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Lucia Ledwaba

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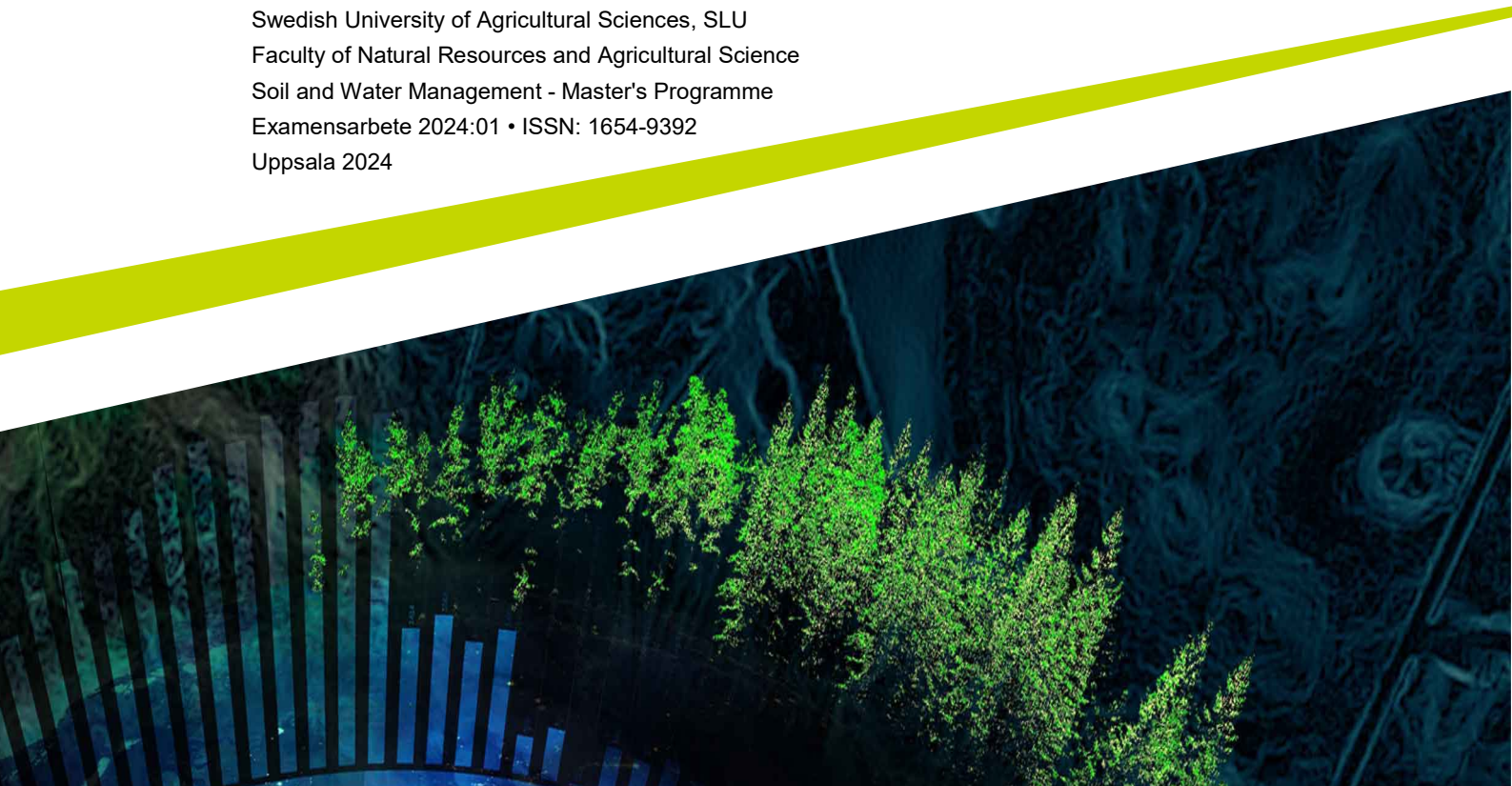

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Supervisor:	**Cecilia Lalander, SLU, Department of Energy and Technology**
Examiner:	Björn Vinnerås, SLU, Department of Energy and Technology

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ABSTRACT

Dry sanitation approach, where there is no need for water-flush, is a low-cost technology that has gained interest in recent years, and the focus in this study is vermicomposting as an on-site toilet system that can be used to reduce the wastewater released into the environment. A toilet design outline was used to illustrate and create a functioning vermicompost unit, whereby faeces was added into the vermicomposting unit on a daily-basis, to simulate an on-site urine diverting toilet. The earthworms used were *Esenia fetida* and the average feeding rate per adult worm of 0.02g/day and this system had a continuous (daily) faecal feeding that began at 80g/day for 3 weeks to 120g/day for 8 weeks. The indicator micro-organisms *Enterococcus* spp. and *Escherichia* spp. and pathogen *Salmonella* spp. were monitored and reduced in terms of concentration from 9 $\log_{10}$, 7 $\log_{10}$, 6.5 $\log_{10}$ respectively to 5 $\log_{10}$, 2.5 $\log_{10}$ and 3 $\log_{10}$. The bacteriophages MS2 and ΦX174 were also evaluated to no significant reduction within the vermicompost unit. A total material added into the vermicompost on a VS basis was 2.5 kg with an output material being that of 0.9 kg which gave a material mass balance reduction of 63 % (VS basis). The continuous faecal loading preceded in a gradual faecal build-up that did not affect the system from achieving this material reduction. Therefore, the vermicomposting system with its material reduction was seen as a viable dry sanitation approach for faecal decomposition.

Keywords: vermicomposting, faeces, dry sanitation*, Esenia fetida*,

Popular summary

When people speak of using their own waste to create a resource, truth be told, most people tend to think of the stereotypical tree-hugging activist. Words like green-toilet, dry toilets, waterless sanitation, composting toilets, vermicomposting toilets, are all words not usually heard or used in conversations. Adapting a new way of thinking and looking at a normal convectional toilet and its effect in the environment, one cannot help but seeking out innovative technologies that can help to counteract these environmental effects. Environmental effects such as that normal toilets require numerous litres (L) of water (per person) to flush and in areas where there is shortage of water, this is concerning. Water can be conserved if innovative, low-cost technologies such as vermicomposting are adapted in such water deprived areas. However, vermicomposting is not a popular toilet system as it has to do with earthworms, which could be consisted slimy to the average person.

In recent years, with the aid of Bill and Melinda Gates foundation, alternative toilets from a typical flush-toilet have been noticeable in acceptance especially with regard to their global “reinvent the toilet” campaign. Urine diverting-vermicomposting toilets can be seen as one of these alternative ways for safe sanitation, where it cooperates the mechanisms from earthworms to degrade the faecal matter and yield a product that has the potential to be used back in the environment. The one noticeable benefit from a vermicomposting toilet unit is that the earthworms are not visually exposed as they lay beneath the surface because they are considered “shy” to light exposure.

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1. Introduction

The global prospective on sanitation can be regarded as the means to provide adequate disposal of human waste and thus improving and protecting public health (Uchegbu, 2015). It incorporates hygiene measures that prevent the spread of bacteria and viruses from the excreta by limiting human contact with the disposed human waste as well as providing clean drinking water. Moreover, sanitation is fundamental to the integrity of human wellbeing and diseases prevention (Esa:United Nations, 2016). According to the World Health Organization (2015), 2.3 billion people still have no access to basic sanitation facilities; of these, 892 million still practice open defecation. In 2015, 2.9 billion people (roughly 39% of the global population) used safely managed sanitation services that ensure excreta is treated or disposed of in a safe manner (World Health Organisation , 2017). Furthermore, 13% of the global population used improved sanitation services such as ventilated latrines (VIP) and toilets where the excreta are disposed of *in situ* (WHO, 2017).

The current conventional approaches for managing human waste falls into two categories: waterborne systems (which requires water use) and dry systems (which require no water). In a conventional sanitation approach, many countries have the toilets at the user side, while the sewage removal is done by waterborne piping operated and/or provided by a municipality or public sector of a government (Buzie-Fru, 2010). This integrated waterborne piping network collects black-water from the toilet units, greywater from households (namely kitchen, laundry and bath water), storm water, industrial wastewater and direct the collected water towards a treatment centre for biological and chemical treatment, to be later discharged into nearby water bodies (Steven A Esrey et al, 1998). According to a study conducted by Baum R et al (2013), approximately 50% of the world's water bodies are being polluted with untreated wastewater. This is from sewage that is not treated before being discharged into the environment. Untreated wastewater that is released into water bodies endangers the health of the surrounding communities by increasing the risk of spreading infectious water-induced diseases such as cholera and typhoid fever through drinking water (Guyton A.C, 1992). It is estimated that the lack of safe drinking water, proper sanitation as well as handwashing is the cause of 0.3 million deaths every year (Lim S.S et al., 2012). The lack of treatment of the excreta causes eutrophication of various waterways, which has an impact on the aquatic ecosystems (Fidjeland, 2015). Generally, most sewage treatment plants around the world – excluding those in most high-income countries – are aimed at removing solids and not nutrients such as nitrogen and phosphorous from the wastewater, which leads to insufficiently treated wastewater being discharged into lakes and streams causing eutrophication, an overload of nutrients in the water. Therefore, lakes and streams often experience toxic algae blooms (FAO, 2017).

In some areas in the world, where there is shortage of water, sewage treatment plants have high volume load of sewage that their facilities cannot handle efficiently over long periods of time (G. Hill et al., 2012). One way to solve this issue could be to reduce the sewage load that comes into these treatment plants by implementing a dry sanitation approach at a household and/or community level thus this approach would reduce the water used in disposing of sewage to these facilities, and thus reduce the sewage load (SuSanA , 2008). The treatment

plants could then effectively treat the loads according to their infrastructural capacity (G. Hill et al., 2012). Dry sanitation can be defined as the disposal of human excreta without the use of water transport, which theoretically produces no wastewater (Niwagaba, 2009). This approach can help to reduce the ground and surface water contamination, should the waste be treated or disposed of correctly, and lessening the risk of spreading diseases and reducing the wastewater nutrient load in receiving water bodies.

A dry sanitation facility is a waterless system such as dehydrating toilets, composting toilets and urine diverting dry toilets (UDDT) (G. Hill et al., 2012). They provide an on-site system that retains both the liquid (urine) and solid excretion where they are produced and then are either composted or dehydrated (Buzie-Fru, 2010). In a composting toilet the faeces or/and the urine are degraded through aerobically decomposition process (Fittschen & Niemczynowicz, 1997).

One of the challenges for managing human excreta at decentralized sites is the lack of standard municipal infrastructural resources such as water supply, financial contribution for road access and technical support (G. Hill et al., 2012). On-site disposal systems currently in use in various places around the world offer a low-cost sanitation solution that goes towards the development of basic and improved sanitation (M. Gonzalez et al, 2010). The solid/faecal fractions collected by either composting, storage or burning or other forms of treatments, can be used for soil amendment or as fertilizer for food production hence a resource (H. Jönsson et al., 2008). The downside of this system is that should these fractions not be treated correctly and then released into the environment, there is a risk of soil contamination, groundwater pollution particularly during high rainfall seeping (Buzie-Fru, 2010). Pathogens that are found in human waste that have been released with the untreated excreta can be transmitted via the faecal-oral route and can have dire consequences to the health of the communities if consumed (Ottoson, 2004). Therefore, fractions that are collected from these toilet systems may be viewed as either a hazard or a resource to the environment, depending on how there are treated and released.

Alternatively, a vermicomposting toilet can be used as a dry sanitation approach, where the urine is diverted from the faeces and the solid fraction treated by vermicomposting (Lalander, Hill, & Vinneräs, 2013). The earthworms facilitate the microbial decomposition of the organic solids by maintain an aerobic environment thereby changing the material within the toilet system (Dominquez & Edwards, 2004).

As human excreta contain disease causing microorganisms (pathogens), the dry system provided must reduce the pathogen concentration during treatment before utilizing the end product (USEPA, 2017). A marked reduction in pathogens such as *Salmonella* spp. and other bacteria of the Enterobacteriaceae family during vermicomposting of aerobic sewage sludge has been demonstrated (Mitchell, 1978). However, studies have been done on sewage sludge and livestock excreta in composting consequently limiting the scientific data research conducted into the vermicomposting system with the use of faecal material as a substrate.

1.1 Aim

The main aim of this study was to investigate the *in-situ* faecal decomposition in a vermicomposting toilet and to evaluate the reduction of pathogenic bacteria and selected indicator microorganisms in a vermicomposting system that was designed to simulate an on-site toilet.

2. Background

2.1 Faecal matter composition

Faeces consists of materials that have passed through the human intestine and can be regarded as undigested material that is mixed with material extracted from the blood stream or fragmented from glands (Guyton A.C, 1992). Faeces can contain a relatively large concentration of pathogenic viruses, bacteria, cysts of protozoa and eggs of helminths (Feachem et al, 1983; WHO, 2006).

The composition of faeces depends largely on the food intake, body weight and health status, and these factors vary from low-middle income countries to higher-income countries (Rose. C. et al, 2015). In industrialized countries, because of high consumption of protein-based diets such as fish and meat, the average daily production of defecation is 80-140 g (wet weight) per person, corresponding to approximately 25–40 g (dry weight) per person per day (Niwagaba, 2009). In low- to middle-income countries such as Thailand, the average wet faecal weight production ranges between 120 to 400 g per person per day, and this higher production is due to the higher consumptions of fibre and vegetable-based diet (Schouw N.L et al, 2002).

Faeces are generally composed of water, undigested fats, polysaccharides, protein, bacterial biomass and undigested food residues. The larges fraction in faeces is water (75%) (Snyder W et al, 1975). The remaining 25% comprises of solid organic material. The solid fraction can be further taken apart to have bacterial biomass, the dead and living bacterial with a combined estimate of 25-54% depending on the diet of the human excreting the faeces. It also has a protein fraction ranging from 2-25%, undigested cellulose, vegetable fibres and other undigested plant matter and undigested lipids (Rose. C. et al, 2015). These fractions strongly dependent on the food intake and the biological availability (Achour L, 2006).

The inorganic fraction of the solid composition is predominately made up of iron phosphate, calcium phosphate and other small amounts of digestive juices or intestinal secretion. In terms of the total amount of nutrients excreted, 10-20% is of nitrogen and potassium and 20-50% of the phosphorus can be found in the faecal fraction, while the rest is found in urine (Niwagaba et al, 2009; Vinnerås et al, 2006). Of the nitrogen found in the faecal fraction, about 17% is contained in bacterial biomass and 10% found as ammonia from degradation of urea, amino acids and peptides. The remaining proportion of the nitrogen is found in different organic compounds such as uric acid and different enzymes or peptides (Vinnerås, 2001)

Due to the low digestion rate of consumed heavy metals, the main proportion will be found in the faecal fraction, where these heavy metals can remain undigested through the intestines (Vahter et al, 1991). Over time heavy metals such as cadmium and mercury are excreted in

urine, trace elements Cu, Zn, Cr are also excreted through sweat in different proportion (Scroeder & Nason, 1971).

Pathogenic bacteria can be present in organic wastes from several different genera (e.g. *Salmonella* spp.) and can be secreted with faeces (Table 1). Many of these pathogenic bacteria are zoonotic micro-organisms, meaning they can carry infectious diseases and can transmit between animals and humans. They can cause symptom-free infectious as well as serious infectious (Elving, 2009). An example is *Escherichia coli* O157, which is a serotype of *E.coli* , causing health problems for humans and has shown to survive up to 10 weeks in faeces. Other human pathogens found in faeces have been implicated in transmissions of various infectious diseases, including typhoid fever caused by *Salmonella* Typhi; and diarrhoeal diseases caused by both viruses, parasites and bacterial pathogens (World Health Organisation, 2017) (Table 1). The transmission of these are commonly through the faecal-oral route, but transmission can also occur via respiratory route. Also, viruses being smaller than bacteria and parasites, they are easily transported in the environment and can cause contamination of groundwater and other water reservoirs (Elving, 2009).

Table 1: Some human carrier pathogens found in faeces and their transmission pathways (not limited to) routes of water; food and from person to person spreading.

	Transmission (pathway)	*Diseases / symptoms*	*Description*
Salmonella Typhi	Water, food	Typhoid fever	Zoonotic Bacteria
Salmonella spp. (non-typhi)	Food, water (rare)	Food poisoning	Zoonotic bacteria
E. coli	Water, food	Diarrhea, gastroenteritis	Zoonotic bacteria
Hepatitis A	Water, food	Infectious hepatitis	Zoonotic virus

(World Health Organization, 2016)

2.2 Vermicomposting process

Vermicomposting is a bio-oxidative process of degrading solid organic matter that involves the interaction of earthworms and micro-organisms (the micro-organisms that are both in the earthworm guts and in the faeces (Dominquez & Edwards, 2004). This method can alter the chemical properties of raw materials with the assistance of enzymes in the gut of the earthworm (Iasha Diana Putri et al, March 2020). The earthworms mix, aerate and fragment the material, which increases the surface area exposing it to further microbial activities (F. Fernando et al, 2012). During this process, important plant nutrients in the faeces, i.e. Ca, P, N and K, are mineralised and converted into a plant available form through the microbial interactions (P.M Ndegwa et al., 2001).

The process of vermicomposting consists of two different periods that relate to the earthworm activity, namely an active vermicomposting phase, in which the earthworm modifies the physio-chemicals characteristics of the material as well as its microbial composition (Lores et al., 2006), followed by a maturation period when the earthworms are displaced and micro-organisms decompose the processed material (M. Aira et al., 2007).

Optimal conditions for a vermicomposting process require a neutral pH and a material moisture content between 75-85% and this is to sustain the worm population within the system (F. Fernando et al, 2012). Unlike thermophilic composting, in which high temperature kills pathogens, vermicomposting is a mesophilic process (Usman Ali et al., 2015). Due to the mesophilic nature of vermicomposting process, it does not pass the guidelines for pathogen destruction for class-A compost – which is said to be satisfactory for general distribution according to the Environmental Protection Agency rules to further Reduce Pathogens (PFRPs) (USEPA, 2017).

The vermicomposting process results in a stabilisation, and reduction in volume, of the material. The vemicompost is usually a finely separated and peat-like with high porosity and water holding capacity that contain nutrients in forms that can be taken up by plants (Dominquez & Edwards, 2004). According to Arancon et al (2014), the vermicompost has shown to increase plant yield when can be used as organic fertilizer.

2.3 Earthworm's characteristics

The most common earthworm used for vermicomposting is *Esenia fetida*, with the common name red wiggler or red worms, although many other species have the potential to degrade organic solids (Dominquez & Edwards, 2004). These earthworms physical characteristics are thread-like, elongated, cylindrical with uniform ring-like structures all along the length of their bodies (Gajalakshmi, 2004). *Esenia fetida* are temperate species with a natural ability to colonize wastes. They have a high rate of consumption, assimilation of organic matter and digestion. They also have a tolerance to a wide range of environmental factors, with optimal moisture levels of 85% (Dominquez & Edwards., 2012). They have a pink-brown colour appearance and the adult sized earthworm is 4-8 mm by 50-100 mm in length with a mean weight of 0.55 g. The lifecycle ranges between 45-51 days, and it takes 28-30 days for the earthworms to mature (Dominquez & Edwards., 2012). The waste ingested by the earthworms is digested and decomposed by proteolytic enzyme in the stomach and various enzymes secreted in the intestine. Calcium that is excreted in the inner-walls of the worm's oesophagus will soften and neutralize the organic waste ingested by the earthworm. Therefore, the earthworms will excrete an un-digested substrate called vermi-cast (Bhat, 2017)

2.4 Vermicomposting of faecal matter

Studies demonstrated by Payal Garg et al (2006) showed that some earthworm's species such as the *Esenia fetida* consume a wide range of organic wastes like sewage sludge, animal dung/waste, industrial refuse and crop residue. Also, it is known that vermicomposting has the ability to degrade other wastes, such as household waste, horse manure, apple and grape pomace used in the fruit tea industry (Garcia-Sanchez et al., 2017). Many studies have been reported on the use of vermicomposting for the processing of different organic wastes, while only few have studied the use in the processing of source-separated human faeces (Puri et al., 2004; and Shalabi et al., 2006). Puri (2004) demonstrated the survivability and reproduction of earthworms in human faeces and reported that the earthworm's survival was possible because of the physio-chemical characteristics of the human waste and the composition of the organic material. Shalabi (2006) attempted vermicomposting with

partially degraded faecal matter by using two different earthworm species. It was concluded that faecal matter could be converted to mature compost by vermicomposting within a period of usually three months, in cases when the temperature was between 20 and 30 °C. It was also found that adding in $CaCO^3$ to faecal matter in a vermicomposting process could lead to a product with advanced maturity in terms of total organic carbon, volatile solids, and respiration activity (Shalabi, 2006)

2.5 Literature remarks on microbial reduction

Temperature and moisture content are to be considered as factors involving the inactivation of bacterial pathogens, however, in a published book by Edwards et al. (2014), it was cited that earthworm activities may lead to a pathogen reduction through competitive interactions of the bacterial organisms and the earthworms themselves, provided the moisture level remain stable (Clive A. Edwards et al,, 2014). It has been demonstrated through the study of Pedersen and Hendriksen (1993) that microbial species and their activities to decrease or increase, changes greatly in the passage through the gut of the earthworms (Pedersen J.C et al., 1993).

The influence of earthworms in the vermicompost process on human pathogens can be complex. Mechanisms stated by Edwards et al. (2010) by which human pathogens may be reduced, involve the effects of mechanical disruption due to ingestion and the grinding action of the earthworms, the destruction of micro-organisms by enzymatic digestion and assimilation, as well as some effects may include the stimulation of other microbial species that may lead to competition, antagonism or phagocytic activity from both within the earthworms and within the materials (Clive A. Edwards et al, 2010). Another possible mechanism for the reduction of human pathogens within the vermicomposting process is suggested by Edwards et al. (2014) and N. Soobhany (2017) which is the production of antimicrobial substances such as humic acids that indirectly effects the pathogens and the increase in the contact from the earthworms and the microbial community within the system (N. Soobhany et al., 2017). In 2012, Yadav et al found a concentration of *Salmonella* spp. to be below detection limit of 1 Log_{10} CFU/g after 6 months of vermicomposting of faecal slurry (Yadav et al., 2012). Rahul Kumar (2011) found similar findings with removal of *Salmonella* spp. that ranged between $12x10^3$ and $17x10^3$ that decreased to $4x10^3$ CFU/g and *Escherichia coli* reduction from $8x10^2$ to $1x10^1$ CFU/g over a duration of 70 days, indicating the selective nature of microbial reduction (Rahul Kumar et al., 2011). In another studies done on cow manure and oat straws, there was a reduction in *E. coli* and *Salmonella* spp. to very low concentrations (Contreras-Ramos SM et al, 2005). In 2001, Eastman et al, published a paper that studied the effectiveness of vermiculture in reducing pathogens, it found a significant improvement in the reduction of *Salmonella* spp. enteric virus and faecal coliforms in comparison to a control system with no earthworms. (Eastman BR et al., 2001). Lalander et al. (2015), published a case study conducted in Kampala and found no active *Salmonella* spp. in vermicomposted material, however, *Salmonella* spp. was found in the untreated materials, which suggested that it was inactivated during the process of vermicomposting (C. Lalander et al, 2015). In the same case study, it was observed that there was no inactivation of *Enterococcus* spp., this was stipulated to be due to recontamination of processed materials by untreated material that was continuously loaded

into the treatment system. Similarly, in a study published in 2011 by Aira et al., on the reduction of pathogens in a continuous loading feed of cow manure, it was found that there was a reduction in faecal enterococci and *E. coli* (Aira M et al., 2011).

3. Material and Methods

3.1 Materials used

Medium to small stones used for the bottom of the system to create a drainage, textural element for the system, were collected in the SLU, Mark Vatten Miljö garden. Wood bark, under the brand name Täckbark Gardol (average TS 28.7%; VS of TS 83.3%) was purchased at Bauhaus (Uppsala, Sweden). Immature food waste compost (average TS 25.2%; VS of TS 82.5%) was received from a household compost (Uppsala, Sweden). Earthworms (*Esienia feotida*) were purchased at Vermigrön (Hofors, Sweden). Vermicomposted material, used as bedding (average TS 17 %; VS of TS 83 %), was received with the worms. Fresh faeces (average TS 25 %; VS of TS 87 %) were collected and kept at -20 °C in dog excreta plastic bags from anonymous donors estimated between early to middle adulthood (ages 22 - 45), in the SLU, Department of Energy and Technology (Uppsala, Sweden). Before the start of the experiment, all collected faeces were thawed, homogenized and separated into 120 g packets that were then kept at -20 °C until use.

Chromocult agar plates were made from heating 500 mL of deionized water mixed with 13 g of chromocult medium (Chromocult®, Merck Millipore). The solution was poured into petri dishes for solidification 20 ml in each. All other microbial media were purchased pre made from the National Veterinary Institute (SVA, Uppsala, Sweden).

3.2 Inoculation

The inoculate solution comprised of *Escherichia coli* (ATCC 8739) and *Salmonella enterica* subspecies – 1 serovar Typhimurium phage type 178 isolated from sewage sludge (Sahlström L et al., 2004) grown in unselective microbial nutrient growth medium (NB, Oxoid AB, Sweden)

3.3 Propagation of phages

2 mL soft agar were taken into a test tube placed in a heating block and 1 mL diluted sample and 1 mL host strain were vortexed and poured gradually onto a Blood agar (BAB) plate to incubate at 37 ℃ for 12 h. The propagation of bacteriophage ΦX174 was cultivated against its host strain *E. coli* (ATCC 13706) and MS2 phage against its host strain *Salmonella* WG49. The host bacteria were cultivated in a nutrient broth and incubated on a shaker at 37 ℃ for 90 min. A ration of 1:1 concentration of phages and bacteria were determined and incubated at 37 ℃ on a shaker for 24 h. After incubation, solution were centrifuged at 4000 rpm for 3 min to sediment the cellular debris and supernatant is clear. The supernatant were collected by sterile filtration into a new container.

3.1 Experimental set-up

3.1.1 The first experimental set-up

Earthworms were placed in a 2 L round white bucket from IKEA (Uppsala, Sweden). Earthworms with bedding were placed directly into the 2 L buckets and no faeces were added. Addition of 80 g of faeces were added into the 2 L bucket 24 h later, to allow the adaption of the earthworms. On the fourth day, 80 g of faeces were added and placed in a heap in the centre of the 2 L bucket. The faeces were gently spread around the surface using the back of a spatula spoon, this was to increase surface area to allow for better consumption by the earthworms. No other measurements were taken and no further feedings were conducted with this system design as all earthworms died.

3.1.2 The second experimental set-up

The aim of the system design was used to simulate a toilet design. A 30 L bucket with an outlet/hole at the side of the bottom to allow for leachate/drainage from the system was used (Figure 1). The size of the bucket was used with reference to the dimensional size of a conventional toilet (438 mm x 375 mm) with an area of 0.15m^2. A plastic liner was placed inside the 30 L bucket to allow for an easier transferring of the vermicompost material from the unit to outside the unit for measurement purposes.

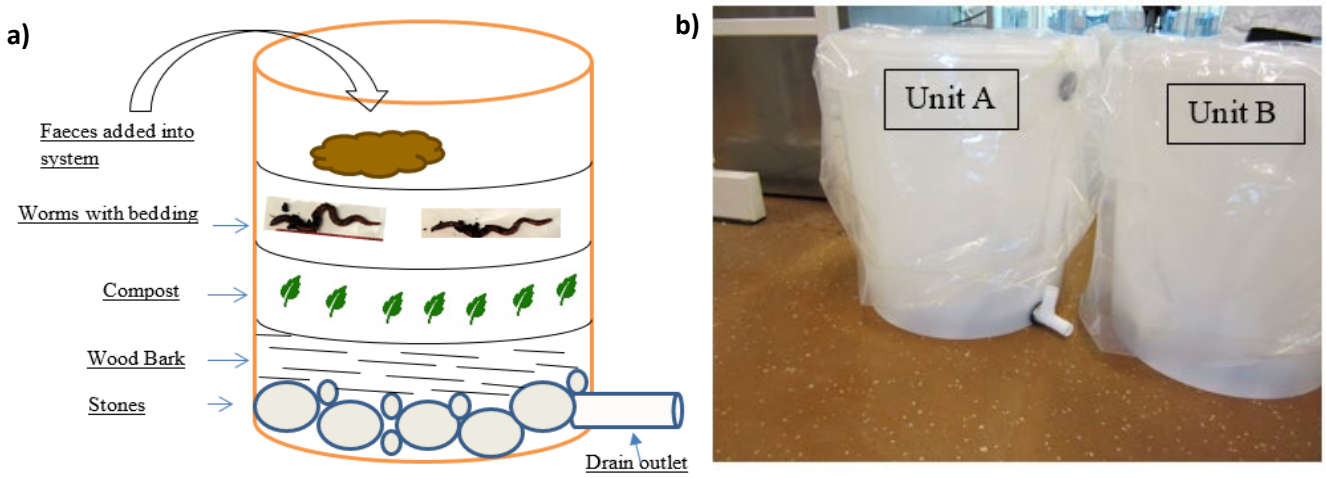


Figure 2: The vermicomposting unit: a) description illustration of the vermicompost; b) picture of replicated vermicomposting unit A, B, C. The daily faecal feed was added above the bedding with the worms.

Upon placing the plastic liner in the 30 L buckets with small to medium stones ranging from 8 cm to 12 cm in size, were placed at the bottom of the bucket to create structure and to allow excess drainage to flow to the outlet.

The first material to be placed into the system were wood bark. The wood bark was used to provide extra bedding for the earthworms and addition of carbon source for the system. The next material placed into the system were immature food waste compost, acting as additional bedding and extra buffer zone for the worms. The vermicompost unit were constructed in

triplicates. There were 2700, 2600 and 2800 number of worms respectively, per replicate (Table 2) and the worms arrived with their own bedding and placed onto the immature compost material. A 24 h time window was introduced between adding the worms and adding faeces into the system to allow for the adaptation of the earthworms to their new environment.

At the start of the experiment, 80 g of faeces contaminated with 1% (w/w) of *Salmonella* spp. and bacteriophages MS2 and ΦX174 were added into the vermicompost system a week into the second experimental set up. Further addition of 80 g of contaminated faeces every second day for three weeks: e.g. on Monday's, Wednesday's and Friday's. The addition of faeces every second day was done to gradually allow the adaptation of the earthworms and to observe and note the degradation of the faeces within the system. At week 1 and 2 the concentration of *Salmonella* spp. was below the detection limit in the faeces and therefore inoculation of salmonella into the faeces was started at week 5.

On the fourth week, the feeding of faeces increased to 120 g per day and this daily feeding rate was kept throughout the 10-week experiment. The faeces were added on the surface of each vermicompost unit. In order to stimulate the conditions in a toilet unit, the daily feeding faeces were placed in a small heap. A total of 4 kg of faeces per unit were added into the system for the duration of the study.

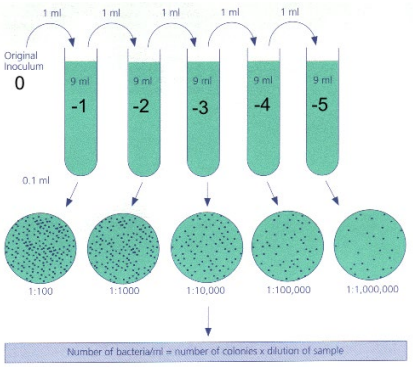
Table 2: Quantitative description of the material added into each initial unit.

Description & Unit	*A*	*B*	*C*
Stone weight (kg)	5.9	5.3	5.4
Wood bark - mulch (kg)	5.4	5.2	5.3
Compost[1] (kg)	1.8	2.0	1.9
Worms with bedding (kg)	1.7	1.6	1.7
Total initial weight (kg)	15	14	14
Worms Biomass (g)	680	640	700
Number of worms	2700	2600	2800
Total amount of faeces (kg)	4	4	4

1: compost with general household waste was already in the process of degradation

3.2 Sample collection

Samples from each unit was taken at random surface location at approximate depth of 5 cm. The hypothesis was that the earthworms would have mixed the faeces into the bedding for degradation. A sample (1 g) was taken before adding the faecal material into the units (A, B and C). Sampling was done every week from the vermicompost units. Thus, each week, test tube samples were taken from each unit for analysis.



3.2.1 Final sampling

On the final week of the experiment (11th week), each unit were separated into three equal layers (as the materials were homogenised) and poured into three smaller containers to determine the density. The earthworms were sorted manually and weighed: the material surface was exposed to light, which caused the earthworms to move towards the bottom of the unit, upon which the material was removed manually. The density of the final vermicompost material was determined by collecting 200 g of sample (a homogenous material found in the vermicompost after separation of worms) placed into a 500 mL container. Enumeration of juvenile/small worms and cocoons were estimated from the density test to extrapolate the concentration to the entire material.

3.3 Sample Analyses

Pathogenic bacteria (*Salmonella* spp.) and indicator organisms (*Escherichia coli*, *Enterococcus* spp., bacteriophages ΦX174 and MS2) were used to model the patterns of reduction for pathogens (disease-causing organisms) during the degradation of faeces in the vermicompost units. The faecal indicators used were *Enterococcus* spp. (a naturally occurring gram-negative cocci in the intestine tract of humans), *Escherichia coli* (which is a form of faecal coliform that is indicative of faecal contamination). *Salmonella* spp. were monitored as pathogens within the system and were selected as they are pathogenic bacteria found in excreta, which can cause symptom-free infectious as well as serious infectious (Elving, 2009). No differentiation was made between *E. faecalis* and native *Enterococcus* spp., thus indicator organisms are easier to sample and to measure (Elving, 2009; Sidhu & Toze, 2009). Bacteriophages (viruses that infect bacteria), which are host specific, were used as indicator organisms for enteric viruses, as bacteriophages have similar morphology, size and structure to that of animal viruses (Bradley. I et al,, 2011).

3.3.1 Microbial analyses

From each 1 g samples taken within the vermicompost and faecal input, a dilution series were made using a 9 mL of buffer solution of 0.9% NaCl peptone water with 0.1% surfactant Tween 80 (pH 7). The samples were vortexed to mix the contents within the test tubes and 1 mL of selected diluted was taken and spread evenly on specified plates for enumeration of bacterial colonies. For analysis of *Enterococcus* spp., 1 mL of diluted samples were spread on Slanetz & Barley agar (SlaBa) plates and incubated at 44 °C for 48 h. All colonies on the plates were enumerated. For the detection of *Escherichia coli*, 1 mL of diluted samples were spread on

chromocult plates, incubated at 37 °C for 24 h prior to enumeration of the CFU. Purple/blue colonies were enumerated. For analysis of *Salmonella* spp, 1 mL of diluted samples were spread on Xylose-Lysin-Deoxycholate (XLD) agar and incubated at 37 ℃ for 24 h. Colonies growth with a black centre were enumerated and counted as *Salmonella* spp.

3.3.2 Phage analyses

For the analysis of bacteriophages, translucent plaques formed on a Blood agar (BAB) plates were counted after incubated at 37 °C for 12 h for each dilution series concentration.

3.3.3 Physico-chemical analyses

The total solids (TS) of the materials was determined after drying samples at 105 °C for 24 h and its total volatile solids (VS) determined after heating at 550 °C for 6 h. The TS and VS were determined from each material added into the system, from the vermicompost and the worms.

3.3.2 Worm analysis

For microbial analysis of the worms, predominately adult worms were used following the procedure described in Lalander et al (2015). Five worms were washed in buffered solution of 0.9% NaCl peptone water with 0.1% surfactant Tween 80 (pH 7) and further bathed in 70% ethanol. The worms were placed at -20 °C for 24 h. The worms were weighed and homogenized using a mortar and pestle. A diluted series were conducted to enumerate the colony forming units of *Salmonella* spp, *Esherichia coli, Enterococcus* spp.

4. Calculations

The rate of biological decomposition is measured by the feeding rate of the individual organisms and their total biomass. The outcome of these two variables provides a criteria for the rate of substrate decomposition per unit volume. Below are calculations used:

- worm density (kg/m^2) = worms (g) x 1000 / area of unit (m^2) (Eq. 1)
- Number of worms = worms (g) / average weight of the adult worm (g) (Eq. 2)
- Actual feeding (g/day) = average faecal feeding (g) / day (Eq. 3)
- Average feeding rate per adult worm (g/day/worm) = actual feeding (g/day) / number of worms (Eq. 4)
- Daily worm feeding rate (g ww faeces/kg-worms/d) = daily worm feeding rate (g-VS/kg-worms-day) / ave faecal TS% x VS of dw % (Eq. 5)

Probable additional feeding rate will take into account the number of small worms produced during the experiment:

- Additional feeding = number of small worms x average feeding rate (g/d/worms)

(Eq. 6)

- Possible feeding rate for the vermicompost unit (g/day/m2) = actual feeding (g/day) + additional feeding/ area unit (m2) (Eq. 7)
- Feeding rate (g faeces-ww/m2) = $\frac{\text{feeding rate of worm g-VS/m2}}{\text{Ave faecal TS\% x dw \%}}$ (Eq. 8)

The average respiration (kg) was calculated by the average taken from the total VS loss (kg) of each unit. The Mol (g/mol) of carbohydrates was 162, oxygen was 32, carbon dioxide was 44 and water was 18 thus the normality of concentration (n) was the molar mass (g/mol) divided by the total VS loss (g) for each unit. It is assumed that the vermicompost material consisted of polysaccharides ($C_6H_{10}O_5$) which are starch and cellulose, with six molecules of O_2 to degrade into six molecules of CO_2 and five molecules of H_2O. It was further assumed that the total mass of the material reduced on an organic content basis had been fully respired into CO_2 and H_2O.

Average respiration (kg) was determined in the following:

- Total VS loss (kg) = n x Molar mass (g/mol) (Eq. 9)
- O_2; H_20; CO_2 was determined by: VS loss(g)/1000 (Eq. 10)

The density (g/L) was calculated using the mass of the materials (g) by the volume (L). The density analysis of vermicompost material was determined by the volume of the container divided by the number of worms within each 500 mL of container. The cocoons determined was taken from the volume of the container divided by the number of cocoons found in each 500 mL container.

Total biomass of worms (g/m^2) was determined by the total biomass divided by the area of the unit.

The total solids (TS) and volatile solids (VS) were determined as:

- Total Solids (%) = (TS) / (wet weight) × 100 (Eq. 11)
- Volatile Solids (% of TS) = 1 - (ash weight) / (TS) × 100 (Eq. 12)

The material reduction was measured on a VS basis where the vermicompost (VC) of the total VS:

- Mat_Red = (Tot.VS_IN) / (Tot.VS_OUT) X 100 (Eq. 13)

where Tot.VS_IN and TotVS_OUT is the total sum of the average of VS (kg) of the materials added to the VC and material out, respectively.

The total VS (kg) for all materials added into the units as inflow and materials of the outflow was estimated by:

- Total_VS = mat_TS% x VS of dry weight % x total weight (Eq. 14)

where the mat_TS is the inflow materials added and the VS of the dry weight and the total weight of the added material. The same is expressed for the outflow materials.

The material reduction on a total solids basis was calculated assuming that the total amount of ash in the IN material will equal the total amount of ash content in the OUT material:

- In_totAsh + WBM_totAshAdded = OUT_totAsh + WBM_totAshHarvested (Eq. 15)

where the In_totalAsh is the total mass of the ash content of the Inflow material and WBM is the worms biomass. The OUT is the total mass of the ash of the material outflow and the harvested from the units. Where the density of the worms was measured per unit volume (Lalander et al, 2015).

The log reduction of the average microbial concentration was calculated as:

- Log reduction = Log_{10} (initial) – Log_{10} (final) (Eq. 16)
- Moisture content (%) = 100 – Total Solids (Eq. 17)

4.1 Statistically Analysis

Statistical analysis of the experimental data was performed using Microsoft Excel. Results were reported as a mean of three. A t-test was performed to compare the bacterial count and a Pearson Product-Moment Correlation (PPMC) was determined for two variables. The probability levels for statistical significance were p-value <0.05 for the tests. Pearson correlation of the experimental duration (time) and average bacterial CFU were to determine correlation between the two variables.

ANOVA with a 95% confidence interval was used to find whether a statistically significant difference existed between the initial CFU/g and two variables: the average CFU/g at 10 weeks and the average CFU/g at 11 weeks. By using the equal variance for the standard error mean, a normal distribution can be indicated which means that although the p-value in the probability plot is <0.005, there is normal theoretically distribution in the data presented.

5. Results

5.1 Material flows in the units

Table 3: Physiochemical results of each material of the inflow and outflow (average of triplicates with standard deviation)

	INFLOW		
	Ave TS (%)	Ave VS (% of TS)	Total weight (kg)
Bedding	**17±0.9**	**83±5.0**	**1**
Wood bark	**28±2.9**	**67±0.6**	**0.5**
Raw compost	**25±0.2**	**83±3.7**	**1.9**
Faeces	**25**	**87**	**4**
Toilet paper [1]	**97**	**40**	**0.05**
1. **(Ververis C et al., 2007)**			
	OUTFLOW		
	TS (%)	Vs of dw (%)	Total weight (kg)
Layer 1	**15±0.9**	**62±1.1**	**4 ±0.6**
Layer 2	**14±1.1**	**65±2.6**	**3±0.4**
Layer 3	**16±2**	**61±1.5**	**3 ±0.05**

*Since the outflow material were homogenized it was difficult to separate into the initial inflow material, however, the end material was separated into three layers to get the average TS and VS.

The inflow material varied in TS% whereas the TS content of the outflow material were consistent between 14 -15 % therefore showed a homogenised in material in the outflow. The average VS % during the study varied throughout the inflow material ranging 67 -87 % which reduced to an average of 63 % VS of dw in the outflow materials.

Throughout the study period, the moisture content varied between 70 – 85 %, as compared to a study of vermicomposting drying process where the moisture content was between 75 and 90% (Kakitis, 2017)

The pH measured throughout the units at the inflow and outflow had an average of 7.5, similarly to a case study by A. Parvaresh (2004) with a pH between 7-8 (A Parvaresh et al, 2004)

Table 4: The worm density analysis taken from the worms going into the vermicompost and the worm's biomass measured at the end of the experiment (average of triplicates with standard deviation)

	Inflow	**Outflow**
Average worm biomass (kg/unit)	0.7±0.02	0.6±0.1
Total feed of faeces (kg)	12	
Average faeces feed per unit (kg)	4	
Average feaces feed per day (g)	120	
Average weight of an adult worm[1]	0.3	
Actual feeding (kg/day)	0.05	
Actual feeding rate (kg/m^2/day)	0.4	
Ave number of adults worms (worms/unit)	2700±45	2500±0.4
Area of unit (m^2)	0.14	
Worm density (kg/m^2)	4.8±0.17	4.5±0.7
Daily worm feeding rate (g VS/kg-worms d)		30
Ave daily worm feeding rate (g ww faeces/kg-worms d)		140
Ave daily area feeding rate (g VS/m^2 d)		140±16
Daily area feeding rate faeces (g ww faeces/ m^2 d)[2]		620±73
Average additional feeding		5.8
Ave Possible faecal feeding rate (g/day)		58
Total average number of small worms (worms/unit)		280±12
Total possible biomass (kg-worms/m^2)[3]		13±1.4
Ave possible daily area feeding rate (g ww faeces/ m^2 d)		1700±200

1 (U.S General Services Administration, National Archives and Records services, 1999)
2 Calculated as the total VS reduction / (77 days of experiment/area of the unit)

3 The combined biomass of the already adult worms and potential adult biomass of the small worms.

Table 4 demonstrates the actual feeding rate of contaminated faeces into the system and the potential feeding rate with the assumption of the rates by the addition of small worms and cocoons that were still to mature. The time-period to full-size maturity of the worms was not considered. The average number of small worms per unit in the vermicompost outflow was 280. There was a slight decrease in earthworm biomass as well as earthworm density over 77 days of the experiment and a decrease in the number of adult worms (Table 4). The average weight of the worms was 0.3 g which gave a worm density averaging at 4.8 kg-worms/m^2 at the end of the experiment at a feeding rate of 0.4 kg/m^2/day. The average feeding rate per adult worm was extrapolated to be 0.02 (g/day-worms). The actual feeding rate was found to be 52 (g/day). If the experiment had taken longer than 77 days, the assumption is that a possible feeding rate of the worms would be 58 (g/day).

The mass balance conducted over the system demonstrated a VS inflow of 2.5 kg, while 0.9 kg of VS was found in the outflow, resulting in an overall VS loss of 1.5 kg (Figure 2). This indicated a 38 % of material within the system remained on the VS basis, meaning that the material reduction was 63 % over 11 weeks.

Table 5: The chemical formula $C_6H_{10}0_5$ was used to calculate the total VS loss (kg) and respiration amount of oxygen (O_2), carbon dioxide(CO_2), water(H_20) of the system, where n is the normality of concentration

Sample A	**$C_6H_{10}O_2$**	**O_2**	**CO_2**	**H_2O**
n	8.0	48	48	40
Total VS loss (kg)	1.3	1.5	2.1	0.7
VS loss (g)	1300	1600	2200	720
Sample B	**$C_6H_{10}O_2$**	**O_2**	**CO_2**	**H_2O**
n	10	63	63	53
VS loss (kg)	1.7	2.0	2.8	0.9
VS loss (g)	1700	2030	2800	950
Sample C	**$C_6H_{10}O_2$**	**O_2**	**CO_2**	**H_2O**
n	9.6	58	58	48
VS loss (kg)	1.6	1.8	2.5	0.9
VS loss (g)	1600	1900	2500	870

From the breakdown of polysaccharides ($C_6H_{10}O_2$) assumed to be mainly in the vermicompost, the estimated amount of oxygen needed for respiration was 1.8 kg and, in the process, the average carbon dioxide (CO_2) generated from the system was 2.5 kg and the water vapour released was 0.9 kg. It is assumed that the worms consume oxygen while feeding on the organic matter and generates carbon dioxide and water vapour which is released (Weidong Wang et al., July 2013)

The system maintained a neutral pH average of 7.5 with a standard deviation of 0.08 and moisture content between 75-85 %.

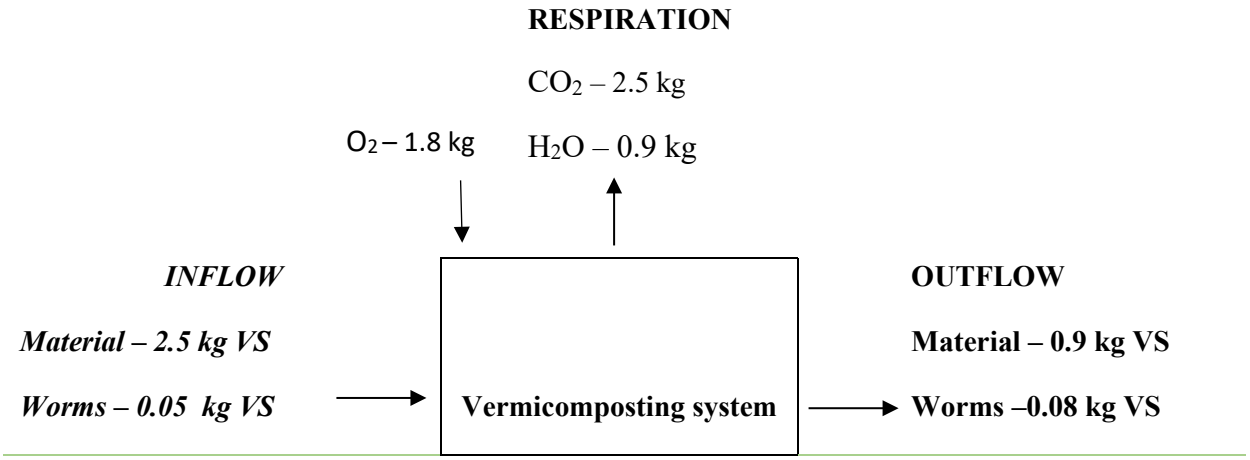


Figure 2: A schematic representation of the carbon flow in the vermicomposting system, with VS in the materials that were added to the system (INFLOW) and the VS in the material measured at the end of the experiment (OUTFLOW).

5.2 Fate of bacteriophages in the vermicompositing system

5.2.1 Evaluation of bacteriophages found within the system

The bacteriophages were monitored over 11 weeks (Figure 3). The concentration of MS2 and ϕx174 bacteriophages in the faeces added into the system were evaluated and compared with the bacteriophages concentration in the vermicomposted material from five weeks onto the 11th week in the outflow material.

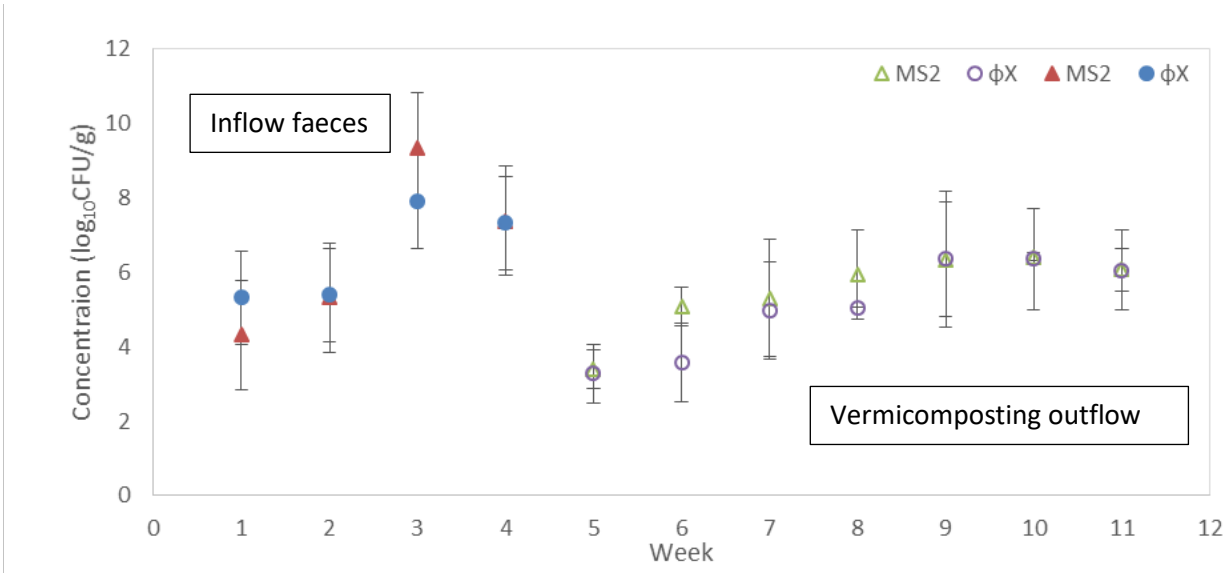


Figure 3: The average log_{10} concentration of bacteriophages of MS2 and ϕx from week 1 to week 4 (with solid fills) of the faecal input and the average log concentration of the vermicompost outflow materials from week five to week ten.

With regards to the bacteriophages monitored within the vermicompost, there was no inactivation with the continuous feeding mode, however, there was a stabilisation in terms of concentration of MS2 and ΦX17 in the material at 5 log10 PFU/g. There was no additional reduction after faecal feeding had stopped.

5.3 Fate of micro-organisms

5.3.1 Inflow material –evaluation of bacteria found within the faeces added to the system

The concentrations of *Enterococcus* spp. and *Escherichia coli* in the system were compared from week 1 to week 8. There was a high concentration of *Enterococcus* spp (Figure 4) within the input material added into the system from the start of week 1 until week 8, as compared to the *Salmonella* spp. and *Escherichia coli* concentrations. The *Escherichia coli* concentration was the lowest over the 8 weeks of the experiment. *Salmonella* spp. was initially not detected in the faecal input material at week 1 but it was detected from week 3, after inoculating *Salmonella spp.* to the faeces.

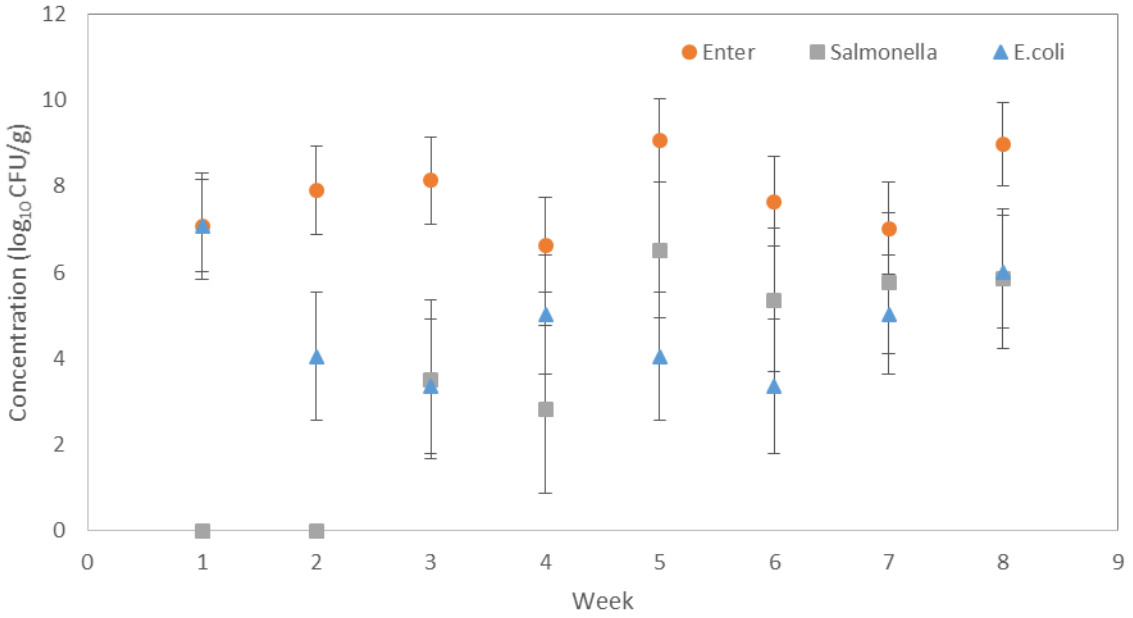


Figure 4: Concentrations (log_{10} CFU/g) of Enterococcus *spp. (orange circles),* Salmonella *spp. (grey squares) and E. coli (blue triangles) in the faeces added into the vermicompost over eight weeks.*

5.3.2 Outflow material - Concentrations of microorganism found in the vermicompost system

To allow for degradation to occur and earthworms to adapt to the continual environment of faecal inputs, samples for microbial levels were taken from week five.

5.3.3 *Salmonella* spp.

The concentration of *Salmonella* spp. in the faecal input (indicated as stars in Figure 5) varied over the course of the experiment. The average concentration found in the vermicomposted material was 4.4 log_{10} CFU/g and although, for the weeks to come, it appeared to increase within the vermicompost. The concentration of *Salmonella* spp. found in the vermicomposted material was lower than the concentration found in the faeces added.

From week 5 to week 6, there was a reduction of 4log_{10} (99.99 % elimination of the pathogen), however, the inconsistency of the inactivation of *Salmonella* spp. throughout the system was better appropriated with the process of dilution.

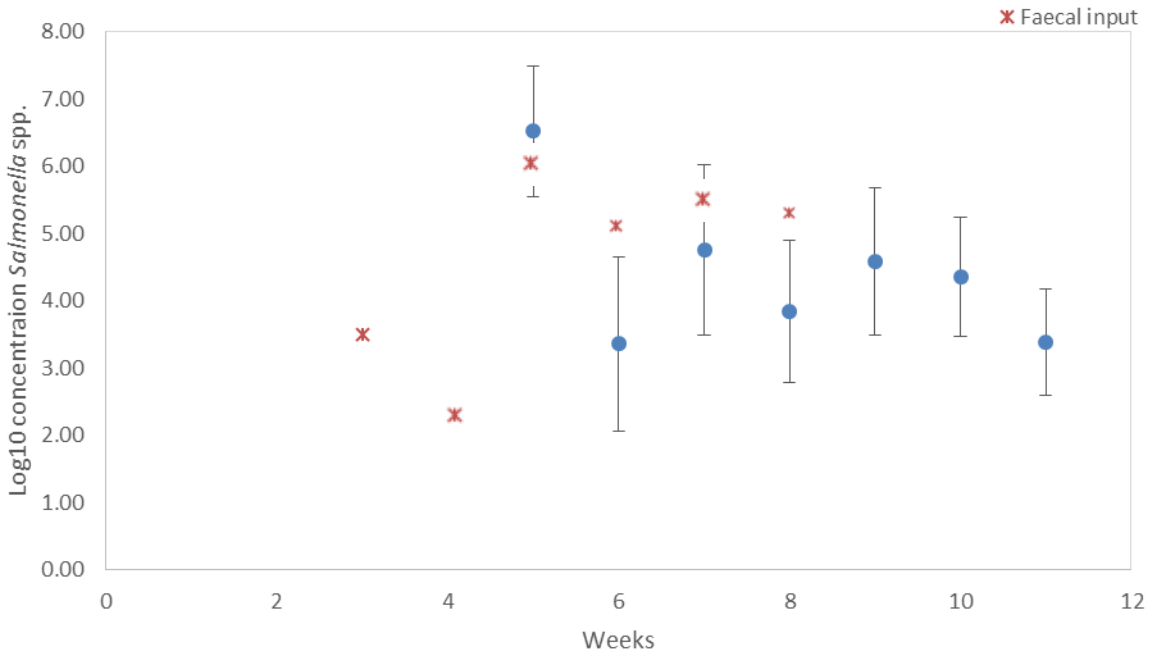


Figure 5: The log concentration of Salmonella *spp. in the vermicompost system over time with the indication of the faecal input concentrations marked with a star symbol. A standard deviation error bars from 3 samples each week.*

There was no consistency in the reduction in *Salmonella* spp. The average decline of S*almonella* spp. in the vermicomposting systems at the final week of adding input material into the system was on average 2.5 $\log_{10}$ (Figure 6). At 11 weeks after continual feeding had stopped there was an average of 3 $\log_{10}$ dilution which was estimated. This indicated a 99% dilution of *Salmonella* spp. in the system during continual feeding.

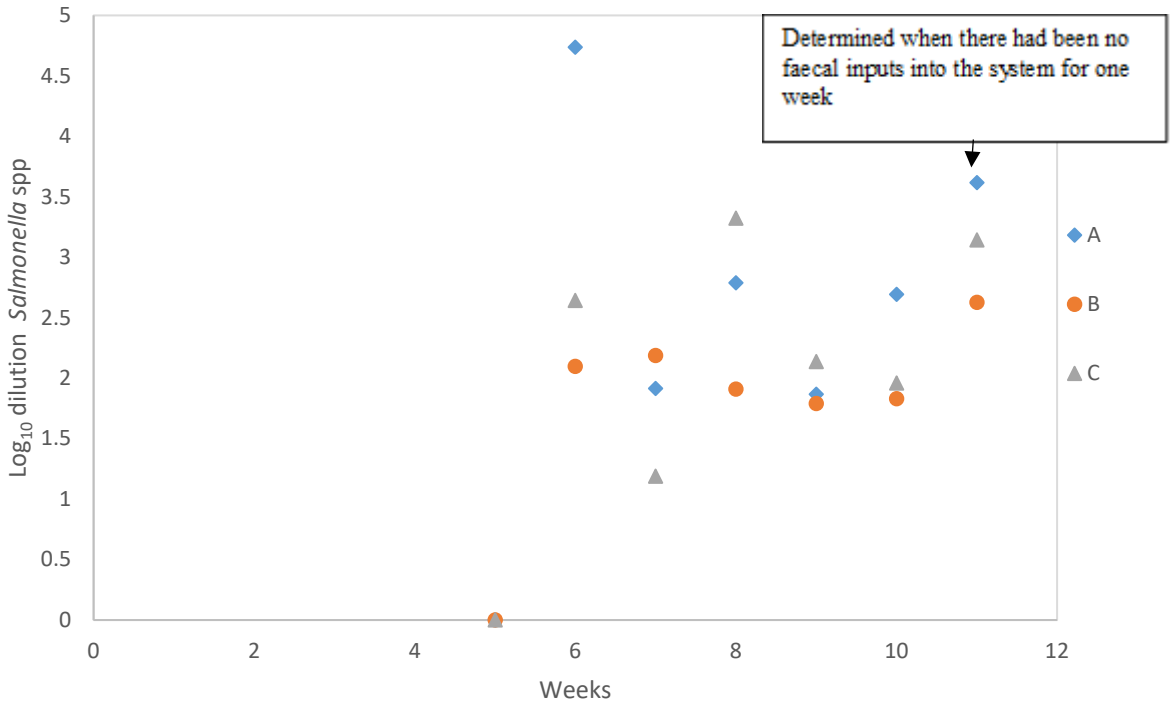


Figure 6: Log_{10} dilution in Salmonella *spp. concentration calculated as the difference in concentration between the inflow faeces and the concentration in the vermicomposting system, in units (A, B and C) over time.*

5.3.4 *Escherichia coli*

In Figure 7, the concentration of *E. coli* in the system was found to be at a higher concentration that the concentrations of the faecal inputs, except in the case at week 8 where the concentration of the faecal input was 6 log_{10}, which was 1 log_{10} unit less than in the vermicompost system when analysis was done at week 5. The highest concentration of *E. coli* found in the system was at week 5 at 7 log_{10} CFU/g, which was the same concentration found in the faecal input at week 1. The lowest concentration of 2.5 log_{10} within the system was at week 11, when there had been no faecal addition.

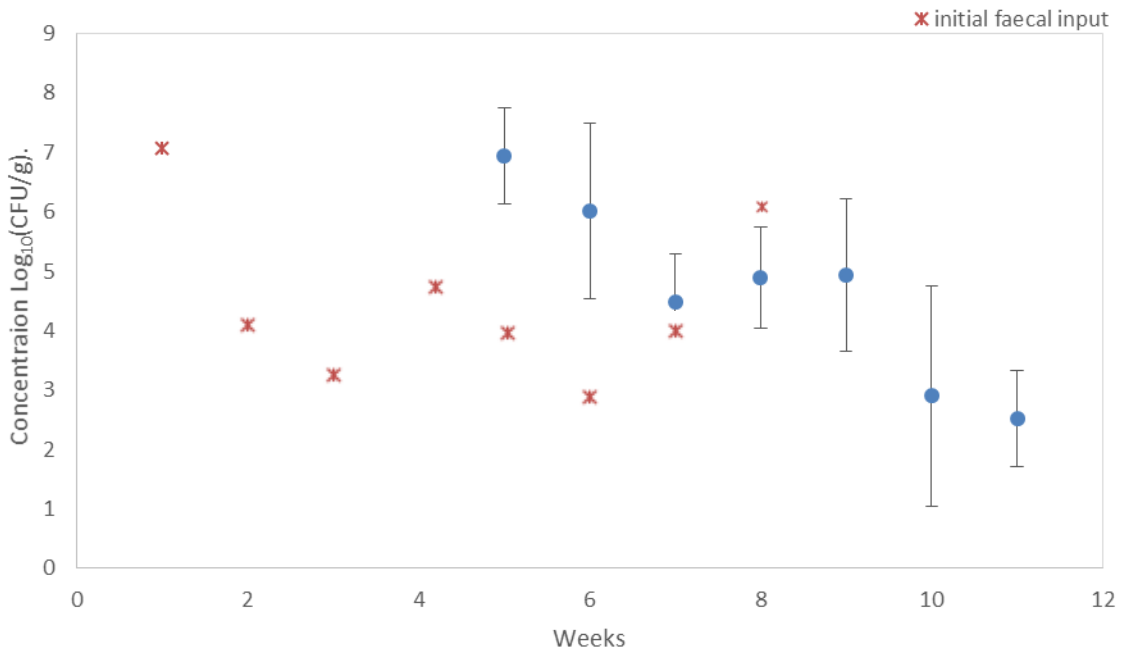


Figure 7: The log concentration of E. coli *found in the vermicompost system over time with the indication of faecal input marked with a star symbol through eight weeks. Standard deviation error bars presented with a total of 3 samples each week.*

5.3.5 *Enterococcus* spp.

The initial concentration of *Enterococcus* spp. found in the faecal input week 1 was 7 $\log_{10}$. The average concentration at week 5 was 9 $\log_{10}$ in the vermicompost system, the same concentration found at week 5 for the faecal input (Figures 4 and 9). There is no significant dilution process in this system from week 5 to the final week, as the concentration of *Enterococcus* spp. was at an average of 5.5 $\log_{10}$ throughout the system.

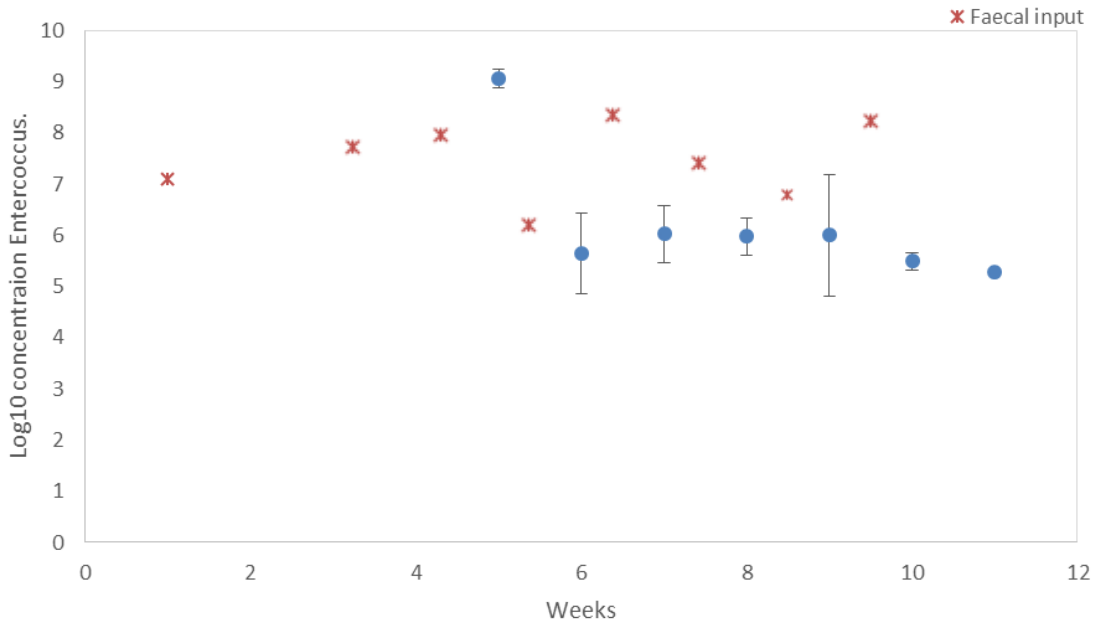


Figure 8: The log concentration of Enterococcus spp. found in the vermicompost system over time with the indication of faecal input represented by the star symbol. Standard deviation error bars with 3 samples per week.

For *Salmonella* spp. and *E. coli* there was a significant difference from the initial faecal inflow concentration measured at week 5 to that at week 10 (Table 6). However, there was no statistically difference between when there was daily faecal feeding into the system to when there was no faecal feeding (week 10 - 11).

Pearson correlation of the average concentration and time was -0.860, this statically analysis showed there is a strong correlation between the time and the average CFU/g of indicator organisms (*Escherichia coli* and *Enterococcus* spp.) and *Salmonella* spp. For micro-organisms with the p-value larger than the significant level of 0.05 indicate that the null hypothesis can be rejected.

Table 6: Summary of log concentration and reduction of micro-organisms in the vermicompost system. Presented as average (n=3) ± standard deviation.

	Week 5 (log_{10} CFU/g)	Week 10 (log_{10} CFU/g)	Week 11 (log_{10} CFU/g)	Log_{10} reduction in continual feeding (weeks 5-10)	Log_{10} reduction in no feeding (weeks 10-11)
Salmonella spp.	$6.5^{\pm 0.6}$	$4^{\pm 0.3}$	$3^{\pm 0.2}$	3 [b]	3 [b]
Escherichia coli	$7^{\pm 0.4}$	$3^{\pm 2}$	$2.5^{\pm 0.16}$	2.7 [a]	4.4 [a]
Enterococcus spp.	$9^{\pm 0.05}$	$6^{\pm 0.9}$	$5^{\pm 0.03}$	3.3 [b]	3.8 [b]
MS2	$3.4^{\pm 0.6}$	$6^{\pm 0.08}$	$6^{\pm 0.02}$		
Φx174	$3.2^{\pm 0.2}$	$6^{\pm 0.02}$	$6^{\pm 0.01}$		

[a] Significant difference from when there was faecal feeding into the system.
[b] No significant difference from faecal feeding.

6. Discussion

The earthworms are sensitive to changes in their environment, so it is important to adjust their environment in a way that protects their survival (Garcia-Sanchez et al, 2017). A system design that has bedding material is critical in the survival of the earthworms, especially when using harsh materials such as faeces in the treatment process. The initial system design did not have an environment to support the survival of the earthworms when feeding fresh faeces, hence the system failed with a complete die-off of the earthworms. The second system design was created in a way to provide an environment adapted for the earthworms to survive as well as provide a habitat that supported all stages of the lifecycle of the earthworm. The materials such as wood mulch and compost added to the second system design which allowed for the earthworms to degrade the materials over time.

The life expectancy of *Eisenia fetida* under controlled conditions varies between 1 to 5 years, with a reproductive lifecycle between 40 to 60 days, if favourable moisture and nutritive conditions are maintained (J.M Venter & A.J Reinecke, 2015). In this experiment, mature worms averaging 2700 per unit, were used in the initial input material and the experiment duration was a total of 77 days which means the juvenile (small) worms, which averaged 280 per unit, did not reach full maturity within the system. This would mean that for the long term of such a system, the reproductive lifecycle of the earthworms should be considered for an efficient vermicompost system. Nevertheless, the decrease of 0.04 kg/unit of the total worm biomass from the start of the experiment to the end of the 11 weeks, can be assumed, it is from the incomplete lifecycle of the earthworms.

The average worm density at the end of the experiment was 4.5 kg/m^2 (Table 4), which was higher compared to P. Ndegwa (2000) with an earthworm density at 1.60 kg/m^2 (P.M Ndegwa et al., 2000). The experimental system proved to degrade faeces at an estimated rate of 630 g faeces/m^2-d at the end of the 77 days experimental trial with an average feeding rate per kg worm biomass was 140 g-ww faeces/kg-worms-d in this study. The average feeding rate can be viewed along with that of (C. Lalander (2015) with an estimation of 400 g-feed/kg-worm-day at the end of the experiment even though the exact feeding rate was not resolute in that study since the entire number of worms of the unit could not be known (C. Lalander et al, 2015). However, stated by (C. Lalander (2015) at a higher feeding rate, substantial part of material in the units were found unprocessed even though a higher feeding rate would generate a greater biomass production.
By comparison of these two case studies, C. Lalander (2015) fed each unit every day to every third day as it was observed that if more waste than what the worms consumed were added to the unit, the temperature within the system increased above the body temperature of the worms which caused the worms to move to the edge and top of the units, leaving unprocessed material in the centre. Whereas, this case study the units were fed daily with the same amount of faecal material and having a lower feeding rate which likely caused the worms to thoroughly process the material. The other contributing factor to the difference in the feeding rate in these two case studies, can be that of the different materials used in C. Lalander (2015), which 80 % of total feed added were one day old manure and food waste which comprised of 20 % of the total feed added. This study, however, used only faecal matter as its input material.

Hartenstein & Hartenstein (1981) experimented on vermicomposting of activated sludge and approximately 1 g worm could convert 4 g of activated sludge in five days, meaning a feeding rate of 0.8 kg-feed/kg-worm/day (R & F Hartenstein et al, 1981). In this experiment the feeding rate was close to 0.14 kg-feed/kg-worm-day, this means this case study had a lower degradation rate in the vermicomposting process as compared to Hartenstein & Hartenstein (1981). One fraction of the inflow material (the compost) was not fully matured in this experiment hence could have affected the efficiency of the degradation rate of the faeces because the worms degrade the compost simultaneously with the faeces, instead of only focusing on degrading the faecal matter therefore the degradation rate would be improved when the worms only have the input materials to degrade. It can be assumed that a matured

compost be used as bedding material for the worms which also acts as a refuge for the worms when the conditions become unfavourable (e.g. high temperature or ammonia concentration (Dominquez J. E., 2010). However, the inflow compost material was accounted for in the calculations of the total reduction. Another aspect contributing to the rate of degradation was the presence of inert material, for instance there was a large bone within one unit that could have affected the adaption of the worms however, it was unlikely that this large bone affected the efficiency of the degradation rate of the system.

With regards to the reduction of VS %, a comparison to a study by A. Parvaresh (2004), were there was a difference in input materials used from dewatered sludge, straw and decaying leaves which had a VS reduction of 92 %, to 45 % in municipal sludge (A Parvaresh et al, 2004). Hartenstein & Hartenstein (1981) reported a 9 % reduction in VS over a period of 4 weeks with sludge decomposition by earthworms (R & F Hartenstein et al, 1981). Whereas this study showed a 63 % material reduction on VS basis over a period of 11 weeks, of all materials in the system. This is assumed that the total mass of material reduced on the basis of organic content has been respired into water and carbon dioxide (C. H. Lalander et al., 2015). Although macronutrients were not analysed in the vermicomposting system, it is assumed, based on similar literature experiments, that earthworms change the chemical characteristics of the compost and as organics are degraded releasing nutrients in the system (Luiz F.S Antunes et al., 2016).

6.1 Build-up of faecal matter

The faeces build up was determined by visual observation. Over the duration of the experiment, it was observed that there was a gradual build-up of faecal volume, likely because there was a lower degradation rate than expected. The volume build-up was not measured during the study. To avoid further build-up, one week of no feeding was conducted at week 11 to allow the earthworms to degrade the materials. During this week, an estimation of the possible feeding rate of the earthworms was conducted to establish whether there would be further degradation in the system if a longer experimental trial was conducted.

At the end of the trial the earthworms degraded the faeces, mixed and homogenised the materials within the vermicompost.

The other noticeable element in a dry toilet system like this is the toilet paper, which did not degrade as rapidly as the other materials in the compost. The toilet paper was not compacted and not wetted in this system. The earthworms generally degrade other substrates such as the faeces, simple carbon complexes which are accessible to them, and after those simple complexes were degraded, more complex carbon chains such as lignin were degraded (Moran-Salazar et al., 2016). The earthworms, along with micro-organisms, specifically from the phylogenetic group of *Basidomycetes* and *Mitosporic fungi*, degrade the lignin and cellulose (H. Insam et al, 2007). Lignin, which the toilet paper contains, has complex carbon chains that take longer to degrade within the system and it is primarily degraded by fungi. Lignin-degrading fungi leave the cellulose, which are accessible simple carbons that the earthworms are then free to degrade. (H.Insam et al., 2007; Moran-Salazar et al.,2016).

6.2 Material reduction potential

The average total amount material in the three replicate units at the outflow (end of the experiment) were compared to those in the inflow to determine the material reduction and changes in the total quantity (weight) in the given area of the units. The input material of the three replicated units had an average total VS of 2.5 kg, and the output material of 0.92 kg (dry-weight basis), yielding a material reduction of 63 % on VS basis. So, there was a loss of mass and volume in the materials added into the vermicompost system. The dry mass of the organic solids decreased owing to micro-organism (biological) degradation and earthworm activity. There was an increase in small worms and cocoons (Table 4) and this added to the degradation potential of the system, as the small worms grow and mature into adults, they begin to feed on the materials hence increasing in degradation. Therefore, an extrapolation was taken from the small worms and cocoons that grew within the vermicompost during the trial thus a potential biomass was determined. This means that the biomass of the already existing worms in the vermicompost along with the potential for the small worms to grow into adult worms, added up to a total of 12 kg-worms- m^2 in the system. With the total potential daily feeding rate extrapolated to 1700 g-ww/faeces/m^2-d it can be assumed that with more time given, there would be a possibility of achieving a higher degradation rate in the systems (C. Lalander et al., 2013; Lalander et al, 2015; Lalander, Hill, & Vinnerås, 2013).

6.3 Bacterial reduction in the system

There was variation in the concentrations of each micro-organism between each week, which means that the collected data varied considerably. This could be from sampling errors made during the duration of the experiment or it could be attributed to the behaviour differences of the micro-organism. Nevertheless, there was a dilution effect of the concentrations of each micro-organism from the initial inflow concentration within the experimental weeks. The study does raise uncertainty on whether there will be further bacterial reduction with time in a continual daily feeding of faecal matter based on the absence of statistical significant from when the feeding stopped.

Faeces of 60 g – 80 g was added for five weeks prior to sampling, and the total material in the units at the beginning weighed 8.2 kg, the faecal input amounted for 7 % of the total material in the units. This would mean an approximate 93 % dilution effect can be extrapolated. At the end this ration would be 30 % and thus the dilution effect would be less than 1 $\log_{10}$. Laboratory-based studies that have been performed under controlled conditions generally produce results that may differ from those in (seasonal) field-based studies that were exposed to external influences from the environment (Christy E. Manyi-Loh et al., 2016). In a study by Kudva et al. in 1998, the findings highlighted that the survival period of pathogenic bacteria was lower in laboratory studies as compared to observations made in field studies, this was due to influences by external fluctuations in ambient temperature (Kudava I.T et al., 1998). A concern would be to point out that laboratory results would be a challenge to apply to field conditions (Soupir M.L et al., 2008). Especially if the end material would be used in land application or disposal, recycling or soil emendation (Watcharasukam M et al., 2009) .

6.3.1 *Salmonella* spp.

There was an accumulation of *Samonella* spp. from the added faeces into the system as more faeces inoculated with *Salmonella* spp. was added. It was present in the inflow faecal material and also found in the degraded vermicompost material. The *Salmonella* spp. at 6 weeks had a concentration of below 7 Log_{10} and then declined to an average of 3 Log_{10} at week 11 (Figure 4). A study by (Lalander C et al., 2013) in a fly larvae treatment, the concentration of the *Salmonella* spp. drastically reduced from 7 Log_{10} CFU/g to below the detection limit (<1 Log_{10} CFU/g) at the end of the experiment. In a system with a continuous feeding load such as in this experiment, it is likely that pathogens are spread out through a dilution process from the top of the system to the bottom of the system, as compared to a case study done by Lalander et al., (2015) that found vermicomposting process significantly reduced the load of *Salmonella* spp. therefore decreasing the hygienic risk of handling and managing the material for the use in the environment (C. Lalander et al, 2015). With this experiment a post-sanitisation treatment would be hygienically better if it were to be managed by humans and safer for the environment than to leave it untreated.

6.3.2 *Escherichia coli*

The final concentration of the system was 2.5 log10 CFU/g, meaning that the *E. coli* concentration decreased below the US EPA guidelines of 3 log10 CFU/g during this vermicomposting process, similarly to (Rodrique, 2020) study that their vermicompost system reduced *E.coli* to undetectable levels in 90 days which was attributed to microbial competition within the system. However, in this study the *E.Coli* was found to not reduce further than the concentration within the faecal matter added into the system, which supports the study found by (L. Henault-Ethier et al., 2016) that reported a decrease of inoculated E. coli within 18 – 21 days in both batch or continuous system but stating that *E. fetida* negatively influenced *E.coli* survival and it did not decrease with increasing earthworm density.

The faecal input concentration from week 1 to week 8 was slightly lower compared to the concentration found in the system however, no significant trend was established which means that the earthworms and other microbial activities of this system did not eliminate *E.coli* by enhancing enzymatic activities in both the earthworm gut and cast associated processes. A decline in concentration began once there was no added faecal inputs into the system. From this, it can be extrapolated that *E.coli* may be less competent competitors for labile C sources than other pathogenic indicators (N. Soobhany et al., 2017)

Cutler (2016) study shows an increase of *E.coli* in thermophilic composting where high levels of nitrogen composition sustained and increased the growth of the *E.coli*, although our study differed in the mesophilic composting, the results showed an increase within the system of *E.coli* at week 7 to week 9 and it can be extrapolated to the availability of nitrogen content, although the nitrogen content was not considered in this study.

6.3.3 *Enterococcus* spp.

The concentration of *Enterococcus* spp. in the faecal input at week 1 was 7 log_{10} and had a considerable higher concentration than other monitored micro-organisms (Figure 4). This can be attributed to the higher concentration found within the faeces intrinsically, as this micro-organism is common in faeces. A reduction (99.9%) in *Enterococcus* spp. concentration was found. The passage of organic residues through the gut of the earthworms have been demonstrated to cause the die-off of *E. faecalis* and other microorganisms (some pathogenic) (Dominquez & Edwards., 2012).

6.3.4 Hygiene quality of the vermicompost unit

In Kumar and Shweta (2011) and M. Aira (2011) study, it reports a reduction in pathogenic micro-organisms through batch systems with retention time that vary from 60 days to six months. In this vermicompost system, it is likely that pathogens are spread through leaching from the top of the unit towards the lower levels of the unit, which diminutions the hygiene quality of this system (Aira M et al., 2011), and could have had an effect on the low reduction of *Enterococcus* spp. in this system, which could be attributed by the constant feeding despite the vermicompost material been well degraded (Lalander et al, 2015). Another hygiene factor to consider in this system is, these pathogenic bacteria are zoonotic micro-organisms, meaning they can carry infectious diseases and can be transmitted to humans. In addition to the direct transmission to the human population, some zoonotic pathogens also infect domestic animals and wildlife causing economic losses (Albihn A & Vinneras B, 2007)

In this system there is a risk of zoonotic disease transmission through the use of earthworms as well as through the potential use of the outflow degraded material (R. Kumar and Shweta., 2011). When there is a potential risk of zoonotic disease transmission or for a closed-loop vermicomposting system, earthworms can be treated in order to lower the risk (C. Lalander et al, 2015). Thus, the hygiene quality of this vermicompost unit can be regarded as low, specifically if the vermicompost material should be used as fertilizer. It would have to be treated prior to any further use to avoid the spread of diseases (C. Lalander et al, 2015). However, a post-stabilisation step of ammonia sanitisation can be a way to improve the hygiene quality and inactivate pathogens including bacteria, in order to increase the fertilizer value of the vermicompost material (Nordin A et al., 2009) Additionally Ndegwa and Thompson (2001) reported pre-composting prior to vermicomposting to meet a safe biological standard and this pre-composting step may result in reduction of harmful pathogens if temperatures are high enough. The shortcoming of this, is that it could also increase the rate of the faecal coliforms if satisfactory temperatures are not met thus the follow-up step would be vermicomposting to ensure the reduction of most harmful pathogens (Rodrique, 2020)

6.3.6 Viability of the system

On a practical sense, for a family of five at an assumption that each individual defecates an average of 120 g per day, a total of 600 g of faeces would be produced per day. Assuming an individual uses the system on an average of 6 days a week, therefore a total of 3400 g of faeces for the household. Since the current system has an average potential daily feeding of 1700 g/ww-faeces/m^2 at an area of 0.14 m^2 (Table 4), the required area needed for the family of five would be 0.38 m^2. Therefore, it can be suggested for the current experimental system to be adapted to a family of five, the area should increase to almost three units. Meaning the current system of 360 mm (internal top width) and 300 mm (internal bottom width) would have to be elongated in size to increase the area suitable for a family of five. It can be viewed that a pit latrine design would be a better fit for the purpose of a family of five with a vermicomposting system. According to (World Health Organisation , 2017) a traditional pit latrine is 1 to 1.2 m in diameter and for a family of six, a depth of 1.8 m will be needed to provide a volume of 0.06 m^3 (of excreta) per person for an anticipated 5 year life of a latrine.

Furthermore, the use of the plastic liner in the system can be practical in a household in transferring and/or changing materials. However, it would be problematic when it comes to removing; separating or keeping the earthworms in the system (e.g. should there be need to replace bedding material). A solution to this would be to have a rotatable bucket, in which the vermicomposting bucket has sections filled of worms and another section of the same bucket without worms, so that when the time comes to remove degraded materials, there is another section of the bucket to accommodate the earthworms. There would have to be a light sensor within the bucket to control the direction the earthworms must move to as they are sensitive to light. This mechanism will require an even greater area to allow for the worms to move from an old section of the bucket to a new section of the bucket. Alternatively, an interchangeable compartment within the bucket where the worms could be placed and replaced by removing the compartment of bedding material.

7. Conclusion

This vermicomposting toilet system can reduce the mass and volume of its initial organic materials whilst physio-chemically transforming the initial materials into a peat-like, odourless, and homogenous material, demonstrating a 63 % material reduction on a VS basis. This vermicomposting system is capable of decreasing faecal pathogens and indicator organisms through dilution, but it can increase the risk of disease transmission, only if there are no management strategies applied. Post-sanitisation steps are required when placing the organic matter back into agricultural land or back into the environment and this is to ensure the production of hygienically safe materials.

This vermicomposting system set-up has materials that are accessible and inexpensive that can help to provide basic sanitation for a community. It can be simple, which means it is robust enough that it can be easily maintained by the on-site toilet user. There is no visual appearance of the earthworms as the worm cycle is within the material of the system. Given the restrain on temperature within a vermicompost process, the organic wastes within the system manage heat dissipation rather than the accumulation of heat. This vermicompost

process can lead to a decline in the micro-organisms monitored without the need to increase the temperature. Moreover, there is an overall reduction in the continual adding of faeces with regards to material reduction (VS %) as well as this system has the potential to decrease indicator organisms and pathogen salmonella.

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