HEALTH AND SAFETY ASPECTS OF THE USE OF PRODUCTS FROM URINE-DIVERSION TOILETS

by

PHASHA MMOLAWA CYNTHIA

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Department of Microbiology and Plant Pathology

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DECLARATIONS

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or part has been submitted at any university for a degree.

Signature: _____

Date: _____

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SUMMARY

The use of human excreta as a fertilizer has been used to a very limited extent. Human excreta has been distributed throughout the environment as a result of faulty sanitation system design, improper use and/or the total absence of any sanitation system. Human excreta contain nutrients in the form of nitrogen (N), phosphorus (P) and potassium (K). Agricultural use of sludge may be an alternative to conventional fertilizers provided that the risk to public health is considered. The application of biosolids to agricultural fields however poses an environmental and health risk since sludge may contain toxic chemicals, pollutant organic compounds and pathogenic parasitic microorganisms of faecal origin. If urine and faeces were separated the risks could potentially be minimized and both of these fractions could possibly be disinfected and utilized.

Dehydrated faeces (humanure), urine, soil, irrigation water and crop samples were analysed for the total coliform, faecal coliform, faecal *Streptococci*, *Salmonella* spp, *Aspergillus* spp and helminth eggs. Moisture content and pH of each sample were also determined. Survival studies of microorganisms in dehydrated faeces using different treatments (ash; NaOH and pasteurization) were performed. Human urine both sterile and non-sterile was stored at $15^{o}C$, $20^{o}C$ and $30^{o}C$ for 50 d to estimate the effect of different storage conditions on the survival of pathogens. Four agricultural plots were prepared for treatment, two for spinach and two for carrot crops. Dehydrated faeces contained total coliform, faecal coliform, Faecal *Streptococci* and *Salmonella* spp. Treatment with NaOH and ash reduced the number of pathogens in dehydrated faeces, but not to safe levels. The number of helminth eggs was higher in dehydrated faeces (humanure) than in the soil and crops. Few helminth eggs survived pasteurization at $60^{o}C$ for 30 min, $70^{o}C$ for 20 min and $90^{o}C$ for 5 min. Pathogens survived after storage of human urine at $15^{o}C$ and $20^{o}C$ for 50 d. Most of the pathogens (total coliform, faecal coliform, *Salmonella* spp and *Aspergillus* spp) were reduced at $30^{o}C$. 62.8% of helminth eggs found in humanure were *Ascaris* eggs and 82% of *Ascaris* eggs found were viable. Application of humanure to soil resulted in the contamination of soil and crops. However the viability of helminth eggs in crops was very low.

No bacteria survived pasteurization at $70^{o}C$ for 20 min and $90^{o}C$ for 5 min. Therefore heat treatment at $70^{o}C$ for 20 min and $90^{o}C$ for 5 min was the most effective disinfection method for the bacteria. The results showed that *Ascaris* spp, *Toxocara* spp, *Trichuris* spp, *Enterobius vermicularis*, *Hymnologies diminutions* and *Taenia* spp were present in soil, carrots, and spinach samples when humanure was applied to soil as a fertilizer. Pathogenic and parasitic microorganisms present in humanure represented a high risk of infection.

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LIST OF ABBREVIATIONS

Cfu: Colony Forming Units

CSIR: Council for Scientific and Industrial Research

DM: Dry matter

EcoSan: Ecological Sanitation

Fc: Faecal coliform

Fs: Faecal *Streptococci*

HM: Humanure

HO: Helminth ova

K: Potassium

N: Nitrogen

NaOH; Sodium hydroxide

NOM: Official Mexican Norm

NV: Non-Viable

P: Phosphorus

RV: Rappaport-Vassiliadis

Tc: Total coliform

TS: Total solid

UD: Urine Diversion

V: Viable

VIP: Ventilated Improved Pit

VIDP: Ventilated Improved Double Pit

WHO: World Health Organisation

XLD: Xylose-Lysine-Desoxycholate

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CHAPTER 1

1.1 Introduction

1.1.1 Ecological Sanitation (EcoSan)

Sanitation is not only a problem in developing countries. Over the decades, the main focus of sanitation has changed from health aspects to the reduction of environment impacts. About two million people die every year due to diarrhoeal diseases. (Langergraber and Muellegger, 2005).

In South Africa many community sanitation schemes have been successfully implemented utilising EcoSan toilets such as the ventilated improved pit (VIP) and ventilated improved double pit (VIDP) toilets (Austin and Duncker, 2002).

Humans produce excreta, which contain chemical elements that are needed and can be used as fertilizer for plants producing human food. Although improper use of human excreta causes hygiene and health problems, human excreta are used frequently as night soil in some areas of the world such as China, Vietnam and Japan without any generally known problems for agricultural productivity (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

A person produces approximately 500 kg/annum of urine and 50 kg/annum of faeces. These faeces contain 10 kg of dry matter, which contain approximately 5.7 kg of nitrogen, 0.6 kg of phosphorus and 1.2 kg of potassium per year (Wolgast, 1993).

According to Wolgast (1993), the annual amount of human excreta of one person corresponds to the amount of fertilizer needed to produce 250 kg of cereal, which almost satisfies the yearly consumption of that person (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

Ecological Sanitation is a potential alternative approach to conventional human waste disposal. A sanitation system based on EcoSan is a closed-loop system, which

closes the gap between sanitation and agriculture (Langergraber and Muellegger, 2005). Human excreta distributed in the environment as a result of faulty sanitation system design, their incomplete implementation, poor operation and improper use (Simpson-Hebert, 1997).

EcoSan uses human excreta as a resource to be recycled rather than as a waste to be disposed of. Recycling nutrients to soil and plants reduces the need for chemical fertilizers and restores soil organisms to protect plants. Agricultural reuse of wastewater offers an additional resource of irrigation water and high soil fertilizing potential (Austin and Duncker, 2002).

Today the alternatives to the conventional wastewater system include systems that separate or divert urine and faeces in order to utilise the nutrients more efficiently. In regions without a sewerage network, nutrient utilisation as well as improved sanitation is possible by not mixing the fractions and minimizing flushwater. If the faecal fraction is kept dry there will be less leaching from e.g. pit latrines and the smell will be reduced (Schönning, 2001). The main reasons to separate urine and faeces are thus to recycle the plant nutrients in urine and to obtain a faecal fraction that is more practical to treat and safer to handle (Schönning, 2001).

Ecological sanitation systems are not well understood and cannot be replicated without a clear understanding of how they function. They have unfamiliar features such as urine diversion pedestals or squatting plates. In addition they require more promotion, support, education and training than ordinary pit or VIP toilets (Esrey *et al*., 1998).

A concern is often expressed that some EcoSan systems are too expensive for low-income households in developing countries. EcoSan systems do not need to cost more than conventional systems. EcoSan systems need not be expensive to build because (1) the entire structure is build above ground, there is thus no need for expensive digging and lining of pits (2) and urine is diverted, no water is used for flushing and the volume of the processing vault is fairly small, as it is emptied periodically (Esrey *et al*., 1998).

Many of the EcoSan alternatives introduced are small-scale systems that demand more personal involvement of the users, including handling of the waste. Thus if not sufficiently treated the possible health risks are higher compared to conventional waterborne waste removal systems. Therefore recommendations for e.g. storage, treatment or reuse practices should be formulated (Höglund, 2001).

The inability of existing sanitation systems to properly manage the increasing volumes of human excreta has contributed much to the worldwide escalation in ecological problems. With rapid population growth, especially in urban areas, the situation will not improve unless there is a significant change in the manner in which sanitation systems are chosen, designed and implemented (Simpson-Hebert, 1997).

Over the past decade the existing wastewater treatment systems have been criticised for their non-sustainability. Toilet waste contains virtually all the plant nutrient humans ingest through food and drink and could theoretically be recycled to plants. Phosphorus is finite resource, with present recoverable reserves calculated to last for less than 200 years, whereas potassium is assumed to last for 300 years (Höglund, 2001).

Ecological sanitation regards human excreta as a resource to be recycled, rather than as a waste to be disposed of. The notion that excreta is waste with no useful purpose is a modern misconception. In nature there is no waste, all the products of living things are used as raw materials by others. Recycling sanitised human urine and faeces by returning them to the soil could potentially restore the natural cycle that has been disrupted by current sanitation practices (Esrey *et al.*, 1998).

There are many reasons for recycling the nutrients in excreta. Recycling prevents direct pollution caused by sewage being discharged or seeping into water resources and ecosystems. A secondary benefit is that recycling returns nutrient to soil and plants, and reduces the need for chemical fertilizers. It restores good soil organisms to protect plants, and it is always available locally, wherever people live (Esrey *et al.*, 1998).

To increase the sustainability of waste disposal, alternatives to conventional treatment have been suggested. These can be called complementary alternative or ecological wastewater systems and the aim is often to reuse the plant nutrients of the wastewater as a fertilizer. One concept is source-separating wastewater systems which include blackwater systems, where the wastewater from toilets is treated separately, urine-separating systems with separate handling of urine and different types of dry systems where the toilet waste is handled without the use of flush water (Höglund, 2001).

Treated sewage sludge, often referred to as biosolids, is an inevitable product of wastewater treatment. Biosolids contain valuable amounts of plant nutrients and trace elements and recycling to agricultural land (as part of an integrated farm management plan) is recognized as the best practicable environmental option for their final disposal (Horan *et al*., 2004).

The application of sewage sludge to agricultural land as an alternative to sacrificial land disposal is not a new concept and has been practised throughout the world for the last few decades (Henning *et al*., 2001). The use of sludge represents the solution of environmental and economic problems associated with the pollution and eutrophication of rivers, lakes and bays where sludge are disposed of and discharged. Therefore there is a need to utilize the fertilizing value of sludge in soil (Jiménez *et al*., 2002).

An advantage of using human urine instead of chemical fertilizers or sewage sludge is the low concentrations of heavy metals found in urine. Desiccated faeces contain fewer nutrients than urine, however it is a valuable soil conditioner. It may be applied to the soil to increase the organic matter content, improve water-holding capacity, increases the availability of nutrients and helps to maintain a healthy population of beneficial soil organisms that actually protect plants from soil-borne diseases (Austin and Duncker, 2002).

Pathogens and the attraction of vectors are major concerns during the final disposal of biosolids after waste stabilization. There are four major types of human pathogenic organisms found in biosolids: (1) bacteria, (2) viruses, (3) protozoa, and

(4) helminths. Suggesting that contaminants and pathogens accumulated in biosoilids need to be removed to a safe level when disposed of for beneficial use, such as land application as a fertilizer or soil conditioner (Hong *et al*., 2004).

If urine and faeces is separated, the two fractions can easily be treated and utilised pathogens (Heinonen-Tanski and Van Wijk-Sijbesma, 2004). The separation must be done so that the urine fraction is totally free from faeces, but a small amount of urine in the faeces fraction is not a problem. Therefore baby pots containing both faeces and urine should be added to the faeces fraction, since the faeces of both babies and adult people can contain the same pathogens (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

It is important to emphasise at this point that excreta contain both dangerous materials (pathogens in faeces) as well as beneficial components (nutrients in urine). The challenge of modern sanitation practice is to find ways to: (a) contain the dangerous part of the excreta in order to prevent transmission of disease; (b) use the beneficial part of the excreta productively; and (c) avoid damage to the natural environment (Austin and Duncker, 2002).

There are limited financial resources in South Africa as well as the urgent need to conserve water and protect the environment. It is essential to look beyond the current restrictions for innovative ways and means of bringing adequate sanitation to the millions of people currently without access to adequate facilities (Austin and Duncker, 2002).

Objectives

The objectives of this study were:

- to evaluate the environmental factors affecting the survival of excreted pathogens in faeces and whether the application of NaOH, ash and pasteurization could reduce the numbers of pathogens in faeces.

- to determine the storage temperature, pH, growth and the survival of pathogens in urine.

- to determine the microbiological risk of applying humanure on carrots and spinach.

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CHAPTER 2

2. Literature review

2.1 Sanitation and public health

The major cause of both morbidity and mortality are diarrhoeal diseases due to lack of sanitation and clean water. The incidences of these diseases are strongly related to unsanitary excreta disposal, poor personal hygiene, domestic hygiene and unsafe drinking water. These diseases are mainly transmitted faecal oral way, i.e. by person-person contact, through water, food, soil, etc. contaminated by faeces and then ingested. In order to reduce the transmission and occurrence of these pathogens improvement in sanitation and personal hygiene are essential (Carlander and Westrell, 1999).

Any disposal of human or animal wastes to land entails some potential risk of introducing diseases agents (viruses, bacteria, and parasites) into the food chain (via animals or plants or the water supply). The present concern is the effect on public health of adding household waste to animal waste and treating both as animal waste. As animals are the chief reservoir of most enteric bacteria that are pathogenic to humans, no additional hazards from bacteria are expected in a mixed waste system (Deng and Cliver, 1992).

The development of a sustainable sanitation system includes the utilisation of nutrients from human urine and faeces in agriculture. However the quality of the sludge is not fully trusted among agricultures and food producers. One uncertainty is the difficulty of guaranteeing the quality of all sludge and the risk of the presence of non-analysed, but hazardous compounds in the sludge (Hanaeus *et al*., 1997).

Inadequate disposal of wastewater can cause flooding of roads, housing, and communal areas where large numbers of people pass through, thus creating further health hazards. To control these wastewater drainage systems should be installed as well as toilet facilities of such a nature as to minimise the danger of transmission of disease. Unsanitary toilet facilities encourage the spread of enteric diseases through

the faecal-oral route or by flies. Where toilets are shared the health risks increase (Barnes and Taylor, 2004).

The increased consumption of raw and sparsely cooked vegetables, coupled with the importation of fresh produce from countries were produce handling is compromised by lower sanitation standards, has generated a heightened concern for food safety (Islam *et al*., 2005).

Protozoa and helminthes are the parasites of primary public health concern for wastewater reuse. An important characteristic of these organisms is the production of a cyst or ova stage, which aids their survival (Erdogrul and Sener, 2004).

Another problem, which is indirectly related to the sewerage system, is the fact that a very large part of the population in a modern urbanised society lives on a comparatively small part of the land. Hence, the food is transported from a large area to a small one and a nutrient such as phosphorus accumulate near the densely populated areas and is not used if it is not transported back to the areas of food production (Hanaeus *et al*., 1997).

2.1.1 Sanitation systems

The success or failure of a sanitation system depends on the interaction of environmental, human and technical factors. The most important environmental aspects are climate, soil and groundwater; these vary from place to place and have a influence on the choice of the most appropriate sanitation system. The technology selected should therefore be adapted to the local environmental conditions (Winblad and Kilama, 1980).

It is better to protect the environment from faecal pollution than to undertake expensive measures to reduce pollution that has already taken place. The approach to the sanitation challenge should therefore be ecologically sustainable. This means that sanitation systems should neither pollute ecosystems nor deplete scarce resources. It further implies that sanitation systems should not lead to a degrading of water or land and should, where possible ameliorate existing problems caused by pollution.

Sanitation systems should also be designed to recycle resources such as water and nutrients present in human excreta (Simpson-Hebert, 1997).

2.1.2 Early reuse of human excreta

Human excreta have traditionally been used for crop fertilisation in many countries. In Japan the recycling of urine and faeces was introduces in the 12th Century and in China human and animal excreta have been composed for thousands of years. In Swedish cities, organised collection and transportation of latrine products to farmers started in the 18th Century. As the population grew, quantities increased and treatment alternatives to facilitate the handling of excreta were developed. A mixture of latrine products and peat provided a fertiliser without smell that could be transported long distances (Höglund, 2001).

Wastewater reuse has been growing over the previous three decades and is now considered an essential management strategy in areas of the world where water is in short supply. Many countries now consider wastewater reuse as a method to secure water resources. The benefits of reclaimed wastewater for irrigation are several, including:

- increased crop yields;
- decreasing the use of fertilisers while providing increases in nutrients and organic matter for soil conditioning;
- soil conservation and potential reduction of desertification;
- improving of the environment by enabling zero-discharge to receiving bodies; and
- enabling the reallocation of freshwater supplies for urban use (Gregory, 1999).

The practice of reuse has also been common in Europe. Dry toilet latrines were emptied and its contents used regularly as fertiliser for arable farming until after the second half of 1900, for example in Finland (Olsson, 2001). The reuse of insufficiently treated human and animal wastes in agriculture may result in transmission of pathogens. Most disease-causing agents in excreta including an array

of viruses, bacteria, protozoa and helminthes are shed in faeces rather than urine (Höglund *et al*., 2002).

Key features of EcoSan are prevention of pollution and disease caused by human excreta, treatment of human excreta as a resource rather than waste, and recovery and recycling of the nutrients. In nature excreta from humans and animals play an essential role in building healthy soils and providing valuable nutrients for plants. Others use products of living things as raw materials. Conventional approaches to sanitation misplace these nutrients, dispose of them and break this cycle (Esrey *et al*., 1998).

To allow the reuse of wastewater and sludge it is desirable to use technologies that minimise the removal of nutrients yet reliably remove the high pathogen concentrations, particularly helminth ova, which present a high health risk (Jiménez-Cisneros *et al*., 2001). Decreasing the risk for transmission of disease is another implication for further refinement of the latrine products. The latrine products can also be mixed with lime to produce limed ammonium nitrate and ammonium sulphate (Höglund, 2001).

Although faeces contain fewer nutrients than urine, they are a valuable soil conditioner. After pathogen destruction through dehydration and decomposition, the resulting inoffensive material may be applied to the soil to increase the organic matter content, improve water-holding capacity and increase availability of nutrients (Esrey *et al*., 1998; Austin, 2001).

If urine is used as a fertilizer, the vegetation will grow and more water will evaporate. The resulting drying of the soil will decrease in relative humidity improve the chances of pathogen survival. In comparison with the use of septic tanks and pit latrines with a mixture of faeces, urine and some water resulting in the contamination of tropical freshwater, the horticultural and agricultural use of urine thus hardly constitutes an increase in risks of disease transmission through helminthes and schistosoma (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

If urine fertilization is done carefully at the correct time, the amount used is moderate, and the urine is incorporated directly into the soil, urine nitrogen has the same agricultural values as nitrogen of commercial mineral fertilizers and the barley absorbs almost all urine nitrogen. Losses through evaporation can be low. For barley the nitrogen needed under Swedish climate conditions was 100 kg/ha for one growth period of 90-110 days. If there are no atmospheric losses there can be no smell and the loss to water will be unimportant. The intake of phosphorus from urine during the first year was 12%, which was better than that from mineral fertilizers (Kirchmann and Petterson, 1995).

2.1.3 Dry sanitation with waste reuse

Dry sanitation with reuse is promoted as an appropriate technology for community settings without sewerage or plentiful water. It has been heralded as solving many of the problems encountered with other sanitation systems. These include fly breeding, smell, ground water contamination, short pit life and pit collapse. It is also claimed to achieve sufficient destruction of disease causing organisms to enable safe handling of compost (Esrey *et al*., 1998).

Few have addressed the problems that can be associated with using these technologies in community settings or have documented the pathogen die-off. The enthusiasm, which this sanitation technology has generated, seems sometimes to have overshadowed. The most important issue of whether the end products from dry sanitation toilets *per se* in community settings are safe to handle and use as soil conditioners and plant fertilisers (Peasey, 2000).

Dry sanitation (South Africa)

The urine diversion 'dry box' toilet is another modification of the Vietnamese double chamber dry toilet. The Council for Scientific and Industrial Research (CSIR) in South Africa saw urine diversion as a possible solution to many problems that had been confronted with the VIP toilets (Peasey, 2000).

A pilot project was carried out in 1997 with a blow moulded plastic toilet seat. The chambers and the superstructure were constructed with locally available materials. The toilet is raised above the ground. The faeces are collected in one container, when container is full it is sealed and other container is used. The urine flows into a soak-pit with the option of converting to collection, should people be willing to try later (Austin and Vuuren, 1999).

Dry sanitation (Mexico)

A modified version of the Vietnamese double chamber dry toilet is promoted by the Mexican NGO, Espacio de Salud AC (ESAC). This modified version of the Vietnamese double chamber dry toilet was designed by Cesar Anorve in Mexico. The squatting slab is replaced by two toilet risers. Conventional-looking urine separating toilet seat is placed on the toilet riser and the toilet is painted in attractive colours. The urine-separating toilet seat has been modified as a result of feed-back from users and a domestic urinal has also been designed. The design has also been adapted for use within (Peasey, 2000).

The SIRDO is a prefabricated solar-heated toilet developed over 15 years ago in Mexico by the Alternative Technology Group (GTA). The SIRDO is promoted as a radical change from the traditional pit latrine transforming faecal material into a biofertilizer free from pathogens (Peasey, 2000).

In Mexico, it is the responsibility of each municipality to provide free public sanitation service via the collection and transportation of solid waste. This waste is limited to those wastes that are catalogued as municipal solid waste, those that are listed in the environmental legislation and are considered to be generated through municipal activities and that are not considered dangerous due to their chemical and physical nature (Buenrostro and Bocco, 2003).

The severe heath related problems, mainly gastrointestinal diseases that are generated due to the irrigation of crops with raw wastewater in Mexico have motivated the National Water Commission to establish sanitation programs that include the treatment of wastewater to remove most of the pathogens contained in it.

Advanced primary treatment (APT: coagulation-flocculation-sedimentation) has been studied, couple with filtration and disinfection. This process has been applied successfully in countries like Norway, Sweden, France, Spain and the United State and more recently it has been proven for removing pathogens from wastewater to comply with international regulations for unrestricted irrigation (Jiménez *et al.*, 2000).

Mexico is experiencing a rapid urbanization process that has surpassed the government's capacity to install an adequate environmental protection regime. In some rural settlements, an urban-fringed environment has been created that has a wide range of social mix and a large mixture of industrial activities that are characteristic to urban canters, such as artisan workshop and small farming communities (Buenrostro *et al.*, 2001).

Dry sanitation (Sweden)

Sweden is probably the country with the most advanced system of collection and reuse of human urine. There are a number of settlements (called eco-villages) or apartment blocks in the country where the residents have ecological sanitation systems with urine diversion toilets. The pedestals are made of porcelain, in both dry and flushing versions. The flushing version is often found in high-density residential apartments or cluster housing. The urine from all houses is collected in large underground tanks and collected by farmers in road tankers and used for fertilising their crops (Austin and Duncker, 2002).

Experience from systems with urine separation is limited. The results from an inventory made by (Hanaeus and Johansson, 1996), March-April 1996 concerning existing systems with urine separation show that there were eleven bigger systems in Sweden. Most of them were new, seven of the eleven systems started to operate during 1995 or later. In spite of some problems with different components in the systems, the overall impression of the inventory is that the systems were working well (Hanaeus *et al.*, 1997).

Until recently most of the companies in Swedish food industry had not considered the reuse of human waste products, including urine in agriculture. Their policies are often aimed at closing the loop but few of them have made any decisions regarding recycling of plant nutrients. Regarding urine, two companies claimed to disapprove of its use whereas one of the largest producers of dairy products would approve of using urine on agricultural products, on condition that there is no risk for faecal contamination which is of course impossible to guarantee and not necessary according to our risk estimates (Berglund, 2001).

There are already municipalities in Sweden which require that urine from all newly built houses not connected to the communal wastewater system be collected for reuse. In 1997, 12% of the municipalities in Sweden had at least some installations for urine-separation and 25% of them expressed an interest in the system. The number of urine-separating toilets that have been installed and the volume of urine that is collected and used in agriculture in Sweden are unknown (Höglund, 2001).

Dry sanitation (developing countries)

In developing countries, 300 million urban residents have no access to sanitation and it is mainly low-income urban dwellers that are affected by lack of sanitation infrastructure. Approximately two-thirds of the population in the developing world have no hygienic means of disposing of excreta and an even greater number lack adequate means of disposing of total wastewater (Gregory, 1999).

Helminth ova are highly resistant organisms that can survive for a long period under a variety of environmental conditions, increasing the risk of infection. For this reason processes that have been used in some countries are not always applicable under those particular conditions of developing countries (Barrios *et al.*, 2004).

Developing countries are currently facing the problem of regulating sludge application and disposal, since the quality of sludge is quite different from that of the nations that usually develop standards. For example helminth ova in sludge generated in Mexico City are found in concentrations of more than 150 ova/g of total

solids, which are much higher than those found in sludge in the City of Chicago, USA (less than 1 ova/g TS) (Barrios *et al.*, 2004).

The most common sanitation system in the world is the pit latrine, which provides on-site sanitation for 20% of the population in developing countries. These latrines protect the population from infectious diseases compared to on the ground excretion; the excreta collected in the pits may contaminate the ground water to transport of pathogens and nitrate leaching (Höglund, 2001).

The risk for handling and reusing the faecal fraction is probably in more acute need of control than the risk related to urine. If the faecal fraction is kept dry and adding the lime or ash raises the pH, enteric pathogens will be inactivated. Education is also crucial in order to get the systems to function hygienically. Apart from decreasing diarrhoeal diseases the management of water and sanitation is also important for reducing cases of e.g. malaria and schistosomiasis (Esrey *et al.*, 1998).

For sustainable agriculture in developing countries it is important to increase the use of organic fertilisers as a complement to mineral fertilisers. Only animal manure and crop residues were mentioned earlier, whereas later reports have emphasised human urine and faeces as being important resources as well (Höglund, 2001).

The low risk for transmission of infections through urine further support the implementation of urine-separation. The higher temperature in many of the developing regions would probably be beneficial for the inactivation of enteric pathogens in the urine. In addition, the more concentrated urine obtained from toilets or latrines that do not use flush water would probably increase the inactivation rate (Höglund, 2001).

2.1.4 Health risks

The benefits of wastewater reuse may be limited by its potential health hazards associated with the transmission of pathogenic organisms from irrigated soil to crops, grazing animals and humans (Erdogrul and Sener, 2004).

In developing countries, excreta-related diseases are common. There are approximately thirty excreta related diseases and many of these are of specific importance in excreta and wastewater use schemes (Strauss, 1994).

The potential risk of bacterial, viral and parasitic diseases that can be transmitted through the cycle of man-sludge-soil-crop-man constitutes the greatest problem associated with the disposal of sludge for agricultural land. Thus the public is reluctant to accept that reuse can be beneficial when associated with problems of bad odours and the attraction of vectors. In developing countries where data of microbiological quality are almost non-existent in spite of the fact that this represents the main problem for disposal (Jiménez *et al*., 2002).

Whether urine-separation and the reuse of urine can be recommended depends on whether the associated health risks are considered to be acceptable. These risks can be balanced against benefits like the fertiliser value of human urine. Higher risks from reuse of waste products may be acceptable in areas where enteric disease is endemic and where it is more often transmitted through poor hygiene and sanitation (Blumenthal *et al*., 2000). In areas where food is scarce, benefits from larger harvests may reduce other risks such as malnutrition that makes the individual more susceptible to infections (Höglund, 2001).

Epidemiological studies on people in contact with source-separated urine would be a reliable way to investigate whether the practice of reusing affect public health. This type of study would not be feasible with the small numbers of people in Sweden who handle urine. Several investigations regarding the impact of wastewater reuse on health of people in the immediate vicinity have been conducted. These have often focused on parasites that are endemic in the area of investigation and that are known to be persistent in the environment (Blumenthal *et al*., 1996).

Even though individual cases of viral infections theoretically could arise from handling urine, they would probably not be recognised by any surveillance system. The risk for an outbreak caused by direct contact with urine is low, since few people are exposed, e.g. compare to a drinking water supply or recreational water (Höglund, 2001).

Regarding the risk for pathogens transmission there is a choice of whether to store the urine at conditions that virtually eliminates pathogens or to account for further inactivation in the field. Risk for potential transmission of zoonotic diseases to animals in the field will be lower. Zoonotic agents may infect e.g. cattle or birds, and either disease or the animal may function as a reservoir or vector, with the possibility of further transmission of the pathogen. Bacterial spores have been recognised as a potential problem for animals in relation to the reuse of other organic wastes. These spores will be present in any organic waste and will also withstand harsh treatments such as pasteurisation (Jönsson *et al*., 2000).

Another risk that has been recognised is that of the uptake and growth of pathogens in plants. This has been reported for bacteria that were present in the seed, for plant grown hydroponically and for damaged plants but does not occur to a sufficiently large extent in the field to be a risk. If applied to a non-food crops the foodborne route of transmission is eliminated, but there is still an infection risk for people involved in the production and processing of crops as well as for humans and animals in the surroundings (Höglund, 2001).

However, the agricultural or aquacultural use of excreta and wastewater can only result in an actual risk to public health if all of the following occur:

- That either an infective dose of an excreted pathogen reaches the field or pond, or the pathogen multiplies in the field or pond to form an infective dose;
- That this infective dose reaches a human host;
- That this host becomes infected; and
- That this infection causes disease or further transmission.

The first three points constitute the potential risk and the last one constitutes the actual risk to public health. If the last one does not occur, the risks to public health remain a potential risk. The actual risks to public health that occur through waste use can be divided into three broad categories: those affecting consumers of the crops grown with the waste (consumer risk), those affecting populations living near to a waste reuse scheme (nearby population risk) (Strauss, 1994).

2.1.5 Epidemiological evidence of pathogens

The actual public health importance of an excreta or wastewater use practice can only be assessed by an epidemiological study to determine whether or not it results in an incidence or prevalence of disease, or intensity of infection, that is measurably in excess of that which occurs in its absence. Such studies are methodological difficult, and there have been only a few well-designed epidemiological studies on human wastes reuse. Most of the available evidence concerns wastewater irrigation, and there is much less information about excreta use in agriculture and about aquaculture use (Strauss, 1994).

Clear evidence of increased infection rates was found in several of the investigations, some of them involving irrigation with untreated or poorly treated wastewater. According to Cooper and Olivieri (1998) there are no recorded incidents of infectious disease transmission associated with reuse of appropriately treated wastewater, possibly because the risk is too low for detection by epidemiological methods. In the risk assessment method, used as an alternative in predicting risks for infection, viruses constituted the highest risk (Höglund, 2001).

2.2 Urinary pathogens

Urinary excreted pathogens are of less concern for environmental transmission than are faecal pathogens. When using a urine separating toilet there is a possibility for faecal material to enter the urine part of the bowl and thus contaminate the urine solution in the collecting tank (Hoglund *et al.*, 1998). Experiments in Sweden have established that, should faecal contamination of source-diverted urine occur, six months of storage time is sufficient for the destruction of pathogenic organisms (Olsson, 1996).

Urine contains few pathogens though may contain *Ascaris* eggs and schistosoma eggs. Urine may also carry the pathogens responsible for typhoid. Experiences from China and Japan suggest that urine not containing these may be used as a plant fertiliser (urine from individuals using antibiotics is also not recommended). Faecal

contamination of the urine can occasionally occur when the user has diarrhoea, by mistake or by children who often find it difficult to correctly use the urine-separator (Peasey, 2000).

Some human parasitic pathogens such as some helminth eggs or *Schistosoma miracidia* can enter the soil through the urine of infected persons. Because the number of infected persons in developing countries in the tropics is high it is obvious that this fact should be considered (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

In a healthy individual the urine is sterile in the bladder. When transported out if the body different types of dermal bacteria are picked up and freshly excreted urine normally contains less than 10 000 bacteria per ml. Pathogens that may be transmitted through urine are rarely sufficiently common to constitute a significant public health problem and are thus not considered to constitute a health risk related to the reuse of human urine in temperate climates (Höglund, 2001).

2.2.1 Bacteria in urine

The pathogens traditionally known to be excreted in urine are *Leptospira interrogans*, *Salmonella typhi*, *Salmonella paratyphi* and *Schistosoma haematobium*. There is a range of other pathogens that have been detected in urine but their presence is not considered significant for the risk of environmental transmission. Leptospirosis is a bacterial infection causing influenza-like symptoms with 5-10% motality that is general transmitted by urine from infected animals (Höglund, 2001).

Human urine is not considered to be an important route for transmission of disease since the prevalence of the infection is low. Infections by *S. typhi* and *S. paratyphi* only cause excretion in urine during the phase of typhoid and paratyphoid fevers when bacteria are disseminated in the blood. This condition is rare in developed countries (Feachem *et al.*, 1983).

Although source separating sewage systems may have several environmental advantages the sanitary demands and regulations have to be met in order to minimise the risk for disease transmission (Höglund *et al.*, 1998).

Mycobacterium tuberculosis and *Mycobacterium bovis* may be excreted in the urine, but tuberculosis is not considered to be significantly transmitted by other means than by air from person to person. *M. tuberculosis* is exceptionally isolated in nature, but was identified in wastewater coming from hospitals (Feachem *et al*., 1983).

2.2.2 Protozoa in urine

Microsporidia are a group of protozoa recently implicated in human disease, mainly in HIV-positive individuals. The infective spores are shed in faeces and urine, and urine is a possible environmental transmission route. Microsporidia have been identified in sewage and in waters, but no water or foodborne outbreaks have been documented although they have suspected (Hass *et al*., 1999; Cotte *et al*., 1999).

Schistosomiasis, or bilharziasis, is one of the major human parasitic infections mainly occurring in Africa. When infected with urinary Schistosomiasis caused by *Schistosoma haematobium*, the eggs are excreted in the urine, sometimes during the whole life of the host (Feachem *et al*., 1983).

2.2.3 Viruses in urine

A few viruses may be excreted through urine but are of minor concern for environmental transmission compared to the large number of enteric viruses that may enter the urine part of the toilet bowl through displacement of faecal material, thus contaminating the urine mixture in the collection tank (Höglund *et al*., 1998; Höglund *et al*., 2002).

Cytomegalovirus (CMV) is excreted in urine, but the transmission of CMV occurs person to person and the virus is not considered to be spread by food and water. CMV infects a large proportion of the population; 50-85% by the age of 40 was reported in USA (Höglund, 2001).

Enteric viruses are a major cause of gastrointestinal infections in humans in developed countries, estimated to be responsible for 80% of the cases in the US and

are assumed to be responsible for many cases where no etiological agent is found. Some 15% of waterborne outbreaks in the US and 27% in Sweden were reported to be caused by viruses (Höglund *et al.*, 2002).

Rotavirus is the most important etiologic agent of severe diarrhoea in infants and young children worldwide. In Sweden 53% of children with gastroenteritis attending hospital and 41% not attending were found to have a rotavirus infection. In studies of adults rotavirus was responsible for 4-17% of diarrhoea cases with known agents (Parashar *et al.*, 1998).

It can be concluded that pathogens that may be transmitted through urine are rarely sufficiently common to constitute a significant public health problem and are thus not considered to constitute a health risk related to the reuse of human urine in temperate climates. The inactivation of urinary pathogens in the environment reduces their ability for transmission.

2.2.4 Inactivation of pathogens in urine

The fate of the enteric pathogens entering the urine collection container is of vital importance for the hygiene risks related to the handling and reuse of the urine. To determine the duration and the conditions for sufficient storage of the urine mixture before its use as fertiliser, it is necessary to estimate the survival of various microorganisms in urine as a function of time. Studies have been performed where different microorganisms were added to the urine and their inactivation followed over time (Schönning, 2001).

Survival of helminth eggs and *Scistosoma miracidia* will be reduced in urine under the influence of urine salts, the increase in pH through ureolysis and the raise in temperature if the urine is preserved under a tropical sun or poured onto tropical top soil which will often have reached or surpassed the critical temperature levels (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

Limited work has been undertaken on urine treatment other than storage, such as acidification, heating and evaporative concentration. For the urine mainly

temperature and the elevated pH (9) in combination with ammonia has been concluded to affect the inactivation of microorganisms. Bacteria like *Salmonella* can be inactivated rapidly, whereas viruses are hardly reduced at all at low temperature (4-5°C) (Höglund, 2001).

2.2.5 Recommendations for the reuse of human urine

For single households the urine mixture is recommended for all type of crops, provided that the crop is intended for the household's own consumption and that one month passes between fertilising and harvesting, i.e. time between last urine application and consumption. This approach can probably be used for any smaller system in developing countries, whereas larger (urban) systems may be adapted. (Höglund *et al*., 2002).

Higher ambient temperatures in many developing country setting will however increase inactivation rates and add in safety. One reason for more relaxed guidelines for single households is that person-to-person transmission will exceed the risk from urine related environmental transmission (Höglund, 2001).

2.3 Faecal pathogens

Human faeces always contain high amounts of enteric microorganisms including many pathogens and opportunistic pathogens. The role of opportunistic pathogens is important since a considerable percentage of the world population i.e. pregnant woman, children, old people and people stressed by sicknesses, malnutrition etc are extra sensitive to their impact (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

From a risk perspective the presence of pathogens in human faeces should always be considered since there are so many different types of enteric infections and the prevalence is unknown for several of them. To ensure the reduction in pathogens, faeces need to be treated or stored under controlled conditions (Höglund, 2001). The potential risk of the principal parasites of faecal origin present in raw sludge is due especially to the fact that they are extremely resistant to certain conditions and to their persistence in an infective state for long periods of time (Jiménez *et al*., 2002).

Waste activated sludge is the second product of the wastewater processing. The nutrient and organic matter contents of sludge could be a resource to maintain soil fertility. The principal environmental concerns are due to the inevitable presence of human pathogens. Sewage sludge generally contains pathogens such as coliforms, *Escherichia coli*, *Klebsiella* sp, *Enterobacter* sp, *Salmonella typhimurium* (Al-Bachir *et al*., 2003).

2.3.1 Bacteria in faeces

Bacteria have generally considered the leading cause of gastrointestinal illness in surveillance systems. Of these bacteria, at least *Salmonella*, *Campylobacter* and enterohaemorrhagic *E. coli* (EHEC) should be considered when evaluating microbial risks from various fertiliser products including faeces, sewage sludge and animal manure (Höglund, 2001).

Faecal coliform are gram-negative bacilli, non-sporulated, heat tolerant, strict aerobes or facultative, with the ability to ferment lactose with formation of gas in 48 hours at 44.5^{o}C. They include the species *E. coli* and certain strains of *Klebsiella pneumoniae* in addition to other enteric bacteria. Therefore they are considered ideal bacteriological indicators of contamination of an intestinal origin (Jiménez *et al*., 2002).

Salmonella choleraesuis is the leading cause of clinical salmonellosis in swine in the United States and has been the second most common *Salmonella* serotype isolated from animal and animal-related sources. In humans choleraesuis infection is uncommon, but when it occurs the results are serious as the organism commonly caused septicemia that results in localized infections such as endocarditis, meningitis, andosteomyelitis and a death rate that is higher than that of typhoid (Feder *et al*., 1998).

S. choleraesuis usually transmitted by contaminated faeces shed from clinically ill and asymptomatic carrier pigs. Relatively low levels of faecal shedding (10^2/g faeces) can result in transmission, and faecal shedding can be detected intermittently

for up to 8 weeks, and the organism remains viable in soil for over a year (Gray *et al*., 1996). *Salmonella typhi* are gram-negative bacilli and motile to their peritrichous flagella, most of them are producers of H_2S. The members of this genus tend to be pathogenic for man or for other homeothermal animals. The most common diseases in humans are typhoid fever and gastroenteritis (Jiménez *et al*., 2002).

The faeces of a healthy person contain large number of commensal bacteria of many species. The most widely used indicator has been the faecal coliform *E. coli*, the main constituent of the enterobacteria, enterococci (Faecal *Streptococci*), and anaerobic bacteria such as Clostridium, Bacteroides and Bifidobacterium (Feachem *et al*., 1983).

This invasion is a characteristic of typhoid infections and other enteric fevers caused by salmonellae. During infections restricted to the gut, bacteria will be passed only in the faeces. When invasion has occurred bacteria may be passed in the urine as well and will also be found in the bloodstream at some stage. In areas with insufficient sanitation, cholera may still occur and constitute a risk for contamination of water (Höglund, 2001).

The pathogenic or potentially pathogenic bacteria are used as indicators. They most commonly enter a new host by ingestion (in water, on food, on fingers, in dirt), but some may also enter through the lungs (after inhalation of aerosol particles) or through the eye (after rubbing the eye with faecally contaminated fingers). Diarrhoea is a major symptom of many bacterial intestinal infections. The bacteria may also invade the body from the gut and cause either generalised or localised infections (Feachem *et al*., 1983).

2.3.2 Protozoa in faeces

Protozoan parasites are pathogens that have developed adaptations that enable them to survive for prolonged periods in the environment. Their hardiness also protects them from destruction by chemical disinfection as used in drinking water production processes. At the same time the two best known protozoan enteropathogens, *Cryptosporidium parvum* and *Giardia lamblia/intestinalis*, have been studied intensively during the last decade partly due to their environmental resistance and have also been shown to be highly infectious in humans, identified as agents for waterborne epidemics. Infectious doses are low especially *Cryptosporidium* have been the cause of several large waterborne outbreaks (Höglund, 2001).

Many species of protozoa can infect man and cause disease. Among them are several species that are harboured in the intestinal track of man and other animals, where they may cause diarrhoea or dysentery. Infective forms of these protozoa are often passed as cysts in the faeces, and man is infected when ingesting them. Only three species of human intestinal protozoa are considered to be frequently pathogenic: *Giardia lamblia, Balantidium coli, and Entamoeba histolytica* (Teunis and Havelaar, 2002).

Pathogenic protozoa they cause clinical cases of diarrhoea or severe gastroenteritis. Because of their capacity to form cysts under adverse conditions, these organisms represent a serious health problem for the population in general. Table 2.1 shows the morphology of some of the protozoa of medical importance (Jawetz *et al*., 1995).

Table 2.1 Morphology and clinical characteristics of cysts of intestinal parasitic protozoa (Jawetz *et al.*, 1995)

Protozoa cysts	Size (µm) and shape	Number of nuclei	Clinical characteristics
Entamoeba coli	14-30 (average 15-20) spherical smooth wall	8 in mature cysts, sometimes 1,2,4 and 16	Diarrhoea, abdominal pain, weigh loss and fever
Entamoeba histolytica	9-14.5 (average 12-15) spherical smooth wall	1-4, 4 in mature cysts	Can evolve into fulminating colitis, perforation and hepatic abscess
Flagellates *Giardia lamblia*	8-14 long 7-10 wide oval	4 (number depend on age), lie close together one end	Intermittent diarrhoea, malabsorption and weight loss

Giardia parasites have been found in sludge in high concentrations in Western Australia where the remain the most common cause of enteric disease. (Hu *et al.*, 1996) have reported that *Giardia* cysts were detectable in sludge that had been stored for over a year. *Cryptosporodium* species and *Giardia* species pose a serious threat to human health as these organisms are difficult to inactivate with disinfectants and their doses in human are very low (Hu *et al.*, 1996; Finch and Belosevic, 2001).

2.3.3 Viruses in faeces

Numerous viruses may infect the intestinal track and be passed in the faeces, they may infect new human hosts by ingestion or inhalation. One gram of human faeces may contain 10^9 infectious virus particles, regardless of whether the individual is experiencing any discernible illness. More than 120 different types of viruses may be excreted in the faeces, with most commonly identified including rotavirus, adenoviruses (including poliovirus), hepatitis A virus, reoviruses, enteric viruses and

diarrhoea-causing viruses and the somatic and f$^+$ coliphage (especially rotavirus) (WHO, 1989).

The somatic and f$^+$ coliphages pertain to the group of bacteriophages that have been proposed as possible models of ideal indicators of enterovirus. This fact is due to their similar physical structure and given that they are among the viruses, the group with greatest resistance in terms of the evaluation of the disease destruction processes such as chlorination. It is still unknown whether their resistance is greater than the resistence of other indicators (Jiménez et al., 2002).

Enteric viruses are now considered to cause the majority of gastrointestinal infections in developed regions. Hepatitis A has also been recognised as a pathogen of concern when applying waste to land and is considered a risk for water- and foodborne outbreaks especially where the sanitary standard are low (Höglund, 2001).

Rotavirus is excreted in high numbers 10^{10}-10^{12} particles/g faeces have been reported, sometimes for periods of up to one month reported 50% infectious dose for rotavirus in adults to be approximately 10 focus forming units (FFU). Rotavirus is more resistant than or at least as stable as other enteric viruses under different environmental conditions (Höglund et al., 2002; Pesaro et al., 1995).

Considerably among different types of virus and even among different strains of the same virus. Inactivation is a rate process, and the removal of infectivity therefore depends on both the efficiency of removal and the numbers initially present. In faeces and sewage these may be higher than 10^6 per gram and 10^6 per litre, respectively (Stroffolin et al., 2001).

Viruses are also present in throat secretions, especially during the early stages of infection. These particles are highly infectious and can remain viable for a considerable period under suitable conditions. Infection takes place when the virus is ingested, possibly in food or water (Feachem et al., 1983).

2.3.4 Helminth in faeces

In developing countries helminth infection are of great concern. Many species of parasitic worms or helminths have human hosts. Some can cause serious illnesses, but a number generate few symptoms. Only those helminths whose eggs or larval forms are passed in the excreta are of concern to this study. Only *Schistosoma haematobium* is voided in the urine, the others examined are all excreted in the faeces i.e. *Ascaris lubricoides, Fasciola hepatica* etc (Feachem *et al.*, 1983).

The eggs of helminth like *Ascaris* are persistent in the environment, and therefore regarded as an indicator of hygienic quality (WHO, 1989). They cause mechanical deterioration, tissue damage, toxic effects and blood loss. Helminth ova especially *Ascaris* spp. Genus are the most commonly found in raw sludge. They present high resistance to chemical and physical conditions and they can be considered the most resistant form of parasites. They have ability to survive long periods of time in raw sludge and soil (up to six years from their original application), much more than bacteria, viruses and molds (Jiménez *et al.*, 2002).

The study of helminth egg contamination is very important as they are found in great concentrations in sludge and is very resistant to most sludge treatment processes. Their presence is associated with sanitary risks when sludge is used as an agricultural fertilizer and processes that are able to eliminate this contamination need to be well known (Asaolu and Ofoezie, 2003).

Human helminth infections are a major cause of morbidity and mortality, are the hardiest of the pathogens of interest in faecal matter intended for handling and reuse. Ascariasis is one of the most common helminthes infections globally, with an estimated one billion cases (Feachem *et al.*, 1983).

Helminth ova are extremely resistant to various chemicals that in normal form destroy bacteria and viruses such as chlorine, ozone and they even present the ability to develop the embryonic stage in 50% sulphuric, nitric and acetic acids, in solutions saturated with copper sulphate, ferrous sulphate and copper acetate. For this reason the eggs of *Ascaris* are often used as indicators organisms in monitoring the

efficiency of treatment or disinfection processes. *Ascaris* spp were able to survive for 20 days at temperatures of -20.9 to -27°C, while *Toxocara canis* survives at temperatures under 38°C (Jiménez *et al*., 2002).

2.3.5 Inactivation of pathogens in faeces

The inactivation of pathogens in faeces is a more complex issue than in urine, due to varying conditions regarding moisture, other climatic factors as well as a larger influence of the construction of the system, e.g. how well the urine is diverted and whether anal cleansing is practised. During faeces collection the addition of other material such as ash or lime also needs to be considered, as it may increase the die-off rate for pathogens. The alkalinity of different types of ashes does however vary, and it may be difficult to predict the final pH and the related pathogen inactivating effect (Höglund, 2001).

Many studies have been undertaken on agricultural reuse of wastewater treated by stabilisation ponds systems. Stabilisation ponds are known as extensive treatment systems, well adapted to low socio-economic conditions in developing countries. It has been demonstrated by several authors that these systems can lead to very satisfactory effluent quality if they are well designed. In Morocco, several studies have been undertaken on stabilisation ponds, such as Microphytic ponds (Kouraa *et al*., 2002).

To reduce the number of microbial pathogens in the sludge, a microbial disinfection process can be implemented. For agricultural purposes, the reduction of pathogens is usually accomplished either by adding lime or by microbial anaerobic digestion, which also eliminates the odours typically associated with raw sewage. Studies in recent years have demonstrated the effectiveness of ionising radiation in the effective removal or inactivation of pathogenic bacteria, viruses and protozoan parasites. Several authors have presented calculations of the cost of irradiating sewage sludge. However an accurate cost for such treatment cannot be given in a straight forward manner, because the pollutants and bacterial contents differ for each kind of waste (Al-Bachir *et al*., 2003).

Different processes are being considered as advanced treatments that achieved destruction of pathogens in sludge allowing unrestricted application in land and most of them are based on increasing of temperature over $50^{\circ}C$, according to the European Union Working Document on Sludge. Lime stabilization, thermophilic digestion and composting processes have some draw back such as the increase in the mass of solids that needs to be transported, in the case of lime stabilization, maintaining adequate operating conditions when using thermophilic processes, and the large requirement of area needed for composting (Barrios *et al*., 2004).

Within all types of treatment disinfection plays a critical role because it guarantees the elimination of indicator organisms (i.e. faecal coliform) and other type of microorganisms. One of the most common types of disinfection is chlorination although the requirements vary considerably depending on the quality and types of microorganisms present (Jiménez *et al*., 2001).

Acid treatment has been sporadically reported to reduce microbial content in water and sludge, when employing organic acids. Some authors have studied the application of peracetic acid to evaluate the impact on different microorganisms. Peracetic acid is mixture of acetic acid and hydrogen peroxide. Advantages of using this acid for disinfection include the shirt retention times required, the absence of disinfection by-products or temperature requirements, and the fact that when applied to sludge does not increase the total mass of solids (Barrios *et al*., 2004).

Pathogenicity must be considered when sewage sludge is applied to agricultural land. Sewage sludge must be stabilized to reduce odour and the potential for attraction of disease vectors before its application. Anaerobic digestion is a popular method for sewage sludge stabilization. For lifting the restrictions further treatment is needed to further reduce the pathogens. Irradiation is a recognized method for reducing or eliminating pathogens in digested sewage sludge. After sludge is irradiated, the recolonizing microorganisms are much less harmful to animal and human health than those originally present (Wen *et al*., 1997).

The conventional wastewater treatment process produces solids during primary sedimentation and biological treatment. Solids are further stabilized in order to

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reduce organics, eliminate offensive odours, and lower pathogen levels (Hong *et al*., 2004).

Several technologies have been developed to simultaneously increase the efficiency of pathogen reduction and enhance anaerobic digestion. Pasteurization is a heat treatment process in which the sludge is pretreated at elevated temperature for a specified period. Pasteurization can be achieved by a number of techniques that employ various combinations of high temperature, extended time and high pH (Strauch, 1988).

The use of microwave/infrared irradiation has risen over the last 10 years. Microwaves have been applied to many areas such as organic decomposition, medical waste sterilization, and killing of pathogens in food, animal manures, and soil. There have been studies on heating mechanisms, disinfection of food, and industrial applications. Many biologists claimed that there was no evidence of a microwave effect and that the biocidal effects of microwaves were either due entirely to heating or were indistinguishable from external heating (Hong *et al*., 2004).

According to the most recent UK survey of sewage sludge disposal activities, 57% of all sludge in the UK was treated by mesophilic anaerobic digestion (MAD) before final disposal. The 1989 UK Code of Practice for Agricultural use of sewage sludge which regulates the treatment of sewage sludge prior to agricultural disposal, recognises MAD as a two-stage process with various operating conditions (Horan *et al*., 2004).

Compliant MAD is defined as a primary sludge digestion stage with the temperature maintained between 3 and 35°C and with a minimum mean hydraulic retention of 12 days, followed by a secondary stage that provides a mean retention period of at least 14 days (Horan *et al*., 2004).

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2.4 Diseases related to sanitation and excreta-related infections

Although implemented for the protection of public health, the convention wastewater treatment systems are not primarily designed for reducing pathogens. Still, they have been fairly effective in reducing transmission of pathogenic microorganisms. This owes both to minimised human contacts with wastewater, the different treatment processes and to dilution of the treated wastewater in the recipient waters (Höglund, 2001).

Municipal wastewater contains a variety of pathogenic organisms of human origin. Diseases caused by these pathogens can occur as a result of ingestion of untreated or improperly treated water, ingestion of infected aquatic food species, skin contact with the contaminated water and with improperly disinfected wastewater effluent in reuse application (Liberti *et al*., 2002).

Reported outbreaks of foodborne illness associated with fresh fruits and vegetables in the USA have nearly tripled since 1973 (Islam *et al*., 2005). Waterborne outbreaks are often caused by sewage-contaminated drinking water. In the Nordic countries known aetiological agents were identified in 36% of waterborne outbreaks in 1975-1992. Of these 46% were bacteria, 43% viral and 11% protozoan. Viruses are considered to be responsible for a major proportion of the outbreaks where no aetiological agent is found. Waterborne and foodborne transmission illustrate a pathogen's ability to survive in and spread through the environment (Höglund, 2001).

Higher incidences of enteric infections in the population have been recorded in epidemiological investigations in areas where wastewater was used on crops (Cifuentes, 1998; Bouhoum and Amahmid, 2000). Foodborne outbreaks caused by wastewater irrigation of vegetables and fruits have been documented (Yates and Gerba, 1998).

The case is made for food-borne infection being a problem, which should be of increasing concern. Global trading and increasing numbers of susceptible individuals are key factors. Further trends in many countries toward eating more raw, or lightly cooked, vegetables to preserve taste and heat labile nutrients may also increase the likelihood of foodborne infections (Erdogrul and Sener, 2004).

Excreta may be related to human disease in two ways. The agents of many important infections escape in the body's excreta eventually to reach others. The first means of relation are excreted infections. In some cases the reservoir of infections escape in the body's excreta, because such infection cannot be controlled through changes in human excreta. The second way in which excreta relate to human disease is through the insect breeding that waste disposal often encourages (Feachem *et al*., 1983).

Diarrhoea disease is one of the leading causes of infact mortality and is closely related to both socio-economic situation and environmental health issues such as access to clean water. The incidence of diarrhoea is used as an indicator to determine the health status of children and identify possible environmental hazards (Barnes and Taylor, 2004).

Insects may be nuisance in themselves, but they may be also mechanically transmit excreted pathogens either on their bodies or in their intestinal tracks and sometimes they may be vectors for pathogens that circulate in the blood. If an excreted infection is to spread, an infective dose of the disease agent has to pass from the excreta of a patient, carrier, or reservoir of the infection to the mouth or some other entryway of a susceptible person. The spread will depend upon the numbers of pathogens excreted, how these numbers change during the particular transmission route or life cycle, and the dose required to infect a new individual. Infective dose is in turn related to the susceptibility of the new host (Feachem *et al*., 1983).

2.5 Transmission routes of pathogens

Health hazards associated with excreta re-use are of two kinds: the occupational hazard to those who handle the excreta, which is direct through different means of

person to person contact and the risk that contaminated products from re-use may subsequently infect humans or animals through consumption or handling, which is indirect and include vehicle-borne (food, water etc), vector-borne, airborne long-distance and parenteral transmission (injections with contaminated syringes) (Höglund, 2001).

Pathogens in excreta

Hands   Flies   Surface water   Solid waste   Soil
                  & Wastewater   (casual/landfill)

Agriculture/Aquaculture   Groundwater & surface water

Food   Water supply   Leisure
                                (e.g. swimming)

Pathogens enter humans
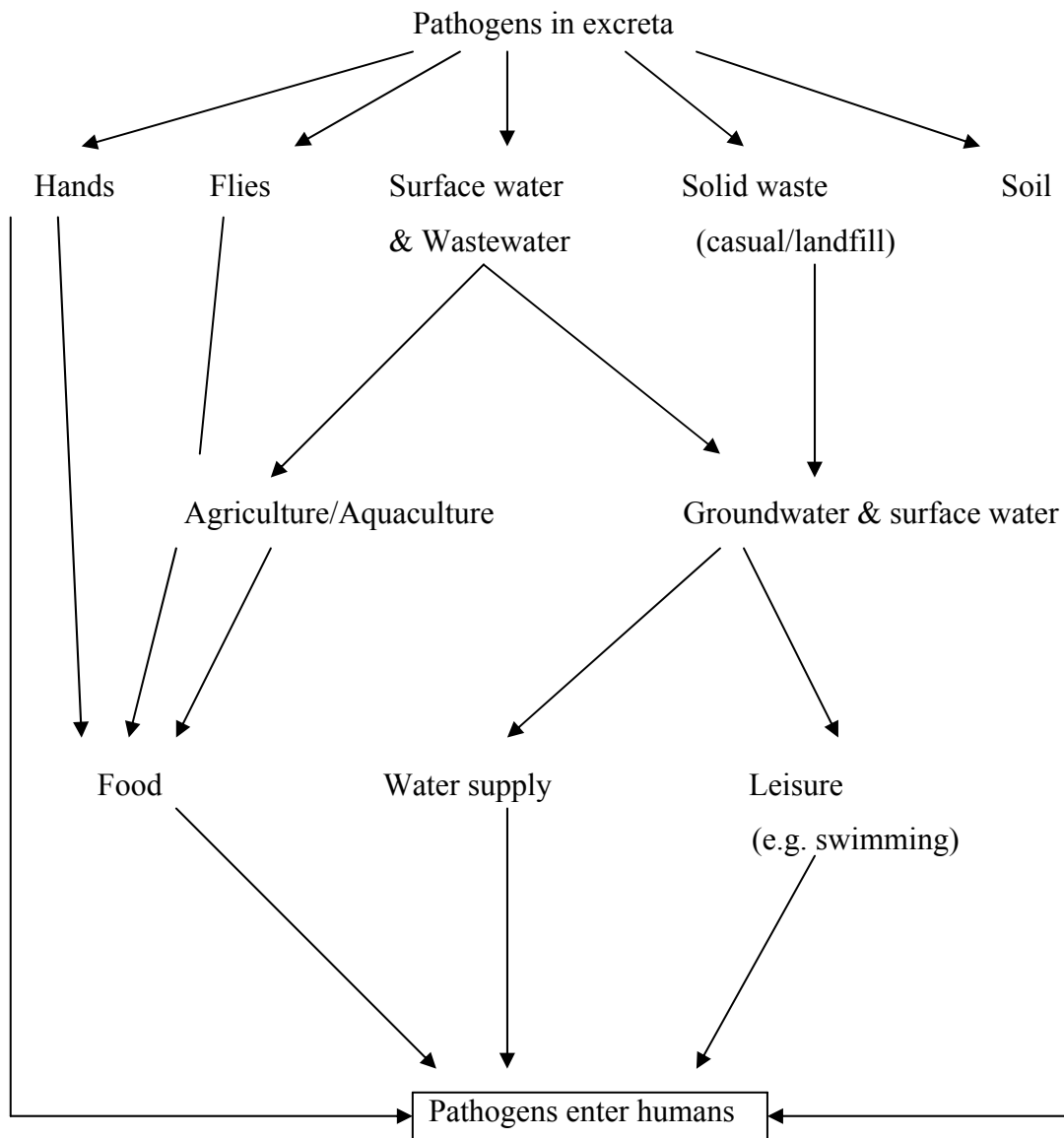
Figure 2.1: Transmission routes for pathogens found in excreta (Franceys *et al*., 1992).

Excreta-related diseases are common in developing countries. Pathogenic organisms can enter the human body by a number of routes as illustrated in Figure 2.1.

It should be noted that poor domestic and personal hygiene, indicated by routes involving food and hands, often diminishes or even negates any positive impact of improved excreta disposal on community health (Feachem *et al*., 1983). Risk assessments have also evaluated the increased risk from wastewater-irrigated crops. Irrigation with wastewater on crops used for energy or industrial purposes may be safer but still involves risks for transmission of disease to humans and animals in the surroundings and transport of pathogens to the groundwater (Carlander *et al*., 2000).

Fruits and vegetables, particularly those eaten raw and without peeling, have been demonstrated to be the vehicle for transmission of a range of parasites. Parasites that have been associated with vegetables or fruit-borne outbreaks of infection, rather than individual cases, include the protozoan parasites *Giardia* and helminth parasites *Ascaris* (Erdogrul and Sener, 2004).

The handling and reuse of all different types of waste products with human or animal origins involve hygiene risks. Whether human excreta (faeces and urine) are reused directly, diluted in wastewater (treated or untreated) that is reused, or are a constituent of sewage sludge used in agriculture, enteric pathogens will be present and able to cause infections by ingestion of waste product or by consumption of crops that have been fertilised (Höglund, 2001). Most faecal-oral infections are transmitted on hands and during food preparation, rather than through drinking contaminated water directly. Faeces also provide a fertile environment for many organisms that cause disease in humans. Improving hygiene practices and providing sanitation facilities could have a direct influence on a number of important public health problems besetting South Africa (Barnes and Taylor, 2004).

2.6 Contamination of crops

The original source of contamination of implicated produce has been identified. Manure from farm animals has been highly suspected as a leading vehicle of pathogen transmission. Sources of microbial pathogens on fresh produce at the pre-harvest stage include faeces, irrigation water, inadequately composted manure, soil, air, animals and human handling (Islam *et al*., 2005). Water contamination may be from human or animal sources. Infected animals have the potential to contaminate

procedure, either as a result of fertilization procedures or during handling, transport or storage of the products in areas in which the infected animals may have contaminated the environment. Pickers, handlers, packers and other individuals involved in the production and processing of products may also have the potential to contaminate procedure (Erdogrul and Sener, 2004).

Rodhe (2003) mentions several methods for determining slurry contamination of a crop. One method is to compare the masses of a large amount of clean crop and of contaminate crop taken from similar sized areas. Another method is to classify the contamination visually, but Rodhe (2003) rejected this method because of its inaccuracy (Rodhe, 2003). Spreading slurry onto grassland can lead to high losses of ammonia. Therefore there is a need for equipment that places the slurry below the cutting height in order to avoid contamination of the crop and reduce nitrogen losses in the form of ammonia after spreading (Rodhe, 2003).

2.7 Survival of microorganisms in the environment

The survival of bacteria in the soil depends on many parameters such as temperature, moisture, pH, soil composition and the presence of other microorganisms. Low temperatures help the survival of bacteria in soil with survival level tending to decrease as the temperature increases. Moisture is fundamental in controlling temperature as water has a high calorific capacity. The alternating cycles of freezing and thawing in winter also have an influence on the survival of pathogens in soil. A low incubation temperature and high soil moisture aid the survival of *E. coli* and *Enterococcus* spp (Cools *et al.*, 2001).

Unfavourable soil, nitrogen and water conditions are often limiting factors in the growth of plants. Environmental factors such as high salinity, fungicides and spells of hot, cold or dry weather can also limit microbiological nitrogen fixation. Although nitrogen is an essential element for all life it can also be a pollutant when applied in a wrong place or concentration. Nitrogen fertilisers in the soil the vulnerable to microbiological losses and to leaching into water. There must be replaced for each yield the risk increases that a surplus pollutes the soil and water environment. Urea-nitrogen pollution originate from human urine was found to be one reason for toxic

producing dinoflagellate blooms in aquaculture which is leading to fish deaths (Glibert and Terlizzi, 1999; Rowell, 1994).

Acidity and alkalinity (pH <6.0 or pH > 8.0) tend to be unfavourable to most bacteria in the soil, while neutral soils generally help the growth and survival of enteric bacteria. The pathogenic organisms *Erysipelothrix*, *Salmonella*, *E. coli*, *S. faecalis* and *Mycobacterium* survive better in soils with a pH between 6.0 and 7.0 and the best inactivation rates for pathogenic microorganisms are found in acidic soils (Gerba *et al*., 2001).

From the time of excretion the concentration of enteric pathogens usually declines by the death or loss of infectivity of a proportion of the organisms. Protozoa and viruses are unable to grow in the environment, thus numbers will always decrease whereas bacteria may multiply under favourable environmental conditions. The ability of a microorganism to survive is defines as its persistence. The persistence of microorganisms in the environment is a field that has been widely investigated (Feachem *et al*., 1983).

2.7.1 Nitrogen content of crops, soil and manure

The primary plant nutrient associated with sewage sludge is N: however sludge also contributes significant amounts of other macro and micronutrients. Nitrogen availability from sewage sludge and sludge compost is reported to range from 0% to 56%. Warman and Termeer (2005) suggested that biosolids compost N could replace one-third of the fertilizer N required by fescue without decreasing yield (Warman and Termeer, 2005).

Nitrogen is an essential element for protein synthesis. Plant dry matter usually contains 1-5% nitrogen. The need for nitrogen is highest at times of vigorous growth when leaf and seed proteins develop. Nitrogen occurs in fresh urine as urea, which is useful for plants and often present in commercial fertilisers. Urea degrades easily by microorganisms to ammonium, which is also useful for plants. In a slightly alkaline solution, part of the ammonium can, however evaporate easily as ammonia. This

evaporation can be noted in the form of unpleasant smell (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

The main factors that influence N availability from sewage sludge are its inorganic N content, digestion process (aerobic vs. anaerobic), C:N ratio, pH, the method and timing of application and soil type and properties (Warman and Termeer, 2005). Excessive applications of sewage sludge beyond crop requirements and the soil's absorptive capacity or applications made in the fall or winter may results in ground water contamination by nitrates, loss of N through denitrification, toxin nitrate concentrations in animals and surface water contamination by P. Therefore there are still questions about the release of essential and non-essential nutrients from sewage sludge and sludge composts and the short and long term economic benefits to the farmer and the community from applying sludge to the land (Warman and Termeer, 2005).

2.7.2 Phosphorous content of crops, soil and manure

Phosphorus is the second element related to vegetative growth. Its need is approximately one tenth of that of nitrogen. There can be shortage of phosphorus in the soil, because all plants use phosphorus for their yields. Typically the need is some 10-20 kg/ha for each yield. This amount of phosphorus should be replaced through fertilisation. In reality the need can be higher in phosphorus poor soils, since part of it is bound to non-soluble salts of iron, calcium or aluminium (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

Phosphorus content in sewage sludge and sludge compost and P availability to crops varies even more widely than N. To some extent, the soil conditions and characteristics of biosolids, which influence N mineralization and plant availability, apply to P, but not to K, which is not organically bound and not subject to mineralization reactions. The use of chemical flocculants in some wastewater treatment processes can decrease P mineralization from sludge and composts while P availability to crops are controlled by soil Fe, Al and Ca content (Warman and Termeer, 2005).

2.7.3 Physiochemical and biological factors that may affect the survival in excreta and reuse systems

Different factors that affect the inactivation of pathogens in the environment include temperature, pH, moisture and competition from naturally occurring microorganisms. To obtain a fertiliser product from excreta that is safe to use it is possible to apply treatment methods utilising any of these parameters in combination with time (Höglund, 2001).

Temperature

Most microorganisms survive well at low temperatures (5°C) and rapidly die-off at high temperatures (more than 40°C). This is the case for different types of media including water, soil, sewage, and crops. Temperature depends on season and storage conditions and dilution on the amount of flushwater used (Feachem *et al*., 1983; Höglund *et al*., 2002).

A temperature of 40°C is an effective barrier to the survival of helminth eggs, but in high ammonia concentrations already a temperature of 30°C was effective. Helminth eggs are rapidly destroyed when the temperature rises, if the relative humidity is low (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

To ensure inactivation in e.g. composting processes, temperature around 55-65°C are needed to kill all types of pathogens (except bacterial spores) within hours. The hardiest organisms are cysts of *Entamoebae histolytica*, *Ascaris* eggs and *Mycobacterium tuberculosis*. Viruses such as bovine parvivirus and *Salmonella typhimurium* phage 28B are also considered to be heat resistant. Temperature effects might especially be of concern in temperate regions where the temperatures are quite low during a large part of the year (Höglund, 2001).

pH

Many microorganisms are generally adapted to a neutral pH (7) even though enteric pathogens need to withstand the acidic conditions in the stomach to cause an infection. Highly acidic or alkaline conditions will have an inactivation effect on most microorganisms by the hydrolysation of cell components or denaturation of enzymes. Bacteria survival is shorter in acid soils (pH 3-5) than in alkaline soils (Feachem *et al*., 1983).

The pH of fresh urine is generally around 6, but rises to approximately pH 9 in the collection and storage tanks due to a rapid conversion of urea to ammonia. In previous studies the elevated pH was shown to have bactericidal and antiprotozoan effects. Most enteric viruses are resistant to inactivation at both low (3.5) and high (10.0) pH, generated at high pH values may act as an inactivating agent for viruses as well as for other microorganisms (Höglund *et al*., 2002).

Moisture

Moisture content is mainly applicable to the survival in soil and in faeces. A moist soil favours the survival of microorganisms and drying may be used as a process to sanitise excreta in dry latrines (Esrey *et al*., 1998).

Virus survival is prolonged under moist conditions. Protozoa cysts are highly sensitive to desiccation, which may also affect their survival on plant surfaces. For *Ascaris* eggs to be inactivated moisture level below 5% is needed (Feachem *et al*., 1983)

Nutrients

If nutrition is available and other conditions are favourable bacteria may grow in the environment. Nutrient deficiencies thus only affect bacteria. Enteric bacteria adapted

to the GI track are not always capable of competing with indigenous bacteria for the scarce nutrients available and the ability to reproduce and even survive in the environment therefore tends to be limited (Feachem., *et al* 1983).

2.7.4 Destruction of pathogens (Die-off or survival)

As the death and survival of excreted pathogens is an important factor influencing transmission, these organisms should be destroyed or otherwise rendered harmless. In principle pathogens die off upon excretion, as environmental conditions outside the human host are generally not conducive to their survival. Exceptions are pathogens whose transitional stages multiply in intermediate hosts such as the miracidia of e.g. *Clonorchis* or *Schistosoma* (Strauss, 1994).

Also, some viruses, although they cannot multiply outside a suitable host cell, may survive for many weeks in certain environments, especially where temperatures are cool (less than 15°C). Environmental factors of importance in the die off rate of pathogens are high temperatures, low moisture content and time. A high temperature especially is the most important consideration as all living organisms, from the simplest to the most complex can survive at temperatures only up to a certain level. Above that level they perish (Feachem *et al*., 1983).

Some treatment processes have little effect on excreted pathogens and simply allow the necessary time for natural die-off to occur. Certain treatment processes create conditions that are particularly hostile to excreted pathogens and that promote their rapid death (Feachem *et al*., 1983). Another important factor is the infective dose of pathogens i.e. the dose required to create the disease in a human host. For helminths, protozoa and viruses, the infective dose is less than 10^2; while for bacteria it is medium between 10^4 to high 10^6 (Strauss, 1994).

2.8 Management of human excreta

Sludge could contain thousands of different compounds. More specifically, components of sewage sludge include organic substances derived from domestic sewage and industry. These contaminant include heavy metals (Zn, pb, Cd), linear alkylbenzene sulfonates (LASs), nonylphenol (NP), phthalates, Polycyclic aromatic

hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (lindane), DDE/DDT, and polychlorinated dibenzodioxins and dibenzofurans (PCDDs/PCDFs). The presence of all the contaminants in sludge is important, and their presence may be sufficiently detrimental to treatment efficiency and the environment, toxicity assessment of sludge samples using bioassays have been performed (Robidoux *et al*., 1998).

The treatment of sludge leads to the production of large amounts of a secondary waste product sewage sludge. While standard method of processing sludge reduces its volume, efficient techniques for utilizing the dewatered sludge are still lacking. One possible solution is to use it as a soil fertilizer, but the use of this is limited (Pempkowiak and Obarska-Pempkowiak, 2002). In the rural areas of central Europe sewage has often been treated in small local plants, usually in septic tanks. In recent years, small biological wastewater treatment plants have been introduced. These are usually ground filters, filter-drainage systems and, for larger amounts of sewage, trickle filters and activated sludge systems. Nevertheless, the primary and secondary sludge from these has to be disposed of (Zwara and Obarska-Pempkowiak, 2000).

2.8.1 Urine-Diversion systems

By collecting the urine and the faecal fractions separately from the rest of the sewage water, the majority of the nutrients are contained in a small fraction relatively unpolluted by heavy metals (Vinnerås, 2002).

To get pure urine the separation should be made in the toilet. If the toilet is a simple pit latrine or VIP latrine, where the person is squatting, most of the urine passed can be diverted by a groove and pipe to a connected jerry can or an irrigation channel and the faeces can fall directly into the pit container. Separation may be easily practised in schools (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

The urine separating system is based on toilets in which the bowl is divided into two parts, where the smaller front bowl collects the urine, which in some cases is flushed with a small amount of flushwater. In the rear bowl the faeces and used toilet paper are collected. A small amount of flush water is used for the urine and the faecal

material is either collected dry or flushed with the normal amount of flush water (4-6 litres) for low-flush toilets used in Sweden. The urine and its flush water are collected in a tank, usually buried in the ground. When the tank is full the urine solution is transported to a storage tank at a farm where later is used as fertiliser on arable land, preferably for cereal crops (Höglund *et al*., 1998).

With a better-closed nutrient loop, many more people, including low income farmers would be able to produce more food and other plant products. It would also reduce the pollution effects from unsafe excreta disposal and surplus use of chemical fertilisers and protect surface and ground water and the air. A more effective utilisation of human excreta would also reduce the waterborne enteric microbiological diseases (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

By using urine-diverting toilets, it is possible to collect the most nutrient containing fraction in a concentrated way. If the urine is collected separately, the main proportion of the nutrients from the household is collected in a small fraction with low heavy metal content (Jönsson *et al*., 2000; Höglund *et al*, 2000).

If the ammonia content is over 1 mg 1^{-1}, the pH is over 8.8 and no fresh urine is added, storage of the urine mixture for one to six months, depending on the temperature, inactivates all non spore-forming pathogens present, so the urine can then be recycled as a fertilizer to agriculture with negligible hygienic risks (Jönsson *et al*., 1997, 2000; Höglund *et al*., 1998; Höglund, 2001).

If the urine is collected uncontaminated by heavy metals, an unpolluted and potent organic fertilizer that contains mineral nutrients is obtained. Field trials and pot experiments have shown that diverted human urine is comparable to mineral fertilisers. Although source separating sewage systems may have several environmental advantages the sanitary demands and regulations have to be met in order to minimise the risks for disease transmission (Vinnerås, 2002).

Human urine is the largest contributor of nutrients in household wastewater; urine contains some 90% of the nitrogen, 50-65% of the phosphorus and 50-80% of the potassium. The higher figures have been published by (Wolgast 1993). They are

based on the human physiology and are often cited in the literature. Albold (2002) based his lower figures on measurements in Alpine leisure areas where chemical precipitation and evaporation reactions may have led to higher nutrient losses. The figure presented above depends on the body weight of the person involved. The climate, water intake and diet characteristics, especially its protein (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

The total quantities of nutrients in human urine are significant also when compared with the quantities of nutrients in the mineral fertilizers used by agriculture. The yearly production of human urine in Sweden equals 15-20% of the mineral fertilizer (Vinnerås, 2002).

The amounts of heavy metals in the collected urine are very low. It contained less than 3.6 mg Cd/kg P, while the chemical fertilisers sold in Sweden 1995 contained approximately 26mgCd/kg P (Olsson, 1995).

2.8.2 Characteristics of source-separated human urine

Collected source-separated human urine (urine mixture) has different characteristics than freshly excreted urine since it is mixed with flushwater and transported through pipes to a tank. Most collection tank are placed outside, usually buried underground. The temperature thus varies depending on climate and season. In Sweden the temperature of urine mixture ranged from 3^oC to 19^oC (Höglund et al., 1999).

The pH of fresh urine is normally between 4.8 and 7.5, but in other samples collected it was around 9.0. The pH is related to the concentration of ammonium. Temperature, pH, dilution, and ammonia are the parameters considered that might affect the persistence of microorganisms in urine tanks (Jönsson et al., 2000).

2.8.3 Hygienic aspects on the reuse of source-separated human urine

The handling and reuse of all different types of waste products with human or animal origins involve hygiene risks. Whether human excreta (faeces and/or urine) are reused directly, diluted in wastewater (treated or untreated) that is reused, or are a

constituent of sewage sludge used in agriculture, enteric pathogens will be present and able to cause infections by ingestion of the waste product or by consumption of crops that have been fertilised (Cooper and Olivieri, 1998).

Cysts and oocysts of protozoa and helminth ova are considered to be of great public health concern since they remain viable for extended periods outside their human host and viruses have received attention due to low infectious doses and difficulties in analysing their presence in waste products (Asano and Levine, 1998).

Many of the ecological sanitation alternatives being introduced are small-scale systems that demand more personal involvement of the users, including handling of the waste. Thus if not sufficiently treated the possible exposure points for pathogens are increasing compared to conventional piped systems. With the main goal of recycling nutrients and minimising utilisation of natural resources, hygiene aspects must be prioritised. To successfully introduce and optimise alternative wastewater systems it is necessary to evaluate hygiene risks and sanitary aspects in accordance with sustainability criteria (Höglund, 2001).

2.8.4 Faecal separation

The second largest contributor of nitrogen and phosphorus to household wastewater is faeces. The plant availability of this nitrogen is probably lower than that of mineral fertiliser since only about 50% of the nitrogen is extractable with water. The remainder of the nitrogen is incorporated into organic matter and bacteria. Of the faecal nitrogen, about 17% is contained in the bacterial fraction and about 10% is found as ammonia from degradation of urea, peptides and amino acids. The remaining nitrogen is found in different organic compound such as uric acid and different enzymes and peptides (Vinnerås and Jönsson, 2002; Vinnerås, 2001).

Source separation and collection of faeces can be done either by collecting the faeces dry or if a water closet is used, by separating the faeces from the flushwater after a short transport. To get a functional system where both the water recipient is protected and the nutrients are collected into reusable fractions, the urine has to be diverted and collected separately at source (Vinnerås, 2001).

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A technique for removing some dry matter and nutrients is to use a three-chamber septic tank. In Sweden, the accumulated sludge is removed once a year. However due to decomposition of organic matter and the reducing environment, the main fraction of the bound nutrients is dissolved into the wastewater and transported down the system (Vinnerås, 2001).

One way to get a dry faecal fraction is by using dry urine-diverting toilets. The urine diversion decreases the volume of the faecal fraction and its smell giving two fractions that are easy to handle. Another technique for obtaining a compact faecal fraction when using a double flushed urine-diverting toilet is to separate faecal particles from the flush-water (Vinnerås and Jönsson, 2002).

There are several commercial separation systems available on the market today. The two main types of systems used are filtration and Aquatron or in some cases a combination of both. The Aquatron system is based upon a combination of a whirlpool effect, surface tension and gravity, some system also have a filter that increases the effect of the separation. The filtration systems available today are based on filter with the filter cake that complements the filter with a retention time of several months. These kinds of system are simple and robust (Vinnerås, 2001).

The plant availability of the faecal phosphorus might be comparable to mineral fertiliser, since a considerable fraction is found in granulated particles mainly bound to calcium. The rest of the phosphorus exists mainly as organic bound material, e.g. bacteria. Only a small fraction of the faecal phosphorus can be found as free phosphate ions (Vinnerås and Jönsson, 2002).

The main idea of faecal separation is to keep the advantage of the flushed toilet and then after a short transport make it possible to recover the faecal nutrients in a fraction as small, undiluted and unpolluted as possible (Vinnerås, 2001).

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CHAPTER 3

The use of coal ash, wood ash, NaOH and pasteurization for the reduction of pathogens in dehydrated faeces**.**

Abstract

The management of human excreta is a concern since the generated volumes are increasing as a result of extended sewage and advanced wastewater treatment systems that have to deal with the ever-increasing population. Human excreta contain nutrients such as nitrogen (N), phosphorus (P) and potassium (K). The application of faeces to land has been suggested as a way of using these nutrients as fertilisers. Unfortunately, human faeces also contain a high concentration of pathogens, which limit its potential use as fertiliser. This study investigated the level of contamination of selected pathogens in faeces with moisture content of 28.9% and how application of coal ash, wood ash, NaOH and pasteurization reduces pathogens. Dehydrated faeces were analysed for total coliform (TC), faecal coliform (FC), Faecal *Streptococci* (FS), *Salmonella* spp, *Aspergillus* spp and helminth eggs. The results show that the amount of colony forming units of FC, TC, FS, and *Salmonella* spp was higher than *Aspergillus* spp. The numbers of pathogens were reduced in all different treatments. The wood and coal ash treatment resulted in 2 log reduction of TC, FC and FS during an 8 d experimental period. No *Salmonella* and *Aspergillus* spp survived treatment with NaOH, wood ash, coal ash, pasteurization at 60°C for 30 min, 70°C for 20 min and 90°C for 5 min. Helminth eggs were found in high numbers in untreated dehydrated faeces. Only helminth eggs survived after pasteurisation at 70°C for 20 min and 90°C for 5 min.

Keywords: Pathogens, Dehydrated faeces, Sewage sludge, Biosolids, Wastewater, Sludge, Pasteurisation, Wood ash, Coal ash, NaOH.

3.1 Introduction

The management of sewage sludge is a major challenge and is becoming a greater concern since the generated volume is increasing as a result of extended sewerage and advanced wastewater treatment (Robidoux *et al*., 1998). The treatment of sewage leads to the production of large amounts of a secondary waste product sewage sludge. The quality of the sludge depends on the wastewater treatment technology and excessive contents of pathogens, heavy metals and organic pollutants are of concern (Pempkowiak and Obarska-Pempkowiak, 2002).

Production of municipal solid waste, including organic waste is increasing while soils are progressively losing organic matter due to intensive cultivation and climatic conditions. This makes the recycling of organic waste as soil amendments a useful alternative to incineration, landfill or rubbish dumps. Wastewater sludge is a valuable source of plant nutrients and organic matter and has been recycled as a fertilizer on agricultural land (Hassen *et al*., 2001). Human faeces, on the other hand are rich in phosphorus and potassium, which are important plant nutrients. More organic matter in soils is important to improve the soil structure in tropical countries so that it becomes more resistant to droughts and to erosion from heavy rains and floods (Engel *et al*., 2001; Smith *et al*., 2001).

In terms of microbiological pollution, wastewater sludge contains various pathogenic agents introduced by wastewater such as bacteria, viruses and parasites. The study of helminth egg contaminations is important as these are found in great concentrations in sludge (Capizzi-Banas *et al*., 2004). Among the pathogen of epidemiological relevance, *Ascaris* eggs are resistant towards most disinfection treatments in sewage sludge, a treatment lasting two months with an initial pH of 12.5 was required to obtain no viable organisms. The presence of eggs is associated with sanitary risks when sludge is used as an agricultural fertilizer. (Vinnerås *et al*., 2003a).

Biosolids may contain pathogens, heavy metals and toxic organic compounds, but may also be of acceptable quality for agricultural use depending on the initial contaminant level, treatment processes applied and their efficiency compared to fertilizers (Franco-Hernandez *et al*., 2003).
The effects of chemical treatment are mainly inactivation of protein or inactivation of the DNA/RNA strand. When choosing chemicals for disinfecting sewage

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products, an additional effect to possibly take advantage of is the potential agricultural value of substances in the treatment, e.g. NH_3, KOH and PO_4^{3-} (Allievi *et al*., 1994).

Faeces contain approximately 10^{10} microorganisms per gram dry matter and some of them are pathogenic (Vinnerås *et al*., 2003b). The risk of pathogens associated with the use of wastewater and sludge for agricultural purposes depends on a group of complex factors including the effectiveness or treatment processes in removing or inactivating pathogens (Robidoux *et al*., 1998).

Before using human faeces as a fertiliser, it must be made safe because the number of enteric bacteria, viruses, protozoa and helminth eggs in faeces can be high (Heinonen-Tanski and Van Wijk-Sijbesma, 2004). Disinfection methods currently available to produce hygienically safe dried faecal material for recycling include pasteurization and chemical methods. Pasteurization is a more reliable method for sanitation and uses heat inactivation. There are a number of methods that can be used for heat production including incineration or anaerobic digestion. One common method for disinfection of faecal matter in developing countries is the use of coal or wood ash for increasing the pH, which disinfects the faeces (Vinnerås *et al*., 2003b).

The efficiency of ash treatment depends on the amount of ash used and the origin of the ash, since the amount of available hydroxide ions may vary. Studies on *Salmonella typhimurium* phage 28B and faeces treated with ash have shown a die-off from $8 \log_{10}$ after three weeks of storage to $2 \log_{10}$ in seven weeks, in different toilets in Vietnam (Carlander and Westrell, 1999).

The objectives of this study were therefore to investigate:
- The level of contamination of selected pathogens in dehydrated faeces collected in South Africa;
- The effect of pasteurisation, application of wood ash, coal ash and NaOH on the inactivation of pathogens in dehydrated faeces.

3.2 Materials and methods

3.2.1 Dehydrated faeces samples

Aussie Austin from the CSIR Building and Construction Technology supplied 1 kg of dehydrated faeces. The faeces were collected from a septic tank at CSIR and it was relatively fresh (floating on top). The faeces were left in the sun for a number of days to dry out. After the samples were dried they were ground into small pieces and transferred to plastic bags. The samples were then transported to the laboratory and kept at room temperature for analysis.

3.2.2 Microbiological analysis

Total coliform, faecal coliform, Faecal *Streptococci*

Dehydrated faeces were analyzed for total coliform, faecal coliform, *Salmonella* spp, Faecal *Streptococci* and *Aspergillus* spp. The organisms were enumerated using the serial dilution technique. 1 g of dehydrated faeces was added to 9 ml sterile buffered peptone water solution and vortex for 10 s using an aseptic technique and 10^{-1} to 10^{-10} dilutions were made. 0.1 ml aliquots were plated on three selective media i.e. m-Endo agar, m-Enterococcus agar (37°C) and m-Fc-agar (44°C) in triplicate. The plates were incubated at 37°C and 44°C for 24 h. The colonies were identified by form and colour. After 24 h the number of typical colonies was recorded.

Aspergillus **analysis**

Fungi were enumerated by serial dilution in sterilized 1/4 strength Ringer's solution. 1 g of dehydrated faeces was added to 9 ml sterilized 1/4 strength Ringer's solution and 10^{-1} to 10^{-10} dilutions were made. Rose-bengal agar amended with 0.1 mg streptomycin-sulphate ml^{-1} was used to enumerate fungi (Parkinson, 1994). The plates were inoculated with 100µl of each dilution (three plates per suspension) and incubated at 25°C for two to four days.

Salmonella **analysis**

1 g of dehydrated faeces samples were added to 9 ml buffered peptone water and vortex for 10 s. Serial dilutions 10^{-1} to 10^{-5} were prepared and incubated at 37°C for 18-24 h. 0.1 ml of the mixture was transferred to 10 ml Rappaport-vassiliadis (RV) enrichment broth and incubated at 37°C for 24 h. The enrichment broth was subcultured by spreading 0.l ml onto the plates of Xylose-Lysine-Desoxycholate (XLD) agar and incubated at 37°C for 24 h. Occurrence of black colonies suggested the presence of *Salmonella* (APHA, AWWA and WPCF, 1995).

3.2.3 Quantitative analysis of helminth eggs

Figure 3.1 is considered as the Mexican Official Norm, NOM-001-ECOL-1996, which is derived from the USEPA method. The NOM method is based on the use of four steps:

- ❖ The dissociation of parasites from organic matter;
- ❖ Parasites sedimentation;
- ❖ Parasites concentration and;
- ❖ Microscope examination.

For viability determination, the samples was rinsed with 0.1 N sulphuric acid and incubated at 26°C for 20 d, with shaking. Aliquots of 0.1 ml of each tube was placed in glass slides or Doncaster disk, covered with 0.1 ml of Safranin dye and microscopically examined for stained or non-viable (NV) and unstained or viable (V) egg. Eggs impermeable (unstained) were recorded as viable and stained eggs as non-viable eggs. Safranin dye was used because it gave the most uniform, clear colours results and always produces good contrast between eggs and background and between NV and V eggs (Figure 3.1).

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┌─────────────────────────────────────────────────┐
│            Dehydrated faeces samples             │
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┌─────────────────────────────────────────────────┐
│   Liquefy X volume corresponding to 2 g TS,      │
│   with 1L (tap water) of 0.1 % tween 80 and      │
│   sediment 3 h or overnight                      │
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┌─────────────────────────────────────────────────┐
│  Aspirate supernatant and filtrate the sediment  │
│          in a 150-160 μm sieve                    │
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┌─────────────────────────────────────────────────┐
│  Rinse filtrate with 1-2 L of water and recover  │
│          in the same container                    │
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┌─────────────────────────────────────────────────┐
│            Sediment 3 h or overnight             │
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                        ▼
┌─────────────────────────────────────────────────┐
│        Centrifuge sediment at 660 g/ 10 min      │
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│  Aspirate supernatant and resuspend the sediment │
│  with 150 ml of zinc sulphate density 1.3        │
│  (Optional magnesium sulphate density 1.3)       │
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│  Centrifuge at 660 g/ 10 min and recover         │
│  supernatant.                                    │
│  Break density with 1 L of distilled water.      │
│  Let settle 3 h or overnight.                    │
│  Optional: Obtain a filtrate in a 20 μm sieve    │
└─────────────────────────────────────────────────┘
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┌─────────────────────────────────────────────────┐
│  Aspirate supernatant and centrifuge at          │
│          660 g/ 10 min                            │
└─────────────────────────────────────────────────┘
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│  Regroup sediment in a 50 mL tube with 15 mL of  │
│  acid alcohol solution and 10 mL of ethyl        │
│  acetate.                                        │
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│  Centrifuge at 660 g/ 5 min. Optional: Filter    │
│  with a 20 μm sieve                               │
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┌───────────────────────┐  ┌──────────────────────────┐
│ Rinse with 0.1 N.     │  │ Add 1 drop of safranin   │
│ sulphuric acid        │  │ into 1mL of the          │
│ Incubate at 26 ºC for │  │ sediment, let rest for   │
│ 20 days, with shaking │  │ 5 min.                   │
└───────────────────────┘  └──────────────────────────┘
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┌─────────────────────────────────────────────────┐
│  Transfer to a Sedgwick-Rafter chamber or        │
│  Doncaster disc and quantify under microscope.   │
│  Report number of viable ova/ gTS.               │
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Figure. 3.1. Official Mexican method (NOM-001-ECOL-1996) for the quantitative analysis of helminth eggs in sludge samples (Jiménez *et al*., in press).

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3.2.4 Physical and Chemical analysis

Moisture content determination

The moisture content was determined using a standard method, procedures as described by (Islam *et al*., 2004).

pH determination

The pH of untreated dehydrated faeces, faeces with NaOH, faeces with wood ash, faeces with coal ash and faeces pasteurized at 60°C for 30 min, 70°C for 20 min and 90°C for 5 min was determined by adding 10 g of the mixture to 250 ml distilled water. The suspension was stirred for 5 min and then allowed to settle for 1 h. The pH of the liquid was determined with a pH meter.

3.2.5 Survival of microorganisms using different treatments

Survival studies in dehydrated faeces were performed with six different pathogens: total coliform, faecal coliform, *Salmonella* spp, Faecal *Streptococci Aspergillus* spp and helminth eggs.

Ash treatment

Wood ash and coal ash samples were obtained from Attridgeville, Pretoria and these were used for the ash treatment. The ash samples were filtered through a 2 mm sieve. 20 g wood ash was mixed with 20 g dehydrated faeces. pH was determined by taking 10 g of the mixture and suspending into 250 ml distilled water. Microbiological analysis was conducted at daily intervals for 8 d. The same procedure was followed for coal ash treatments.

NaOH treatment

20 g of dehydrated faeces samples were treated with 2g NaOH. 10 g of this mixture was suspended in 250 ml of distilled water and the pH was determined as previously described and the number of surviving microorganisms was determined immediately and at 1 d intervals for 8 d.

Pasteurization

Pasteurization was carried out by adding 1 g of dehydrated faeces in test tubes containing 9 ml buffered peptone or ringer's solution. The test tubes containing the solutions were placed in a water-bath at 60^oC for 30 min, 70^oC for 20 min, and 90^oC for 5 min. The numbers of surviving microorganisms were determined after each temperature/time treatment.

3.3 Results and discussion

3.3.1 Fungi and indicator bacteria

The dehydrated faeces contained all the selected pathogens and fungi analysed (Table 3.1).

Table 3.1 Comparison of the different treatments for the reduction of microorganism numbers in dehydrated faeces immediately after mixing.

	Untreated dehydrated faeces (Control)	Faeces with NaOH	Faeces with coal ash	Faeces with wood ash	Faeces heated at 60°C for 30 min	Faeces heated at 70°C for 20 min	Faeces heated at 90°C for 5 min
n	3	3	3	3	3	3	3
Fungi (cfu/g)	6.00×10^{2}	NG	NG	NG	NG	NG	NG
Total coliform (cfu/g)	1.05×10^{4}	NG	8.30×10^{3}	5.60×10^{3}	9.00×10^{2}	NG	NG
Faecal coliform (cfu/g)	1.69×10^{4}	NG	5.10×10^{3}	4.20×10^{3}	7.00×10^{2}	NG	NG
Faecal *Streptococci* (cfu/g)	1.57×10^{6}	6.90×10^{5}	1.06×10^{6}	9.20×10^{5}	1.30×10^{4}	NG	NG
Salmonella spp. (cfu/g)	6.00×10^{1}	NG	NG	NG	NG	NG	NG

Cfu: Colony forming units

NG: No growth

The disinfection effect of NaOH

The NaOH treatment resulted in the reduction of Faecal *Streptococcus* numbers, from 10^6 to 10^5 cfu/g, giving a 1 log reduction (Table 3.1). No total coliform, faecal coliform, *Salmonella* and fungi survived NaOH treatment (Table 3.1). NaOH was used for the treatment of dehydrated faeces, because NaOH solubilize municipal waste activated sludge at ambient temperature.

The disinfection effect of coal ash

Coal ash contains potentially toxic elements such as As, Cd, Cr, Hg and Se, and may present significant hazards to organisms (Rowe, 1998). The coal ash treatment resulted in the reduction of total coliform and faecal coliform numbers, from 10^4 to 10^3 cfu/g, giving a 1 log reduction (Table 3.1). There was no effect on Faecal *Streptococcus* numbers. No *Salmonella* and fungi survived coal ash treatment (Table 3.1).

The disinfection effect of wood ash

Recycling of wood ash to soil has been suggested as a means to maintain and improve the nutrient balance of soil. The wood ash act as a source of base cations, raising the soil pH, as well as mineral nutrients (Steenari *et al*., 1999). Wood ash can also serve as a liming agent (Demeyer *et al*., 2001). The wood ash treatment resulted in the reduction of Faecal *Streptococci* numbers, from 10^6 to 10^5 cfu/g, giving a 1 log reduction (Table 3.1).

The total coliform and faecal coliform was reduced by 1 log (10^4 to 10^3 cfu/g), which is lower that that from urine-diverting latrines in Vietnam (A >7 log reduction of faecal coliform was recorded after three months of storage according to Schönning, 2001). This implies higher amount of ash to faeces be added and more time of storage is needed to obtain high reduction of pathogens. No *Salmonella* and fungi survived wood ash treatment (Table 3.1). Advantage of using ash to treat pathogens is that any heavy metals in the sewage sludge are expected to be bound in the form of insoluble metal hydroxides in the ash. (Reynolds *et al*., 2002) showed that the ash

acts both as a bulking agent and a buffer to maintain the pH above 12 in the initial processing. It is also plays an important role in odour control as the residual carbon in the product absorbs odours organics.

The disinfection effect of temperature

60°C for 30 min

The pasteurization treatment at 60°C for 30 min resulted in a 2 log (10^6 to 10^4 cfu/g) reduction of Faecal *Streptococci* numbers. The Faecal coliform and total coliform decreased from (10^4 to 10^2 cfu/g) giving a 2 log reduction, which is higher reduction compare to biosolids a 1 log reduction of total coliform and no difference in faecal coliform numbers was obtained according to Franco-Hernandez *et al*., (2003). (Table 3.1). No *Salmonella* and fungi survived pasteurisation at 60°C for 30 min (Table 3.1).

70°C for 20 min

No organism survived pasteurization at 70°C for 20 min (Table 3.1).

90°C for 5 min

No organism survived pasteurization at 90°C for 5 min (Table 3.1).

Table 3.2 Initial helminth egg counts and pH on dehydrated faeces samples

3.3.2 Helminth ova

Sample	pH	Total solid (%)	*Ascaris* spp eggs count/gTS	*Toxocara* spp egg count/gTS	*Trichuris* spp egg count/gTS	*Taenia* spp eggs count/gTS	Total eggs count/gTS
Untreated dehydrated faeces (control)	6.5	70.2	5.9	0.5	0.8	0.3	7.5
Faeces with NaOH	12.1	70.6	4.8	0.3	0.3	0	5.4
Faeces with coal ash	9.9	66.2	5.7	0	0.3	0.3	6.3
Faeces with wood ash	10.1	65.8	4.4	0.5	0.3	0	5.2
Faeces at 60°C, 30min	7.9	70.2	4.8	0.5	0.0	0	5.3
Faeces at 70°C, 20min	8.4	70.2	3.4	0.3	0	0	3.7
Faeces at 90°C, 5min	8.9	70.2	2.8	0	0.3	0	3.1

TS: Total Solid

Dehydrated faeces contained high numbers of helminth eggs (Table 3.2). The helminth eggs recovered from dehydrated faeces were *Ascaris* spp (5.9 eggs/gTS),

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Toxocara spp (0.5 eggs/gTS)*, Trichuris* spp (0.8 eggs/gTS) and *Taenia* spp (0.3 eggs/gTS). The total helminth eggs (7.5/gTS), which are comparable to eggs found in sludges and contaminated wastewater from South Africa (ranging from 3-8 eggs/gTS, according to Austin and Duncker, (2002). *Taenia* spp was found in both untreated dehydrated faeces and faeces treated with coal ash (Table 3.2). NaOH treatment resulted in the reduction of helminth eggs from 7.5 to 5.4 eggs/gTS. The coal and wood ash treatment reduced the numbers from 7.5 to 6.3 eggs/gTS and from 7.5 to 5.2 eggs/gTS which is higher reduction compare to that from urine-diverting latrines in Vietnam a 1% survival of *Ascaris* eggs was recorded after three months of storage according to Schönning (2001). Pasteurization treatment resulted in the reduction of helminth eggs from 7.5 to 5.3 eggs/gTS (60^oC, 30 min); 7.5 to 3.7 eggs/gTS (70^oC, 20 min) and 7.5 to 3.1 eggs/gTS (90^oC, 5 min). Heinonen-Tanski and Van Wijk-Sijbesma (2004) showed that a temperature of 40^oC is an effective barrier to the survival of helminth eggs and eggs are rapidly destroyed when the temperature rises. This implies that the storage time used in different temperatures be increased for high reduction of helminth eggs. Results from a study of urine-diverting latrines in Vietnam indicate that it is possible to within the stored faeces achieve a significant reduction of viable *Ascaris* ova within a six-months period (Schönning, 2001).

3.3.3 Survival of bacterial indicators over time
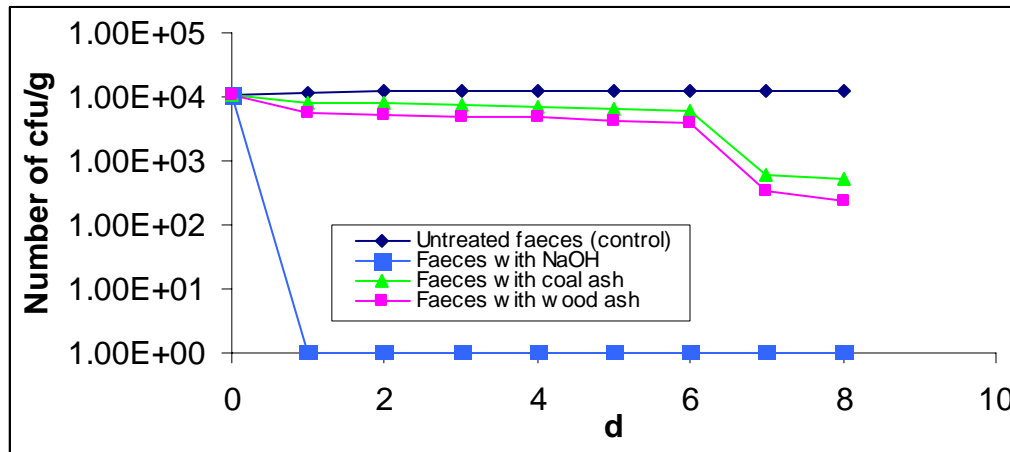
Figure 3.2 Microbiological trend of total coliform in untreated dehydrated faeces (the control), faeces treated with NaOH, faeces treated with wood ash and faeces treated with coal ash between 1 and 8 d of incubation (25°C).

No total coliform survived NaOH treatment. Treatment with wood ash resulted in the reduction of total coliform numbers from 1.05×10^{4} to 2.40×10^{2} cfu/g and coal ash from 1.05×10^{4} to 5.40×10^{2} cfu/g, giving a 2 log reduction. Total coliform numbers showed no effect in untreated dehydrated faeces and the numbers were ranging between 1.05×10^{4} and 1.27×10^{4} cfu/g during 8 d of incubation (Fig 3.2).
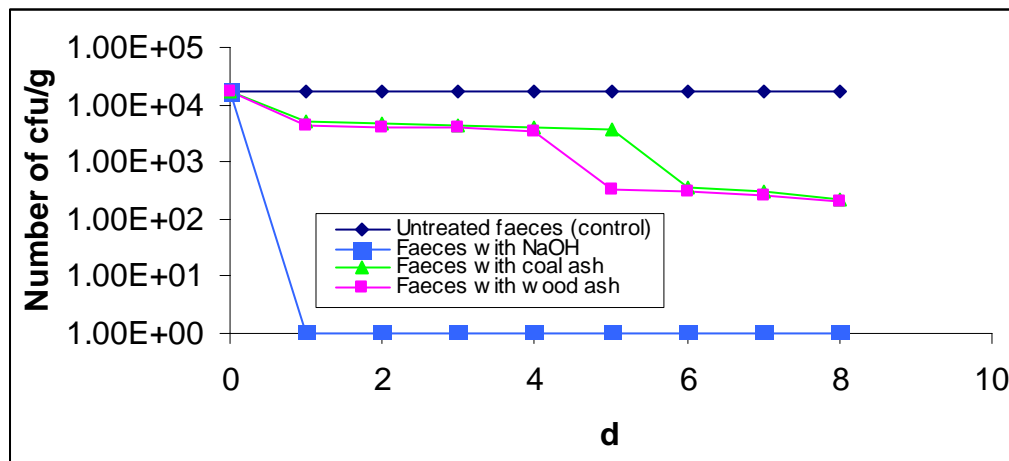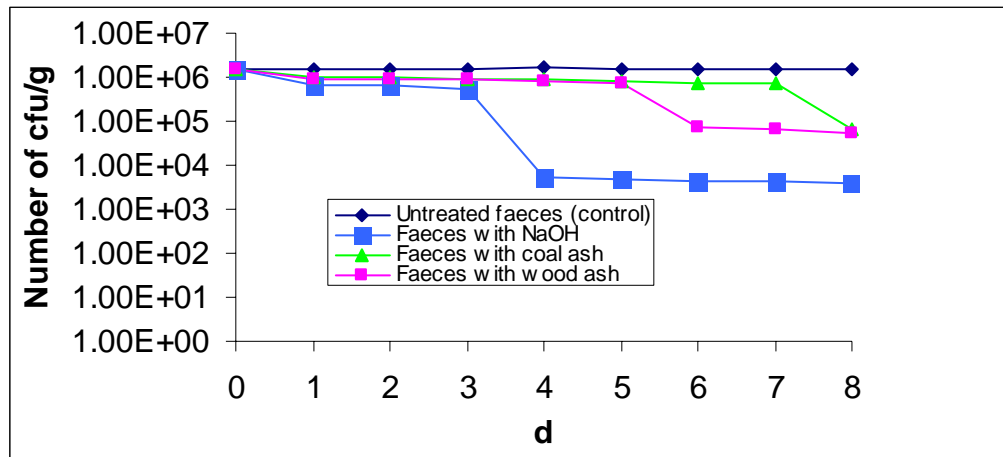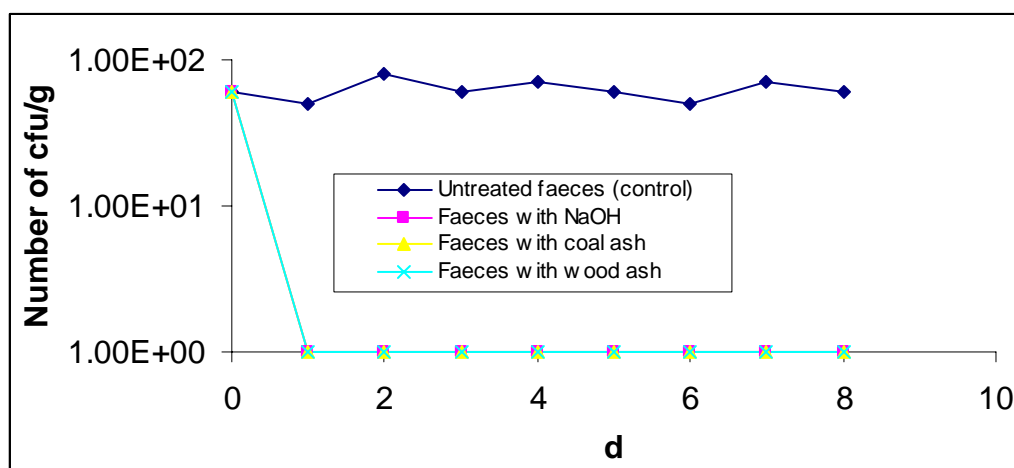

Figure 3.3 Microbiological trend of faecal coliform in untreated dehydrated faeces (the control), faeces treated with NaOH, faeces treated with wood ash and faeces treated with coal ash between 1 and 8 d of incubation (25°C).

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No faecal coliform survived NaOH treatment (Fig 3.3). Treatment with wood ash resulted in the reduction of faecal coliform numbers from 1.69×10^4 to 2.10×10^2 cfu/g and coal ash from 1.69×10^4 to 2.20×10^2 cfu/g, giving a 2 log reduction. No difference in faecal coliform numbers was observed in untreated dehydrated faeces and the numbers were ranging between 1.68×10^4 and 1.72×10^4 cfu/g during 8 d of incubation (Fig 3.3).

Figure 3.4 Microbiological trend of Faecal *Streptococci* in untreated dehydrated faeces (the control), faeces treated with NaOH, faeces treated with wood ash and faeces treated with coal ash between 1 and 8 d of incubation (25^oC).

The NaOH treatment resulted in the reduction of Faecal *Streptococci* numbers from 1.57×10^6 to 3.70×10^3 cfu/g, giving a 4 log reduction (Fig 3.4). Wood ash resulted in the reduction of Faecal *Streptococci* numbers from 1.57×10^6 to 5.40×10^4 cfu/g and coal ash from 1.57×10^6 to 6.30×10^4 cfu/g, giving a 2 log reduction. No difference in Faecal *Streptococci* numbers was observed in untreated dehydrated faeces and the numbers were ranging between 1.55×10^6 and 1.62×10^6 cfu/g during 8 d of incubation (Fig 3.4).

Figure 3.5 Microbiological trend of *Salmonella* spp in untreated dehydrated faeces (the control), faeces treated with NaOH, faeces treated with wood ash and faeces treated with coal ash between 1 and 8 d of incubation (25°C).

No *Salmonella* survived NaOH, coal ash, wood ash treatments during 8 d of incubation (Fig 3.5). No difference in *Salmonella* numbers was observed in untreated dehydrated faeces and the numbers were ranging between 5.00×10^{1} and 8.00×10^{1} cfu/g during 8 d of incubation (Fig 3.5). Feachem *et al*. (1983) showed that *Salmonella typhi* survived for 3 to 52 d in faeces stored at room temperature in sealed cans. Time needed for a certain reduction or elimination is however hard to predict since the conditions may vary significantly. The ambient temperature, moisture and storage container will affect the inactivation of microorganisms (Schönning, 2001).

3.3.4 Survival of *Aspergillus* spp over time

Figure 3.6 Microbiological trend of *Aspergillus* spp in untreated dehydrated faeces (the control), faeces treated with NaOH, faeces treated with wood ash and faeces treated with coal ash between 1 and 8 d of incubation (25°C).
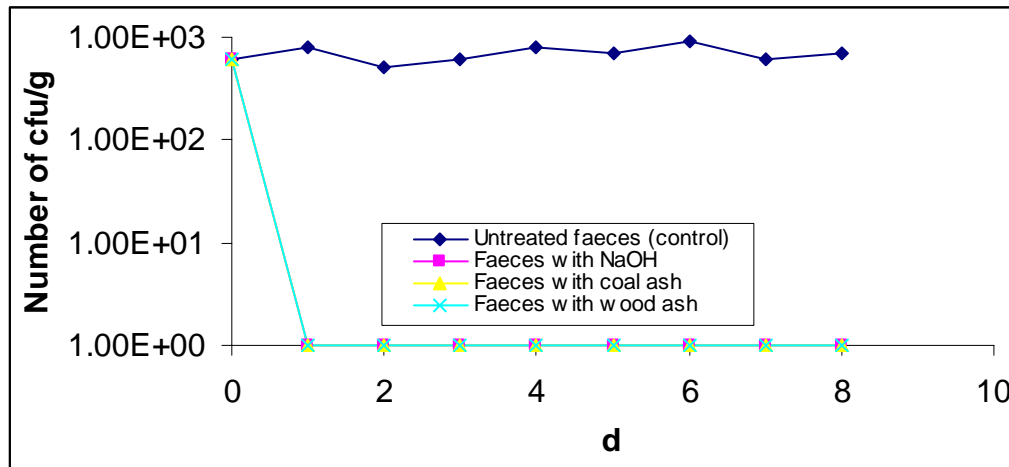
No fungi survived NaOH, wood ash and coal ash treatment during 8 d of incubation (Fig 3.6). *Aspergillus* numbers showed no difference in the control with numbers ranging between 5.00×10^{2} and 9.00×10^{2} cfu/g (Fig 3.6). Low numbers of fungi were found in untreated faeces. This was not surprising since fungi do not occur in high numbers in the intestinal tract.

3.4 Conclusions

- Heat treatment at 70°C for 20 min and 90°C for 5 min was the most effective disinfection method for the microorganisms.
- Treatment with NaOH, wood ash and coal ash acted as biocide as indicated by the decrease in the numbers of microorganisms over the 8 d experimental period. NaOH treatment was more effective than wood and coal ash.
- The number of helminth eggs were still present in dehydrated faeces after pasteurization at 60°C for 30 min, 70°C for 20 min and 90°C for 5 min. Suggesting that the storage time used in different temperatures be

increased for high reduction of helminth eggs.

- High temperature and pH (alkaline) reduces the number of helminth eggs in dehydrated faeces.

- Due to the presence of pathogens in dehydrated faeces, it is recommended that dehydrated faeces need to be disinfected before use as fertilizer or soil conditioner.

- It is suggested that the ash to faeces ratio be increased and/or ash be re-applied in order to treat all of the remaining pathogens.

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CHAPTER 4

Storage temperature, pH and the survival of pathogens in urine

Abstract

The aim of this study was to determine the biocidal effect of urine in relation to storage temperature and pH over a period of 50 d. The numbers of faecal indicator organisms were determined in naturally contaminated urine samples and filter sterilized urine spiked with different organisms. The survival of the organisms in human urine was determined at 15, 20 and 30°C. No reduction in the number of pathogens was observed during the first 8 d period of storage at 15, 20 and 30°C. At 30°C most of the pathogens were inactivated after 50 d of storage whereas at 15°C and 20°C some of the pathogens survived after 50 d of storage. Total coliform, Faecal *Streptococci* and *Salmonella* were found in both spiked and unfiltered naturally contaminated human urine samples, while faecal coliform and *Aspergillus niger* were found in relatively lower numbers. A higher temperature and an alkaline pH had a biocidal effect on these pathogens.

Keywords: Source separation, urine diverting, urine

4.1 Introduction

Source separating sewage systems have been promoted as a part of a sustainable future in Sweden. Using a urine-diverting (UD) toilet, the urine is easily collected separately at source. The other fraction worth recycling, according to its nutrient content, is the faeces (Vinnerås, 2001).

Source separation of human urine is based on toilets equipped with two bowls, a front one for the collection of urine and a rear one for faecal material. Both dry sanitation toilets and water closets are possible to equip in this way. Thus the urine is supposed to be collected separately and led through a sewer system to a collection tank for urine (Hellstrom *et al.*, 1999).

Human urine is the largest contributor of nutrients in household wastewater. Sundberg (1995) estimate that 60% of the phosphorus and 80% of the nitrogen in household wastewater comes from urine. The total quantities of nutrients in human urine are significant also when compared with quantities of nutrients in the mineral fertilizers used in agricultural practices (Jönsson *et al.*, 1997).

Urine contains nitrogen in the form of urea. Its beneficial effects on plant production have been studied intensively. The chemical industry produces it artificially on a large scale and farmers all over the world use urea. It is equally possible to use pure urine for fertilisation (Heinonen-Tanski and Van Wijk- Sijbesma, 2004).

The reuse of source-separated human urine as a crop fertiliser has been advocated in Sweden due to its high contribution of nutrients to household wastewater. At the same time it constitutes less than 1% of the total wastewater volume. Thus it is possible to collect a relatively concentrated fertiliser by separating urine from the wastewater (Höglund, 2001; Höglund *et al*, 2002).

In a healthy individual, the urine is sterile in the bladder. When transported out of the body, different types of dermal bacteria are picked up and freshly excreted urine normally contains <10 000 bacteria per ml. By urinary track infections, which is more than 80% of cases are caused by *E. coli*, significantly higher amounts of bacteria are excreted (Höglund, 2001).

In terms of N, P and K the yearly production of human urine in Sweden equals 15-20% of the mineral fertilizer consumption 1993 in the country. Furthermore, the amounts of heavy metals in the collected urine are very low. It contained less than 3.6 mg Cd/kg P while the chemical fertilisers sold in Sweden 1995 contained approximately 26 mg Cd/kg P (Höglund *et al.*, 1998).

The toilet fractions contain the major part of the nutrients from the household. In a sustainable society these nutrients have to be recycled. These fractions are of high risk for pathogenic content. Diverted urine contains only small amounts of pathogens that mostly originate from faecal contamination; the urine can easily be disinfected by storage (Vinnerås *et al.*, 2003).

Compared with other organic fertilisers, the proportion of directly plant available nitrogen in source-separated urine is large (Kirchmann and Pettersson, 1995). Compared with other sewage products, source separated urine may also have hygienic advantage because only few pathogens are excreted through urine and partly because of rapid die-off of many pathogenic bacteria in the collection and storage tanks (Olsson *et al*., 1996; Olsson, 1995)

Its high nutrients content and hygienic quality makes urine a much more valuable resource for fertilisation than faeces. The urine fraction utilised in agriculture for human food plants must in that case be free of faeces. The terminal simplicity of its application makes it possible for households to use it with only a hand-hoe and watering can, implements that are typically used by small-scale farmers and vegetable gardeners, especially by woman (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

Systems with urine separation toilets have been installed in urban areas as well as in rural areas. An extended use of urine separation toilets will results in an increased amount of transport of urine solution. This could results in a considerably negative environmental impact if the existing systems for transportation are used (Hellstrom *et al*., 1999; Hanaeus *et al*., 1997).

Contamination with faecal material is considered to the primary factor determining hygienic risk. Even a small amount of contaminating faecal material leads to a comparatively high concentration, since the dilution is much lower than in a conventional wastewater collection system (Jönsson *et al*., 1997). Risk associated with the handling and reuse of urine will partly be dependent on the inactivation of microorganisms during storage. Bacterial and protozoan have previously been shown to be inactivated in stored urine (Höglund *et al*., 2002; Höglund and Stenstrom, 1999).

The Swedish practice has sometimes been to store urine for some months before use in order to wait for the die-off of possible enteric microorganisms. Because the survival time of microorganisms is always shorter at higher temperatures, the storage

time of urine up to the time when it is needed as fertiliser can be minimised in tropical climates (Heinonen-Tanski and Van Wijk- Sijbesma, 2004).

To determine the duration and conditions for sufficient storage of the urine solution before its use as fertiliser it is necessary to estimate the survival of various microorganisms in urine as functions of time. The parameters that may vary in the urine collection and storage tanks are especially dilution, temperature and pH (Höglund *et al.*, 1998).

The aims of this study were:

- To quantify the faecal contamination that occurs in human urine;
- To determine the survival of pathogens in the urine in relation to storage temperature and pH.

4.2 Materials and methods

4.2.1 Sampling

An early morning urine sample was collected from four different individuals using 100 ml sterile bottles. The samples were transported to the laboratory and stored at 4^oC and analysed within 24 h.

4.2.2 Microbiological indicator analysis

Non-sterile urine samples

The urine samples were homogenized into a 200 ml sterile bottle and diluted as a 1 in 10 serial dilution series (between 10^{-1} and 10^{-10}). For each step three parallel agar plates were streaked with 0.1 ml of the suspension. The media used were the following:

- For quantification of total coliform m-Endo Agar Les was used. The plates were incubated at 37^oC and the numbers of typical colonies were recorded after 24 h of incubation.
- Faecal coliform were enumerated on m-Fc Agar plates incubated at 44^oC for 24 h.
- For Faecal *Streptococci* m-Enterococcus Agar was used, plates incubated at 37^oC for 24 h.
- *Salmonella* spp Rambach agar was used; the plates were incubated at 37^oC for 24 h.
- *Aspergillus* spp Rose-bengal agar was used. The plates were incubated at 25^oC for three to four days and the number of typical colonies was then recorded after 24 h.

Sterile urine samples (Control)

An early morning urine sample was collected from four different individuals using 100 ml sterile bottles. The samples were transported to the laboratory within one hour. In the laboratory the samples were homogenized into a 200 ml sterile bottle. The samples were then filtered through a 0.2 µm filter to eliminate microorganisms that could be present and interfere with the analysis. The microorganisms used in this study were chosen on the basis that they are known to be uropathogens. They were grown on Nutrient agar at 37°C for 24 h and collected with a sterile loop.

4.2.3 Survival experiments

Temperature

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┌──────────────────────────────────────────┐
│   Naturally contaminated urine samples     │
└──────────────────────────────────────────┘
                     │
                     ▼
┌──────────────────────────────────────────┐
│               Homogenised                  │
└──────────────────────────────────────────┘
        │                          │
        ▼                          ▼
┌──────────────────┐   ┌──────────────────────────────┐
│ Naturally        │   │ Filtered through a 0.2µm filter│
│ contaminated     │   └──────────────────────────────┘
│ urine samples    │                  │
└──────────────────┘                  ▼
        │              ┌──────────────────────────────┐
        │              │ 15ml of the filtrate was       │
        │              │ transferred into 25ml sterile  │
        │              │ bottles                        │
        │              └──────────────────────────────┘
        │                              │
        ▼                              ▼
┌──────────────────┐   ┌──────────────────────────────┐
│ Incubation took  │◄──│ Each bottle was inoculated with│
│ place at 15, 20  │   │ E.coli, Faecal Streptococci,   │
│ and 30°C         │   │ Salmonella and Aspergillus niger│
└──────────────────┘   └──────────────────────────────┘
        │
        ▼
┌──────────────────┐
│ Microbiological  │
│ analysis         │
└──────────────────┘
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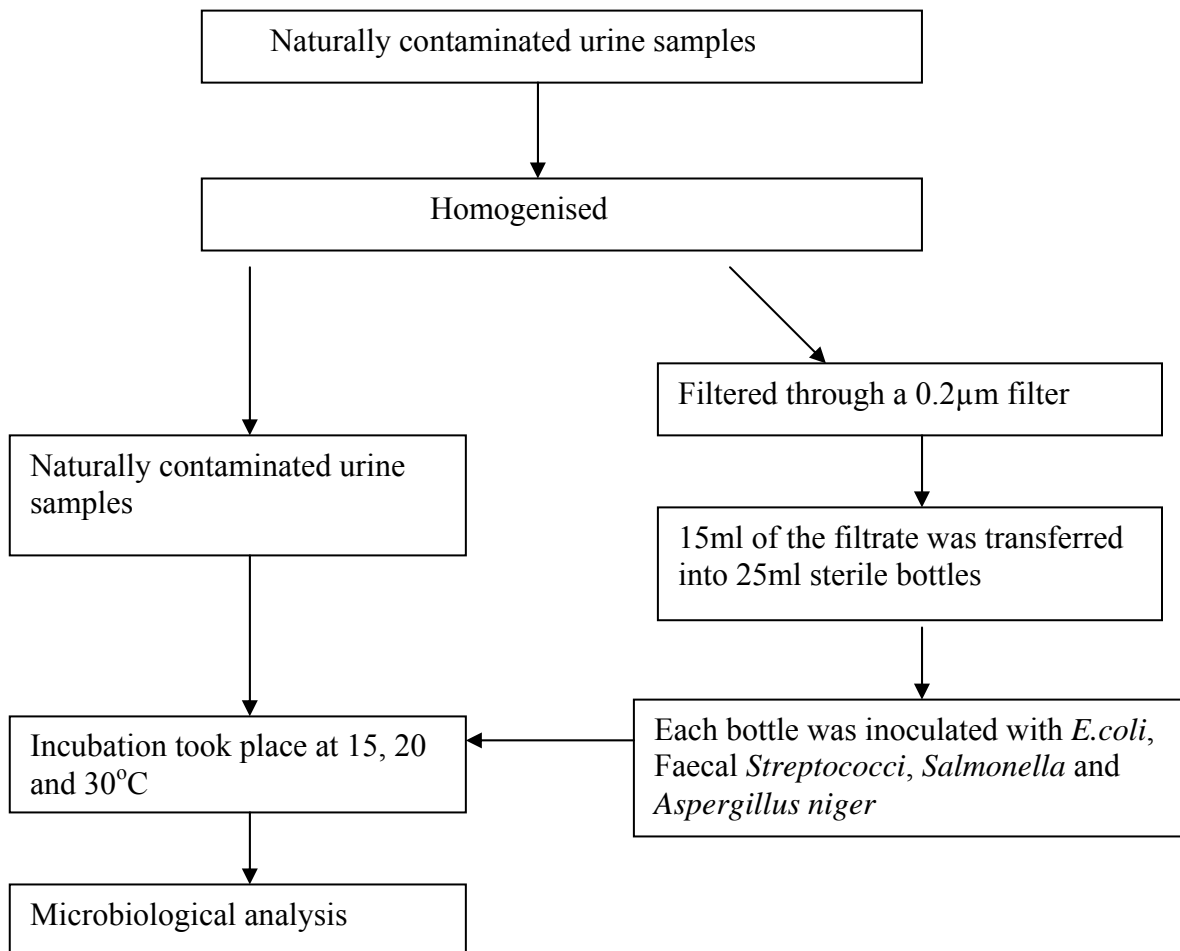
Figure 4.1 Flow diagram of naturally contaminated and spiked human urine samples

Two different experiments were conducted to estimate the effect of different storage conditions on the survival of pathogens in human urine. In the first experiment naturally contaminated urine samples were homogenised in sterile sealed glass bottles and stored at 15, 20 and 30°C. The analysis was done three times during the first week and followed by 10 d intervals. In the second experiment sterile urine samples was transferred into 25ml sterile bottles. For each bottle *E coli*, *Salmonella typhimurium*, Faecal *Streptococci* and *Aspergillus niger* were inoculated and incubated at 15, 20 and 30°C.

4.3 Results and discussion

4.3.1 Microbiological analysis

Table 4.1 Enumeration of total coliform, faecal coliform, Faecal *Streptococci*, *Salmonella* spp and *Aspergillus* spp in naturally contaminated human urine samples immediately after mixing.

Organism	Cfu/ml
Total coliform	$<10^1$
Faecal coliform	$<10^1$
Faecal *Streptococci*	$<10^1$
Salmonella spp	$<10^1$
Aspergillus spp	3.00×10^5

cfu= Colony forming units

No total coliform, faecal coliform, Faecal *Streptococci* and *Salmonella* spp were initially found in naturally contaminated human urine at pH 6.3. The total *Aspergillus* count was 3.00×10^5 (Table 4.1).

Table 4.2 Enumeration of total coliform, faecal coliform, Faecal *Streptococci*, *Salmonella* spp and *Aspergillus* spp in filtered human urine immediately after spiking with pathogens.

Organisms	Cfu/ml
Total coliform	$<10^1$
Faecal coliform	$<10^1$
Faecal *Streptococci*	$>3.00 \times 10^7$

100

|	
Salmonella spp	>3.00x10^7
Aspergillus niger	5.50x10^6

No total coliform and faecal coliform were initially found in spiked human urine at pH 6.3. The total *Salmonella* and Faecal *Streptococci* count was >3.00x10^7 cfu/ml. The number of *Aspergillus niger* was 5.50x10^6 cfu/ml (Table 4.2).

Exposure of unspiked and spiked human urine samples to different temperatures had no biocidal effect over the first 8 d of the experimental period (Appendix 2, Table 2.1 and 2.2).
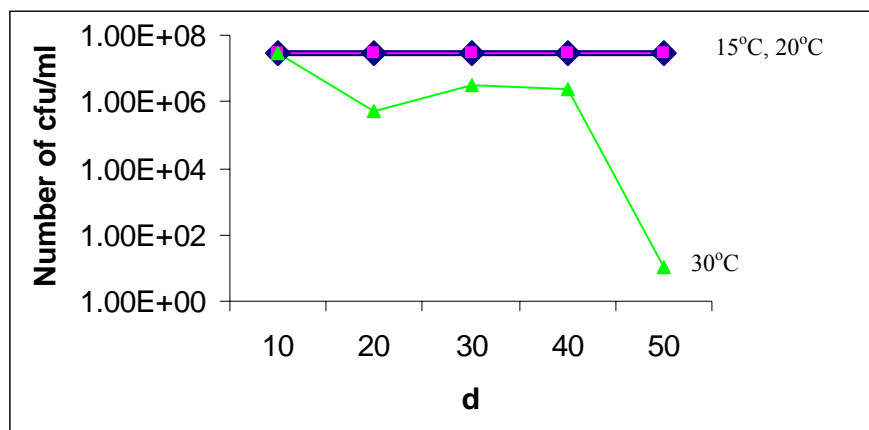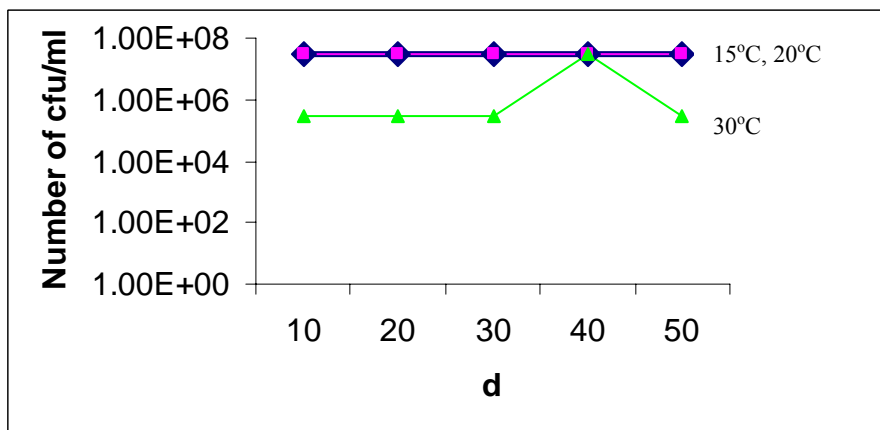

Figure 4.2 Inactivation of total coliform and influence of storage temperature on spiked filtered human urine samples over a 50 d experimental period.

At 15°C and 20°C, no difference in the number of total coliform was observed over the 50 d experimental period. At 30°C the total coliform numbers decreased after 20 d of storage by 6 log (10^7 to <10^1 cfu/ml) (Fig 4.2). The pH in all the samples remained with the range of 6.3 to 7.6 at 15°C; 6.4 to 8.9 at 20°C and 6.5 to 9.3 at 30°C.
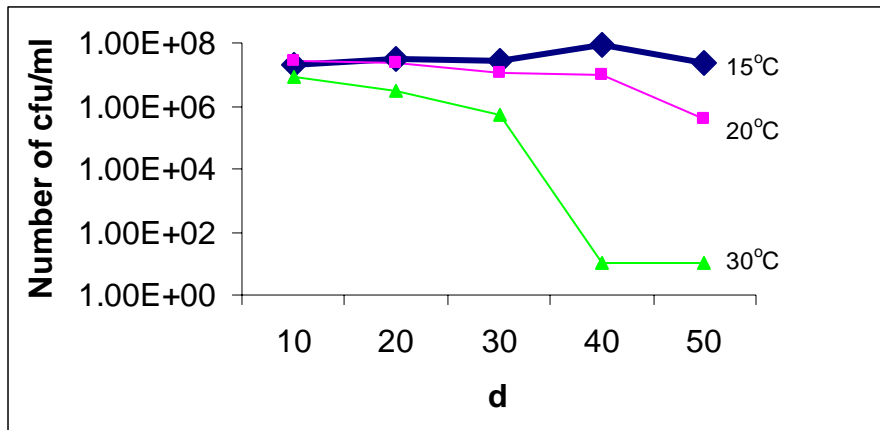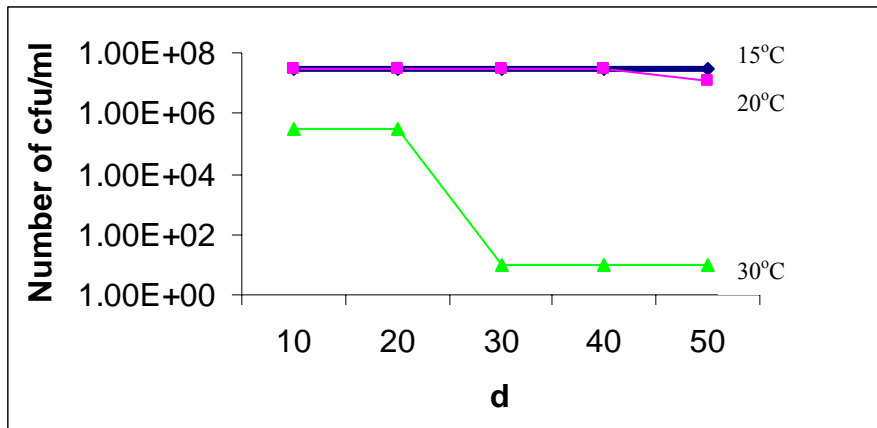
Figure 4.3 Inactivation of faecal coliform and influence of storage temperature on spiked filtered human urine samples over a 50 d experimental period.

Faecal coliform numbers decreased after 40 d of storage at 20^oC from 10^7 to 10^5 cfu/ml and after 30 d at 30^oC from 10^6 to 10^1 cfu/ml whilst these numbers remained the same at 15^oC (Fig 4.3).
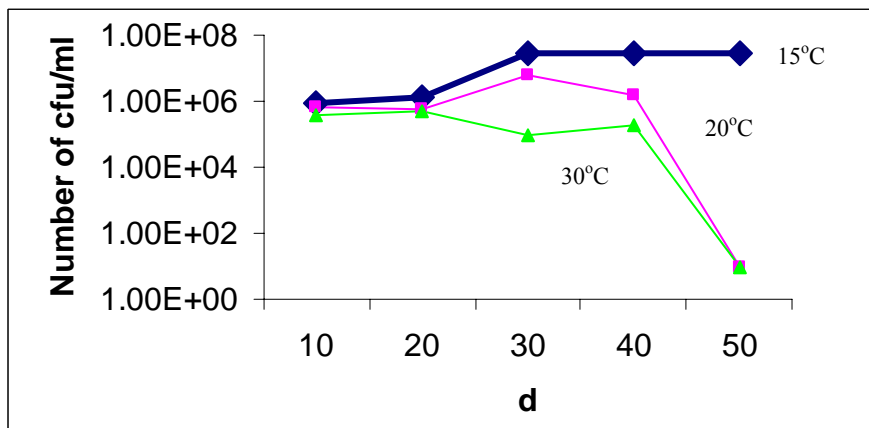

Figure 4.4 Inactivation of Faecal *Streptococci* and influence of storage temperature on spiked filtered human urine samples over a 50 d experimental period.

At 15, 20 and 30^oC no difference in the number of Faecal *Streptococci* was observed over the 50 d experimental period (Fig 4.4). Experiments of human source separated urine showed that no reduction of Faecal *Streptococci* occurred during 40 d after an initial 1 log reduction (Höglund, 2001).

Figure 4.5 Inactivation of *Salmonella* spp and influence of storage temperature on spiked filtered human urine samples over a 50 d experimental period.

At 15^oC and 20^oC, no difference in the number of *Salmonella* was observed over the 50 d experimental period. At 30^oC the numbers decreased by 6 log (10^7 to $<10^1$ cfu/ml) (Fig 4.5). (Höglund *et al.*, 1998) showed that *Salmonella typhimurium* survived for more than 20 d in human source separated urine at 20^oC.
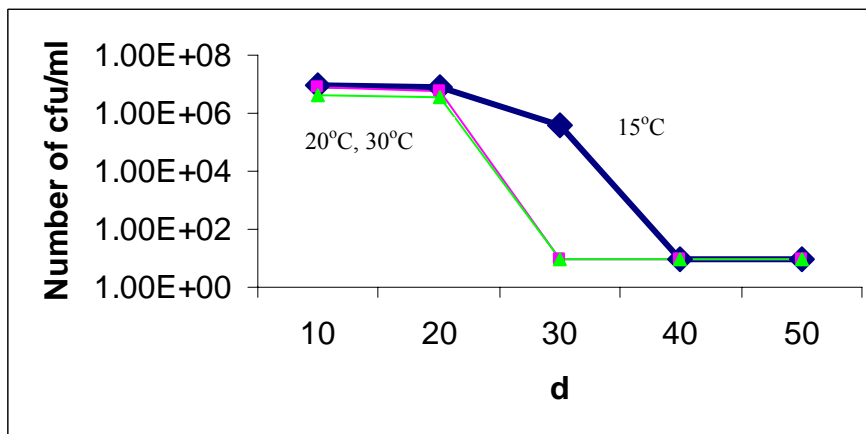

Figure 4.6 Inactivation of *Aspergillus* spp and influence of storage temperature on spiked filtered human urine samples over a 50 d experimental period.

 At 15^oC *Aspergillus* number increased from 10^5 to 10^7. At 20 and 30^oC the *Aspergillus* numbers decreased (10^5 to $<10^1$ cfu/ml), giving a 4 log reduction (Fig 4.6).
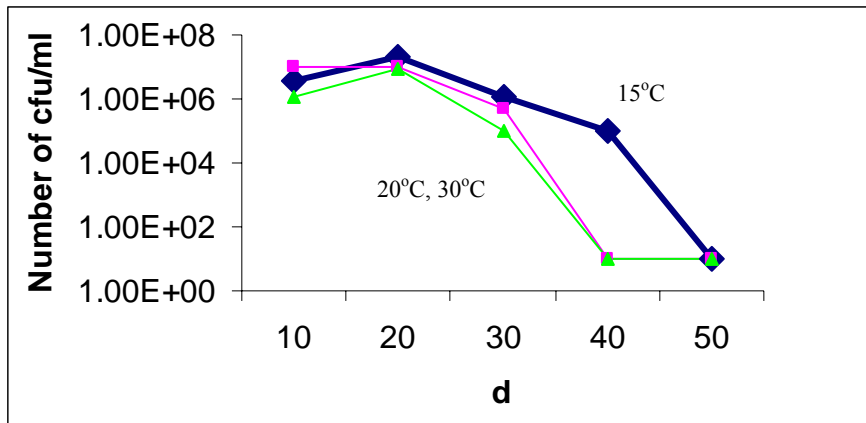
Figure 4.7 Inactivation of total coliform and influence of storage temperature on unfiltered naturally contaminated human urine samples over a 50 d experimental period.

The total coliform numbers decreased by 5 log (10^6 to $<10^1$ cfu/ml) at 15, 20 and 30°C over a 50 d of experimental period (Fig 4.7). The pH in all the samples remained with the range of 6.4 to 7.3 at 15°C, 7.2 to 8.7 at 20°C, 8.6 to 9.2 at 30°C.

Figure 4.8 Inactivation of faecal coliform and influence of storage temperature on unfiltered naturally contaminated human urine samples over a 50 d experimental period.
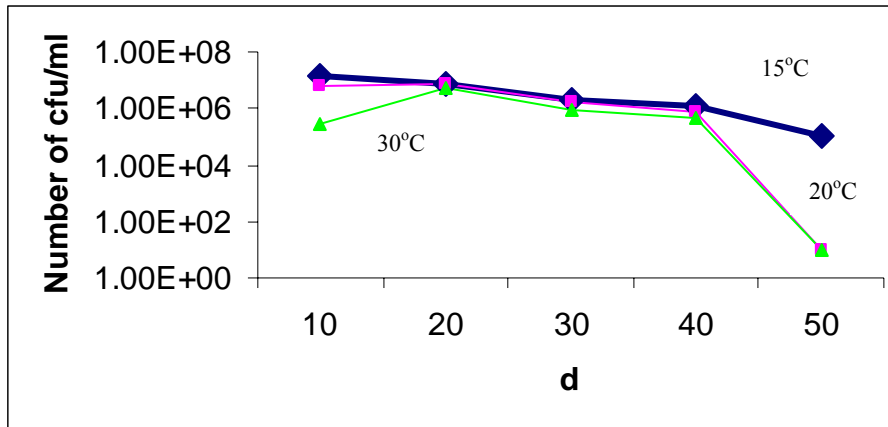
The numbers of faecal coliforms decreased from 10^6 to $<10^1$ cfu/ml, giving a 5 log at 15, 20 and 30°C over a 50 d of experimental period (Fig 4.8). Faecal coliforms showed reduction after 20 d at 15, 20 and 30°C, which is more time than that from human source separated urine (5 d at 20°C) (Höglund *et al*., 1998).

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Figure 4.9 Inactivation of Faecal *Streptococci* and influence of storage temperature on unfiltered naturally contaminated human urine samples over a 50 d experimental period.

At 15 °C the Faecal *Streptococci* numbers decreased from 10^7 to 10^5 cfu/ml, giving a 2 log reduction during a 50 d experimental period, which is comparable to the results in human source separated urine (no reduction of Faecal *Streptococci* occurred during 40 d after an initial 1 log reduction at 4°C and 20°C (Höglund *et al*., 1998). Higher reduction of Faecal *Streptococci* occurred at 20°C from 10^6 to 10^1 cfu/ml, giving a 5 log reduction and at 30°C 10^5 to 10^1 cfu/ml, giving a 4 log reduction (Fig 4.9).

Figure 4.10 Inactivation of Faecal *Streptococci* and influence of storage temperature on unfiltered naturally contaminated human urine samples over a 50 d experimental period.
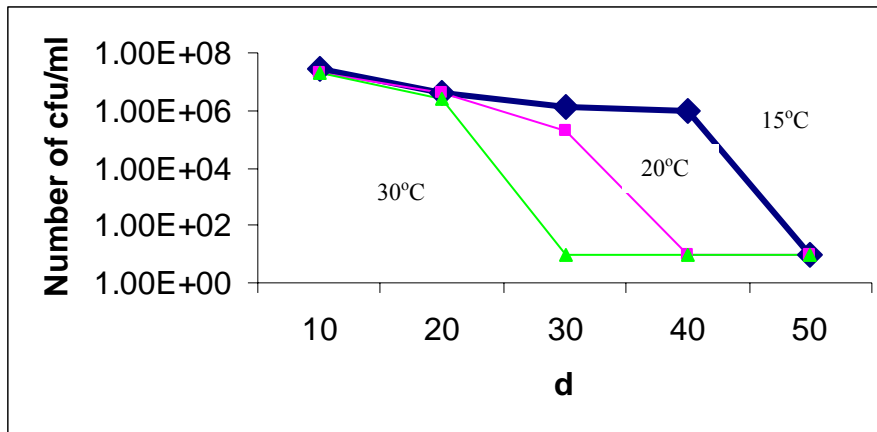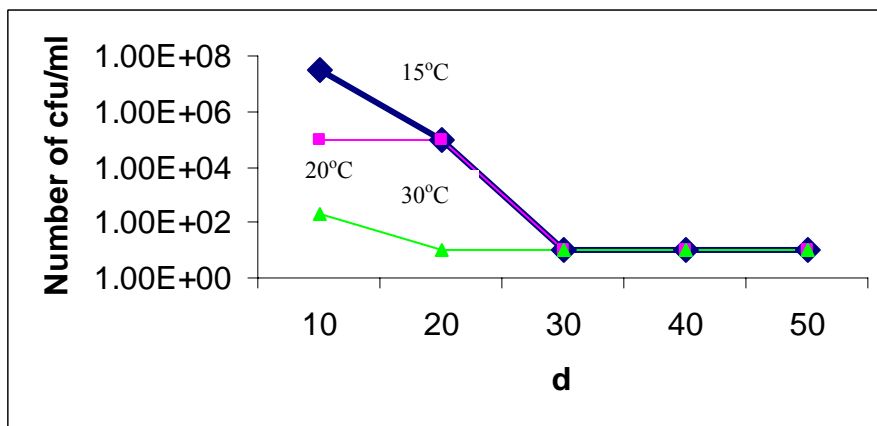
At 15^oC, 20^oC and 30^oC the *Salmonella* numbers decreased by 6 log (10^7 t0 $<10^1$ cfu/ml) over a 50 d experimental period (Fig 4.10).

Figure 4.11 Inactivation of *Aspergillus* spp and influence of storage temperature on unfiltered naturally contaminated human urine samples over a 50 d experimental period.

The *Aspergillus* numbers decreased from 10^5 to 10^1 cfu/ml giving a 4 log reduction at both 15^oC and 20^oC. No difference in the number of *Aspergillus* was observed at 30^oC over 50 d of experimental period (Fig 4.11). Experiments in Sweden have established that should faecal contamination of source-diverted urine occur, six months of storage time is sufficient for the destruction of pathogenic organisms according to (Austin and Duncker, 2002). Suggesting that more time of storage is needed for reduction of pathogens in human urine.

4.4 Conclusions

- Faecal *Streptococci* showed a longer survival at 15, 20 and 30°C in spiked human urine samples compared to the other microorganisms.

- The number of total coliform and faecal coliform decreased after 10 d of storage in spiked human urine at 30°C whereas *Aspergillus* spp and *Salmonella* numbers showed a reduction after 20 d of storage at 30°C.

- Naturally contaminated urine samples showed a reduction of total coliform, faecal coliform and Faecal *Streptococci* after 20 d of storage, except for *Salmonella* and *Aspergillus* numbers, which were reduced after 10 d at all temperatures. This indicated that a low risk for transmission of infectious disease caused by pathogens when handling urine.

- It appears that human urine used in this study was found to be contaminated after 50 d of storage due to faecal contamination. Therefore it is suggested that urine should be stored up to six months before reuse as a fertilizer. The recommended period of storage is dependent on the temperature.

- It is recommended that pit latrines must be improved to allow the separation of urine and faeces so that the urine fraction is clean from faeces. The use of pure urine need to be promoted as an effective and free fertilizer.

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CHAPTER 5

Potential pathogenic contamination of carrots and spinach after applying humanure

Abstract

Ecological sanitation (EcoSan) are decentralized sanitary facilities that allow for storage and partial treatment, recycling and reuse of excreta. By reusing EcoSan sludge as soil conditioner, crop productivity may increase without representing an excessive cost for the farmers. The reuse of sludge has been limited due to the high amount of pathogenic and parasitic microorganisms of a faecal origin that they contain. This study investigates the risk of polluting spinaches and carrots with total coliform, faecal coliform, Faecal *Streptococci*, *Salmonella*, *Aspegillus* spp and helminth ova when humanure is applied to soil as fertilizer. Four plots were prepared for the crops at the University of Pretoria agricultural farm, two for spinach and two for carrot. Helminth eggs detected in humanure samples were *Ascaris* spp (22.7/gTS); *Trichuris* and *Toxocara* spp (2.3/gTS); *Enterobius vermicularis* and *Hymenolepis diminuta* (1.2/gTS). Total helminth eggs in humanure were found to be 29.5/gTS and 82% of *Ascaris* eggs were viable. No helminth eggs were detected in the water used for irrigation. Higher numbers of helminth ova were found in leaves (spinach and carrots), compared to the roots suggesting that the ova adhere preferably to the leaves. Viable helminth eggs found in crop samples was lower compared to viable eggs in soil samples. These results highlight the potential for transmission of helminth eggs when humanure is applied in soil to fertilize crops.

Keywords: Vegetables, Carrots, EcoSan, Spinach, Humanure, Irrigation water, Quantitative analysis, Viability, Helminth eggs, *Ascaris* spp, *Toxocara* spp, *Trichuris* spp, *Taenia* spp, *Enterobius vermicularis*, *Hymenolepis diminuta*

5.1 Introduction

The technology of ecological sanitation or dry box toilets has been used successfully for years in different countries, e.g. China, Ecuador, El Salvador, Ethiopia, Guatemala, Mexico, Sweden, Vietnam, Zimbabwe and South Africa. The most important difference between this technology and that of composting is the moisture content in the faeces receptacle. The urine and faeces can be separated and both of these fractions can easily be treated and utilised (Austin and Duncker, 2002).

Animal manure is not specifically treated to reduce their bacterial content and large numbers of bacteria are applied to soil together with the manure. A number of human enteric pathogens have been associated with animal manures and the use of manures as fertilizer can expose humans to enteric pathogens through food, water or direct contact (Gagliardi and Karns, 2002).

Application of contaminated irrigation water to soil is also a possible source of contamination. Many outbreaks of infection have been associated with water or food directly or indirectly contaminated with animal manure. Contaminated manure can contact the produce directly through its use as a soil fertilizer or indirectly through infiltration or irrigation water or water used to wash the produce (Islam *et al*., 2005).

Organic farms largely use animal manure or compost to fertilize their crops. Manure or improperly composted manure used on the farm can be a source of pre-harvest pathogen contamination of produce. Many outbreaks of infection have been associated with water or food directly or indirectly contaminated with animal manure (Islam *et al*., 2004).

Agricultural use of sludge may be an alternative to conventional fertilizers provided that the risk to public health is considered. The reuse of sludge has been limited due to the high amount of pathogenic parasitic microorganisms of a faecal origin that they contain (Gaspard and Schwartzbrod, 2003). Sewage sludge has been spread on the land for decades, and there are many reports from all over the world showing that

sewage sludge can be effectively used as a source of fertilizer for crop yield and for improving physical and chemical properties of soil (Qasim *et al*., 2001).

Benefits of the application of sewage sludge to land are limited due to the harmful elements such as heavy metals and human pathogens. According to the 1997 guidelines (WRC, 1997), the current standards for the unrestricted use of sludge on agricultural soils cannot be attained within a reasonable framework of applied technology. Henning *et al*. (2001) reported that none of the wastewater treatment works in South Africa complied due to the heavy metals in sludge. Investigations that illustrate the benefits of sludge are important, since there is still a general reluctance among agriculturists to recognise the economic value of sewage sludge to improve the soil organic status without contaminating the environment (Henning *et al*., 2001). The farmers have used sewage sludge in South Africa to fertilize crops especially in the rural areas. Henning *et al*. (2001) showed that adding sludge to the soil promotes plant growth significantly more than when commercial fertilizer is added.

By reusing sludge as a soil amendment, crop productivity may increase without an excessive cost to the farmer. Therefore there is a need to utilize the fertilizing value of sludge in soil, instead of destroying them with costly processes, or prevent the eutrophication of water bodies with nitrate or phosphate that sludge contains (Jiménez *et al*., 2002).

Some cities in the developing world treat only about 10% of their sewage. Even in South Africa, reports have indicated that some sewage waste does not reach treatment plants, but flows without being treated into rivers and oceans (Austin and Duncker, 2002).

In terms of microbiological pollution, sludge frequently contains various pathogenic microorganisms introduced by wastewater such as bacteria, viruses and parasites. Among them *Ascaris* eggs are the most resistant to different kinds of disinfection. The potential risks associated with the spreading of such sludge must be addressed in order for this to be a viable disposal route. Currently, the presence of heavy metals, organic molecules and pathogenic microorganisms raised questions about sludge being spread on land (Capizzi-Banas *et al*., 2004).

Treated and untreated sewage sludge is used for crop irrigation in Mexico. Recent epidemiological studies have demonstrated real and potential risks for human health associated with the presence of viable helminth eggs in such wastewaters. Responding to this health risk the Mexican government in 1993 established a limit of <1 viable helminth egg per litre in irrigation waters (Buitron and Galvan, 1998).

The use of wastewater for irrigation purposes has caused many disease outbreaks caused by bacteria, protozoa, parasitic helminth and viruses (Barnes and Taylor, 2004). Non-composted or improperly composted manure can contaminate vegetables when used as a fertiliser or soil amendment. Recent outbreaks of foodborne disease associated with fresh produce have raised concern that these foods may be an increasing source of foodborne infections. (Islam *et al*., 2004).

In developing countries, some water from sources contaminated with human and animal faeces to irrigate vegetables and fruits have been responsible for their high rates of contamination with helminth eggs. Considering that some vegetables grown in these countries are exported increases the risks to other countries (Kozan *et al*., 2005). The WHO technical report (1989) emphasized that in cases of irrigation with untreated wastewater, intestinal nematodes constituted a high risk (Gaspard and Schwartzbrod, 1995).

Fruits and vegetables, eaten raw and without peeling can transmit a range of parasites. Parasites that have been associated with vegetables and fruit-borne outbreaks of infection include helminth parasites like *Ascaris*. Helminths are the parasites of primary public health concern for wastewater reuse. An important characteristic of these organisms is the production of an ova stage, which helps them to survive (Özlem and Hakan, 2005).

Helminth infections constitute one of the most important public health problems around the world, especially in the tropical and sub-tropical regions of Africa, Asia, Central and South America (Asaolu and Offoezie, 2003). The trend toward using wastewaters for multiple purposes poses an urgent need to develop adequate

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methods for the detection of helminth eggs and reliable systems for elimination of these organisms (Buitron and Galvan, 1998).

Failure to adhere to hygienic standards on the farm and in the kitchens of the institutions like schools, hospitals, restaurants and hotels can lead to widespread helminthic infections to humans as a result of consumption of improper washed vegetables used as salad ingredients (Kozan *et al*., 2005).

The objective of this study was:

- To determine the microbiological risk in spinach and carrot when humanure is applied to soil as fertilizer.

5.2 Materials and methods

5.2.1 Sampling

25 kg of humanure (EcoSan sludge) were supplied by Aussie Austin from the CSIR Building and Construction Technology. The initial content of helminth ova, faecal coliform, total coliform, Faecal *Streptococci*, *Aspergillus* spp and *Salmonella* spp was determined.

5.2.2 Preparation of site

Table 5.1 Experimental plots

Spinach		Carrots	
Plot 57 (Experimental)	Plot 59 (Control)	Plot 61 (Experimental)	Plot 63 (Control)
2.2g LAN (A)		64.28gLAN (A)	
7.5kg H.M (B) 1.1g LAN		7.0kg H.M (B) 0g LAN	
37.2g LAN (C)		12.8g LAN (C)	161.1g LAN (G)
3.8kg H.M (D) 18.6g LAN	321.3g LAN (G)	2.5kg H.M (D) 6.4g LAN	
69.2g LAN (E)		22.2g LAN (E)	
0.25kg H.M (F) 34.6g LAN		1.5kg H.M (F) 11.1gLAN	

H.M= Humanure

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LAN=LAN (Limestone Ammonium Nitrate)

Four plots were prepared, numbered plot 57, 59, 61 and 63. Two for spinach (plot 57 and 59) and the other two for the carrots (plot 61 and 63). Plots 59 and 63 were controls, only LAN from the Agricultural farm was applied. Plot 57 and 61 were experimental, both humanure from the CSIR and LAN were applied. Plot 57 and 61 were divided into six blocks each, named block A-F. Shaded blocks sized 1mx2m (both humanure and LAN were applied). Unshaded blocks sized 2mx2m (only LAN was applied). Different loading of manure was applied in each of the four plots (Table 5.1).

5.2.3 Vegetable production conditions

Carrots and spinach were produced on the Agricultural farm of the University of Pretoria according to production practices outlined in the Handbook for Vegetable growers (Lonez and Maynard, 1988). These crops were considered because they are usually eaten in South African rural areas and are often consumed raw. A split-plot block design plan was followed for each crop. Humanure was applied with different rates of applications and mixed with soil for each block in relation to the quantity of total nitrogen and helminth ova added (Table 5.1). To determine the amount of sludge to be added, the following criteria were taken into account: (a) the nitrogen demand by crops (50 kgN/ha for carrots and 100 kgN/ha for spinach); (b) application rates above and below 8 ton/ha, which is the value established as the South African norm and (c) three different Helminth ova rates.

Following these criteria, sludges were applied on carrots at 0, 7, 12.5 and 35 tons/ha corresponding to 0, 1, 1.7 and 4.8 HO/cm^2, while for spinach 0, 1.3, 19 and 37.5 ton/ha equivalent to 0, 0.18, 2.6, and 5.1 HO/cm^2 were used. Sludge was applied to soils and mixed to a dept of 10 cm. Seeds were planted during the second week of November 2004. The pattern within the blocks was in rows 300 mm apart. The seeds were spaced 50 mm apart within the rows. A fork was used to firm the soil over the seeds which were planted at a depth of 5 cm. Different concentration of LAN were applied on top of the soil after the seeds were sowed in all plots (Table 5.1).

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5.2.4 Sampling of soil and vegetables

Approximately 400 g of soil was aseptically collected in sterile plastic bags 10 cm deep. For each plot 100 g of soil were collected before sowing, after three weeks and after harvesting. Analysis of carrots and spinach began at week 7 for the spinach and after three months for the carrots when the vegetables were large enough for sampling. From each plot in each block, the whole plant was removed from the soil and weighed to measure the yield. The roots and leaves were collected separately from the plant, 30 g of spinach and carrots leaves; 30 g of spinach roots and carrots were collected from the farm in sterile plastic bags as plant samples to measure the content of Helminth ova. 5 g of both crop samples were taken for the analysis of faecal coliform, total coliform, Faecal *Streptococci*, *Aspergillus* spp and *Salmonella* spp. The samples were brought to the laboratory from the field in a cooler with ice, placed into a cold room in cooler at $4^{o}C$ within 5 h of collection and analyzed within 24 h.

5.2.5 Water samples

Irrigation water samples were collected in sterile screw-capped bottles and transported to the laboratory in an ice box and analysed within 2 h. Isolation and enumeration of bacteria were done using selective growth media used in chapter three and four. One sample was collected and surface plates were made in duplicate in selective media. For bacterial enumeration, spread plates were used to determine the number of colony forming units (cfu/ml; 37 and $44^{o}C/$ 24 h).

5.2.6 Quantitative analysis of helminth eggs from humanure, irrigation water, soil and crop samples

Helminth ova were monitored due to their high persistence in the environment and because they are considered as quality indicators for most of the reuse practices (WHO, 1989). In a sterile bag 200 ml of tween water was added to 30 g of spinach and carrot samples and were homogenized in a stomacher lab-blender 400 for 1 h. The same procedure of helminth analysis used in chapter three was followed (Jiménez *et al*., in press).

5.2.7 Isolation of bacteria and fungi from soil and vegetable samples

Total coliform, faecal coliform, Faecal *Streptococci* and *Aspergillus* spp analysis

Total coliform, faecal coliform and Faecal *Streptococci* are considered as good indicators of faecal pollution (Feachem *et al*., 1983). *Aspergillus* spp was used because it is an opportunistic pathogen. One gram of each soil, mixture of soil and manure samples were mixed with 9 ml of buffered peptone for bacteria and ringer's solution for pathogenic fungi in a sterile test tube and vortexed for 10 s. 45 ml of buffered peptone water was added to 5 g of each carrot; carrot leave; spinach root and spinach leave samples and rinsed by rubbing and vigorously agitating by hand for 30 sec. Enumeration of bacteria and pathogenic fungi in soil and vegetable samples were determined as described below. Serial dilutions (10^{-1} to 10^{-10}) of each sample were prepared and 0.1 ml portions of each dilution was spread onto selective media. Plates were incubated at 25°C (*Aspergillus* spp) for two to three days (Parkinson, (1994), 37°C (total coliform and Faecal *Streptococci*) for 24 h and 44°C (faecal coliform) for 24 h, and the number of typical colonies was counted

***Salmonella* analysis**

One gram of sample (soil, mixture of soil and humanure) was placed in 9 ml buffered peptone water and vortexed for 10 s. Serial dilutions 10^{-1} to 10^{-5} of each sample were prepared and incubated at 37°C for 18-24 h. 0.1 ml of the mixture was transferred to 10 ml Rappaport-vassiliadis (RV) enrichment broth and incubated at 37°C for 24 h. The enrichment broth was subcultured by spreading 0.l ml onto the plates of Xylose-Lysine-Desoxycholate (XLD) agar and incubated at 37°C for 24 h. Occurrence of black colonies suggested the presence of *Salmonella* (APHA, AWWA and WPCF, 1995).

5.2.8 pH, total nitrogen and moisture analysis

The pH of the manure, irrigation water, soil and crops was determined by adding 1 g of soil or manure to 25 ml of distilled water. The suspension was stirred for 5 min and allowed to settle for 1 h. The pH of the supernatant was determined with a pH meter. Moisture content of manure, soil and crop samples were determined by drying 5 g of each sample at 105°C for 24 h in an oven and then weighing the residue (Islam *et al*., 2004). Total nitrogen of humanure was determined by the Kjeldhal method (Bremner and Keeney, 1996).

5.3 Results and discussion

Table 5.2: Chemical parameters and pathogenic microorganisms in EcoSan sludge samples

Parameter	Value
pH	6.9
TS content (%)	43.0
N content (%)	0.2
Total coliform, cfu/gTS	2.2×10^6
Aspergillus spp, cfu/gTS	3.9×10^3
Faecal *Streptoccoci*, cfu/gTS	2.1×10^6
Faecal coliform, cfu/gTS	1.8×10^6
Salmonella spp, cfu/gTS	2.2×10^5
Total *Ascaris* ova /gTS	22.6
Total Helminths ova /gTS	29.6

TS: Total Solids

The number of cfu of total coliform, faecal coliform, Faecal *Streptococci* and *Salmonella* spp were higher than *Aspergillus* spp numbers (Table 5.2).

The TS content of EcoSan sludges was 43.0% with the N content of 0.2. The N content (0.2%) was within the normal rate for domestic sludges (0.2-0.6%).

The number of helminth ova (29.6 ova/gTS) and *Ascaris* ova (22.6 ova/gTS) were higher. This indicates that the numbers are as high as for sludges from developed countries (Jiménez and Wang, 2004).

Table 5.3: Chemical parameters and pathogenic microorganisms in soil samples

	Soil before sowing	Soil after three weeks
pH	7.8	7.5
TS content (%)	85.6	85.7
Total coliform, cfu/gTS	8.1×10^3-2.7×10^5	1.2×10^2-2.6×10^4
Faecal *Streptococci,* cfu/gTS	0, absent	0-1.1×10^2
Faecal coliform, cfu/gTS	2.6×10^3-1.1×10^4	0-2.1×10^4
Salmonella spp, cfu/gTS	0, absent	0, absent
Aspergillus spp, cfu/gTS	0-7×10^1	0-1.1×10^2
Total helminths ova /gTS	0.4	0.2

The pH of soil samples remained constant over a three week period (Table 5.3).

Total coliform numbers decreased by 1 log, Faecal *Streptococci* numbers increased by 2 log and *Aspergillus* spp numbers increased by 1 log after three weeks. No S*almonella* spp were found in any of the samples and Faecal *Streptococci* were only found in soil after three weeks (Table 5.3).

The numbers of helminth ova decreased in all the samples over the three week period (0.4 and 0.2 HO/gTS). *Ascaris* eggs were found in high numbers and *Toxocara* spp was also found, but in low numbers (Appendix 3.1). Feachem *et* al (1983) showed that *Ascaris* eggs were found in field soil where sewage sludge was applied for fertilization or irrigation and the eggs survived for years in the soil.

Table 5.4 Viable helminth egg counts in Ecosan slugde samples

Organism	Viable eggs/gTS	Non-viable eggs/gTS	Total eggs/gTS
Ascaris spp	18.6	4.1	22.7
Toxocara spp	0.6	1.7	2.3
Trichuris spp	1.7	0.6	2.3
Enterobius vermicularis	0	1.2	1.2
Hymenolepis diminuta	0	1.2	1.2

High numbers of helminth eggs were found in EcoSan slugde (Table 5.4). An equal number of *Toxocara* and *Trichuris* spp (2.3 eggs/gTS) were found in EcoSan sludge samples. *Enterobius vermicularis* and *Hymenolepis diminuta* were also found in equal numbers (1.2 eggs/gTS). Eighty two percent of *Ascaris* eggs were viable, emphasizing the prevalence of *Ascaris* eggs in EcoSan sludge samples (Table 5.4). The percentage of viable eggs in samples treated according to the Official Mexican Method for the quantitative analysis of helminth eggs in sludge samples (Fig 3.1) (Jimenez *et al.*, in press) was higher. This indicates that neither the acid nor ethyl acetate caused any decrease in eggs viability. The other reason for the viability might be due to the thick cell wall of helminth eggs.

Table 5.5 pH, helminth egg counts and bacterial counts in irrigation water samples.

Date	pH	Helminth eggs count/5L	Total coliform cfu/ml	Faecal coliform cfu/ml	Faecal *Streptococci* cfu/ml	*Salmonella* spp cfu/ml	*Aspergillus* spp cfu/ml
11-15-04	7.2	$<10^1$	3.6×10^1	2.2×10^1	$<10^1$	$<10^1$	1.0×10^1
11-29-04	6.6	$<10^1$	2.4×10^1	5.0×10^1	1.0×10^1	$<10^1$	1.0×10^1
12-13-04	6.6	$<10^1$	2.8×10^1	3.0×10^1	$<10^1$	$<10^1$	1.0×10^1
12-20-04	6.7	$<10^1$	3.2×10^1	5.0×10^1	1.0×10^1	$<10^1$	2.0×10^1
01-10-05	8.8	$<10^1$	1.2×10^1	9.0×10^1	$<10^1$	$<10^1$	1.0×10^1

Water used to irrigate came from a well and was stored in open tanks for the irrigation of crops. The pH values in all the samples of irrigation water remained within the range of 6.6 to 8.8 (Table 5.5). Low numbers of pathogens were found in irrigation water. Total coliform ranged from 1.2×10^1 to 3.6×10^1 cfu/ml, faecal coliform from 3.0×10^1 to 2.2×10^1 cfu/ml, Faecal *Streptococci* from 0 to 1.0×10^1 cfu/ml. The number of *Aspergillus* spp showed no difference over three months period ranging from 1.0×10^1 to 2.0×10^1 cfu/ml. Low number of *Aspergillus* were found in irrigation water samples than total and faecal coliform. No *Salmonella spp* nor helminth ova were found in any of the five samples analyzed (Table 5.5). Nevertheless the results showed that the irrigation water was contaminated with microorganisms. The reason for this might have been as a result of birds drinking water from the open tank (Table 5.5). Total coliform, faecal coliform and Faecal *Streptococci* were found in higher numbers than other pathogens.

Table 5.6 Helminth egg counts and bacterial counts in spinach soil samples after harvesting.

Treatment	Total coliform, cfu/gTS	Faecal coliform, cfu/gTS	Faecal *Streptococci*, cfu/gTS	*Salmonella* spp, cfu/gTS	*Aspergillus* spp, cfu/gTS	Viable eggs/gTS	Non-viable eggs/gTS	Total helminth ova/gTS
Control	$2.10x10^3$	$7.38x10^2$	$3.43x10^4$	$<10^1$	$2.08x10^3$	0.04	0.4	0.5
$0.18HO/cm^2$	$9.90x10^3$	$8.70x10^3$	$1.10x10^3$	$1.20x10^1$	$8.90x10^3$	0.5	0.5	1.1
$2.6HO/cm^2$	$3.40x10^3$	$<10^1$	$1.20x10^4$	$<10^1$	$3.00x10^2$	0.7	0.5	1.3
$5.1HO/cm^2$	$<10^1$	$<10^1$	$3.50x10^2$	$2.30x10^1$	$5.00x10^2$	0.8	0.6	1.5

Table 5.7 Helminth egg counts and bacterial counts in carrot soil samples after havesting.

Treatment	Total coliform, cfu/gTS	Faecal coliform, cfu/gTS	Faecal *Streptococci*, cfu/gTS	*Salmonella* spp, cfu/gTS	*Aspergillus* spp, cfu/gTS	Viable eggs/gTS	Non-viable eggs/gTS	Total helminth ova/gTS
Control	$1.83x10^3$	$2.83x10^3$	$1.13x10^2$	$<10^1$	$<10^1$	0.02	0.3	0.4
$1.0HO/cm^2$	$2.50x10^3$	$1.00x10^3$	$2.10x10^2$	$<10^1$	$<10^1$	0.3	0.3	0.6
$1.7HO/cm^2$	$1.60x10^3$	$1.40x10^3$	$9.00x10^3$	$<10^1$	$1.00x10^1$	0.4	0.4	0.8
$4.8HO/cm^2$	$1.80x10^7$	$8.80x10^4$	$4.40x10^4$	$<10^1$	$7.00x10^1$	0.5	0.5	1.1

The application of humanure to soil resulted in an increase of pathogens (Table 5.6 and 5.7). Viable helminth eggs in spinach soil samples decreased from 0.8 eggs/gTS to 0.04 eggs/gTS (Table 5.6) and from 0.5 eggs/gTS to 0.02 eggs/gTS viable helminth eggs in carrot soil samples (Table 5.7). The lower number of viable eggs in carrot soil samples was attributed to the longer time taken to grow the carrot (12 weeks) than spinach (7 weeks) resulting in the damage of eggs because of humanure dehydration. *Ascaris* eggs have been reported to survive for up to two years in soil that has been irrigated with sewage sludge and for this reason they pose a serious health risk (Strauch, 1991).

The number of Faecal *Streptococci* increased by 2 log, total coliform increased by 4 log, faecal coliform increased by 1 log after application of humanure to soil. No increase in the number of *Aspergillus* and *Salmonella* spp was found after application of humanure in carrot soil samples (Table 5.7). The numbers of total coliform, faecal coliform, Faecal *Streptococci* and *Aspergillus* spp in spinach soil decreased after application of humanure. This indicates that the soil was already polluted when humanure was applied to soil.

**Table 5.8** Viable helminth egg counts in carrot leaf samples.

	Viable eggs/gTS	Non-viable eggs/gTS	Total helminth ova/gTS
Control	0.01	0.5	0.5
1.0HO/cm^2	0.02	0.6	0.7
1.7HO/cm^2	0.2	0.7	0.9
4.8HO/cm^2	0.4	1.1	1.5

There was an increase in viable eggs as the amount of humanure was added to the plots (Table 5.8). The initial number of 0.01 eggs/gTS in the control sample increased to 0.02 eggs/gTS when 1.0HO/cm^2 was applied followed by 0.2 eggs/gTS when 1.7 HO/cm^2 was applied and to 0.4 when 4.8HO/cm^2 was applied. This viability represents 17.5% of the total helminth eggs found in carrot leaf samples (Table 5.8). Suggesting that the eggs present in carrot leaf were inactive, reducing the risk to spread the disease.

Table 5.9 Viable helminth egg counts in carrot samples.

|	Viable eggs/gTS	Non-viable eggs/gTS	Total helminth ova/gTS
Control	0.006	0.3	0.3
$1.0HO/cm^2$	0.1	0.3	0.4
$1.7HO/cm^2$	0.1	0.5	0.6
$4.8HO/cm^2$	0.2	0.8	1.1

Viable helminth eggs in carrot samples were found in low numbers than in carrot leaves (Table 5.9). The initial number of 0.006 eggs/gTS in the control sample increased to 0.1 eggs/gTS and 0.2 eggs/gTS when 1-1.7 HO/cm^2 and $4.8HO/cm^2$ were applied, respectively. This viability represents 16.6% of the total helminth eggs found in carrot samples. This indicates that the eggs were also inactive (Table 5.9). The contamination of vegetables by *Ascaris* eggs, following the use of sludge for fertilization has been a major concern of parasitologist. Carrot irrigated with sewage sludge was reported to be contaminated with *Ascaris* eggs and 36% of the eggs were viable according to Feachem *et al.* (1983).
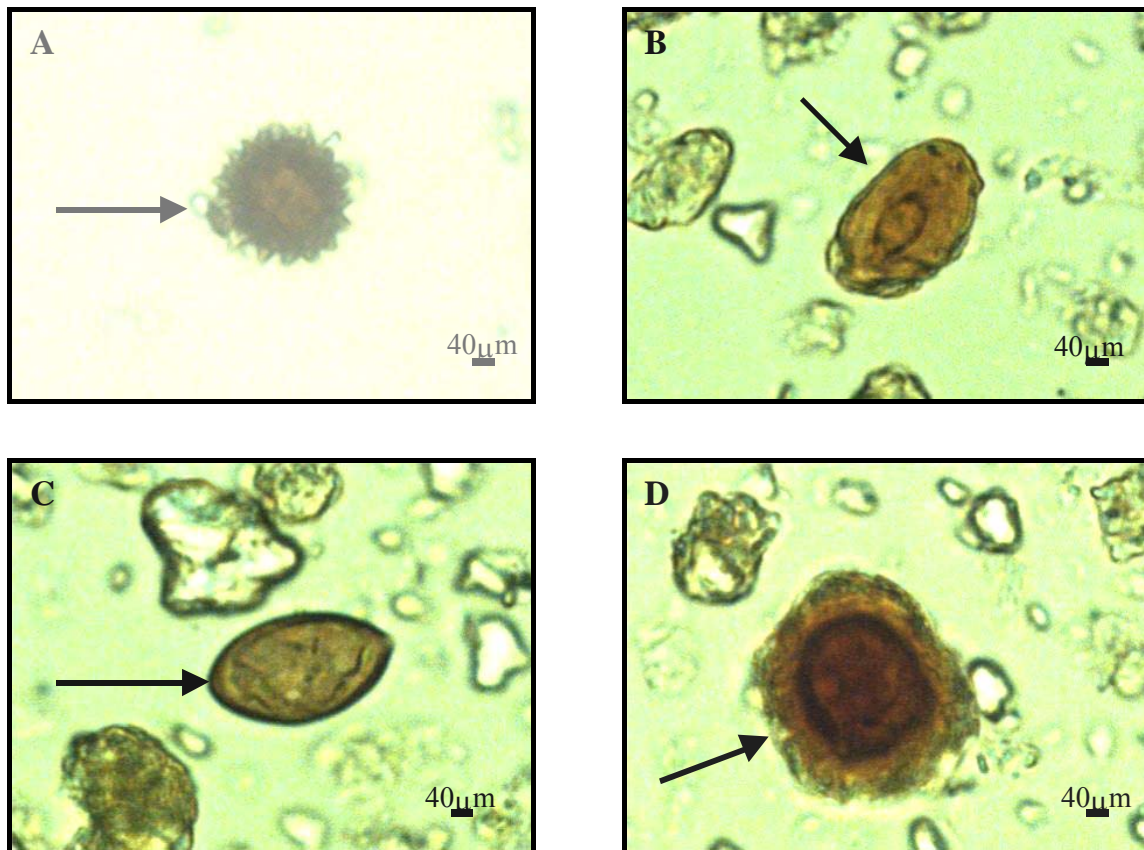
Figure 5.1: Photomicrograph of helminth eggs after incubation at 26°C for 20 d (A) *Ascaris lumbricoides* from spinach, (B) *Hymenolepis diminuta* from humanure, (C) *Trichuris* spp from humanure (D) *Ascaris lumbricoides* from soil samples.

Helminth eggs were recovered in humanure, soil and crop samples (Fig 5.1). The species identified were mostly *Ascaris* spp (Fig 5.1 a, d). Humanure samples contained different species of helminth eggs including *Hymenolepis diminuta* and *Trichuris* spp (Fig 5.1 b, c).
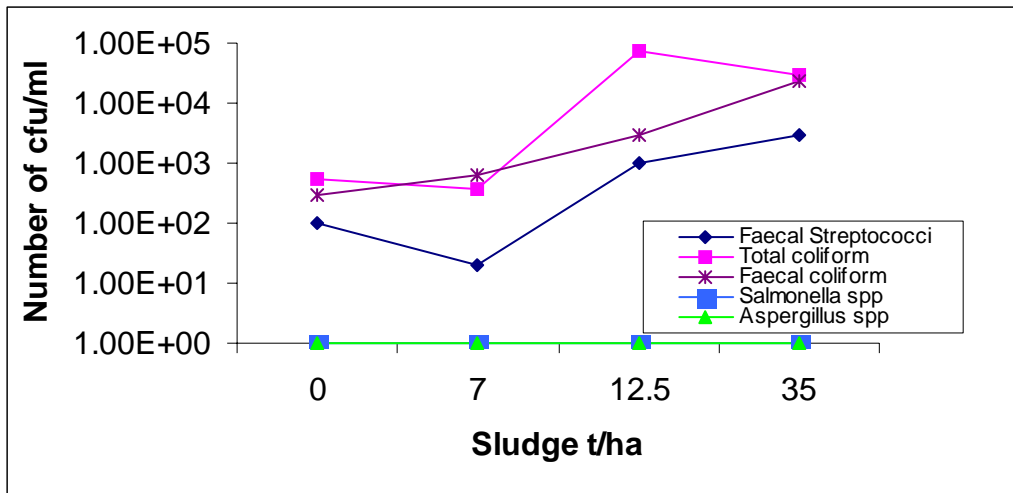
Figure 5.2 Microbiological analyses of pathogens on carrot leaves

The number of Faecal *Streptococci* in carrot leaves increased by 1 log from 10^2 to 10^3 cfu/ml after the application of humanure. Total coliform and faecal coliform numbers increased by 2 log from 10^2 to 10^4 cfu/ml after application of humanure (Fig 5.2). No *Salmonella* spp were found in carrot leaves. Some microorganisms such as *E. coli* showed preferential attachment to the interior of damaged vegetables rather than on the surface, as the juice within the vegetables provide a good growth medium (Takeuchi and Frank, 2000).
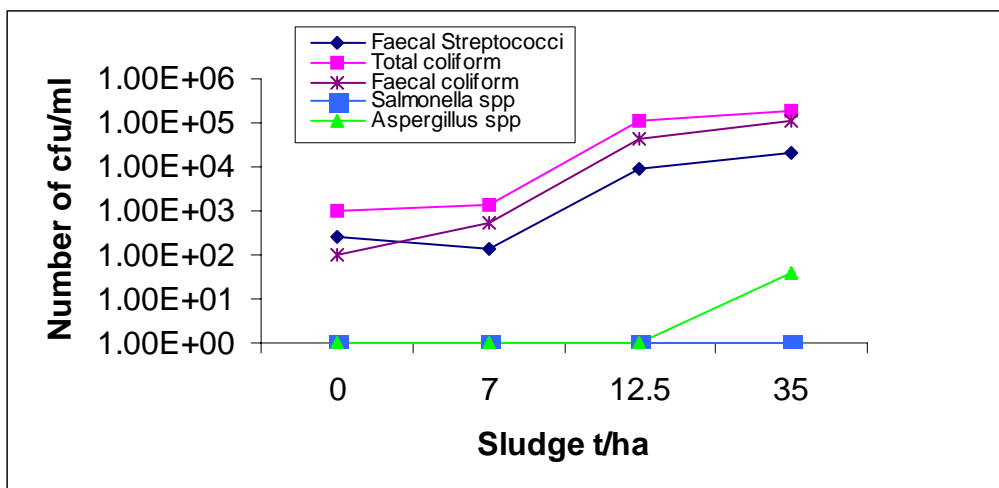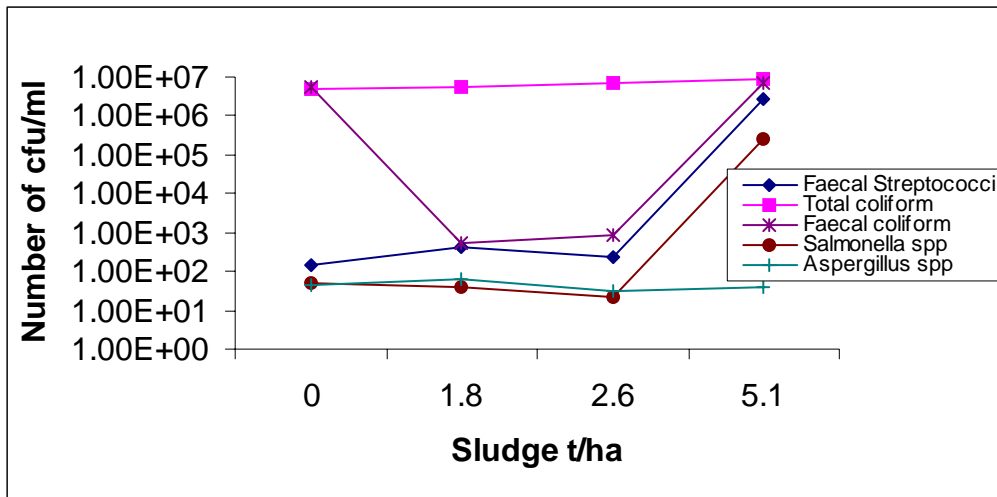

Figure 5.3 Microbiological analyses of pathogens on carrot

Total coliform and faecal coliform numbers increased by 3 log from 10^2 to 10^5 cfu/ml and the Faecal *Streptococci* numbers increased from 10^2 to 10^4 cfu/ml after the application of humanure (Table 5.3). *Aspergillus* spp increased from zero to 10^1 cfu/ml. No *Salmonella* spp were found in carrot samples (Fig 5.3). A microorganism such as *E.coli* is capable of entering the plant (carrot) through the root system and migrates to the edible portion of the plant (Solomon *et al*., 2002). The ability of *E.coli* to penetrate plant tissue has also been reported by (Takeuchi and Frank, 2000). *E. coli* was found in carrot samples, a 3 log increase was obtained after application of humanure to soil.
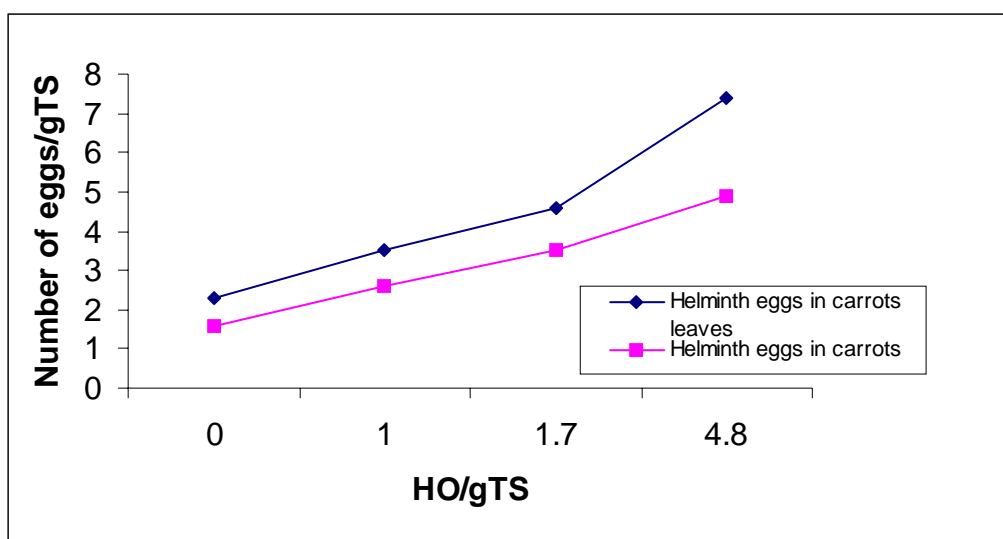
Figure 5.4 Helminth egg counts on infected carrot samples

Application of Ecosan sludge increased the number of helminth eggs in carrot samples. Helminth eggs found in carrot leaf samples increased from 2.3 to 7.4/gTS and helminth eggs in carrot increased from 1.6 to 4.9/gTS after EcoSan application (Fig 5.4). Carrot leaves were found contaminated with *Ascaris* spp, *Trichuris* spp and *Toxocara* spp. Carrots were also contaminated with *Ascaris* spp, *Trichuris* spp, *Toxocara* spp, *Taenia* spp and *Enterobius vermucularis* (Fig 5.4). Crop contamination with *Ascaris* was also reported by Ayres *et al*. (1992).

Figure 5.5 Microbiological analyses of pathogens on spinach leaves

Faecal *Streptococci* numbers in spinach leaf samples increased by 4 log from 10^2 to 10^6 cfu/ml after EcoSan application. There was no effect in total coliform, faecal coliform and *Aspergillus* spp numbers after the application of humanure. The number of *Salmonella* spp increased by 4 log from 10^1 to 10^5 cfu/ml (Fig 5.5). Petterson *et al*. (2001) reported that microorganisms such as *E.coli* were found to grow on the inside of vegetables irrigated by sewage.
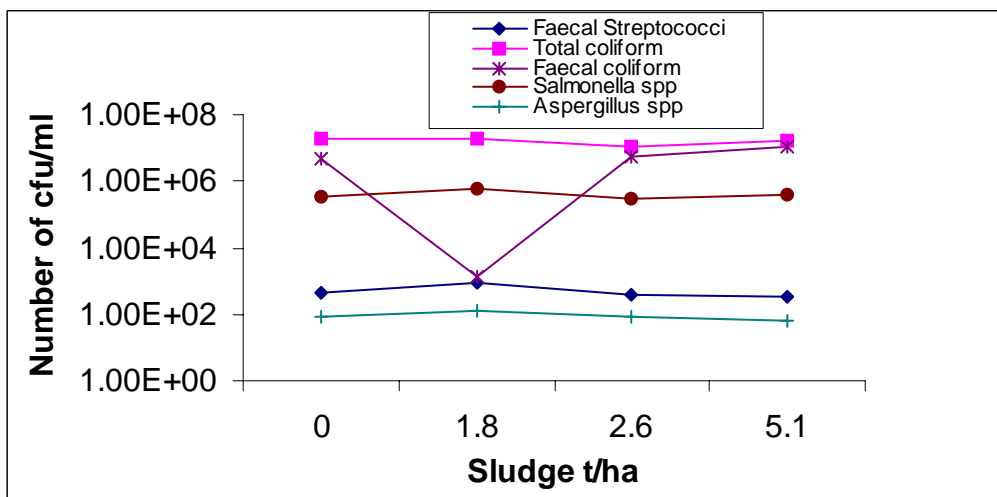
Figure 5.6 Microbiological analyses of pathogens on spinach roots

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The faecal coliform numbers increased by 1 log from 10^6 to 10^7 cfu/ml after application of EcoSan (Fig 5.6). There was no difference in the number of Faecal *Streptococci*, total coliform, *Salmonella* and *Aspergillus* spp after application of humanure (Fig 5.6). Research in the US has already highlighted cases in which *E. coli* infected people after eating raw vegetables fertilizer with sludge (Scotsman, 1998).
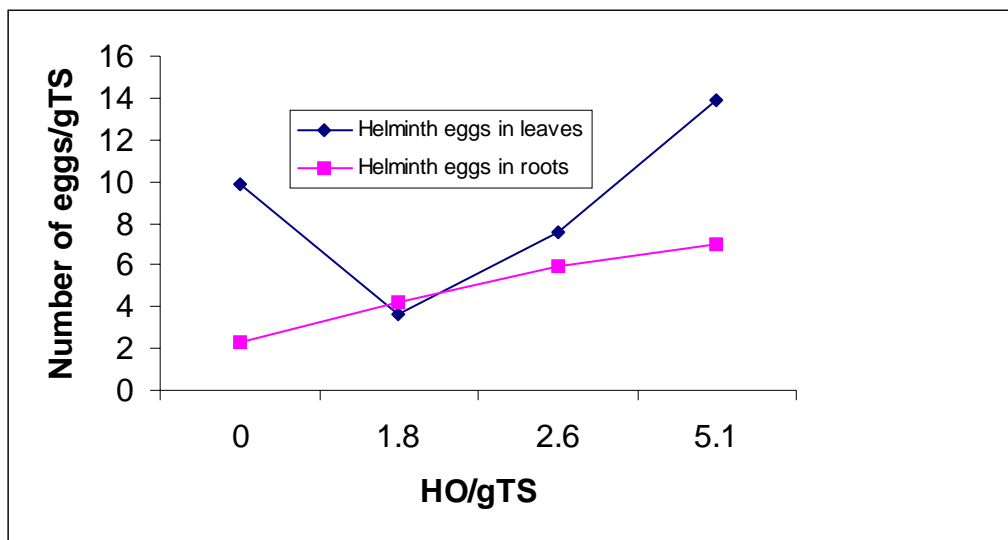
Figure 5.7 Helminth egg counts on infected spinach samples

Helminth eggs numbers in spinach leaf samples increased from 9.9 to 13.9/gTS and helminth eggs in spinach roots increased from 2.3 to 7.0/gTS (Fig 5.7). Spinach leaves were contaminated with *Ascaris* spp, *Trichuris* spp and *Enterobius vermicularis* after the application of humanure to soil. Spinach roots were also contaminated with *Ascaris* spp, *Toxocara* spp and *Trichuris* spp (Fig 5.7). Ayres *et al*. (1992) recovered viable *Ascaris* eggs from vegetables such as lettuce irrigated with sewage. Viable *Ascaris* eggs were also recovered from both lettuce and tomatoes irrigated with sewage (Gaspard and Scwartzbrod, 1993). Özlem and Hakan, (2005) recovered *E. vermicularis* and *Ascaris* eggs in spinach.

5.4 Conclusions

- EcoSan contained high numbers of helminth eggs (29.6 ova/gTS), indicating a high risk of contamination when EcoSan is applied to soil as fertilizer.

- Application of EcoSan sludges at different rates to soil resulted in high numbers of total coliform, faecal coliform, Faecal *Streptococci*, *Salmonella*, *Aspergillu*s spp and helminth eggs in spinach and carrot samples.

- The results of this study showed that *Ascaris* spp, *Toxocara* spp, *Trichuris* spp, *Enterobius vermicularis*, *Hymenolepis diminuta* and *Taenia* spp may come in contact with soil, carrot and spinach when humanure is applied to soil as fertilizer.

- Humanure used, as crop fertilizer should be treated before application to soil to eliminate pathogen contamination.

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CHAPTER 6

General discussion

The primary aim of sanitation is to prevent the transmission of excreta-related diseases. There is a risk of disease transmission related to the handling and reuse of all different types of waste products of human or animal origin (Hoglund, 2001). The separation of urine and faeces must be done so that the urine fraction is totally free from faeces and both of these fractions can be treated separately (Heinonen-Tanski and Van Wijk-Sijbesma, 2004). Source-separated urine has hygienic advantages because few pathogens are excreted through urine and can be disinfected easily by storage (Vinnerås *et al*., 2003). Treatment of human and animal excreta is the most important way to prevent the spread of pathogens in the environment.

In spiked urine samples, storage at 30°C for 50 d was effective for killing all the pathogens tested ($<10^{1}$ cfu/ml), except for Faecal *Streptococci,* which had a count of (>3.00x10^{7} cfu/ml). Storage of naturally contaminated urine samples was more effective for killing pathogens compared to spiked urine samples. Pathogens present in naturally contaminated urine samples were killed after 50 d of storage at all the temperatures tested (15, 20 and 30°C). Hoglund *et al*. (1998) reported that Faecal *Streptococci* died off in urine (reduction was observed after 25-30 d at 20°C). This was similar to the results obtained in this study. Schonning (2001) suggested that urine should be stored (up to six months) before reuse as a fertilizer. The recommended period of storage was however dependent on the temperature of the treatment.

Treatment of dehydrated faeces with NaOH, wood ash and coal ash decreased the number of total coliform, faecal coliform, Faecal *Streptococci*, *Aspergillus*, *Salmonella* and helminth eggs over the 8 d experimental period. NaOH treatment at a pH of 12.1 was more effective than treatments with wood ash at a pH of 10.1 and coal ash at a pH of 9.9. However, relatively large numbers of pathogens were still present in dehydrated faeces after treatment with ash. A 1 log reduction of faecal and total coliform was observed after treatment with wood and coal ash over the 8

d period, rendering the treatment insufficient for use as a disinfection technique. A study of wood ash and faeces mixture at a pH of 9 resulted in a 7 $\log_{10}$ reduction of faecal coliforms and 1% survival of *Ascaris* eggs after three months (Schonning, 2001). Coal ash analysed in the same study with a pH of 7 in the mixed material resulted in the inactivation of *Ascaris* within 120 d (Schonning, 2001), suggesting that a long storage time is required to obtain an effective reduction of pathogens.

Helminth eggs are extremely hardy pathogens and the most difficult to destroy. Pasteurization treatment reduced the number of helminth eggs from 7.5 to 3.7 eggs/gTS at 70°C, 20 min and from 7.5 to 3.1 eggs/gTS at 90°C, 5 min. *Ascaris* eggs have been reported to rapidly die at temperatures over 40°C in different types of media including water, sewage, soil and crops (Feachem *et al*., 1983). The storage time depend on the temperature used in combination with other negative environmental conditions such as high ammonia content or low moisture (Heinonen-Tanski and Van Wijk-Sijbesma, 2004).

No bacteria in dehydrated faeces survived pasteurization at 70°C for 20 min and 90°C for 5 min. Pasteurization was the most effective treatment for killing microorganisms present in dehydrated faeces. This implies that pasteurization at high temperatures was an effective disinfection.

Helminth ova in humanure were found in higher numbers compared to ova in soil, carrots and spinach. No helminth ova were found in irrigation water samples. The numbers of *Ascaris* eggs in humanure were higher than eggs in soil, carrot and spinach samples with the viability of 82%, suggesting a high risk of humanure contamination. Application of EcoSan sludge to soil resulted in the contamination of carrot and spinach with pathogens at levels, which pose a health risk to the consumer.

A microorganism such as *E.coli* is capable of entering the plant (carrots) through the root system and migrates throughout to the edible portion of the plant (Solomon *et al*., 2002). *Ascaris* eggs, *Trichuris* spp, *Enterobius vermicularis* and *Toxocara* spp ova were found in spinach samples. The number of ova was higher in leaves than in the roots, suggesting that the ova adhered preferentially to the leaves. A

study of Özlem and Hakan (2005) reported that spinach was found to be contaminated with *E. vermicularis* and *Ascaris* eggs. Helminth ova were also found on carrots, but in lower numbers than on the leaves. Viability of helminth ova in both crops was very low; although present they were mainly in an inactive state reducing the risk of causing infection. Ayres *et al*. (1992) recovered viable *Ascaris* eggs from vegetables such as lettuce irrigated with sewage.

Recommendations

- Further research is needed, especially regarding the inactivation of pathogens in faeces using different treatment methods.
- Cost effective pasteurization methods need to be developed.
- A higher ratio of ash to faeces should be investigated as a method to improve the disinfection efficiency.
- Storage at high temperature has been considered as a viable treatment option for the pathogens.
- Crops need to be monitored over a longer time to allow further reduction of pathogens due to factors such as microbial activity and UV-light.
- Methods to minimize exposure to pathogens should be encouraged e.g. people handling the excreta should wear gloves for personal protection.

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Appendix 1

1.1: Microbiological analysis of dehydrated faeces

Organism	Storage time (days)	Untreated dehydrated faeces (cfu/g)	Faeces with NaOH (cfu/g)	Faeces With coal ash (cfu/g)	Faeces with wood ash (cfu/g)	Faeces at 60°C, 30 min (cfu/g)	Faeces at 70°C, 20 min (cfu/g)	Faeces at 90°C, 5 min (cfu/g)
Total coliform	0	1.05×10^4	1.05×10^4	1.05×10^4	1.05×10^4	1.05×10^4	1.05×10^4	1.05×10^4
	2	1.21×10^4	$<10^1$	8.10×10^3	5.40×10^3	8.00×10^2	$<10^1$	$<10^1$
	4	1.20×10^4	$<10^1$	7.00×10^3	4.90×10^3	1.20×10^3	$<10^1$	$<10^1$
	6	1.27×10^4	$<10^1$	6.00×10^3	3.80×10^3	6.00×10^2	$<10^1$	$<10^1$
	8	1.25×10^4	$<10^1$	5.40×10^2	2.40×10^2	7.00×10^2	$<10^1$	$<10^1$
Faecal coliform	0	1.69×10^4	1.69×10^4	1.69×10^4	1.69×10^4	1.69×10^4	1.69×10^4	1.69×10^4
	2	1.68×10^4	$<10^1$	4.70×10^3	4.10×10^3	7.00×10^2	$<10^1$	$<10^1$
	4	1.70×10^4	$<10^1$	4.10×10^3	3.50×10^3	9.00×10^2	$<10^1$	$<10^1$
	6	1.71×10^4	$<10^1$	3.50×10^2	3.00×10^2	1.100×10^3	$<10^1$	$<10^1$
	8	1.68×10^4	$<10^1$	2.20×10^2	2.10×10^2	1.00×10^3	$<10^1$	$<10^1$
Faecal *Streptococci*	0	1.57×10^6	1.57×10^6	1.57×10^6	1.57×10^6	1.57×10^6	1.57×10^6	1.57×10^6
	2	1.58×10^6	6.30×10^5	9.90×10^5	8.80×10^5	1.20×10^4	$<10^1$	$<10^1$
	4	1.62×10^6	5.10×10^3	8.70×10^5	8.20×10^5	1.60×10^4	$<10^1$	$<10^1$
	6	1.59×10^6	4.50×10^3	7.60×10^5	7.00×10^4	1.00×10^4	$<10^1$	$<10^1$
	8	1.58×10^6	3.70×10^3	6.30×10^4	5.40×10^4	1.10×10^4	$<10^1$	$<10^1$
Salmonella spp	0	6.00×10^1	6.00×10^1	6.00×10^1	6.00×10^1	6.00×10^1	6.00×10^1	6.00×10^1
	2	8.00×10^1	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	4	7.00×10^1	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	6	5.00×10^1	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	8	6.00×10^1	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
Aspergillus spp	0	6.00×10^2	6.00×10^2	6.00×10^2	6.00×10^2	6.00×10^2	6.00×10^2	6.00×10^2
	2	5.00×10^2	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	4	8.00×10^2	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	6	9.00×10^2	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$
	8	7.00×10^2	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$	$<10^1$

Appendix 2

2.1: Influence of storage temperature on spiked human urine samples

Organism	Storage time (days)	Number of cfu/ml		
		15^oC	20^oC	30^oC
Total coliform	2	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	4	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	6	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	8	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
Faecal coliform	2	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	4	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	6	$>3.00 \times 10^7$	$>3.00 \times 10^7$	2.90×10^7
	8	$>3.00 \times 10^7$	$>3.00 \times 10^7$	1.93×10^7
Faecal *Streptococci*	2	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	4	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	6	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	8	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
Salmonella spp	2	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	4	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	6	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	8	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
Aspergillus spp	2	$>3.00 \times 10^7$	8.10×10^6	2.70×10^6
	4	$>3.00 \times 10^7$	7.30×10^6	2.30×10^6
	6	$>3.00 \times 10^7$	2.10×10^6	7.00×10^7
	8	$>3.00 \times 10^7$	5.60×10^6	5.00×10^7

Exposure of the spiked urine samples to different temperatures had no biocidal effect (Table 4.3)

2.2: Influence of storage temperature on naturally contaminated human urine samples

Organism	Storage time (days)	Number of cfu/ml		
		15°C	20°C	30°C
Total coliform	2	>3.00x10^7	>3.00x10^7	>3.00x10^7
	4	>3.00x10^7	>3.00x10^7	>3.00x10^7
	6	>3.00x10^7	>3.00x10^7	>3.00x10^7
	8	2.83x10^7	1.95x10^7	1.29x10^7
Faecal coliform	2	>3.00x10^7	>3.00x10^7	>3.00x10^7
	4	>3.00x10^7	>3.00x10^7	>3.00x10^7
	6	>3.00x10^7	>3.00x10^7	2.90x10^7
	8	2.74x10^7	2.02x10^7	9.30x10^6
Faecal *Streptococci*	2	>3.00x10^7	>3.00x10^7	>3.00x10^7
	4	>3.00x10^7	>3.00x10^7	>3.00x10^7
	6	>3.00x10^7	>3.00x10^7	>3.00x10^7
	8	1.84x10^7	1.11x10^7	6.40x10^6
Salmonella spp	2	>3.00x10^7	>3.00x10^7	>3.00x10^7
	4	>3.00x10^7	>3.00x10^7	>3.00x10^7
	6	>3.00x10^7	>3.00x10^7	>3.00x10^7
	8	>3.00x10^7	>3.00x10^7	>3.00x10^7
Aspergillus spp	2	>3.00x10^7	8.10x10^6	2.70x10^6
	4	5.70x10^6	7.30x10^6	2.30x10^6
	6	3.70x10^6	2.10x10^6	7.00x10^7
	8	9.00x10^5	1.60x10^6	3.00x10^7

Exposure of the naturally contaminated urine samples to different temperatures had no biocidal effect (Table 4.4).

2.3: Influence of storage temperature on spiked filtered human urine samples

Organism	Storage time (days)	Number of cfu/ml		
		$15^{o}C$	$20^{o}C$	$30^{o}C$
Total coliform	10	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	20	$>3.00 \times 10^7$	$>3.00 \times 10^7$	5.00×10^5
	30	$>3.00 \times 10^7$	$>3.00 \times 10^7$	3.10×10^6
	40	$>3.00 \times 10^7$	$>3.00 \times 10^7$	2.30×10^6
	50	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$<10^1$
Faecal coliform	10	2.31×10^7	2.54×10^7	8.20×10^6
	20	$>3.00 \times 10^7$	2.20×10^7	2.80×10^6
	30	2.69×10^7	1.16×10^7	5.00×10^5
	40	8.50×10^6	9.90×10^6	$<10^1$
	50	2.47×10^7	4.00×10^5	$<10^1$
Faecal *Streptococci*	10	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	20	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	30	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	40	$>3.00 \times 10^7$	$>3.00 \times 10^7$	2.88×10^7
	50	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
Salmonella spp	10	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	20	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$>3.00 \times 10^7$
	30	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$<10^1$
	40	$>3.00 \times 10^7$	$>3.00 \times 10^7$	$<10^1$
	50	$>3.00 \times 10^7$	1.19×10^7	$<10^1$
Aspergillus spp	10	9.00×10^5	7.00×10^5	4.00×10^5
	20	1.40×10^6	6.00×10^5	5.00×10^5
	30	$>3.00 \times 10^7$	5.90×10^6	1.00×10^5
	40	$>3.00 \times 10^7$	1.60×10^6	2.00×10^5
	50	$>3.00 \times 10^7$	$<10^1$	$<10^1$

2.4: Influence of storage temperature on unfiltered naturally contaminated human urine

Organism	Storage time (days)	Number of cfu/ml		
		15°C	20°C	30°C
Total coliform	10	3.70x10^6	9.90x10^6	1.10x10^6
	20	1.95x10^7	9.40x10^6	8.80x10^6
	30	1.10x10^6	5.00x10^5	1.00x10^5
	40	1.00x10^5	<10^1	<10^1
	50	<10^1	<10^1	<10^1
Faecal coliform	10	9.40x10^6	7.70x10^6	4.50x10^6
	20	8.10x10^6	5.30x10^6	3.40x10^6
	30	4.00x10^5	<10^1	<10^1
	40	<10^1	<10^1	<10^1
	50	<10^1	<10^1	<10^1
Faecal *Streptococci*	10	1.31x10^7	6.30x10^6	3.00x10^5
	20	7.40x10^6	7.30x10^6	5.40x10^6
	30	2.10x10^6	1.70x10^6	9.00x10^5
	40	1.20x10^6	7.00x10^5	5.00x10^5
	50	1.00x10^5	<10^1	<10^1
Salmonella spp	10	>3.00x10^7	2.14x10^7	2.08x10^7
	20	4.20x10^6	4.00x10^6	2.6x10^6
	30	1.40x10^6	2.00x10^5	<10^1
	40	1.00x10^6	<10^1	<10^1
	50	<10^1	<10^1	<10^1
Aspergillus spp	10	3.00x0^5	1.00x10^5	2.00x10^1
	20	1.00x10^5	1.00x10^5	<10^1
	30	<10^1	<10^1	<10^1
	40	<10^1	<10^1	<10^1
	50	<10^1	<10^1	<10^1

Appendix 3

3.1: Determined helminth eggs in soil samples

Plots	Before sowing		After three weeks
	Ascaris spp/gTS	*Toxocara* spp/gTS	*Ascaris* eggs/gTS
57	0.4	0	0.2
59	0.3	0	0.1
61	0.4	0.04	0.1
63	0.3	0.04	0.1

3.2: Determined helminth eggs in soil spinach after harvesting

	Ascaris spp/gTS	*Trichuris* spp/gTS	*Enterobius vermicularis* spp/gTS
Control	0.5	0	0.04
0.18HO/cm^2	1.1	0	0
2.6HO/cm^2	1.2	0.04	0
5.1HO/cm^2	1.3	0.1	0

3.3: Determined helminth eggs in soil carrot after harvesting

	Ascaris spp/gTS	*Toxocara* spp/gTS	*Trichuris* spp/gTS	*Taenia* spp/gTS	*Enterobius vermicularis* spp/gTS
Control	0.3	0.004	0.01	0	0
1.0HO/cm^2	0.6	0.02	0.02	0	0
1.7HO/cm^2	0.7	0.02	0.04	0	0.02
4.8HO/cm^2	1.0	0.02	0.04	0.02	0.02

3.4: Determined helminth eggs in spinach samples

	Spinach leaves			Spinach roots		
	Ascaris spp/gTS	*Trichuris* spp/gTS	*Enterobius vermicularis* spp/gTS	*Ascaris* spp/gTS	*Trichuris* spp/gTS	*Toxocara* spp/gTS
Control	0.3	0.003	0	0.07	0	0
0.18HO/cm^2	0.1	0.01	0	0.14	0	0
2.6HO/cm^2	0.2	0.01	0	0.18	0.01	0
5.1HO/cm^2	0.4	0.04	0.02	0.21	0.01	0.01

3.5: Determined helminth eggs in carrots leaves samples

	Ascaris spp/gTS	*Toxocara* spp/gTS	*Trichuris* spp/gTS	*Enterobius vermicularis* spp/gTS
Control	0.16	0	0.003	0
1.0HO/cm^2	0	0	0.003	0
1.7HO/cm^2	0.003	0	0.02	0
4.8HO/cm^2	0.02	0	0.04	0

3.6: Determined helminth eggs in carrots samples

	Ascaris spp/gTS	*Toxocara* spp/gTS	*Trichuris* spp/gTS	*Enterobius vermicularis* spp/gTS	*Taenia* spp/gTS
Control	0.19	0.003	0	0	0
1.0HO/cm^2	0.28	0.006	0.003	0	0
1.7HO/cm^2	0.35	0.003	0.01	0.003	0
4.8HO/cm^2	0.47	0.01	0.01	0	0.01

3.1: Carrot from the Agricultural farm in the University of Pretoria

3.2: Carrots in the irrigated field in the Agricultural farm

3.3: Spinach in the irrigated field in the Agricultural farm

3.4: Preparation of plots for the planting of carrot and spinach