Chapter 3

The Microbiological Quality of Sewage Sludge in South Africa

3.1. Introduction

The practice of using sewage sludges in agricultural land is attractive to many farmers and water authorities (Carrington *et al*., 1982). It provides nutrients for crop growth as well as organic matter for soil conditioning (Melloul and Hassani, 1999). In the UK approximately 70% of sludge produced is deposited on land (Carrington *et al*., 1982). This is practiced primarily for economic reasons (Kelley *et al*., 1984; Bouwer, 1992) and also as an alternative means of disposal since the ban on sea disposal (EPA, 1999). In South Africa, agricultural soil is often degraded through erosion and the nutrient and carbon content are low. Land application therefore appears to be a beneficial and environmentally sustainable sludge management option (Sidhu *et al*., 1999) for South Africa.

However, sludge contains microorganisms that could pose a health hazard to humans. The types of organisms present in sludge are determined by the microbiological quality of wastewater from which the sludge is generated. These organisms include bacteria, viruses, protozoa and helminths (Burge and Marsh, 1978; Strauch, 1991).

At present, very few South African wastewater treatment plants disinfect their sewage sludge. Techniques commonly used in South Africa include aerobic stabilization by increasing the sludge age in the activated sludge process and anaerobic digestion in either mesophylic or heated digesters. These techniques are not capable of adequately disinfecting sewage sludge, resulting in a product that contains a large number of pathogens and can still have a high potential for vector attraction. Techniques such as lime stabilisation and composting which yield sewage sludge of improved microbiological quality (WRC, 1997), which can be safely applied to agricultural land are not common practice in South Africa.

The South African sewage sludge guidelines classify sewage sludge at four levels (Type A, B, C and D), rated in the order of improving microbiological quality. Type A is of low microbiological quality and may not be used for agricultural use. Type B sludge is typically an anaerobically digested sludge or waste activated sludge. This sludge type may be used in agricultural practice, but with strict control to minimize the exposure of humans to pathogens. As this sludge type is used extensively, the rest of the thesis focuses on the agricultural use of a type B sludge. The Types C and D are of acceptable microbial quality, with Type D being produced for unrestricted use, provided the levels of metal and inorganic content are kept at the limits set in the guidelines (WRC, 1997).

Most environmental concerns about land application of sewage sludge have focused on effects of nutrients especially nitrogenous (N) and phosphorus-containing (P) compounds and effects of heavy metals (Hyde, 1976). Microorganisms from sludge are often low on the priority list. To assess the threat posed by different microorganisms in sludge intended for soil conditioning, the types of organism present in the sludge must be determined. This chapter addresses the microbial quality of sewage sludge to be used for soil amendment purposes. The secondary aim of this chapter is to estimate the quantity of microorganisms (Faecal coliforms, *E.coli*, *Salmonella* spp and *Ascaris*) in sewage sludge, as the persistence of these organisms will be followed during the green house experiments.

3.2. Material and Methods

3.2.1 Sample Collection

Sludge samples (n=78) were collected at selected wastewater treatment plants. Three rounds of sampling were done to include seasonal variation, i.e winter, summer and autumn. These samples were analysed for microbiological content.

3.2.2 Microbiological Analysis

Two different South African laboratories were selected for the analyses: the East Rand Water Care Company Laboratory (ERLAB) and the Agricultural Research Council (ARC) Institute for Soil, Climate and Water (ISCW) laboratory. No accredited laboratory for the analysis of sludge could be found. For this reason, an inter-laboratory extraction and analysis train was set up. This was done to utilize the expertise of both laboratories to the optimum. Samples were collected and transported to the laboratories with a maximum delay of 72 hours. Organisms analysed were *Ascaris* ova (viability was not established), faecal coliforms and *Salmonella*, using methods as indicated in Table 3.1.

Table 3.1 Methods used in the analysis of microorganisms in sludge

Organism	**Method**
Ascaris ova	ERLAB Ascaris ova method. 2003
Salmonella spp	Bridson, 1998
Faecal coliforms	Difco Laboratories. 1998

3.2.3 Microbial Diversity

Additional sludge samples were obtained from two of the Waste Water Treatment Plants (WWTP) in the Gauteng province; namely Rondebult, a high metal sludge (HMS) and Olifantsfontein, a low metal sludge (LMS) (Table 3.2). These sludges are products of aerobic treatment (using aerator). Three samples were obtained for each of the sludge types. The sludges from these two plants were used as soil amendment in experimental trials. The microbial component in these sludge samples was determined using the Analytical Profile index and the Biolog technique.

Table 3.2 Metal content of sludge from Rondebult and Olifantsfontein (ERWAT, Sludge Analysis Report, 2003)

Metal	Rondebult (HMS) mg/kg	Olifantsfontein (LMS) mg/kg
Cr	308	31
Cu	167	42
Ni	138	21
Pb	155	47
Zn	1334	1036
Cd	11	5
Co	51	7

i Analytical Profile Index

Organisms present in each of the sludge samples (three HMS and three LMS) were identified using the Analytical Profile Index (API) according to the manufacture's instructions (BioMérieux, South Africa). The API system uses 21 miniature reaction compartments (cupules) that produce 23 biochemical reactions and is standardized for rapid identification of microorganisms. These tests and related reagents are indicated in Appendix A. The relevant API was chosen based on the Gram stain (-ve or +ve) reaction and the bacterial morphology.

The low metal sludge (LMS) and the high metal sludge (HMS) samples were collected from two wastewater treatment plants situated in the eastern Gauteng region, South Africa. Two samples comprising 1 g of each sludge type were emulsified in 9 ml of bacteriological peptone and incubated at 35 ^{o}C overnight. As the samples appeared concentrated, serial dilutions were made prior to transferring to petri dishes. A pour plate was made using plate count agar and incubated at 35 ^{o}C for 18 - 24 hrs. Following incubation, different colonies were picked up and transferred by streaking onto plate count agar, using an inoculating loop, and incubated at 35 ^{o}C for 18 - 24 hrs. Well-defined colonies were picked using an inoculating loop. Gram staining was done by using crystal violet dye, iodine and acetone (Eikelboom, 2000).

(a) Identification of the *Enterobacteriaceae*

The API 20E is an identification system for *Enterobacteriaceae* and other gram-negative bacteria (Juang and Morgan, 2001). Well-isolated colonies were picked off from the plate and suspended in 5ml sterile water. The suspension was carefully emulsified to achieve a homogeneous bacterial suspension. Bacterial identification tests were done according to the manufacturer's instructions (API 20E, BioMerieux, South Africa).

(b) Identification of the *Staphylococci*

For identification of the microorganisms belonging to the genus *Staphylococcus*, the API Staph was used (Ligozzi *et al*., 2002). Well-isolated colonies were picked off from the plate and suspended in 5ml API STAPH medium. The microtubes of the API Staph strip were filled with the bacterial suspension. The bacterial identification tests were done according to the manufacturer's instructions (API Staph, BioMerieux, South Africa).

ii Microbial Identification Using the Biolog Technique

The Biolog system was used to identity gram positive and gram negative bacteria isolated from the three LMS and three HMS sewage sludge samples. Bacteria typed by gram staining were inoculated onto appropriate Biolog media and subsequently onto specific Biolog 96-well microtiter plates for identification as outlined in the Biolog user manual. Gram postive cocci and rods were inoculated onto BUG (Biolog Universal Growth) + 5% sheep blood and BUG + glucose media, respectively prior to suspension in the supplied inoculating fluid. Gram negative bacteria were inoculated onto TSA with 5% sheep blood prior to suspension in the supplied inoculating fluid. Biolog microplates were inoculated with bacteria and incubated at 30 ^{o}C for 24 hrs according to the manufacturer's instructions. Colour formation in the individual cells of the microtitre plates was measured at 590 nm using microplate reader to determine the extent of reduction of the tetrazolium violet dye included with

the individual substrate in each microplate well. Readings were taken at 6 hrs and at 24 hrs following incubation.

3.3 Results and Discussion

3.3.1 Incidence of Organisms in Sludges from WWTPs in South Africa

Figure 3.1 indicates the faecal coliform counts from sludge samples studied between 2001 to 2003.

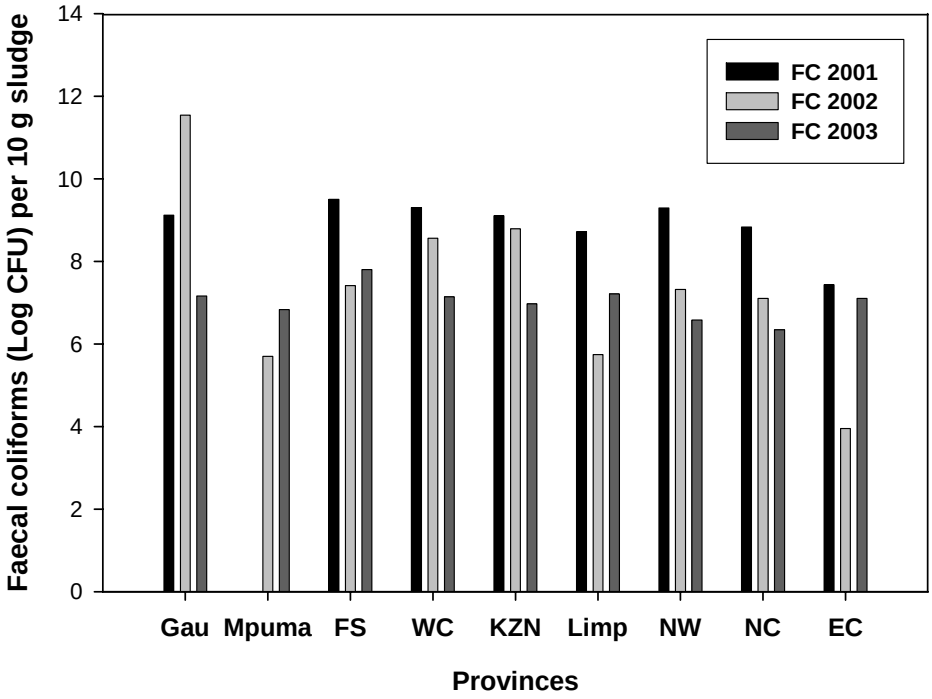

Figure 3.1 Incidence of faecal coliforms detected in sewage sludge from each of the South African provinces. Gau (Gauteng Province), Mpuma (Mpumalanga), FS (Free State), WC (Western Cape), KZN (KwaZulu-Natal), Limp (Limpopo), NW (North West), NC (Northern Cape) and EC (Eastern Cape).

Table 3.3 details the number of sludge samples that tested positive for *Salmonella* spp in each of the provinces. The results indicate that all provinces in South Africa need to manage or at least monitor prevalence of *Salmonella* spp in the wastewater sludges.

Figure 3.3 indicates the numbers of *Ascaris* ova detected in samples obtained from the WWTPs studied. Samples form Gauteng, Kwazulu-Natal and the Northern Cape had the highest number of *Ascaris* ova.

Table 3.3 Incidence of *Salmonella* spp in WWTPs at different South African Provinces.

Provinces	WWTP per Province	% WWTP with *Salmonella* 2001	% WWTP with *Salmonella* 2002	% WWTP with *Salmonella* 2003	% WWTP without *Salmonella* 2001-2003
Gauteng	20	60%	40%	30%	25%
Mpumalanga	2	0	50%	0	50%
Free State	5	40%	40%	80%	20%
Western Cape	15	80%	13%	13%	13%
KwaZulu-Natal	10	40%	40%	60%	20%
Limpopo	5	100%	60%	20%	0
North West	7	43%	57%	29%	14%
Northern Cape	4	75%	50%	50%	0
Eastern Cape	4	0	0	25%	75%

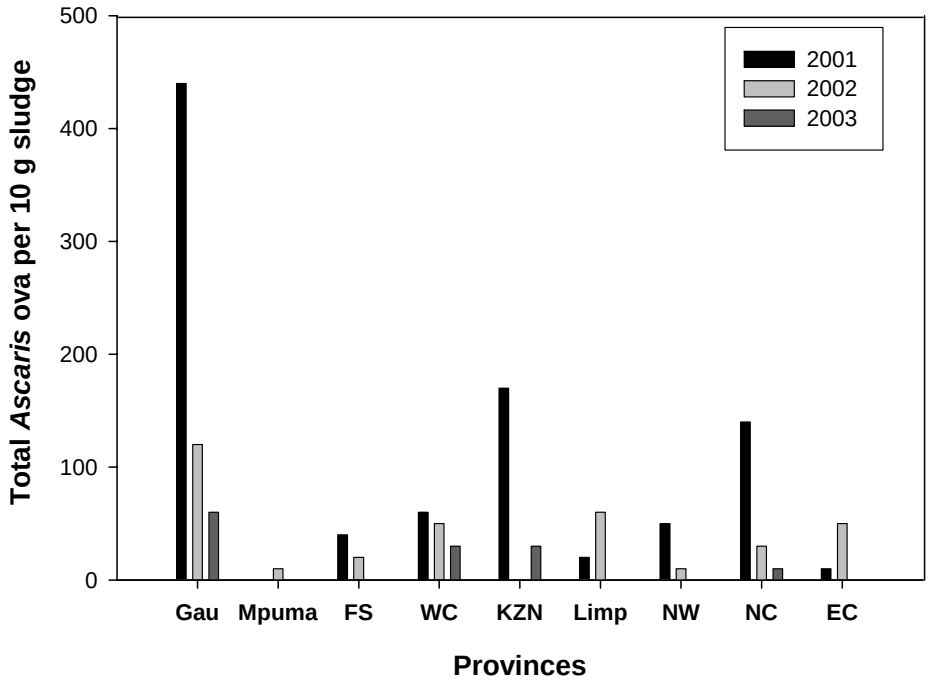

Figure 3.2 Incidence of *Ascaris* ova in sludge samples collected in all the provinces between 2001 and 2003.

The KwaZulu-Natal province has the largest number of people, with 20.3% of the population living in this area (Dorrington *et al*., 2002). However, a large proportion of people in KwaZulu-Natal live in rural areas which do not receive sanitation services. The WWTPs studied from this region service urban areas, which also yielded high prevalence of *Ascaris* ova and *Salmonella* spp.

Gauteng is the second largely populated province after KwaZulu-Natal, with 19.4% of people living in this province (Dorrington *et al*., 2002; Census, 2001). Gauteng is the heartland of the country's economy, and a province with the highest incidence of urbanization as people move into this area for improved quality of life. This migration results in increased number of informal settlements with poor sanitation facilities that may have resulted in the high incidence of *Ascaris* and *Salmonella* infections as noted in the sludge samples from the Gauteng region.

Other than a couple of belt press sludge samples, samples obtained from the Western Cape were mainly from drying beds or compost which show less

prevalence of microorganisms, as a result of the unfavourable dry conditions. Most of the WWTPs studied in the Western Cape service affluent communities. This could also explain the limited prevalence of *Salmonella* spp and *Ascaris* ova in this region. Samples from Mpumalanga were also collected from drying bed and showed limited prevalence. The prevalence of pathogens in the Eastern Cape appears limited, as a large proportion of people in this province live in rural dwellings that do not receive any sanitation services.

3.3.2 Microorganisms identified using API and the Biolog technique

Two known pure bacterial cultures were used as positive controls to validate the proficiency of the Biolog technique and were positively identified (Table 3.4).

Table 3.4 Validation of the proficiency of Biolog

Culture	Species
Control A	*Serratia marcescens*
Control B	*Photobacterium logei*

Table 3.5 Microorganisms occurring in sewage sludge, as found in this study and elsewhere

Microorganism	Reference	This Study Technique (Sludge Type)
Escherichia coli	Strauch, 1991	API (LMS and HMS)
Serratia spp.	Strauch, 1991	API (LMS and HMS)
Salmonella spp.	Carrington *et al*., 1982	API (LMS and HMS)
Citrobacter	Strauch, 1991	API (LMS and HMS)
Klebsiella ornilytica	Dudley *et al*., 1980	API (LMS and HMS)
Shigella spp	Strauch, 1991	**X**
Yersinia enterocolitica	Strauch, 1991, Pell, 1997	**X**
Clostridium spp	Strauch, 1991	**X**
Leptospira spp	Strauch, 1991	**X**
Mycobacterium spp	Strauch, 1991	**X**
Vibrio cholerae	Strauch, 1991	**X**
Streptococcus	Strauch, 1991	**X**
Enterobacter	Strauch, 1991	Biolog (LMS)
Serratia	Strauch, 1991	Biolog (LMS)
Proteus	Strauch, 1991	**X**
Providencia	Pelczar *et al*., 1993	**X**
Listeria monocytogenes	Strauch, 1991	**X**
Staphylococcus lentus	Dudley *et al*., 1980	API (LMS)
Achromobacter spp	Pelczar *et al*., 1993 Prazmo *et al*., 2003	Biolog (LMS)
Chromobacterium violaceum	Pelczar *et al*., 1993 Prazmo *et al*., 2003	Biolog (LMS)
Pseudomonas spp	Pelczar *et al*., 1993 Prazmo *et al*., 2003	Biolog (HMS)

Table 3.5 Continued

Microorganism	Reference	This Study Technique (Sludge Type)
Pseudomonas aeruginosa	Pelczar *et al*., 1993	Biolog (HMS)
Pantoea agglomerans	Pelczar *et al*., 2003	Biolog (HMS)
Serpens flexibilis		Biolog (LMS)
Oligella urethralis		Biolog (LMS)
Raoutella terrigena		Biolog (HMS)
Brevibacterium liquefaciens		Biolog (HMS)
B. mcbrellneri		Biolog (HMS)
B. linens		Biolog (HMS)
B. otitidis		Biolog (HMS)
Leclercia adecarboxylata		Biolog (HMS)
Rhodococcus australis		Biolog (HMS)
Cellulomonas hominis		Biolog (HMS)
Acitenobacter calcoaceticus		Biolog (HMS)
Exiguobacterium acetylicum		Biolog (HMS)

X = these organisms were not detected in the present study.

Bold = present in the current study, but not reported elsewhere.

This study used sensitive tests and detected microorganisms that are not commonly found in sludge. However these organisms are not indicated as human pathogens, but are mainly associated with the environment and, thus, may have originated from water or soil.

Most of the organisms identified using Biolog are not known to cause disease in healthy people. However, they may cause opportunistic infections in people who have weakened immune systems such as those undergoing antibiotic

therapy, cancer treatment or those with HIV/AIDS. For instance *Brevibacterium* strains usually present on the skin (Pelczar *et al*., 1993) have been implicated in bloodstream infections in HIV/AIDS patients (Brazzola *et al*., 2000).

Some organisms such as *Achromobacter*, *Chromobacterium* and *Pseudomonas* identified in this study, have been reported as frequently occurring in sewage (Pelczar *et al*., 1993; Prazmo *et al*., 2003). These bacteria are responsible for denitrification in soil (Drysdale *et al*., 1999).

Achromobacter xylosoxidans often found in aqueous environmental sources (Clermont *et al*., 2001) is an opportunistic pathogen that has been implicated in serious infections (Ramos *et al*., 1996; Hernandez *et al*., 1998). *Oligella urethralis* is an organism of the genital and urethral tracts and has been implicated in urinary tract infection (Mammeri *et al*., 2003) and meningitis (Graham *et al*., 1990), *Serratia marcescens* and *Pseudomonas aeruginosa* with respiratory infections (Kirschke *et al*., 2003), *Chromobacterium violaceum* is the causal agent of septicaemia (Perera *et al*., 2003) while *Acinetobacter* species are often associated with nosocomial infections (McDonald *et al*., 1998).

Children with chronic granulomatous disease are predisposed to infection caused by *Chromobacterium violaceum* (Macher *et al*., 1982). Although infections caused by *C. violaceum* are rare, when they occur they are responsible for a high incidence of mortality (Ti *et al*., 1993).

The presence of the opportunistic pathogens in sludge may have serious implications for the consumers if such sludges are used for soil amendment. This is particularly pertinent for young children, expectant women, the elderly and those infected with HIV/AIDS, as their immune systems are compromised.

The primary route of exposure to pathogens is by ingestion. If sludge is to be used in the production of food crops, then there is a chance of exposure

through ingestion. Consequently, there is a greater need to reduce pathogen numbers prior to soil application.

Biochemical profiling using API has become popular in recent years (Bezuidenhout *et al*., 2002). This technique although qualitative, identifies organisms based on their biochemical reactions and it provides rapid identification thereof (Juang and Morgan, 2001).

Organisms identified with API were common between the LMS and HMS except for the *Staphylococcus lentus*, which was only identified in the LMS (Table 3.3). Dudley *et al*., (1980) also found *Klebsiella* spp, *Salmonella* spp, and *Staphylococcus* spp in the Texas sewage sludge. *Salmonella* and *E.coli* are some of the most common organisms in sewage sludge (Jones, 1980; Carrington *et al*., 1982; Strauch, 1991; Bouwer, 1991; Jones 1999).

Bezuidenhout and colleagues (2002) in KwaZulu-Natal, South Africa also used API to identify similar species, namely *E.coli*, *Serratia* spp., and *Klebsiella* spp. in contaminated water. They however did not detect any *Salmonella* spp in this region. Generally *Salmonella* spp frequently occur in wastewater and sewage sludge and this organism has been reported to persist in various environments due to its ability to withstand stressful conditions (Strauch, 1991). For this reason outbreaks of Salmonellosis occur frequently worldwide (Melloul and Hassani, 1999). Carrington and colleagues (1982) have reported that *Salmonella* spp may multiply in sludge in the absence of competition from other microorganisms.

3.4 Conclusion

Testing of sludge samples showed large numbers of Faecal coilforms, indicating that intensive treatment of sludge from WWTPs across the country is required to meet the type C and D class South African guidelines.

It is likely that the large numbers of *Ascaris* in the Gauteng area could be related to increased urbanization in this province.

The microbial population determined for LMS was similar to the population of HMS except for the presence of *Staphylococcus lentus* in LMS.

Due to the presence of potentially noxious pathogens in the sewage sludge, it is recommended that sewage sludge need to be adequately disinfected prior to use in agricultural land.

Further research on the microbial quality in South African water and soil in the Gauteng region will be necessary to establish the types of organisms present in these environments.

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