CHAPTER 4
FIELD TRIALS: MICROBIOLOGICAL EFFECTS ON FOOD CROPS FERTILISED WITH FAECAL MATERIAL FROM URINE-DIVERSION TOILETS

4.1 INTRODUCTION

One of the advantages of ecological sanitation is that faecal material can be advantageously applied to soils (Esrey et al 1998). At present, however, faecal material from urine-diversion (UD) toilets in South Africa has not been classified in the current South African norms (WRC 2006). Considering the widespread use of urine-diversion technology in South Africa, it has become important to assess the possible uses or acceptable disposal methods of the faecal material produced, and for that reason information concerning the quality of this material and of its effects under different management options is important.

This chapter is based largely on chapter 3 of the Water Research Commission (WRC) publication entitled "*Use of human excreta from urine-diversion toilets in food gardens: agronomic and health aspects*" (Mnkeni et al 2006). This publication emanated from the WRC project number K5/1439 entitled "*Strategy for the furtherance of knowledge and good practice of ecological sanitation (ecosan) technology in South Africa*." The writer was the overall project leader and also co-author/editor of this particular chapter.

4.2 BACKGROUND AND PURPOSE OF INVESTIGATION

Urine-diversion sanitation technology involves the storage of faeces in dry conditions that inactivate the microorganisms to such an extent that a safe soil conditioner is produced (Esrey et al 1998). When applied to land, this material increases agricultural yields (Esrey et al 1998; Austin and Duncker 2002, Kouraa et al 2002), because human excreta contain organic matter, phosphorus and nitrogen compounds that are essential plant nutrients. The use of dry human excreta is not new. It was documented in the 12th century in China (Schönning 2001c) and until the second half of the 19th century in Finland (Olsson 2001). Because this material originates in toilets based on the principle of separating urine from faeces, it dehydrates quite rapidly. Compared to traditional latrine sludge (and even to sludge produced in conventional wastewater treatment systems) faecal material from urine-diversion toilets displays a lower moisture content that contributes to microorganism inactivation (Esrey et al 1998; Schönning 2001b).

Recycling excreta to soils reduces the need for chemical fertilisers; however, pathogens are recycled to humans if improper agricultural practices are followed (Höglund 2002). Concerns about using faecal material include higher pathogenic content in developing countries compared to that in developed countries (Jimenez et al 2002 & 2004). This material, as well as that from other sanitation alternatives in small-scale systems, demands more personal involvement from the users (including handling of the waste),

which constitutes a higher human exposure level compared to that from conventional piped systems. Nevertheless, it is considered that where the material can improve agricultural productivity, it can contribute to improving the nutritional status of the population, thus improving public health (Höglund 2001; IWMI 2003). According to Peasey (2002), although ecosan technology is spreading all over the world, and with it the recycling of excreta to soils, only a few researchers have addressed the problems associated with the revalorization practice or documented the pathogen die-off. Moreover, little data about the microbial quality of ecosan faecal material from developing countries (where the health risks are the highest) are available. The objective of this research was thus to investigate the potential health risks of using faecal material in agriculture by determining the pathogen uptake on the surfaces of the edible portions of the crops.

4.3 METHODOLOGY

A 25kg composite sample of dry faecal material mixed with some topsoil was collected from the main heap of UD faecal material in eThekwini (described in chapter 5 of this thesis). This material had been extracted from UD toilets and left in a heap exposed to the elements for four months. For microbial characterisation, four bacteria, one fungus as well as helminth ova were measured. Total coliforms were measured due to their presence in faeces, faecal coliforms and faecal *streptococci* because they are considered as good indicators of faecal pollution by most authors (e.g. Feachem et al 1983) and S*almonella spp* because they are often considered in sludge regulations. For fungi there is not a universal indicator; *Aspergillus spp.* were used because they are opportunistic pathogens belonging to a group of moulds that is found worldwide. Finally, helminth ova were monitored due to their high persistence in the environment and because they are considered as quality indicators for most faeces use practices. The most important health effects associated with helminth infections are anaemia (hookworm), rectal prolapse (*Trichuris*) and intestinal obstruction and malnutrition (*Ascaris*). *Ascaris* and *Trichuris* alone infect over one third of the population in developing countries (WHO/UNICEF 2000). Helminths are commonly associated with sanitary risks when sludge is used as an agricultural fertilizer (Asaolu and Ofoezie 2003).

To analyse total coliform, faecal coliform, faecal *streptoccoci*, *Aspergillus spp.*, *Salmonella spp* and helminth ova, serial dilution techniques as described below (4.3.1 – 4.3.4) were used (Islam et al 2004 & 2005):

4.3.1 Total coliform, faecal coliform and faecal *streptococci*

1g of faecal material was added to 9ml of sterile buffered peptone water solution and vortexed for 10s using an aseptic technique. 10^{-1} to 10^{-10} dilutions were made. 0,1mℓ aliquots were plated on three selective media, i.e. m-Endo agar, m-Enterococcus agar (37°C) and m-Fc agar (44°C) in triplicate. The plates were incubated at 37°C and 44°C for 24h. After 24h the numbers of typical colonies were recorded; the colonies were identified by form and colour.

4.3.2 *Aspergillus spp*

Fungi were enumerated by serial dilution in sterilised ¼ strength Ringer's solution. 1g of faecal material was added to 9mℓ of the solution and 10^{-1} to 10^{-10} dilutions made. Rose-

bengal agar amended with 0,1mg streptomycin-sulphate per mℓ was used to enumerate the fungi. The plates were inoculated with 100µℓ of each dilution (three plates per suspension) and incubated at 25°C for 2 to 4 days.

4.3.3 *Salmonella spp*

1g of faecal material was added to 9mℓ buffered peptone water and vortexed for 10s. 10^{-1} to 10^{-5} serial dilutions were prepared and incubated at 37°C for 18 to 24h. 0,1mℓ of the mixture was transferred to 10mℓ Rappaport-Vassiliadis (RV) enrichment broth and incubated at 37°C for 24h. The broth was sub-cultured by spreading 0,1mℓ onto plates of Xylose-Lysine-Desoxycholate XLD) agar and incubated at 37°C for 24h. Occurrence of black colonies suggested the presence of *Salmonella*.

The technique used for *Salmonella* was replaced during the second phase of the study with a standard technique (APHA, AWWA, WEF 1995). TS (total solids), pH, and nitrogen were also determined using standard techniques (APHA, AWWA, WEF 1995).

4.3.4 Helminth ova

For helminth ova (HO) detection the Ayres technique modified by Maya, Jiménez and Schwartzbrod (2006 – in press at the time the experiment was performed) was used. This was preceded by adding 200mℓ of 0,01% tween solution (a mild detergent) to 30g spinach and carrot samples in a sterile bag and pummelling in a stomacher lab-blender 400 for 1h. Once the helminth ova were enumerated the viability was determined by incubating the sample at 26°C for 3-4 weeks. The viability (larva formation) was observed under a microscope.

4.3.5 Procedure

Analyses were performed to characterize the following:

- the faecal material prior to its application;
- the soil before sowing and after harvesting;
- the irrigation water during the study; and
- the crops after harvesting.

These analyses were performed for the purpose of establishing the measurable microbial effects of the faecal material and irrigation water on the natural soil, as well as the microbial effects on the crops themselves.

To assess the microbial effects of the faecal material in agriculture, two kinds of crops were selected, namely spinach and carrots. These crops were considered because they are eaten everywhere in South Africa, are often consumed raw, and for spinach the edible parts grow above the ground, while for carrots they grow below the ground. 2m x 9m plots at the experimental farm of the University of Pretoria were used (Figure 4.1). Each crop was planted in two plots, one being used as control while the other was divided into three sections. Each plot was treated with a different application rate of faecal material, except for the control plot where no faecal material was added (negative control).

Figure 4.1: Spinach and carrot crops at the University of Pretoria's experimental farm, January 2005

To determine the amount of faecal material to be added, the following criteria were taken into account:

- the nitrogen demand of crops (50kg N/ha for carrots and 100kg N/ha for spinach);
- application rates above and below 8t/ha, which is the maximum permissible value in South Africa; and
- three different helminth ova rates.

Following these criteria, the material was applied on carrots at 0; 7; 12,5 and 35t/ha (tons per hectare) corresponding to 0; 1; 1,7 and 4,8 HO (helminth ova)/cm^2, while for spinach 0; 1,3; 19,0 and 37,5t/ha equivalent to 0; 0,18; 2,6 and 5,1 HO/cm^2 were used.The helminth rate was defined as the quantity of total helminth ova applied per square centimetre. The material was mixed to a depth of 100mm. Seeds were planted to a depth of 50mm in the second week of November 2004 (summer). The pattern within the blocks was in rows 300mm apart and the seeds were spaced 50mm apart within the rows. Spinach was harvested in January 2005 (after 7 weeks) and carrots in March 2005 (after 12 weeks). In each case, the whole plant was pulled from the soil and cut to collect roots and leaves separately. For faecal material and soil analyses 1g samples were used. To analyse bacteria and fungi, 5g of crop samples were taken, while for helminth analyses the sample size was 30g.

4.4 RESULTS AND DISCUSSION

4.4.1 Characterisation of faecal material (Table 4.1)

While the TS content was high (43 ± 2%) and hence the moisture content low, the N content (0,2 - 0,34%) was within the common range for domestic sludges (0,2 - 0,6%) if the N contribution that would have been due to urea was subtracted (90% of the value according to Metcalf and Eddy 2003). The N content was low compared with other sludges, however, and this implied that higher quantities of material needed to be added

to fulfil the nutrient demand of the crops. This would not have been important if the material had no microbial content, but this was not the case.

Regarding helminths, the value found (29,8 ± 2,9 total helminths/gTS) indicated that the concentrations were not as high as could be expected for sludges from developing countries (ranging from 67 to 735 ova/gTS according to Jiménez and Wang 2005) and were even comparable to those obtained from anaerobic digester sludges in South Africa (2 to 40 *Ascaris*/gTS according to Snyman and van der Walt 2003). However, the faecal material had already been stored in a heap exposed to the weather for about four months, which accounted for much of the pathogen die-off that had already taken place.

Table 4.1: Faecal material characterisation

Parameter	Mean value (n=3)
N content, %	0,2 - 0,34
Total coliforms, CFU/gTS	$2{,}2x10^{6}$
Aspergillus spp, CFU/gTS	$3{,}9x10^{3}$
Faecal *streptococci*, CFU/gTS	$2{,}1x10^{6}$
Faecal coliforms, CFU/g TS	$1{,}8x10^{6}$
Salmonella spp, CFU/gTS	$2{,}2x10^{5}$
Total *Ascaris*, ova/gTS	25,3 ± 4,4
Total helminths, ova/gTS	29,8 ± 2,9
Viability of helminths, %	88,8 ± 0,5

Table 4.2: Original soil characteristics

Parameter	Mean value (n=3)
pH	7,7 ± 0,21
TS content (moisture)	86% ± 2 (14 ± 2)
Total coliforms	$8{,}1x10^{3}$ - $2{,}7x10^{5}$
Faecal *streptococci*	0, absent
Faecal coliforms	$2{,}6\ x10^{3}$ - $1{,}1x10^{4}$
Salmonella spp	0, absent
Aspergillus spp	0 - $7\ x\ 10^{1}$
Total helminths, ova/gTS	1,4 ± 0,5
Viability of helminths, %	0 - 10%

4.4.2 Irrigation water

Water used for irrigation came from a borehole and was stored in open tanks. The water was not disinfected and birds were often observed drinking water from the tanks. This is possibly the reason why some microbial pollution was found, although at very low concentrations. Total coliforms ranged from 0,1 to 0,3 CFU/100ml, faecal coliforms from 0,2 to 0,9 CFU/100ml, faecal *streptococci* from 0 to 0,1 CFU/100ml, while *Salmonella spp* and helminth ova were not detected in any of the five samples analysed. 5 litre samples were used for the analysis.

4.4.3 Original soil characteristics (Table 4.2)

Soils were slightly alkaline (pH 7,7) and contained microorganisms such as total and faecal coliforms. The first are commonly considered to be native in soils, while the second have been reported as native in water in high-temperature countries and therefore could be present in soils (Hazen and Toranzos 1990). Faecal *streptococci* and *Salmonella* (using the APHA, AWWA, WEF method) were not found in the soil while helminth ova were present in low concentrations (1,4 ± 0,5 HO/gTS) and with very low viability (0 to 10%). According to the records of the farm, the plots had not received any manure application (which can contain helminth ova) for at least 1,5 years. Regarding the genus, almost all the eggs found were *Ascaris* although *Toxocara* was sometimes also found.

4.4.4 Crop results

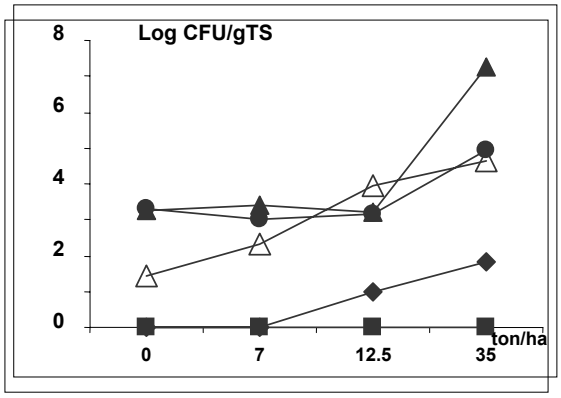

Figure 4.2(a): ▲Total Coliform; ●Faecal Coliform; ΔFaecal *Streptococci*; ■*Salmonella spp.*; and ♦*Aspergillus spp* in carrot soil after harvesting

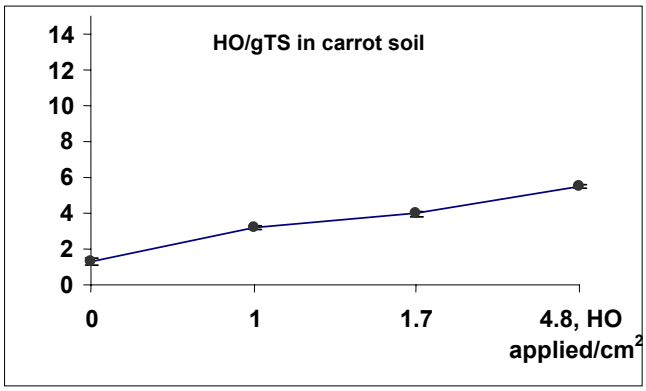

Figure 4.2(b): Helminth ova content in carrot soil after harvesting

The quantities of faecal material applied were equivalent in terms of the actual **viable** helminth eggs to application rates of 0,9; 1,5 and 4,3 HO/cm^2 for carrots and 0,2; 2,3 and 4,5 HO/cm^2 for spinach. Figure 4.2 shows the microbial effects of the application of faecal material on carrot soil. Total coliforms and faecal coliforms (Figure 4.2(a)) were present in the soil in similar concentrations for all the application rates, and only for the highest value a noticeable increase can be seen, while faecal *streptococci* increased correspondingly with increasing faecal material. Similar results were obtained for spinach soil, although the increase for the highest sludge application rate was less noticeable. In the case of *Salmonella*, the results in spinach soil were erratic, indicating that the Islam et al (2004) analytical technique was not appropriate. For carrots using the APHA, AWWA, WEF (1995) technique, *Salmonella* results were negative in all cases. Helminth ova in soils for both carrots and spinach (Figure 4.2(b), only for carrots) show a clear correlation with the rate of faecal material applied: the larger the sludge application rate the greater the number of helminth ova found in soils.

There was a diminishing helminth ova viability from the original value of 88,8% to 52 ± 3% for spinach soil and to 39 ± 7% in carrot soil. The greater decrease of viability in carrot soil was likely due to the longer time taken to monitor carrot soil (12 weeks) than for spinach soil (7 weeks). The decrease in viability can be explained, although not conclusively, by the high temperature registered during the summer time in Pretoria (ranging from 27-38^{o}C during the day) but also to possible prior damage suffered by the eggs during their earlier dehydration in the UD toilets as well as during further exposure to the elements in the heap, as mentioned above and as described in chapter 5. *Ascaris* have been reported to die rapidly at temperatures over 40^{o}C in different types of media including water, soil, sewage and crops (Feachem et al 1983). The temperature limit could be less if high temperatures are combined with other negative environmental conditions such as high ammonia content or low moisture (Heinonen-Tanski and Van Wijk-Sijbesma 2004).

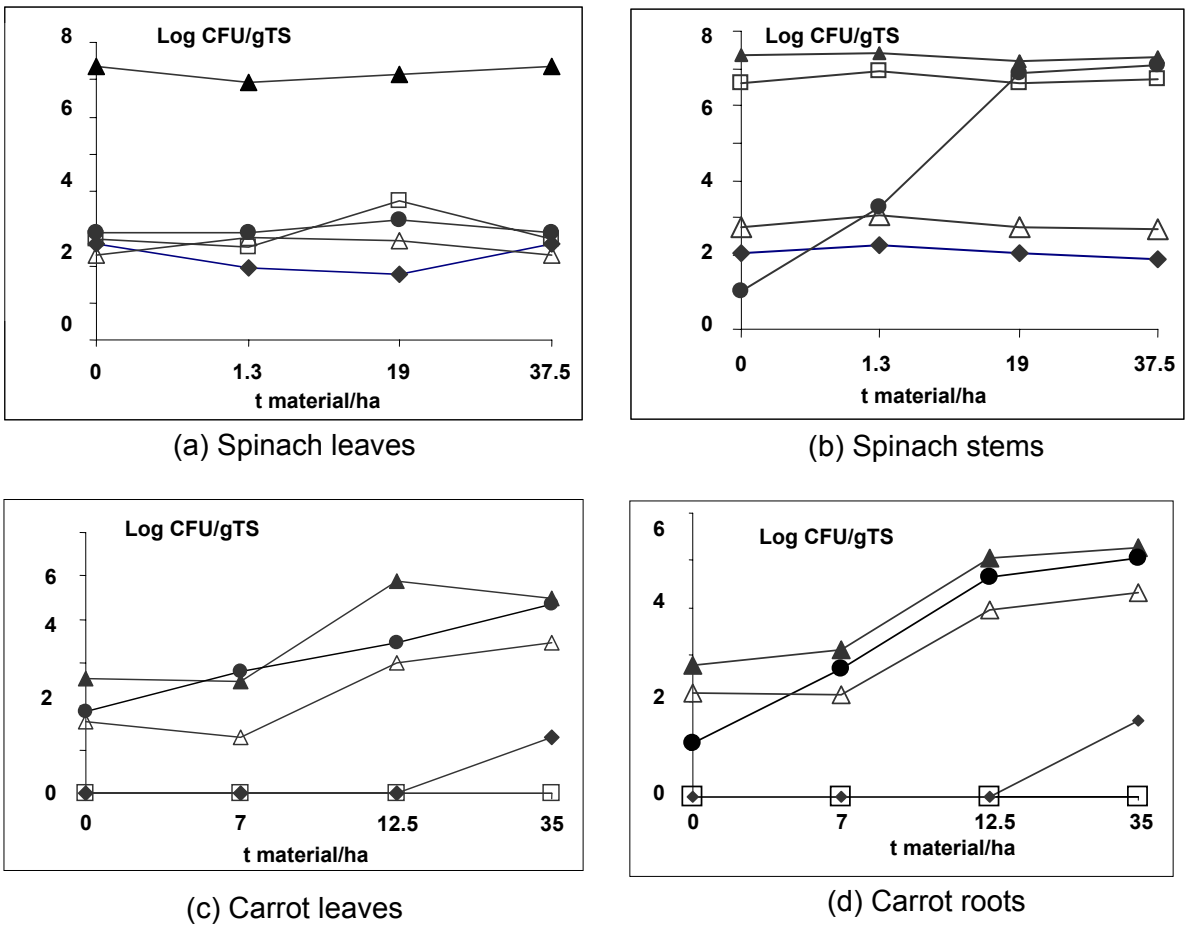

Figure 4.3: ▲Total Coliform; ●Faecal Coliform; ΔFaecal Streptococci; ■*Salmonella spp.*; ♦*Aspergillus spp.* on crops after harvesting

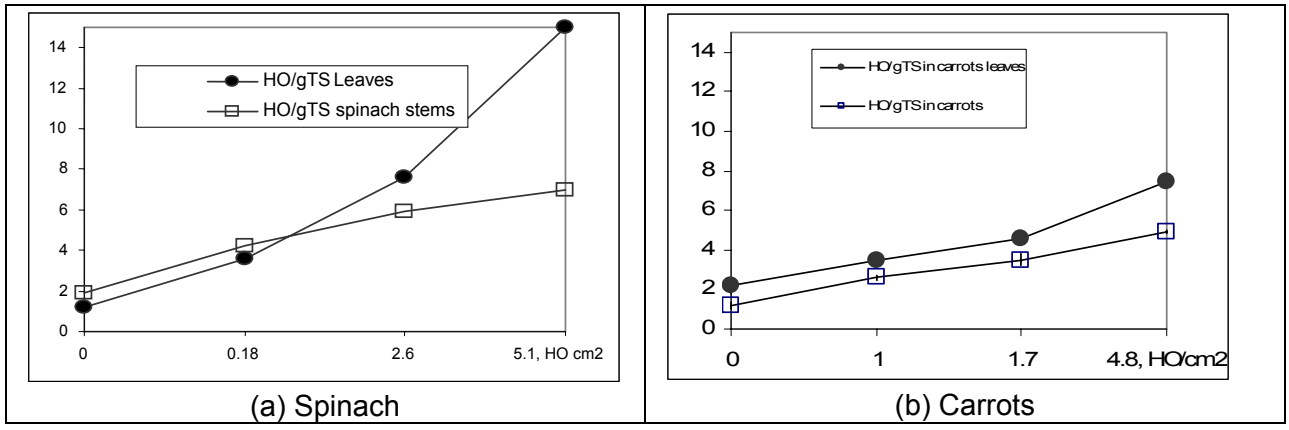

Figure 4.4: Helminth ova content in crops

Figure 4.3 shows the results of the bacteria numbers in spinach leaves (a) and stems (b). There was not a clear relationship between the quantity of faecal material applied and the total microbial number in leaves or stems. For faecal coliforms in stems, the results seem to indicate that bacteria can survive underground but not on top of the soil where UV sunlight is available to kill the organisms. In carrot leaves (c) and roots (d), total and faecal

coliforms as well as faecal *streptococci* increased as the faecal material application rate increased. *Aspergillus spp* and *Salmonella spp* were present in low numbers at all the different treatments, although the *Salmonella* results in spinach are suspect due to the testing technique used during the first phase of the experiment, as described in section 4.3.3. Regarding helminth ova, increasing concentrations were found in both stems and leaves (Figure 4.4(a) and (b)) as the quantity of material applied (and hence that of helminths) increased. Contamination is seen to be more important in leaves than in stems (roots), seeming to indicate that helminth ova are preferentially attached to parts of the plants above ground rather than to soil.

Although these results show that crops were polluted even using the smallest application rate, understanding the health significance would require proper epidemiological or toxicological studies that consider the probability of microorganisms, especially helminth ova, actually infecting the host. This would depend on the viability of eggs, the quantity of microorganisms consumed by a person through conventional daily diets in the region and the infective dose. Concerning the viability of helminth ova, for spinach crops the data were not obtained, but in carrot leaves it was 25 ± 5% while in carrot roots it was 20 ± 8%. This indicated that although present, they were mainly in an inactive state, thus reducing the risk of spreading the disease through consumption.

4.5 CONCLUSIONS FROM THIS EXPERIMENT

Faecal material was extracted from urine-diversion toilets in the eThekwini region of South Africa and left in a heap exposed to the weather for four months. Applying different rates of material to spinach and carrots, two common edible crops, it was found that the bacteria and fungi content were only noticeable for the higher rates (>35 t/ha), while the helminth ova content varied, both in leaves and stems, depending on the quantity of material applied. Helminth ova content was, for both crops, more prevalent in leaves, suggesting that the ova adhere preferentially to plants rather than soil.

To assess the actual health risk of helminth ova consumption, the final viability on crops needs to be considered as well as the infective dose for farmers and consumers and the daily diet of vegetables in the region. The actual age and storage conditions of the faecal material used are also important considerations.

It is clear, however, that there is a health risk involved in growing edible crops in soils amended with ecosan biosolids. Even if in this case the spinach and carrots were cooked before consumption, normal handling of the crops during harvesting and preparation could have caused infection if personal hygiene was unsatisfactory. It is thus of utmost importance that crop growers and consumers, as well as proponents of biosolids use, are aware of the storage and treatment requirements for ecosan biosolids before these are applied to soils where crops are grown. These aspects are investigated further in chapter 5.