCOD, sCOD, NH_4^+,

protein, carbohydrates, VFAs, and heavy metals do not have a significant effect on AA content of prepupae (*F*-test; $P > 0.05$, see Appendix G). Table 5-4 shows the heavy metal and micro-nutrient, and the bioaccumulation factor of prepupae reared on FS. The results indicate that Cadmium, Manganese, Mercury, and Zinc all have bioaccumulation factors of over 1.0, meaning the prepupae absorbed the heavy metals into their bodies.

Table 5-4 Mean heavy metal and mineral concentration and bioaccumulation factor, including 95% CI, of prepupae reared on faecal sludge

Element	Periodic Symbol	Heavy metal concentration		Bioaccumulation	
		Mean (mg kg^{-1})	95% CI	Mean (BAF)	95% CI
Aluminium	Al	4182	(2749 – 5615)	0.49	(0.41 – 0.57)
Antimony	Sb	0.2	(0.1 – 0.2)	0.60	(0.44 – 0.77)
Arsenic	As	0.7	(0.5 – 1.0)	0.55	(0.47 – 0.64)
Cadmium	Cd	2.2	(1.0 – 3.4)	5.07	(4.14 – 6.01)
Chromium	Cr	43	(31 – 54)	0.29	(0.22 – 0.37)
Cobalt	Co	5.2	(3.4 – 7.0)	0.39	(0.31 – 0.46)
Copper	Cu	62	(48 – 76)	0.84	(0.74 – 0.93)
Iron	Fe	3434	(2007 – 4862)	0.33	(0.27 – 0.39)
Lead	Pb	7.4	(3.2 – 11.5)	0.65	(0.49 – 0.81)
Manganese	Mn	539	(450 – 627)	1.08	(0.95 – 1.20)
Mercury	Hg	0.1	(0.0 – 0.2)	1.72	(1.28 – 2.16)
Molybdenum	Mo	3.1	(2.7 – 3.4)	0.62	(0.55 – 0.70)
Nickel	Ni	23	(17 – 28)	0.35	(0.28 – 0.42)
Selenium	Se	1.6	(1.4 – 1.9)	0.86	(0.76 – 0.97)
Tin	Sn	2.6	(2.2 – 3.0)	0.75	(0.66 – 0.85)
Vanadium	V	9.4	(5.0 – 13.8)	0.32	(0.26 – 0.38)
Zinc	Zn	762	(611 – 912)	1.02	(0.81 – 1.24)

5.4.3. Residue analysis

Table 5-5 summarises the chemical characteristics of FS before the experiment, and the remaining residue afterwards. These results show that only tCOD and protein were changed significantly by the presence of BSFL, with the presence of BSFL resulting in an increased tCOD reduction, and decreased protein reduction. The presence of BSFL did not significantly affect the percentage change of sCOD, NH_4^+, carbohydrates and VFA.

Table 5-6 summarises residue mineral content, however there was no significant difference between the mineral content of interventions in the presence and absence of BSFL. This

implies that the feeding action of the BSFL does not significantly decrease the fertiliser value of the residue, compared to FS which has only undergone aerobic digestion.

Table 5-5 Summary statistics of chemical characteristics; including mean and 95% CI, of faecal sludge and residue, and mean percentage change; parameters followed by [a] indicate the % reduction in parameter is significantly different between replicates with and without BSFL (Mann-Whitney test; *P* < 0.05)

		Faecal Sludge			Residue		% Change
Parameter	units	Mean	95% CI	BSFL Present?	Mean	95% CI	Mean
Total solids [a]	%	28.9	(27 – 31)	No	51	(46 – 56)	42
				Yes	62	(57 – 67)	52
pH		7.2	(7.12 – 7.28)	No	8.56	(8.35 – 8.77)	16
				Yes	8.54	(8.31 – 8.77)	17
tCOD [a]	g kg^{-1} TS	788	(616 – 901)	No	253	(165 – 340)	-67
				Yes	129	(83 – 174)	-82
sCOD	g kg^{-1} TS	103	(82 – 125)	No	6	(4 – 9)	-91
				Yes	7	(5 – 9)	-89
NH_4^+	mg kg^{-1} TS	122	(99 – 146)	No	28	(19 – 37)	-74
				Yes	31	(23 – 39)	-72
Protein [a]	g kg^{-1} TS	91	(79 – 102)	No	98	(73 – 124)	20
				Yes	61	(44 – 78)	-25
Carbohydrates	g kg^{-1} TS	774	(712 – 836)	No	779	(708 – 851)	2
				Yes	763	(689 – 837)	2
VFA	mg kg^{-1} TS	15	(13 – 17)	No	22	(13 – 32)	12
				Yes	28	(17 – 40)	54

Table 5-6 Mineral analysis of residue, mean and 95% CI, remaining after interventions with, and without BSFL

		without BSFL		with BSFL		regression model		
Nutrient	Unit	Mean	95% CI	mean	95% CI	*F*	df	*P*
Nitrogen	% TS	1.9	(1.5 – 2.4)	1.4	(0.9 – 1.8)	3.89	1, 52	0.054
Phosphorus	% TS	3.2	(2.7 – 3.6)	3.0	(2.5 – 3.6)	0.23	1, 52	0.63
Potassium	% TS	1.8	(1.4 – 2.1)	1.7	(1.3 – 2.1)	0.15	1, 52	0.70
Calcium	% TS	5.6	(4.9 – 6.3)	5.4	(4.7 – 6.1)	0.25	1, 48	0.62
Magnesium	% TS	1.4	(1.3 – 1.6)	1.4	(1.2 – 1.6)	< 0.01	1, 50	0.99
Sodium	mg kg^{-1}	7859	(6370 – 9347)	7700	(5730 – 9670)	0.02	1, 52	0.90
Iron	mg kg^{-1}	8852	(5806 – 11899)	12587	(8514 – 16660)	2.26	1, 51	0.14
Copper	mg kg^{-1}	72	(65 – 79)	76	(66 – 86)	0.38	1, 52	0.54
Zinc	mg kg^{-1}	1112	(858 – 1367)	1145	(871 – 1419)	0.03	1, 52	0.86
Manganese	mg kg^{-1}	530	(440 – 619)	572	(460 – 683)	0.37	1, 51	0.55
Boron	mg kg^{-1}	27	(21 – 33)	35	(27 – 43)	2.59	1, 52	0.11
Aluminium	mg kg^{-1}	7770	(5590 – 9951)	9126	(6914 – 11339)	0.81	1, 52	0.37
Sulphur	% DM	0.7	(0.6 – 0.8)	0.7	(0.6 – 0.8)	0.05	1, 52	0.83

There was a significant difference in nitrogen, potassium, sodium and copper concentrations in different layers (Table 5-7). However, there was no significant change in phosphorus, calcium, magnesium, iron, zinc, manganese, boron, aluminium, or sulphur with increasing depth. Nitrogen concentrations were highest in residue of the first 50cms of layers, while potassium, sodium, and copper levels were highest in residue from the first 100cms of layers. There was a significant reduction in concentration of all four nutrients from the first 50cms of residue compared with residue from 100 – 150cm layers.

Table 5-7 Mineral analysis of faecal sludge residue significantly affected by layer, including arithmetic mean and 95% CI

					Regression model		
Nutrient	Layer	Mean	95% CI	*P*	F	Df	P
Nitrogen (% TS)	0 – 20cm	2.3	1.6 – 2.9	0.7	4.97	4, 49	0.0019
	21 – 50cm	2.4	1.7 – 3.1	-			
	51 – 100cm	1.1	0.3 – 2.1	0.005			
	101 – 150cm	1.1	0.5 – 1.6	0.002			
	Combined	1.3	0.8 – 1.8	0.01			
Potassium (% TS)	0 – 20cm	2.3	1.7 – 2.9	-	2.77	4, 49	0.038
	21 – 50cm	1.8	1.3 – 2.3	0.17			
	51 – 100cm	1.8	1.0 – 2.5	0.21			
	101 – 150cm	1.1	0.6 – 1.6	0.002			
	Combined	1.7	1.0 – 2.5	0.14			
Sodium (g kg^{-1})	0 – 20cm	11.2	8.8 – 13.7	-	7.47	4, 49	0.0001
	21 – 50cm	9.4	6.7 – 12.2	0.23			
	51 – 100cm	8.7	5.8 – 11.6	0.13			
	101 – 150cm	4.2	2.5 – 5.9	< 0.001			
	Combined	5.6	3.2 – 8.0	< 0.001			
Copper (mg kg^{-1})	0 – 20cm	76	70 – 83	0.27	3.01	4, 49	0.027
	21 – 50cm	86	69 – 102	-			
	51 – 100cm	69	55 – 82	0.081			
	101 – 150cm	59	44 – 74	0.003			
	Combined	80	65 - 95	0.53			

5.4.4. Feasibility model

A business feasibility study was conducted by the HAAS Business School, University of California, Berkeley, in 2011 (see Appendix A) on the commercial viability of a decentralised BSFL FSM plant. Using data gathered from field interviews in Dar es Salaam, Tanzania, internet resources, research reports and publications, key assumptions were made to evaluate the

business model. Critical assumptions include the cost of emptying latrines, bioconversion rates, prepupal crude fat content, price of protein rich prepupal animal feed, percentage residue remaining, and price of residue as fertiliser (Table 5-8). The business model was recalculated using data collected in this study and updated product value estimates (Table 5-8).

Table 5-8 Key assumptions included in HAAS feasibility study, Sources: this study, current market prices for fishmeal and fertiliser, and estimated latrine emptying price (Still 2002)

Key assumptions	HAAS Model	Whole latrine FS	Top layer FS	Optimised model
Latrine emptying cost	\$327 day^{-1}	\$327 day^{-1}	\$327 day^{-1}	\$216 day^{-1}
Bioconversion rate (wet FS to wet BSFL)	11.5%	5.9%	6.3%	7.5%
Prepupae lipid content	30%	4.2%	4.6%	15%
Price / kg high grade BSF feed	\$1	\$1	\$1	\$1.8
Waste (residue) leftover after BSF processing	40%	45%	35%	40%
Price / ton of fertiliser	\$50	\$50	\$50	\$100

Table 5-9 Business models for decentralised faecal sludge management plants, with varying key assumptions for latrine emptying costs, bioconversion, prepupal lipid content, faecal matter reduction (FMR), and cost/produce values

Model	Fertiliser sold?	Best case / worst case	HAAS Model	This Study (whole latrines)	This study (top layer)	Optimum
			Fixed investment breakeven and setup time (years)			
1	Yes	Best	0.90	0.94	1.05	0.54
		Worst	2.59	3.02	5.56	0.72
	No	Best	1.82	2.62	2.61	0.72
		Worst	-	-	-	1.22
2	Yes	Best	0.85	1.17	1.33	0.60
		Worst	1.95	5.09	12.75	0.84
	No	Best	1.23	3.87	3.76	0.81
		Worst	13.51	-	-	1.46
3	Yes	Best	0.53	0.50	0.56	0.39
		Worst	22.62	2.26	-	0.45
	No	Best	-	-	-	0.51
		Worst	-	-	-	1.26

Best Case Scenario: Assumes highest revenues & lowest costs + upfront capital expenditure, Worst Case Scenario: Assumes lowest revenues & highest costs + upfront capital expenditure

Three models were evaluated two times, with and without sale of residue as fertiliser: Model 1 – Crude Oil and BSFL feed, Model 2 – Biodiesel and BSFL feed, and Model 3 – BSFL feed only. Four situations were assessed for each model using key assumption data described above: original HAAS model, whole latrine FS, top layer FS, and an optimised model. Success of the model was determined by the fixed investment breakeven and setup time, and the best and worst case scenarios for each model are presented (Table 5-9). The situation with the fasted fixed investment breakeven and setup time was in an optimum situation, Model 3), where residue was sold as a fertiliser.

5.5. Discussion

The study presented here shows that the presence of BSFL had a significant effect on waste reduction, while layer had a significant effect on FMR, prepupal ash and crude protein content. Layer, change in pH, tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, and heavy metals had no significant effect on prepupal production, prepupal dry weight and bioconversion, or prepupal nutritional value, including: total solids, ash, crude protein, crude fat crude fibre, gross energy, and amino acid concentrations. There was no significant difference in residue mineral contents, whether BSFL had been present or not.

5.5.1. Faecal matter reduction

It is important to note that the FS depth had a significant effect on FMR efficiency. Importantly, there was no significant difference in wet weight FMR for the first metre of FS that was excavated from the pit latrines. This implies that BSFL could efficiently reduce the top metre of FS removed from a pit latrine, providing the FS was suitable. The highest wet weight FMR was approximately 65%. This was higher than BSFL FMR of top layer pit latrine material (≈58%) (see Chapter 4), fresh human faeces (55%) (Banks *et al.* 2014), and chicken manure (≈50%) (Sheppard *et al.* 1994). It is also far higher than *Musca domestica* reduction of chicken manure (≈30%) and cow manure (≈25%) (Morgan *et al.* 1975). However, the FMR is still lower

than BSFL reduction of MOW (≈76%) (Diener *et al.* 2011), and far below the FMR capability of Tiger worms (96%) (Furlong *et al.* 2014). It is important to note that the FMR on the combined FS layer, although lower than some layers, was still approximately 50%, comparable to previous research mentioned above. The implications of BSFL capacity to reduce waste will be discussed later.

The differences between FMR of FS in the presence or absence of BSFL were slightly smaller (≈8%) than in previous studies on pit FS (8.7%) (see Chapter 4), and far lower than previously reported by Lalander *et al.* (2013) when BSFL fed on fresh faeces (≈30%). The possible reasons for this were discussed previously (see Chapter 4). Additionally, in this study, several invasive species, including house flies (sp. *Musca domestica*), drain flies (family Psychodidae), and thrips (order Thysanoptera), laid eggs, which developed into larvae, in FS replicates without BSFL. The presence of the invasive species was not ascertained until a week into the experiment, subsequently all containers were covered in netting. It is interesting to note that there were no invasive larvae in the replicates which contained BSFL. It cannot be concluded that filth flies did not oviposit in replicates containing BSFL, however it can be established that no invasive larvae survived. This conclusion is supported by previous research that demonstrates the presence of BSFL reduces the ovipositing, or prevents larval development, of filth flies (Furman *et al.* 1959, Sheppard 1983, Bradley *et al.* 1984). Although the cause of BSFL inhibiting filth fly oviposition has not been firmly clarified, there are a number of suggestions. Kilpatrick *et al.* (1959) proposed that the presence of BSFL altered the feeding environment, making it unsuitable for the development of filth flies. While Bradley *et al.* (1984) suggested that interspecific semiochemical communication between BSFL and gravid *M. domestica* females reduces oviposition. Additionally, it has been shown how the presence of BSFL significantly reduces volatiles, attractive to *M. domestica*, produced by human faeces after 11 days of feeding (Banks 2010). However, the infestation which occurred in the current study happened within the first week, with 6 days old BSFL, 10 days younger than used in the

previous study (Banks 2010). The 6 day old BSFL were too small to effectively alter the FS, resulting in no noticeable difference in odour reduction between paired replicates with and without BSFL (Banks, LSHTM, personal observation). This supports the theory of semiochemical communication between BSFL and filth flies rather than a reduction in attractive volatiles produced by the FS. Considering this study was not designed to test attractiveness of FS containing BSFL, it is important that future work is conducted to further investigate the subject. It is also important to investigate how BSFL as a FSM method, if correctly managed, could help control filth fly populations, and it may be possible to identify filth fly repellent semiochemicals that could be used to help control these mechanical disease vectors.

The results show that the change in chemical characteristics, pH, tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, and heavy metals, had no significant effect on the FMR efficiency of BSFL when developing on FS. This is an original study, and the results suggest that BSFL are capable of efficiently reducing FS within a wide range of the characteristics tested (Table 5-5). However, future work is recommended to further investigate the effect of these characteristics on FMR efficiency of BSFL, specifically characteristics known to inhibit dipteran larval growth. Although the chemical characteristics fall within a similar range to FS identified by previous research (see Chapter 3), it is important to determine the upper or lower tolerances of BSFL.

It was also found that the BSFL significantly reduce the tCOD content of FS by approximately 82%, and sCOD content by approximately 89%. This is comparable to BSFL when feeding on organic leachates (Popa *et al.* 2012), where COD was reduced by approximately 79%. The reduction of COD has also be observed by another novel FSM technology, the Tiger worm, shown to remove between 81 – 87% COD from FS (Wang *et al.* 2011, Furlong *et al.* 2014).

Reporting dry weight FMR is more accurate than wet weight FMR, because it excludes the varying water concentrations in the FS and residue. However, an anomaly occurred during this study as described previously (see Chapter 4). Once again the authors could not identify the

source of the anomaly, and the wet weight FMR data has been presented in this study, resulting in less accurate indications of FMR.

5.5.2. Prepupal production

The results presented here show that there was no significant difference in prepupal weight for larvae reared on FS from the top metre or pit latrines or on the combined layer of FS. This implies that if FS from the top metre of a latrine, or the entire latrine emptied and mixed together, is used as a BSFL rearing material at a feeding rate of 50 mg larvae^{-1} day^{-1}, the prepupae that develop will be a uniform size. Unfortunately, the dry weight of the prepupae is approximately 2.5 times smaller than when BSFL are reared on MOW (Diener *et al.* 2011), and in previous studies on pit latrine FS (see Chapter 4). It is possible to increase prepupal size by increasing feeding rate (Myers *et al.* 2008, Diener *et al.* 2009, Banks *et al.* 2014). However, this would result in lower FMR and bioconversion levels, but increased growth rate. Again, the bioconversion rates produced by the experiment are similar across the first three layer depths, 0 – 100cm, and the combined layer. The bioconversion rates were approximately half that of MOW (Diener *et al.* 2011), and a third of fresh human faeces (Banks *et al.* 2014), however they were approximately 30% higher than swine manure (Newton *et al.* 2005) and chicken manure (Sheppard *et al.* 1994). Unfortunately the lowest depth of FS had very low bioconversion. This is due to the BSFL being unable to develop successfully on the lowest depth of FS, with FS from two latrines producing 100% mortality, therefore no prepupae. This is vital when considering what quantity of FS to empty from pit latrines.

The proximate analysis of the prepupae once again indicates that the depth of FS can affect the prepupal value as crude protein content was highest in prepupae reared on the top metre of FS. The prepupae had approximately a third lower crude protein content than in previous studies (Hale 1973, Booram *et al.* 1977, St-Hilaire *et al.* 2007b). However the protein content is comparable to prepupae reared on chicken layer mash at a higher feeding rate (Diener *et al.*

2009). Importantly, the total solids, crude fat, crude fibre, and gross energy contents of the prepupae do not vary significantly with depth of FS. While the crude fibre is comparable to previous studies (Booram *et al.* 1977), the crude fat content is ten times lower, and the ash content almost three times higher (Hale 1973, Booram *et al.* 1977, St-Hilaire *et al.* 2007b). The fat content of insects is largely dependent on their diet (Stanley-Samuelson *et al.* 1983), and it has been demonstrated that it is possible to increase prepupal fat content through the addition of high-lipid content fish offal (St-Hilaire *et al.* 2007a). This suggests that it could be possible to boost the lipid content of BSFL feed in FS through the addition of high-lipid wastes, such as used cooking oil which would otherwise be disposed of.

The AA content of BSFL (Table 5-10) reared on FS is comparable to previous research where BSFL were reared on swine manure (St-Hilaire *et al.* 2007b), and to *M. domestica* pupae (Calvert *et al.* 1969), another alternative protein source suggested for animal nutrition (Pieterse *et al.* 2014). Animal feed produced using *M. domestica* as a protein source has shown that the AAs present have a high bioavailability, which can be utilised efficiently by broiler chickens feeding. Considering that the AA composition of BSFL is similar to that of *M. domestica*, it is suggested that animal feeds composed of BSFL could be similarly suitable as a protein source for animals. It is suggested that future work in conducted to study the suitability of BSFL as a protein source for animals, however, and BSFL reared on human faeces must be sufficiently sterilised to remove any dangers presented by pathogen transmission.

5.5.2.1. Heavy metals

The concentrations of heavy metals in BSFL prepupae reared on FS are important when considering the use of BSFL as a source of animal protein. There are regulations inside the European Union (EU) that limit the concentrations of heavy metals in animal feed (Table 5-11). The current study found that the mean arsenic and mercury concentration is lower than, or

equal to the current animal feed regulations. However, the cadmium and lead concentrations are higher.

Table 5-10 Amino acid composition of BSF prepupae reared on faecal sludge (the present study), swine manure (St-Hilaire *et al.* 2007b), and house fly pupae reared on CSMA fly medium (Calvert *et al.* 1969); values reported as % of total protein, essential amino acids are marked with *

	This study (BSF prepupae)	(St-Hilaire *et al.* 2007b) (BSF prepupae)	(Calvert *et al.* 1969) (House fly pupae)
Amino Acid		Mean (% total)	
Histidine*	2.5	3.0	3.5
Serine	4.9	4.3	4.4
Arginine	4.0	6.8	5.7
Glycine	10.5	5.9	5.3
Aspartic acid	13.2	9.6	11.6
Glutamic acid	11.4	9.7	14.7
Threonine*	4.5	4.6	4.6
Alanine	7.1	7.8	5.7
Proline	5.9	6.2	4.2
Cysteine	0.4	-	0.5
Lysine*	4.9	6.7	7.1
Tyrosine	5.4	7.9	6.7
Methionine*	1.6	1.9	3.5
Valine*	6.8	7.2	4.6
Isoleucine*	4.6	5.2	4.8
Leucine*	7.6	8.0	7.2
Phenylalanine*	4.7	5.1	5.7

Table 5-11 Regulated heavy metal concentrations, mean ppm, found in BSFL prepupae reared on faecal sludge

Heavy metals	This study		(EU 2002)
(mg kg^{-1})	Mean	Range	Animal feed regulations
Arsenic	0.7	0.2 – 2.5	2
Cadmium	2.2	0.4 – 15	2
Lead	7.4	1.3 – 47.6	5
Mercury	0.1	0.01 – 0.9	0.1

The concentrations of cadmium and mercury could potentially limit the use of BSFL prepupae as a source of protein in animal feed when considering the bioaccumulation factor (BAF). Cadmium and mercury have a BAF over 1.0, 5.07, and 1.72 respectively (Table 5-4). The BAF is an indication of the concentration of heavy metals in the prepupae compared to the FS they

were reared in, where a value over 1.0 indicates the prepupae absorbed more heavy metal than excreted (Walker 1990).

It is possible to reduce the heavy metal concentrations in prepupae by avoiding contaminated food sources, however this is not always possible, especially considering the variation found in human faeces and FS (see Chapter 3). While it is difficult to reduce the heavy metal concentrations of individuals' excreta, it could be possible to reduce concentrations in FS. It is known that batteries are a major source of heavy metal contamination (EC 2002), while there are reports that batteries are added to reduce FS volumes in rural and urban Tanzanian pit latrines (Biran 2010a, Biran 2010b). It is suggested that in areas where BSFL FSM is proposed, it could be possible to combine pit latrine emptying/BSFL in-situ treatment, with educational information and advice, recommending that batteries, and other sources of heavy metals, are no longer dumped into pit latrines. It is also recommended that future work continues to investigate into heavy metal contamination of prepupae, and also methods for separating the heavy metals from valuable fractions of the prepupae.

This study has shown BSFL reared on FS produce prepupae significantly smaller than in previous studies. However, their protein value and AA composition is comparable to alternative animal feed protein sources. The fat content of BSFL reared on FS is far lower than when reared on other food sources, however, it is suggested that the fat content could be increased by addition of high lipid wastes. The protein and fat content ultimately determine the value of the prepupae, which will be discussed further below.

5.5.3. Residue analysis

The results show how the pH of FS changes from neutral to alkaline over the course of the experiment, although there is no difference in final pH in replicates with and without BSFL. This is different to previous research which showed the presence of BSFL resulted in higher pH compared to controls without BSFL (Newton *et al.* 2005, Popa *et al.* 2012). However, in the

study presented, the controls were found to be infested with filth fly larvae. *Musca domestica* are known to increase the pH of the medium they are reared in (Takahashi *et al.* 1966), therefore their presence could have led to similar changes in residue pH. Similar changes in pH of FS have been found in vermifiltration systems using tiger worms (Furlong *et al.* 2014), thought to be due to ammonia excretion by the worms.

The reduction of NH_4^+ could be explained by the ammonia-ammonium equilibrium, which is determined by pH and temperature (Emerson *et al.* 1975). As pH increases, more ammonium is converted to ammonia. Increased levels of ammonium in FS treatments are caused by the presence of BSFL (Green *et al.* 2012). However, a previous study showed how the presence of BSFL resulted in an increase in total ammonium-nitrogen (Lalander *et al.* 2013), while increased ammonia production has also been recorded when *M. domestica* are used to manage pig manure (Čičková *et al.* 2012). An alternative suggestion is that the ammonium is oxidised into nitrites by ammonia-oxidising bacteria, and subsequently oxidised into nitrates by nitrite-oxidising bacteria {Hatzenpichler, 2012 #1388}.

The presence of BSFL has also been previously found to lower chemical oxygen demand compared to controls without BSFL (Popa *et al.* 2012). In a number of FS samples, the presence of BSFL increased the sCOD content compared to controls without BSFL. This could be due to the physical activity of the BSFL, creating a finer particulate compared with controls. This could permit more water to dissolve COD compounds.

There was a significantly lower protein content in the residue which contained BSFL, compared to residue without BSFL. This is unsurprising, as the consumption of proteins is vital to the successful development of insect larvae (Bennett 2000, Simpson *et al.* 2006). There was no difference in carbohydrates between residue with and without BSFL, and there is no significant change between the carbohydrate contents of FS and the residue. Two explanations have been suggested for these results. Firstly, BSFL have high activities of α-amylase, lipase, protease and

trypsin-like protease (Kim *et al.* 2011), resulting in efficient treatment of food-waste and organic materials, however, no lignin-modifying enzymes were identified, suggesting that BSFL are not able to digest lignocellulose. The second explanation is that there is an issue with the method used to determine carbohydrates. It is likely that both contribute to the lack of change in carbohydrates, although the method to determine carbohydrates has only been used once on FS (Irish *et al.* 2013), which also reported anomalous results, where carbohydrates content up to 100% of total solids.

5.5.4. Implications and feasibility

The study presented here has shown that BSFL are efficient at reducing the mass of FS from a variety of layers excavated from a pit latrine. It has also been shown that, while BSFL can develop on a variety of layers of FS, the bioconversion rates, and lipid content are reduced compared to previous studies. These factors are important when considering how to utilise BSFL FSM. There are a number of different techniques suggested, including in-situ BSFL and decentralised BSFL treatment plants.

This study has shown that an in-situ BSFL treatment solution is a theoretically viable method of treating FS. The results demonstrate that BSFL are capable of reducing FS from a variety of depths within a pit latrine, and a wide range of chemical and physical characteristics. Pit latrines with suitable characteristics, as discussed previously (see Chapter 4), could be seeded with BSFL. Once established, the results from this study suggest that BSFL will be able to reduce a large proportion of FS stored within latrines. In an optimum system, BSFL will consume all viable material, and then survive on fresh excreta which are deposited daily by users. Research still needs to be conducted into how BSFL prepupae can be harvested, and how the BSFL population is maintained, either through regular inoculation of BSFL into a pit, or luring female BSF to lay in the pit.

For decentralised BSFL FSM methods, the original HAAS business model was shown to be feasible under a number of best case scenarios (Table 5-9), where highest revenues and lowest costs are assumed. When residue was not sold as fertiliser, lipids were extracted and converted into biodiesel onsite, and prepupae were sold as animal feed, fixed investment breakeven and setup time would be in 1.23 years. When residue is sold as a fertiliser, all three business models are feasible, with the optimum being when only prepupae are sold as animal feed, taking on 0.53 years to breakeven and setup.

The reason why only selling prepupae can be profitable is due to the increased capital and operational costs involved with crude oil and biodiesel extraction and conversion. When assumptions were altered to incorporate values from the current study for bioconversion, prepupal crude fat content, and FMR, imitating a situation where FS from a whole latrine was excavated, Model 3 including the sale of fertiliser broke even in half a year (Table 5-9). When values for bioconversion, prepupal crude fat content, and FMR from only the top 20cm of FS were used in the model, imitating a situation where pit latrines were emptied more frequently, or only the top layer was excavated, once again Model 3 including the sale of fertiliser had the shortest breakeven time, with 0.56 years (Table 5-9). Surprisingly the return on investment was lower for the top 20cm of FS, even at the “best” scenario, than when FS from the whole latrine was used. However, upon closer review, it was determined that this was due to an increase in FMR of the top layer of FS, resulting in a reduced mass of residue to be sold as a fertiliser.

The optimum situation used achievable values for bioconversion rates, enhanced prepupal crude fat content (St-Hilaire *et al.* 2007a), FMR, increased product values based on current market prices for high-grade fishmeal and vermicompost organic fertiliser. Additionally, the optimum situation reduced the cost associated with emptying individual latrines. Under this situation all models, with and without selling fertiliser, were economically viable, ranging from

0.39 – 1.46 years (Table 5-9). It could be argued that an optimum scenario is a fabrication, however, considering the conservative assumptions made by the model for products sale value, and operational costs, and also considering methods of increasing bioconversion and enhancing fat content, it can be argued that an optimum scenario is feasible. Additionally, charging a latrine emptying cost lower than previously reported for similar services (Still 2002) will still benefit low-income households.

What can be established is that the sale of residue must be considered a virtually vital aspect to the successful BSFL FSM business model. This is largely due to the increased revenues gained by the sale of the residue as a fertiliser, but also due to the lack of an operational cost for disposal of residue. Considering the use of BSFL to manage FSM is the overall aim of developing it as a new technology, it is vital that research is conducted into management of the residue which is produced after BSFL feeding occurs. It is also recommended that alternative business models are investigated, which incorporate reduced operational costs associated with pit latrine emptying.

5.6. Conclusion

The study presented here has shown that BSFL are efficient at reducing FS excavated from the top 100cm of a pit latrine, and a combined layer representative of an entire pit latrine, with a range of physical and chemical characteristics. The prepupae that develop have a nutritious value comparable to BSFL reared on cow manure, and *M. domestica*. Although the crude fat content was lower than previous studies, further investigation is required into whether it is possible to enhance lipid content by addition of waste food oil to FS. The study also provides supporting evidence that there are semiochemicals produced by BSFL which could be used as a method of controlling filth flies, such as *M*. *domestica,* recommending further investigation.

The results gathered in this study have helped demonstrate that correct conditions, in-situ and decentralised BSFL FS treatments are viable methods of FSM. Also, under optimum situations,

a decentralised BSFL treatment plant has a strong business model. Solutions suggested in this study could help improve sanitation for billions of people around the world. However, further investigation is required to determine potential problems that may arise from the use of FS fed BSFL as an animal feed source, such as pathogen transmission, or heavy metal bioaccumulation. Further investigation into the composition of the residue and its use as a fertiliser or soil conditioner is highly recommended, as it is a vital source of income produced by FSM using BSFL. Additionally, investigation into liquid effluent quality produced by BSFL FSM is important, focusing on the potential hazard of nitrites and nitrification.

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Chapter 6) The effect of non-excreta additives in pit latrine faecal sludge on black soldier fly mortality

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6.1. Abstract

OBJECTIVES: To determine whether cleaning chemicals previously reported in pit latrine faecal sludge (FS) increase mortality of black soldier fly larvae (BSFL), and to discuss how any effect may influence BSFL faecal sludge management (FSM) methods.

METHODS: Serial dilutions (n = 6) of four cleaning chemicals used by householders were assessed: Jeyes Fluid, Madubula, High Test Hypochlorite (HTH), Pine antiseptic, together with a permethrin-based positive control and a distilled water negative control. Solutions were added to FS with 25 BSFL (replicates n = 4), at a feeding rate of 100mg larvae^{-1} day^{-1}, until 50% were prepupae. Mortality was measured, and dosage-mortality regression equations were calculated using PROBIT analysis to estimate a 50% lethal dose (LD_{50}) and a 20% lethal dose (LD_{20}).

RESULTS: Results showed Jeyes Fluid, Madubula, and the positive control had a significant influence on BSFL mortality ($P < 0.05$). While the HTH and Pine antiseptic had no significant effect on BSFL mortality ($P > 0.05$).

CONCLUSION: Although BSFL mortality can be influenced by cleaning chemicals in FS, the concentrations required to increase mortality over natural levels are far higher than manufacturer's guidelines and household owners reported use. It is unlikely that commonly used cleaning chemicals will affect BSFL FSM, although further work is recommended to determine the effects of other compounds, including: pesticides, insecticides, herbicides, pharmaceuticals, and hormone, which could potentially accumulate further down the food chain.

6.2. Introduction

The implementation of on-site sanitation is recommended (WHO/UNEP 2006) to improve public health (Esrey *et al.* 1991, Fewtrell *et al.* 2005) by preventing disease (Mara *et al.* 1999). With on-site sanitation, the emptying, transportation, and treatment of faecal sludge (FS) can cause problems (Helmer *et al.* 1997, Kariuki *et al.* 2003, WHO/UNEP 2006). These problems can be solved by managing FS on-site. One such method is to use the larvae of the black soldier fly (BSFL), *Hermetia illucens* (L.). The BSFL efficiently reduce FS from a range of pit latrines (see Chapter 5). The final larval stage of the BSFL, known as the prepupae, has an intrinsic value. The prepupae contain proteins and fats which can be used in animal feeds (Hale 1973, Newton *et al.* 1977, Bondari *et al.* 1987, St-Hilaire *et al.* , Hem *et al.* 2008). Although, it has been shown that BSFL reared on pit latrine FS have lower protein and fat (see Chapter 5) levels than found in previous studies (Hale 1973, Booram *et al.* 1977, St-Hilaire *et al.* 2007, Diener *et al.* 2009), although still suitable to be used in animal feed (Diener *et al.* 2009).

However, pit latrine FS contains more than just decomposing human faeces. It is known that pit latrine owners in South Africa use cleaning chemicals such as Jeye's Fluid, Madubula, chlorine, and pine disinfectant (see Chapter 3)(Buckley *et al.* 2008, Nwaneri 2009). While in Tanzania and Vietnam is has been reported that pit latrine owners use additives, such as ash, lime, and kerosene to prevent odours, insects, and to reduce sludge volumes (Biran 2010a, Biran 2010b, Biran 2010c). Cleaning chemicals are made up of various constituents, including: phenolic compounds, alcohols, and surfactants. Phenolic compounds have been found to reduce larval growth in herbivorous insects (Duffey *et al.* 1981, Kubo 1993), and surfactants have been shown to inhibit growth and increase mortality in aquatic dipteran larvae (Lewis *et al.* 1983, Lewis 1991, Ostroumov 2010).

The top four reported chemicals from a previous survey of South African latrine owners (see Chapter 3) were selected for testing: Jeyes Fluid (53%), Madubula (21%), Chlorine (15%), and

Pine antiseptic (11%). Jeyes Fluid and Madubula are both Tar Acid (carbolic acid) based disinfectants. While Madubula only lists Tar Acid as an active ingredient, Jeyes fluid contains Tar Acid, 4-chloro-m-cresol, Propan-2-ol, and Terpineol, and has a pH of 8.0 – 10.0. Tar Acids are phenols that have a wide spectrum of microbiocidal, fungicidal, and virucidal and are commonly used in areas with heavy soiling, such as on farms or for veterinary work (Jeffrey 1995). The chlorine that was reported to be used was High Test Hypochlorite (HTH)/Calcium Hypochlorite (Arch Chemicals, South Africa), a popular swimming pool water sanitiser. HTH granules contain 65 – 70% available chlorine, at low concentrations HTH is recommended by the WHO as a way to disinfect drinking water, with higher concentrations used to disinfect surfaces. Finally, pine antiseptics were reported to be used. Pine antiseptics contain pine oil, which is a phenolic disinfectant that is an effective microbicide, fungicide, but not an effective virucide.

The requirement for an alternative faecal sludge management (FSM) technology is necessary to help improve sanitation solutions around the world. The use of BSFL to manage faFS has been shown to effectively reduce FS under different rearing parameters (see Chapter 4), and from a range of latrines with variations in physical and chemical characteristics (see Chapter 5). However, it is important to consider what potentially dangerous chemicals could be present in pit latrine FS that could reduce the efficiency of BSFL. The present study aims to determine whether previously reported cleaning chemicals are lethal to BSFL feeding on FS. Dose response curves of chemicals reported in a survey conducted in South Africa (see Chapter 3) will be obtained to provide an indication of whether the quantities of chemicals reported to be used have a detrimental effect on BSFL.

6.3. Methods

6.3.1. Faecal sludge

Faecal sludge for the experiments was collected from non-chemical, portable toilet placed at the experimental site. In order to ensure that no chemicals were present in the FS, clear instruction regarding its use were provided, and the investigators emptied the toilet every two days, removing any toilet paper. Faecal sludge was homogenised using a drill with a paint mixer bit. Following mixing, the FS was divided into labelled batches of 500g, and frozen at -20^{O}C for 48hrs to kill any fly eggs/larvae present. The FS was stored in a refrigerator until required.

6.3.2. Non-excreta additives

In order to test toxicity of Jeyes Fluid, Madubula, HTH, and Pine antiseptic to BSFL, a serial dilution of each chemical was created (A – F). Additionally, two further concentrations were assessed during the experiment: the manufacturers recommended use (G), 1.2% Jeyes Fluid, 1% Madubula, 0.1% HTH, and 3% Pine antiseptic, and the household owners reported use (H), 0.12% Jeyes Fluid, 0.15% Madubula, 0.15% HTH, and 2% Pine antiseptic. A negative control (distilled H_2O) was tested, as well as a serial dilution of a positive control, Doom, a permethrin-based insecticide. A pyrethroid-based insecticide was selected based on a study which previously demonstrated BSFL susceptibility (Tomberlin 2001), however this study was conducted in 2001, with no subsequent research conducted. Table 6-1 shows the final concentration (g kg^{-1} FS), of cleaning chemicals, and the positive and negative controls tested. After preparing the solutions, a third party allocated randomly generated numbers to each solution, blinding the results from the investigators. The solutions were kept refrigerated at 4°C before use.

Table 6-1 Concentration, g kg^{-1}, of solutions tested on BSFL, including four cleaning chemicals reported to be used in South African latrines, Doom, a permethrin-based insecticide positive control, and distilled water negative control.

	Concentration (ID)							
Chemical	A	B	C	D	E	F	G†	H‡
Jeyes Fluid	503	50.3	5	0.5	0.05	0.005	6	0.60
Madubula	503	50.3	5	0.5	0.05	0.005	5	0.75
Pine Antiseptic	503	50.3	5	0.5	0.05	0.005	15	10
Chlorine	25	2.5	0.3	0.025	0.0025	0.00025	0.025	0.038
Doom (Positive Control)	5	1	0.1	0.01	0.001	0.0001		
Water (Negative Control)	503							

† Manufacturers recommended concentration, ‡ Household owners reported use

6.3.3. Black soldier fly larvae

The BSFL used in the experiments were collected from a colony at the Mariendahl Experimental Farm, maintained by AgriProtein Technologies and the University of Stellenbosch, South Africa. The method of BSF egg collection and early-stage larval rearing is described in a previous study (see Chapter 4). Twenty five larvae were counted and placed in containers for each replicate to be used in the experiment described below.

6.3.4. Experimental setup

A FS feeding rate of 100mg $larvae^{-1}$ day^{-1} was selected for the experiment, as it is suitable for the successful development of BSLF (see Chapter 4). The quantity of FS added was 17.5 grams per week, based on 25 larvae. Labelled 100ml plastic containers had 17.5g of FS added to them, then 8.8ml of chemical solution was mixed in, and the pH measured with a handheld meter (PHH-5012, Omega, UK). The addition of liquid raised the moisture content of the FS to approximately 75%. The moisture content was selected as it had significantly higher faecal matter reduction (FMR) than 65% and 85% (see Chapter 4). Also, it was found in preliminary experiments that FS with a higher moisture content (>75%) stimulated larval crawl-off. Each container had 25 larvae added to them. Four replicates, each containing 25 larvae, were tested for each solution (n=39), a total of 100 BSFL for each chemical concentration (WHO 2006). Each 100ml container was placed in a 1 litre plastic tub and covered with netting, preventing other flies from ovipositing. The experimental containers were stored in a controlled

environment, approximately 27°C, 70% RH, and a 12:12 day/night light cycle. Quantities of FS and chemical solution were premixed and added to replicates every 7 days which prevented mixing inside the 100ml containers, potentially damaging the larvae. The FS in the feeding containers was examined for surviving BSFL before re-feeding, and 24 hours after feeding. This procedure continued until BSFL started to develop into prepupae in over 50% of the chemical solutions tested. At this point all prepupae, larvae, and residues were counted and weighed.

6.3.5. Data analysis

Larval and prepupal count data were entered into Excel 2013 (Microsoft, Washington, USA). A PROBIT analysis, using SPSS Statistics 20 (IBM, New York, USA), was used to determine dosage-mortality regression equations plot a dose response curve for each chemical and calculate 50% lethal dose (LD_{50}) and 20% lethal dose (LD_{20}) for each chemical. Data were unblinded post-analysis.

6.3.6. Ethical clearance

Ethical approval for this study was granted by LSHTM Observational/Interventions Research Ethics Committee (#5972, amendment #A394) (see Appendix B).

6.4. Results

Mortality obtained in the negative control of distilled water was 20% (95% CI; -2.7 – 42.7). All results presented are adjusted for this mortality. The presence of Jeyes Fluid had a significant effect on BSFL mortality (*Z*-test = 7.3; $P < 0.001$), LD_{50} of 103g kg^{-1} (95% CI 47.1 – 229.5), and LD_{20} of 44.5g kg^{-1} (95% CI 9.1 – 84.5. Madubula had a significant effect on BSFL mortality (*Z*-test = 7.5; $P < 0.001$), LD_{50} of 134.5g kg^{-1} (95% CI 74.9 – 326.2), and LD_{20} of 82.8g kg^{-1} (95% CI 38.9 – 155.6). HTH and pine antiseptic had no significant effect on BSFL mortality, *Z*-test = 1.1; $P = 0.27$, $Z = 1.5$; $P = 0.13$, respectively. The Doom positive control had a significant effect on larval mortality ($Z = 2.5$; $P = 0.012$), however no confidence intervals could be predicted for the LD_{50} of 6.0g kg^{-1}, or LD_{20} of 2.0g kg^{-1}. The highest concentration of Doom, 5g kg^{-1}, only achieved a

58% (SE ± 9.3) mortality. Table 6-2 displays mean mortality, and 95% confidence intervals, for all chemical solutions tested. Starting pH of the negative control with water was 8.15. The pH of FS with chemicals ranged between 6.78 and 8.94, and had no significant effect on mortality (F = 2.24; df = 1, 37; P = 0.14).

Table 6-2 Mean mortality, including 95% CIs, for different concentrations of commonly used cleaning chemical solutions tested (n=39), plus pyrethroid-based insecticide positive control, and distilled water negative control.

	Concentration ID																	
	A		B		C		D		E		F		G		H			
Chemical	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Jeyes Fluid	100	(100 - 100)	19	(2.3 - 35.7)	25	(4.6 - 45.4)	11.5	(-13.5 - 60.0)	6	(8.0 - 46.0)	10.7	(1.9 - 70.1)	12.3	(-9.1 - 69.1)	11	(-16.0 - 54.0)		
Madubula	100	(100 - 100)	10	(-6.0 - 26.0)	42	(7.3 - 76.7)	20	(-2.7 - 42.7)	22	(-2.1 - 46.1)	36	(27.0 - 45.0)	47	(26.0 - 68.0)	17	(-9.7 - 43.7)		
Pine Antiseptic	69	(55.9 - 82.1)	26	(-4.5 - 56.5)	26	(-7.1 - 59.1)	26	(-7.9 - 59.9)	21	(-15.5 - 57.5)	33	(-1.6 - 67.6)	20	(1.3 - 38.7)	28	(-13.2 - 69.2)		
Chlorine	38	(13.3 - 62.7)	18	(4.8 - 31.2)	13	(-12.1 - 38.1)	32	(-8.3 - 72.3)	15	(-6.0 - 36.0)	18	(-16.7 - 52.7)	32	21.6 - 42.4)	33	(12.0 - 54.0)		
Doom (Positive Control)	58	(28.4 - 87.6)	25	(-0.7 - 50.7)	8	(-10.0 - 26.0)	28	(-3.6 - 59.6)	38	(11.8 - 64.2)	26	(-16.4 - 68.4)						
Water (Negative Control)	20	(-2.7 - 42.7)																

6.5. Discussion

The study presented here has shown that some commonly used cleaning chemicals have a significant impact on the mortality of BSFL when added to FS. It is evident that Jeyes Fluid and Madubula have a significantly negative affect on BSFL mortality. Low doses of Jeyes Fluid and Madubula, 44.5g kg^{-1} and 82.8g kg^{-1} respectively, can cause BSFL mortality equal to a negative control of distilled H_2O. However, the quantities of chemicals required to cause an increase in mortality is far higher than used in practice under user conditions. Manufacturers' guidelines recommend approximately 7.5 times lower concentration of Jeyes Fluid, and 16.5 times lower concentration of Madubula, to be used while cleaning. This implies that household owners would have to use the manufacturers recommended amount of Jeyes Fluid once a day, or twice a day for Madubula, to have a significant effect on BSFL mortality. Furthermore, pit latrine owners reported using 75 times lower concentration of Jeyes Fluid than was found to increase mortality, and 110 times lower concentration than Madubula. Therefore, it can be assumed that the use of Jeyes Fluid and Madubula would not have a negative effect on BSFL mortality if pit latrine owners continued to use it, and the FS was then fed to BSFL.

The results indicate that the use of HTH granules and pine antiseptic as cleaning chemicals is very unlikely to increase mortality of BSFL. The concentrations of both chemicals investigated never produced a 100% mortality result, which is why the PROBIT model could not estimate lethal doses. Considering that the highest concentration of HTH investigated was 10,000 times higher than the manufacturers recommended amount, and 650 times higher than pit latrine owners reported use, it is safe to assume that HTH granules will not have a detrimental effect on BSFL mortality. The same can be assumed for pine antiseptic, where the highest concentration investigated was 33 times higher than the manufacturers recommended amount, and 50 times higher than pit latrine owners reported use. However, chlorine users reported adding up to 27 litres of water along with the chlorine. This addition of water could

lead to the FS containing unsuitably high moisture content, resulting in the sludge needing to be dewatered before being fed to BSFL.

It must be considered that Jeyes Fluid, Madubula, HTH and pine antiseptic contain chemicals that are found in other cleaning chemicals which could potentially affect BSFL. It is known that phenolic compounds can reduce the larval growth of herbivorous insects (Duffey *et al.* 1981, Kubo 1993). It is important to increase how much is known about the effects of phenolic compounds on BSFL. Also, further work is necessary concerning surfactants which are found in pine antiseptics, and thousands of other cleaning products, and are known to inhibit growth of aquatic dipteran larvae (Ostroumov 2010).

The negative control produced a mortality of 20% (SE ± 7.1), equivalent to mortalities when BSFL were fed on dairy manure at a similar feeding rate (≈ 23%) (Myers *et al.* 2008). However, the mortality was high compared to a previous study that investigated the life history traits of BSFL, including mortality, at various relative humilities (≈ 3%) (Holmes *et al.* 2012). The positive control of a pyrethroid based insecticide powder, diluted in water, was expected to be capable of causing 100% mortality at most concentrations. This is because previous research had shown BSFL are susceptible to insecticides (Furman *et al.* 1959, Kilpatrick *et al.* 1959, Axtell *et al.* 1970), including pyrethroid-based insecticides (Tomberlin 2001). However, the positive controls produced curious results, with none of the concentrations causing 100% mortality. It is possible that the insecticide dust used, which was presented in a water solution, did not dissolve fully. This could explain how the reported results are from far lower concentrations than calculated. Alternatively, it is also possible that the BSFL have evolved a resistance to the insecticide, since South Africa manufactured over R700,000,000 (≈$100,000,000) and imported R850,000,000 (≈$120,000,000) worth of pesticides, insecticides, fungicides or herbicides in 2011 for use in crop protection, household and garden use, the evolution of an insecticide

resistance is feasible (StatsSA 2013). Further research is required to determine the levels of insecticides that are present in FS, and the susceptibility of BSFL to them.

The method of BSFL feeding on FS is important to consider when discussing the issues of chemical induced mortality, since this study was conducted in laboratory conditions, very different to real world situations. If the BSFL are utilised as an in-situ solution, where BSFL are inoculated into pit latrine vaults then later harvested for protein and fats, then the addition of cleaning chemicals will be highly varied. When a pit latrine owner cleans their latrines, and pour the excess chemicals and water into the vault, the chemicals will likely be concentrated over a small surface area, rather than uniformly spread throughout the FS. If chemicals cause specific areas to become unsuitable for BSFL, they will avoid feeding in them. Such behaviour has been seen in the past where BSFL have avoided areas of stagnating liquid (Diener *et al.* 2011), and preferentially fed around them. The BSFL are known to be tolerant of harsh environments, whether they are conducive for development or not. It is possible that the BSFL could adapt to the presence of chemicals, although this could extend development time. It has been reported that pit latrine owners add kerosene, ash (Biran 2010c), lime and charcoal (Biran 2010a) to reduce odours emanating from pit latrines. However, previous research determined BSFL feeding on fresh human faeces significantly reduce the concentration of volatiles released, compared to controls where BSFL were absent (Banks 2010). There is also anecdotal evidence that residue, post-BSFL treatment, have far lower odour intensities compared to residue which did not contain BSFL (*personal observation*). This implies that in an in-situ BSFL treatment situation, the action of the BSFL could help reduce odours emanating from a pit latrine, increasing user acceptance, while potentially reducing egg laying of disease carrying filth flies, such as *Musca domestica*. Future work is necessary to further investigate what effect BSFL have on pit latrine odours, and user acceptance.

However, if FS is excavated from vaults, and processed at a decentralised BSFL treatment plant, it is important to consider the effect chemicals could have on mortality. It can be assumed that after emptying, FS would be mixed, and any excess garbage removed. The FS may also have water added, or be dewatered, to achieve an appropriate moisture content for BSFL feeding. During these processes, it is expected that any chemicals that may be present in concentrated areas, will be uniformly spread throughout the FS. The results reported here suggest that at recommended and reported concentrations, the chemicals investigated here would not increase the mortality of BSFL. Additionally, although the chemicals added to the FS altered the pH, the range was similar to the pH of FS found in previous studies which were suitable for BSFL development (see Chapter 5). However, the chemicals investigated here are just a small percentage of possible cleaning chemicals which could end up in FS. It is important for future work to determine what range of cleaning chemicals is in use in areas where BSFL FSM could be implemented.

6.6. Conclusion

This study showed that BSFL mortality can be influenced by cleaning chemicals in FS. However, the quantities required to increase mortality over natural levels are so high that it is improbable they would ever be added to latrines by the users. Even if household owners follow manufacturers' guidelines, then the presence of cleaning chemicals in FS should not affect BSFL mortality implying no change in behaviour would be required from household owners. It is also important to continue research into the effects of cleaning chemicals and other non-excreta additives, such as ash, kerosene, lime, or pit additives used to reduce pit filling have on BSFL. In addition, future work needs to be conducted to determine whether more pervasive compounds, including: pesticides, insecticides, herbicides, pharmaceuticals, and hormones, could have a negative impact on BSFL mortality, and potentially accumulate further down the food chain. Faecal sludge potentially has a wide variety of components which could affect BSFL. Therefore it is important to consider them in respect to FSM. However, this

study has provided initial evidence that commonly used chemicals have a small effect on BSFL, further providing evidence for the use of black soldier fly larvae as a novel human FSM system.

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Chapter 7) Discussion and Conclusion

7.1. General discussion

Improved sanitation is vital to improving social and economic development in low- and middle income countries (Mara *et al.* 2010). On-site sanitation is recommended as the most suitable method (WHO/UNEP 2006) to improve sanitation globally, however, issues arise when pit latrines become full and require emptying. The lack of faecal sludge (FS) treatment and dumping sites means there is a requirement for novel faecal sludge management (FSM) technologies.

The use of black soldier fly larvae (BSFL) has been suggested for a number of reasons, including: suitability for artificial rearing (Sheppard *et al.* 2002), ability to develop on and efficiently reduce, a variety of organic materials, (Newton *et al.* 2005, Diener *et al.* 2011), yielding a valuable prepupae high in protein and fat (Newton *et al.* 2005) which can be used as a protein source for animals (Hale 1973, Newton *et al.* 1977, Bondari *et al.* 1987, St-Hilaire *et al.* 2007b, Hem *et al.* 2008), or have fats transformed into biodiesel (Li *et al.* 2011a, Li *et al.* 2011b, Zheng *et al.* 2012a, Zheng *et al.* 2012b), the BSFL have also been shown to reduce pathogens in chicken and dairy manure (Erickson *et al.* 2004, Liu *et al.* 2008), and human faeces (Lalander *et al.* 2013), and are known to develop in pit latrines (Kilpatrick *et al.* 1956, Irish *et al.* 2013), and on fresh human faeces (Banks 2010, Lalander *et al.* 2013).

For these reasons, it was suggested that BSFL could be an environmental, scalable, and suitable FSM technology, which could reduce indiscriminate dumping and disease, and provide an income for entrepreneurs. However, a number of questions have to be answered before BSFL can be used in FSM. Previous research has shown that they can digest fresh human faeces (Banks 2010, Lalander *et al.* 2013), but there was little data on how key rearing parameters affected their development. There was also little data on their ability to develop on, and digest, pit latrine FS. It was vital to determine how effectively BSFL were at faecal matter

reduction (FMR) and prepupal biomass production while feeding on fresh faeces and pit latrine FS under different key rearing parameters. It was also a priority to determine variations in the physical characteristics, and chemical components of different layers of pit latrine FS, and what effects they had on BSFL development. Finally, it was important to understand the effect of commonly used cleaning chemicals, which could be present in FS, on the mortality of BSFL.

This study has shown that BSFL can develop successfully on fresh human faeces. Growth rate plasticity means that larvae are capable of successfully developing on a range of resources that may be transient in nature (Metcalfe *et al.* 2001, Tu *et al.* 2003, Wright *et al.* 2003, Dmitriew *et al.* 2005, Dmitriew 2011), and implies that BSFL could be capable of consuming FS with a range of nutritional contents, while still being capable of developing into valuable prepupae. The study has shown that BSFL are effective at FMR. Furthermore bioconversion rates of human faeces into prepupal biomass are more efficient than when reared on swine manure, chicken manure and municipal organic waste (MOW) (Sheppard *et al.* 1994, Newton *et al.* 2005, Diener *et al.* 2011). The results supported the use of BSFL as a novel FSM solution. However more research was needed to determine BSFL ability to consume pit latrine FS.

It was necessary to determine the physical and chemical characteristics of FS from pit latrines in a country which could benefit from a novel technology. The study showed that physical and chemical characteristics of FS collected in this study were comparable to research also conducted in South Africa (Bakare 2014), and Tanzania (Irish *et al*., 2013, Torondel, LSHTM, unpublished data). The results indicate similar trends in the total solids (TS), total chemical oxygen demand (tCOD), protein, volatile fatty acids (VFAs) and carbohydrates, as depth increased to previous studies in South Africa (Bakare 2014), Tanzania, and Vietnam (Torondel, LSHTM, unpublished data). The current study found that FS collected had lower admissible heavy metal concentrations than European Union (EU) standards for FS which can be used in agriculture, except copper and mercury (EU 1986). It was found that the physical and chemical

characteristics of the FS were suitable for BSFL development (Fatchurochim *et al.* 1989, Popa *et al.* 2012). The study implies that, although there is variation in FS between, and within, latrines, which was to be expected, there will be regularity between different countries, with FS suitable for BSFL development. These results can be extrapolated to suggest that BSFL as a novel FSM technology could be suitable for many countries around the world, depending on their ability to develop successfully on FS.

Prior to testing the ability of BSFL to develop on a range of different FS, it was important to identify how key rearing parameters affected BSFL FMR and prepupal production when reared on homogenised top layer FS. This study showed that the most effective FMR (≈58%) was comparable to BSFL FMR of chicken manure (≈50%) (Sheppard *et al.* 1994), and higher than common houseflies, *Musca domestica,* FMR of chicken manure (≈30%) and cow manure (≈25%) (Morgan *et al.* 1975). This indicated that the use of BSFL to manage human FS would effectively reduce the mass of the FS by half, resulting in easier residue management, post-processing. The study showed that key rearing parameters had a significant effect on FMR, and prepupal production, of BSFL when they developed on FS. Moisture content, FR and LD can be controlled in a decentralised FSM plant. Moisture content can be controlled by the drying, or addition of water, of FS. The FR used in a FSM plant will depend on what the desired outcome of the plant is. If a treatment plant's primary aim is to reduce the mass of FS to a safer residue, then low a feeding rate will result in more efficient FMR, but lower prepupal production. Conversely, if prepupal production is the primary aim of a BSFL treatment plant, and FMR secondary, then a higher FR would be beneficial. The study also suggests that the purpose of a decentralised BSFL treatment plant can be tailored by adjusting the key parameters investigated. When considering using an in-situ BSFL treatment method, FS MC of a pit latrine must be considered before implementation. Additionally, the water table, and seasonality must be considered before implementation. A rising water table, due to seasonal rainfall, could cause flooding of pit latrines, raising the MC of the FS to inappropriate levels. Feeding

rate and LD of BSFL in-situ would be difficult to determine. However, the results presented indicate that lower FRs result in increased FMR. This is important when the main aim of an in-situ treatment solution is to improve FMR, and increase the life span of a pit latrine. Additionally, the design of a specialised BSFL toilet will be heavily influenced by all three parameters. However, a significant amount of research and engineering must be undertaken before a BSFL toilet is an achievable FSM technology. This study has shown that key rearing parameters can affect FMR and prepupal production of BSFL when reared on pit latrine FS. Due to the variation in how BSFL can be utilised to manage FS, the results indicate that the key parameters can be adjusted depending on what the primary aim is, FMR or prepupal production.

One of the key aims of this study was to determine how BSFL developed on FS from various layers of a pit latrine, with varying physical and chemical characteristics. It was found that FS depth had a significant effect on FMR efficiency. With the highest FMR in FS excavated from the first metre of pit latrines. The highest wet weight FMR (≈65%) was higher than homogenised top layer pit latrine FS (≈58%) (see Chapter 4), fresh human faeces (55%) (Banks *et al.* 2014), and chicken manure (≈50%) (Sheppard *et al.* 1994). It is also far higher than the reduction caused by *M. domestica* feeding on chicken manure (≈30%) or cow manure (≈25%) (Morgan *et al.* 1975). However, the FMR is still lower than BSFL reduction of MOW (≈76%) (Diener *et al.* 2011), and far below the FS FMR capability of Tiger worms (96%) (Furlong *et al.* 2014). Although Tiger worms provide a low maintenance, affordable, effective and safe replacement for septic tanks, no valuable products are produced, whereas BSFL FSM methods benefit from the production of protein and fat rich prepupae. It is important to note that the FMR on the combined FS layer, although lower than some layers, was still approximately 50%. The study has shown that the change in chemical characteristics, pH, tCOD, sCOD, NH_4^+, protein, carbohydrates, VFAs, and heavy metals, had no significant effect on the FMR efficiency of BSFL when developing on FS. This suggests that BSFL are capable of efficiently

reducing FS within a wide range of the characteristics tested. This study showed BSFL reared on FS produce prepupae significantly smaller than previous studies (Diener *et al.* 2011)(see Chapter 4). However, it is possible to increase prepupal size by increasing feeding rate (Myers *et al.* 2008, Diener *et al.* 2009, Banks *et al.* 2014)(see Chapter 4). Additionally, the protein value and amino acid composition of prepupae is comparable to alternative animal feed protein sources (Diener *et al.* 2009, Pieterse *et al.* 2014). Although the fat content of BSFL reared on FS is far lower than when reared on other food sources (Hale 1973, Booram *et al.* 1977, St-Hilaire *et al.* 2007b), it was suggested that the fat content could be increased by addition of high lipid wastes (St-Hilaire *et al.* 2007a). The concentrations of heavy metals in BSFL prepupae reared on FS are important when considering the use of BSFL as a source of animal protein. Regulations inside the European Union (EU) limit the concentrations of heavy metals in animal feed, and the current study found that the mean arsenic and mercury concentration is lower than, or equal to the current animal feed regulations. However, the cadmium and lead concentrations are higher. It is possible to reduce the heavy metal concentrations in prepupae by avoiding contaminated food sources. It has been shown that, while BSFL can develop on a variety of layers of FS, the bioconversion rates are reduced compared to previous studies (Diener *et al.* 2011, Banks *et al.* 2014). These factors are important when considering different FSM approaches, including in-situ BSFL and decentralised BSFL FSM plants. In an optimum in-situ BSFL treatment solution, BSFL will consume all viable FS, and then survive on fresh excreta which are deposited daily by users. For a decentralised BSFL approach, an optimum scenario was economically feasible, by increasing bioconversion rates to achievable values, enhancing prepupal crude fat content (St-Hilaire *et al.* 2007a), reducing FMR, reducing operational costs, and increasing product values to realistic values. It could be argued that an optimum scenario is a fabrication, although considering the conservative assumptions made by the model for products sale value, and operational costs, and also considering methods of increasing bioconversion and enhancing fat

content, it can be argued that an optimum model is feasible. What can be established is that the sale of residue must be considered a vital aspect to the successful BSFL FSM business model. Solutions suggested in this study could help improve sanitation for billions of people around the world, however it was important to investigate the potential problems caused by hazardous substances in FS.

The study showed how some commonly used cleaning chemicals have a significant impact on the mortality of BSFL when added to FS. Jeyes Fluid and Madubula had a significantly negative effect on BSFL viability. However, the results implied that household owners would have to use the manufacturers recommended amount of Jeyes Fluid once a day, or twice a day for Madubula, to have a significant effect on BSFL mortality. Therefore, it can be assumed that the use of Jeyes Fluid and Madubula would not have a negative effect on BSFL mortality if pit latrine owners continued to use it, and FS fed to BSFL. The results also showed how chlorine (HTH) granules and pine antiseptics are very unlikely to increase mortality of BSFL. It must be considered that Jeyes Fluid, Madubula, HTH and pine antiseptic contain chemicals that are found in other cleaning products which could potentially affect BSFL. It is known that phenolic compounds can reduce the larval growth of herbivorous insects (Duffey *et al.* 1981, Kubo 1993), and surfactants found in pine antiseptics, and thousands of other cleaning products, are known to inhibit growth of aquatic dipteran larvae (Lewis *et al.* 1983, Lewis 1991, Ostroumov 2010). Therefore it is important to increase how much is known about the effects of these compounds, and others, on BSFL. The method of BSFL feeding on FS is important to consider when discussing the issues of chemical induced mortality, since the study was conducted in laboratory conditions, very different to real world situations. If the BSFL are utilised as an in-situ solution, where BSFL are inoculated into pit latrine vaults then later harvested for protein and fats, then the addition of cleaning chemicals will be highly varied. If chemicals cause specific areas to become unsuitable for BSFL, they will avoid feeding in them. This behaviour has been seen in the past where BSFL have avoided areas of stagnating liquid (Diener *et al.*

2011), and preferentially fed around them. However, if FS is excavated from vaults, and processed at a decentralised BSFL FSM plant, it is important to consider the effect chemicals could have on mortality. It is expected that any chemicals that are present will be uniformly spread throughout the FS. The results reported suggest that at recommended and reported concentrations, the chemicals investigated would not increase the mortality of BSFL. Faecal sludge potentially has a wide variety of components which could affect BSFL viability. Therefore it is important to consider them in respect to FSM. However, this study has provided initial evidence that commonly used chemicals have little effect on BSFL, further providing evidence for the use of BSFL as a novel FSM system.

7.2. Limitations of study

There were a number of issues which arose throughout the course of the current study. In Chapter 4 and Chapter 5, the dry weight FMR calculation was subject to an anomaly which resulted in a net gain of total solids. Weighing of FS and residue was conducted by the author, or under the author's supervision, ensuring that correct weighing protocols were followed, and although the data and calculations were thoroughly checked, it is unknown what caused the anomaly. The reporting of dry weight FMR is preferential, as it removes the affect variations in FS and residue moisture content has on the results, giving a more accurate representation of BSFL FMR capacity. Therefore, due to this anomaly, the FMR capacity of BSFL has only been reported as wet weight.

In Chapter 5, a problem developed when filth flies infested replicates which did not contain BSFL. The infestation occurred within the first 7 days of the experiment, after which netting was used to cover the replicates. However this was too late, with the infestations established, removing 100% of the filth fly larvae was not possible. Due to the wide spread number of infestations it was not possible to remove the infested replicates from the experiment, as there would not have been sufficient remaining for analysis. It was also not possible to restart

the experiment again due to time constraints. However, the intensity of the infestation varied between replicates, and it was decided to keep infested replicates in the experiment. Fortunately, this problem produced thought-provoking data. Even though the replicates were infested within the first 7 days of the experiment, only replicates not containing BSFL were infested, albeit at an early stage of their development. Due to small size of the BSFL, the physical characteristics of the FS had not changed significantly compared to a paired replicate without BSFL. Furthermore, there was no detectable difference in foul odours released by both replicates, although this was only determined using the experimenter's sense of smell. These results provide further evidence of semiochemical communication between BSFL and filth flies, which inhibits adult oviposition or larval development, warranting further investigation.

In chapter 6 an issue occurred when the pyrethroid-based insecticide positive control failed to cause 100% mortality, even at the highest concentrations. It is thought that this was due to the insecticide powder being insoluble in water, resulting in uneven distribution and dilutions. It is also suggested that the BSFL could have developed a resistance to pyrethrioid insecticides due to high usage in South Africa. Additionally, in chapter 6 the chlorine and pine disinfectant tested did not cause 100% mortality, resulting in insufficient data to calculate lethal doses using PROBIT analysis.

There are also a number of improvements which could have been made to the study. Ideally, a larger number of pit latrines would have been surveyed and the layers of FS excavated. Additionally, physical and chemical characteristic analysis could have been conducted, gathering more data on variation and trends in FS. It would also have been worthwhile to obtain FS mechanically emptied from pit latrines managed by local municipalities, and compared the composition to those manually emptied. Furthermore, the identification of insects found in FS would have gathered data on species variation found in pit latrines, a topic which lacks in-depth investigation. It would also have been useful to conduct in depth analysis

of FS for hazardous substances, including pesticides, insecticides, hormones and organic compounds, which could potentially bioaccumulate in prepupae. In regards to the survey, it would have been worthwhile to ask more in-depth questions about the use of BSFL as a FSM technology, and people's opinions on use of FS reared BSFL as a product.

The experiments described in this study were performed in a retro-fitted experimental room. The environmental conditions in the room were controlled using a heater and humidifier, and the space was limited. With a larger, purpose built room, variations in temperature and humidity would not occur, and there would be more space available. With the extra space an increased number of replicates could be performed, improving the statistical strength of the data obtained. With a larger space, it would also have been possible to conduct larger scale experiments to test mass rearing of BSFL on FS, and to test more levels of moisture content and feeding rates in the experiment conducted in Chapter 4. The increase in space could also have allowed more than 1 feeding rate to be evaluated in the experiment described in Chapter 5. Finally, Chapter 6 could have been improved by testing more chemicals, including cleaning chemicals and pit latrine additives used to decrease pit fill-up rate, such as lime, ash, and commercially sold additives.

During the work conducted in this study, a number of lessons were learnt by the authors. Locating suitable pit latrines was harder than anticipated. The experimental work was conducted in the Western Cape Province of South Africa, which has high sanitation coverage provided by the municipal government. It was found that the common design of pit latrines used by the government prevented manual emptying, due to a solid concrete slab and superstructure. Mechanical emptying was viable, however this method would have made it impossible to empty the FS in specific layers. In order to locate suitable latrines it was necessary to travel to the Eastern Cape Province, which had lower government provided

sanitation coverage. However, it still took 5 days to locate suitable pit latrines, even with the help of local government officials and non-government organisations.

It is also interesting to note that pit latrine owners who were surveyed were very willing to be interviewed once the project had been fully explained. This emphasises how important the topic of sanitation is to people who suffer from a lack of suitable FSM technologies, with the owners of pit latrines which were excavated being very gracious. However, it was found that not all questionnaires were answered correctly, with the main inconsistency being in the reporting of using the pit latrine to dispose of household waste. It is perhaps understandable that people who are advised not to dispose of waste into pit latrines are reluctant to report the behaviour.

During the planning of the experiments, the authors estimated how long it would take to setup and terminate the experiments, and weigh the FS and BSFL periodically. However, the time it took to perform each of these tasks was far longer than anticipated, especially when terminating the experiments. Even a relatively "simple", but well planned, experimental design can result in a larger number of work-hours than anticipated, resulting in the requirement to employ assistants. This was an invaluable lesson learned which will be considered in all future experimental designs, and budgets.

7.3. Future work

The study presented has major implications on how BSFL can be used as a FSM method. On-site applications include in-situ BSFL, and the development of a dedicated BSFL toilet, which could be used in areas where the off-site method is unfeasible. The study has shown that fresh excreta deposited into pit latrines, or BSFL toilets, can be effectively reduced, degrading the waste and decreasing fill-up rates. Also, BSFL can effectively reduce FS already present in pit latrines, reducing FS with a wide range of physical and chemical characteristics. The study indicates that commonly used cleaning chemicals should not have a negative impact on BSFL

viability if they are used in-situ. Implying little behaviour change would be required of pit latrine owners in regards to hygiene practices, with the presence of BSFL within a pit not reducing the cleanliness of a latrine.

In situations where on-site BSFL FSM methods are unsuitable, it is suggested that an off-site decentralised method could be utilised if economically viable. This study has shown how BSFL can effectively reduce homogenised FS with a wide range of physical and chemical characteristics. A further advantage of off-site BSFL FSM is that water can be added or removed from FS which has unsuitable moisture content for BSFL development. Furthermore, by adjusting feeding rates and larval densities, it is possible to match the objectives of a BSFL FSM plant to a desired outcome, focussing on FMR, prepupal production, or a combination of both.

However, before any of these BSFL FSM methods can be fully implemented, there are still a large number of questions which must be answered. To be able to implement the use of BSFL in-situ, it is vital to assess BSFL ability at reducing FS in a real pit latrine. This will indicate whether BSFL are capable of slowing pit latrine fill-up rates, ultimately extending the life span of the pit latrine. It is also important to investigate devices which could be used to retrieve prepupae from within pits, for example the “Kone” or “Daisy Chain”, described previously (see Chapter 1). These devices must be tested to determine prepupal collection efficiency, and the nutritional content of prepupae harvested must be ascertained. It is also important to determine user acceptability of BSFL as a method of FSM. An alternative on-site FSM method suggested is the development of a dedicated BSFL toilet. However, this technology is still at a very early stage of development, with a large amount of research required to obtain a feasible prototype, which would then require development and testing.

One problem which is shared by both on-site solutions is how to sustain a BSFL population. It has been proposed that in communities where on-site BSFL FSM is suitable, local

entrepreneurs could provide a service of collecting prepupae from latrines, while maintaining the BSFL populations. However, this business model needs in-depth investigation, and may not be suitable for BSFL toilets located in crisis situations or remote locations. It has been suggested that BSFL populations could be maintained by adult BSF from the surrounding environment. However, research must be conducted on how to ensure adults oviposit in suitable locations. This could be by using man made oviposition sites, an oviposition attractant semiochemical which lures female BSF into egg laying, or a combination of both.

The implementation of off-site BSFL FSM also requires investigation. There is currently a pilot BSFL FSM plant being run in South Africa by The BioCycle, described previously (see Chapter 1). It is important that the feasibility of this model is examined, with investigation into how the model could be expanded in South Africa, and other countries. It is important to determine the size range of communities that can be covered by decentralised BSFL plants, as the number of latrines in a community will be a limiting factor for an economically feasible business model. The current study has suggested that varying the feeding rate and larval density in an off-site BSFL plant could provide flexibility depending on a desired outcome: FMR, prepupal production, or a balance of both. It is therefore vital that this adaptability is investigated, with thorough research into what outcome thresholds are achievable, variations in between, and how the result affect the economic viability of the business model.

The HAAS business model (see Appendix A) indicates that a decentralised BSFL FSM business model is financially feasible under correct conditions. The model was designed for Dar es Salaam, Tanzania, and was tailored to provide a free pit latrine emptying service. It is recommended that future work is conducted into refining the model, and determine location specific data for low- and middle- income countries which would benefit from BSFL FSM technologies. Also, the study could incorporate other business models, such as Sanergys end-to-end approach of excreta storage, collection, transport and treatment (Sanergy 2013). This

would reduce overheads, and potentially change the model significantly. The business models are also dependant on data gathered on the value of prepupae and residue, however these issues will be discussed below.

Regardless of which BSFL FSM method is implemented, there are a number of challenges, and opportunities, which need to be investigated. Considering the wide range of physical and chemical characteristics of FS, the limitations at which BSFL can successfully develop, including upper and lower moisture content, and the quantities of organic material in FS, needs further attention.

An incidental finding of the present study was that it provided anecdotal evidence, which supports previous research, for BSFL controlling filth fly populations. It is recommended that the topic is rigorously researched, as the control of disease spreading filth flies would be beneficial to BSFL FSM methods, and the surrounding communities. Additionally, it has been suggested that a semiochemical is responsible for filth fly control. If correct, a synthetic version could be manufactured and used to control filth flies populations, helping reduce disease.

It is also important to determine what environmental characteristics could limit the successful rearing of BSFL, especially in low- and middle-income countries where power and water sources may be inadequate to maintain constant rearing conditions. A lot of research must be conducted into how to successfully setup and maintain adult BSF colonies, specifically in resource poor areas mentioned above. Currently there is a number of organisations researching into the mass rearing of BSFL, including AgriProtein (AgriProtein 2014) and The BioCycle (BioCycle 2014) in South Africa, EnviroFlight (EnviroFlight 2014) and ESR International (ESRI 2014) in the U.S.A, EAWAG (EAWAG 2014) and Bioflytech (Bioflytech 2014) in Europe, and PROteINSECT, who work with 12 partners from 7 countries around the world (PROteINSECT 2014). These organisations successfully maintain BSF colonies, however a lot of research is required to help limit any wastage associated with mass rearing. Methods of egg

collection could be improved through investigation into semiochemical oviposition lures. Early larval mortality can be improved by examination into early stage larval requirements, such as diet and environment, and how they differ from older BSFL. It is also important to consider how genetic diversity can affect the mass rearing of BSFL, specifically how variations in phenotypes could alter size, egg production, and development time.

There are also major gaps in knowledge about the products of BSFL FSM, namely the residue and the prepupae. It is vital to investigate the value of these products. The value of the residue is as a fertiliser, with future work needing to determine the nutrient content, and value in plant growth trials. Research must be conducted into how to safely treat the residue to remove pathogens, while retaining its value. Alternative methods of residue treatment should also be investigated, such as conversion to charcoal for soil amendment, known as biochar. A major issue that requires investigation is the potential contamination of residue by hazardous components, including: heavy metals, pesticides, insecticides, herbicides, pharmaceuticals, and hormones. These contaminates find their way into FS through human excretion or environmental leaching, and many are endocrine disrupting compounds (EDC), known to cause sterility in men and increase cancer risks (WHO/UNEP 2012). Furthermore, it is recommended that an investigation is conducted into liquid effluent produced by BSFL FSM. This is in order to determine concentrations of nitrites, which could lead to nitrification of ground water. A thorough investigation is required to determine the presence of these contaminants in FS and residue, and whether they could potentially limit the use of residue in the food chain. Additionally, the same research must be carried out on prepupae which have been reared on FS. Research must focus on determining the prepupal value, in regards to proteins and fats, methods of safely removing pathogens, acceptability as a protein replacement in animal feeds, potential as a source of biodiesel, enhancing prepupal crude fat contents, and the potential contamination by hazardous compounds.

7.4. Overall conclusion

A novel FSM system is required to help improve sanitation in low- and middle-income countries which currently lack access to suitable FSM. The work conducted in this study shows how BSFL could potentially increase the life-span of pit latrines, reducing the frequency of emptying, therefore reducing the economic cost on pit latrine owners. Additionally, with further investigation, the harvesting of prepupae could provide a source of income for latrine owners, or entrepreneurs who offer to service BSFL pit latrines or toilets. Additionally, this study has shown that an off-site BSFL FSM plant is economically feasible. By adjusting feeding rates and larval densities, it is possible to match the objectives of a BSFL FSM plant to a desired outcome, focussing on FMR, prepupal production, or a combination of both. This flexibility is important as it means the technology can be tailored to a variety of locations, while this adaptability could be central to the successful implication of BSFL as an off-site FSM method.

Although there are still many topics which need to be investigated, the study presented here demonstrates that BSFL are an appropriate method of FSM, both on-site and off-site. They are capable of transforming FS into prepupal protein, and a partially-treated residue, providing a valuable economic product for low- and middle-income communities. With continued investigation, the use of BSFL as a method of FSM could be applied around the world, helping improve sanitation, health, and standard of living for billions of people.

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Appendix A) HAAS Business Model

University of California
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Haas School of Business

Black Soldier Fly - Feasibility Study

Prepared by Nitin Agrawal, Marissa Chacko, Meena Ramachandran & Min Thian
May 2011

1. Data for this model have been collected from various sources which include field interviews, web sources, research reports and publications.

2. This model can support processing capacity of approximately 13,000 liters of waste per day. For additional processing, it is advisable to increase the necessary cost inputs.

3. There are three proposed models -- Model I, II & III. For each model, there is a revenue and a cost component.

4. The breakeven analysis is driven by the results from each of these models.

5.To estimate business feasibility for new geographies, the assumptions tab would need to be updated with data pertaining to the country under study. All the figures in blue are assumptions for the user of this spreadsheet to input/ change.

Key Assumptions

**All critical assumptions to this model are highlighted in grey*

General Assumptions			Unit	Data source
Working days / year		300		Based on # of working days in Tanzania
# months / year		12		
# weeks / year		52		
Kg / ton		1000		
Interest rate for a small business loan		18.0%		Tanzania Investment Bank
Company registration and legal fees		$500	USD	Mafuta Sasa Interview
Company setup time		4	months	Mafuta Sasa Interview

Collection Assumptions			Unit	Data source
Population assumptions				
City population		3,000,000	people	Tanzania Census
% Population living in unplanned unserviced areas		80.0%		Tanzania Census
% Population using latrines		75.0%		Tanzania Census
Average # of users per latrine		9	people	Temeke Interview
Average # of latrines emptied / day		3	latrines	Assumption
Average pit latrine width		1.25	meters	World Health Organization - Chap. 8 Sanitation
Pit latrine depth		3.5	meters	World Health Organization - Chap. 8 Sanitation
Permanent labor / month	$	200	USD	Feed production interview
Temp worker salary / month	$	60	USD	Feed production interview
Latrine emptying expense	$	67	USD	Dar es Salaam quantitative report
Latrine pick-up / loading expense	$	2		
Cost to rent transport per day / latrine (up to 1500 L capacity per truck)	$	120	USD	Biodiesel center interview

Processing Assumptions			Unit	Data source
Conversion rate from wet waste (kg) to wet BSF (kg)		11.5%		Banks 2010 - *Determination of physical and biochemical changes* with 50% reduction for pit latrine material
Conversion rate from wet waste (kg) to low grade dry BSF (kg) *(Model III)*		5.0%		Diener 2009 - *BSF Financial Analysis in Costa Rica*
Conversion rate from wet waste (kg) to high grade dry BSF (kg) *(Model I & II)*		3.5%		Diener 2009 - *BSF Financial Analysis in Costa Rica* and 30% Lipid Content Loss Li 2010
Biodiesel conversion from crude oil (%)		96%		Li 2010 - *From Organic Waste to Biodiesel*
Lipid content in BSF Larvae		30.0%		Li 2010 - From Organic Waste to Biodiesel
Labor (per month)				
Production Manager / Engineer	$	350	USD	Mafuta Sasa Interview
Inventory / Insectory Manager	$	165	USD	Mafuta Sasa Interview
Salesperson	$	240	USD	Mafuta Sasa Interview
Accountant	$	250	USD	Mafuta Sasa Interview
Temp worker / month	$	60	USD	Best Animal Food Interview
Property & plant rental cost (per month per square meter)	$	1	USD	*Mafuta Sasa Interview*
Telephone, electricity, water (per month)	$	42	USD	Diener 2009 - *BSF Financial Analysis in Costa Rica*
Infrastructure maintenance (per month)	$	167	USD	Diener 2009 - *BSF Financial Analysis in Costa Rica*
Capital improvements to facility	$	1,500	USD	Mafuta Sasa Interview
Other fixed startup expenses	$	400	USD	Diener 2009 - *BSF Financial Analysis in Costa Rica*
Insectory	$	5,000	USD	www.thebiopod.com + assumption
Commercial used oven	$	7,000	USD	Diener 2009 - *BSF Financial Analysis in Costa Rica*
Generator	$	2,000	USD	Mafuta Sasa Interview
Oil extractor (grinder + separator)	$	70,000	USD	Best Animal Food Interview - Sunflower oil extractor + assumption
Containers needed for oil / biodiesel storage/shipping		100		
Truck rental / Delivery (per day) (1500 L)	$	40	USD	Mafuta Sasa Interview

Black Soldier Fly - Feasibility Study

Deliveries / week		1		
Feed Assumptions			**Unit**	**Data source**
Grinder	$	280	USD	UN Report on Biofuels in Sub-Saharan Africa
Packaging machine	$	750	USD	Alibaba.com / Chinese manufacturer
Variable costs / pack	$	1	USD	Assumption
Additional labor costs (per month)	$	60	USD	Mafuta Sasa Interview
Additional expendables (per month)	$	50	USD	Diener 2009 - *BSF Financial Analysis in Costa Rica*
Price / kg of high grade BSF feed (lipids removed) *Models I & II	$	1.0	USD	Best Animal Feed interview & http://www.organicvaluerecovery.com/our_process/our_process_product_breakdown.htm
Price / kg of low grade BSF feed (lipids unremoved) *Model III	$	0.7	USD	Best Animal Feed interview
Packaging size	$	50	kg	Industry standard
Biodiesel Assumptions			**Unit**	**Data source**
Price % discount for BSF crude oil (compared to diesel)		60%		Mafuta Sasa Interview
Capital expenditure for biodiesel processing line	$	30,000	USD	Mafuta Sasa Interview *(Extreme Biodiesel - Capacity of 10,000 L per week)*
Cost / container for storing and shipping biodiesel	$	18	USD	Alibaba.com / Chinese manufacturer
Additional property & plant rental cost (per month)		200	USD	Mafuta Sasa Interview *(Additional space needed for 251 sq meter biodiesel facility,*
Additional expendables (per month)		50	USD	Diener 2009 - *BSF Financial Analysis in Costa Rica*
Variable cost of production (per liter)	$	0.25	USD	Mafuta Sasa Interview
Price of diesel / liter	$	1.33	USD	Dar es Salaam rate as of May 2011
Price % discount for BSF biodiesel (compared to diesel)		30%		Mafuta Sasa Interview
Time to set up Biodiesel line		4.00	months	Mafuta Sasa Interview
Byproduct Assumptions			**Unit**	**Data source**
Waste leftover after BSF processing		40%		http://www.organicvaluerecovery.com/our_process/our_process_products.htm
Glycerin production as a % of oil		20%		Mafuta Sasa Interview
Price / liter of glycerin		$1.70	USD	
Price / ton of low grade fertilizer		50	USD	Conservative assumption on low grade fertilizer in Tanzania

Breakeven & Profitability Analysis

Model #	Scenario		Revenues ($) (with fertilizer)	Revenues ($) (without fertilizer)	Costs ($)	Capital Expenditure ($)	Profit / Loss ($)	Return on Investment (%)	Fixed Investment breakeven (in years)	Fixed Investment breakeven + setup time
	High	10%								
	Medium	(10%)								
Model I, Crude Oil and Feed										
	High	10%	311,757	226,757	212,715	95,590	14,042	15%	6.81	7.14
	Medium	-	283,416	206,142	193,377	86,900	12,765	15%	6.81	7.14
	Low	(10%)	255,074	185,528	174,039	78,210	11,489	15%	6.81	7.14
	Best Case Scenario		311,757	226,757	174,039	78,210	52,717	67%	1.48	1.82
	Worst Case Scenario		255,074	185,528	212,715	95,590	-27,187	(28%)	-	-
Model II, Biodiesel and Feed										
	High	10%	414,654	329,653	259,955	128,590	69,698	54%	1.84	2.51
	Medium	-	376,958	299,685	236,323	116,900	63,362	54%	1.84	2.51
	Low	(10%)	339,262	269,716	212,691	105,210	57,026	54%	1.84	2.51
	Best Case Scenario		414,654	329,653	212,691	105,210	116,962	111%	0.90	1.23
	Worst Case Scenario		339,262	269,716	259,955	128,590	9,761	8%	13.17	13.51
Model III, Feed Only										
	High	10%	233,752	148,751	190,429	18,348	-41,677	(227%)	-	-
	Medium	-	212,502	135,229	173,117	16,680	-37,888	(227%)	-	-
	Low	(10%)	191,252	121,706	155,805	15,012	-34,099	(227%)	-	-
	Best Case Scenario		233,752	148,751	155,805	15,012	-7,054	(47%)	-	-
	Worst Case Scenario		191,252	121,706	190,429	18,348	-68,723	(375%)	-	-

Best Case Scenario: *Assumes highest revenues & lowest costs + upfront capital expenditure*
Worst Case Scenario: *Assumes lowest revenues & highest costs + upfront capital expenditure*

Black Soldier Fly Revenue Model I - Crude Oil & Feed

Total Number of Latrines in the City		
City Population	3,000,000	
* % of population in unplanned unserviced areas	80%	
= Total population in unplanned unserviced areas	2,400,000	
* % population using latrines	75%	
= Population using latrines	1,800,000	
/ Average number of users / latrine	9	
= Estimated number of latrines in the city		**200,000**

Amount of Waste Collected per day		
# of latrines serviced	3	
* Average volume of full latrine (m^3)	4.3	
= Total volume of Latrine collected / day (m^3)	12.9	
* 1000L / m^3	1,000	
=Total volume of waste collection per day (L)		**12,879**

Annual Crude Oil Production		
Total wet waste collected / day (L)	12,879	
* Conversion rate for BSF	12%	
= Total BSF produced /day (kg)	1,481	
* % Lipid component in BSF	30%	
= Total crude oil produced from BSF / day (L)	444	
* Number of working days / year	300	
= Annual crude oil production (L)		**133,297**

Annual Revenue from Crude Oil			
Total oil produced from BSF / day		444	
* Average price for diesel / liter	$	1.33	
* Price discount for biodiesel feedstock		60%	
= Revenue from oil / day	$	236	
* Number of working days / year		300	
= Annual revenue from crude oil			**$ 70,914**

Annual BSF Feed Production		
Total waste collected / day (L)	12,879	
* Conversion rate to dry BSF	4%	
= Total BSF feed produced / day (kg)	451	
* Number of working days / year	300	
= Annual BSF feed production (kg)		**135,229**

Annual Revenue from BSF feed			
Total BSF feed produced / day (kg)		451	
* Average price / kg	$	1	
= Revenue from BSF feed / day	$	451	
* Number of working days / year		300	
= Annual revenue from BSF feed			**$ 135,229**

Annual BSF Byproduct - Fertilizer		
Total waste collected / day	12,879	
* # of working days / year	300	
* % of leftover waste as fertilizer	40%	
= Total fertilizer produced / year		**1,545,469**

Revenues from BSF Byproduct - Fertilizer			
Total fertilizer produced / year		1,545,469	
/ # of kg / ton		1,000	
= Total fertilizer produced in tons / year		1,545	
* Price / ton of low grade fertilizer	$	50	
= Annual revenue from fertilizer			**$ 77,273**

Total Annual Revenue from BSF Harvest - Model I			
			$ 283,416

Black Soldier Fly Cost Model I - Crude Oil & Feed

Start-up Costs		
Commercial Used Oven	$ 7,000	
+ Grinder + oil separator *(oil)*	$ 70,000	
+ Biodiesel conversion equipment kit *(Biodiesel)*	$ -	
+ Capital Improvements	$ 1,500	
+ Commercial Used Oven	$ 2,000	
+ BSF Insectory	$ 5,000	
+ Laboratory Equipments	$ 500	
+ Company registration & legal fees	$ 500	
+ Miscellaneous fixed expenses	$ 400	
= Total Start-up costs *(Loan or seed investment)*		**$ 86,900**

Interest Expense		
Loan (Start-up costs)	$ 86,900	
* Annual interest rate	18%	
= Annual interest expense		**$ 15,642**

Annual Operating Costs		
Annual Waste Collection Expenses *(All Activities Outsourced)*		
Extraction Labor / latrine	$ 67	
* Number of latrines serviced / day	3	
= Total labor cost for waste extraction / day	$ 201	
+ Pick-up / loading expense / latrine	$ 6	
+ Truck rental cost / day	$ 120	
= Total collection costs / day	$ 327	
* Number of working days / year	300	
= Annual collection expenses		**$ 98,100**

Annual Waste Processing Expenses		
Property & plant rental cost (per month)	$ 3,661	
+ Telephone, electricity, water (per month)	$ 42	
+ Expendables (per month)	$ 400	
+ Infrastructure maintenance (per month)	$ 167	
+ Labor		
Production Manager / Engineer	$ 350	
Accountant	$ 250	
Inventory / Insectory Manager	$ 165	
Salesperson	$ 240	
2 Temporary Labor	$ 120	
= Monthly processing expenses	$ 5,395	
* Number of months / year	12	
= Annual processing expenses / year		**$ 64,741**

Total Annual Operating Expense		
Collection expense	$ 98,100	
+ Processing expense	$ 64,741	
=Total operating expenses		**$ 162,841**

Other Production Related Costs		
Annual Crude Oil Related Expenses		
Deliveries / week	1	
* Weeks / year	52	
* Cost of truck rental / day	$ 120	
= Total delivery expense / year	$ 6,240	
+ Containers expense	$ 1,800	
Total expenses related to crude oil		**$ 8,040**

Feed Related Expenses		
Annual feed production	135,229	
/ kg / bag	50	
= Number of packs	2705	
* Variable cost / pack	$ 1	
= Total variable expense / 50 pack	$ 2,705	
+ Packaging machine	$ 750	
+Expendables	$ 600	
+ Delivery expenses / year *(1 delivery/ week)*	$ 2,080	
+ Annual labor expense	$ 720	
Annual feed related expenses		**$ 6,855**

Total Annual Production Cost		
Annual interest expense	$ 15,642	
+ Operating expenses	$ 162,841	
+ Crude Oil related expense	$ 8,040	
+ Feed related expense	$ 6,855	
=Total annual production cost		**$ 193,377**

Black Soldier Fly Revenue Model II - Biodiesel & Feed (Biodiesel produced in-house)

Total Number of Latrines in the City		
City Population	3,000,000	
* % of population in unplanned unserviced areas	80%	
= Total population in unplanned unserviced areas	2,400,000	
* % population using latrines	75%	
= Population using latrines	1,800,000	
/ Average number of users / latrine	9	
= Estimated number of latrines in the city		**200,000**

Amount of Waste Collected per day		
# of latrines serviced	3	
* Average volume of full latrine (m^3)	4.3	
= Total volume of Latrine collected / day (m^3)	12.9	
* 1000L / m^3	1,000	
=Total volume of waste collection per day (L)		**12,879**

Annual Biodiesel Production		
Total waste collected / day	12,879	
* Conversion rate for BSF	11.5%	
Total BSF produced /day	1,481	
* % Lipid component in BSF	30%	
= Total oil produced from BSF / day	444	
* Number of working days / year	300	
= Annual oil production	133,297	
* Biodiesel conversion rate (%)	96%	
= Annual biodiesel production		**127,965**

Annual Revenue from Biodiesel			
Total oil produced from BSF / day		444	
* Biodiesel conversion rate (%)		96%	
= Biodiesel production / day		427	
* Number of working days / year		300	
= Annual biodiesel production		127,965	
* Price / liter of diesel	$	1.33	
* Discount for biodiesel sale		30%	
= Annual biodiesel revenue			**$ 119,135**

Annual BSF Feed Production		
Total waste collected / day (L)	12879	
* Conversion rate to dry BSF	3.5%	
= Total BSF feed produced / day (kg)	451	
* Number of working days / year	300	
= Annual BSF feed production (kg)		**135,229**

Annual Revenue from BSF feed			
Total BSF feed produced / day (kg)		451	
*Average price / kg	$	1	
= Revenue from BSF feed / day	$	451	
* Number of working days / year		300	
= Annual revenue from BSF feed			**$ 135,229**

Total Byproduct Output from BSF - Glycerin		
Total oil produced / year	133,297	
* % of oil converted to glycerin	20%	
= Total glycerin produced		**26,659**

Revenues from BSF byproduct - Glycerin			
Total glycerin produced		26,659	
* Price / liter of glycerin	$	1.70	
= Annual revenue from glycerin			**$ 45,321**

Annual BSF Byproduct - Fertilizer		
Total waste collected / day	12,879	
* # of working days / year	300	
% of leftover waste as fertilizer	40%	
= Annual fertilizer produced		**1,545,469**

Revenues from BSF byproduct - Fertilizer			
Total fertilizer produced / year		1,545,469	
/ # of kg / ton		1000	
= Total fertilizer produced in tons / year		1,545	
* Price / ton of low grade fertilizer	$	50	
= Annual revenue from fertilizer			**$ 77,273**

Total Annual Revenue from BSF Harvest - Model II			
			$ 376,958

Black Soldier Fly Cost Model II - Biodiesel & Feed

Start-up Costs		
Commercial used oven	$ 7,000	
+ Grinder + oil separator *(oil)*	$ 70,000	
+ Biodiesel conversion equipment kit *(Biodiesel)*	$ 30,000	
+ Capital improvements	$ 1,500	
+ Commercial Used Oven	$ 2,000	
+ BSF insectory	$ 5,000	
+ Laboratory equipment	$ 500	
+ Company registration & legal fees	$ 500	
+ Miscellaneous fixed expenses	$ 400	
= Total start-up costs *(Loan or seed investment)*		**$ 116,900**

Interest Expense		
Loan (Start-up costs)	$ 116,900	
* Annual interest rate	18%	
= Annual interest expense		**$ 21,042**

Annual Operating Costs		
Annual Waste Collection Expenses *(All Activities Outsourced)*		
Extraction Labor / latrine	$ 67	
* Number of latrines serviced / day	3	
= Total labor cost for waste extraction / day	$ 201	
+ Pick-up / loading expense / latrine	$ 6	
+ Truck rental cost / day	$ 120	
= Total collection costs / day	$ 327	
* Number of working days / year	300	
= Annual collection expenses		**$ 98,100**

Annual Waste Processing Expenses		
Property & plant rental cost (per month)	$ 3,862	
+ Telephone, electricity, water (per month)	$ 42	
+ Expendables (per month)	$ 400	
+ Infrastructure maintenance (per month)	$ 167	
+ Labor		
2 Production Managers / Engineers	$ 700	
Accountant	$ 250	
Inventory / Insectory Manager	$ 165	
Salesperson	$ 240	
3 Temporary Labor	$ 180	
= Monthly processing expenses	$ 6,006	
* Number of months / year	12	
= Annual processing expenses / year		**$ 72,070**

Total Annual Operating Expense	
Collection expense	$ 98,100
+ Processing expense	$ 72,070

Alternative Scenario Related Costs		
Annual Biodiesel Related Expenses		
Amount of biodiesel produced / year (L)	127,965	
* Other variable expenses / liter	$ 0.25	
= Total annual variable expense	$ 31,991	
Deliveries / week	1	
* Weeks / year	52	
* Cost of truck rental / day	$ 120	
= Total delivery expense / year	$ 6,240	
+ Containers expense	$ 25	
Total expenses related to biodiesel		**$ 38,256**
Feed Related Expenses		
Annual feed production	135,229	
/ kg / bag	50	
= Number of packs	2,705	
* Variable cost / pack	$ 1	
= Total variable expense / pack	$ 2,705	
+ Packaging machine	$ 750	
+Expendables	$ 600	
+ Delivery expenses / year *(1 delivery/ week)*	$ 2,080	
+ Annual labor expense	$ 720	
= Annual feed related expenses		**$ 6,855**

Total Annual Production Cost	
Annual interest expense	$ 21,042
+ Operating expenses	$ 170,170
+ Biodiesel related expense	$ 38,256
+ Feed related expense	$ 6,855

Black Soldier Fly Revenue Model III - Feed only

Total Number of Latrines in the City		
City Population	3,000,000	
* % of population in unplanned unserviced areas	80%	
= Total population in unplanned unserviced areas	2,400,000	
* % population using latrines	75%	
= Population using latrines	1,800,000	
/ Average number of users / latrine	9	
= Estimated number of latrines in the city		**200,000**

Amount of Waste Collected per day		
# of latrines serviced	3	
* Average volume of full latrine (m^3)	4.3	
= Total volume of Latrine collected / day (m^3)	12.9	
* 1000L / m^3	1,000	
= Total volume of waste collection per day (L)		**12,879**

Annual BSF Dry Feed Production		
Total wet waste collected / day (L)	12,879	
* % conversion to dry weight BSF	5%	
= Total BSF produced /day (kg)	644	
* Number of working days / year	300	
= Annual BSF feed production (kg)		**193,184**

Annual Revenue from BSF Feed			
Total BSF feed produced / day (kg)		644	
*Average price / kg	$	0.70	
=Revenue from BSF feed / day	$	451	
*Number of working days / year		300	
=Annual revenue from BSF feed			**$ 135,229**

Annual BSF byproduct - Fertilizer		
Total waste collected / day	12,879	
* # of working days / year	300	
* % of leftover waste as fertilizer	40%	
= Total fertilizer produced / year		**1,545,469**

Revenues from BSF byproduct - Fertilizer			
Total fertilizer produced / year		1,545,469	
/ # of kg / ton		1,000	
= Total fertilizer produced in tons / year		1,545	
* Price / ton of low grade fertilizer	$	50	
= Annual revenue from fertilizer			**$ 77,273**

Total Annual Revenue from BSF Harvest - Model III	**$ 212,502**

Black Soldier Fly Cost Model III - Feed Only

Start-up Costs		
Commercial Used Oven	$ 7,000	
+ Grinder (feed)	$ 280	
+ Biodiesel conversion equipment kit *(Biodiesel)*	$ -	
+ Capital Improvements	$ 1,500	
+ Commercial Used Oven	$ 2,000	
+ BSF Insectory	$ 5,000	
+ Laboratory Equipments	$ -	
+ Company registration & legal fees	$ 500	
+ Miscellaneous fixed expenses	$ 400	
= Total start-up costs *(Loan or seed investment)*		**$ 16,680**

Interest Expense		
Loan (Start-up costs)	$ 16,680	
* Annual interest rate	18%	
= Annual interest expense		**$ 3,002**

Annual Operating Costs		
Annual Waste Collection Expenses *(All Activities Outsourced)*		
Extraction Labor / latrine	$ 67	
* Number of latrines serviced / day	3	
= Total labor cost for waste extraction / day	$ 201	
+ Pick-up / loading expense / latrine	$ 6	
+ Truck rental cost / day	$ 120	
= Total collection costs / day	$ 327	
* Number of working days / year	300	
= Annual collection expenses		**$ 98,100**
Annual Waste Processing Expenses		
Property & plant rental cost (per month)	$ 3,661	
+ Telephone, electricity, water (per month)	$ 42	
+ Expendables (per month)	$ 400	
+ Infrastructure Maintenance (per month)	$ 167	
+ Labor		
Production Manager / Engineer	$ 350	
Accountant	$ 250	
Inventory / Insectory Manager	$ 165	
Salesperson	$ 240	
1 Temporary Labor	$ 60	
= Monthly processing expenses	$ 5,335	
* Number of months / year	12	
= Annual processing expenses / year		**$ 64,021**

Total Annual Operating Expense		
Collection expense	$ 98,100	
+ Processing expense	$ 64,021	
= Total operating expenses		**$ 162,121**

Alternative Scenario Related Costs		
Annual Biodiesel Related Expenses		
Amount of biodiesel produced / year (L)	-	
* Other variable expenses / liter	$ 0.25	
= Total annual variable expense	$ -	
+ Packaging expenses / year	0	
+ Delivery expenses / year *(1 delivery / week)*	$ -	
= Annual biodiesel related expenses		**0**
Feed Related Expenses		
Annual feed production	193,184	
/ kg / bag	50	
= Number of packs	3,864	
* Variable cost / pack	$ 1	
= Total variable expense / 50 pack	$ 3,864	
+ Packaging machine	$ 750	
+Expendables	$ 600	
+ Delivery expenses / year *(1 delivery/ week)*	$ 2,080	
+ Annual labor expense	$ 700	
Annual feed related expenses		**$ 7,994**

Total Annual Production Cost		
Annual interest expense	$ 3,002	
+ Operating expenses	$ 162,121	
+ Biodiesel related expense	$ 0	
+ Feed related expense	$ 7,994	
=Total annual production cost		**$ 173,117**

Appendix B) LSHTM Ethics Approval

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM
Application number: **5830**

Name of Principal Investigator	**Ian Banks**
Faculty	**Infectious and Tropical Diseases**
Head of Faculty	**Professor Simon Croft**

Title: **Effect of fly density on the development of black soldier fly larvae (H. illucens) using different amounts of human faeces as a food source**

This application is approved by the Committee.

Chair of the Ethics Committee .....[redacted]

Date .....12 November 2010.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

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www.lshtm.ac.uk

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Observational / Interventions Research Ethics Committee

Ian Banks
Research Degree Student
DC / ITD
LSHTM

19 March 2013

Dear Mr. Banks,

Study Title:	**To assess the impact of black soldier fly (Hermetia illucens) larvae on faecal reduction in pit latrines**
LSHTM ethics ref:	**5972**
LSHTM amend no:	**A394**

Thank you for your application of 20 February 2013 for the amendment above to the existing ethically approved study and submitting revised documentation. The amendment application has been considered by the Observational Committee.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above amendment to research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

Approval is dependent on local ethical approval for the amendment having been received, where relevant.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
LSHTM amendment application	n/a	20/02/2013
Protocol	1.4	20/02/2013
Consent Form		20/02/2013
Questionnaire		20/02/2013

After ethical review

Any further changes to the application must be submitted to the Committee via an E2 amendment form. The Principal Investigator is reminded that all studies are also required to notify the ethics committee of any serious adverse events which occur during the project via form E4. At the end of the study, please notify the committee via form E5.

Yours sincerely,

[redacted]

Professor Andrew J Hall
Chair
ethics@lshtm.ac.uk
http://intra.lshtm.ac.uk/management/committees/ethics/

Appendix C) Informed Consent Form

Pit Latrine Survey

Introduction:
Hello. I am Ian Banks, a student from London School of Hygiene and Tropical Medicine, England. I am working with Stellenbosch University, and a company called AgriProtein. Together we are looking at ways to improve the emptying of pit latrines using an insect called the black soldier fly. The black soldier fly is an insect that does not spread disease, and the young black soldier fly (larvae) eat lots of waste. These larvae feed on animal and human faeces and may be used to improve hygiene and sanitation in communities in South Africa by reducing diarrheal disease. The objective of the study is to survey your pit latrine, and if it is suitable I will take samples of the pit waste. I will then feed the waste to the larvae to see how effective they are at eating it. I would like to take a small sample from the pit now, and maybe return to empty it later if it is suitable.

Time
It will take approximately 30 minutes to fill in the questionnaire, measure dimensions of the pit, and take photographs of the pit latrine. If you are happy for me to take samples then that will take a further 15 minutes. If I return to empty the pit later it may take a whole day to empty.

Household
I will not ask any questions that make you upset. If you are not happy you do not need to give a response during the interview and the survey will stop. All of your information provided to us will be kept confidential. A copy of this consent form will be given to you and one copy kept by the student.

Contact information:
If you have any question after interviewing, please contact the below information.

Researcher
- Ian Banks
- Mariedahl Experimental Farm, Elsenberg, Stellenbosch, 7607
- 072 6113 869
- ian.banks@lshtm.ac.uk

Do you want to participate in this study? If yes,

_____ _____ _____
Interviewer (***Translator name***) Interviewee Date

Appendix D) Pit Latrine Questionnaire

Pit ID	
Date	
Time	
Province	
Municipality	
Township	
Village	
Latitude	
Longitude	
Elevation	
Type of latrine?	Family/Communal
Name of Latrine Owner	
Owner Contact Number	
Average daily users?	
How many people in family under 5?	
How many people in family between 5 & 15?	
How often do you eat meat? (inc. fish)	3daily/2daily/1daily/weekly/monthly/never
How often do you eat stampmielies & beans?	3daily/2daily/1daily/weekly/monthly/never
How often do you eat Maize meal?	3daily/2daily/1daily/weekly/monthly/never
How often do you eat fruit & vegetables?	3daily/2daily/1daily/weekly/monthly/never
When was pit constructed?	
When was the pit last emptied?	
Is urine separated?	YES/NO
What is used for anal cleansing?	Water/Paper/Leaves/ Other:_____
If paper/leaves are used, where are they disposed?	In vault/separated/Other:_____
Is the vault content used in agriculture?	YES/NO
If yes, roughly how often?	
Is other waste disposed of in the pit latrine?	YES/NO
Quantity?	
How Frequently?	
If so what?	Kitchen waste/Wastewater/ All waste/Soil/Other:_____
Does the family use specific cleaning products or chemicals?	YES/NO
If so, what?	Bleach/Chlorine/Other:_____
Quantity?	
How Frequently?	
Does the Family use something to make the pit perform better?	YES/NO
What?	Soil/Bleach/Ash/Lime/ Other:_____
Quantity?	
How Frequently?	

Questionnaire		
Pit ID		
Latrine design?	Slab/VIP/UD/Other _____	
Number of vaults?		
Shape of vault(s)	Circle/Square/Other:_____	
Distance from top of the pit to sludge		Metre
Top layer of sludge	Solid/Liquid	
Reported depth of Vault?		
Is sludge at least 150cm Deep?	YES/NO	
Is the pit suitable for emptying?	YES/NO	

BSF Perception	
Would you feed your livestock on BSFL that have eaten pit latrine waste?	YES/NO
Would you eat livestock that have been fed BSFL which have eaten pit latrine waste?	YES/NO
Would you eat eggs produced by chickens fed BSFL which have eaten pit latrine waste?	YES/NO
IF feeding livestock on BSFL that have eaten pit latrine waste increased their value, would this change your perception?	YES/NO

Pit Odour	
<u>In Superstructure</u>	
Skatole – Odour Intensity	0/1/2/3/4/5/6
Ammonia – Hedonistic Tone	+4/+3/+2/+1/0/-1/-2/-3/-4
<u>In Pit</u>	
Skatole – Odour Intensity	0/1/2/3/4/5/6
Ammonia – Hedonistic Tone	+4/+3/+2/+1/0/-1/-2/-3/-4

Pit Emptying		
Is the vault lined?	YES/NO	
What is the vault lined with?	Bricks/Concrete Plaster/Other:_____	Rings/Mud/
Is the bottom of the vault lined?	YES/NO	
What is the bottom of the vault lined with?	Bricks/Mud/Plaster/Other:_____	
Depth of Sludge (metres)		Metre(s)
Dimensions of pit (metres) W x H x D		Metre(s)

Notes

Appendix E) Latin-squares

000	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	7	2	8	5	1	4	3	6
2_{BSFL}	1	5	3	2	7	8	6	4
3_{BSFL}	4	6	2	1	3	7	5	8
4_{BSFL}	2	7	1	8	6	5	4	3
1_{noBSFL}	6	4	5	7	8	3	1	2
2_{noBSFL}	5	3	7	6	4	2	8	1
3_{noBSFL}	8	1	4	3	2	6	7	5
4_{noBSFL}	3	8	6	4	5	1	2	7

100	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	1	2	6	3	4	5	7	8
2_{BSFL}	2	7	1	4	3	6	8	5
3_{BSFL}	4	8	5	1	2	7	6	3
4_{BSFL}	6	4	8	5	7	1	3	2
1_{noBSFL}	8	3	2	7	1	4	5	6
2_{noBSFL}	3	6	7	8	5	2	1	4
3_{noBSFL}	7	5	3	2	6	8	4	1
4_{noBSFL}	5	1	4	6	8	3	2	7

200	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	4	3	6	1	7	2	8	5
2_{BSFL}	6	8	5	3	2	7	4	1
3_{BSFL}	3	2	1	6	5	8	7	4
4_{BSFL}	1	5	8	7	4	3	2	6
1_{noBSFL}	5	6	7	8	3	4	1	2
2_{noBSFL}	7	1	4	2	8	6	5	3
3_{noBSFL}	2	7	3	4	1	5	6	8
4_{noBSFL}	8	4	2	5	6	1	3	7

001	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	7	4	6	5	3	2	1	8
2_{BSFL}	8	3	1	4	5	6	7	2
3_{BSFL}	5	2	4	7	6	3	8	1
4_{BSFL}	6	5	2	1	8	7	3	4
1_{noBSFL}	4	8	5	3	2	1	6	7
2_{noBSFL}	3	1	7	6	4	8	2	5
3_{noBSFL}	2	7	3	8	1	5	4	6
4_{noBSFL}	1	6	8	2	7	4	5	3

101	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	4	6	2	3	1	7	5	8
2_{BSFL}	5	4	6	7	8	3	2	1
3_{BSFL}	2	1	8	6	7	5	3	4
4_{BSFL}	1	2	7	8	3	4	6	5
1_{noBSFL}	8	7	1	5	6	2	4	3
2_{noBSFL}	6	5	3	4	2	8	1	7
3_{noBSFL}	3	8	4	2	5	1	7	6
4_{noBSFL}	7	3	5	1	4	6	8	2

201	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	2	6	5	4	8	7	3	1
2_{BSFL}	6	5	1	8	3	2	7	4
3_{BSFL}	3	4	2	6	1	5	8	7
4_{BSFL}	5	3	8	7	4	6	1	2
1_{noBSFL}	7	8	4	1	2	3	6	5
2_{noBSFL}	8	7	3	2	5	1	4	6
3_{noBSFL}	1	2	7	3	6	4	5	8
4_{noBSFL}	4	1	6	5	7	8	2	3

002	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	6	3	2	5	8	1	7	4
2_{BSFL}	3	2	8	6	5	7	4	1
3_{BSFL}	8	5	4	3	1	6	2	7
4_{BSFL}	5	8	1	4	7	3	6	2
1_{noBSFL}	4	6	7	1	3	2	8	5
2_{noBSFL}	7	1	5	2	4	8	3	6
3_{noBSFL}	1	7	6	8	2	4	5	3
4_{noBSFL}	2	4	3	7	6	5	1	8

102	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	4	2	1	8	5	6	7	3
2_{BSFL}	6	7	8	3	1	5	2	4
3_{BSFL}	1	8	6	5	7	3	4	2
4_{BSFL}	2	6	5	7	3	4	1	8
1_{noBSFL}	7	5	4	2	6	8	3	1
2_{noBSFL}	8	4	3	1	2	7	5	6
3_{noBSFL}	5	3	2	6	4	1	8	7
4_{noBSFL}	3	1	7	4	8	2	6	5

202	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	5	8	3	1	4	2	7	6
2_{BSFL}	6	4	7	2	8	3	1	5
3_{BSFL}	2	3	1	4	6	5	8	7
4_{BSFL}	3	5	4	6	7	1	2	8
1_{noBSFL}	7	6	8	3	2	4	5	1
2_{noBSFL}	8	1	2	7	5	6	3	4
3_{noBSFL}	1	7	6	5	3	8	4	2
4_{noBSFL}	4	2	5	8	1	7	6	3

010	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	2	7	6	3	5	4	1	8
2_{BSFL}	1	4	3	8	7	2	5	6
3_{BSFL}	7	6	5	2	4	3	8	1
4_{BSFL}	6	5	1	7	2	8	3	4
1_{noBSFL}	4	2	8	1	6	5	7	3
2_{noBSFL}	8	3	2	6	1	7	4	5
3_{noBSFL}	3	1	4	5	8	6	2	7
4_{noBSFL}	5	8	7	4	3	1	6	2

110	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	1	5	2	8	7	4	3	6
2_{BSFL}	7	1	5	4	6	3	2	8
3_{BSFL}	3	6	7	5	2	8	1	4
4_{BSFL}	5	3	4	2	1	6	8	7
1_{noBSFL}	8	2	3	7	4	5	6	1
2_{noBSFL}	6	4	8	1	5	2	7	3
3_{noBSFL}	2	8	1	6	3	7	4	5
4_{noBSFL}	4	7	6	3	8	1	5	2

210	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	4	5	8	6	1	7	2	3
2_{BSFL}	1	2	6	4	7	3	5	8
3_{BSFL}	3	7	4	2	8	6	1	5
4_{BSFL}	7	4	5	1	3	8	6	2
1_{noBSFL}	2	6	3	7	5	1	8	4
2_{noBSFL}	5	1	7	8	4	2	3	6
3_{noBSFL}	6	8	1	3	2	5	4	7
4_{noBSFL}	8	3	2	5	6	4	7	1

011	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	6	3	8	2	5	4	7	1
2_{BSFL}	1	5	2	7	4	6	3	8
3_{BSFL}	5	8	4	3	7	2	1	6
4_{BSFL}	8	4	1	5	3	7	6	2
1_{noBSFL}	2	6	7	4	1	3	8	5
2_{noBSFL}	3	7	6	8	2	1	5	4
3_{noBSFL}	4	1	3	6	8	5	2	7
4_{noBSFL}	7	2	5	1	6	8	4	3

111	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	7	3	4	8	1	5	2	6
2_{BSFL}	4	1	3	5	8	6	7	2
3_{BSFL}	8	2	1	4	6	3	5	7
4_{BSFL}	2	4	8	1	3	7	6	5
1_{noBSFL}	5	7	6	3	4	2	8	1
2_{noBSFL}	1	5	2	6	7	4	3	8
3_{noBSFL}	3	6	5	7	2	8	1	4
4_{noBSFL}	6	8	7	2	5	1	4	3

211	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	6	3	4	5	7	1	2	8
2_{BSFL}	8	1	7	2	4	5	3	6
3_{BSFL}	4	7	3	6	1	8	5	2
4_{BSFL}	7	6	1	8	3	2	4	5
1_{noBSFL}	2	8	5	3	6	4	7	1
2_{noBSFL}	3	2	8	1	5	7	6	4
3_{noBSFL}	1	5	6	4	2	3	8	7
4_{noBSFL}	5	4	2	7	8	6	1	3

012	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	8	5	1	3	6	4	2	7
2_{BSFL}	5	8	6	1	2	3	7	4
3_{BSFL}	3	7	2	4	5	6	8	1
4_{BSFL}	2	3	4	6	7	5	1	8
1_{noBSFL}	4	1	8	7	3	2	5	6
2_{noBSFL}	7	6	5	2	8	1	4	3
3_{noBSFL}	6	4	7	5	1	8	3	2
4_{noBSFL}	1	2	3	8	4	7	6	5

112	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	2	8	1	7	4	6	3	5
2_{BSFL}	4	6	5	3	2	7	1	8
3_{BSFL}	1	3	8	2	6	5	4	7
4_{BSFL}	8	7	2	1	3	4	5	6
1_{noBSFL}	5	2	6	4	7	1	8	3
2_{noBSFL}	7	5	3	8	1	2	6	4
3_{noBSFL}	3	1	4	6	5	8	7	2
4_{noBSFL}	6	4	7	5	8	3	2	1

212	Position							
Replicate	A	B	C	D	E	F	G	H
1_{BSFL}	7	6	3	4	1	2	8	5
2_{BSFL}	8	5	2	1	7	3	6	4
3_{BSFL}	1	4	6	2	3	8	5	7
4_{BSFL}	5	3	4	7	6	1	2	8
1_{noBSFL}	4	7	1	5	8	6	3	2
2_{noBSFL}	2	1	8	3	5	4	7	6
3_{noBSFL}	6	2	5	8	4	7	1	3
4_{noBSFL}	3	8	7	6	2	5	4	1

020		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	5	4	7	2	1	6	8	3
	2_{BSFL}	2	5	8	3	6	7	1	4
	3_{BSFL}	4	8	2	6	7	3	5	1
	4_{BSFL}	7	2	4	5	8	1	3	6
	1_{noBSFL}	3	1	5	8	4	2	6	7
	2_{noBSFL}	1	6	3	7	2	8	4	5
	3_{noBSFL}	6	7	1	4	3	5	2	8
	4_{noBSFL}	8	3	6	1	5	4	7	2

120		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	4	2	5	8	3	7	6	1
	2_{BSFL}	7	4	3	1	5	2	8	6
	3_{BSFL}	2	1	6	5	4	8	3	7
	4_{BSFL}	3	5	1	7	2	6	4	8
	1_{noBSFL}	1	8	2	3	6	5	7	4
	2_{noBSFL}	8	6	4	2	7	3	1	5
	3_{noBSFL}	6	3	7	4	8	1	5	2
	4_{noBSFL}	5	7	8	6	1	4	2	3

220		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	5	3	2	4	7	1	6	8
	2_{BSFL}	8	1	5	3	2	4	7	6
	3_{BSFL}	1	2	7	6	4	8	5	3
	4_{BSFL}	3	4	1	2	5	6	8	7
	1_{noBSFL}	6	7	4	8	1	3	2	5
	2_{noBSFL}	2	6	3	7	8	5	1	4
	3_{noBSFL}	4	5	8	1	6	7	3	2
	4_{noBSFL}	7	8	6	5	3	2	4	1

021		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	7	4	5	1	8	3	6	2
	2_{BSFL}	5	8	7	6	2	1	3	4
	3_{BSFL}	2	5	3	8	4	7	1	6
	4_{BSFL}	3	7	6	5	1	2	4	8
	1_{noBSFL}	4	1	2	7	5	6	8	3
	2_{noBSFL}	8	2	1	3	6	4	7	5
	3_{noBSFL}	1	6	4	2	3	8	5	7
	4_{noBSFL}	6	3	8	4	7	5	2	1

121		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	7	1	5	8	4	3	6	2
	2_{BSFL}	6	5	8	1	2	7	3	4
	3_{BSFL}	1	3	6	2	5	4	8	7
	4_{BSFL}	5	8	3	4	7	6	2	1
	1_{noBSFL}	2	7	4	3	1	8	5	6
	2_{noBSFL}	3	2	7	6	8	1	4	5
	3_{noBSFL}	4	6	2	7	3	5	1	8
	4_{noBSFL}	8	4	1	5	6	2	7	3

221		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	4	3	2	1	6	7	5	8
	2_{BSFL}	8	7	3	6	2	1	4	5
	3_{BSFL}	5	2	1	7	4	8	6	3
	4_{BSFL}	6	1	7	2	8	5	3	4
	1_{noBSFL}	1	6	4	8	5	3	2	7
	2_{noBSFL}	3	8	5	4	1	6	7	2
	3_{noBSFL}	2	3	8	3	7	4	1	6
	4_{noBSFL}	7	4	6	5	3	2	8	1

022		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	4	7	8	5	3	1	6	2
	2_{BSFL}	5	8	2	7	6	3	4	1
	3_{BSFL}	6	1	7	2	4	8	3	5
	4_{BSFL}	3	5	6	8	1	2	7	4
	1_{noBSFL}	7	2	1	6	5	4	8	3
	2_{noBSFL}	8	6	3	4	2	5	1	7
	3_{noBSFL}	2	3	4	1	8	7	5	6
	4_{noBSFL}	1	4	5	3	7	6	2	8

122		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	4	5	6	8	1	3	2	7
	2_{BSFL}	2	1	8	5	4	6	7	3
	3_{BSFL}	5	2	7	4	3	1	6	8
	4_{BSFL}	7	3	2	1	6	8	5	4
	1_{noBSFL}	6	7	4	3	2	5	8	1
	2_{noBSFL}	1	6	5	7	8	4	3	2
	3_{noBSFL}	8	4	3	6	7	2	1	5
	4_{noBSFL}	3	8	1	2	5	7	4	6

222		Position							
		A	B	C	D	E	F	G	H
Replicate	1_{BSFL}	5	3	4	1	8	6	2	7
	2_{BSFL}	4	5	6	8	7	2	1	3
	3_{BSFL}	7	2	1	6	3	8	4	5
	4_{BSFL}	2	1	3	7	5	4	6	8
	1_{noBSFL}	3	8	7	4	2	1	5	6
	2_{noBSFL}	1	4	8	3	6	5	7	2
	3_{noBSFL}	6	7	2	5	4	3	8	1
	4_{noBSFL}	8	6	5	2	1	7	3	4

Appendix F) Multiple regression analysis

```
. regress arc_ww_wr ib1.cont_treat##i.FR##ib1.MC##i.LD
```

Source	SS	df	MS		
Model	6480.42763	53	122.27222	Number of obs	214
Residual	843.275823	160	5.27047389	F(53, 160)	23.20
				Prob > F	0.0000
				R-squared	0.8849
				Adj R-squared	0.8467
Total	7323.70346	213	34.3835843	Root MSE	2.2958

arc_ww_wr	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
cont_treat						
Control	-5.018328	1.623341	-3.09	0.002	-8.224267	-1.812389
FR						
100	-8.889363	1.623341	-5.48	0.000	-12.0953	-5.683424
200	-6.357283	1.623341	-3.92	0.000	-9.563223	-3.151344
cont_treat#FR						
Control#100	-3.188114	2.295751	-1.39	0.167	-7.721996	1.345769
Control#200	.2115983	2.389494	0.09	0.930	-4.507417	4.930613
MC						
65	-6.008372	1.623341	-3.70	0.000	-9.214311	-2.802432
85	-6.854683	1.623341	-4.22	0.000	-10.06062	-3.648744
cont_treat#MC						
Control#65	-2.665669	2.295751	-1.16	0.247	-7.199552	1.868214
Control#85	-5.666241	2.295751	-2.47	0.015	-10.20012	-1.132358
FR#MC						
100#65	3.845828	2.295751	1.68	0.096	-.6880548	8.379711
100#85	2.528643	2.295751	1.10	0.272	-2.00524	7.062526
200#65	-5.916296	2.295751	-2.58	0.011	-10.45018	-1.382413
200#85	5.266155	2.295751	2.29	0.023	.7322727	9.800038
cont_treat#FR#MC						
Control#100#65	2.773949	3.246683	0.85	0.394	-3.63793	9.185827
Control#100#85	7.12934	3.246683	2.20	0.030	.7174614	13.54122
Control#200#65	4.858056	3.313632	1.47	0.145	-1.68604	11.40215
Control#200#85	7.460955	3.313632	2.25	0.026	.9168586	14.00505
LD						
800	-14.49764	1.623341	-8.93	0.000	-17.70358	-11.2917
1200	-4.999303	1.623341	-3.08	0.002	-8.205242	-1.793364
cont_treat#LD						
Control#800	.3936444	2.389494	0.16	0.869	-4.32537	5.112659
Control#1200	-2.232135	2.295751	-0.97	0.332	-6.766018	2.301748

FR#LD						
100#800	15.70746	2.295751	6.84	0.000	11.17358	20.24135
100#1200	5.459943	2.295751	2.38	0.019	.9260603	9.993826
200#800	12.40228	2.295751	5.40	0.000	7.868393	16.93616
200#1200	-6.846466	2.295751	-2.98	0.003	-11.38035	-2.312583
cont_treat#FR#LD						
Control#100#800	5.143142	3.313632	1.55	0.123	-1.400954	11.68724
Control#100#1200	4.838376	3.246683	1.49	0.138	-1.573502	11.25025
Control#200#800	-.4477586	3.379254	-0.13	0.895	-7.121453	6.225936
Control#200#1200	3.721878	3.313632	1.12	0.263	-2.822218	10.26597
MC#LD						
65#800	9.347903	2.295751	4.07	0.000	4.81402	13.88179
65#1200	.7838446	2.295751	0.34	0.733	-3.750038	5.317727
85#800	10.99396	2.295751	4.79	0.000	6.460073	15.52784
85#1200	-.4957289	2.295751	-0.22	0.829	-5.029612	4.038154
cont_treat#MC#LD						
Control#65#800	2.760451	3.313632	0.83	0.406	-3.783645	9.304547
Control#65#1200	4.015414	3.246683	1.24	0.218	-2.396465	10.42729
Control#85#800	8.253919	3.313632	2.49	0.014	1.709822	14.79801
Control#85#1200	9.779585	3.246683	3.01	0.003	3.367707	16.19146
FR#MC#LD						
100#65#800	-7.368683	3.246683	-2.27	0.025	-13.78056	-.9568042
100#65#1200	-5.862691	3.246683	-1.81	0.073	-12.27457	.5491879
100#85#800	-11.11488	3.246683	-3.42	0.001	-17.52675	-4.702998
100#85#1200	3.294888	3.246683	1.01	0.312	-3.11699	9.706767
200#65#800	-2.958557	3.246683	-0.91	0.364	-9.370436	3.453321
200#65#1200	9.104547	3.246683	2.80	0.006	2.692669	15.51643
200#85#800	-17.3121	3.246683	-5.33	0.000	-23.72398	-10.90023
200#85#1200	-5.315762	3.246683	-1.64	0.104	-11.72764	1.096117
cont_treat#FR#MC#LD						
Control#100#65#800	-3.458935	4.639084	-0.75	0.457	-12.62067	5.7028
Control#100#65#1200	-2.524908	4.591503	-0.55	0.583	-11.59267	6.542857
Control#100#85#800	-10.78533	4.639084	-2.32	0.021	-19.94707	-1.623599
Control#100#85#1200	-8.889094	4.591503	-1.94	0.055	-17.95686	.1786715
Control#200#65#800	-3.253792	4.686183	-0.69	0.488	-12.50854	6.000957
Control#200#65#1200	-6.590887	4.639084	-1.42	0.157	-15.75262	2.570848
Control#200#85#800	-7.413255	4.686183	-1.58	0.116	-16.668	1.841494
Control#200#85#1200	-10.07403	4.639084	-2.17	0.031	-19.23576	-.9122923
_cons	49.36373	1.147876	43.00	0.000	47.09678	51.63067

Appendix G) Supplementary results

Factor		Wet weight faecal matter reduction		
		F	df	*P*
pH		0.04	4, 36	0.99
tCOD		0.08	5, 34	0.99
sCOD		0.13	6, 34	0.99
NH_4^+		0.20	7, 31	0.98
protein		0.49	1, 58	0.49
carbohydrates		0.02	1, 58	0.89
VFAs		0.40	7, 31	0.89
Aluminium	Al	0.60	5, 34	0.70
Antimony	Sb	0.29	5, 31	0.91
Arsenic	As	0.43	7, 31	0.88
Cadmium	Cd	0.79	3, 36	0.51
Chromium	Cr	0.71	7, 31	0.66
Cobalt	Co	0.18	6, 33	0.98
Copper	Cu	0.28	3, 35	0.84
Iron	Fe	0.71	6, 32	0.64
Lead	Pb	0.30	7, 29	0.95
Manganese	Mn	0.11	6, 32	0.99
Mercury	Hg	0.82	6, 33	0.56
Molybdenum	Mo	0.43	5, 35	0.82
Nickel	Ni	0.25	6, 33	0.95
Selenium	Se	0.16	6, 32	0.98
Tin	Sn	0.86	6, 31	0.53
Vanadium	V	0.63	8,30	0.75
Zinc	Zn	1.26	6, 32	0.30

Factor		Prepupal dry weight		
		F	df	*P*
pH		2.41	3, 14	0.11
tCOD		0.38	1, 22	0.54
sCOD		3.85	1, 22	0.06
NH_4^+		1.27	1, 22	0.27
protein		0.71	1, 22	0.41
carbohydrates		2.21	1, 22	0.15
VFAs		3.09	1, 22	0.10
Aluminium	Al	0.63	1, 22	0.43
Antimony	Sb	0.57	1, 21	0.46
Arsenic	As	1.16	1, 22	0.29
Cadmium	Cd	0.36	1, 22	0.56
Chromium	Cr	0.35	1, 22	0.56
Cobalt	Co	3.81	1, 22	0.64
Copper	Cu	2.11	1, 22	0.16
Iron	Fe	0.46	1, 22	0.51
Lead	Pb	1.49	1, 21	0.24
Manganese	Mn	4.21	1, 22	0.05
Mercury	Hg	0.50	1, 22	0.49
Molybdenum	Mo	0.98	1, 22	0.33
Nickel	Ni	0.86	1, 22	0.36
Selenium	Se	3.93	1, 22	0.06
Tin	Sn	0.07	1, 21	0.79
Vanadium	V	0.89	1, 22	0.35
Zinc	Zn	0.55	1, 22	0.46

Factor		Bioconversion dry weight		
		F	df	*P*
pH		2.61	4, 19	0.07
tCOD		0.62	6, 17	0.71
sCOD		2.31	5, 24	0.08
NH_4^+		1.35	8, 15	0.30
protein		0.52	1, 28	0.48
carbohydrates		< 0.01	1, 28	0.98
VFAs		1.88	7, 16	0.14
Aluminium	Al	2.55	1, 25	0.12
Antimony	Sb	0.06	1, 27	0.81
Arsenic	As	2.31	1, 28	0.14
Cadmium	Cd	0.17	1, 28	0.69
Chromium	Cr	1.27	1, 28	0.27
Cobalt	Co	3.63	1, 24	0.07
Copper	Cu	1.69	1, 28	0.20
Iron	Fe	0.96	1, 28	0.34
Lead	Pb	0.07	1, 27	0.79
Manganese	Mn	2.72	1, 28	0.11
Mercury	Hg	<0.01	1, 28	0.96
Molybdenum	Mo	3.03	1, 28	0.09
Nickel	Ni	2.13	1, 28	0.16
Selenium	Se	0.54	7, 16	0.79
Tin	Sn	0.70	1, 27	0.41
Vanadium	V	0.84	1, 28	0.37
Zinc	Zn	1.15	1, 28	0.29

Factor		Prepupal ash		
		F	df	*P*
pH		1.05	4, 16	0.41
tCOD		0.37	6, 14	0.88
sCOD		1.01	1, 25	0.33
NH_4^+		2.61	1, 25	0.12
protein		0.12	1, 25	0.73
carbohydrates		< 0.01	1, 25	0.99
VFAs		1.07	7, 13	0.44
Aluminium	Al	1.31	1, 25	0.26
Antimony	Sb	0.68	1, 24	0.42
Arsenic	As	2.16	1, 25	0.15
Cadmium	Cd	1.61	1, 25	0.22
Chromium	Cr	0.88	6, 14	0.53
Cobalt	Co	1.02	6, 14	0.45
Copper	Cu	0.71	4, 16	0.60
Iron	Fe	0.85	1, 25	0.36
Lead	Pb	2.50	1, 24	0.13
Manganese	Mn	1.03	6, 14	0.45
Mercury	Hg	0.89	1, 25	0.35
Molybdenum	Mo	0.46	5, 15	0.80
Nickel	Ni	1.00	5, 15	0.45
Selenium	Se	0.26	7, 13	0.96
Tin	Sn	< 0.01	1, 24	0.95
Vanadium	V	1.42	1, 25	0.24
Zinc	Zn	0.56	1, 25	0.46

		Prepupal crude protein		
Factor		*F*	df	*P*
pH		0.80	4, 18	0.54
tCOD		0.79	6, 16	0.59
sCOD		2.87	1, 27	0.10
NH_4^+		1.73	8, 14	0.18
protein		0.32	1, 27	0.32
carbohydrates		< 0.01	1, 27	0.97
VFAs		3.56	1, 27	0.07
Aluminium	Al	2.27	1, 27	0.14
Antimony	Sb	0.48	1, 26	0.49
Arsenic	As	3.59	1, 27	0.07
Cadmium	Cd	2.46	1, 27	0.13
Chromium	Cr	3.95	1, 27	0.06
Cobalt	Co	0.89	7, 15	0.54
Copper	Cu	3.57	1, 27	0.07
Iron	Fe	0.86	1, 27	0.36
Lead	Pb	0.28	7, 14	0.95
Manganese	Mn	0.70	7, 15	0.67
Mercury	Hg	< 0.01	1, 27	0.97
Molybdenum	Mo	0.58	6, 16	0.74
Nickel	Ni	0.48	7, 15	0.83
Selenium	Se	0.61	7, 15	0.74
Tin	Sn	0.06	1, 26	0.81
Vanadium	V	1.38	1, 27	0.25
Zinc	Zn	0.29	1, 27	0.60

		Prepupal total solids		
Factor		*F*	df	*P*
Layer		1.74	4, 21	0.18
pH		0.37	1, 24	0.55
tCOD		0.08	1, 24	0.78
sCOD		0.59	1, 24	0.45
NH_4^+		1.24	1, 24	0.28
protein		3.03	1, 24	0.09
carbohydrates		2.58	1, 24	0.07
VFAs		0.28	1, 24	0.60
Aluminium	Al	0.01	1, 24	0.92
Antimony	Sb	2.23	1, 23	0.15
Arsenic	As	0.02	1, 24	0.88
Cadmium	Cd	0.03	1, 24	0.87
Chromium	Cr	0.43	1, 24	0.52
Cobalt	Co	0.02	1, 24	0.88
Copper	Cu	0.57	1, 24	0.46
Iron	Fe	0.01	1, 24	0.92
Lead	Pb	0.68	1, 23	0.42
Manganese	Mn	0.12	1, 24	0.73
Mercury	Hg	0.84	1, 24	0.37
Molybdenum	Mo	0.34	1, 24	0.34
Nickel	Ni	0.47	1, 24	0.50
Selenium	Se	0.02	1, 24	0.89
Tin	Sn	0.05	1, 23	0.82
Vanadium	V	< 0.01	1, 24	0.98
Zinc	Zn	0.04	1, 24	0.84

Factor		Prepupal crude fat		
		F	df	*P*
Layer		0.71	4, 22	0.59
pH		1.91	2, 24	0.17
tCOD		1.46	2, 25	0.24
sCOD		0.80	1, 25	0.59
NH_4^+		1.98	1, 25	0.17
protein		3.15	2, 24	0.06
carbohydrates		3.14	1, 25	0.09
VFAs		4.00	1, 24	0.06
Aluminium	Al	0.49	1, 25	0.49
Antimony	Sb	0.03	1, 24	0.86
Arsenic	As	1.42	1, 25	0.24
Cadmium	Cd	0.06	1, 25	0.80
Chromium	Cr	3.75	1, 25	0.06
Cobalt	Co	0.49	1, 25	0.49
Copper	Cu	0.72	1, 25	0.40
Iron	Fe	1.15	1, 25	0.29
Lead	Pb	0.14	1, 24	0.72
Manganese	Mn	< 0.01	1, 25	0.96
Mercury	Hg	0.39	1, 25	0.54
Molybdenum	Mo	0.40	1, 25	0.80
Nickel	Ni	3.16	2, 24	0.06
Selenium	Se	4.44	1, 25	0.05
Tin	Sn	0.15	1, 24	0.71
Vanadium	V	0.43	1, 25	0.52
Zinc	Zn	1.54	1, 25	0.23

Factor		Prepupal crude fibre		
		F	df	*P*
Layer		0.42	4, 13	0.79
pH		3.61	1, 16	0.08
tCOD		0.02	1, 16	0.90
sCOD		4.25	1, 16	0.06
NH_4^+		< 0.01	1, 16	0.98
protein		0.11	1, 16	0.74
carbohydrates		2.22	1, 16	0.16
VFAs		0.14	1, 15	0.72
Aluminium	Al	3.22	1, 16	0.09
Antimony	Sb	1.69	1, 15	0.21
Arsenic	As	1.11	1, 16	0.31
Cadmium	Cd	0.50	1, 16	0.49
Chromium	Cr	0.01	1, 16	0.92
Cobalt	Co	0.37	1, 16	0.55
Copper	Cu	1.22	1, 16	0.29
Iron	Fe	0.30	1, 16	0.59
Lead	Pb	0.63	1, 16	0.44
Manganese	Mn	0.30	1, 16	0.59
Mercury	Hg	1.08	1, 16	0.31
Molybdenum	Mo	< 0.01	1, 16	0.99
Nickel	Ni	0.01	1, 16	0.93
Selenium	Se	0.21	1, 16	0.65
Tin	Sn	0.43	1, 16	0.52
Vanadium	V	0.02	1, 16	0.88
Zinc	Zn	1.93	1, 16	0.18

		Prepupal gross energy		
Factor		*F*	df	*P*
Layer		2.38	4, 16	0.10
pH		4.53	1, 19	0.05
tCOD		1.88	1, 19	0.19
sCOD		0.27	1, 19	0.61
NH_4^+		0.26	1, 19	0.61
protein		0.55	1, 19	0.47
carbohydrates		0.01	1, 19	0.94
VFAs		0.57	1, 18	0.46
Aluminium	Al	0.33	1, 19	0.57
Antimony	Sb	1.33	1, 18	0.26
Arsenic	As	0.26	1, 19	0.62
Cadmium	Cd	1.14	1, 19	0.30
Chromium	Cr	< 0.01	1, 19	0.96
Cobalt	Co	0.11	1, 19	0.74
Copper	Cu	1.30	1, 19	0.27
Iron	Fe	0.12	1, 19	0.73
Lead	Pb	1.59	1, 18	0.22
Manganese	Mn	0.23	1, 19	0.63
Mercury	Hg	0.07	1, 19	0.80
Molybdenum	Mo	< 0.01	1, 19	0.96
Nickel	Ni	0.03	1, 19	0.88
Selenium	Se	0.34	1, 19	0.57
Tin	Sn	0.01	1, 18	0.92
Vanadium	V	0.01	1, 19	0.92
Zinc	Zn	0.72	1, 19	0.41