

Chapter 6

Microbial Risk Assessment of Using Sewage Sludge for Soil Enrichment

6.1 Introduction

Changes in agricultural practice have over the years raised concern regarding the risk of ingesting pathogens from vegetables irrigated with wastewater or sewage sludge (Rudolfs *et al.*, 1951; Brent *et al.*, 1995; Shuval *et al.*, 1997). The occurrence of foodborne diseases remains a widespread problem in both the developing and developed world (Zwietering and van Gerwen, 2000). A systematic evaluation of safety is therefore important to control the risk of foodborne diseases. It is for this reason that worldwide, many initiatives are being taken to develop and apply microbial risk analysis (Blumenthal *et al.*, 1989; Rose and Gerba, 1991; Zwietering and van Gerwen, 2000). While sewage sludge contaminated soil has been shown to potentially lead to contaminated crops (Rudolfs *et al.*, 1951), in parts of Africa including South Africa, contaminated soil on its own is a hazard as deliberate and direct soil ingestion is common in these areas, especially by pregnant women in rural and peri-urban communities (Hunter, 1993; Walker *et al.*, 1997).

There is an increasing interest in the application of quantitative risk analysis in the production of microbiologically safe products (Notermans and Teunis, 1996; Petterson *et al.*, 2001). However, the quantitative evaluation of food safety is very complex, especially since in many case specific parameter values are difficult to obtain (Zwietering and van Gerwen, 2000). Scarcity of data often leads to qualitative assessments.

Individual adverse health effects related to microbial pathogens usually result from a single acute exposure, rather than long term chronic exposure (Farber *et al.*, 1996). Attempts of microbial risk assessment have generally used thermotolerant

faecal coliforms (Al-Nakshabandi *et al.*, 1997; Shuval *et al.*, 1997). However, unlike many other hazards, risk assessment of bacterial pathogens is influenced by a number of factors, including growth and possible inactivation from processing steps such as cooking in the case of vegetables or desiccation in the case of soil. Microorganisms are dynamic and adaptable. They can lose or acquire virulence-associated characteristics and can also adapt to the control measures set to manage microbial risks (Voysey and Brown, 2000). Also, consumption patterns may vary between individuals. These differences may have strong demographic components such as sex, age, culture and health status (Farber *et al.*, 1996).

A risk assessment provides a means of estimating the probability of adverse effects associated with measured or estimated levels of hazardous agents, and a tool for predicting the extent of potential health effects (Genthe, 1998). It involves a process that scientifically evaluates the probability of occurrence and severity of known or potentially adverse health effects resulting from human exposure to foodborne hazards (Zwietering and van Gerwen, 2000).

Risk assessments normally consist of four distinguishable but interacting phases generally referred to as:

- Hazard identification;
- Exposure assessment;
- Dose-response assessment and
- Risk characterization.

These were detailed in Chapter 2 section 2.10.1

6.2 Health Considerations for Consumption of Contaminated vegetables

In assessing microbial risk, the benchmark of 1 infection in 10 000 people per year is regarded as an acceptable level (Haas, 1996). This estimation is also supported by the United States Environmental Protection Agency (Rose and Gerba, 1991; Macler and Regli, 1993).

Infection with microbial hazard is complicated by a number of factors, that include the fact that:

- Microorganisms are capable of replicating;
- The virulence and infectivity of microorganisms can change depending on their interaction with the host and the environment;
- Genetic material can be transferred between microorganisms leading to the transfer of characteristics such as antibiotic resistance and virulence factors;
- Microorganisms can be spread through secondary and tertiary transmission;
- The onset of clinical symptoms can be substantially delayed following exposure;
- Microorganisms can persist in certain individuals leading to continued excretion of the microorganism and continued risk of spread of infection and
- Low doses of some microorganisms can cause a severe effect (Buchanan *et al.*, 2000).

Although sewage sludge contains various microorganisms as was indicated in earlier chapters, for the purpose of this study, the risk assessment was carried out only for *Escherichia coli*, *Salmonella* spp and *Ascaris* based on the laboratory analysis outlined in Chapter 4.

6.3 Assumptions

The approach used in the health risk assessment in this study involved a descriptive approach, which relies on estimating the frequency and severity of exposure to health hazards. Some common assumptions include:

- i The population is equally susceptible to an exposure.
- ii Exposure is from consumption of contaminated crops (potatoes as a worst case scenario) grown in sewage sludge amended soil. Individuals may also be exposed to pathogens by accidentally or deliberately ingesting contaminated soil (Walker *et al.*, 1997).
- iii Pathogenic microorganisms from sludge used are homogeneously distributed in the soil.
- iv It was assumed that there would be a certain degree of pathogen die-off and/or removal from the sludge and soil until the final ingestion by an individual in the home. These factors include settling, adsorption into soil, biological competition, UV irradiation from sunlight and a degree of removal and/or inactivation by washing of the vegetables. While other workers have indicated a rapid die-off of microorganisms following wastewater irrigation of soil (Rudolfs *et al.*, 1951), a possible re-growth of bacteria on vegetables have also been reported (Armon *et al.*, 1995).
- v While cooking or boiling of vegetables would reduce microorganisms, it was assumed that cross contamination could occur during food preparation.
- vi The risk of being infected by microbiological pathogens correlates with the level of contamination and the amount of contaminated vegetables

consumed. Higher numbers of microorganisms will indicate a higher risk of contracting microbial infection.

- vii All microorganisms ingested with the vegetable (or with the soil) are infective.

6.4 Methodology

Due to the scarcity of epidemiological data, assessment of the risk to health from the use of sewage sludge is based on a potential risk. This is based on accidental consumption of contaminated soil during the growing season and also on detection of microorganisms on the crops at the time of harvesting. The application rates of 8 tons/ha in high metal sludge and 16 tons/ha in low metal sludge were used.

Models used in this study are the beta-distribution infectivity probability model for bacteria and the single hit exponential model for parasites (Rose and Gerba, 1991).

β -distribution infectivity model

$$p = 1 - (1 + (N/\beta))^{-\alpha} \dots\dots\dots (1)$$

Single-hit exponential model

$$p = 1 - \exp(-rN) \dots\dots\dots (2)$$

where p = probability of infection from a single exposure or daily risk of infection

N = exposure or number of organisms ingested per exposure

α, β, r = parameters characterized by dose response curves

In addition to the single exposure risk or daily infection, weekly, monthly and yearly risk were calculated as 7, 30 or 365 days of exposure respectively, where

$$P_t = 1 - ((1 - P_{\text{calc}})^t)$$

t = 7, 30 or 365

A risk of 1 in 10 000 per year is considered acceptable risk of infection.

6.4.1 E. coli

The presence of *E. coli* was established by culture method. The quantity of *E. coli* present in 1 g of sample (soil or potatoes) was determined (Chapter 4). Contaminated potatoes (16) were those obtained from the LMS16 in chapter 4. This concentration was used to determine the *E. coli* present in 200 mg soil.

Risk estimation was based on the Beta-distributed “infectivity probability” model. The α and β values (0.1705 and 1.16) for *E. coli* are those proposed by Pepper *et al.*, 1996). Hypothetical values were also used to assess what levels of exposure are associated with certain levels of risk, and to estimate what quantity of *E. coli* in a (1) gram of potato or in 200 mg of soil would constitute acceptable risk.

6.4.2 Salmonella spp

The presence of *Salmonella* spp was determined by making use of culture method as described in Chapter 4. Results were based on the presence (positive test) and absence (negative test) of the organisms.

As the method used for *Salmonella* spp identification did not involve enumeration, hypothetical numbers were used in the analysis. The α and β values (0.33 and 139.9) for *Salmonella* spp are those proposed by Rose and Gerba (1991).

6.4.3 Ascaris ova

At present, there appears to be no information on the dose-response data for *Ascaris*. Therefore, there are no estimates for the r value for this organism. The value ($r = 0.0199$) for *Gardia* (Rose and Gerba, 1991), another protozoan was used in estimating the probability of infection as they have the same infective dose (Brooks *et al.*, 1991). Hypothetical numbers were used in analysis.

6.5 Results and Discussion

Although the present study assumed that all exposed individuals stand an equal chance of infection, risk of infection will vary between individuals depending on a number of factors. That is, a particular meal may pose no risk or a very high risk to an individual depending on the processing and handling of contaminated crops. Also factors such as the age, sex, previous exposure and immunocompetence of an individual influence the risk of infection (Buchanan *et al.*, 2000). Carneiro *et al.* (2002) could establish a link between rates of infection and socioeconomic status. In their study, children with less intense infection came from affluent households with higher socioeconomic and schooling profiles, while children from crowded dwellings had most infections (Carneiro, *et al.*, 2002). Farm workers could be among those at a high risk of infection, as a result of continued exposure at the work place.

6.5.1 E. coli

Of the 224 contaminated soil samples studied throughout the experiment, 71 were found not to have *E.coli*. Estimates of risk of infection from accidentally or deliberately ingesting 200 mg of soil contaminated with sewage sludge at different treatment options are tabulated in Tables 6.1 to 6.4. For 8 tons/ha high metal sludge (HMS), the risk is reduced by week six due to pathogen die-off (Table 6.1). The HMS at an application rate of 16 tons/ha shows far less risk than both LMS 8 tons/ha and 16 tons/ha (Tables 6.3 and 6.4). Application rate of 16 tons/ha LMS

shows a greater risk of infection when compared the other treatment options (HMS 8 tons/ha, HMS 16 tons/ha and LMS 8 tons/ha).

Using high metal sludge at 8 tons/ha resulted in a quick pathogen die-off as there were no microorganisms detectable after the sixth week of planting. All the subsequent weeks showed no risk of infection (Table 6.1). At harvest, no microorganisms were detected from potatoes grown in the HMS for both 8 tons/ha and 16 tons/ha. The HMS shows low probability of infection, probably due to the inhibitory role of heavy metals in this sludge (Tsai and Olson, 1990). Although pathogen die off appears an answer for controlling crop contamination, some researchers (Byrd *et al.*, 1991; Amman and Ludwig, 2000; Buchanan *et al.*, 2000) argue that for microorganisms that release toxins and those that may be non-culturable, the absence of viable pathogens may not necessarily imply microbiologically safe produce.

Table 6.1 Risk of ingestion of *E.coli* associated with accidental or deliberate ingestion of 200 mg soil contaminated with High Metal Sludge applied at 8 tons/ha

Time (Weeks)	Organisms CFU/200 mg	P(Single Exposure)	P(Weekly)	P(Monthly)	P(Yearly)
0	89.25	5.24E-01	9.94E-01	1.00E+00	1.00E+00
2	1172.25	6.93E-01	1.00E+00	1.00E+00	1.00E+00
4	314.5	6.16E-01	9.99E-01	1.00E+00	1.00E+00
6	352500	8.84E-01	1.00E+00	1.00E+00	1.00E+00
8	0	0	0	0	0
10	0	0	0	0	0
12	0	0	0	0	0

Table 6.2 Risk of ingestion of *E.coli* associated with accidental or deliberate ingestion of 200 mg soil contaminated with High Metal Sludge applied at 16 tons/ha

Time (Weeks)	Organisms CFU/200 mg	P(Single Exposure)	P(Weekly)	P(Monthly)	P(Yearly)
0	309.5	6.14E-01	9.99E-01	1.00E+00	1.00E+00
2	3982.5	7.50E-01	1.00E+00	1.00E+00	1.00E+00
4	1398250	9.08E-01	1.00E+00	1.00E+00	1.00E+00
6	427511.2	8.88E-01	1.00E+00	1.00E+00	1.00E+00
8	1.5	1.32E-01	6.29E-01	9.86E-01	1.00E+00
10	1.25	1.17E-01	5.82E-01	9.70E-01	1.00E+00
12	0	0	0	0	0

Of the 64 potatoes studied from all the treatment options, 16 (25%) were found to be contaminated. These potatoes were grown in LMS 16 tons/ha. Risks of infection for exposure to *E. coli* calculated based on consumption of 1 g of contaminated potato grown in LMS 16 tons/ha soil are shown in Table 6.3. The average of the counts obtained from cleaned potato peels was 18 000 CFU/g. This number of organisms yielded a high probability of exposure of 8.07 E01 for a single exposure. The weekly, monthly and yearly exposures yielded even higher risk of infection (100% probability). At harvest, none of the potatoes from HMS 8 tons/ha, HMS 16 tons/ha or LMS 8 tons/ha had any *E. coli*. The probability of exposure to microorganisms from potato using these application rates is zero.

Table 6.3 Risk of ingestion of *E. coli* associated with accidental or deliberate ingestion of 200 mg of soil contaminated with Low Metal Sludge applied at 16 tons/ha

Time (Weeks)	Organisms CFU/200mg	P(Single Exposure)	P(Weekly)	P(Monthly)	P(Yearly)
0	5050	7.60E-01	1.00E+00	1.00E+00	1.00E+00
2	5987.5	7.67E-01	1.00E+00	1.00E+00	1.00E+00
4	2040000	9.14E-01	1.00E+00	1.00E+00	1.00E+00
6	325895	8.82E-01	1.00E+00	1.00E+00	1.00E+00
8	136.9	5.57E-01	9.97E-01	1.00E+00	1.00E+00
10	32.9	4.38E-01	9.82E-01	1.00E+00	1.00E+00
12	20325	8.11E-01	1.00E+00	1.00E+00	1.00E+00
12	18 000 (in 1 g potato)	8.07E-01	1.00E+00	1.00E+00	1.00E+00

Table 6.4 Risk of ingestion of *E.coli* associated with accidental or deliberate ingestion of 200 mg soil contaminated with Low Metal Sludge applied at 8 tons/ha

Time (Weeks)	Organisms CFU/200mg	P(Single Exposure)	P(Weekly)	P(Monthly)	P(Yearly)
0	435.75	6.36E-01	9.99E-01	1.00E+00	1.00E+00
2	5272500	9.27E-01	1.00E+00	1.00E+00	1.00E+00
4	925000	9.01E-01	1.00E+00	1.00E+00	1.00E+00
6	0	0	0	0	0
8	750	6.68E-01	1.00E+00	1.00E+00	1.00E+00
10	1355	7.00E-01	1.00E+00	1.00E+00	1.00E+00
12	45.25	4.67E-01	9.88E-01	1.00E+00	1.00E+00

Hypothetical numbers of *E. coli* were fitted to the model to predict what level of contamination would produce a corresponding risk of 1 infection in 10 000 (Table 6.5). These numbers were used to determine risk of infection ranging from a single

exposure to yearly exposure, and to estimate the quantity of organism that would constitute acceptable risk (Rose and Gerba, 1991).

Table 6.5 Risks associated with hypothetical exposures to potato or soil contaminated with *E. coli* (Beta Distribution Model)

N (Number of organisms)	P(Single Exposure)	P(Weekly)	P (Monthly)	P (Yearly)
10 000	7.87E-01	1.00E+00	1.00E+00	1.00E+00
1 000	6.84E-01	1.00E+00	1.00E+00	1.00E+00
100	5.33E-01	9.95E-01	1.00E+00	1.00E+00
10	3.20E-01	9.33E-01	1.00E+00	1.00E+00
1	1.01E-01	5.24E-01	9.58E-01	1.00E+00
0.1	1.40E-02	9.40E-02	3.45E-01	9.94E-01
0.01	1.46E-03	1.02E-02	4.30E-02	4.14E-01
0.001	1.47E-04	1.03E-03	4.40E-03	5.22E-02
0.0001	1.47E-05	1.03E-04	4.41E-04	5.35E-03
Number of organisms required for an acceptable risk				
6.83X10⁻⁴	1.00E-04			
9.75X10⁻⁵		1.00E-04		
2.27X10⁻⁵			1.00E-04	
1.87X10⁻⁶				1.00E-04

The present study has shown that for a daily consumption of vegetation grown from contaminated soil (or ingestion of soil), the number of *E. coli* should be less than 6.83×10^{-4} (Table 6.5) for the risk of infection to meet the requirements suggested by US EPA of 1 in 10 000 acceptable risk (Rose and Gerba, 1991; Macler and Regli, 1993). For the annual risk of infection to be less than 1 in 10 000, the number of organisms should be less than 1.87×10^{-6} . The presence of 1 CFU/g *E. coli* is likely to bring about a probability of infection of 1.01×10^{-1} , which is approximately 1 in 10. This risk increases for a weekly, monthly and yearly infection

with corresponding risks of 5.24E-01 (5 in 10), 9.58E-01 (9 in 10) and 100% probability respectively (Table 6.5).

Handling of potatoes has been associated with *E. coli* infection in the United States (Armstrong *et al.*, 1996). Jones (1999) also indicated the potential health risks associated with the persistence of *E. coli* in agricultural environments, yielding a high incidence of human infections in the UK.

Figure 6.1 shows that even small numbers of bacteria could result in a high probability of infection. Considering the high prevalence of HIV/AIDS in South Africa (Dorington *et al.*, 2002), the use of inadequately treated sludge could pose serious health hazards in individuals with compromised immune systems.

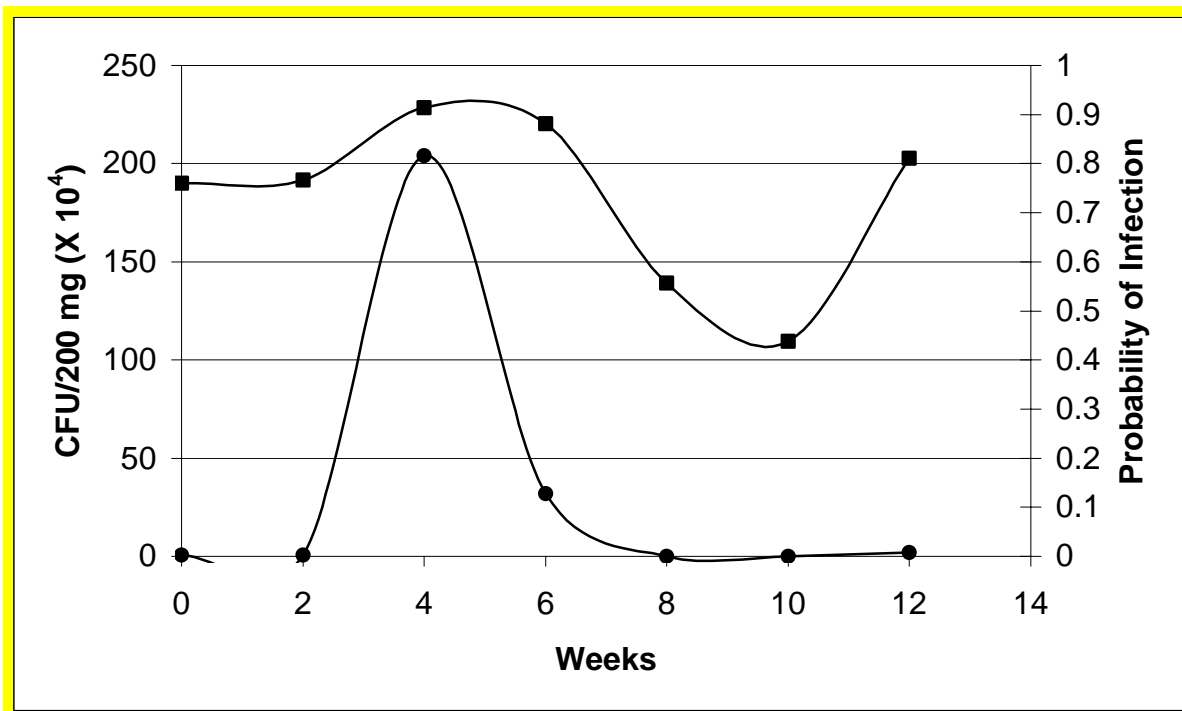


Figure 6.1 The relationship between the number of bacteria in 200 mg of contaminated soil amended with 16 tons/ha LMS, and the probability of infection (• = CFU/200 mg; ■.= Probability of infection).

Low metal sludge yielded high risk of infection throughout the duration of the experiment based on the accidental ingestion of 200 mg of contaminated soil (Figure 6.1; Table 6.3 and 6.4). This study assumed that people might be infected from accidentally ingesting soil (WRC, 1997) or deliberately and willingly ingesting soil (Hunter, 1993; Walker *et al.*, 1997; Smith, 2002). In Africa, eating soil (geophagia) dates from the 18th century as observed in Nigeria, Ghana and Sierra Leone (Hunter, 1993). It has become a common practice and spread to other countries, namely Malawi, Zambia, Swaziland and South Africa (Walker *et al.*, 1997). Although the main reason for consuming soil is uncertain, it has been associated with poverty and poor eating habits, and has been found to be prevalent in pregnant women, those with poor nutrition and those with a family history of geophagia (Geissler *et al.*, 1998; Smith, 2002). The use of sewage sludge either in their gardens or in the farms could have serious health effects for communities who practice geophagia. Geissler and colleagues (1998) studied a relationship between geophagy in school children in Western Kenya, and helminthes, and found that 77% of the children ate soil daily and 48% of the soil samples they tested were contaminated with *Ascaris*.

Potatoes are rarely consumed raw, however there is evidence that contaminated potatoes can lead to serious infections (Seals *et al.*, 1981; Brent *et al.*, 1995). Although some organisms such as *Clostridium botulinum* can survive baking (Brent *et al.*, 1995) the prevalence of microorganisms in potato dishes is likely to be due to a number of factors that include handling which could lead contamination of utensils and surfaces. As microorganisms have a potential to replicate, such cross contamination may eventually contaminate the finished product.

6.5.2 *Salmonella* spp

As the results for *Salmonella* spp were only based on the presence and absence of this organism, risk assessment based on the beta distribution model for consumption of *Salmonella* spp was based only on hypothetical numbers (Table 6.6). For this organism, to attain a risk of infection to be less than 1 in 10 000, there need to be less than 4.25×10^{-2} organisms per g for daily exposure and 1.17×10^{-4} for yearly exposure. If one ingests a single organism, the probability of infection is about 2 in a 1000 (2.35×10^{-3}) for a single exposure and about 2 in 100 (1.63×10^{-2}), 7 in 100 (6.81×10^{-2}) and 6 in 10 (5.76×10^{-1}) for weekly, monthly and yearly exposures respectively.

Table 6.6 Risks associated with hypothetical exposures to *Salmonella*
(Beta Distribution Model)

N (Number of organisms)	P(Single Exposure)	P(Weekly)	P (Monthly)	P (Yearly)
10 000	7.57E-01	1.00E+00	1.00E+00	1.00E+00
1 000	5.00E-01	9.92E-01	1.00E+00	1.00E+00
100	1.63E-01	7.12E-01	9.95E-01	1.00E+00
10	2.25E-02	1.47E-01	4.95E-01	1.00E+00
1	2.35E-03	1.63E-02	6.81E-02	5.76E-01
0.1	2.36E-04	1.65E-03	7.05E-03	8.25E-02
0.01	2.36E-05	1.65E-04	7.07E-04	8.57E-03
0.001	2.36E-06	1.65E-05	7.08E-05	8.61E-4
0.0001	2.36E-07	1.65E-06	7.08E-06	8.61E-05
Number of organisms required for an acceptable risk				
4.25×10^{-2}	1.00E-04			
6.08×10^{-3}		1.00E-04		
1.42×10^{-3}			1.00E-04	
1.17×10^{-4}				1.00E-04

Risk from infection due to *Salmonella spp* contaminated food was indicated by Walls and Scott (1997) in USA where 1.2 million cases of *Salmonella spp* infection are reported per year. Based on their assessment and the number of cases reported, they concluded that on any given day, 3190 individuals might become infected with *Salmonella* (Wall and Scott, 1997).

6.5.3 Ascaris

Ascaris ova were detected in the soil only at the beginning of the experiment (1/g soil for HMS in week 2, and 4/g soil for LMS in week 4) (Chapter 4). No *Ascaris* were detected in samples analysed from the sixth week of the experimental period.

Table 6.7 Risks associated with hypothetical exposures to *Ascaris*

(Exponential Model)

N (Number of organisms)	P(Single Exposure)	P(Weekly)	P (Monthly)	P (Yearly)
10 000	1.00E+00	1.00E+00	1.00E+00	1.00E+00
1 000	1.00E+00	1.00E+00	1.00E+00	1.00E+00
100	8.50E-01	1.00E+00	1.00E+00	1.00E+00
10	1.73E-01	7.36E-01	9.97E-01	1.00E+00
1	1.88E-02	1.25E-01	4.34E-01	9.99E-01
0.1	1.90E-03	1.32E-02	5.54E-02	5.00E-01
0.01	1.90E-04	1.33E-03	5.68E-03	6.70E-02
0.001	1.90E-05	1.33E-04	5.70E-04	6.91E-03
0.0001	1.90E-06	1.33E-05	5.70E-05	6.93E-04
Number of organisms required for an acceptable risk				
5.27X10⁻³	1.00E-04			
7.55X10⁻⁴		1.00E-04		
1.76X10⁻⁴			1.00E-04	
1.45X10⁻⁵				1.00E-04

According to the model, using hypothetical numbers, if only a single *Ascaris* is consumed, there is a probability of infection is 1.88E-02 (approximately 2 in 100) for daily exposure and 1.25E-01 (1 in 10), 4.34E-01 (4 in 10) and 9.99E-01 (9 in 10) for weekly, monthly and yearly exposures respectively (Table 6.7). If less than 5.27×10^{-3} organism is consumed (from soil or vegetable), the risk of infection is acceptable (< 1 in 10 000). For yearly exposure, infection will be less than 1 in 10 000 if less than 1.45×10^{-5} organisms are consumed.

Although no *Ascaris* ova were detected on the potato peel at the time of harvest, the possibility of infection from these organisms may not be ignored. Recently, Carneiro *et al.* (2002) reported prevalence of *Ascaris lumbricoides* infection from consumption of contaminated water in Brazil. It has been earlier reported that *Ascaris* infects approximately 25% of the world's population annually (Crompton, 1988).

6.6 Conclusions and Recommendations

This risk assessment was based on the accidental or deliberate ingestion of contaminated soil during the planting, growing or harvesting following sewage sludge application, and also on the consumption of contaminated potato post-harvest. Risk estimation was based on the quantity of organisms in the soil and on the surface of potato.

It has clearly been shown that even very low numbers of pathogens may present a high risk of infection from *E. coli*, *Salmonella* spp and *Ascaris* to those individuals exposed to these pathogens. Risk assessment for these organisms required that their numbers present on the crop should be extremely low to correspond to a less than 1 in 10 000 annual risk of infection. Haas and colleagues (2000) who validated their results with reference to two outbreaks have pointed out that comparison of real world situations with the predictions these models are highly plausible.

This study recommends the use of *E. coli* as an indicator for safe use of sewage sludge in agricultural land.

Using HMS at 8 tons/ha resulted in limited risk of infection as pathogens die long before crops are harvested. However, other agents such as heavy metals and organic chemicals should be put into consideration when there is intended use of sewage sludge.

Considering the risk associated with exposure to heavy metals, the LMS at 8 tons/ha appears a better option to use, if preceded by intense treatment.

The risk estimated in this study is based on the pathogens studied. Considering that sewage sludge contains numerous pathogens (Chapters 2 and 3) including viruses, undoubtedly the potential for sludge use in agricultural land to cause gross health effects far exceeds the estimates made in this study.

The risk assessment is a useful tool to illustrate that management practices could play an important role in reducing the health risk associated with the use of sewage sludge on agricultural land.

Due to the prevalence of HIV/AIDS in the country and the poor hygienic practices of most people including those living in informal settlements, use of inadequately treated sludge in agricultural land used for crops meant for human consumption holds potential to yield countless infections, and could pose a serious health hazard for such communities.

Intensive pathogen reduction in sewage sludge will be necessary prior to using the product as soil conditioner. This will ensure that sewage sludge to be applied to land starts with low numbers that may eventually die off.

As this study was based on conservative assumptions, and has estimated a high risk, further studies especially those based on epidemiological data are recommended.

This study was based on potato, which is one of the high risk crops, further research on other crops will need to be investigated for evaluation of risk of infection.

6.7 References

- Al-Nakshandi, G.A., Saqqar, M.M., Shatanawi, M.R., Fayyad, M. and Al-Horani, H. 1997. Some Environmental Problems Associated with the Use of Treated Wastewater for Irrigation in Jordan. *Agriculture Water Management*. **34**(1). 81-86
- Amann, R. and Ludwig, W. 2000. Ribosomal RNA-targeted Nucleic Acid Probes for Studies in Microbial Ecology. *FEMS Microbiology Reviews*. **24**. 555-565
- Armon, R., Dosoretz, C.G., Azov, Y. and Shelef, G. 1995. Residual contamination of crops Irrigated with Effluent of Different Qualities: a Field Study. *Water Science and Technology*. **30**(9). 239-248
- Armstrong, G.L., Hollingsworth, J. and Morris J.G. 1996. Emerging Foodborne Pathogens: *Escherichia coli* O157:H7 as a Model of Entry of a New Pathogen into the Food Supply of the Developed World. *Epidemiological Reviews*. **18**. 28-51
- Blumenthal, U.J., Strauss, M., Mara, D.D. and Cairncross, S. 1989. Generalised model of the Effect of Different Control Measures in Reducing Health Risks from Waste Reuse. *Water Science and Technology*. **21**. 567-577
- Brent, J., Gomez, H., Judson, F., Miller, K., Rossi-Davis, A., Shillam, P., Hatheway, C. McCroskey, L., Mintz, E., Kallander, K., McKee, C., Romer, J., Sinlgeton, E., Yager, J. and Sofos, J. 1995. Botulism from Potato Salad. *Dairy, Food and Environmental Sanitation*. **15**(7). 420-422
- Brooks, G.F., Butel, J.S. and Ornston, L.N. 1991. Medical Microbiology. 9th Edition. 488pp

Buchanan, R.L., Smith, J.L. and Long, W. 2000. Microbial Risk Assessment: Dose-Response Relations and Risk Characterization. *International Journal of Food Microbiology*. **58**. 159-172

Byrd, J.J., Xu, H.S. and Colwell, R.R. 1991. Viable but non-culturable bacteria in drinking water. *Applied Environmental Microbiology*. **57**. 875-878

Carneiro, F.F., Cifuentes, E., Tellez-Rojo, M.M. and Romieu, I. 2002. The Risk of *Ascaris lumbricoides* Infection in Children as an Environmental Health Indicator to Guide Preventive Activities in Caparaó and Alto Caparaó, Brazil. *Bulletin of the World Health Organization*. **80**(1). 40-46

Crompton, D.W.T. 1988. The Prevalence of Ascariasis. *Parasitology Today*. **4**. 162-168

Dorrington, R., Bradshaw, D. and Budlender, D. 2002. HIV/AIDS Profile in the Provinces of South Africa. Indicators for 2002. Medical Research Council, South Africa. 31pp

Farber, J.M., Ross, W.H. and Harwig, J. 1996. Health Risk Assessment of *Listeria monocytogenes* in Canada. *International Journal of Food Microbiology*. **30**. 145-156

Geissler, P.W., Shulman, C.E., Price, R.J., Mutemi, W., Mzani, C., Friis, H. and Lowe, B. 1998. Geophagy, Iron Status and Anaemia Among Pregnant Women on the Coast of Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **92**(5). 549-553

Genthe, B. 1998. Specialist Study on the Potential Health Impacts of the Proposed wastewater treatment facility on the West Bank of East London. Environmentek, CSIR. 20pp

Haas, C.N. 1996. Acceptable Microbial Risk. *Journal of the American Water Works Association*. **88**(12). 8

Haas, C.N., Thayyar-Madabusi, A., Rose, J.B. and Gerba, C.P. 2000. Development of a Dose-Response relationship for *Escherichia Coli* O157:H7. *International Journal of Food Microbiology*. **1748**. 153-159

Hunter, J.M. 1993. Macroterm Geophagy and Pregnancy Clays in Southern Africa. *Journal of Cultural Geography*. **14**. 69-92

Hyde, H.C. 1976. Utilization of Wastewater Sludge for Agricultural Soil Enrichment. *Journal of Water Pollution Control Federation*. **48**(1). 77 - 90

Jones, D.L. 1999. Potential Health Risks Associated with the Persistence of *Escherichia coli* O157:H7 in Agricultural Environments. *Soil Use and Management*. **15**. 76-83

Macler, B.A. and Regli, S. 1993. Use of Microbial Risk Assessment in Setting US drinking Water Sandards. *International Journal of Food Microbiology*. **19**. 245-256

Notermans, S. and Teunis, P. 1996. Quantitative Risk Analysis and the Production of Microbiologically safe Food: An Introduction. *International Journal of Food Microbiology*. **30**. 3-7

Pepper, I., Gerba, C.P. and Brusseau, M. 1996. Pollution Science. Academic Press, San Diego. 397pp

Petterson, S.R., Ashbolt, N.J. and Sharma, A. 2001. Microbial Risks from Wastewater Irrigation of salad Crops: A Screening-Level Risk Assessment. *Water Environment Research*. **72**(6) 667-672

Rose, J.B., and Gerba, C.P. 1991. Use of Risk Assessment for Development of Microbial Standards. *Water Science and Technology*. **24**(2). 29-34

Rudolfs, W., Falk, L.L. and Ragotzkie, R.A. 1951. Contamination of Vegetables Grown in Polluted Soil. *Sewage and Industrial wastes*. **23**. 992-1000

Seals, J.E., Snyder, J.D., Edell, T.A., Hatheway, C.L., Johnson, C.J., Swanson, R.C. and Hughes, J.M. 1981. Restaurant-Associated Type A botulism: Transmission by Potato Salad. *American Journal of Epidemiology*. **113**. 436-444

Shuval, H., Lampert, Y and 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

Smith, B. 2002. Eating Soil. *Planet Earth Autumn*. 21-22

Tsai, Y-L. and Olson, B.H. 1990. Effects of Hg^{2+} , CH_3-Hg^+ , and Temperature on the Expression of Mercury Resistance Genes in Environmental Bacteria. *Applied and Environmental Microbiology*. **56**(11). 3266-3272.

Voysey, P.A. and Brown, M. 2000. Microbiological Risk Assessment: A New Approach to Food safety Control. *International Journal of Food Microbiology*. **58**. 173-179

Walker, A.R.P., Walker, B.F., Sookaria, F.I. and Canaan, R.J. 1997. Pica. *Journal of Roy Health*. **117**. 280-284

Walls, I. And Scott, V.N. 1997. Use of Predictive Microbiology in Microbial Food Safety Risk Assessment. *International Journal of Food Microbiology*. **36**. 97-102

WRC, 1997. Permissible Utilisation and Disposal of Sewage Sludge. Water Research Commission. TT 85/97

Zwietering, M.H and van Gerwen, S.J.C. 2000. Sensitivity Analysis in Quantitative Microbial Risk Assessment. *International Journal of Food Microbiology*. **58**. 213-221