

Chapter 2

Literature Review

2.1 Introduction

The use of human excreta for fertilizer, ranging from night soil application to irrigation with sewage has been a world-wide practice for many years, especially in highly populated countries such as China and India (Rudolfs *et al.*, 1950). It is especially advantageous because it recycles nutrients back to the land and can be economically attractive (Zenz *et al.*, 1976).

In South Africa sludge production is increasing rapidly, and at the same time the soil condition has deteriorated markedly. As sludge contains high levels of organic matter and nutrients (Hu *et al.*, 1996), use of this product in agricultural land could provide an alternative means of disposal, and also benefits the poor soil quality of most of South Africa's agricultural land. It is believed that when treated properly, and provided certain industrial contaminants are restricted from entering the sewage, the resultant sewage sludge can become a relatively innocuous organic fertilizer and soil conditioner of significant value for growing trees, grass and certain crops (WRC, 1997).

The beneficial use of sludge for soil amendment in South Africa was also recently shown by Snyman and colleagues (1998). At present, sewage sludge is used for crop growing but limited only to fenced areas to restrict access to unauthorized persons as well as milk-, meat- and egg producing animals (WRC, 1997). Other recommendations suggest that application may only be done with planting and during the period subsequent to harvesting and prior to the next growing season. Snyman and Van der Waals (2003) reported that South African farmers, noting the increased crop production as a result of enhanced soil properties from sewage sludge use, are in favour of using sewage sludge and show adherence to the recommended dosage of 8 ton/ha as stipulated in the guidelines (WRC, 1997).

Elsewhere, application of sewage sludge to deteriorated soil, resulted in increased yields (Tester and Parr, 1983). Consequently, municipal sewage sludges are routinely utilized on agricultural lands in various parts of the world. In Canada, it is becoming a common practice such that as much as 43% of the produced sewage sludge is applied to land. By comparison, the United States and Europe apply approximately 60% and 34% respectively of their sewage sludge to agricultural land (EPA, 1999; Apedaile, 2001).

2.2 Metals and Toxic Organic Pollutants in Sludge

The composition of wastewater sludge may be highly variable depending on the quantity and quality of contributions from industrial and domestic sources. The types of constituents include among others, chlorinated hydrocarbons, polynuclear aromatic hydrocarbons and metals (Brown *et al.*, 1991). Hyde (1976) pointed out that heavy metals are retained in soils following sludge application and can accumulate to the point at which they are toxic to plants. Thus, due to their uptake by crops, they may also be toxic to humans and animals. This observation was confirmed by Rost and colleagues (2001) who recently reported that heavy metals have long lasting adverse effects on biological functions in soil. The heavy metal of major concern, because of its possible phytotoxicity and danger to the human food chain, is cadmium (Cd). Other heavy metals of importance are copper (Cu), nickel (Ni) and zinc (Zn), and they are also known to be phytotoxic (Hyde, 1976; Purves, 1990).

Organic compounds such as pesticides, polychlorinated biphenyls, halogenated aliphatics, ethers and aromatic hydrocarbons are the products of industrial wastewater which could land up in wastewater sludges (Korentajer, 1991; Vorobieva *et al.*, 1996; Kouloumbis *et al.*, 2000). The concentration of these compounds needs to be monitored and limited by implementing source reduction.

2.3 Socio-economic Issues Regarding Sludge Use

Farmers and the food industry have expressed their concern that agricultural use of untreated sludge may affect the safety of food products and the sustainability of agricultural land, and may carry potential economic and liability risks (NRC, 1996). There is also concern that the use of contaminated sewage sludge for crop production could negatively affect the export market. For fear of foodborne illness, some countries may refuse importation of vegetables and foods produced under such agricultural practices (Sobsey, 1996; Doyle, 2000).

There has been increased public scrutiny of the potential health and environmental consequences of land spreading of sewage sludge. It appears that once fear of pathogens, odours, nuisances and possible environmental deterioration have been generated in a community, people have great difficulty in accepting the risks, even if there aren't any, of applying sewage sludge to agricultural land (Hyde, 1976; Tauxe, 1997). Thus, it is essential that aesthetic characteristics and matters affecting both long-term quality of the land and the public health must be thoroughly understood before using sewage sludge on farmland.

In spite of the increasing concerns, in their recent report to the United States Environmental Protection Agency, the National Research Council pointed out that there is no evidence that proper use of wastewater treatment sludge on land has any detrimental effect on either the people working at the site, on the population surrounding the land application site, or on people eating the crops grown in the sludge-amended soil (NRC, 2002). Vesilind (2003) is of the opinion that the aversion to sludge use emanates from the knowledge of its origin and not necessarily from diseases linked to sludge use.

Kirby (2001) pointed out that exposure to potentially lethal pathogens is linked to social factors such as class, education and income. Carneiro and his colleagues (2002) have observed that less intense *Ascaris* infection came from affluent

households with higher socio-economic profile. In many African countries including South Africa, a large percentage of the population live in poverty (Parliamentary Bulletin, 1996), thus it can be expected that these households would be intensely affected by contaminated crops. The high incidence of HIV/AIDS (Human Immunodeficiency Virus/ Acquired Immune Deficiency Syndrome) infection in the country could translate into more pathogenic infections due to their depressed immune systems, if such communities are exposed to contaminated crops. South Africa is a comparatively large country, covering 1,221,042 square kilometers and with an estimated population of about 40 million. It has been estimated that 14.2% of people in South Africa have been infected with HIV/AIDS (Dorrington *et al.*, 2002). The routine surveillance conducted by the Department of Health has shown that among pregnant women attending public health clinics for antenatal care, the prevalence has increased from less than 1% in 1990 to 26.5% in 2002 (Dorrington *et al.*, 2002). Overall, it is estimated that 23.3% of men and 23.5% of women are infected whereas the prevalence amongst the male and female youth is 5.8% and 21.6% respectively (Dorrington *et al.*, 2002).

2.4 Microorganisms Encountered in Sewage Sludge

Infectious diseases are transmitted primarily through human and animal excreta, particularly faeces. If there are active cases or carriers in the community, then faecal contamination of water sources will result in the causative organisms being present in water. Pathogens in domestic sewage are primarily associated with insoluble solids. Many of these organisms become bound to solids following wastewater treatment and are transferred to wastewater sludge (Bitton, 1994). As the wastewater treatment processes concentrate these solids into sewage sludge, the sewage sludge has higher quantities of pathogens than incoming wastewater (EPA, 1999). However, the transmission of pathogens can be minimized by reducing the infectivity of sludges through effective treatment processes (Smith, 1996).

The actual species and quantity of pathogens present in sewage sludge from a particular municipality depend on the health status of the local community and may vary substantially at different times (EPA, 1999). The four major types of human pathogenic organisms, namely bacteria, viruses, protozoa and helminths may all be present in sludge. These organisms can cause infection or disease if humans or even animals are exposed to sufficient levels. The infective dose, that is, the number of pathogenic organism to which a human must be exposed to become infected, varies depending on the organism and on the health status of the exposed individual (EPA, 1999). While some pathogens may cause infections in a susceptible host by a single organism, others may require several hundreds to be present before an infection can be initiated. Symptoms may vary in severity from mild gastroenteritis to severe and sometimes fatal diarrhoea, dysentery, hepatitis or typhoid depending on the type of pathogen and pathogen load. Thus, when reclaimed water or sludge is used on fields producing food crops, it is critical to protect public health.

In the sections that follow, the major bacterial, viral and parasitic organisms found in wastewater sludge are described.

2.4.1 Bacteria

Wastewater normally contains many bacterial species, and strains (Vilanova *et al.*, 2002) that may end up in the wastewater sludge. If such sludge is not adequately treated and used in agricultural land, crop contamination may be imminent. As Buber and colleagues (1999) have pointed out, contamination of food material does not only occur during food processing, but may also begin with the production of raw food materials in the environment.

Faecal coliforms and enterococci have been used widely as faecal pollution indicators (Vilanova *et al.*, 2002). Both bacterial groups include several species. For example, the genus *Enterococcus* contains 19 recognized species (Manero and Blanch, 1999). *Salmonella* spp, *Shigella* spp, *Campylobacter* spp, *Yersinia*

spp, *Leptosporia* spp and *Escherichia coli* are bacterial pathogens of primary concern in sludge. *Escherichia coli* is particularly abundant in human and animal faeces, where numbers may reach 10^9 g⁻¹ of faeces (Bitton, 1994). The major bacterial groups or species are tabulated in Table 2.1 and some of these (*) are discussed in sections i to viii. Several case studies have been cited in these sections. These case studies do not necessarily detail outbreaks due to wastewater sludge use, but are indicators of what the effects could be if the pathogens manage to survive and infect a receptor, as a worst-case scenario.

Table 2.1 Bacterial pathogens to be expected in sewage sludge (Source: EPA, 1999; Strauch, 1991)

Pathogen	Disease
<i>Salmonella</i> spp *	Salmonellosis (gastroenteritis)
<i>Shigella</i> spp *	Bacillary dysentery
<i>Escherichia coli</i> *	Urinary infection; diarrhoea
<i>Yersinia enterocolitica</i> *	Yersniosis (gastroenteritis)
<i>Clostridium</i> spp *	Gas gangrene
<i>Leptospira</i> spp	Leptospirosis
<i>Mycobacterium</i> spp	Tuberculosis and leprosy
<i>Vibrio cholerae</i> spp	Cholera
<i>Staphylococcus</i> spp	Osteomyelitis
<i>Streptococcus</i> spp	Rheumatic fever; glomerulonephritis
<i>Klebsiella</i> spp	Pneumonia; urinary tract infection
<i>Enterobacter</i> spp	Urinary tract infection
<i>Serratia</i> spp	Meningitis; endocarditis
<i>Citrobacter</i> spp	Neonatal meningitis
<i>Proteus</i> spp	Urinary tract infection
<i>Providencia</i> spp *	Urinary tract infection
<i>Listeria monocytogenes</i> *	Listeriosis

i *Escherichia coli*

Escherichia coli is normally found in the gastrointestinal tract of humans and other warm-blooded animals (Brooks *et al.*, 1991) and is the most common cause of foodborne illness. Foods that have been implicated with *E. coli* include cheese, beef, fish, poultry, apple cider and lettuce (Reis *et al.*, 1980; Kornacki and Marth, 1982; Ackers *et al.*, 1998).

Escherichia coli, depending on the infective strain, can cause a variety of illnesses that include infantile diarrhoea, traveler's diarrhoea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombocytopenic purpura (TP) (Pell, 1997; Penner, 1998). Hemorrhagic colitis is a severe illness and is characterized by bloody diarrhoea and severe abdominal cramps while HUS is characterized by bloody diarrhoea followed by renal failure. Thrombocytopenic purpura yield symptoms similar to those of HUS but the central nervous system is also affected. Death often occurs in patients with HUS and TP (Pell, 1997). Hemolytic uremic syndrome can be a serious complication in children and is a leading cause of acute kidney failure (Penner, 1998).

ii *Salmonella* spp

Salmonellosis was normally associated with contamination of food of animal origin, but in recent years, it has been indicated that *Salmonella* spp contamination may also occur in foods of plant origin. For instance *Salmonella* spp outbreaks have been associated with consumption of celery, watercress, watermelon, lettuce, cabbage, tomatoes, potatoes and carrots (Wells and Butterfield, 1997; Guo *et al.*, 2000). These organisms have also been implicated in infections due to wastewater spreading (Melloul and Hassani, 1999). *Salmonella* spp are capable of surviving and multiplying in fruits and vegetables. Asplund and Nurmi (1991) have demonstrated that tomatoes can provide a favourable environment for growth of *S. enteitidis*, *S. infantis* and *S. typhimurium* in spite of their low pH value, showing that the high acidity is not necessarily

effective enough to inhibit *Salmonella* spp growth. *Salmonella* spp can grow and multiply at temperatures of 22°C (Asplund and Nurmi, 1991) suggesting that once produce has been contaminated, microorganisms may continue to grow on the shelf in retail stores increasing the risk of infection.

Enteric fever is caused by the microorganism *S. typhosa*, in which the organism, ingested along with food finds its way into the bloodstream. Another organism, *S. cholera-suis* causes septicemia resulting in blood poisoning. The *S. typhimurium* and *S. enteritidis* cause gastroenteritis, an infection very commonly associated with contaminated food. Symptoms of Salmonellosis include nausea, vomiting, headache, chills, diarrhoea, fever and can even lead to reactive arthritis. In most cases the disease is short-lived, but salmonellosis can be fatal (Penner, 1998). Infants, once infected, frequently become long-term carriers and cause family outbreaks (Burge and Marsh, 1978).

iii *Listeria* spp

Listeriosis is rare in non-pregnant healthy adults, however, adults with conditions such as type 1 diabetes, cardiovascular disease, renal transplant, neoplasm, alcoholism and AIDS are more susceptible (Penner, 1998). Due to its ability to survive for long periods and its capability to grow at refrigerator temperature (Penner, 1998) this organism poses a serious threat in regard to foodborne illness. Healthy animals can be asymptomatic carriers of *L. monocytogenes* (Pell, 1997).

Listeria monocytogenes is a human and animal pathogen capable of causing nausea, vomiting, headache, fever, and severe infections like septicemia, encephalitis and meningitis, especially in immunocompromised individuals, newborns and pregnant women where it can result in stillbirths. About 100 cells of *L. monocytogenes* are sufficient to cause illness (Brooks *et al.*, 1991). In the USA this organism has a fatality rate of 20 – 40% (Penner, 1998).

Several outbreaks have been associated with contaminated commercial foodstuffs, such as vegetables, milk and meat products on which these bacteria can multiply even at low temperatures (Bubert *et al.*, 1999). Both *L. monocytogenes* and *L. innocua* have been isolated from various environmental samples such as soil, vegetation and human and animal faeces (Bubert *et al.*, 1999).

iv *Yersinia enterocolitica*

Yersinia enterocolitica causes yersiniosis and is found in a variety of animals, particularly pigs. It will grow at refrigerator temperatures, but grows best at room temperature. Infection with this organism yields symptoms that range from a mild gastroenteritis to severe conditions of polyarthritis and meningitis (Prescott *et al.*, 2002).

v *Shigella* spp

Shigellosis is caused by bacteria of the genus *Shigella* (Brooks *et al.*, 1991). This disease is characterized by diarrhoea, abdominal pain, vomiting and fever. As few as 10 to 100 microorganisms are sufficient to cause an illness (Penner, 1998). *Shigella* are readily killed by heat and do not survive well in acidic environments (Prescott *et al.*, 2002).

vi *Clostridium* spp

Clostridium botulinum produces a neurotoxin that cause botulism. After the toxin is absorbed, it binds to nerve endings and causes vomiting and diarrhoea, fatigue, dizziness and headache. Later there may follow constipation, double vision, difficulty speaking and swallowing, involuntary muscles may become paralyzed leading to cardiac and respiratory failure and eventually death. (Penner, 1998). The *C. botulinum* spores are heat resistant (Brooks *et al.*, 1991).

Clostridium perfringens produces toxins that cause diarrhoea and severe abdominal pain. However, death is uncommon. Although spores of this organism are common in raw foods and they are heat resistant, large numbers of vegetative cells of *C. perfringens* are necessary for an illness to occur (Penner, 1998).

vii *Campylobacter jejuni*

Campylobacter jejuni causes campylobacteriosis characterized by cramps, nausea, diarrhoea, headache and fever. Onset of the disease following consumption of contaminated food is within two to five days. Prolonged illness may lead to complications such as meningitis, urinary tract infection and reactive arthritis, but death occurs rarely (Penner, 1998). The high incidence of *C. jejuni* infections in persons infected with the human immunodeficiency virus points to the widespread transmission of low levels of this organism (Blaser, 1996). *Campylobacter* cells survive for several weeks at temperatures even as low as 4 °C (Waage *et al.*, 1999). The infective dose of *C. jejuni* is very small, it has been estimated that about 500 cells of this organism can cause human illness (Black *et al.*, 1988). Also, *Campylobacter* cells may enter a viable but non-culturable state due to starvation and physical stress, making them even more difficult to detect (Brooks *et al.*, 1991).

viii *Providencia* spp

Providencia spp are members of the normal intestinal flora. They cause urinary tract infection and are often resistant to antimicrobial therapy (Brooks *et al.*, 1991).

2.4.2 Persistence of Bacteria in Soil

The survival of microorganisms added to soil is influenced by a number of factors that include, water-holding capacity of soil, temperature, rainfall, sunlight, organic material in soil and the hydrogen ion (Rudolfs *et al.*, 1950; deRopp, 1999).

Faecal coliforms can survive for several years under optimum conditions, and the *Salmonella* spp may survive for a year in rich, moist organic soil (deRopp, 1999). The survival period of *Salmonella* spp has been reported to be as long as between 15 – 117 weeks in contaminated soil (Rudolfs *et al.*, 1950; Jones, 1980; Strauch, 1991; Sidhu *et al.*, 1999; Baloda *et al.*, 2001). The *L. monocytogenes* grows well in sewage and survives for long periods in soil (Strauch, 1991). Other bacteria such as *Streptococcus jaecelis*, *Clostridium botulinium*, *Clostridium tetani*, *Clostridium perfringes* and butyl-butyric *Clostridia* spp were found in small numbers 7 months after sludge application (Hyde, 1976).

Campylobacters spp are not capable of proliferating in the environment, but may remain dormant and survive in the environment for several weeks at low temperatures (Waage *et al.*, 1999). However, the infective dose is very small which increases the risk of infection (Black *et al.*, 1988).

One of the most important factors influencing the survival of pathogenic bacteria in soil is competition with the existing soil microflora. In soils with low microbial activity, the newly added microorganisms may persist for much longer (Bitton, 1994). Thus the application of large quantities of sludge to soil with low existing microbial activity will increase the ability of the pathogens to persist in soil environment and hence increase the potential risk for transfer of pathogens to crops grown in the soil. On the other hand, in biologically active soils, microorganism numbers are rapidly reduced due to competition (Penner, 1998). The soils in South Africa are typically biologically active, which could be advantageous due to the fact that introduced microorganisms are rapidly out

competed. However, as a result of high microbial activity, the organic material in agricultural soil is low (Korentajer, 1991).

Microorganisms may move through the contaminated soil as a result of rainfall or irrigation (Gerba *et al.*, 1975). Gagliardi and Karns (2000) have indicated that if soil pores do not become clogged, *E. coli* can travel below the top layers of soil for more than two months. They also indicated that *E.coli* from manure applied to soil could survive, replicate and move vertically in the soil (Gagliardi and Karns 2000). While soil contaminated with sewage sludge could lead to crop contamination, it has been indicated that water bodies such as groundwater, storm-water and rivers could be contaminated following rainfall or irrigation as a result of runoff from contaminated agricultural land (Lee and Jones-Lee, 1993; Bilgrami and Kumar, 1998).

2.4.3 Viruses

Sludge from wastewater treatment may contain demonstrable numbers of viruses even after anaerobic digestion (Damgaard-Larsen *et al.*, 1977). Some of the viruses that can be expected in sewage sludge are tabulated in Table 2.2.

Human enteric viruses are excreted in faeces, and can be shed in high numbers (10^8 to 10^{10} particles per gram of faeces) by infected individuals (Abbaszadegan *et al.*, 1999). The persistence of enteroviruses in sludge and sludge-amended soil was demonstrated by Damgaard-Larsen *et al.* (1977) and by Straub *et al.* (1994). The virus of greatest potential concern appears to be Hepatitis A, a disease with potential for long-term liver damage (Pahren *et al.*, 1979).

Table 2.2 Viruses that can be expected in sewage sludge (Sources: EPA, 1999; Strauch, 1991; Bofill-Mas *et al.*, 2000)

Pathogen	Disease
Enteroviruses	
Coxsackievirus A	Acute hemorrhagic conjunctivitis
Coxsackievirus B	Meningoencephalitis
Echovirus	Acute hemorrhagic conjunctivitis
Poliovirus	Poliomyelitis
Adenovirus	Respiratory and systemic infections
Reovirus	Acute respiratory infections
Hepatitis A virus	Infectious hepatitis
Rotavirus	Acute gastroenteritis
Astrovirus	Gastroenteritis
Calicivirus	Acute gastroenteritis
Coronavirus	Gastroenteritis
BK virus	Uretal stenosis and hemorrhagic colitis
JC virus	Multifocal leukoencephalopathy
Norwalk and Norwalk-like viruses	Acute gastroenteritis

Human polyomaviruses JC virus and BK virus were also indicated as being present in urban sewage obtained from widely divergent geographical areas in Europe and Africa (Bofill-Mas *et al.*, 2000). The JC virus is aetiologically associated with a fatal demyelinating disease known as progressive multifocal leukoencephalopathy, which has emerged as a frequent complication of AIDS in HIV infected individuals. Infection with BK virus has been associated with diseases of the urinary tract including hemorrhagic cystitis and ureteral stenosis (Bofill-Mas *et al.*, 2000).

Virus inactivation under natural conditions is a slow process (Damgaard-Larsen *et al.*, 1977). Viruses may become eluted and travel through the soil (Damgaard-Larsen *et al.*, 1977) which includes both vertical and lateral migration (Straub,

1995). For instance, other enteroviruses such as the coxsackie B3 virus have been isolated 18m below the soil surface after wastewater recharge (Straub *et al.*, 1995). Rainfall and irrigation events may contribute to viral transport (Straub *et al.*, 1995). Viruses readily adsorb to soil particles, and this has been reported to prolong their survival (WHO, 1979). However these viruses remain as infectious to humans as free viruses.

Viruses can survive for up to six months in cold weather and for three months in warm weather. Enteric viruses can survive up to 170 days in loamy and sandy soil. Poliomyelitis virus has been detected in soil irrigated with infected sewage sludge and effluent after 96 days in winter and 11 days in summer in the UK, and on the surface of mature vegetables 23 days after irrigation had ceased (Tierney *et al.*, 1977; WHO, 1979). Viral survival on crops may be shorter than in the soil if viruses on crops surfaces are directly exposed to detrimental environmental factors such as sunlight and desiccation (Pahren *et al.*, 1979; WHO, 1979). The warm climate in some regions of South Africa may reduce the survival of these viruses. However, more prolonged survival can be expected in the moist or more protected parts of plants, such as within the folds of leafy vegetables, in deep stem areas and on rough cracked surfaces of edible roots. It is also likely that viruses can penetrate damaged roots and under certain conditions enter the stem and leafy parts of edible plants (Pahren *et al.*, 1979).

Once crops are harvested, enteric viruses can survive for prolonged periods during commercial and household storage at low temperature. The risk of human infection associated with virus-contaminated crops is greatest in the case of fruits and vegetables consumed raw (WHO, 1979).

2.4.4 Parasites

Parasites are a group of foodborne pathogens that have received relatively little attention. Parasites that are usually encountered in sludge are indicated in Table 2.3, and some of these (*) are briefly discussed.

Table 2.3 Parasites that can be expected in sewage sludge (EPA, 1999; Strauch, 1991)

Pathogen	Disease
<i>Entamoeba histolytica</i>	Amebiasis
<i>Giardia lamblia</i> *	Giardiasis
<i>Toxoplasma gondii</i>	Toxoplasmosis
<i>Sarcocystis</i> spp	Intestinal infection
<i>Taenia</i> spp *	Taeniasis
<i>Diphyllobothrium latum</i>	Pernicious anaemia
<i>Echinococcus granulosus</i>	Echinococcosis
<i>Ascaris</i> spp *	Ascariasis
<i>Toxocara</i> spp	Pneumonic symptoms
<i>Trichuris trichiura</i>	Trichuriasis
<i>Toxoplasma gondii</i>	Toxoplasmosis
<i>Cryptosporidium</i> *	Cryptosporidiosis

Some of the common types of parasites that have been detected in fresh fruits and vegetables include *Giardia lamblia*, *Entamoeba histolytica* and *Ascaris* spp. (Brackett, 1987). As little as 10 or fewer *Giardia* cysts are sufficient to cause illness (Brooks *et al.*, 1991). Ayres and colleagues (1992) recovered viable *Ascaris* eggs from lettuce irrigated with raw sewage, while Gaspard and Schwartzbrod (1993) recovered viable *Ascaris* from both tomatoes and lettuce following raw sewage irrigation. It has also been demonstrated that farm workers may be infected with enteric parasites as a result of occupational exposure (Clark *et al.*, 1984). It should be noted that these incidents were associated with the irrigation of raw sewage and not wastewater sludge. However, it does give an indication of potential risk.

The parasites most often found in sludge are *Ascaris* species such as *A. lumbricoides* (human intestinal roundworm) and *A. suum* (pig's roundworm) as

well as some *Toxocara* and *Trichuris* species (Bitton, 1994; Gaspard *et al.*, 1995).

Ascaris eggs and certain larval stages of trichostrongylids can survive for over a year in soil that has been irrigated with sewage sludge (Strauch, 1991), and the eggs of *Cryptosporidium parvum* and *Taenia saginata* are known to survive in sewage for more than 12 months (NRC, 1996). *Cryptosporidium* species and *Giardia* species pose a serious threat to human health as these organisms are difficult to inactivate with disinfectants and their infective doses in humans are very low (Finch and Belosevic, 2001).

Protozoan parasites, such as *Giardia* spp have been found in sludge in Western Australia where they remain the most common cause of enteric disease (Hu *et al.*, 1996). The most noxious are the *Ascaris* eggs and coccidial oocysts as they have high resistance (Pahren *et al.*, 1979; Gaspard and Schwartzbrod, 1993). Helminths larvae are usually killed by composting, but often remain viable in slurry during storage (Shuval *et al.*, 1984).

Also encountered in sludge are the organisms of the genus *Cryptosporidium* (Kuczynska and Shelton, 1999; EPA, 1999). Of the *Cryptosporidium* species, *C. parvum* is the agent of clinical cryptosporidiosis in humans and livestock. The *C.parvum* oocysts are shed by infected mammals and are known to be resistant to standard disinfectants (Champlaud *et al.*, 1998). Waterborne *C. parvum* oocysts may remain viable for several months (Kuczynska and Shelton, 1999).

Table 2.4 indicates the concentrations of pathogens as indicated by other countries.

Table 2.4 Concentrations of pathogens in sludge from other countries (Jimenez *et al.*, 2002)

Pathogen	Concentration	Country
Fecal coliforms (MPN/gTS)	$3.6 \times 10^4 - 1.4 \times 10^6$	United Kingdom
	$2.3 \times 10^7 - 9.3 \times 10^{10}$	Mexico
	2.0×10^7	United States
<i>E.coli</i> (PFU/gTS)	$1.0 \times 10^6 - 1.9 \times 10^6$	Mexico
	1.3×10^5	United States
<i>Ascaris</i> /gTS	2.40 – 8.98	United Kingdom
	66 – 136	Mexico
	1.4 – 9.7	United States
	0.60 – 2.4	France

2.5 Disinfecting Treatment Processes

Previous sections provided detailed discussions on the occurrence of microorganisms in sludge and their potential presence in crops if inadequately treated sludge is used for land application. However, the transmission of pathogens can be minimized by reducing the infectivity of sludges through effective treatment processes (Smith, 1996). Various techniques are used to eliminate or reduce the number of microorganisms to levels that do not threaten human health (EPA, 1999).

Many of these treatment processes are applied either to stabilize the sludge, i.e reduce its vector attraction potential and odour or render the sludge easier to handle, store and transport by reducing the volume or drying the wastewater sludge. Additional treatment technologies need to be employed to reduce the viable content. Some of these techniques recommended in the US Part 503 rule are indicated in Table 2.5.

If effective treatment is not available, long term storage could be used to accelerate inactivation and thus reduce the number of infective species before

sludge is spread onto soil (Jenkins *et al.*, 1999). Jenkins and colleagues (1999) warned that although storing prior to spreading could be an effective management practice for reducing infective oocyst load, spreading of sludge during the cold season may have the opposite effect by sustaining the survival of *C. parvum* oocysts and positioning them for transport in surface runoff (Jenkins *et al.*, 1999).

Table 2.5 Techniques listed in the 40 CFR Part 503 and their effectiveness in removing pathogens (EPA, 1999)

R = Reduction, E = Elimination, ✓ = effective in pathogen reduction/elimination and ✗ = not effective in pathogen reduction /elimination

Technique	Description	Effectiveness in Eliminating Pathogens					
		Viruses		Bacteria		Parasites	
		R	E	R	E	R	E
Aerobic Digestion	Sewage sludge is agitated with air or oxygen to maintain aerobic conditions	✓	✗	✓	✗	✗	✗
Air Drying	Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum duration of 3 months	✓	✗	✓	✗	✗	✗
Anaerobic digestion	Sewage sludge is treated in the absence of air at a specific temperature. The values of the temperature shall be between 15 days at 35 °C and 60 days at 20 °C	✓	✗	✓	✗	✗	✗
Composting	Using either the within-vessel, static aerated pile, or widow composting methods. The temperature of sewage sludge is raised to 40 °C or higher and remains at 40 °C or higher for 5 days. Fours in the 5 day period, the temperature in the compost pile exceeds 55 °C	✓	✗	✓	✗	✓	✗
Lime Stabilization	Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 for 2 hrs.	✓	✗	✓	✗	✗	✗
Thermal Treatment	Sewage sludge is heated to a temperature of 180°C or higher for 30 minutes	✓	✗	✓	✓	✓	✗

2.6 Treatment and Sewage Sludge Classification in South Africa

Snyman and colleagues (2003) documented the treatment technologies employed by South African wastewater treatment plants. According to this study, 57% of the sludge that is produced employs anaerobic digestion of primary and humus sludge (Snyman *et al.*, 2003). The sludge types generated from these plants are presented in figure 2.1.

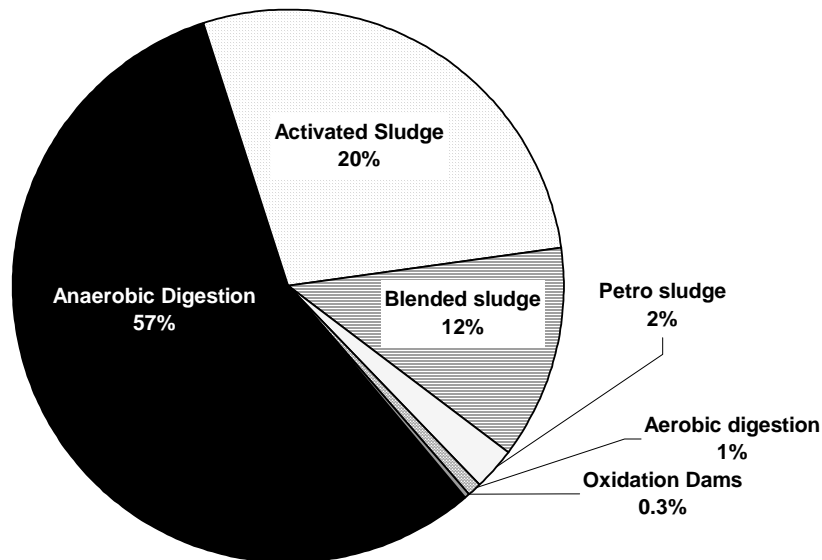


Figure 2.1 Sludge types produced by the wastewater treatment plants surveyed in South Africa on a mass percent basis. The blended sludge represents primary and activated sludge blended before or after digestion (Snyman *et al.*, 2003).

Figure 2.2 illustrates the tertiary and additional stabilisation technologies employed by the wastewater treatment plants surveyed in South Africa. The majority (74% mass) of the sludge producing treatment plants surveyed do not treat the sludge further than the traditional anaerobic digestion and activated sludge treatment. Composting is used by both metropolitan city councils and plants in smaller town councils while pelletisation is only employed by large metropolitan councils (Snyman *et al.*, 2003). Aerobic digestion is employed as an

additional treatment method after anaerobic digestion in one major site (Snyman *et al.*, 2003).

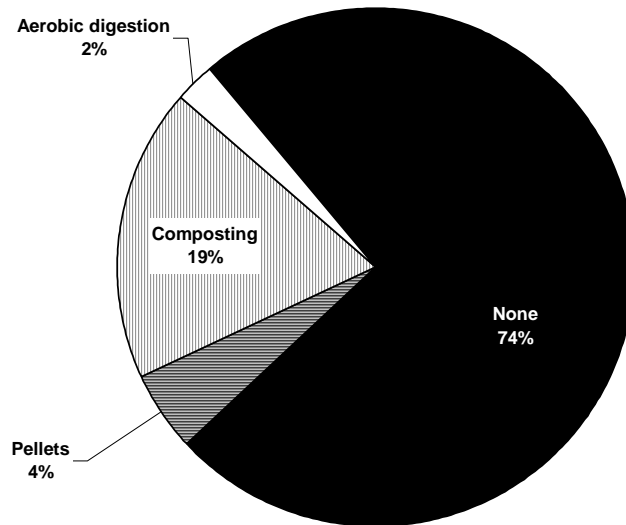


Figure 2.2 The tertiary and additional stabilisation technologies employed by the wastewater treatment plants surveyed in South Africa on a mass percent basis (Snyman *et al.*, 2003).

The sewage sludge produced from treatment plants in South Africa is used for a number of activities, including application onto golf courses and use by municipalities for lawn cultivation, while some is collected by farmers for agricultural use. The disposal and beneficial use of sewage sludge in South Africa are summarized in figure 2.3.

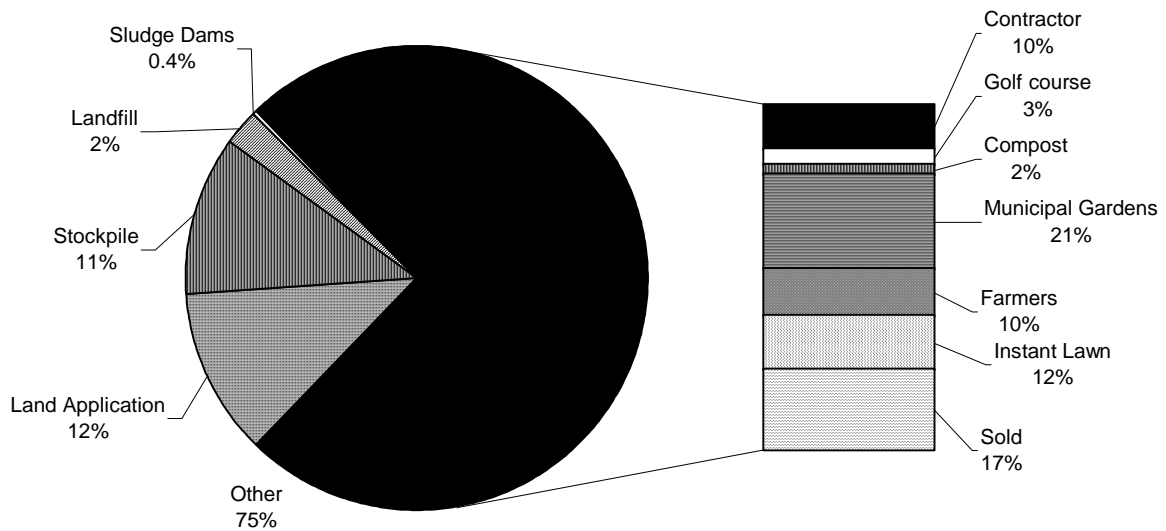


Figure 2.3 the major disposal methods employed by the wastewater treatment plants surveyed in South Africa on a mass percent basis (Snyman *et al.*, 2003).

Table 2.6 summarizes the classification of sewage sludge indicated in the South African sludge guidelines. The South African guidelines classify sludge at three levels (Types A, B and C) and a fourth category (Type D) that stipulates ceiling limits for pollutants is added. Although the hygienic quality of Type D is similar to Type C, the Type D sludge is produced for unrestricted use on land at maximum application of 8 tonnes per hectare per year, the levels of metals and inorganic content are limited to acceptable low levels (WRC, 1997).

Table 2.6 Classification of Sewage Sludge to be used or disposed off on Land (WRC, 1997)

Sewage Sludge	Treatment	Characteristics-Quality of Sewage sludge
Type A Sludge	Cold digested sludge Septic tank sludge Oxidation tank sludge	Usually unstable and can cause odour nuisances and fly-breeding Contains pathogenic organisms Variable metal and inorganic content
Type B Sludge	Anaerobic digested sludge Surplus activated sludge Humus tank sludge	Fully or partially stabilized – should not cause significant odour nuisance or fly-breeding Contains pathogenic organisms Variable metal inorganic content
Type C Sludge	Pasteurised sludge Heat treated sludge Lime-stabilised sludge Composted sludge Irradiated sludge	Certified to comply with the following quality requirement: Stabilized – should not cause odour nuisances or fly-breeding Contains no viable <i>Ascaris</i> ova per 10 gram of dry sludge Maximum 0 <i>Salmonella</i> organisms per 10 gram dry sludge Maximum 1000 Faecal coliform per 10 gram dry sludge, immediately after treatment (disinfection/sterilization) Variable metal and inorganic content
Type D Sludge	Pasteurised sludge Heat-treated sludge Lime-stabilised sludge Composted sludge Irradiated sludge	Certified to comply with the following quality requirement: Stabilized – should not cause odour nuisances or fly-breeding Contains no viable <i>Ascaris</i> ova per 10 gram of dry sludge Maximum 0 <i>Salmonella</i> organisms per 10 gram dry sludge Maximum 1000 Faecal coliform per 10 gram dry sludge, immediately after treatment (disinfection/sterilization) Has specific limits for metal and inorganic content (summarized in WRC, 1997) Product must be registered in terms of Act 36 of 1947 if used for agricultural activities

2.7 Resistance of Microorganisms to Disinfection

The previous section discussed disinfecting techniques employed by wastewater treatment plants to reduce or eliminate the numbers of infective species. If the sewage sludge used is not adequately treated, there is potential for crop contamination.

Studies have shown that once fruits and vegetables have been contaminated, it may be difficult to disinfect them (Maxy, 1982; Takeuchi *et al.*, 2000; Wachtel *et al.*, 2002a; Wachtel *et al.*, 2002b). Some microorganism such as *E. coli* show preferential attachment to the interior of damaged fruits and vegetables than on the surface (Takeuchi *et al.*, 2000) as the juice within the vegetable provides good growth medium (Maxy, 1982). Itoh and coworkers (1998) found that *E. coli* was internalized when radish sprouts were produced from contaminated seeds and therefore would be protected from surface decontamination treatment. *E. coli* is capable of attachment to the interior of stomatal pores (Seo and Frank, 1999; Takeuchi and Frank, 2000; Takeuchi and Frank, 2001) and has a tendency to form aggregate associations (Wachtel *et al.*, 2002a). These attachment sites and aggregation tendencies may cause bacterial resistance to physical methods of surface disinfection as well as chemical treatment such as chlorination (Wachtel *et al.*, 2002b).

2.8 Protecting the Public and Environment through Regulatory Management

Most countries adopt a similar approach to protect the public from infection due to pathogens originating from wastewater sludge. The use of wastewater sludge is regulated and these regulations stipulate how the sludge should be disinfected and/or how to minimize the chance of infection through prescribed management practices. In the United States, the use and disposal of treated sewage sludge is regulated under CFR Part 503 (EPA, 1999).

The regulation protects public health and the environment through requirements designed to reduce the potential for contact with disease-bearing pathogens in

sewage sludge applied to the land or placed. These requirements are divided into:

- Requirements designed to control and reduce pathogens in treated sewage sludge and
- Requirements designed to reduce the ability of the treated sewage sludge to attract vectors (insects and other living organisms that can transport sewage sludge pathogens)

It includes both performance and technology-based requirements. Wastewater plants have the freedom to modify conditions and combine processes with each other to meet the requirements.

At present in South Africa humans and the environment are protected under the National Water Act 36 of 1998 (NWA), National Environmental Management Act 107 of 1998 (NEMA), Water Services Act 108 of 1997(WSA), the Constitution of the Republic of South Africa (Act 108 of 1996) and the Health Act 63 of 1977.

The Department of Water Affairs and Forestry is the custodian of water resources in South Africa. The guidelines for sewage sludge classification and application are summarized in a document on permissible utilization of sewage sludge (WRC, 1997; WRC, 2002). If the sludge reuse or disposal method does not comply with the requirements detailed for the applicable classification its reuse or disposal requires permission, which could be in a form of a licence or permit (WRC, 2002).

In South Africa, there aren't any specified restricted techniques for sludge treatment, but the chosen technologies need to yield the sludge quality as required in the guidelines (WRC, 1997).

2.9 Public Perception

The benefits of sewage sludge are well understood by the scientific community, and through consultation, most governments around the world recognize the benefits of using sludge in crop production. It is for this reason that a number of countries have since engaged in utilizing sewage for land application purposes.

Despite the advancements in sludge use in agriculture, the main recipients of these services have often been neglected. This often led to fear and rejection of sewage sludge among some members of the public as a result of misinformation due to media coverage (Sunday Times, 2003). Due to lack of scientific knowledge, the public will generally reject any association with a product or service if it is linked to odour or discolouration (Small Wright, 2002). Tyson (2002) reported that if sewage sludge did not smell, the public probably would not complain.

In a small preliminary survey done in South Africa, it has emerged that only a small percentage (39%) of low income earners were aware of what sewage sludge was (Snyman and Van der Waals, 2003). Snyman and Van der Waals (2003) also noted that the respondents did not understand the risks associated with using sewage sludge for agricultural soil amendment. Of the respondents from a higher income bracket, 79% were found to have knowledge of sewage sludge and its potential benefits. The majority of the respondents from this group also expressed their willingness to purchase vegetables from a sewage sludge fertilized farm, with 45% prepared to consume vegetables grown on sewage sludge (Snyman and Van der Waals, 2003). It appears from this survey that if members of the general public are informed of the benefits of sludge, reception of the use of sewage sludge might increase in the future. It is thus the responsibility of the sludge producers together with the governments to introduce mechanisms of educating the public of sewage sludge and its use in agriculture.

2.10 Assessing Human Risk Exposure

The sections preceding indicate that many wastewater plants generate sludges that still contain pathogens. However, these sludges are still used in agricultural practices. The question to address therefore is “What is the risk associated with this practice?” If the risk of using such sludge is unacceptable, what management practices should be adapted to reduce this risk to an acceptable level?

While complete elimination of pathogens from sludge is ideal, it has been indicated that if the numbers of pathogens in sludge are reduced to an acceptable level, the use of such sludge in agricultural land does not appear to result in unacceptable risk to human health (Apedaile, 2001; Tanner *et al.*, 2003). According to Vesilind (2003), coming into contact with small doses of pathogens is the “sufficient challenge” our bodies need to stay healthy as our enhanced health comes not from zero exposure, but from a sufficient exposure to pathogens. Although this is true for healthy individuals, this could be different for the South African population, as a large percentage of the population is HIV positive and therefore immunocompromised (Dorrington *et al.*, 2002).

One of the concerns often raised regarding sludge application is the emission of pathogenic aerosols during land application (Pillai *et al.*, 1996). The risk of release rises as the pathogenic content in sludge increases. Raw sludge from municipal sewage would be more likely to release airborne pathogens than those that have been treated to reduce the pathogens (Straub *et al.*, 1993). Tanner and colleagues (2003) evaluated the potential for bio-aerosols from sludge application, and concluded that the risk of adverse public health effects from bio-aerosols following land applied sludge is low. Forcier (2002) indicated that although quantities of bio-aerosols could be released during storage, loading and land application, they are diluted and scattered through atmospheric dispersion in ambient air. The survival of and the potential for infection from these organisms are lessened by the natural processes of attenuation such as ultra-violet radiation

and desiccation (Forcier, 2002). Bio-aerosol emissions are also lessened when applied sludge is subsequently incorporated into the soil (Straub *et al.*, 1993). It appears that the methods used for sludge land application do not result in airborne release of biological agents to the same extent as in wastewater treatment facilities (Apedaile, 2001).

Tools exist to measure the risk to human health associated with the use of sewage sludge that contains pathogens in agricultural practices. The following section details one of the tools used in this thesis.

2.10 .1 Health Risk Assessment

The health risk assessment provides a means to estimate the probability of adverse effects associated with measured or estimated levels of the hazardous agents, and a tool for predicting the extent of potential or probable health effects. The protocol was originally developed for carcinogen assessments. However, current trends favour the application of similar procedure to establish the risk of microbiological hazards. The process as defined by the US EPA, is comprised of four distinguishable but interacting phases, namely:

- Hazard identification;
- Exposure assessment;
- Dose-response assessment and
- Risk characterisation (Zwietering and van Gerwen, 2001)

The interrelation of these phases is depicted in Figure 2.4.

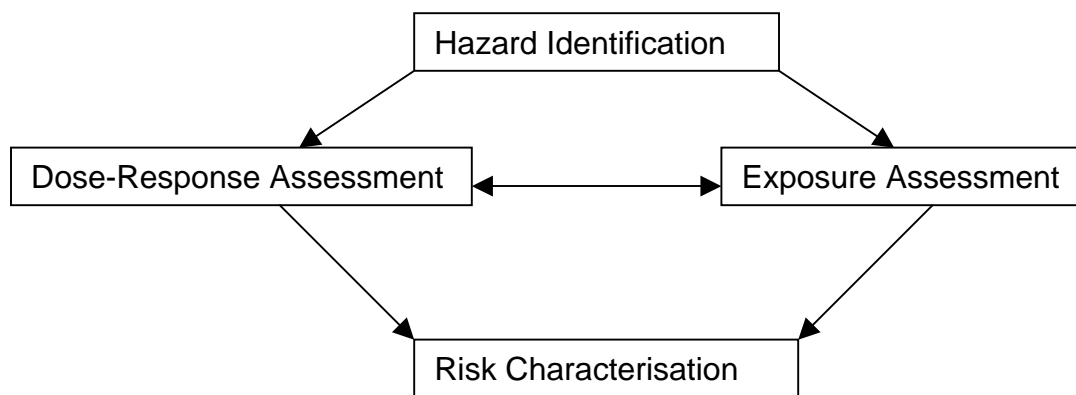


Figure 2.4 The interrelation of the risk assessment phases (Genthe 1998).

i Hazard Identification

This involves the identification of biological, chemical and physical agents capable of causing adverse health effects and that may be present in a particular food or group of foods (Rocourt *et al.*, 2001). Once the health hazard has been identified, the remainder of the process encompasses the description of the properties of the hazardous agent and the identification of both acute and chronic health effects (Genthe, 1998).

ii Hazard Characterisation

This involves the qualitative and or quantitative evaluation of the nature of the adverse health effects associated with the hazard present in food. It provides description of the severity and duration of adverse effects that may result from ingestion of a microorganism in food. This involves a dose response assessment by establishment of a relationship between the dose of an agent and the rate of infection. Dose response assessment is considered a key ingredient of quantitative risk assessment as it is supposed to provide the link between exposure to a hazardous agent and the probability of ensuing health effects (Teunis and Havelaar, 2000).

Some microorganisms when present at sufficient levels are capable of causing disease, while others may produce toxins that contribute to the development of a

disease (Brooks *et al.*, 1991). Toxins produced by bacteria are generally classified into two groups, exotoxins and endotoxins. Exotoxins are excreted by living cells, while endotoxins are released on bacterial death (Brooks *et al.*, 1991).

iii Exposure Assessment

This involves the qualitative and or quantitative evaluation of the likely intake of biological, chemical and physical agents via food, as well as exposure from other sources if relevant (Rocourt *et al.*, 2001). It is usually defined as a process of measuring or estimating the intensity, frequency and duration of human exposure to a contaminant. The task of exposure assessment is to provide the actual exposure conditions required to predict risk, and to identify and predict the effects of the proposed control options (Genthe, 1998).

iv Risk characterisation:

This involves the qualitative and or quantitative estimation, including attendant uncertainties of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterization and exposure assessment (Rocourt *et al.*, 2001). Risk characterisation has been defined as the process of calculating the incidence of the health effect under the conditions of exposure described in exposure assessment. A major component of risk assessment is an evaluation of all assumptions used and all sources of uncertainty (Genthe, 1998). In risk characterisation all results of the former steps are integrated, bringing together all inaccuracies from the former steps (Zwietering and van Gerwen, 2000). Thus risk characterization is defined as the process of estimating the likelihood or probability of experiencing the adverse effects of an identified hazard, the impact or consequences of those effects and describing the attendant uncertainty of the estimates.

2.11 Factors Affecting Management of Sewage Sludge Use in South Africa

South Africa has unique factors that could influence management of land application of sewage sludge. These factors include population density, high incidence of HIV/AIDS, unique climatic conditions and soil quality, amongst others. A detailed description of these factors will be provided in later sections to indicate how they influence management of sludge use in South Africa.

2.12 Conclusion

Sewage sludge could be used beneficially in agricultural practices, especially in South Africa's carbon depleted soils. It appears there are vast agronomic and economic benefits to sludge use, particularly as the cost of fertilizers are on the increase.

However, pathogens do occur in a large percentage in what is regarded as sewage sludge ready for agricultural use. In South Africa, little information is available on the risks associated with using sewage sludge that has not been disinfected.

International authors have investigated and quantified these risks. As a result of the factors that are unique to South Africa, it would not be appropriate to adopt work from other countries. These factors justify an investigation to assess the risks associated with the use of pathogen rich sewage sludge in agricultural practices.

A high risk crop was chosen to illustrate a worst case scenario. It was therefore decided to investigate the prevalence of microorganisms in a crop grown in sewage sludge amended soil. A risk assessment will provide 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.

Based on our understanding and findings a functional management plan for sewage sludge application to agricultural land can be formulated.

2.13 References

Abbaszadegan, M., Stewart, P. and Lechevallier, M. 1999. A Strategy for detection of viruses in groundwater by PCR. *Applied and Environmental Microbiology*. **65**(2). 444 - 449

Ackers, M-L., Mahon, B.E., Leahy, E., Goode, B., Damrow, T., Hayes, P.S., Bibb, W.F., Rice, D.H., Barrett, T.J., Hutwagner, L., Griffin, P.M. and Slutsker, L. 1998. An Outbreak of *Escherichia coli* O157:H7 Infections Associated with Leaf Lettuce Consumption. *The Journal of Infectious Diseases*. **177**. 1588-1593

Apedaile, E. 2001. A Perspective on Biosolids Management. *The Canadian Journal of Infectious Diseases*. **12**(4)
http://www.pulsus.com/Infdis/12_04/aped_ed.htm

Asplund, K. and Nurmi, E. 1991. The Growth of *Salmonellae* in Tomatoes. *International Journal of Food Microbiology*. **13**. 177-182

Ayres, R.M., Stott, R., Lee, D.L., Mara, D.D. and Silva, S.A. 1992. Contamination of Lettuce with Nematode Eggs by Spray Irrigation with Treated and Untreated Wastewater. *Water Science and Technology*. **26**(7-8) 1615-1623

Baloda, S.B., Christensen, L. and Trajcevska, S. 2001. Persistence of a *Salmonella enterica* Serovar Typhimurium DT12 Clone in a piggery and in Agricultural Soil Amended with *Salmonella*-Contaminated Slurry. *Applied and Environmental Microbiology*. **67**(6). 2859-2862

Bilgrami, K.S. and Kumar, S. 1998. Bacterial Contamination in Water of the River Ganga and its Risk to Human Health. *International Journal of Environmental Health Research*. **8**. 5-13

Bitton, G. 1994. Wastewater Microbiology. Wiley-Liss, New York. 478pp

Bofill-Mas, S., Pina, S. and Girones, R. 2000. Documenting the Epidemiologic Patterns of Polyomaviruses in Human Populations by Studying their Presence in Urban Sewage. *Applied and Environmental Microbiology*. **66**(1). 238 - 245

Black, R.E., Levine, M.M., Clement, M.L., Hughes, T.P. and Blaser, M.J. 1988. Experimental *Campylobacter jejuni* Infection in Humans. *Journal of Infectious Diseases*. **157**. 472-479

Blaser, M.J. 1996. How Safe is Our Food? *The New England Journal of Medicine*. **334**(20). 1324-1325

Brackett, R.E. 1987. Microbiological Consequences of Minimally Processed Fruits and vegetables. *Journal of Food Quality*. **10**. 195-206

Brooks, G.F., Butel, J.S. and Ornston, L.N. 1991. Medical Microbiology. 9th Edition. New York. 488 pp

Brown, K.W., Thomas, J.C. and Donnelley, K.C. 1991. Bacterial Mutagenicity of Municipal Sewage Sludge. *Journal of Environmental Science and Health* **A26**(3). 395-413

Bubert, A., Hein, I., Rauch, M., Lehner, A., Yoon, B., Goebel, W. and Wagner, M. 1999. Detection and Differentiation of *Listeria* spp. By a Single Reaction Based on Multiplex PCR. *Applied and Environmental Microbiology*. **65**(10). 4688 - 4692

Burge, W.D. and Marsh, P.B. 1978. Infectious Disease Hazards on Land spreading Sewage Wastes. *Journal of Environmental Quality*. **7**(1) 1-9

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 Caparao and Alto Caparao, Brazil. *Bulletin of the World Health Organization*. **80**(1). 40-46

Champlaud, D., Gobet, P., Naciri, M., Vagner, O., Lopez, J., Buisson, J.C., Varga, I., Harly, G., Mancassola, R. and Bonnin, A. 1998. Failure to Differentiate *Cryptosporidium parvum* from *C. meleagridis* Based on PCR Amplification of Eight DNA Sequences. *Applied and Environmental Microbiology*. **64**(4). 1454 - 1458

Clark, C.S., Linnemann, C.C., Clark, J.G. and Gartside, P.S. 1984. Enteric Parasites in Workers Occupationally Exposed to Sewage. *Journal of Occupational Medicine*. **26**(4). 273 - 275

Damgaard-Larsen, S., Jensen, K.O., Lund, E. and Nissen, B. 1977. Survival and Movement of Enterovirus in Connection with Land Disposal of Sludges. *Water Research*. **11**. 503 – 508

DeRopp, 1999. Worms and Disease. In: Humanure Handbook. Jenkins, J (Ed.) Jenkins Publishing, PA. www.weblife.org/humanure/chapter7.html

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

Doyle, M.P. 2000. Reducing Foodborne Disease: What Are the Priorities? *Nutrition*. **16**(7/8). 647-649

Easton, J.S. 1983. Utilisation and Effects of Anaerobically Digested Sludge on a Red Sandy Soil of Natal. *Water SA*. **9**(2). 71 - 78

EPA, 1999. Environmental Regulations and Technology. Control of pathogens and vector attraction in sewage sludge. U.S. Environmental Protection Agency. EPA/625/R-92-013. 111pp

Finch, G.R. and Belosevic, M. 2001 Controlling *Giardia* spp. and *Cryptosporidium* spp. in drinking water by microbial reduction processes. *Canadian Journal of Civil Engineering*. **28**(Suppl. 1). 67-80

Forcier, F. 2002. Biosolids and Bioaerosols: The Current Situation. Biosolids and Bioaerosols Solinov. Quebec Ministry of Environment. 22pp

Gagliardi, J.V. and Karns, J.S. 2000. Leaching of *Escherichia coli* O157:H7 in Diverse Soils Under Various Agricultural Management Practices. *Applied and Environmental Microbiology*. **66**(3). 877-883

Gaspard, P. and Schwartzbrod, J. 1993. Determination of the Parasitic Contamination of Irrigated Vegetables. *Water Science and Technology*. **27**(7-8). 295-302

Gaspard, P. G., Wiart, J. & Schwartzbrod, J. 1995. Urban sludge reuse in agriculture: Waste treatment and parasitological risk. *Bioresearch and Technology*. **52**: 37-40

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

Gerba, C.P., Wallis, C. and Melnick, J.L. 1975. Fate of Wastewater Bacteria and Viruses in Soil. *Journal of Irrigation and Drainage Engineering*. **101**. 157-174

Guo, X., Chen, J., Beuchat, L.R. and Brackett, R.E. 2000. PCR Detection of *Salmonella enterica* Serotype Montevideo in and on Raw Tomatoes Using Primers Derived from *hilA*. *Applied and Environmental Microbiology*. **66**(12). 5248 – 5252

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

Hu, C.J., Gibbs, R.A., Mort, N.R., Hofstede, H.T., Ho, G.E. and Unkovich, I. 1996. *Giardia* and Its Implications for Sludge Disposal. *Water Science and Technology*. **34**(7-8). 179-186

Itoh, Y., Sugita-Konoshi, Y., Kasuga, F., Lwaki, M., Hara-Kudo, Y., Saito, N., Noguchi, Y., Konuma, H. and Kumagai, S. 1998. Enterohemorrhagic *Escherichia coli* O157:H7 Present in Radish Sprouts. *Applied and Environmental Microbiology*. **64**(4). 1532-1535

Jimenez, B., Maya, C., Sanchez, E., Romero, A., Lira, L. and Barrios J.A. 2002. Comparison of the Quantity and Quality of the Microbiological Content of Sludge in Countries with Low and High Content of Pathogens. *Water Science and Technology* **46**(10) 17-24

Jenkins, M.B., Walker, M.J., Bowman, D.D., Anthony, L.C. and Ghiorse, W.C. 1999. Use of a Sentinel system for field Measurements of *Cryptosporidium parvum* oocyst inactivation in soil and animal waste. *Applied and Environmental Microbiology*. **65**(5). 1998-2005

Jones, P.W. 1980. Health hazards associated with the handling of animal wastes. *Veterinary Record*. **106**. 4-7

Kirby, J.B. 2001. Exposure, resistance, and recovery: a three-dimensional framework for the study of mortality from infectious disease. *Social Science and Medicine*. **53**. 1205-1215

Korentajer, L. 1991. A review of the agricultural use of sewage sludge: Benefits and potential hazards. *Water SA*. **17**(3). 189-196

Kornacki, J.L. and Marth, E.H. 1982. Foodborne Illness Caused by *Escherichia coli*: A Review. *Journal of Food Protection*. **45**(11). 1051-1067

Kouloumbis, P., Rigas, F. and Mavridou, A. 2000. Environmental Problems from the Disposal of Sewage Sludge in Greece. *International Journal of Environmental Health Research*. **10**. 77-83

Kuckzynska, E. and Shelton, D.R. 1999. Method for Detection and Enumeration of *Cryptosporidium parvum* Oocysts in Feces, Manures and Soils. *Applied and Environmental Microbiology*. **65**(7). 2820 - 2826

Lee, G.F. and Jones-Lee, A. 1993. Public Health Significance of Waterborne Pathogens in Domestic Water Supplies and Reclaimed Water. Report to state California Environmental Protection Agency Comparative Risk Project, Berkley, CA, 27pp

Manero, A. and Blanch, A.R. 1999. Identification of *Enterococcus* spp. with a biochemical key. *Applied and Environmental Microbiology*. **65**. 4425-4430

Maxy, R.B. 1982. Fate of Microbial Contaminants in Lettuce Juice. *Journal of Food Protection*. **45**(4). 335-339

Melloul, A.A and Hassani, L. 1999. *Salmonella* Infection in Children from the Wastewater-spreading Zone of Marrakesh City (Morocco). *Journal of Applied Microbiology*. **87**. 536-539

NRC, 1996. Use of Reclaimed Water and Sludge in Food Crop Production. Committee on the Use of Treated Wastewater Effluents and Sludge in the Production of Crops for Human Consumption. Water Science and Technology Board. Commission on Geosciences, Environment and Resources. National Research Council. National Academy of Press, Washington, DC

NRC, 2002. Biosolids Applied to land. National Research Council. National Academy of Press, Washington, DC

Pahren, H.R., Lucas, J.B., Ryan, J.A. and Dotson, G.K. 1979. Health risks associated with land application of municipal sludge. *Journal of Water Pollution Control Federation*. **51**(11). 2588 - 2601

Pell, A.N. 1997. Manure and Microbes: Public and Animal Health Problem? *Journal of Dairy Science*. **80**. 2673-2681

Penner, K. 1998. Microorganisms and Foodborne Illness. Food Safety. <http://www.oznet.ksu.edu>

Pillai, S.D., Widmer, K.W., Dowd, S.E. and Ricke, S.C. 1996. Occurrence of Airborne Bacteria and Pathogen Indicators during Land Application of Sewage Sludge. *Applied and Environmental Microbiology*. **62**(1). 296 - 299

Parliamentary Bulletin, 1996. Poverty in South Africa – Poverty Week Debate. 21 October 1996. Parliamentary Bulletin No. 7. Republic of South Africa

Prescott, L.M., Harley, J.P. and Klein, D.A. 2002. Microbiology 5th ed. McGraw-Hill. New York. 1026pp

Purves, D. 1990. Toxic Sludge. *Nature*. **346**. 617 - 618

Recke, H., Schnier, H.F., Nabwile, S. and Qureshi, J.N. 1997. Responses of Irish Potatoes (*Solanum Tuberosum* L.) to Mineral and Organic Fertilizer in Various Agro-Ecological Environments in Kenya. *Experimental Agriculture*. **33**(1). 91-102

Reis, M.H., Vasconcelos, J.C. and Trabulsi, L.R. 1980. Prevalence of Enterotoxigenic *Escherichia coli* in Some Processed Raw Food from Animal Origin. *Applied and Environmental Microbiology*. **39**(1). 270-271

Rocourt, J., Hogue, A., Toyofuku, H., Jacquet, C. and Schlundt, J. 2001. Listeria and listeriosis: Risk assessment as a new tool to Unravel a multifaceted problem. *American Journal of Infection Control*. **29**(4). 225-227

Rost, U., Joergensen, R.G. and Chander, K. 2001. Effects of Zn enriched sewage sludge on microbial activities and biomass in soil. *Soil Biology and Biochemistry*. **33**. 633-638

Rudolfs, W., Falk, L.L. and Ragotzkie, R.A. 1950. Literature review on the occurrence and survival of enteric, pathogenic, and relative organisms in soil, water, sewage, sludges, and on vegetation. *Sewage and Industrial Wastes*. **22**. 1261-1281

Seo, K.H. and Frank, J.F. 1999. Attachment of *Escherichia coli* O157:H7 to Lettuce Leaf Surface and Bacterial Viability in Response to Chlorine Treatment as Demonstrated by Using Confocal Scanning Microscopy. *Journal of Food Protection*. **62**(1). 3-9

Shuval, H.I., Yekutieli, P. and Fatal, B. 1984. Epidemiological evidence for Helminth and Cholera transmission by Vegetables Irrigated with Wastewater: Jerusalem – a case study. *Water Science and technology*. **17**. 442-443

Sidhu, J., Gibbs, R.A., Ho, G.E. and Unkovinch, I. 1999. Selection of *Salmonella* Typhimurium as an Indicator for Pathogen Regrowth Potential in Composted Biosolids. *Letters in Applied Microbiology*. **29**. 303-307

Small Wright, M. 2002. Sewage Sludge/Biosolids. A Health and Environmental Crisis and Scandal. <http://www.pereladra-ltd.com/AB1473/webpage.cfm>

Smith S. R. 1996. Agricultural recycling of sewage sludge and the environment. Biddles Ltd., Guildford. 446pp

Snyman, H.G., De Jong, J.M. and Aveling, T.A.S. 1998. The Stabilization of Sewage Sludge Applied to Agricultural Land and the Effects on Maize Seedlings. *Water Science and Technology*. **38**(2). 87-95

Snyman, H.G., Terblanche, J.S. and Van der Westhuizen J.L.J. 1999. Management of Land Disposal and Agricultural Reuse of Sewage Sludge Within the Framework of the Current South African Guidelines. Procedures of Specialised Conference on Disposal and Utilization of Sewage Sludge: Treatment Methods and Application Modalities. Athens, Greece

Snyman, H.G. and Van der Waals, J. 2003. Laboratory and field scale evaluation of agricultural use of sewage sludge. Final Report of WRC Project Number K5/1210

Snyman, H.G., Herselman, J.E. and Kasselmann, G. 2003. A Metal Content of South African Sewage Sludge and an Evaluation of Analytical Methods for their Determination in Sludge. WRC Report

Sobsey, M.D. 1996. Microbial Aspects of Water Quality and Health in the Developed and Developing World. Stockholm Water Symposium 1996. Safeguarding Water Resources for Tomorrow: New Solutions to Old Problems. Stockholm. 195-197

Straub, T.M., Pepper, I.L. and Gerba, G.L. 1993. Hazards from Pathogenic Microorganisms in Land-disposed Sewage Sludge. *Rev of Environmental Contamination and Toxicology*. **132**. 55-91

Straub, T.M., Pepper, I.L., Abbaszadegan, M. and Gerba, C.P. 1994. A Method to Detect Enteroviruses in Sewage Sludge-Amended Soil Using the PCR. *Applied and Environmental Microbiology*. **60**(3). 1014 - 1017

Straub, T.M., Pepper, I.L. and Gerba, C.P. 1995. Comparison of PCR and Cell

Culture for Detection of Enteroviruses in Sludge-Amended Field Soils and Determination of their Transport. *Applied and Environmental Microbiology*. **61**(5). 2066 - 2068

Strauch, D. 1991. Survival of Pathogenic Microorganisms and Parasites in Excreta, Manure and Sewage Sludge. *Revue Scientifique et Technique office International des Epizooties*. **10**(3). 813 - 846

Sunday Times, 2003. Putting Our Pooh to Good Use. Location: Sunday 24 August 2003. Gauteng News

Tanner, B., Brooks, J., Josephson, K., Gerba, C. and Pepper I. 2003. Evaluation of the Potential for Bioaerosols from Land Applied Biosolids. Proceedings of the IWA Biosolids 2003 Conference, wastewater Sludge as a Resource. 23-25 June 2003. Trondheim. Norway

Takeuchi, K., Matute, C.M., Hassan, A.N. and Frank, J.F. 2000. Comparison of the Attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella typhimurium* and *Pseudomonas fluorescens* to Lettuce Leaf. *Journal of Food Protection*. **63**(10). 1433-1437

Takeuchi, K. and Frank, J.F. 2000. Penetration of *Escherichia coli* O157:H7 into Lettuce Tissue as Affected by Inoculum Size and Temperature and the Effect of Chlorine Treatment on Cell Viability. *Journal of Food Protection*. **63**(4). 434-440

Takeuchi, K. and Frank, J.F. 2001. Quantitative Determination of the Role of Lettuce Leaf Structures in Protecting *Escherichia coli* O157:H7 from Chlorine Disinfection. *Journal of Food Protection*. **64**(2). 147-151

Tauxe, R.V. 1997. Emerging foodborne diseases: an evolving public health challenge. *Emerging Infectious Diseases*. **3**. 425-434

Tester, C.F. and Parr, J.F. 1983. Intensive Vegetable Production Using Compost. *Biocycle*. **24**(1). 34-36

Teunis, P.F.M. and Havelaar, A.H. 2000. The Beta Poisson Dose-Response Model is not a Single-Hit Model. *Risk Analysis*. **20**(4). 513- 520

Tierney, J.T., Sullivan, R. and Larkin, E.P. 1977. Persistence of Poliovirus 1 in Soil and on Vegetables Grown in Soil Previously Flooded with Inoculated Sewage Sludge or Effluent. *Applied and Environmental Microbiology*. **33**(1). 109-113

Tyson, J.M. 2002. Perceptions of Sewage Sludge. *Water Science and Technology*. **46**:373-380

Vesilind, P.A. 2003. Pathogens in Sludge: A Case of Sufficient Challenge. Proceedings of the IWA Biosolids 2003 Conference, wastewater Sludge as a Resource. 23-25 June 2003. Trondheim. Norway

Vilanova, X., Manero, A., Cerdà-Cuéllar, M. and Blanch, A.R. 2002. The effect of a sewage treatment plant effluent on the faecal coliforms and enterococci populations of the reception river waters. *Journal of Applied Microbiology*. **92**. 210-214

Vorobieva, L.V., Selyuzhitskii, G.V. and Chernova, G.I. 1996. Ecologic and Hygienic Evaluation of Lake Ladoga as a Source of Drinking Water. *Hydrobiologia*. **322**. 137-141

Waage, A.S., Vardund, T., Lund, V. and Kapperud, G. 1999. Detection of Small Numbers of *Campylobacter jejuni* and *Campylobacter coli* Cells in Environmental Water, Sewage and Food Samples by a Seminested PCR Assay. *Applied and Environmental Microbiology*. **65**(4). 1636-1643

Wachtel, M.R., Whitehead, L.C. and Mandrell, R.E. 2002a. Association of *Escherichia coli* O157:H7 with Preharvest Leaf Lettuce Upon Exposure to Contaminated Irrigation Water. *Journal of Food Protection*. **65**(1). 18-25

Wachtel, M.R., Whitehand, L.C. and Mandrell, R.E. 2002b. Prevalence of *Escherichia coli* Associated with a Cabbage Crop Inadvertently Irrigated with Partially Treated Sewage Wastewater. *Journal of Food Protection*. **65**(3). 471-475

Wells, J.M. and Butterfield, J.E. 1997. *Salmonella* Contamination Associated with Bacterial Soft Rot of Fresh Fruits and Vegetables in the Marketplace. *Plant Disease*. **81**. 867-872

WHO, 1979. Human Viruses in Water, Wastewater and soil. Report of a WHO Scientific Group. World Health Organization Technical Report Series 639. World Health organization, Geneva. 19 -20

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

WRC, 2002. Addendum to the Guideline on Permissible Utilisation and Disposal of Sewage Sludge. Edition 1 Water Research Commission. TT 154/01

Zenz, D.R., Peterson, J.R., Brooman, D.L. and Lue-Hing, C. 1976. Environmental Impacts of Land Application of Sludge. *Water Pollution Control Federation*. **48**(10) 2332-2342

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