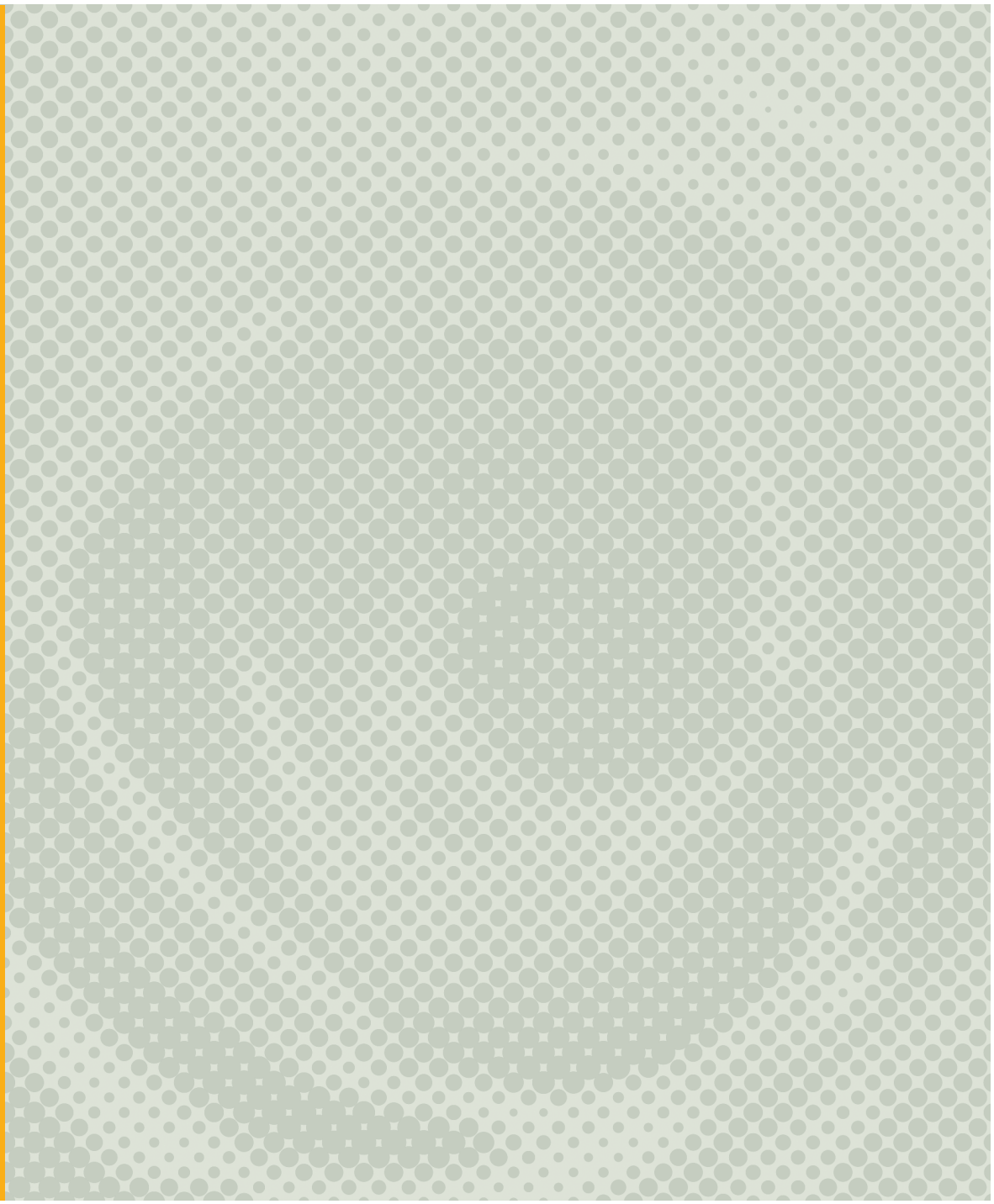
Quantitative microbial risk assessment of agricultural use of fecal matter treated with urea and ash

Jørgen Fidjeland

Norwegian University of Life Sciences
Department of mathematical sciences and technology
Master Thesis 30 credits 2010



Abstract

Today about 2,6 billion people lack access to improved sanitation and over 2 billion people use pit latrines. This results in spreading of pathogens from excreta into the environment and drinking water sources, causing severe illnesses. In developing countries the health impact of this is, however, overshadowed by malnutrition. Sustainable sanitation can prevent spreading of pathogens to the environment, as well as ensure safe reuse of the nutrients in excreta for food production in agriculture.

A planned sanitation system in Kampala, Uganda is aiming to sell fecal matter treated for two months with urea, as a fertilizer product. Using urea, the most common mineral fertilizer in the world, to treat fecal matter will inactivate most of the pathogens, and increase the fertilizer value of the fertilizer product. Ash could also be used in combination with urea to enhance the pathogen inactivation.

The objective of this research was to assess the health risk for the farmer applying the fertilizer product, and for the food consumer eating crops grown in these fields. The evaluation of the urea and ash treatment was conducted by using quantitative microbial risk assessment for identifying the health risk, applying relevant data for the conditions in Kampala.

Ascaris was identified as the main threat to human health. Assuming that the farmer is not using protective clothing, 4 % w/w urea is needed to achieve an annual risk of *Ascaris* infection less than 10^{-3}, which is suggested to be tolerable. Health risk associated with consuming raw carrots and spinach grown in soil amended with treated fecal matter were acceptable if 3 % w/w urea or more was used in the treatment. Using ash in addition to urea increased the *Ascaris* inactivation, but was not very favorable since water addition is needed to maintain the moisture, extending the volumes needed during treatment.

An inactivation study of *Salmonella* typhimurium phage 28B in fecal matter treated with urea and ash, showed no difference in inactivation at different pH levels. Because both *Salmonella* typh. phage 28B and adenovirus are dsDNA viruses, raising the pH will probably not affect the adenovirus inactivation either. However, a pH increase would increase the rotavirus inactivation. More research is on their inactivation and fate in the soil is needed to evaluate whether or not they represent a significant health risk in this context.

Sammendrag

Omtrent 2,6 milliarder mennesker mangler tilgang til forbedrede sanitærsystemer og over 2 milliarder bruker enkle latriner. Som følge av dette blir patogene mikroorganismer spredd i miljøet og drikkevannskilder, noe som medfører alvorlige sykdommer. I utviklingsland er likevel konsekvensene av feil- og underernæring mye større. Bærekraftige sanitærløsninger kan forhindre spredning av patogener, i tillegg til å sørge for at næringsstoffer i ekskrementene kan brukes på en trygg måte til matproduksjon i jordbruk.

Et planlagt sanitærprosjekt i Kampala, Uganda, har som mål å selge fekalt materiale som jordforbedringsprodukt, etter to måneder behandling med urea. Ved å bruke urea, som er den vanligste kunstgjødselen i verden, vil de fleste patogene mikroorganismer bli inaktivert, samtidig som gjødselverdien til jordforbedringsproduktet økes. Aske kan også brukes i kombinasjon med urea for å forsterke inaktiveringen av patogener.

Målet med denne studien var å vurdere helserisikoen for bonden som bruker jordforbedringsproduktet på åkrene, og for matkonsumenten som spiser mat dyrket på disse åkrene. Evalueringen av urea- og askebehandlingen ble gjort ved å bruke kvantitativ mikrobiell risikovurdering, der relevante data for situasjonen i Kampala ble brukt.

Ascaris ble funnet å representere den største helserisikoen. Forutsatt at bonden ikke bruker beskyttende bekledning, må 4 vektprosent urea brukes i behandlingen for at den årlige risikoen for *Ascaris* infeksjon for bonden skal være mindre enn 10^{-3}, som er foreslått som akseptabel risiko. Dersom 3 vektprosent urea eller mer ble brukt i behandlingen, vil konsumering av rå gulrøtter og spinat også resultere i akseptabel risiko. Bruken av aske sammen med urea vil øke inaktiveringen av *Ascaris*, men er ikke veldig hensiktsmessig siden vann må tilsettes for å bevare fuktigheten, noe som øker lagringsvolumene til behandlingen.

Et forsøk der inaktiveringen av *Salmonella* typhimurium fag 28B ble studert, viste ingen forskjell i inaktivering ved forskjellige pH-nivå. Fordi både *Salmonella* typh. fag 28B og adenovirus er dsDNA virus, vil en økning av pH verdien i behandlingen av fekalt materiale ikke påvirke inaktiveringen av adenovirus heller. Imidlertid vil en pH økning kunne akselerere inaktiveringen av rotavirus. Mer forskning på inaktivering av disse virusene, og hva som skjer med dem når de kommer i jorda, trengs for å vurdere om de utgjør en betydelig helserisiko i denne sammenhengen.

Acknowledgements

First of all I want to thank my supervisor Arve Heistad for guiding me through this research. Your constructive feedback has been very motivating and helpful. And thanks to my co-supervisor Razak Seidu for helping me with the QMRA modeling, and for always keeping smiling.

Thanks to Karsten Gjefle in Susan Design for suggesting interesting aims for my research and for providing very much relevant information. Your commitment working with the sanitation challenge is really inspiring.

I am grateful to Jon Fredrik Hanssen for his excellent guidance on the laboratory work. I am also very thankful to Siri Merete Grønhovd and Else Marie Aasen who patiently helped me a lot at the lab.

I want to thank Charles Niwagaba at Makerere University and Onesmus Semalulu in NARO for taking the time to give valuable information about agriculture and food habits in Uganda.

Thanks to my good friend Kristian Fjørtoft, who introduced me to Susan Design, for many good discussions, your valuable advices and your positive and optimistic attitude.

Special thanks to my mother for always encouraging me, and to my father who gave me the interest for nutrients recycling through a variety of garden composting systems.

And to my fiancée Kari, thank you so much for always being there for me and supporting me.

Ås, July 2010

Jørgen Fidjeland

Jørgen Fidjeland

Table of contents

List of figures

List of tables

Abbreviations

CCE	Calcium carbonate equivalent
DALY	Disability Adjusted Life Years
DM	Dry matter
HO	Helminth Ova
ID_{50}	Median infectious dose, the number of pathogens ingested that result in a 50 % probability of getting infected
NB	Nutrient broth
NA	Nutrient agar
QMRA	Quantitative Microbial Risk Assessment
SIP	Soil improvement product
T_{90}	The time required for 90 % reduction of pathogens, usually given in days
WHO	World Health Organization

1. Introduction

1.1 The sanitation challenge

Today about 2.6 billion people lack access to proper sanitation, and over 2 billion people uses pit latrines. This results in spreading of pathogens from excreta into the environment and drinking water sources such as ground water and rivers, causing severe illnesses. Diarrhea is widespread in the developing world, and is the main cause of death in children under the age of 5 in sub-Saharan Africa. Measured in disability adjusted life years (DALY), unsafe water, sanitation and hygiene contributes to about 3,7 % of the total disease burden in the low- and middle-income countries. The health impact of water-related diseases is however overshadowed by mal- and undernutrition, and child underweight for age accounts for 8,7 % of the disease burden in the same regions (Lopez et al. 2006).

In order to increase food production, the recycling of nutrients from human excreta into agricultural food production is promoted under the term sustainable sanitation. The degree of sustainability of a system depends on the proportion of nutrients that is recycled, the amount of water, materials, chemicals and energy that is used, and the economic and socio-cultural viability of the system. As for all sanitation systems, the main concern is to avoid the transmission of pathogenic microorganisms through the fecal-oral route.

There are several drivers for the implementation of sustainable sanitation systems. Where the economic input among farmers is too low to buy commercial fertilizers, the reuse of nutrients can provide a cheaper alternative. Furthermore, the worlds remaining sources of mineral phosphorus is decreasing, and the world reserves of phosphorus rock are expected to last for 80-100 years, stressing the need for nutrient recycling in both developed and developing countries (Berg et al. 2005). Increased economic value of phosphorous may be of greater importance in developing countries, due to lower economic input in farming systems. In areas with scarce water resources, the use of conventional water closets is undesirable. Systems that use less or no water are thus preferred, and this also reduces the amount of wastewater that has to be treated.

In order to be accepted by the people who use and maintain the sanitation service, the system must be socio-culturally adapted to the local setting. Ideally, the sanitation services should be locally financed to be economically sustainable. In order to achieve this, the

willingness to pay for improved sanitation is important to consider when choosing system. Furthermore, the possibility to sell treated excreta as fertilizer products to farmers can be used as a source of income.

The excreta intended to use in agriculture must be treated to avoid health risk implications when used for food production. The aim of the treatment is to reduce the number of pathogenic microorganisms. Urine-diverting toilets, which separate urine from the fecal matter, reduce the amount of fecal sludge that needs to be treated. Urine contains few pathogens, and after some weeks or months of storage, the urine can be used as a fertilizer without significant health risk. The fecal matter contains most of the pathogenic microorganisms, and inactivation of the pathogens is crucial in order to recycle the nutrients for use in agriculture. The fecal sludge can be treated in different ways, but the most suitable treatments for developing regions are composting, storage, drying, solar heating, anaerobic digestion, alkaline treatment, ammonia-based treatment, acidic treatment, or combinations of these.

1.2 Situation in Kampala, Uganda

Shallow groundwater wells are an important water source in the densely populated settlements in Kampala, Uganda. The microbiological quality of this water is deteriorated quickly after rainfall events, caused by poorly designed and maintained pit latrines and insufficient sanitation coverage (Howard et al. 2003). Due to this the population suffers from continuous disease epidemics from cholera, dysentery and gastrointestinal worms (Niwagaba et al. 2009; Wendo 2003). Diseases caused by poor sanitation are estimated to account for 80 % of the outpatient attendance in Uganda (Wendo 2003).

Susan Design is an NGO that is establishing sustainable sanitation concepts, and has designed a sanitation concept to be implemented in Kampala, Uganda. The two aims of the project are 1) to develop a concept that provides slum inhabitants with sanitation facilities that are economically affordable, and 2) to recycle the nutrients in excreta back to the agriculture in a safe way. In order to make a sanitation system that can spread without too extensive funding, Susan Design has focused on willingness to pay for proper sanitation alternatives and fertilizer products. Products like urinals and squatting pans are designed to be both esthetical and functional, so that people are willing to pay for improved sanitation.

Stored urine and treated fecal matter is supposed to be sold to farmers outside the city and be a source of income for the project. Urine only needs storage for about 1-4 weeks to be sanitized, while the fecal matter needs a more extensive treatment. Urea is a common mineral fertilizer which is suggested to be used as a sanitizing agent in fecal matter (Vinnerås et al. 2003). In addition to provide a safe soil improvement product (SIP), the added urea will increase the fertilizer value of the end product. In the sanitation system planned by Susan Design, urea is added to fecal matter from urine-diverting toilets.

The Peepoo bag is a small bag made of degradable material that can be used for collection of both urine and fecal matter. It is self-sanitizing because it contains a dose of urea (Vinnerås et al. 2009). For the case of the Susan Design project in Kampala, both the fecal matter amended with urea and the Peepoo bags are planned to be stored two months before they are sold to farmers.

The agriculture in Uganda is diverse, including large-scale farmers exporting both food and cash crops to Europe, medium-scale farmers selling their crops at local markets, and small-scale farmers growing food for their families. Uganda has one of the lowest agro-chemical usages in Africa (ACODE 2006), and most small scale farmers cannot afford to buy mineral fertilizers, while large-scale farmers fertilize with both macro and micro nutrients. Fecal sludge are by the farmers generally considered safe if it is dry and without odor, and most of the farmers are willing to use excreta for food production if it is cheaper than commercial fertilizers, although they are concerned that some people may not want to buy this food (Schröder 2010).

The sanitation situation in Kampala leaves little doubt that the Susan Design project will make a significant positive impact on the health situation there. The situation for the farmers and the food-consumers is different, since the use of human excreta potentially can have a negative health impact. Farmers often do not use protective clothing, and accidental ingestion of treated excreta during application and gardening can cause health risk. Children are likely to take part in the application work even if the farmers are discouraged to include them (O. Semalulu, pers. comm.), and they are much more exposed to severe health impacts caused by pathogens.

For a sanitation project in an urban slum which is selling the fertilizer products to farmers outside the city, a responsibility of ensuring that all of the recommended health protection measures are followed, will expand the responsibility domain for the sanitation project. Verification of proper application can be done by giving the application job to a professional, trained team, or one professional can lead the application work and be responsible for that protective clothing is used and that children are not included in the work. Farmers can be recommended to not grow certain crops when they are buying this type of fertilizer products, but the crop restrictions are almost impossible to control due to long distances.

1.3 Assessment of health risk

The *WHO guidelines for the safe use of wastewater, excreta and greywater* (2006) give recommendations on treatment and management in order to avoid unacceptable health risk. It is based on the Stockholm framework, which is a harmonized approach to control water related diseases. Different exposures and diseases are compared through the Disability Adjusted Life Years (DALY) unit, which is a measure of the years lost due to premature death, diseases and chronic effects. The DALY unit enables cross-sectional cost-efficiency comparison of health initiatives.

Acceptable risk is suggested to be 10^{-6} DALY per person per year which is equivalent of being ill 32 seconds per year. This should be adjusted according to local conditions and in relation to other water-related risk factors. According to the Stockholm framework, health risk assessment should be done in relation to acceptable risk, prior to the setting of health based targets. The health risk assessment includes both QMRA and epidemiological surveys.

Several health based targets are set in the guidelines, including treatment performance targets, quality targets, and management practices. While the performance and quality targets are mainly focused on the pathogen content, the management practices are more concerned about reducing the exposure of pathogens to the individuals, aiming at creating multiple barriers to break the fecal-oral cycle. Some of the recommended health interventions for the use of fecal sludge are treatment, working sludge into soil, use of protective clothing, crop restriction, hygienic food handling, produce washing and processing, and hygiene promotion.

1.3.1 Quantitative microbial risk assessment

Quantitative microbial risk assessment (QMRA) is a tool for calculating the health risk as a result of exposure to pathogenic microorganisms. It is comparable to risk assessments for chemical substances, but the uncertainties are in general much larger for the microbial risk assessment. The risk assessment is the first of three steps of a risk analysis; the other two are risk management and risk communication. These are defined as follows by Haas et al. (1999):

> *Risk assessment – the qualitative or quantitative characterisation and estimation of potential adverse health effects associated with exposure of individuals to hazards (materials or situations, physical, chemical and/or microbial agents).*
>
> *Risk management – the process for controlling risks; weighing policy alternatives and selecting the most appropriate action taking into account risk assessment, values, engineering, economics and legal, social and political issues.*
>
> *Risk communication – the communication of risks to managers, stakeholders, public officials and the public; includes public perception and ability to exchange scientific information.*

The QMRA process as described by Haas et al. (1999) has the following four steps:

1. Hazard identification
   - Identify relevant pathogens
2. Dose-response assessment
   - Relationship between doses and negative health effect on exposed population
3. Exposure assessment
   - Estimate frequency, amount and duration of exposure for relevant transmission routes
4. Risk characterization
   - Estimate public health risk, and evaluate uncertainty and sensitivity

QMRA should not be overall conservative, but try to give the best possible estimate of the actual health risk. It is an iterative process, where the quantifications should be re-estimated

when new and better data are available. Epidemiology is a tool that can give information on the compliance between the risk assessment and the health outcome, and estimates from QMRA should be compared with relevant epidemiological data.

1.4 Inactivation of pathogens

The inactivation of pathogens is the key point to achieve safe fertilizer products from urine and fecal matter. Since the health based targets often are set as inactivation requirements, the most resistant pathogens are chosen to verify inactivation.

Inactivation of pathogens is crucial to achieve safe use of excreta in agriculture. Pathogen inactivation can generally be assumed to follow first-order kinetics, which can be described by the following equation:

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \quad (1)$$

where C_0 is the initial pathogen concentration, C_t is the pathogen concentration after a given time t, and k is the reduction coefficient.

Inactivation of bacteria and bacteriophages has in some cases been observed to have an initial faster die-off at the beginning of the treatment (Niwagaba et al. 2009; Vinnerås et al. 2008). For *Ascaris* inactivation the opposite has been observed, with a lag-phase in the beginning of treatment with low inactivation rate, also referred to as shouldered inactivation (Nordin et al. 2009a; Pecson et al. 2007). However, first-order kinetics can generally be applied for the purpose of inactivation description without too much error.

1.4.1 Factors affecting inactivation

Temperature is the most important factor for pathogen inactivation. Most pathogens die at high temperatures (>40 ℃) , and temperatures of around 55-65 ℃ will kill all pathogens except *Chlostridia* spores within hours (Haug 1993, cited in Niwagaba 2007).

pH is, second to temperature, the single most important factor for pathogen inactivation in many ecological sanitation systems. Most microorganisms are adapted to neutral pH, though the enteric microorganisms have to resist the acid condition in the stomach to be infective. Many enteric viruses are resistant to both high and low pH.

Ammonia is known to be toxic to many microorganisms, especially for concentrations above 50 mM, and is showed to cause inactivation of bacteria, viruses, protozoa and helminthes (Jenkins et al. 1997; Nordin et al. 2009a; Vinnerås et al. 2009; Ward & Ashley 1977).

Drying may inactivate most bacteria, while some viruses, spores and helminthes are quite resistant to such treatment. A moisture content of less than 5 % is needed to inactivate *Ascaris* (Jimenez et al. 2006).

Competing microorganisms and predation may be the most important inactivation mechanism for some systems where fecal matter is mixed with soil or manure (Deng & Cliver 1995; Niwagaba et al. 2009).

Exposure to UV light reduces the number of viable pathogens, and thus pathogens have shorter survival times on the surface of soil and plants than below the soil.

Aerobic conditions favor the inactivation of viruses, since air has a denaturating effect on the proteins.

1.5 Ammonia treatment with urea

Urea degrades into ammonia and carbonates in a hydrolysis reaction called ureolysis, see reaction (2). This process is catalyzed by the enzyme urease which is produced by many common bacteria and fungi.

$$CO(NH_2)_2 + 3\ H_20 \rightarrow 2\ NH_4^+ + OH^- + HCO_3^- \qquad (2)$$

Addition of 2 % w/w urea in fecal matter with 17 % dry matter content, yields a pH value of about 9 and approximately 230 mM uncharged ammonia (Nordin et al. 2009a). Uncharged ammonia is shown to be toxic to most bacteria, viruses, protozoa and helminthes.

Urea is the most common nitrogen mineral fertilizer in the world, and using it as a disinfection agent in fecal matter will increase the fertilizer value in the SIP (Nordin et al. 2009b). The ammonia is not consumed in the process, thus there is no reduction in the disinfection potential over time and the fertilizer value will be sustained, given that the storage facility is gas-tight.

Ammonia is not dependant of extensive mixing since it migrates well through fecal matter, especially when dry matter (DM) content is low. With DM content of 5 %, the diffusion of

ammonia is faster than 2,5 cm h^{-1}, while it may take 3 days to reach 5 cm at 15 % DM content (Vinnerås et al. 2009).

The ammonia equilibrium is very important since it is the uncharged ammonia (NH_3) that is toxic to micro-organisms. The fraction of uncharged ammonia is increased with higher temperature and higher pH, see figure 1. The equilibrium constant pKa can be calculated by the following formula (Clement & Merlin 1995):

$$pKa = 0.09018 + 2729.92/T \quad (3)$$

where T is the temperature given in °K. The NH3-percentage can then be calculated with the following formula:

$$\% - NH_3 = \frac{100}{1+10^{(pKa-pH)}} \quad (4)$$

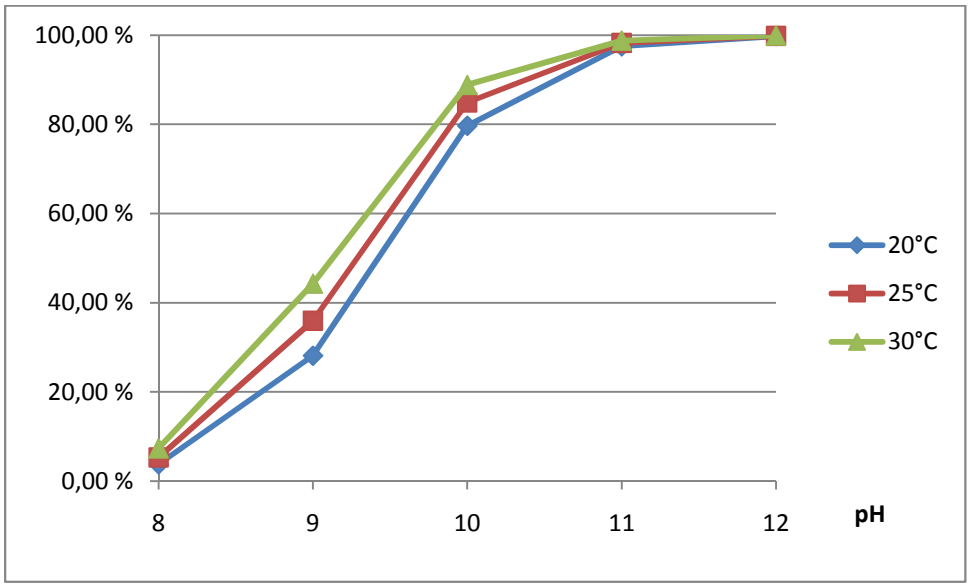


Figure 1: Fraction of ammonia that is in uncharged state (NH_3)

1.5.1 Bacteria

Salmonella Typhimurium and *E.coli* are both gram-negative bacteria and major agents of foodborne disease, but are rapidly inactivated by ammonia (Nordin et al. 2009b; Vinnerås et al. 2003). Other gram-negative pathogens like *Vibrio Cholerae, Helicobacter pylori* and *Campylobacter,* which all are associated with wastewater irrigation, were also assumed to be inactivated rapidly and thus insignificant in this context. The gram-positive bacteria

Enterococcus is more resistant to ammonia than the gram-negative, but is less resistant than the viral indicators.

The spores of *Clostridium* are not inactivated by ammonia, but the health impact of clostridium spores is not believed to be important when it comes to agricultural use of fecal matter (Sherpa et al. 2009).

1.5.2 Viruses

Diarrhea is yearly responsible for the deaths of 1.87 million children below 5 years, representing 19 % of all children deaths. Studies have found viral agents in 43% in samples from diarrhea in developing countries (Ramani & Kang 2009). Viruses are in general persistent to high pH values, and may survive long in the environment. Enteric viruses are especially resistant to low pH, being adapted to the environment in the gastrointestinal tract, and are often resistant to high pH as well. Viruses causing other diseases, like respiratory diseases, may also be shed in feces and cause severe health effects.

The virucidal effect of ammonia is suggested to be RNA and protein cleavage (Cramer et al. 1983; Ward & Ashley 1977). Non-enveloped single stranded RNA viruses belonging to the picorna- and caliciviridae are sensitive to ammonia and rapidly inactivated.

Hepatitis A virus is a picorna-virus shown to transmit through crops irrigated with fecally contaminated water (Hernandez et al. 1997), but is assumed to inactivate rapidly by ammonia since other picorna-viruses are rapidly inactivated by ammonia.

Norovirus is the main source of foodborne disease worldwide, and has a low infectious dose (Teunis et al. 2008). Mourine norovirus, which is closely related to human norovirus, is inactivated with approximately 1 $\log_{10}$ unit per week in solution at room temperature at neutral pH (Cannon et al. 2006). This should cause an approximate 8 $\log_{10}$ reduction in 2 months, and norovirus is thus not important in this setting.

Rotavirus is the main cause of childhood diarrhea in developing countries, and is responsible for the mist severe form of viral gastroenteritis in humans, resulting in huge economic impact on society (Gerba et al. 1996). It has a very low infectious dose, is quite resistant to high pH, and no significant inactivation effect of ammonia was found for the ammonia

concentrations in urine (Höglund et al. 2002). Food contaminated with rotavirus from fecally contaminated water have been reported (Hernandez et al. 1997).

After rotavirus, enteric adenovirus (type 40 and 41) is reported to be the second most common viral agent of childhood diarrhea (Crabtree et al. 1997). Adenoviruses are in general very persistent in the environment, and are often recovered in large numbers in sewage sludge (Bofill-Mas et al. 2006). An ability to use host cell repair enzymes to repair DNA damaged by UV radiation may also prolong the survival in the environment (Thurston-Enriquez et al 2003, cited in Mena & Gerba 2009). *Salmonella* typhimurium phage 28B, which has to similar genome structure to enteric adenovirus, is slowly inactivated, but a significant effect of ammonia treatment is observed (Nordin et al. 2009b).

1.5.3 Protozoa

Protozoa are of major impact on foodborne disease, especially for the consumption of fresh fruit and vegetables. The most common species are *Cryptosporidium*, *Giardia* and *Cyclospora*. *Cryptosporidium* is commonly used as a model for protozoa inactivation, being more resistant to treatment than *Giardia* (Olson et al. 1999). Inactivation of *Cryptosporidium parvum* by ammonia has been studied by Jenkins et al. (1998), and shows that the oocysts were rapidly inactivated, with 5 $\log_{10}$ reduction at less than 6 days at an ammonia-concentration of 148 mM, which is equivalent to 1 % urea treatment. Uncharged NH_3 ions are suggested to be able to pass the membrane and raise the internal pH. Charged OH^- ions are unable to penetrate the cysts explaining the resistance to high pH values. The cyst wall will also be more permeable when pH is elevated, allowing the ammonia to enter more readily.

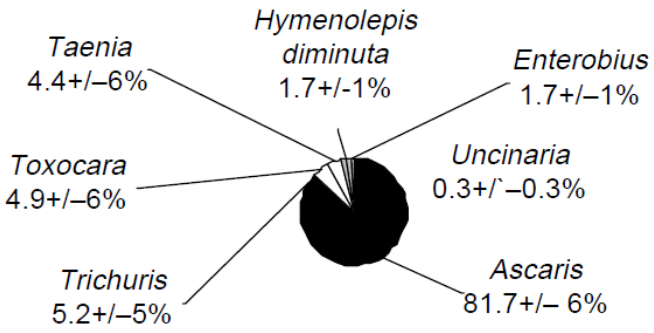
According to Dawson (2005), the largest outbreaks of foodborne disease are associated with *Cyclospora*. Unfortunately, inactivation data for this parasite is lacking.

1.5.4 Helminthes

Ascaris the most widespread soil-transmitted helminth and is estimated to have infected 1,4 billion people in the world. It has a low infectious dose, and *Ascaris* eggs are considered as one of the most persistent helminth ova, due to a multilayered structure of lipid and chitin (Schönning 2001). There are other helminthes that cause severe diseases, but studies indicate that *Ascaris* is at least as persistent as other helminthes in the environment, see

figure 2. *Ascaris* infection can have serious physical and mental consequences, especially for children. This can be cognitive and societal impairment, growth failure, malnutrition and higher susceptibility to infection (Pecson & Nelson 2005).

a)



b)

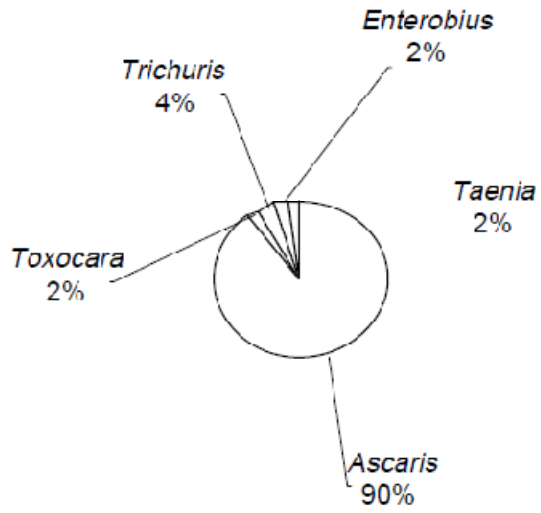


Figure 2: Helminth ova distribution in a) ecosan sludge and b) crops grown in ecosan sludge. (South Africa). Reference: Jimenez et al.(2006)

Schistosoma haematobium is widespread in Africa, but is dependent on nearby water bodies to survive due to a complex parasitic life-cycle. Tchounwou et al. (1991) showed that the *Schistosoma miracidiae* was being rapidly inactivated (2 $\log_{10}$ after 4 hours) at 1 % urea addition in water.

1.6 Alkaline treatment with ash

Using ash as an additive to sanitize fecal matter increases the pH value. The increase is dependent on temperature and the chemical composition of ash, mainly the content of carbonates and hydroxide content. The chemical characteristics of ash from firewood

combustion vary a lot depending on type of firewood and combustion temperature. The calcium carbonate equivalent (CCE) from wood-fired boiler-ash samples has been reported to vary from 13 to 92 %, with a median value of 48 % (Vance 1996, cited in Demeyer et al. 2001). According to Naylor & Schmidt (1986, cited in Etiegni & Campbell 1991), carbonates and bicarbonates predominate when the combustion temperature is below 500°C, while oxides become more prevalent with temperatures over 1000°C which is common for industrial woodfired boilers. The alkaline effect of ash can be highly temperature dependent. In a study where 1 litre ash was added per kg fecal matter, the initial pH value was 10-10.5 at 24℃ and 12.7-12.8 at 34℃ (Nordin et al. 2009a). It can be difficult to maintain a stable pH during the treatment period, and a decline in pH is often observed due to chemical or biological reactions (Pecson et al. 2007).

Using ash as an additive increases the dry matter content and reduces the moisture in the fecal matter, causing inactivation of several pathogens. On the other hand, the low moisture content results in non-homogeneous distribution of the hydroxide ions, reducing the effectiveness of the high pH. This explains why inactivation of pathogens may be low even with pH values above 12 (Moe & Izurieta 2003). Adding water to sustain the normal dry matter content (approx 17 %) has been done in several inactivation studies where ash is added (Nordin et al. 2009a), and a similar practice could be used in full-scale to improve the distribution of hydroxide ions in large scale sanitation as well.

1.6.1 Combination of alkaline and ammonia treatment

According to Nordin et al. (2009), addition of ash prior to urea is not recommended, since the increase of pH resulting from the ash inhibits the hydrolysis of urea. However, it should be possible to add the urea first, and then add ash when the urea was degraded. Addition of ash increases the pH which yields a higher proportion of uncharged ammonia, thus enhancing the inactivation process. On the other hand, the prevention of odor and insects by adding ash at the toilet would be lost due to such management. Furthermore, water has to be added in order to maintain the original moisture content of approximately 83 % in the fecal matter, which will enlarge the storage volumes needed. The effect of an increased proportion of uncharged ammonia will be partly lost due to dilution with water.

Other types of alkaline treatment could be used in combination with ammonia treatment, such as lime or potassium hydroxide (KOH). Since ash is commonly used in ecological sanitation systems in Uganda, this was chosen as alkaline agent in this study.

1.7 Aims of study

- Evaluate different treatment alternatives combining urea and ash treatment, and their impact on pathogen inactivation and health risk.
- Assessing the health risk associated with application of treated fecal sludge using QMRA, assuming that the farmers and the children are doing the work without using protective clothing.
- Assessing the risk of foodborne transmission by consuming crops grown in soil amended with treated fecal sludge using QMRA. Carrots and spinach were chosen since they are consumed raw and where the edible parts grow below and above soil, respectively.
- Evaluate the potential for enhancing the ammonia-based inactivation of dsDNA viruses by increasing pH, by studying the inactivation of *Salmonella* typhimurium phage 28B in fecal matter treated with urea and ash.

2. Methods

2.1 Hazard identification

Several pathogens were evaluated to consider which ones to include in the QMRA. The main criteria for selection were infective dose, prevalence in developing countries generally and Uganda specifically, and resistance to alkaline and ammonia treatment and temperature inactivation.

Table 1: Infective dose and ammonia-based inactivation of some pathogens

Pathogen		ID_{50}	t_{90} [days]	NH_3 [mM]	Medium	pH	Temp
Salmonella	Gram -	23600[a]	<1[f]	102-177	Feces	9	24
EHEC	Gram -	208[b]	<0.7[g]	ca 1100	Feces	9.3	24
Enterococcus	Gram +		14[f]	102-177	Feces	9	24
Clostridium	Spores		NI[g]	ca 1100	Feces	9.3	24
Poliovirus type 1	ssRNA	76[a]	<<1[h]	50	Buffer	9.5	21
Coxsackievirus	ssRNA	48[a]	<<1[h]	50	Buffer	9.5	21
Echovirus 11	ssRNA		<<1[h]	50	Buffer	9.5	21
Rotavirus	dsRNA	6.17[a]					
Adenovirus	dsDNA	1.7*[c]	61**[i]	NR	Sewage	NR	22
Cryptosporidium	Protozoa	165[d]	<2[j]	60	Buffer		24
Ascaris	Helminth	859[e]	23.5[k]	130	Feces	8.9	24

NI: No inactivation ; NR: Not reported; * type 4; ** type 5
References: [a]Haas et al. (1999), [b] Schönning et al. (2007), [c] Mena & Gerba (2009), [d] Haas et al. (1996), [e] Navarro et al. (2009), [f] Nordin et al. (2009b), [g] Vinnerås et al. (2003), [h] Ward & Ashley (1977), [i] Bofill-Mas et al. (2006), [j] Jenkins et al. (1998), [k] Nordin et al. (2009a).

Based on the data presented in table 1 and epidemiological data, *Ascaris* was included in the QMRA, while no bacteria or protozoa were included. Health impact of Rotavirus and Adenovirus could be significant, but complete risk assessment could not be done due to lack of data. The impact of different treatment options on rotavirus inactivation will be discussed in section 4.4.3, while an inactivation study was conducted to study the inactivation of *Salmonella* typhimurium phage 28B. Since this is a dsDNA virus, it could be an indicator of the inactivation of adenovirus.

2.2 Dose-response assessment

A dose-response distribution for *Ascaris* established by Navarro et al. (2009) based on epidemiological surveys among children <15 years in Mexico was applied. The dose-response relationship is described by the Beta-Poisson distribution, given in equation (5):

$$P_i(d) = 1 - \left[1 + \frac{d}{N_{50}} \cdot (2^{1/\propto} - 1)\right]^{-\propto} \quad (5)$$

where P_i is the infection risk per exposure, N_{50} is 859 and α = 0,104. The annual risk is calculated with equation (6):

$$P_{year} = 1 - (1 - P_i)^n \quad (6)$$

where n is the number of exposures per year.

2.3 Exposure assessment

2.3.1 Exposure on farm site

For the exposures on the farm, 10 days of application work was assumed. The amount of SIP accidently ingested per day assuming protective clothing is not used, was modeled with a triangular distribution with minimum value 50 mg, most likely value 150 mg, and max value 480 mg. This is based on quantifications reported in Gerba et al. (2002).

Aerosol transmission was not assumed to be significant, since the agriculture is not mechanized and tractors are not common. Accidental exposure when gardening was considered insignificant compared with the application due to the dilution in soil. Vectorborne transmission was not included due to lack of adequate modeling possibilities.

2.3.2 Foodborne transmission

A study by Jimenez et al. (2006) showed a linear relationship between Helminth Ova (HO) content in soil and HO content in carrots and spinach leaves. The HO content in soil was measured as HO/cm^2, and the HO on crops as HO/g dry matter. The HO attachment to vegetables was quantified by applying linear regression on the data reported, giving estimates of 0.7 and 2.5 $\frac{\text{HO/g TS vegetable}}{\text{HO/cm}^2}$ for carrots and spinach respectively.

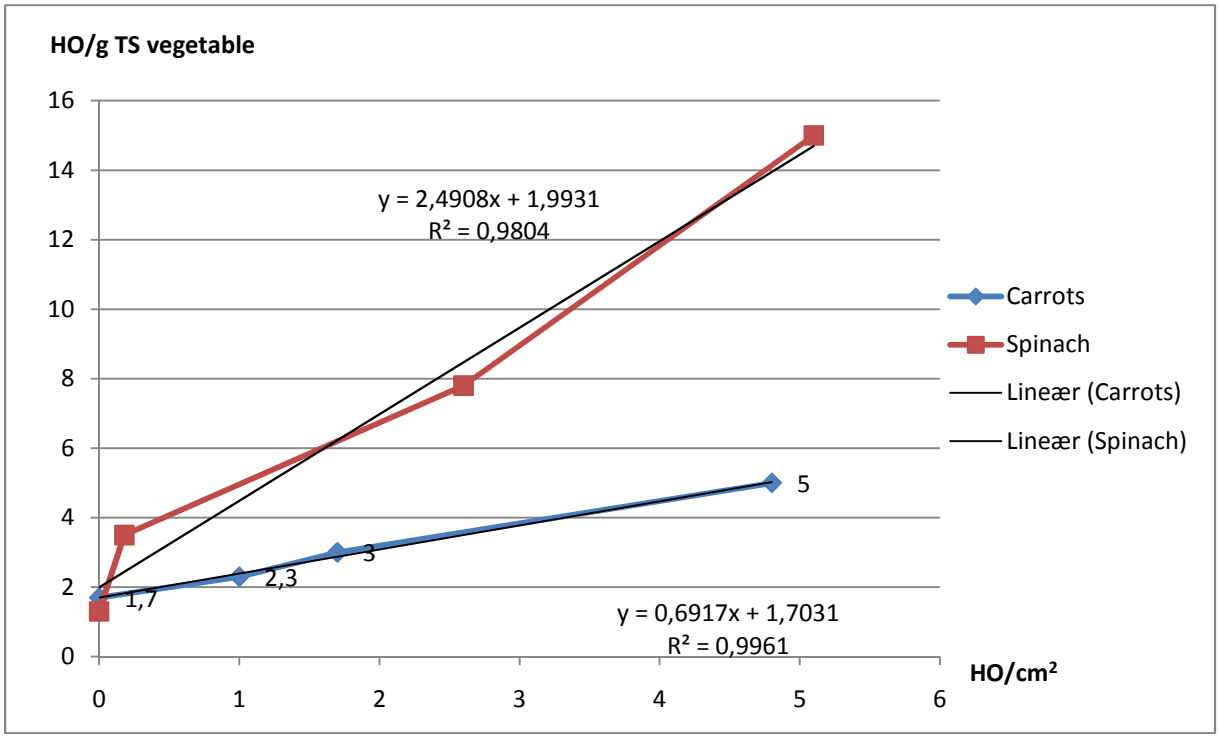


Figure 3: Attachment of HO to crops, based on data reported by Jimenez et al. (2006)

Several estimates have been used in QMRA's for the amount and frequency of vegetable consumption (see table 2). In this risk assessment, the US EPA estimates for childrens consumption were applied as a uniform distribution, and the estimate from Shuval et al. (1997) was applied as a deterministic estimate for grown-ups consumption. Lettuce consumption rates were used since spinach consumption rates are not available.

Table 2: Estimates on vegetable consumption used in QMRA

Vegetable	Amount [g]	Frequency [year-1]	Place	Reference
Carrot	43	121	US	US EPA 1997*
Carrot	28-38	52	US, Children	US EPA 2002*
Lettuce	65	121	US	US EPA 1997*
Lettuce	30-54	52	US, Children	US EPA 2002*
Lettuce	10-12	208	Urban Ghana	Seidu et al. 2008
Lettuce	11.5	365		Ashbolt 2004
Lettuce	100	150	Israel	Shuval et al. 1997

*** cited in Navarro et al. (2009)**

A 1 $\log_{10}$-reduction of *Ascaris* due to crop washing was assumed for spinach (WHO 2006), while a 2 $\log_{10}$ reduction was assumed since carrot is commonly peeled in Uganda (C. Niwagaba, pers. comm.). If the SIP was applied in furrows, the pathogenic contamination of spinach was assumed to be reduced with 2 $\log_{10}$ (Schönning et al. 2007).

For 4 % urea dosage, 6 and 12 tonne sludge/ha will be sufficient to achieve 50 and 100 kg N/ha, which is recommended for carrots and spinach, respectively (Jimenez et al. 2006). Since the fertilizer use in Uganda is very low (O. Semalulu, pers. comm.), the sludge application rate was modeled by a triangular distributions with range 1-2-3 tonne/ha and 2-4-6 tonne/ha for carrots and spinach, respectively.

2.3.3 Prevalence

The pathogen prevalence has a rather large impact on the health risk associated with the use of human excreta, since higher prevalence yields a higher probability that the fecal matter accidently ingested comes from an infected individual. For the estimation of average pathogen prevalence, age distribution was combined with age-specific prevalence data. The age-distribution for Uganda is showed in table 3.

Table 3: Age distribution in Uganda

Age	Population
0-14	50 %
15-64	47.9 %
65+	2.1 %

Reference: CIA (2010)

Ascaris prevalence is strongly dependent on age, with a much higher prevalence among young people. Based on data presented in table 4, prevalence rates of 17 % and 3 % was chosen for the population groups 0-14 years and 15+, respectively.

Table 4: Prevalence of soil-transmitted helminthes

	Age	Ascaris	Trichuris	Hookworm
Primary schools in Kampala, Uganda[1]	10-15	17 %	28 %	12.9 %
Schools at shore of Lake Victoria, Uganda[2]	6-10	9.3 %	12.9 %	2.4 %
Children in urban slum, Karachi, Pakistan[3]	1-5	16.5 %	-	-
Pregnant women in Entebbe, Uganda[4]	14-47	2.30 %	9 %	44.50 %

References: [1] Kabatereine et al. (1997), [2] Standley et al. (2009), [3] Mehraj et al. (2008), [4] Woodburn et al. (2009)

The number of *Ascaris* eggs in fecal matter from an infected individual was modeled as e^x, where x is normal distributed with μ=9,5 and σ=0,6. This is derived from data reported by Holland et al. (1989, cited in Nordin et al. 2009a).

2.3.4 Inactivation

Inactivation rate of *Ascaris* during ammonia and alkaline treatment has been widely studied. *Ascaris suum*, which infects pigs, is often used as a model organism, but the inactivation rates are reported to be equal to *Ascaris Lumbricoides*, which infects humans (Ghiglietti et al. 1995, cited in Nordin et al. 2009a).

To find the inactivation rate as a function of the temperature, the data on inactivation of *Ascaris* reported by Nordin et al. (2009a) and Pecson et al. (2007) was studied. Inactivation rates for batches with similar pH and NH_3 concentrations but with different temperature was tested against different equations to find the temperature impact.

In order to describe the inactivation rate as a function of pH and ammonia concentration, *Ascaris* inactivation rates with different pH, ammonia concentrations and temperature reported by Ghiglietti et al. (1997), Pecson et al. (2007) and Nordin et al. (2009a) was systemized and tested for several mathematical equations using JMP Statistical Software (SAS Institute, Inc., Cary, NC). One of the equations was chosen, trying to both minimize the sum of squared prediction residuals, and keep the number of regression parameters at a minimum.

Inactivation rate is by Ghiglietti et al. calculated by using a formula for shouldered inactivation. In order to compare the inactivation rate with data from Nordin et al. and Pecson et al. who is assuming first-order kinetics, the decay rate k was recalculated assuming first-order kinetics. The ammonia concentration in ammonium hydrate used in the study by Ghiglietti et al. was not reported, so this was implemented as an unknown variable in the regression. K rates from different temperatures were adjusted by using the formula found to describe the temperature effect. Reported inactivation rates with $[NH_3] < 40$ mM was not included in regression. See appendix A for background data used in regression study.

Inactivation on secondary treatment unit

The inactivation in the secondary treatment unit was calculated using the equation found by regression. Three different urea doses, 2, 3 and 4 % w/w, with and without addition of 100 g ash/kg, resulted in 6 treatment alternatives summarized in table 5. Water was assumed added when ash is added, to maintain a dry-matter content of 17 %, resulting in a dilution of the ammonia with a factor 1,6. The pH value of 9,5 are based on interpolation of data from

the research described in section 2.5, and was assumed to be similar for different urea doses, while the pH increase by ash addition was assumed to be 0,1 unit at 19°C. pH values for 3 and 4 % urea treatments were estimated by logarithmic interpolation of literature data, see appendix B.

The treatment period was divided into 2 intervals of different storage temperatures to give a rough estimate of the ambient temperature distribution. Based on the temperature for the coldest month July reported by World Weather Information Service and variation of temperature through the day shown by the weather forecast service yr.no, the 60 days of storage were modeled as 50 days of 19°C and 10 days of 24°C.

In addition to the ammonia from the urea, a natural ammonia content of 0,711 mM per gram dry matter was assumed, which is based on Swedish values (Jönsson et al. 2005). An ammonia-loss of 10 % during storage was assumed. The amount of free uncharged ammonia was calculated using equation (3) and (4).

Table 5: pH and ammonia concentration for the three treatment alternatives

Treatment	pH at 24°C	pH at 19°C
4 % urea + ash	9,5	9,2
4 % urea	9,1	9,1
3 % urea + ash	9,5	9,15
3 % urea	9,05	9,05
2 % urea + ash	9,5	9,1
2 % urea	9,0	9,0

The Peepoo bags contain a urea dose of 4 grams per bag. Assuming a medium weight of 200 g fecal matter per defecation, this is equal to about 2 % urea dose. It is suggested to use a shredder/grinder to divide the Peepoo bags and thus ensure an almost homogenous concentration of ammonia. If the Peepoo bags are frequently used for urine, there will be some dilution of the urea, but this was not studied in detail in this risk assessment.

Inactivation in soil

The high ammonia-content and pH in the SIP will inactivate HO eggs even after application. If the SIP is applied on top of soil, there will be a huge ammonia loss to the atmosphere, due to the high pH and high ammonia-concentration. *Ascaris* is also known to be resistant to UV-

radiation and to survive long in soil, T_{90} times of 625 days has been applied in QMRAs (Schönning et al. 2007), and a T_{90} of 420 days at 20 °C and pH 7 was reported by Pecson et al. (2007).

If the SIP is applied in closed furrows, the effect of ammonia and pH will last longer due to reduced ammonia loss, but the migration of ammonia will reduce the concentration over time. The ammonia loss strongly depend on the time before the furrow is closed, while the ammonia migration depends on soil properties like texture, water holding capacity and cation exchange capacity, and also on irrigation scheme and rainfall events. Ammonia migration is favored if the soil is wet, while advection will cause some dilution of the ammonia in dry soil. A given application rate of 5 tonne/ha corresponds to an average 0,5 mm deep layer, which is equal to a 5 mm deep layer if only 10 % of the area is covered. Because of the fast ammonia migration, no inactivation due to pH and ammonia was assumed for neither top-soil application nor furrow application.

2.4 Risk characterization

Monte Carlo simulations with 10,000 iterations were run using @RISK version 5.5.1 (Palisade Corporation, Newfield, New York). An method similar to the approach used by Hamilton was applied (Karavarsamis and Hamilton 2009; Benke and Hamilton 2008, both cited in Mara, D. & Sleigh, A. 2009). For each exposure of fecal matter, the probability of coming from an infected person where applied using pathogen prevalence, and variations in initial pathogen concentration where simulated for each exposure. Sludge application rate were not assumed to vary for exposure within the same farm and were only resimulated for each iteration and not for each exposure. Framework for the calculations of the QMRA model is shown in figure 4.

Acceptable infection risk for *Ascaris* was set to 10^{-3} per year, assuming an acceptable DALY loss of 10^{-5} per year, a $8{,}25 \cdot 10^{-3}$ DALY loss per case of ascariasis (Chan 1997) and as a worst-case scenario a disease/infection ratio of 1 (Mara & Sleigh 2009).

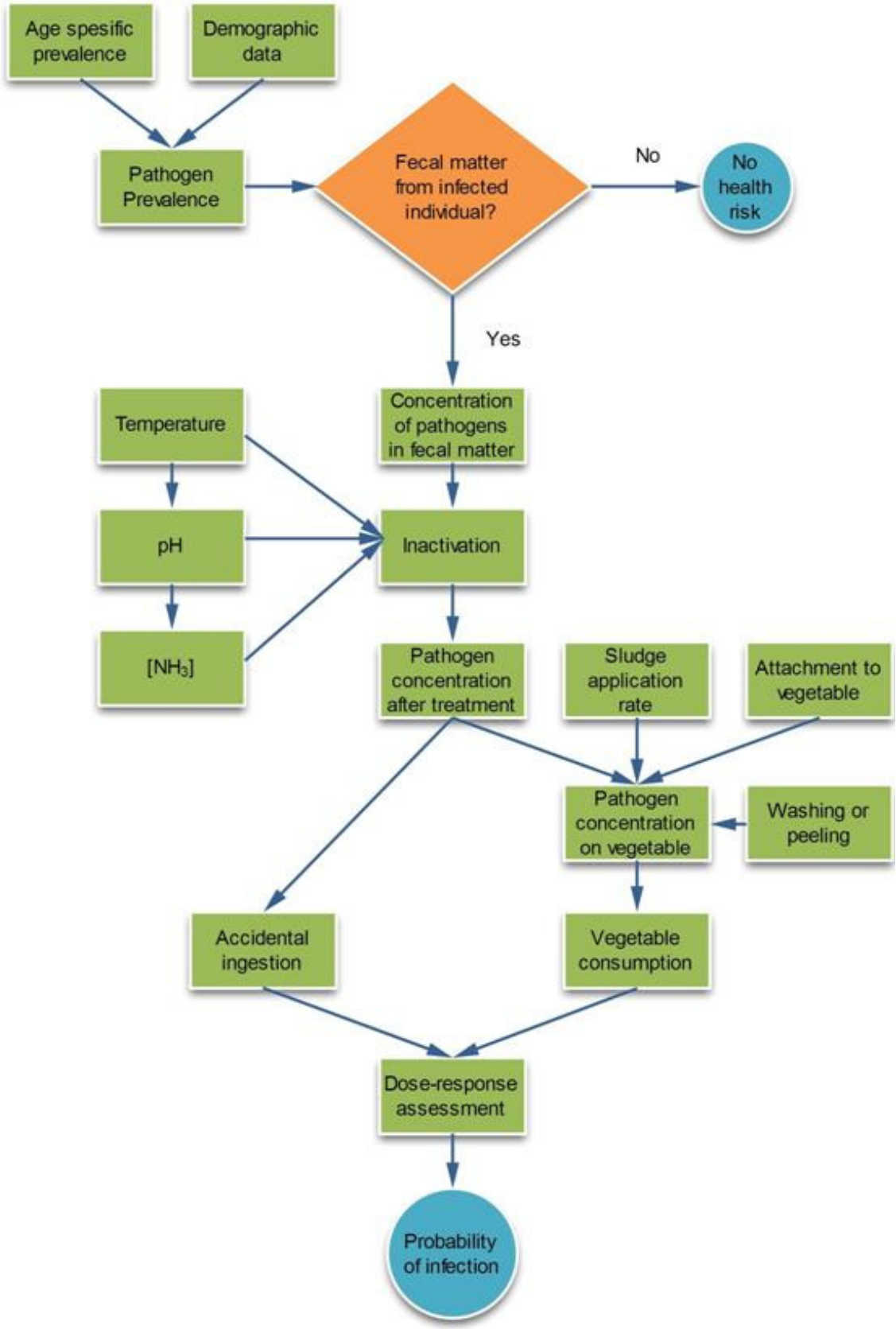


Figure 4: Overview over infection risk calculation

2.5 Inactivation of *Salmonella* typhimurium phage 28B

Fecal matter was spiked with *Salmonella* typhimurium phage 28B and treated with urea and ash. The inactivation of viruses was quantified by 4 plaque assays during 45 days of treatment.

2.5.1 Propagation of bacteriophages

1 ml of overnight culture of *Salmonella* typhimurium type 5 was diluted 1+9 in nutrient broth (NB) and incubated shaken at 37℃ for 2.5 hours. 1 ml diluted phage suspension was added. After 10 minutes of shaken incubation at 37°C, the suspension rested for 5 minutes allowing flocculation of the phages, and was then transferred to bigger e-flask, and diluted with pre-heated NB to total 250 ml suspension. After further 4.5 hour of incubation, 2.5 ml of chloroform was added and reacted for 10 minutes stirred (200 rpm). The suspension was then stored cooled to next day, and then centrifuged at 3000 rpm for 30 minutes.

2.5.2 Preparation of batches

Fecal matter from students was collected in separate plastic bags and stored cold (4℃) until use, maximum two weeks. The fecal matter was homogenized with a grinder, filled into 250 ml flasks (see figure 5 a), and then mixed with urea diluted in water to final urea concentration of 4 % and 12-17 % dry matter. The urea hydrolyzed to next day, and then ash was added to the batches. The ash was collected from several household stoves, sieved through a 4.8 mm mesh, and mixed well to avoid differences in chemical properties. Finally, 5 % wt/wt of phage suspension was added and the batch was homogenized by shaking. The flasks were airtight, and were stored at 25℃ during the experiment. Phage suspension was diluted 1:20 with 250 ml 0,1 M PBS-buffer as a control.

2.5.3 Extraction and plaque assay

For extraction of the viruses from the sludge, an extraction method originally recommended by the US EPA in 1992, and used by Schlindwein et al. (2009) and Belguith et al. (2006), was slightly modified. 2.5 (+- 0.05 gram) gram of sludge was diluted with 22.5 ml 0,1 M phosphate-buffered 10 % beef extract, and the mixture was homogenized by stirring for 30 minutes, see figure 5 b). A decontamination step used by Mignotte et al. (1999) was applied; 8 ml of chloroform was added and reacted stirred for 10 minutes. After centrifugation at 5 000 g for 30 min at 4°C, 18 ml of the supernatant was diluted in tap water and used for plaque assay, see figure 5 c).

a)  b) 

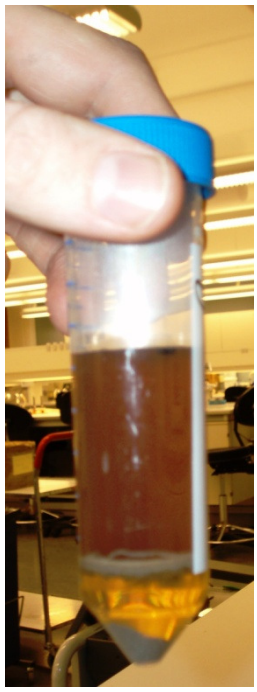
c) 

Figure 5: Batch of fecal matter spiked with phage 28B. b) Virus extraction with 10% beef extract. c) After centrifugation; chloroform phase and solids are located in the bottom of the tube.

0.5 ml of diluted virus suspension was mixed with 4 ml of soft agar (45 ℃) with 1.4 % blood agar base (CM0055, Oxoid) and 0.25 ml of an overnight host culture. This was evenly spread on the top of nutrient agar plates with 4 % blood agar base, and incubated at 37 ℃ for 18 hours. Visible plaques were then counted.

2.5.4 pH and dry-matter measurement

5 g of sludge was diluted in 45 ml deionized water on day 1 and day 45 for pH measurement. The dry matter content was measured by drying at 105°C for 7 hours.

2.5.5 Statistical analysis

The ammonia concentration was calculated by the formula (3) and (4), and by assuming 0,711 mM per gram fecal dry matter. The inactivation rate k was calculated using Microsoft® Excel 2007.

3. Results

3.1 Inactivation of *Salmonella* typhimurium phage 28B

The dilution effect of the water added to maintain moisture content for the ash addition was compensated for by the higher pH in these batches, yielding a higher proportion of uncharged ammonia. Thus the different batches had about the same concentration of uncharged ammonia (

table 6), making it possible to differentiate ammonia effect from pH effect. However, the inactivation study showed no effect of increasing pH in the range 9-11, see figure 6.

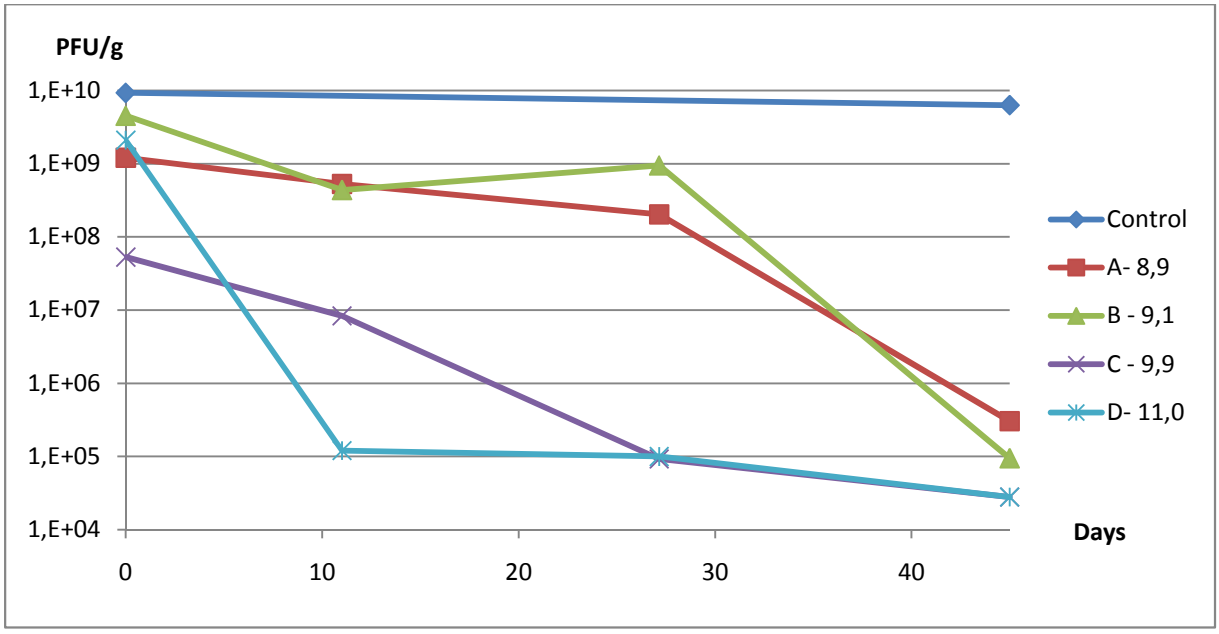


Figure 6: Inactivation of *Salmonella* typhimurium phage 28B with different pH

Table 6: pH range, NH3 concentration, dry-matter content and inactivation rates

Batch	pH	Ash [g/L]	NH_3 [mM]	DM	k	R^2	T_{90}	Extraction pH
A	8,9	0	421	12 %	0,077	0,84	13,0	7,7
B	9,1-8,8	50	454	14 %	0,093	0,76	10,8	7,5
C	9,9-10,1	200	563	15 %	0,077	0,94	13,0	7,3
D	11,0-10,9	400	452	17 %	0,090	0,63	11,1	7,4

3.2 Inactivation of *Ascaris*

The following equation gave a good fit with the inactivation rates for different temperatures:

$$k(T) = (T \cdot f)^{(T+g)} \quad (7)$$

where k(T) is the inactivation rate k as a function of temperature T, and f and g are regression coefficients.

The reported inactivation rates gave a good fit with the following equation:

$$\mathrm{k}([\mathrm{NH_3}], \mathrm{pH}, \mathrm{T}) = \left[(\mathrm{pH} - \mathrm{a})^{\mathrm{b}} \cdot [NH_3]\text{^}c + d\right] \cdot \mathrm{e} \cdot (\mathrm{T} \cdot \mathrm{f})^{(\mathrm{T+g})} \quad (8)$$

where $[NH_3]$ is the concentration of uncharged ammonia given in mM, a and b are regression parameters determining the interpolation of the pH effect, c is a regression parameter determining the extrapolation of the NH_3 effect, d is a regression parameter representing the effect of the constant temperature dependant inactivation, e is regression parameter, and f and g are regression parameters from equation (7). For ammonia and pH level corresponding to 2 % urea, formula (8) gave a k-value that was 15 % higher than the value reported by Nordin et al. (2009a), and thus the parameter e was adjusted so these values became similar. The values of the regression coefficients are given in table 7.

Table 7: Regression parameters

Parameter	Value
a	8,84
b	0,332
c	0,893
d	7,83
e	2,92E+31
e adjusted	2,53E+31
f	7,30E-03
g	2,15E+01

The formula resulted in estimated inactivation rates and *Ascaris* content in fecal matter from infected individual as mentioned in table 8. Compared to the guideline value of <1 egg/g wet weight, 4 % urea treatment will be sufficient for all of the fecal matter, while the for 3 % urea treatment, the average concentration of *Ascaris* will be <1 egg/g.

Table 8: *Ascaris* inactivation and concentration after treatment

Treatment	*Ascaris* inactivation (log_{10} reduction in 60 days)	Mean *Ascaris* content (eggs/g wet weight)	Median *Ascaris* content in sludge from infected individual (eggs/g wet weight)	*Ascaris* content in sludge from infected individual, 95 % percentile (eggs/g wet weight)
4 % urea + ash	5,6	0,004	0,03	0,09
4 % urea	5,5	0,005	0,04	0,10
3 % urea + ash	4,2	0,09	0,8	2,1
3 % urea	3,9	0,2	1,7	5
2 % urea + ash	3,0	1,8	14	38
2 % urea	2,5	5	41	109

Since the addition of ash did not increase the *Ascaris* inactivation very much, the ash treatment alternatives are not included in section 3.3.

3.3 Health risk assessment

3.3.1 Accidental ingestion during application

The health risk of accidental ingestion during application of SIP is shown in figure 7:

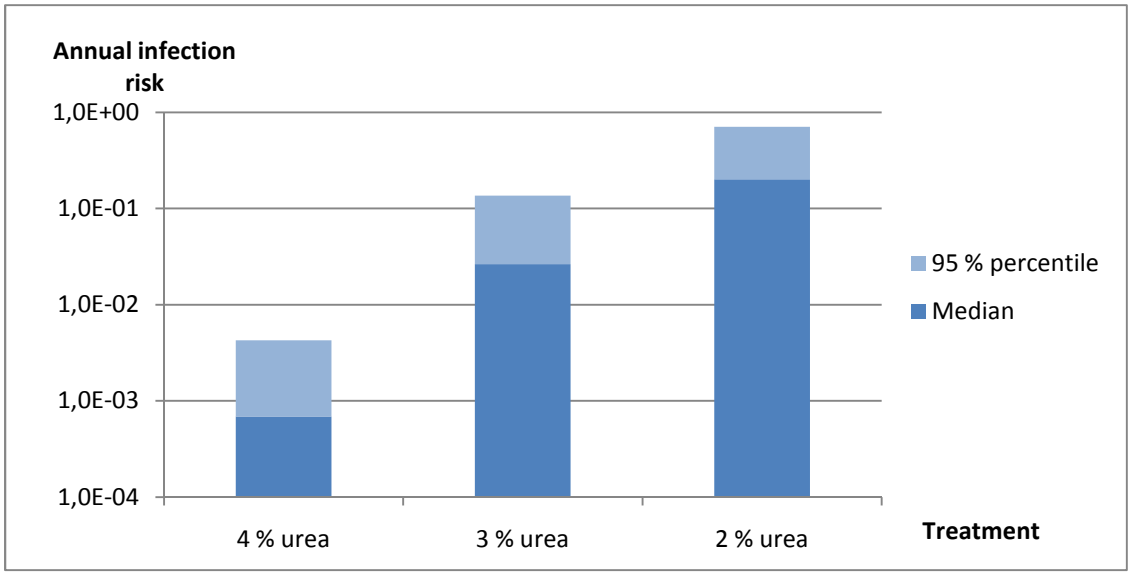


Figure 7: Annual *Ascaris* infection risk for accidental ingestion during application of SIP

Considering a 10^{-3} limit for acceptable infection risk, only the 4 % urea treatment alternative resulted in acceptable risk, given that protective clothing is not used. If the farmers are using protective clothing and gloves, the amount of SIP accidentally ingested will be reduced, and the direct risk associated with application is lowered.

3.3.2 Foodborne exposure

The health risks for foodborne exposure calculated by QMRA are showed in figure 8:

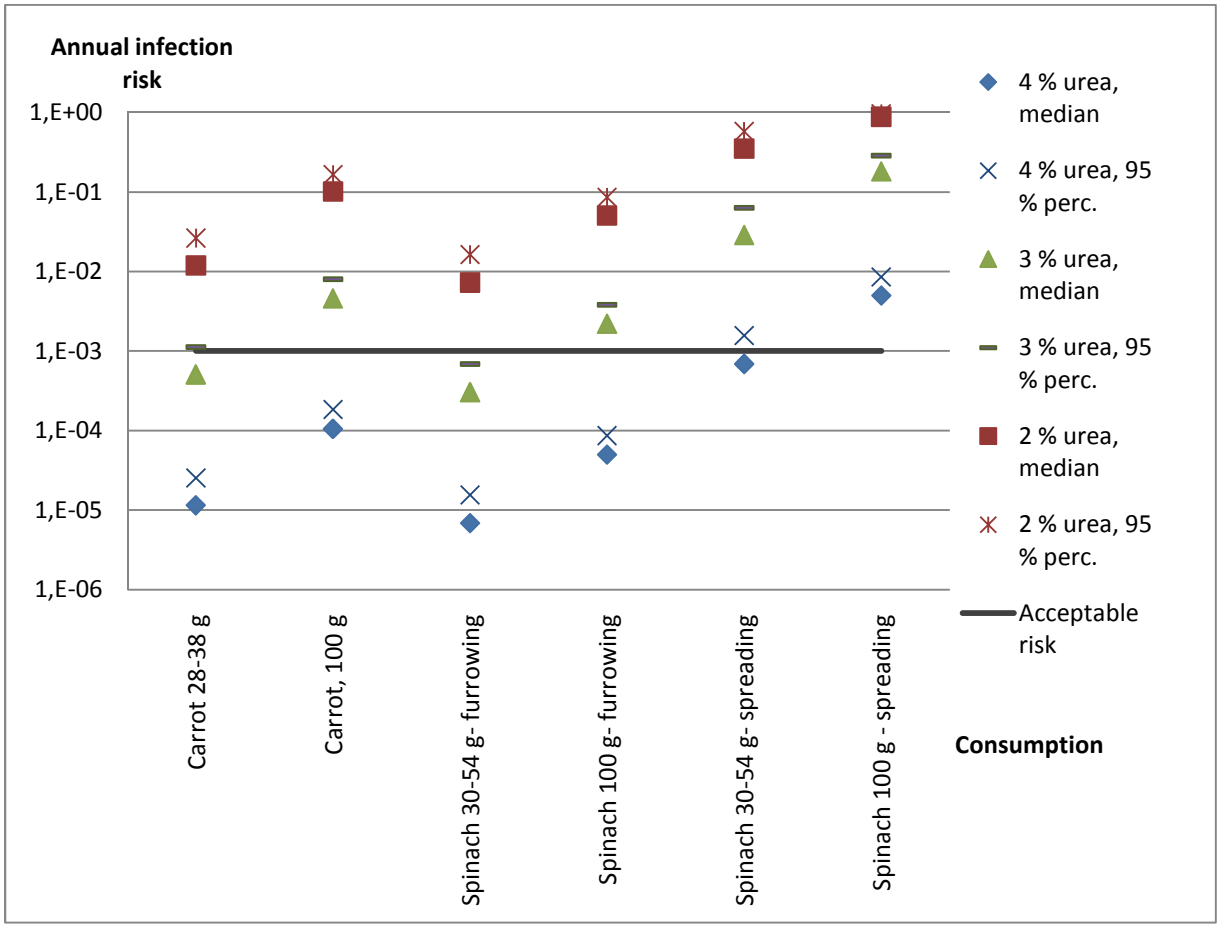


Figure 8: Annual *Ascaris* infection risk for consumption of vegetables

For the case of carrot and spinach consumption, only the 4 % urea treatment resulted in sufficient inactivation to avoid unacceptable risk when grown ups consumption rate is considered, assuming that the SIP is applied in furrows. 3 % urea treatment is sufficient for childrens consumption rate.

4. Discussions

4.1 Professional team

Experiences from other countries show that farmers do not always use the protective clothing even if it is provided. In Ghana, only 13 % of farmers used provided protective clothing, and gloves were not used consistently but were sometimes taken off (Seidu & Stenström 2010 (submitted)). On the other hand is farmer's perception of the safety of fecal sludge strongly related to smell and humidity, and they will probably be more careful when handling urea treated sludge due to the strong ammonia smell and high moisture content compared to dried fecal sludge.

Due to poor hygiene and lack of hand washing, transmission of pathogens from the fields to the household can cause infections in individuals not taking part in the application. Even if protective clothing is used, transmission to the household can happen since the washing of the protective clothing is done by girls or mothers who transfer the pathogens to children in the household (Seidu 2010). So even if children are not directly exposed to the fecal sludge, using a professional team would probably reduce their exposure to pathogens.

4.2 Crop restrictions

Since it is children <15 years that are most exposed to *Ascaris* infections, they are of main concern. For older people, the risk of infection will be lower and the consequences of infection less. Assuming that children < 15 years in Uganda eat vegetable amounts in agreement with the assumptions used in this study, 3 % should be sufficient to avoid unacceptable risk of foodborne *Ascaris*, even though figure 8 recommends 4 % urea with respect to grown ups consumption.

The health risk associated with lettuce cropping is strongly related to the application method. Spreading the SIP on the top of the soil surface will be unfavorable concerning ammonia-loss, and it is likely that farmers will be aware of this and follow recommendations for furrow application if this is communicated properly. Thus the risk of contamination will probably be acceptable even if the farmers do the application work themselves.

If 2 % urea is chosen for the treatment and vegetables consumed raw is not recommended growing in the sludge, it is not sure that this recommendation is followed. Still, because of

the high moisture level and the ammonia smell, it is more likely that these restrictions are followed. Farmers selling crops with high pathogen content on the markets could be a problem, but whether this is significant compared to contamination from food handlers is not sure.

4.3 Uncertainty and limitations of method

4.3.1 *Ascaris* inactivation formula

Formula (8) is an attempt to find the best possible extrapolation of ammonia concentrations and interpolation of the pH effect to describe inactivation of *Ascaris*. However, the choice of input formula determines the shape of both the extrapolation and interpolation, represented as coefficients b and c, respectively. The inactivation rate is especially sensitive to the extrapolation of the effect of ammonia since the ammonia levels in the data are much lower than input data used to estimate *Ascaris* inactivation at 4 % urea.

The inactivation formula for *Ascaris* should not be used uncritically, especially not for treatment periods that are shorter than the background data used for the regression. This is because of the lag-phase of the inactivation, which can last for at least two weeks at 24° and 1% urea dose, and even longer at lower temperatures and lower dosages. The accuracy of the formula is also very limited the lag phase is not accounted for. Adding a specific k-rate for the lag-phase and a parameter for the number of days the lag-phase lasts, could make a better description of the actual inactivation, but there is not sufficient data available to do this. Many other parameters could also been used in the regression, resulting in a better fit with the observed k-rates. However, due to lag-phases and differences in treatment period lengths, the number of parameters was kept at a minimum.

The formula reflects the fact that the *Ascaris* inactivation is highly dependent on temperature. Temperature logging would thus give a much more precise estimate of the inactivation, especially if the data is used in a model with more time steps then only 2 which is used in this model. However, it remains to see if splitting up the treatment period into periods with different temperature gives a good estimate of the actual inactivation under varying temperature.

The formula is also very sensitive to pH, and small pH-changes are causing large differences in the inactivation estimate. This is supposedly reflecting the actual nature of the

inactivation mechanism, but is a major source of uncertainty since pH is difficult to measure precisely, both in lab and in a treatment facility. If ash is used in addition to the urea, pH measurements should be done at the treatment facility to give a more precise estimate of the inactivation, since the alkalinity of ash is varying a lot. This is not so critically if urea is the only disinfectant.

For the case of ecosan sludge where the treatment is based on ash treatment, storage, composting or drying, it has been concluded that it would be inappropriate to generalize conditions to ensure pathogen die-off (Strauss and Blumenthal, 1990, cited in Jimenez et al. 2006). Since the reported inactivation rates for ammonia-based treatment are in agreement with each other and the physical parameters are more stable, the situation differs from that of ecosan sludge. Keeping in mind that the alternative is to measure the actual *Ascaris* inactivation, which is very costly or not possible in many cases, it would actually be more appropriate to generalize factors such as ammonia concentration, pH and temperature in many situations.

4.3.2 Accuracy of assumptions

The health risk assessment depends strongly on several variables that are more or less a matter of guessing. The estimation of the foodborne exposure has a linear relationship with the sludge application rate used for cropping. Furthermore, the consumption rates and frequency are decisive for the health risk calculation. In order to make a better estimate of the health risk, interviews with farmers and food consumers will provide better data than using data from other countries.

It is important to have gas-tight storage facility for ammonia-based treatment. In this calculation, an ammonia loss of 10 % was assumed. Higher losses than this would have a significant impact on the pathogen inactivation and fertilizer value. Exposure to sunlight and highly varying ambient temperature may increase the pressure inside the container on mid-day and reduce it on night, and containers that are only half-full or non-gas-tight containers could experience high ammonia loss, depending on the container quality.

4.4 Viral risk

4.4.1 *Salmonella* typhimurium phage 28B

The pH in batch A was surprisingly low, indicating that the hydrolysis of the urea might not be complete. Hydrolysis was unfortunately not confirmed with measurements of the actual batches, but a parallel batch. Reporting the inactivation as a function of ammonia content could thus overestimate the ammonia concentration. However, the observed results are in agreement with other results reported, see figure 9.

The PBS buffer that was used for the extraction of the viruses was too diluted to reach pH of 7, and the pH of the diluted sludge was measured to 7,5 ± 0,2. Although the optimum for extraction of viruses with beef extract is reported to be 7,2, the extraction potential is not believed to be significant lower for the pH values measured (Berg & Sullivan 1988). The same buffer dilution was used throughout the study, keeping the fraction of the viruses eluted approximately constant.

There was some variation in the dry-matter content due to a wrong measurement of the dry-matter content of the fecal matter in advance of the batch preparation. This might affect the inactivation of phages, and studies has shown that the fecal matter act as a protective matrix and reduces the inactivation compared to inactivation in urine (Nordin et al. 2009b).

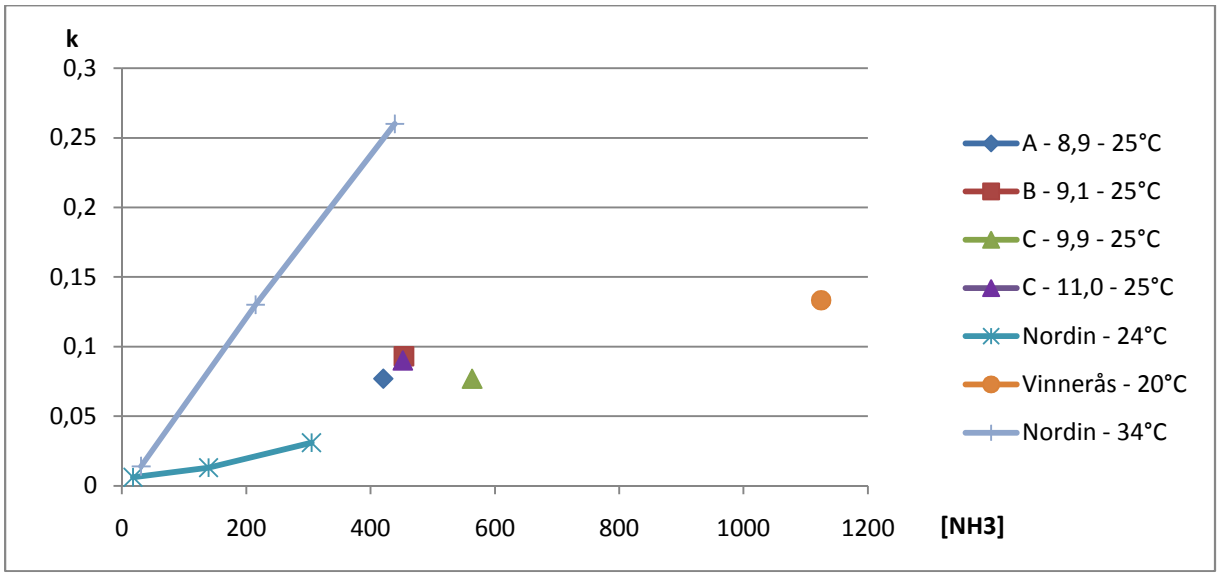


Figure 9: Comparison of inactivation rates from literature and laboratorie test. References: (Nordin et al. 2009b; Vinnerås et al. 2003)

4.4.2 Adenovirus inactivation

Bofill-Mas et al. (2006) studied the inactivation of human adenovirus 5 in sewage and - observed a t_{90} of 61 days at 22°C, and Enriquez et al. (1995) reported the t_{90} in primary wastewater at 15°C to be 40 and 43 days for human adenovirus 40 and 41, respectively. Nordin et al. (2009b) observed a t_{90} for *Salmonella* typh. phage 28B of 164 days at 24°C for fecal sludge (ca 17 % DM, $[NH_3]$ = 0-36 mM), and 78 days when 1 % urea was added ($[NH_3]$ = 102-177 mM). Höglund et al. (2002) reported the t_{90} for *Salmonella* typh. phage 28B to be 71 days in urine at 20°C. The NH_3 concentration in the urine is not reported, but is probably around 30-40 mM. These result are not easily compared due to unreported ammonia content in sewage, and because the protective effect of the fecal matrix. However, the data seems to indicate that 28B is even more conservative than the human adenoviruses. It would then be inappropriate to assess the health risk based on 28B inactivation as a model for adenoviruses. But it can be assumed that also adenovirus inactivation will not be significantly affected by increased pH values in the range 9-11.

4.4.3 Rotavirus inactivation

Ward and Asley (1977) observed a 1 $\log_{10}$ inactivation of Reovirus type 3 in wastewaters sludge with pH = 9.5 in 24 hours at 21℃, and 1.5 $\log_{10}$ inactivation at the same pH with a 290 mM concentration of free ammonia. However, it must be noted that only 55-62 % of initial reovirus in this study was viable after 24 hours in neutral buffers, which shows that not all loss of infectivity was due to inactivation. The result is in agreement with Estes et al. (1979) who reported that 50 % of simian rotavirus was inactivated in an M-glycine buffer at pH 10 at 22℃ in 60 minutes. Höglund et al. (2002) observed no significant effect of neither pH nor ammonia at pH 9 and $[NH_3]$ = 66 mM, resulting in slow inactivation in urine (T_{90} = 35 days). According to this, a conservative estimation of rotavirus inactivation would be to assume T_{90} to be 35 days where pH was below 9,5, while a 0,75 $\log_{10}$ reduction per day can be assumed where pH is above 9,5. If concentration of uncharged ammonia is greater than 290 mM when pH is above 9,5, a 1.2 $\log_{10}$ reduction per day can be assumed. However, it is most likely that this will result in an underestimation of the rotavirus inactivation when pH is between 9 and 9,5. The fast inactivation at 9,5 indicates that the actual threshold is lower than 9,5. Furthermore, it is possible that the ammonia concentrations that correspond to 2 or 4 % urea will have a significant effect on the inactivation of rotavirus even at pH of 9. Such thresholds for ammonia-based inactivation are seen for other pathogens. And even the

difference was not significant, the inactivation study comparing urine and the buffer at pH 9 actually showed a slightly faster rotavirus in the urine.

4.4.4 Fate of viruses in soil and on plants

The potential health risk of foodborne viruses from crops grown in fecal sludge depends on whether or not the viruses are exposed to sunlight on the soil surface, attached to crops or transported to the groundwater. The fate of the viruses depends on soil characteristics, climatic conditions, and irrigation and application practice. Since fecal sludge is usually applied shortly before seeding, a withholding time will normally reduce the probability of viral foodborne transmission. 5-30 days are needed for 1 $\log_{10}$ reduction of rotaviruses in soil at ~20°C (WHO 2006), so two months of treatment and two months of resting should result in at least a 4 $\log_{10}$ reduction of rotavirus, reducing the health risk.

Farmers have sometimes told that they worry about the groundwater quality when using treated excreta on the field. Trials in soil columns show that there is a potential for pathogen migration from land-applied biosolids into the groundwater (Horswell et al. 2009). So even if the risk of foodborne transmission of viruses is insignificant, the inactivation of viruses could still be of important, but this depends strongly on other factors such as soil characteristics and hydrogeological conditions.

4.5 Treatment process alternatives

4.5.1 Ash

According to the results in this study, the use of ash is not very favorable considering the *Ascaris* inactivation and increased storage volumes. This is due to the necessity of water dilution to maintain the moisture content. Even though the proportion of ammonia that is uncharged increases, the dilution will to a large extent neutralize this benefit. However, the value of 17% may not represent the optimum dry matter content, and a sufficient migration of ammonia and hydroxide may happen at a higher dry matter content. Furthermore, the assumptions on pH values are not based on real data, and actual pH values might be higher. Furthermore, the effect of ash may be underestimated since only two temperature intervals were used in the calculation. Higher resolution could result in a higher benefit of the pH increase.

The Peepoo bags are suggested grinded before storage to achieve a homogenous ammonia-concentration in the container. To increase the inactivation of *Ascaris* during treatment, ash could be mixed with the fecal matter in this grinding process, since the urea is already supposedly hydrolyzed. Furthermore, the Peepoo bags may contain significant amounts of urine, increasing the moisture content. Thus, the addition of ash will not yield any unnecessary dilution.

The use of Potassium hydroxide as a disinfectant should be considered, since it does not contain any dry matter. This could enhance inactivation without increasing storage volumes significantly.

4.5.2 Dry matter additives

Since the ammonia and hydroxide migration is dependent on moisture content, fly- and smell preventing additives should replaced with ventilation and fly screens. Toilet paper should maybe be diverted from the fecal matter and be incinerated. According to Swedish valued, the dry matter content of toilet paper is of same magnitude as the fecal matter, and is therefore substantially increasing the dry-matter content in the fecal sludge (Jönsson et al. 2005).

4.5.3 Black paint

Some dehydrating systems use black painting to absorb more heat from the sun and thus increase the temperature (Sherpa et al. 2009). This could be very efficient for ammonia-based treatment as well, since *Ascaris* inactivation is favored by higher temperature. The fraction of ammonia that is present as uncharged (NH_3) also increases with higher temperature. However, the increased temperature variations can cause higher ammonia loss, depending on the container quality.

4.6 Acceptable health risk

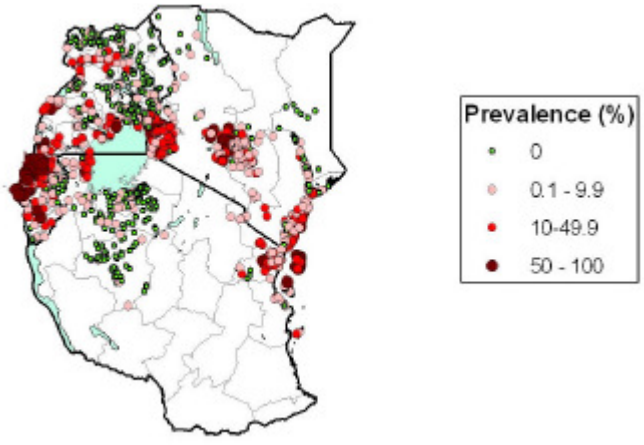
Factors such as age, nutrition and immune system do strongly affect the dose-response relationships. Thus, dose-response relations from industrialized countries are not suitable to use in developing countries. For the case of *Ascaris*, the dose-response relationship is based on data from an area in Mexico with high *Ascaris* prevalence, and is therefore probably closer to the reality in Uganda than a dose-response from a developed country would have been.

Even if there is significant health risk associated with the reuse of nutrients in excreta, the total health impact can be positive. This is due to the fact that increased food production can improve nutrition status and the immune system and thus reduce the risk of getting infected. This is especially important in areas where there is a high prevalence of infections such as *Ascaris* due to other reasons than use of excreta.

Increasing urea addition enhances pathogen die-off, but makes the nutrients available in fecal matter more expensive. For the farmers who are using chemical fertilizers on a regular basis, this is not a problem. But for the small-scale farmers who cannot afford to buy chemical fertilizers, increased urea-dosage will make the nutrients in the fecal matter more expensive. For these farmers will also the extra cost associated with a professional team for the application of the SIP, make it less affordable to buy. Therefore the project management has to consider the different options not only with respect to health risk but also to economic aspects.

4.6.1 Difference in pathogen prevalence

For *Ascaris* and other soil-transmitted helminthes, the prevalence rate is varying a lot within relative short geographic distances, see figure 10. A study in Malawi showed that the infection prevalence of soil-transmitted helminthes was significantly higher in urban areas than in rural areas (Phiri et al. 2000), while the opposite has been observed for hookworm, with higher prevalence rates in rural areas (Mehraj et al. 2008).



.

Figure 10: *Ascaris* prevalence in East African countries. Reference: Brooker et al. (2009)

In many cases will other transmission routes be more important for pathogen exposure than the exposures evaluated here. However, when the treated fecal matter is sold from an urban slum to population groups that live outside the city and are less exposed to certain pathogens, the use of excreta can cause significant health risk. Thus, higher inactivation of pathogens is required to avoid significant health risks when using excreta that are transported from areas with higher pathogen prevalence to areas with low prevalence, compared to local use.

5. Conclusion

Using ash in addition to urea will increase *Ascaris* inactivation to some extent, but because of the large volumes needed due to water dilution and the relative low increase in inactivation, this is not very favorable. If the combination of ash and urea treatment is implemented, pH and temperature should be monitored during storage. These data may result in an estimation of the effect of ash addition that favors the ash treatment more than what is estimated in this research.

If the fecal matter is treated with 4 % w/w urea, the risk for a farmer applying the treated fecal matter on the fields without using protective can be considered acceptable. Use of protective clothing could reduce this risk. On the other hand, other household individuals not working at the fields may be exposed to pathogens. If less than 4 % urea is used, the risk of applying the treated fecal matter exceeds 10^{-3}, and a professional team are recommended to do the application job.

If 3 % w/w urea of more is added before the treatment period will the consumption of carrots and spinach grown in the soil fertilized with the fecal matter result in an annual infection risk less than 10^{-3}. For the case of spinach, this is highly dependent on that the fecal sludge is applied in furrows that are closed, which will probably be done anyway to avoid ammonia loss. If less than 3 % urea is used, vegetables eaten raw should not be grown in soil fertilized with treated sludge.

The laboratory study of *Salmonella* typhimurium phage 28B inactivation under different pH values showed that increasing the pH in the range 9-11 do not increase the inactivation of dsDNA viruses, except the effect of increased proportion of uncharged NH_3. This is probably true also for the case of adenovirus which is also dsDNA virus.

Rotavirus and adenovirus may represent a health risk, but this is not sure. Rotavirus may be inactivated by the urea, but there is a possibility that an additional pH increase is necessary to achieve a significant inactivation by ammonia. The impact of ammonia on adenovirus inactivation is not reported, but based on other inactivation data is adenovirus assumed to be less persistent than *Salmonella* typh. phage 28B. Using ash to increase pH to 9,5 will yield a higher inactivation of rotavirus. How important this is in the context of agricultural reuse of

nutrients from fecal matter, is difficult to predict since data on rotavirus inactivation and the fate of viruses in the soil is lacking.

The results from this QMRA cannot necessarily be adapted to other similar treatment systems. When deciding the doses of urea, ambient temperatures should be applied because of the huge temperature sensitivity of *Ascaris* inactivation. Furthermore, inactivation targets could also be adjusted in a local setting based on personal hygiene, helminth infection prevalence and consumptions habits.

5.1 Further research

In order to get better data on the actual treatment conditions, temperature should be monitored, the ammonia loss should be measured and the pH resulting from different urea and ash-doses should be measured. More knowledge about the consumption habits and farming practice could provide better estimates of the actual health risks.

Thresholds for pH and ammonia concentration for rotavirus inactivation should be investigated by inactivation studies. Adenovirus inactivation by ammonia should be quantified and compared with *Salmonella* typh. phage 28B. The fate of these viruses in the soil and their attachment to crops should also be studied to find out if they represent a risk for foodborne transmission.

Dose-response relationships for other types of adenoviruses should be established, preferably from individuals in a developing country, since the virus is more prevalent there. This is especially crucial for enteric adenovirus.

6. References

ACODE. (2006). Status of Organic Agriculture Production in Uganda. Kampala: Advocates Coalition for Development Environment (ACODE).

Ashbolt, N. J. (2004). Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology*, 198 (1-3): 229-238.

Belguith, K., Hassen, A. & Aouni, M. (2006). Comparative study of four extraction methods for enterovirus recovery from wastewater and sewage sludge. *Bioresource Technology*, 97 (3): 414-419.

Benke, K. K. & Hamilton, A. J. (2008). Quantitative microbial risk assessment: uncertainty and measures of central tendency for skewed distributions. *Stochastic Environmental Research and Risk Assessment*, 22 (4): 533-539.

Berg, G. & Sullivan, G. (1988). Optimum pH levels for eluting enteroviruses from sludge solids with beef extract. *Applied and Environmental Microbiology*, 54 (7): 1880-1881.

Berg, U., Donnert, D., Ehbrecht, A., Bumiller, W., Kusche, I., Weidler, P. G. & Nuesch, R. (2005). "Active filtration" for the elimination and recovery of phosphorus from waste water. *Colloids and Surfaces a-Physicochemical and Engineering Aspects*, 265 (1-3): 141-148.

Bofill-Mas, S., Albinana-Gimenez, N., Clemente-Casares, P., Hundesa, A., Rodriguez-Manzano, J., Allard, A., Calvo, M. & Girones, R. (2006). Quantification and stability of human adenoviruses and polyomavirus JCPyV in wastewater matrices. *Applied and Environmental Microbiology*, 72 (12): 7894-7896.

Brooker, S., Kabatereine, N. B., Smith, J. L., Mupfasoni, D., Mwanje, M. T., Ndayishimiye, O., Lwambo, N. J. S., Mbotha, D., Karanja, P., Mwandawiro, C., Muchiri, E., Clements, A. C. A., Bundy, D. A. P. & Snow, R. W. (2009). An updated atlas of human helminth infections: the example of East Africa. *International Journal of Health Geographics*, 8: 11.

Cannon, J. L., Papafragkou, E., Park, G. W., Osborne, J., Jaykus, L. A. & Vinje, J. (2006). Surrogates for the study of norovirus stability and inactivation in the environment: A comparison of murine norovirus and feline calicivirus. *Journal of Food Protection*, 69 (11): 2761-2765.

Chan, M. S. (1997). The global burden of intestinal nematode infections - Fifty years on. *Parasitology Today*, 13 (11): 438-443.

CIA. (2010). *The World Factbook*. Available at: https://www.cia.gov/library/publications/the-world-factbook/geos/ug.html.

Clement, B. & Merlin, G. (1995). The contribution of ammonia and alkalinity to landfill leachate toxicity to duckweed. *Science of the Total Environment*, 170 (1-2): 71-79.

Crabtree, K. D., Gerba, C. P., Rose, J. B. & Haas, C. N. (1997). Waterborne adenovirus: A risk assessment. *Water Science and Technology*, 35 (11-12): 1-6.

Cramer, W. N., Burge, W. D. & Kawata, K. (1983). Kinetics of virus inactivation by ammonia. *Applied and Environmental Microbiology*, 45 (3): 760-765.

Dawson, D. (2005). Foodborne protozoan parasites. *International Journal of Food Microbiology*, 103 (2): 207-227.

Demeyer, A., Nkana, J. C. V. & Verloo, M. G. (2001). Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. *Bioresource Technology*, 77 (3): 287-295.

Deng, M. Y. & Cliver, D. O. (1995). Persistence of inoculated Hepatitis-A virus in mixed himan and animal wastes. *Applied and Environmental Microbiology*, 61 (1): 87-91.

Enriquez, C. E., Hurst, C. J. & Gerba, C. P. (1995). Survival of the enteric adenovirus-40 and adenovirus-41 in tap, sea, and waste-water. *Water Research*, 29 (11): 2548-2553.

Estes, M. K., Graham, D. Y., Smith, E. M. & Gerba, C. P. (1979). Rotavirus stability and inactivation. *Journal of General Virology*, 43 (MAY): 403-409.

Etiegni, L. & Campbell, A. G. (1991). Physical and chemical characteristics of wood ash. *Bioresource Technology*, 37 (2): 173-178.

Gerba, C. P., Rose, J. B., Haas, C. N. & Crabtree, K. D. (1996). Waterborne rotavirus: A risk assessment. *Water Research*, 30 (12): 2929-2940.

Gerba, C. P., Pepper, I. L. & Whitehead, L. F. (2002). A risk assessment of emerging pathogens of concern in the land application of biosolids. *Water Science and Technology*, 46 (10): 225-230.

Ghiglietti, R., Rossi, P., Ramsan, M. & Colombi, A. (1995). Viability of Ascaris suum, Ascaris lumbricoides and Trichuris muris eggs to alkaline pH and different temperatures. *Parassitologia*, 37: 229-232.

Ghiglietti, R., Genchi, C., DiMatteo, L., Calcaterra, E. & Colombi, A. (1997). Survival of Ascaris suum eggs in ammonia-treated wastewater sludges. *Bioresource Technology*, 59 (2-3): 195-198.

Haas, C. N., Crockett, C. S., Rose, J. B., Gerba, C. P. & Fazil, A. M. (1996). Assessing the risk posed by oocysts in drinking water. *Journal American Water Works Association*, 88 (9): 131-136.

Haas, C. N., Rose, J. B. & Gerba, C. P. (1999). *Quantitative microbial risk assessment*. New York: Wiley.

Hernandez, F., Monge, R., Jimenez, C. & Taylor, L. (1997). Rotavirus and Hepatitis A virus in market lettuce (Latuca sativa) in Costa Rica. *International Journal of Food Microbiology*, 37 (2-3): 221-223.

Holland, C. V., Asaolu, S. O., Crompton, D. W. T., Stoddart, R. C., Macdonald, R. & Torimiro, S. E. A. (1989). The epidemiology of Ascaris Lumbricoides and other soil-transmitted helminths in primary-school children from Ile-Ife, Nigeria. *Parasitology*, 99: 275-285.

Horswell, J., Hewitt, J., Prosser, J., Van Schaik, A., Croucher, D., Macdonald, C., Burford, P., Susarla, P., Bickers, P. & Speir, T. (2009). Mobility and survival of Salmonella Typhimurium and human adenovirus from spiked sewage sludge applied to soil columns. *Journal of Applied Microbiology*, 108 (1): 104-114.

Howard, G., Pedley, S., Barrett, M., Nalubega, M. & Johal, K. (2003). Risk factors contributing to microbiological contamination of shallow groundwater in Kampala, Uganda. *Water Research*, 37 (14): 3421-3429.

Höglund, C., Ashbolt, N., Stenstrom, T. A. & Svensson, L. (2002). Viral persistence in source-separated human urine. *Advances in Environmental Research*, 6 (3): 265-275.

Jenkins, M. B., Anguish, L. J., Bowman, D. D., Walker, M. J. & Ghiorse, W. C. (1997). Assessment of a dye permeability assay for determination of inactivation rates of Cryptosporidium parvum oocysts. *Applied and Environmental Microbiology*, 63 (10): 3844-3850.

Jenkins, M. B., Bowman, D. D. & Ghiorse, W. C. (1998). Inactivation of Cryptosporidium parvum oocysts by ammonia. *Applied and Environmental Microbiology*, 64 (2): 784-788.

Jimenez, B., Austin, A., Cloete, E. & Phasha, C. (2006). Using Ecosan sludge for crop production. *Water Science and Technology*, 54 (5): 169-177.

Jönsson, H., Baky, A., Jeppsson, U., Hellström, D. & Kärrman, E. (2005). Composition of urine, faeces, greywater and biowaste for utilisation in the URWARE model. *Urban Water Rapport*.

Kabatereine, N. B., Kemijumbi, J., Kazibwe, F. & Onapa, A. W. (1997). Human intestinal parasites in primary school children in Kampala, Uganda. *East African Medical Journal*, 74 (5): 311-314.

Karavarsamis, N. & Hamilton, A. J. (2009). Estimators of annual probability of infection for quantitative microbial risk assessment. *Journal of Water and Health*, 8 (2): 365-373.

Lopez, A. D., Mathers, C. D., Ezzati, M., Jamison, D. T. & Murray, C. J. L. (2006). Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet*, 367 (9524): 1747-1757.

Mara, D. D. & Sleigh, A. (2009). Estimation of Ascaris infection risks in children under 15 from the consumption of wastewater-irrigated carrots. *Journal of Water and Health*.

Mehraj, V., Hatcher, J., Akhtar, S., Rafique, G. & Beg, M. A. (2008). Prevalence and Factors Associated with Intestinal Parasitic Infection among Children in an Urban Slum of Karachi. *Plos One*, 3 (11): 6.

Mena, K. D. & Gerba, C. P. (2009). Waterborne Adenovirus. In Reviews of Environmental Contamination and Toxicology, vol. 198 *Reviews of Environmental Contamination and Toxicology, Vol 198*, pp. 133-167. New York: Springer.

Mignotte, B., Maul, A. & Schwartzbrod, L. (1999). Comparative study of techniques used to recover viruses from residual urban sludge. *Journal of Virological Methods*, 78 (1-2): 71-80.

Moe, C. & Izurieta, R. (2003). *Longitudinal study of double vault urine diverting toilets and solar toilets in El Salvador*. Proceedings of the 2nd International Symposium on Ecological Sanitation, Incorporating the 1st IWA Specialist Group Conference on Sustainable Sanitation, 7-11 April 2003, Lubeck: GTZ.

Navarro, I., Jimenez, B., Cifuentes, E. & Lucario, S. (2009). Application of Helminth ova infection dose curve to estimate the risks associated with biosolid application on soil. *Journal of Water and Health*, 7 (1): 31-44.

Naylor, L. M. & Schmidt, E. J. (1986). Agricultural use of wood ash as a fertilizer and liming material. *Tappi Journal*, 69 (10): 114-119.

Niwagaba, C. (2007). *Human Excreta Treatment Technologies – prerequisites, constraints and performance*. Uppsala: SLU, Department of Biometry and Engineering. 70 p.

Niwagaba, C., Kulabako, R. N., Mugala, P. & Jonsson, H. (2009). Comparing microbial die-off in separately collected faeces with ash and sawdust additives. *Waste Management*, 29 (7): 2214-2219.

Nordin, A., Nyberg, K. & Vinnerås, B. (2009a). Inactivation of Ascaris Eggs in Source-Separated Urine and Feces by Ammonia at Ambient Temperatures. *Applied and Environmental Microbiology*, 75 (3): 662-667.

Nordin, A., Ottoson, J. R. & Vinnerås, B. (2009b). Sanitation of faeces from source-separating dry toilets using urea. *Journal of Applied Microbiology*, 107 (5): 1579-1587.

Olson, M. E., Goh, J., Phillips, M., Guselle, N. & McAllister, T. A. (1999). Giardia cyst and Cryptosporidium oocyst survival in water, soil, and cattle feces. *Journal of Environmental Quality*, 28 (6): 1991-1996.

Pecson, B. M. & Nelson, K. L. (2005). Inactivation of Ascaris suum eggs by ammonia. *Environmental Science & Technology*, 39 (20): 7909-7914.

Pecson, B. M., Barrios, J. A., Jimenez, B. E. & Nelson, K. L. (2007). The effects of temperature, pH, and ammonia concentration on the inactivation of Ascaris eggs in sewage sludge. *Water Research*, 41 (13): 2893-2902.

Phiri, K., Whitty, C. J. M., Graham, S. M. & Ssembatya-Lule, G. (2000). Urban/rural differences in prevalence and risk factors for intestinal helminth infection in southern Malawi. *Annals of Tropical Medicine and Parasitology*, 94 (4): 381-387.

Ramani, S. & Kang, G. (2009). Viruses causing childhood diarrhoea in the developing world. *Current Opinion in Infectious Diseases*, 22 (5): 477-482.

Schlindwein, A. D., Simoes, C. M. O. & Barardi, C. R. M. (2009). Comparative study of two extraction methods for enteric virus recovery from sewage sludge by molecular methods. *Memorias Do Instituto Oswaldo Cruz*, 104 (4): 576-579.

Schröder, E. (2010). Marketing Human Waste - A study of possible ways to dispose of urine and faeces from slum settlements in Kampala, Uganda.: German Technical Cooperation (GTZ)

Schönning, C. (2001). *Evaluation of microbial health risks associated with the reuse of source-separated human urine*. Stockholm: Royal Institute of Technology (KTH), Swedish Institute for Infectious Disease Control (SMI).

Schönning, C., Westrell, T., Stenstrom, T. A., Arnbjerg-Nielsen, K., Hasling, A. B., Hoibye, L. & Carlsen, A. (2007). Microbial risk assessment of local handling and use of human faeces. *Journal of Water and Health*, 5 (1): 117-128.

Seidu, R., Heistad, A., Amoah, P., Drechsel, P., Jenssen, P. D. & Stenstrom, T. A. (2008). Quantification of the health risk associated with wastewater reuse in Accra, Ghana: a contribution toward local guidelines. *Journal of Water and Health*, 6 (4): 461-471.

Seidu, R. (2010). *Disentangling the risk factors and health risk associated with faecal sludge and wastewater reuse in Ghana*. Ås: Norwegian University of Life Sciences, Department of Mathematical Sciences and Technology

Seidu, R. & Stenström, T. A. (2010 (submitted)). Occupational health risk of cake sludge application on northern ghana.

Sherpa, A. M., Byamukama, D., Shrestha, R. R., Haberl, R., Mach, R. L. & Farnleitner, A. H. (2009). Use of faecal pollution indicators to estimate pathogen die off conditions in source separated faeces in Kathmandu Valley, Nepal. *Journal of Water and Health*, 7 (1): 97-107.

Shuval, H., Lampert, Y. & Fattal, B. (1997). Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Water Science and Technology*, 35 (11-12): 15-20.

Standley, C. J., Adriko, M., Alinaitwe, M., Kazibwe, F., Kabatereine, N. B. & Stothard, J. R. (2009). Intestinal schistosomiasis and soil-transmitted helminthiasis in Ugandan schoolchildren: a rapid mapping assessment. *Geospatial Health*, 4 (1): 39-53.

Strauss, M. & Blumenthal, U. J. (1990). Use of human wastes in agriculture and aquaculture. No 08/90, Duebenforf, Switzerland: IRCWD.

Tchounwou, P. B., Englande, A. J., Malek, E. A., Anderson, A. C. & Abdelghani, A. A. (1991). The effects of ammonium-.sulfate and urea upon egg hatching and miacidal survival of schistosoma-mansoni. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, 26 (2): 241-257.

Teunis, P. F. M., Moe, C. L., Liu, P., Miller, S. E., Lindesmith, L., Baric, R. S., Le Pendu, J. & Calderon, R. L. (2008). Norwalk virus: How infectious is it? *Journal of Medical Virology*, 80 (8): 1468-1476.

Thurston-Enriquez, J. A., Haas, C. N., Jacangelo, J., Riley, K. & Gerba, C. P. (2003). Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. *Applied and Environmental Microbiology*, 69 (1): 577-582.

U.S.EPA. (1997). *Exposure Factors Handbook, Vol II Food Ingestion Factors*. Washington DC, USA: Environmental Protection Agency.

U.S.EPA. (2002). *Child-Specific Factor Handbook*. Washington DC, USA: National Centre for Environmental Assessment.

Vance, E. D. (1996). Land application of wood-fired and combination boiler ashes: An overview. *Journal of Environmental Quality*, 25 (5): 937-944.

Vinnerås, B., Holmqvist, A., Bagge, E., Albihn, A. & Jonsson, H. (2003). The potential for disinfection of separated faecal matter by urea and by peracetic acid for hygienic nutrient recycling. *Bioresource Technology*, 89 (2): 155-161.

Vinnerås, B., Nordin, A., Niwagaba, C. & Nyberg, K. (2008). Inactivation of bacteria and viruses in human urine depending on temperature and dilution rate. *Water Research*, 42 (15): 4067-4074.

Vinnerås, B., Hedenkvist, M., Nordin, A. & Wilhelmson, A. (2009). Peepoo bag: self-sanitising single use biodegradable toilet. *Water Science and Technology*, 59 (9): 1743-1749.

Ward, R. L. & Ashley, C. S. (1977). Identification of virucidal agent in wastewater-sludge. *Applied and Environmental Microbiology*, 33 (4): 860-864.

Wendo, C. (2003). Uganda, World Bank, and DFID launch sanitation campaign. *Lancet*, 362 (9385): 716-716.

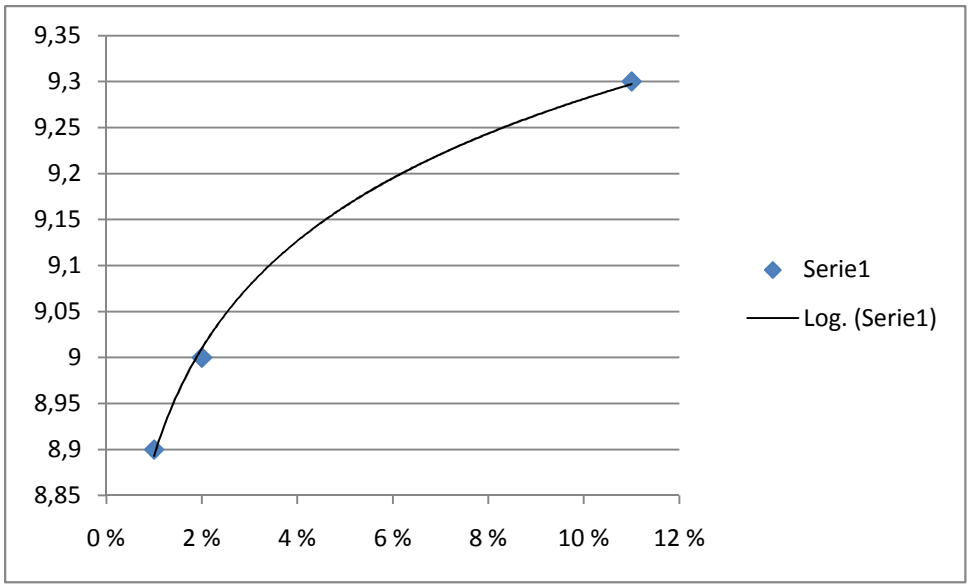
WHO (ed.) (2006). *Excreta and greywater use in agriculture*. WHO guidelines for the safe use of wastewater, excreta and greywater, vol. IV. France.

Woodburn, P. W., Muhangi, L., Hillier, S., Ndibazza, J., Namujju, P. B., Kizza, M., Ameke, C., Omoding, N. E., Booth, M. & Elliott, A. M. (2009). Risk Factors for Helminth, Malaria, and HIV Infection in Pregnancy in Entebbe, Uganda. *Plos Neglected Tropical Diseases*, 3 (6).

Appendix A – Regression data for *Ascaris* inactivation

Reference	pH	NH_3	t	TS	k	k estimate	k estimate adjusted	Included in regression analysis
Ghiglietti	9,8	24	22	10 %	0,0100	0,0190	0,0165	no
Ghiglietti	10	40	22	10 %	0,0308	0,0278	0,0240	yes
Ghiglietti	10	53	22	10 %	0,0336	0,0342	0,0295	yes
Ghiglietti	10,5	75	22	10 %	0,0456	0,0495	0,0428	yes
Ghiglietti	10,5	120	22	10 %	0,0742	0,0722	0,0624	yes
Nordin	9	230	24	17 %	0,0714	0,0825	0,0714	yes
Nordin	8,9	130	24	17 %	0,0426	0,0410	0,0354	yes
Nordin	10	220	24	17 %	0,1538	0,1449	0,1253	yes
Nordin	10,5	57	24	17 %	0,0571	0,0543	0,0469	yes
Nordin	8,3	20	24	17 %	0,0270	-	-	no
Nordin	9	440	34	17 %	0,5263	0,9743	0,8428	no
Nordin	8,9	250	34	17 %	0,488	0,463	0,400	yes
Nordin	12,8	71	34	17 %	0,541	0,573	0,496	yes
Pecson	12	94	20	6,5 %	0,053	0,053	0,046	yes
Pecson	12	399	20	6,5 %	0,18	0,18	0,16	yes
Pecson	12	17	30	6,5 %	0,38	0,08	0,07	temperature est. only
Pecson	12	93	30	6,5 %	0,28	0,28	0,24	no
Pecson	12	398	30	6,5 %	0,96	0,95	0,82	temperature est. only
Pecson	12	13	40	6,0 %	1,8	0,8	0,7	temperature est. only
Pecson	12	393	40	6,0 %	12	12	10	temperature est. only

Appendix B – Interpolation of pH values



Urea dose	pH	Reference
1 %	8,9	Nordin 2009
2 %	9	Nordin 2009
11 %	9,3	Vinnerås 2003