FAECAL SOURCE TRACKING AND WATER QUALITY IN THE EASTERN CAPE, SOUTH AFRICA

A thesis submitted in fulfilment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

of

RHODES UNIVERSITY

by

CATHERINE DIANE LUYT

DECEMBER 2012

ABSTRACT

Water quality is concerning as many still lack access to safe drinking water. Alternate sources such as rivers (FC up to 1600 CFUs/100 mL) and rainwater are often polluted. Rainwater tanks require maintenance to improve water quality, but could be used for non-potable purposes or irrigation. Grahamstown infrastructural failures initiate deviations from DWAF 1996 domestic water guidelines for microorganisms within the distribution system. Frequent testing can decrease risks of waterborne diseases. Limitations to this are inaccessibility of rural areas, distances from testing centres and costs. The low cost H_2S strip test able to be used onsite by communities, may aid in risk assessment. H_2S strip test results are not affected by sulphate (14 to 4240 mg/L) or nitrite (up to 47 mg/L). Transportation of the H_2S strip tests between 10 and 32°C does not modify results significantly. Similarly to other studies: *Klebsiella spp.; Enterobacter spp.* and *Serratia spp.* were isolated from H_2S strip tests. The mH_2S strip test corresponds best with HPC in treated water, while in untreated river water it has approximately 90% correspondence with FCs, while survival of FC causes discrepancies with the H_2S test after 22 days. A faecal coliform inactivation rate of 0.1 CFUs/ day, may be longer than many pathogens. Faecal source tracking, not currently practised in South Africa, could aid health risk assessments for disaster management, which would improve the NMMP programme. Bacterial survival times could propose the time period for which water is unsafe. Bifidobacteria and *Rhodococcus* are proposed to help identify the faecal pollution source. But enumeration of *Rhodococcus* is too lengthy (21 days). The tracking ratio of bifidobacteria (between 0.1 to 6.25) is not source definitive. The bifidobacteria survival rate, could indicator the time since faecal pollution. The bifidobacteria average survival rate is 2.3 CFUs per day for both groups. The culturability and selectivity of agar is still poor, with total bifidobacteria less selectively culturable. Enterococci overgrowth of TB was decreased by Beerens media. SUB is still useful to identify potential human faecal inputs. A single tracking method is thus not suitable alone, but requires a combination of techniques.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

I would like to express my appreciation to my sponsors - The Water Research Commission of South Africa; the Henderson Scholarship from Rhodes University; John Gilliam and the Financial Aid staff at Rhodes University - for making this project possible.

Special mention should go to my supervisor Dr R. Tandlich for his guidance and Dr S. Khamanga for his help. To all the lecturers, who have mentored me throughout my university career, thank you.

I would like to thank the Grahamstown Veterinary Unit, especially Morris Makepe, for all their help testing tap water. I would like to acknowledge the students, lecturers, IWR and others who assisted me by collecting water and by providing support, guidance and equipment.

I must convey my thanks to my family for their mentorship and encouragement. Without them, I would not have been able to achieve this. Thank you William and Helen for providing a loving home environment over this period.

To my truly amazing friends Ian, Beverley and Megan: Thank you for supporting and encouraging me through tough times.

Most importantly I would like to thank God for being my strength (Philippians 4:13).

LIST OF ABBREVIATIONS

°C	Degrees Celsius
µm	Micrometre
ABM	Adjusted Beerens Media
AIC	Akaike Information Criterion
ATP	Adenosine Triphosphate
BDL	Below Detection Limit
Bifido	Bifidobacteria
BSAE	Bovine Serum Albumin Equivalent
BSTFA	N,O-Bis (trimethylsilyl) trifluoroacetamide
$CaCO_3$	Calcium carbonate
CFUs	Colony Forming Units
Chla	Chlorophyll A
Cl^-	Chloride
COP	Coprostanol
DAF	Disaster Management Advisory Form
DAFF	Department of Agriculture, Fishery and Forestry
DALYs	Disability Affected Life Years
df	Degrees of Freedom
DO	Dissolved Oxygen
DWA	Department of Water Affairs
DWAF	Department of Water Affairs And Forestry
DWQ	Drinking Water Quality
E. coli	*Escherichia coli*
EC	Electrical Conductivity
EPA	Environmental Protection Agency (in the USA)
et al.	And Others
FC	Faecal Coliforms
$FC_{Extract}$	Faecal Coliform Concentration in the 75 mL of Ringer solution used to Extract Stone Biofilm Bacteria (MPN Cells/100 mL)
$FC_{Sediment}$	The Faecal Coliform Concentration in the Sediment (MPN Cells/mg Sediment Dry Weight),
FC_{Water}	Faecal Coliforms found in the Water Column (MPN Cells/100 mL)

F-RNA	Male Specific F (Fimbriae) RNA (Ribonucleic Acid) Coliphages
FS	Faecal Streptococci
g	Gram
GC	Gas Chromatography
GIS	Geographic Information System
GIT	Gastrointestinal Tract
GRASS	Gaian Revolutions And Social Solutions Society
GWW	Global Water Watch
h	Hours
H_2S	Hydrogen Sulphide
HBSA	Human Bifidobacteria Sorbitol Agar
HPC	Heterotrophic Plate Count
HPLC	High Performance Liquid Chromatography
HST	Health Systems Trust
IGCDM	Intergovernmental Committee on Disaster Management
IRIN	Integrated Regional Information Networks
IU	International Units
JMP	Joint Monitoring programme
kg	Kilogram
L	Litre
LOQ	Limit Of Quantification
Ltd.	Limited
lx	LUX
m	Meter
M	Molar
MBM	Modified Beerens Media
MDG	Millennium Development Goal
mg	Milligram
GE	
mH_2S	Modified Hydrogen Sulphide Test
min	Minute
mL	Millilitre
MPN	Most Probable Number

mS/m	Millisiemens
MTF	Multiple Tube Fermentation
NADH	Nicotinamide Adenine dinucleotide
ND	Not Determined
NGOs	Non-Governmental Organizations
NH_4^+	Ammonium
NHA	National Health Act
NMMP	National Microbial Monitoring Programme
NO_3^-	Nitrate
NTU	Nephelometric Turbidity Units
NTU	Nephelometric Turbidity Units
NWA	National Water Act
NWP	National Water Policy
NWRS	National Water Resources Strategy
OB	Optical Brighteners
OG	Overgrown
Per capita	Per Person
Per se	In Itself
PFUs	Plaque Forming Units
PO_4^{3-}	Phosphate
PSFC	Percentage Suspendable Faecal Coliforms
psu	Practical Salinity Scale Unit
Pty.	Proprietary
PVC	Polyvinyl chloride
RHP	River Health Programme
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RSA	Republic of South Africa
RU	Rhodes University
s	Second
SABS	South African Bureau of Standards
SALGA	South African Local Government Association
SANS	South African National Standards

SMSes	Short Message Services
SO_4^{2-} (mg/L)	Sulphates
spp.	Species
ss-RNA	Single stranded Ribonucleic acid
SUB	Sorbitol Utilising Bifidobacteria
T (ºC)	Temperature
TA	Total Alkalinity
TB	Total Bifidobacteria
TC	Total Coliforms
TDS	Total Dissolved Solids
TF	triphenyl formazan
TSS	Total Suspended Solids
TTC	2, 3, 5-triphenyltetrazolium chloride
UCT	University of Cape Town
UNHCR	United Nations High Commission for Refugees
UNMDG	United Nations Millennium Development Goal
USA	United States of America
USEPA	United States Environmental Protection Agency
UV	Ultraviolet Light
VBNC	Viable But Non-culturable
VSI	Voluntary Sector Initiative
w/v	Weight per volume e.g. g/L or mg/mL
WASH	Water, Sanitation And Hygiene Campaign Initiated By The Water Supply And Sanitation Collaborative Council
WHO	World Health Organisation
WMA	Water Monitoring Area
WRC	Water Research Commission
WSA	Water Services Act
WWTW	Wastewater Treatment Works
WWW	World Water Watch
ZAR	South African Rand (currency)

LIST OF PUBLICATIONS FROM THIS THESIS

LUYT, C. D., TANDLICH, R., MULLER, W. J. & WILHELMI, B. S. 2012. Review: Microbial Monitoring of Surface Water in South Africa: An Overview. *Int. J. Environ. Res. Public Health,* 9**,** 2669-2693.

LUYT, C. D., MULLER, W. J. & TANDLICH, R. 2011. Factors influencing the results of microbial surface water testing in South Africa. *In:* HACKER, J. (ed.) *DGHM 2011.* Essen, Germany: International Journal of Medical Microbiology.

LUYT, C. D., MULLER, W. J. & TANDLICH, R. Low cost tool for monitoring drinking water and environmental water quality in selected parts of South Africa. 5th Public Health Association of South Africa Conference, 2009 Durban, South Africa. PHASA, 88.

LUYT, C. D., MULLER, W. J. & TANDLICH, R. 2011. Low cost tools for microbial quality assessment of drinking water in South Africa. *Special Issue of the IOMC 2011 Conference Abstracts.* Conference e-Proceedings: International Journal of Collaborative Research on Internal Medicine & Public Health.

TANDLICH, R., LUYT, C. D., SRINIVAS, S. C. & GORDON, A. K. 2012. Concentrations of indicator organisms in the stored rainwater in the Makana municipality, South Africa. *Aerul şi Apa : Componente ale Mediului***,** 89-96.

LUYT, C. D., MULLER, W. J., WILHELMI, B. S. & TANDLICH, R. Health implications of flood disaster management in South Africa. Annual Conference of the International Emergency Management of Society, 2011 Bucharest, Romania.

ZUMA, B. M., LUYT, C. D. & TANDLICH, R. Disaster Management System in South Africa. TIEMS, 2011.

CHAPTER 1: LITERATURE REVIEW OF WATER QUALITY MONITORING AND THE SOUTH AFRICAN CONTEXT

1.1 INTRODUCTION

South Africa is a country located at the southern tip of Africa. Made up of 9 provinces, it is a water-stressed country (Seckler et al., 1999; Quinn et al., 2011), with scarcity predicted by 2025 (Otieno and Ochieng, 2004). Water-stress is defined as having less than 1700 m^3 water per person per year, while water scarcity implies water quantities of less than 1000 m^3 per person per year (Rijsberman, 2006). However this measurement of water access has its flaws, such as not measuring the poor utilisation of water, lack of infrastructure and agricultural uses (Rijsberman, 2006).

South Africa has a legacy of poorly developed areas, with poor infrastructures (Schreiner and van Zyl, 2006). The Eastern Cape is one such province (Leibbrandt and Woolard, 1999; Momba et al., 2009a).Grahamstown is a water scarce region in the Eastern Cape (DWAF, 2004; DWAF, 2006). The provincial lack of infrastructure poses several challenges to those living in Grahamstown (Mamba, 2008; Statistics South Africa, 2012d).

1.2 MILLENNIUM DEVELOPMENT GOALS

The United Nations Millennium Development Goals (UNMDG) were designed to alleviate poverty and increase access to health services, including safe water (United Nations, 2012). Goal 7A is aimed at environmental sustainability and 7B at halving the proportion of people without access to safe drinking water and basic sanitation by 2015 (United Nations, 2012). In 2010, 89.3% of the South African population had access to drinking water within 200 m of their homes (Statistics South Africa, 2011a). This is an improvement from the 84.5 % in 2002 (Statistics South Africa, 2011a). Piped water was still unavailable to 10.5% of the South African population in 2011 (Statistics South Africa, 2011d; Statistics South Africa, 2012d). Without access to distribution or tap water, people are forced to use water of inadequate quality (Momba et al., 2006b; Tandlich and Muller, 2008; Luyt et al., 2012).

According to survey data, the MDG goals for access to water have been reached (Clasen, 2012). The Joint Monitoring Committee (JMP) has stated that there are limitations to their data collection as they do not directly test the water, relying instead on indicators which can be observed by minimally trained interviewers, limiting accuracy (WHO and UNICEF, 2004; Clasen, 2012). The assumption that improved water sources are safe may result in an overestimation of the actual number of people using safe water (Clasen, 2012). Surveys have not assessed the actual quality, quantity or reliability of the water supply (Clasen, 2012), meaning the number of people with access to and use of safe water may be overestimated by surveys (Clasen, 2012).

Nine per cent of the South African population uses river, dam or spring water for their drinking water, equating to 20% of the population in the Eastern Cape (Statistics South Africa, 2012a). South African households have experienced water outages due to pipe breaks, 36.2% of which lasted longer than 15 days (Statistics South Africa, 2011c). This illustrates another scenario in which surveys would have identified access to safe water when, in fact, there was none. Numerous pipe breaks (PMG, 2009a) causing interruptions in supply (Statistics South Africa, 2011d) also contribute to poor water quality and possible gastrointestinal disease due to microbes entering the distribution system (Nygård et al., 2007; PMG, 2009a; Besner et al., 2011). But with frequent infrastructure problems and interruptions in water supply due to pipe bursts and inadequate servicing of community taps (PMG, 2009b), inadequate operation of water treatment plants and skills shortages (Momba et al., 2006b; Momba et al., 2009a) could lead to inadequate drinking water quality. The lack of water pressure has been associated with increased risks of gastrointestinal disease, due to microbes entering the distribution system during these periods (Nygård et al., 2007; Besner et al., 2011). Thus the distribution water is not always safe (Lee and Schwab, 2005).

The South African government is trying to rectify this problem. It is also trying to improve the safety of water by introducing the Blue Drop system, which provides an incentive to municipalities who comply with Department of Water Affairs and Forestry (DWAF) and South African National Standards (SANS) 241:2006 regulations (DWA, 2011a).

Drinking water is monitored by water boards and municipalities in South Africa. The water boards are government-owned entities which operate dams, water infrastructure, and, sometimes, wastewater treatment works (Department of Water Affairs, 2012b). There are 12 water boards in South Africa at present, however different DWA sites have different numbers, as they have not all been updated. The Blue Drop system, implemented in 2008, was designed to provide an incentive for municipalities to comply with regulations (DWA, 2011a). But this only affected the testing of water in the drinking water distribution system. The Blue Drop system is an assessment of water quality in the catchment, distribution system, laboratories used and plans for water management. Blue drop score are calculated by the compliance to all the criteria (DWAF, 2011).

1.3 WATERBORNE DISEASE

Waterborne disease is not only a problem in developing countries, although these areas are more prone due to lack of clean water, poor sanitation and hygiene, education levels of the population, disasters such as droughts and floods and regional political or economic status (Mitchell and Gu, 2010). Many developing countries are in tropical regions, where conditions are more favourable for microbial growth. Disease vectors, pathogens and parasites are found in higher concentrations in developing nations (Mitchell and Gu, 2010). A large proportion of the global disease burden is related to the quality of water and is not just waterborne (Mitchell and Gu, 2010). Waterborne disease can be transmitted via multiple methods such as drinking urine or faecal contaminated water containing helminths, parasites, pathogenic viruses or bacteria (Montgomery and Elimelech, 2007).

Waterborne disease can also be spread indirectly via insects such as mosquitoes which may also carry malaria (Montgomery and Elimelech, 2007) which could be a problem for people collecting water or storing water ineffectively. Toxins from bacteria or viruses can also cause diarrhoeal diseases (Montgomery and Elimelech, 2007). Waterborne disease is caused by the ingestion of water containing pathogenic organisms in a quantity exceeding the infectious dose (Leclerc et al., 2002).

In 1996 Grabow stated there was little information on waterborne disease in South Africa (Grabow, 1996), and this problem has continued with a lack of studies being performed in the country. Waterborne disease is often caused by rotavirus, *Vibrio cholerae* (serovar O1 and O139), *Salmonella enterica* subsp. (*enterica serovar Paratyphi*; *enterica serovar Typhi* and *enterica serovar Typhimurium*)*; Shigella dysenteriae; Shigella flexneri; Shigella boydii; Shigella sonnei, Campylobacter* and *Escherichia coli* (serotypes: O148, O157 and O124) (Sinton et al., 1998; Ishii and Sadowsky, 2008; Cabral, 2010). Other waterborne disease include Hepatitis A and E, Norwalk viruses, enteroviruses, poliomyelitis, *Acanthamoeba, Giardia duodenalis, Entamoeba histolytica, Cyclospora cayetanensis, Toxoplasma gondii, Cryptosporidium parvum, Enterotoxigenic E. coli, Enterohaemorrhagic E. coli, Yersinia spp., Francisella tularensis, Helicobacter pylori, Mycobacteria spp*. (Hunter, 2003; Madigan and Martinko, 2006; Mitchell and Gu, 2010; Hagedorn et al., 2011). From these *Salmonella, Campylobacter*, certain *E. coli* strains and protozoans such as *Cryptosporidium* and *Giardia* are found in human and animal excrement and pathogenic to both groups (Hagedorn et al., 2011). This is by no means a comprehensive list and new pathogens are continuously emerging. Other problems are hepatitis and cryptosporidiosis (Grabow et al., 1994; Grabow, 1996).

Another problem is the immunocompromised population that is steadily increasing with HIV and cancers (Obi et al., 2006; Karim et al., 2009). They are more susceptible to waterborne pathogens and are thus more likely to suffer from disease (Oberholster and Ashton, 2008; Momba et al., 2010). The developing world has massive population growth and a strained health budget due to HIV/AIDS, amongst other diseases, which often have to cover hygiene education, resulting in a lowered capacity to alleviate this problem (Pletschke et al., 2006). Lack of suitable sanitation, overflowing wastewater treatment plants and poor river water quality exacerbate the problem (Momba et al., 2006a). Dumping of raw sewage into rivers is a major problem, while rainwater runoff from areas of open defecation causes toxic water conditions (Jagals et al., 1995; Jagals and Grabow, 1996b; Sinton et al., 1998). There is also need for an early warning system and continuous water monitoring.

1.4 SAFETY OF WATER SOURCES

Safe drinking water is defined by the World Health Organisation (WHO) guidelines as water which does not contribute to any substantial health risk over a lifetime of consumption (WHO, 2011). Microbial concentrations differ depending on the source of water and are thus divided into unimproved and improved groups.

The WHO classification is use to differentiate the sources into improved and unimproved sources. Improved water sources include piped water to dwellings, yards, public taps, protected dug wells, springs and rainwater collection (WHO and UNICEF, 2010a). Unimproved sources are unprotected wells, unprotected springs (with no protection from human or animal contamination at the collection point), water transported to the site, surface water (e.g. rivers, streams and dams) and bottled water (UNICEF and WHO, 2010). Many female children miss school to collect water, while women are neglecting their children to fetch water, both practises undermining other Millennium Development Goals (MDGs) (WHO and UNICEF, 2005; Clasen, 2012). Other problems arising from carrying heavy water loads include the development of musculoskeletal disorders, such as muscular strains and sprains, lower back pain and spinal and joint problems (Geere et al., 2010a; Geere et al., 2010b; Sharma and Singh, 2012). Problems associated with collecting and carrying water could be eliminated by making 50 L per capita per day available to each household (WHO and UNICEF, 2005). Increased access at households would prevent hygiene being compromised (WHO and UNICEF, 2005). Health promotion is still necessary to ensure hygiene practises are followed and to prevent water contamination during collection and storage. (WHO and UNICEF, 2005; Wilkinson et al., 2012).

1.5 HOUSEHOLD STORAGE

Contamination of water from improved sources leaves many people drinking contaminated water (Nath et al., 2006). Such contamination occurs due to seepage in the distribution system, unhygienic transportation methods or poor handling in the home (Wright et al., 2004; WHO and UNICEF, 2005; Maraj et al., 2006). Simple home treatment techniques include chlorination, solar disinfection and the use of filters and combined flocculation/chlorination powders (WHO and UNICEF, 2005; Lantagne et al., 2006; Sobsey et al., 2008). Not only water quality, but quantities need to be sufficient to improve health within a community (WHO and UNICEF, 2005).

Agenda 21, the sustainable development action plan proposed by the 1992 Rio Earth summit, states that a person requires a minimum of 20 L of water per day (WSSCC-2000). Without adequate quantities of water, hygiene is neglected. Good hygiene habits significantly decrease the risk of diarrhoeal diseases within homes (WHO and UNICEF, 2005). Developing countries have the added burdens of unsafe water, lack of sanitation and poor hygiene (WHO, 2012). Some piped water systems in these countries only work for short periods during the day or provide unsafe water (Bartram and Cairncross, 2010).

Developing countries have many people suffering from malnutrition and are therefore more susceptible to gastrointestinal diseases. It has been proposed that improving sanitation and providing safe water are the least expensive ways to save lives and to improve public health (Montgomery and Elimelech, 2007).

Studies have shown that improving the quantity and quality of water helps prevent diarrhoeal diseases (Esrey et al., 1991; Fewtrell et al., 2005; Clasen et al., 2006). Hygiene not only plays an important role in preventing gastrointestinal diseases, but can also decrease respiratory and skin infections (Esrey et al., 1991). Gastrointestinal diseases being referred to here and are associated with waterborne pathogens, causing symptoms such as diarrhoea and vomiting. The young - 90% diarrhoeal deaths are from children under 5 years (WHO and UNICEF, 2005) - and elderly are at greater risk of waterborne diseases, especially in unsanitary conditions (WHO, 2011). Thus water treatment such as boiling is necessary.

In 2008, 78% of South Africa's rural population had access to improved water sources, while access in the urban population was estimated at 99% (UNICEF and WHO, 2010). In 2011, 91.2% of the population had access to tap water (Statistics South Africa, 2012b), with 46.3% coming from household taps and 6.2% coming from a community tap farther than 200 metres from their home (Statistics South Africa, 2012b). The vast improvement in access to drinking water, the introduction of the Blue Drop certificate by the Department of Water Affairs (DWA) and immunisation against the rotavirus have led to a decline in the number of children younger than 5 years suffering from diarrhoeal diseases (HST, 2011; Health Systems Trust, 2012). But diarrhoea still ranks among the top four causes of disability affected life years (DALYs) in South Africa (HST, 2012). DALYs are the sum of the potential life years lost due to premature death or the productive lives lost due to disability (Rushby and Hanson, 2001; Guerrant et al., 2002).

The number of South Africans dying from intestinal infectious diseases rose by 61.8% between 2000 and 2007 (Stats SA, 2011a; HST, 2012). The overall increase in deaths from intestinal infections is partially related to the unreliability of safe drinking water supply.

The number of immunocompromised citizens has increased by approximately 15% over the same time period (Stats SA, 2011b). So despite increased access to safe drinking water, citizens are still at high risk of mortality from intestinal diseases, especially *Campylobacter jejuni* (Obi et al., 2003; Luyt et al., 2012). With interruptions in water supply citizens are turning to alternate sources like springs, streams and rivers for domestic use due to interruptions, while a continuous supply of safe water is required (Laurent, 2005).

The public health risk posed by this water is measured by the concentration of indicator bacteria including thermotolerant/faecal coliforms and *Escherichia coli* (*E. coli*) (SABS, 2006). Raw water usually needs minimal treatment to meet public health requirements. Treatments include boiling, adding bleach or sand filtration (Murray et al., 2004). However the cost of bleach and the time and cost of boiling result in them being neglected (Monyai, 2004). Thus the risk of waterborne disease outbreaks, especially from hepatitis E and cholera, is elevated (Grabow et al., 1994; Dalsgaard et al., 2001; Paulse et al., 2009). This demonstrates the importance of microbial monitoring of surface water in South Africa as a tool for prevention of public health outbreaks.

Access to safe water sources without sanitation facilities greatly hinders the prevention of waterborne diseases. The MDG goal for sanitation still needs to be achieved. Currently, faeces could contaminate once-safe wells under flood or by runoff. Out of the South African population 5.2% has no access to toilets, while 12.7% of the population in the Eastern Cape still lack toilets (Statistics South Africa, 2012b). The use of pit latrines has decreased to 28.1% of the South African population (Statistics South Africa, 2012b). Water borne disease can be contracted by poor hygiene and direct contact with faeces, making sanitation an important factor in eliminating waterborne disease. Despite the progress we have made with the MDGs, sanitation still falls far short of the target (United Nations, 2012).

1.6 MONITORING OF MICROBIAL WATER QUALITY

1.6.1 Indicator Bacteria

Indicator bacteria are used for routine water quality assessment as a surrogate for pathogen testing. Pathogens are often found to be present in minute quantities in the environment. They thus require efficient concentration steps in order to isolate them and complex media owing to their specific nutrient requirements to culture them (Fleisher, 1990; Grabow, 1996; Rompré et al., 2002). Pathogens are prohibitively expensive to identify by means of molecular methods, and interpretation of results are sometimes a problem, while culturing requires extended time periods (Sartory and Watkins, 1998; Taguchi et al., 2005; Field and Samadpour, 2007; Bosch et al., 2008). Indicators are therefore used to denote potential pollution instead (Rompré et al., 2002).

An ideal indicator organism has been defined as an organism that mimics the presence of a pathogen by being present when the pathogen is present and absent when it is not.
They should have a similar survival rate in similar environmental conditions to the pathogens they represent (Genthe and Franck, 1999; Tandlich and Muller, 2008). The indicator should be simply and cost effectively detected, while being a member of the warm-blooded intestinal micro-flora (Grabow, 1996; Sartory and Watkins, 1998). South Africa uses total coliforms (TC), faecal coliforms (FC), *E. coli*, enterococci/faecal streptococci (FS) and heterotrophic plate count (HPC; Tandlich and Muller, 2008) as some of its indicators. The presence of indicators above regulatory levels suggests inadequate treatment or potential faecal pollution with possible health risks associated (DWAF, 1996c; Grabow, 1996; Grabow, 2001; SABS, 2006).

The indicator bacteria mostly come from the Enterobacteriaceae family, of which the total coliform group is a subset consisting of *Escherichia, Citrobacter*, *Enterobacter* and *Klebsiella* (Rompré et al., 2002; José Figueras and Borrego, 2010). The coliform group mostly inhabits human and animal intestinal tracts, and are aerobic and facultative anaerobic, Gram negative, non-spore forming, rod-shaped bacteria which produce gas on lactose fermentation at 35°C within 48 hours of incubation (Rompré et al., 2002; Madigan and Martinko, 2006). Coliforms tend to survive longer than some pathogens (Madigan and Martinko, 2006).

However, their presence is an indicator of potential faecal pollution in water bodies or of regrowth, due to the ineffective treatment of distribution systems (Rompré et al., 2002; José Figueras and Borrego, 2010). Total coliforms can regrow on many surfaces (pipe walls, storage bottles and in biofilms) and within drinking water distribution systems (LeChevallier et al., 1996; Cunliffe et al., 2003; Sakyi et al., 2012). Physicochemical conditions affect their concentration (Fujioka et al., 1998). Biofilm formation in storage containers, which resist chlorine disinfection (LeChevallier et al., 1987) and growth in eutrophic conditions may produce false alerts (Jagals et al., 2003). High concentrations of heterotrophic bacteria may also prevent their growth on certain media, leading to false low concentrations (LeChevallier and McFeters, 1985; Pletschke et al., 2006).

Faecal coliforms are thermotolerant and grow at 44.5°C (DWAF, 1996c). The FC group contains mainly *E. coli*, but non-faecal bacteria such as *Klebsiella, Enterobacter* and *Citrobacter* have been isolated from faecal coliform media (Schraft and Watterworth, 2005). *Escherichia coli*, which has been shown to be more reliable than total coliforms (APHA, 1998; Murray et al., 2004). They are easily cultured on media (Grabow, 1996).
However *E. coli* cannot be used to identify the source of faecal pollution, as they are found in animal, human and environmental sources (APHA, 1992). *E. coli* is far more useful in tropical environments when compared to other coliforms, which tend to multiply as a result of high temperatures (Byamukama et al., 2000; Mara and Horan, 2003; Long et al., 2005; Mosley and Sharp, 2005). *Escherichia coli* has only been reported to grow in tropical river water (Solo-Gabriele et al., 2000) and not to originate from non-faecal sources (Tallon et al., 2005), while some strains may be capable of growing in soil environments (Topp et al., 2003; Ishii and Sadowsky, 2008). *E. coli* is considered an independent, as it is less likely from non-faecal sources, as 95% of thermotolerant coliforms excreted in animal faeces are accounted for by *E. coli* (Mara and Horan, 2003).

Microbial quantification and identification can be determined using multiple methods e.g. (a) culture-based, (b) enzymatic and (c) molecular methods (Venter et al., 1998; de Boer and Beumer, 1999; Venter, 2000; Rompré et al., 2002). Molecular and enzyme methods have become more sensitive and sample preparation methods less cumbersome and are continuously improved. But the cost of molecular and enzymatic methods is still great without any major advantages over culture methods (Luyt et al., 2012).

1.6.2 Culture Based Methods

Culture based methods require the growth of the micro-organism in/on a medium. The medium requires sufficient nutrients for growth, while selecting and differentiating organisms by using additional additives or nutrient exclusion. A wide arrange of available media formulations are found in the "Handbook of Microbiological Media" (Atlas, 2010). Culture based methods are also limited by the biological functions of the organisms.
Thus methods for culturing fungi, bacteria and viruses differ. The cultivation environment is also dictated by the organism's sensitivity to elements such as oxygen, thus requiring either anaerobic or aerobic conditions. The main culture based methods for bacteria are spread-plating, pour plating, membrane filtration and the most probable number (MPN) technique. Samples are either concentrated using membrane filtration or are diluted directly in saline or concentrated via the MPN technique.

Direct plating involves spread-plating a 100µL aliquot of sample onto solidified agar plates. These plates are then incubated at the appropriate temperature for the required period. Pour plating is also used, where a 1 mL aliquot of sample is mixed with molten agar at 45 °C and allowed to solidify (Maier et al., 2000). Spread plating is advantageous as it allows the morphology of the colony to be visualised. Membrane filtration (0.45 or 0.22 µm pore size filter paper) allows samples with low bacterial concentrations to be enumerated by concentrating large volumes on a membrane filter which nutrients are able to permeate.

The MPN method is statistical, using dilutions to identify approximate concentrations of bacteria within a sample. The MPN method uses dilutions of samples by factors of ten until extinction (Mara and Horan, 2003). Dilutions are based on previous experience and results are interpreted using MPN tables. Five replicates per dilution are most commonly used, but 3, 10 and 12 are also used (Mara and Horan, 2003). It estimates the concentration of bacteria present statistically by binomial distribution (Mara and Horan, 2003). The accuracy of the test depends on the number of replicate tubes used, with the results working on a Poisson distribution (APHA, 1998; Sartory and Watkins, 1998). Precision is improved by increasing the number of replicates per dilution and by using microplates with a higher number of replicates (Mara and Horan, 2003). Direct plating and counting tends to yield more reliable results due to large confidence intervals using 5 replicates (Mara and Horan, 2003).

Disadvantages to the membrane filtration technique include the small volume sizes and labour intensity.

Coliforms are enumerated after 18-24 hours on different selective agars, with temperatures varying from 35 to 37°C (Brenner et al., 1993; Grabow, 1996; Van Poucke and Nelis, 2000; Farnleitner et al., 2001). MacConkey and m-Endo agars were traditionally used to identify coliforms (Grabow and du Preez, 1979; Grabow et al., 1981; McFeters et al., 1982; Rompré et al., 2002).

The enumeration of coliforms on lauryl sulphate media is affected by high concentrations of heterotrophic bacteria (WHO, 2003) The MPN method used to enumerate coliforms consist of three parts: the presumptive, the confirmative and the completed tests (APHA, 1992). The presumptive test uses lauryl sulphate broth (A1 Media) with possible additives to increase selectivity (APHA, 1992). One MPN techniques is alternatively known as the multiple tube fermentation technique (MTF) due to its positive result arising due to fermentation. Coliforms ferment lactose, producing acid and gas, visualised as bubbles in inverted Durham tubes within the media (APHA, 1992; APHA, 1998). Positive tests need to be confirmed for thermotolerant coliforms using an indole test (APHA, 1998), with a total analysis time ranging between 48 and 96 hours (Grabow, 1996; Geissler et al., 2000; Rompré et al., 2002).

Thermotolerant coliforms are grown on m-FC agar at 44.5 ± 0.5°C and are presumed faecal coliforms (Grabow, 1996). *Escherichia coli* can be confirmed by indole production in 1% tryptone broth using 4-(Dimethylamino)benzaldehyde with n-butanol in concentrated hydrochloric acid (Kovac's reagent) (Smith and Rockliff, 1982; Sartory and Watkins, 1998; Kamel, 2006). But the use of fluorogenic (hydrolysis to form a fluorophore/ flourochrome, e.g. HiCrome™ ECD Agar with MUG for microbiology (Sigma Aldrich) and chromogenic (hydrolysis to form a colour for instance Chromocult agar (Merck, Darmstadt, Germany) and CM1046 (Oxoid, Hampshire, England)) media can eliminate the confirmation step (Sartory and Watkins, 1998; Geissler et al., 2000).

The Colilert®18 system, an MPN technique requiring 18 hours incubation at 35°C, is used for *E. coli* enumeration (Chao et al., 2004; Murray et al., 2004). It is therefore a rapid, sensitive detection method for faecal coliforms, with sensitivity of 0 CFUs/ 100 mL (Chao et al., 2004) and a shorter incubation period than the usual 24 to 48 hour period (DWAF, 1996c; Tandlich

and Muller, 2008). This shorter incubation time is an advantage for public health decisions. Colilert™ was compared to the m-FC membrane filtration system in 1996, using 50 samples (du Preez and Genthe, 1996).

In the 1996 comparison, the Colilert™ had a false positive rate of 7.2% and a false negative of 12.5% for *E. coli* (du Preez and Genthe, 1996). False positives are undesired positive reactions to the presence of non-target organisms. False negatives refer to results that lack a positive reaction when the target organism is in fact present in the sample (Sobsey and Pfaender, 2002; Luyt et al., 2012). The Colilert™ system had a higher recovery rate (96%) compared to m-FC agar (80%), using the membrane filtration method (du Preez and Genthe, 1996).

A comparison between Colilert™ and lauryl sulphate broth membrane filtration was conducted in 2000 by Sundram et al (2000) using 318 samples, 250 of which were treated water (Sundram et al., 2000). The Colilert™ system produced 65 positives versus the membrane system's 63 for *E. coli*, which was not statistically different. But the Colilert system is more prone to false positives (Sundram et al., 2000). The South African National Ring trial of the Colilert™ system was conducted on 602 water samples in 7 accredited laboratories, where it was shown to be user friendly (Jackson et al., 2002).
The Colilert™ system most closely correlated to the membrane filtration method on lauryl sulphate broth, with a 4.23% difference (Jackson et al., 2002). Chromocult (Merck, South Africa) had a relative difference to the Colilert™ system of 15.73% (Jackson et al., 2002). m-FC had the highest relative difference (62.37%) to the Colilert™ system (Jackson et al., 2002).

The Colilert™ and Colilert®-18 systems have similar methods, but differ in the incubation period for enumeration. The Colilert™ system is incubated for 24 hours (IDEXX Laboratories Inc., 2001; Jackson et al., 2002). The South African data was generated for the Colilert™ system, but should be transferrable to the Colilert® 18 system which is used in international studies (Luyt et al., 2012). From international literature, the false positive rates for the Colilert®-18 system were 7.4% and false negatives 3.5% under subtropical conditions (Chao et al., 2004). Tropical freshwater showed a 36.4% rate of false positives, while these rates were equal to 27.3% in a Florida study (Pisciotta et al., 2002). The Colilert™ system

correctly identified 51.4 - 56.8% of the *E. coli* strains tested, which included samples from the South African Institute for Medical Research (Maheux et al., 2008). Maheux et al. (2008) and Martins et al. (1993) showed the Colilert^{TM} system has the lowest ability to detect *β*-glucuronidase activity. So the Colilert^{TM} system is susceptible to false positives and negative results (du Preez and Genthe, 1996; Maheux et al., 2008).

The type of water may affect the rate of false reports, as well as the media composition which Martins et al highlighted as possible missing an ingredient to improve growth and the incubation temperature (Luyt et al., 2012).

The Colilert^{TM} system and Colilert 18 MPN results could be one order of magnitude higher than the membrane filtrations technique (Cho et al., 2010). This possibly explains the higher *E. coli* recoveries and concentrations in the Colilert system to those found in the membrane filtration method when tested in South Africa (Luyt et al., 2012). The high number of false positives and negatives resulting from the Colilert system may lead to public health warnings being overemphasised, due to overestimated *E. coli* concentrations in surface water (Luyt et al., 2012). This could lead to government resources being used unnecessarily on non-existent problems.

Alternatively, the culturing technique seems to be sensitive and fairly selective (Lazcka et al., 2007). The media is user-friendly, requiring minimal training for effective use and, beneficially, requires little preparation time (Jackson et al., 2002). The cost of the media, however, is still high, increasing routine analysis costs (Jackson et al., 2002) in an already-budget constrained environment. The membrane filtration method currently used is 5.63 times cheaper than the Colilert^{TM} system (Sundram et al., 2000). Using an expensive test may deplete economic resources which could be better used to monitor more sites (Luyt et al., 2012).

Another drawback is the inability of *E. coli* alone to identify the source of contamination. Identifying the source of contamination would help with prioritisation and remediation of waterborne disease outbreaks, while identifying the risk of the pollution (Luyt et al., 2012). As stated above the source of pollution influences its risk to human health.

The use of presence/absence techniques is useful to low-literate users and serves as an early warning system. The hydrogen sulphide test (H_2S test) uses this presence /absence technique with a visual colour change from yellow to black (Sobsey and Pfaender, 2002). A positive result is visualised by the formation of an iron sulphide precipitate which causes the medium to turn black (Genthe and Jagals, 2003). The precipitate is due to a reaction between hydrogen sulphide and ferric ammonium citrate (Venkobachar et al., 1994; Genthe and Franck, 1999; Sobsey and Pfaender, 2002; Hirulkar and Tambekar, 2006; McMahan et al., 2012).

The hydrogen sulphide strip test (H_2S strip test) was designed to be used in rural settings at ambient temperature (25°C), thus an incubator is not required, and requires minimally trained people (Genthe and Franck, 1999; Genthe and Jagals, 2003). The H_2S strip test has had many different incarnations, and no version is standard (Sobsey and Pfaender, 2002). Microbes, belonging to the Enterobacteriaceae group, produce hydrogen sulphide by the reduction of sodium thiosulphate (Sobsey and Pfaender, 2002). The method has been used in other countries like Bangladesh (Gupta et al., 2008). It is well-suited to tropical and temperate areas (Sobsey and Pfaender, 2002).

The H_2S producing bacteria associated with faecal contamination are members of the Enterobacteriaceae group and include: *Aeromonas spp.*(species); *Citrobacter spp.* (Ratto et al., 1989)*; Clostridium spp.* (Castillo et al., 1994; Sobsey and Pfaender, 2002)*; Enterobacter spp.*(Castillo et al., 1994)*; Escherichia spp.*(Sobsey and Pfaender, 2002)*; Klebsiella spp.*(Manja et al., 1982)*; Proteus spp.* (Manja et al., 1982; Nagaraju and Sastri, 1999) and *Salmonella spp*. (Manja et al., 1982; Castillo et al., 1994; Mosley and Sharp, 2005). The H_2S producing bacteria overlap with the faecal coliforms. The incubation time is up to 72 hours at room temperature (between 18 and 22°C) (Sobsey and Pfaender, 2002). There are a number of different versions worldwide, with one method standardised in South Africa. The H_2S strip test costs 10.00 ZAR or less per sample including expenses such as transportation and labour costs (1.32 USD or less). This test is far cheaper than culturing or enzymatic methods (Sobsey and Pfaender, 2002; Hirulkar and Tambekar, 2006; Luyt et al., 2011a). It does not require highly trained technicians to perform the test which can be done on site with no incubation equipment required (Sobsey and Pfaender, 2002; Genthe and Jagals, 2003); see Chapter 4. One drawback is that the coliforms are not specific to human pollution as some of the microbes are found in the environment (Sobsey and Pfaender, 2002). The DWA Blue

Drop certifications say the H_2S test can be used when no other methods are possible, but positive results must be confirmed with Colilert^{TM} (DWAF, 2009). False positives are less likely to occur, however *E. coli* does not cause a positive result. The reliable detection limit is 2 cells/100 mLs, however detection to 0 cells/100 mL is possible.

None of these culturing methods have overcome the problems of bacteria going into viable, non-culturable bacteria (VBNC) states (Barer and Harwood, 1999). Stressed bacteria grow slowly or fail to grow as a result of inhibitory compounds or microorganisms present in the media (Rompré et al., 2002). Incubation times could lead to water being used before its microbial quality has been established. But these problems could only be resolved through enzymatic or molecular techniques. Molecular techniques are far more costly than culturing methods in terms of material.

It was estimated in 2001 that the time for identification and preparation would make PCR cheaper than traditional methods (480 ZAR as opposed to 660 ZAR) of identifying pathogens, but an estimate of the cost of indicator bacteria enumeration in 2003 was cheaper, ranging between 90 ZAR and 130 ZAR.

1.6.3 Regulatory Authorities in South Africa

DWAF guidelines (DWAF, 1996c), WHO and SANS guidelines (SABS, 2006; World Health Organization, 2011) are used to identify the water quality. Compliance with the DWAF guidelines for domestic drinking water require 0 CFUs/100 mL Faecal coliforms (FC), less than 5 CFUs (Colony forming units)/100 mL total coliforms (TC), and less than 100 CFUs/mL heterotrophic bacteria (HPC). WHO guidelines require 0 CFUs/100 mL *Escherichia coli* (*E. coli*) or thermotolerant coliforms (WHO, 2011). The SANS 241: 2006 states that *E. coli* or thermotolerant coliforms should be below 1 CFUs/100 mL in 95% of samples and a maximum of 10 thermotolerant coliform colonies in 100 mL in 1% of samples (South African Bureau of Standards, 2006).

The Agricultural water guidelines by the DWAF state that water containing 1 *E. coli* CFUs/100 mL can be used for all irrigation purposes, while up to 1000 CFUs/100 mL can be used on crops not eaten raw (DWAF, 1996b). Faecal coliform concentration greater than 1000 CFUs/100 mL should only be used for non-edible plants

e.g. tree plantations, and should not come into contact with humans (DWAF, 1996b). Recreational guidelines state that between 0 and 130 CFUs/100 mL is likely to have gastrointestinal effects in full contact, e.g. swimming, while this range is extended to 1000 CFUs/100 mL in water skiing or wind surfing when experienced people are involved. The enterococci range is 0 to 30 CFUs/100 mL for negligible effects and up to 60 CFUs/100 mL being a slight risk with negligible exposure. Above 100 CFUs/100 mL is considered a risk especially if the water is ingested (DWAF, 1996d).

1.6.4 The Distribution System Water Monitoring in South Africa

Water boards and certain testing facilities in South Africa use Colilert as a rapid indicator of water quality. Monitoring distribution systems is conducted by water boards, municipal laboratories and DWA approved or ISO/IEC 17025: 2005 accredited laboratories (DWAF, 2009; DWAF, 2011). There are approximately 58 SANS accredited laboratories in South Africa and only one in the Eastern Cape (Balfour et al., 2011). The current monitoring system requires these organs to report to the Department of Water Affairs.

Water quality is tested using *E. coli* and total coliforms as indicator bacteria, but HPC is also useful to identify treatment efficiencies (DWAF, 2009). DWAF guidelines allow for a maximum of 100 heterotrophic bacteria (HPC) per millilitre, 5 colony forming units (CFUs) per 100 mL of total coliforms and no faecal coliforms per 100 mL (DWAF, 1996c). Tap water is required to meet, amongst others, the Department of Water Affairs and Forestry (DWAF, 1996c), WHO and SANS 241 standards to be acceptable for drinking. Municipalities are required to test water on a monthly basis. An incentive system, called the Blue Drop system, was developed to encourage municipal compliance with drinking water regulations (DWAF, 2009). Implemented in 2009 (DWAF, 2009), the Blue Drop score is calculated based on the following requirements: a water safety plan (A water safety plan is a risk assessment plan and procedures to facilitate safety of the water from catchment to tap (DWAF, 2011)) must be in place; maintenance and running of the system; results must be published; results must be submitted monthly to the DWA; how well the results comply with the SANS 241 requirements and the incident management protocol to solve system failures (DWAF, 2009). But the Blue Drop score does not directly correlate to the microbial safety of the water. Pipe breaks lead to increased microbial risks (Allen et al., 2004; Nygård et al., 2007; Luyt et al., 2011a; Chowdhury, 2012) and old infrastructures are prone to pipe breaks.

Pipe breaks often allow soil to enter the distribution system, affecting water's taste, colour and odour. These characteristics play a large role in the public's perception of water quality (DWAF, 2009; Wright et al., 2012a).

If no water is available from the pipe distribution system, or if the public does not trust the water provided, they start using alternatives like bottled, spring, borehole or rain water. Commercially available bottled water has been shown to be safe in one South African study (Ehlers et al., 2004). Other countries studies have shown that bottled water does not comply with their water quality regulations (Scoaris et al., 2008; Zamberlan da Silva et al., 2008).

1.6.5 Rainwater

Rainwater harvesting (RWH) involves the collection of, the storage from roof tops and land catchments and the subsequent use of rainwater for potable and non-potable purposes (Kahinda et al., 2007; Sharma, 2007). Domestic rainwater harvesting refers to collection from roof tops, yards and hard surfaces for storage in onsite tanks being used for domestic purposes such as drinking and irrigation during municipal interruptions and drought periods (SOPAC, 2007; Kahinda et al., 2007; Sharma, 2007).

Buildings often used for domestic rainwater harvesting (DRWH) include houses, community centres and schools (Salukazana et al., 2005). Rainwater is considered a potable water source in the rural areas of developing countries, though its actual water quality has not been tested (Amin and Han, 2011) and should be established before water is drunk (Tandlich et al., 2011). There are doubts as to whether it meets the countries' drinking water guidelines (Amin and Han, 2011). Studies have shown DRWH is often not suitable for drinking without treatment (Amin and Han, 2011). It does provide a source of gardening watering, toilet flushing, house cleaning, similar to grey water. It should be noted that there may be risks associated with human contact in the houses, thus gardening on vegetation which will not be consumed without cooking is the safest use. This raw water should at least be sand filtered, boiled or have bleach added before ingestion (Murray et al., 2004) in order to minimise health risks. Household level treatment is expensive and time consuming and is thus not often practised (Monyai, 2004). General costs of treating rainwater are high, but rainwater tanks have been used on farms, at teaching institutions and in rural areas for the poor (Mwenge Kahinda and Taigbenu, 2011).

If rainwater and bottled or tapped water are unavailable, such as in the case of rural areas, then river, spring water is used.

1.7 SOUTH AFRICAN MONITORING OF RIVERS

South Africa currently has 19 Water Management Areas (WMAs) (Murray et al., 2004). It has been proposed to decrease this to 9 WMAs (Nkondo et al., 2012). The responsibility of microbial monitoring lies across various levels within government departments.

The National Health Act (NHA) places the responsibility with district and local municipalities as these institutions include environmental pollution and water monitoring amongst their services (NHA, 2008). While the National Water Act (NWA, 1998; Chapter 14, part 1, Paragraph 137) states that the Minister of Water and Environmental Affairs (changed from the Minister of Water Affairs and Forestry in April 2009) is responsible for establishing water monitoring systems of microbial surface water and for appointing a national coordinator and developing a central database (Murray et al., 2004; DWA, 2010), the National Monitoring Programme (NMMP) developed for surface water was based on research by du Preez et al. (1999; 2001; 2002).

The South African National Monitoring Programme (NMMP) for surface water is the microbial water quality monitoring programme for areas highly prone to faecal contamination and uses pH, turbidity and *E. coli* concentrations as indicators (Murray et al., 2004). It is beneficially based on long term research (Portfolio Committee on Human Settlements, 2010). Resultant data can be used to match the DWA and National Department of Human Settlements activities, which are responsible to the National Department of Health for sanitation, in turn responsible for public health, which is affected by the actions of the WMA (Luyt et al., 2012). It can also be used to identify the impact of service delivery in the water and sanitation arenas on public health.

The system needs catchment management forums to succeed (Munnik et al., 2011). Catchment management forums are non-statutory bodies which are part of the catchment management approach, involving participation from all stakeholders in water resource management, to ensure all views are understood (Harpe et al., 2006). Each WMA has a regional coordinator, employed by the DWA, and is responsible for sample collection and

analysis on a biweekly basis, with reports produced bimonthly (Murray et al., 2004; DWA, 2010).

The NMMP has established a publicly accessible, centralised data collection for microbial water quality, which has bimonthly reports allowing identification of surface water quality trends (DWA, 2012a).

South Africa has 278 tertiary catchments, posing a logistical problem. Prioritisation is the solution to assess faecal contamination, especially when considered within the country's financial constraints (DWAF, 2000). One prioritisation criterion is the use to which land in the vicinity of the water source is put, e.g. the type of settlements and agricultural activities nearby, including livestock watering (Luyt et al., 2012). The second criterion is the nature of the population which may interact with or use the water source (Murray et al., 2004; Luyt et al., 2012).

Monitoring based on *E. coli* concentrations takes into account the majority of faecal microbes excreted from warm-blooded animals, cattle and humans (Medema et al., 2003; Murray et al., 2004; Luyt et al., 2012). But *E. coli* cannot be used to distinguish between pollution originating from humans and animals, as it is found in both groups. *E. coli* concentrations have been related to gastroenteritis occurrences resulting from contact with contaminated water and by drinking water with *E. coli* (Prüss, 1998). Soil, with negligible concentrations, is the only possible environmental contributor, making *E. coli* a good indicator bacteria (Hardina and Fujioka, 1991; Desmarais et al., 2002). The method of choice for enumeration of *E. coli* is Colilert -18 (Murray et al., 2004).

A standardised, cost-effective source tracking tool is required to monitor rivers effectively. Up to 30% of surface water resources are not monitored regularly (Rivett et al., 2009).

1.8 FAECAL SOURCE TRACKING

Faecal source tracking is divided into microbial and chemical. The microbial source tracking methods consist of two groups. The first is the library-dependent method relying on a database of antibiotic profiles and the phenotypes or genotypes of bacteria groups (Simpson et al., 2002; Seurinck et al., 2005; Ahmed et al., 2007; Field and Samadpour, 2007; Mott and Smith, 2011).

Second are the library- independent methods, such as source-specific bacteria (*Bifidobacterium, Rhodococcus, Bacteroidales*) , bacteriophages and viruses (Simpson et al., 2002; Noble et al., 2003; Field and Samadpour, 2007; Stoeckel and Harwood, 2007; Lee et al., 2011; Wuertz et al., 2011). Source tracking using enteric pathogens seems reliable, but there are limitations to these methods, other than their expense (Blanch et al., 2006; Ahmed, 2007; Wonga et al., 2012).

1.8.1 Viral Methods

1.8.1 1 Bacteriophage Methods

Bacteriophages are a group of viruses which can infect bacteria (Grabow, 2001). Coliphages are a sub-group. These infect *E. coli* and some other host bacterial species (Hagedorn et al., 2011).

Bacteriophages are constantly excreted by humans and other endothermic animals (Grabow, 2001). The main two investigated groups are coliphages infecting *E. coli* and closely-related bacteria, and *Bacteroides fragilis* HSP40 bacteriophages (Grabow, 2001).

Male specific or F- specific bacteriophages infect their hosts through receptors on the F pilli of host bacteria (Hagedorn et al., 2011). Male specific F (Fimbriae) RNA (Ribonucleic Acid) Coliphages (F-RNA) bacteriophages are further subdivided into four genogroups (Hagedorn et al., 2011). F-RNA specific coliphages morphologically resemble enteric viruses such as Astroviruses, Caliciviruses, Enteroviruses, and Hepatitis A and E (Hagedorn et al., 2011) and are thus better indicators of viruses than bacteria indicators (Grabow, 2001).

Bacteriophages cannot multiply outside host cells and need a temperature of at least 30°C (32 - 40°C) for multiplication, limiting the likelihood of them replicating in surface waters, especially in South Africa (Grabow et al., 1998; Grabow, 2001). F pilli development in *E. coli* is temperature dependent and will not occur below 25°C, thus preventing F-RNA from multiplying in the water environment (Hagedorn et al., 2011).

Viruses are known to be affected by sunlight and temperature, however their survival does not seem to be majorly affected by sunlight, but by different environmental conditions (Bettarel et al., 2009). The lack of replication of the phage may be due to stress from the activation of the SOS response and release of lysogenic strains.

The release of faecal coliform or other enteric bacteria into the environment will cause environmental, low nutrient and heat shock stress. This stress may activate the SOS repair response (Janion, 2001), while starvation of bacteria has been reported to initiate the SOS response (Janion, 2001). The lack of glucose which has been shown to be low in river waters can initiate the SOS response with starvation (Janion, 2001). The exposure to ultraviolet radiation from the sunlight could also lead to DNA damage, which has been shown to cause DNA damage. The activation due to sunlight may take about 24 hours and longer to deregulate in more sunlight damaged bacteria compared to those less sunlight damaged (Sinton et al., 2002). The damage of DNA or stalling of replication is the usual signal for the SOS response to occur (Madigan and Martinko, 2006). The SOS response is the process initiated to repair the DNA through a number of processes (Madigan and Martinko, 2006). The SOS response uses different polymerases and can lead to multiple mutations as it is not done according to a template (Madigan and Martinko, 2006).

The initiation of the SOS response could slow down the metabolism of the cell for survival. The bacterium will be the host for bacteriophages. Thus stress or the SOS response being initiated in the bacterial host will be detrimental to the phages in the host. Thus it would limit the likely hood of replication occur in South African rivers. The SOS response has been reported to be activated by some lytic phages in *Salmonella enterica* (Campoy et al., 2006) and previously irradiated phage (Janion, 2001). The SOS response has been reported to occur in *E. coli* due to mutant filamentous phage (Higashitani et al., 1992). This may be a defence mechanism of the host bacterium to prevent infection with heteroimmune lytic phages (Campoy et al., 2006). The activation of the SOS response would increase the die-off of the

phage and the survival of the phage. This would also call into question its use as a faecal source tracking tool. The temperature of South African river water is never close to 30°C, thus replication of the phage when the host bacterium is outside of its host is unlikely, due to the damage of the host bacterium. During the SOS repair process lytic phages would likely lyse themselves from the host cell or may be cut out of the DNA by the repair process which would also decrease the survival and increase the unlikely occurrence of replication.

1.8.1.2 Somatic Coliphages

Somatic coliphages infect *E. coli* and are closely related Enterobacteriaceae, interacting with their cell wall attachment sites, which are always present. Somatic phages can attach to dead bacteria, and cause lysis of the bacterial cell due to their lytic nature (Grabow, 2001).

These somatic phages include families of *Microviridae, Myoviridae, Podoviridae and Siphoviridae* and can contain DNA or RNA (Grabow, 2001). Some of these somatic coliphages replicate in aquatic environments and are detectable by inexpensive equipment and rapid plaque assays using large volume direct plaque assays or qualitative presence/ absence (P-A) techniques (Grabow et al., 1998; Green et al., 2000; Grabow, 2001).

The problems are the lack of technical staff and the dirge of technology to practise these techniques (Murray et al., 2004). Instead, most of the research on source identification in South Africa has used viral biomarkers, which are more difficult to isolate (Grabow et al., 1998; Sundram et al., 2006; Momba et al., 2009b).

1.8.1.3 Male Specific F-RNA Coliphages

F-RNA coliphages are the simplest single-stranded Ribonucleic acid (ss-RNA) phages with the viral receptor at the shaft F-fimbriae (fertility) which interacts with the host's fertility (sex) fimbriae and infect *E. coli* and the closely related Enterobacteriaceae (Grabow et al., 1998; Grabow, 2001). F-DNA coliphages have their viral receptor at the tip of the F-fimbriae shaft and so have the potential to be used for source tracking, as they are excreted simultaneously with F-RNA coliphages by humans (Grabow, 2001; Chao, 2006). It should also be noted that the F-DNA coliphages may not be able to infect the host strains, (for example *E. coli* K-12 and *Salmonella typhimurium* WG49), used in isolating them. Despite this, they have not been used in South Africa (Luyt et al., 2012).

F-RNA coliphages can be separated by serotyping and genotyping into 4 distinct groups named I, II, III and IV (Grabow, 2001). Groups I and IV originate primarily from animals while groups II and III have been associated with humans (Schaper et al., 2002b; Sundram et al., 2006). The problem is the overlapping of the F-RNA coliphage serotypes/genotypes found in swine, animal and human faeces (Blanch et al., 2006; Momba et al., 2009b). Genotype I has been reported as being found in municipal sewage, possibly due to animal faeces contamination, but there could also be an unknown human group (Hagedorn et al., 2011). Making matters more difficult was the presence of all serotype/genotypes in wastewater treatment plant effluent and wastewaters from rural communities (Schaper et al., 2002b). Instances such as this increase the possibility of misidentifying contamination sources.

Studies have been conducted on hospital and slaughterhouse wastewaters as well as surface water pollution and samples of animal faeces (Hsu et al., 1995; Schaper et al., 2002b; Harwood et al., 2005; Sundram et al., 2006; Momba et al., 2009b; Hagedorn et al., 2011). Schaper et al. (2002b) studied the applicability of F-RNA coliphages.

Samples were collected in South Africa from Pretoria Academic hospital; Gauteng farms sewage from Zeekogat and Baviaanspoort wastewater treatment plants near Pretoria; rural communities such as Atteridgeville, Soshanguwe and Botshabello; abattoir wastewater and secondary effluent from Daspoort wastewater treatment plant (Schaper et al., 2002b). Schaper et al. (2002b) had human faecal samples with 10% containing F-RNA coliphages, while 70% of poultry farm animals faeces, 68% of pig faeces, 33% of cattle faeces had F-RNA coliphages. Out of the 10% of human faeces containing F-RNA coliphages, 90% were serotype/genotype II and 10% serotype/genotype III (Schaper et al., 2002b). All the cattle F-RNA coliphages were serotype/genotype I; with poultry being 80% serotype/genotype I and 20% serotype/ genotype IV (Schaper et al., 2002b). Swine faeces contained 53% serotype/genotype I, 28% serotype/genotype II and 19% serotype/ genotype IV (Schaper et al., 2002b). Sundram et al (2006) detected serotypes I, III and IV from wastewater associated with chicken farming in the Umgeni catchment. Serotypes II and IV were identified from the piggery (Sundram et al., 2006). Serotypes II and III have previously been identified in pigs, possibly due to human faecal contact when feeding (Hsu et al., 1995).

Knowing these groups work in a broad sense, do they work in the South African population? South African studies have shown human faecal samples, collected from individuals, to contain no more than 15% F-RNA coliphages (Grabow et al., 1995). Momba et al (2009b) showed that, in the Eastern Cape area of Alice, Dimbaza, Fort Beaufort and East London, the average F-RNA concentrations in treated wastewater ranged from 2.43×10^3 to 1.82×10^5 PFUs (Plaque Forming Units) /100 mL. The somatic coliphages average concentrations in treated wastewater were higher, ranging from 3.88×10^5 to 2.45×10^6 PFUs/100 mL (Momba et al., 2009b; Luyt et al., 2012).

Bacteriophages are not found in the faeces of the entire South African human population thus the lack of detection is not an indicator of no contamination (Luyt et al., 2012). This is also a problem with other microbial source tracking methods, while bifidobacteria seem to be more universally excreted.

Another possible reason for their lack of detection in rivers with faecal inputs may be their survival rate. Grabow et al. (1998) investigated the survival rate and the environmental fate of the above bacteriophages in comparison to indicator bacteria and enteric viruses and found them to be similar. Other survival studies have taken place on F-RNA bacteriophages have shown differences between survival rates (Brion et al., 2002; Schaper et al., 2002a; Allwood et al., 2003; Allwood et al., 2005; Hagedorn et al., 2011). Groups IV and III are the least resistant to extreme environmental conditions (e.g. chlorine, salinity, pH and temperature), while group I is the most resistant (Schaper et al., 2002a). Thus their lack of presence in rivers is not due to survival rates as they survive as well as enteric viruses and this would indicate that the water lacks viral pathogens.

Growth in enrichment media favours group I and decreases the representation of diversity in the sample (Stewart-Pullaro et al., 2006; Hagedorn et al., 2011). Enrichment media been reported as the most sensitive culture method (Scott et al., 2002). F-RNA coliphages were enumerated via the double-agar plaque assay using bacterial hosts of *Salmonella typhimurium* WG49 and *E. coli* HS(pFamp)R, which is reliable if the ISO method is followed carefully (ISO, 1995; Mooijman et al., 2002). *Salmonella typhimurium* WG49 hosts gave consistently higher counts of F-RNA coliphages than *E. coli* HS(pFamp)R in the Klip River in Gauteng (Grabow et al., 1998).

Somatic coliphages often out-number F-RNA coliphages by a factor of 5 in water environments, but resemble human viruses more closely (DWAF, 1996c; WHO, 2006). Their detection methods are more complex compared to recovery of somatic phages (DWAF, 1996c; World Health Organisation, 2006). Laboratory costs for this analysis and the effectiveness of viral recovery were cited as reasons this method would not be used for routine water testing (Chao, 2006).

1.8.1.4 *Bacteroides* Bacteriophages

The second group mentioned was the *Bacteroides fragilis* HSP40 bacteriophages. The bacterial *Bacteroides spp*. is Gram negative, non-spore forming and obligate anaerobes, and thus die quickly when exposed to oxygen and are unlikely to multiply in the environment (Allsop and Stickler, 1984; Allsop and Stickler, 1985; Grabow, 2001; Mara and Horan, 2003). No replication has been observed for *Bacteroides fragilis* HSP40 phage B40-8 in freshwater environments between 22 and 30°C (Grabow, 2001). Bacteriophages survive well in unfavourable environmental conditions (Tartera and Jofre, 1987; Tartera et al., 1988).

Bacteroides spp. have been found in both humans and animals, although the *Bacteroides fragilis* HSP40 bacteriophages has been identified as originating in humans only (Tartera et al., 1989; Sinton et al., 1998; Grabow, 2001). *Bacteroides fragilis* HSP40 excretion rate in human faeces is between 5 (Kai et al., 1985) and 10% (Tartera and Jofre, 1987) of the international population and unknown for the South African population (Luyt et al., 2012). In a South African study by Grabow et al. (1995), *Bacteroides fragilis* HSP40 was not isolated in the animal faeces sampled, and isolated in only 13% of the human faeces sampled, while 11% of the human samples in a French study were positive (Gantzer et al., 2002).
Thus it is not as useful as indicator bacteria which are excreted by the whole population or as coliphages excreted by a larger percentage of the population (Mara and Horan, 2003).

Four other *Bacteroides* somatic bacteriophages used in faecal source tracking are *Bacteroides tethaioataomicron* (GA17) used in Southern Europe, but not applicable in Great Britain; *Bacteroides ovatus* (GB124) applicable in Great Britain; *Bacteroides fragilis* (HB13) in Spain and Bacteroides spp., (HB73) in Hawaii (Hagedorn et al., 2011). These have been used for human source tracking (Hagedorn et al., 2011). Methods used to concentrate F-RNA bacteriophages can be used for *Bacteroides fragilis* (Hagedorn et al., 2011). *Bacteroides*

tethaioataomicron (GA17), *Bacteroides ovatus* (GB124) and *Bacteroides fragilis* HSP40 were all found in secondary effluents (Hagedorn et al., 2011). Bacteriophage infecting GA17 indicative of faecal sources, and are found in similar proportions to other bacteriophage indicators used (Blanch et al., 2006; Hagedorn et al., 2011). They have been identified in rivers with human faecal contamination (Hagedorn et al., 2011).

They have been detected in a study by Payan 2006 in 65% of the bathing sites tested with secondary effluent contamination with E. coli levels below 100 CFUs/100 mL and 53% of sites with 0 CFUs/100 mL (Blanch et al., 2006). Bacteriophages which infect GB124 have been detected in municipal wastewater and animal faeces, with higher concentrations in water contaminated with sewage effluent than sites where nonhuman pollution occurs (Hagedorn et al., 2011).

Isolation of the phage is important for monitoring. Literature has reported many problems with the use of *Bacteroides fragilis* HSP40 bacteriophages for routine monitoring; including the high variability in (MPN) phage Plague Forming Units (PFUs) (Grabow et al., 1998). The problem of maintaining a viable *Bacteroides fragilis* HSP40 inoculum can be overcome by the addition of oxyrase (*E. coli* isolated mono and dioxygenases which allow anaerobic cultivation under aerobic conditions) (Puig et al., 1999) to the tripe agar technique (Grabow et al., 1998; Puig et al., 1998). Grabow et al. (1998) proposed the use of the presence/absence test (DWAF, 1996c) to overcome these problems, but this is highly prone to contamination, preventing it from being used for monitoring. ISO methods are available for their enumeration and GA17 and GB124 (Hagedorn et al., 2011). A PCR method is more sensitive for identification of *Bacteroides fragilis* HSP40 than plaque assays (Grabow, 2001). These methods require skilled technicians as the enumeration technique requires anaerobic conditions to be strictly maintained, using complex media with antibiotics (Grabow, 2001; Murray et al., 2004). The scarcity of skilled technicians will prohibits use of the phage and viral methods as regular monitoring techniques (Grabow, 2001; Murray et al., 2004).

1.8.2. Bacterial Methods

Different bacteria have been used to identify the source of faecal pollution (Scott et al., 2002; Meays et al., 2004; Blanch et al., 2006; Savichtcheva and Okabe, 2006; Cimenti et al., 2007; Field and Samadpour, 2007; Lee et al., 2011). These methods are either library dependent or independent depending on the bacterial species used. Certain indicator organisms can only be used for tracking with reference to a database detailing differences between the animal and human organisms, using genetic structure, phenotypic or antibiotic resistance profiles (Scott et al., 2002; Moore et al., 2005; Anderson et al., 2006; Vantarakis et al., 2006; Field and Samadpour, 2007). Statistical methods, for example the ratio of faecal coliforms to faecal streptococci, have also been developed (Scott et al., 2002; Simpson et al., 2002; Meays et al., 2004).

1.8.2.1 *Escherichia coli*

Escherichia coli (E. coli) is an indicator organism currently used to identify faecal pollution in water (Scott et al., 2002; Murray et al., 2004). It is relatively easy to culture, but it could possibly grow in the environment under tropical conditions, rendering it less suitable under these conditions (Scott et al., 2002). *E. coli* accounts for a large proportion of the faecal coliforms excreted by faeces (Medema et al., 2003). *E. coli* is excreted by both warm blooded animals and humans (Ishii and Sadowsky, 2008), and so cannot be used as a sole indicator to distinguish contamination sources.

Phenotyping and genotyping allow sources to be identified. But these rely on a library or database listing the differences between the animal and human groups within a geographic area or water body. These methods are therefore known as library dependent methods (Griffith et al., 2003; Stoeckel et al., 2004). Databases are created with antibiotic resistance profiles in different animals and carbohydrate fermentation (Harwood et al., 2003; Stoeckel and Harwood, 2007). The NMMP has databases in each WMA of the isolated *E. coli* properties (Stoeckel and Harwood, 2007). These can be consulted to identify the faecal contamination source, using jack-knife analysis or other statistical approaches (Stoeckel and Harwood, 2007). The source will help establish the possible public health risk, as human faecal contamination is more likely to carry pathogens than animal faecal sources (Curtis et al., 2000; Scott et al., 2002).

Antibiotic resistance analysis, or profiling, works on the theory that normal gut flora are exposed to differing concentrations of different antibiotics and so develop different resistance patterns (Scott et al., 2002; Simpson et al., 2002). Identifying resistances in animal faeces and human faeces could thus help identify the source. Multiple animal sources can be identified through runoff analysis (Hagedorn et al., 2011). The techniques used to identify antibiotic resistance or sensitivity are the Kirby-Bauer antibiotic disk diffusion and replica plating methods (Hagedorn et al., 2011).

Antibiotic resistance profiles have been established for environmental samples through South African studies (Lin et al., 2004; Said et al., 2005; Doughari et al., 2011), but no profiles have been created for different animal groups or humans. Doughari et al (2011) researched the antibiotic resistance profiles of *E. coli* using the disc diffusion method.

Samples were collected from abattoir wastewaters, wastewater treatment plants and the Plankenberg River in Cape Town. The antibiotic resistance profiles were not linked to specific groups e.g. animal or human. Lin et al. (2004) studied the antibiotic resistance profiles of *E. coli* from the Mhlathuze River, KwaZulu-Natal. A correlation between the antibiotic resistance and disease has been established (Obi et al., 2007). Obi et al (2007) used the Kirby Bauer disk diffusion and polymerase chain reaction methods, using heat labile primers for amplification, to identify the enteric pathogens and their antibiotic resistances. The study isolated *Salmonella spp.*, *Shigella spp.* and *E. coli spp.* (Obi et al., 2007). The bacteria species were identified in the environment and from faeces, establishing its virulence and these organisms were isolated from patients both with and without diarrhoea (Obi et al., 2007). Diarrhoea amongst HIV positive patients may be due to decreased immunity. It does however provide a good antibiotic resistance profile for *E. coli* strains found in humans.

Problems associated with library dependent methods include geographic differences. This applies to both antibiotic resistance profiles and molecular profiles (Hagedorn et al., 2011). One such example was noted in Europe, where enterococci antibiotic resistance profiles were not representative of the entire area (Ebdon and Taylor, 2006) South Africa, as with other large areas, may require more than one library (Hartel et al., 2002; Wiggins et al., 2003).

Another problem is that molecular changes occur and antibiotic resistance profiles change over time (Gordon, 2001; Anderson et al., 2005), meaning libraries would require regular

monitoring and recalibration. This will hinder its use, as generating a library is expensive and time consuming. But they remain relatively easy and inexpensive to use when compared to other methods (Hagedorn et al., 2011).

Library independent methods do not require a library of phenotype or genotype difference between the animal and human groups. Instead they rely on bacteria to be specific to one group and not to be excreted by the other group, such as *Rhodococcus* which is only found in animal faeces (Oragui and Mara, 1983; Sinton et al., 1998; Blanch et al., 2004; Plummer and Long, 2007).

1.8.2.2 Bifidobacteria

Another method is selective cultivation such as bifidobacteria (Hagedorn et al., 2011).
The current NMMP framework cannot identify the source of bacterial contamination. Based on international literature data, subgroups of *Bifidobacterium spp.* can easily be used to identify contamination sources relatively cheaply (Blanch et al., 2006). So the methods most likely suitable for source tracking appear to be bifidobacterial tracking ratios or antibiotic-resistance spectra of *E. coli* (Luyt et al., 2012).

Bifidobacteria are obligate anaerobic Gram-positive, non-filamentous, coryneform cells and are commonly non-spore forming, non-motile, smooth colonies and thick pleomorphic rods (Mitsuoka, 1990; Nebra et al., 2003; Bonjoch et al., 2004; Salminen et al., 2004; Bonjoch et al., 2005; Madigan and Martinko, 2006). Bifidobacteria are part of the natural gut flora of warm-blooded animals and humans and are abundant in the human intestinal tracks (Mara and Oragui, 1983; Mitsuoka, 1990; Bonjoch et al., 2005). They grow in the large intestine in a fairly anaerobic environment, producing lactic and acetic acid which lowers the pH of the area and prevent attachment of other bacteria. This is helped by the excretion of bacteriocins and other antimicrobial agents from some species (Wilson, 2005). They are possibly involved with the production of vitamins (Wilson, 2005). *Bifidobacterium spp.* can ferment a large range of sugars and amino acids as well as utilise milk products and proteins (Wilson, 2005). They are prominent in human guts from an early age. Human faeces have reported concentrations of 7.9×10^4 to 2.5×10^{13} CFUs /g dry weight (Mara and Horan, 2003), while Tannock (1997) reported 10^9 to 10^{10} CFUs /g. Bovines are unlikely to contribute to the

bacteria load due to their digestion of bacteria in their abomasum (Resnick and Levin, 1981a).

Bifidobacterium spp. are useful for source tracking as they fulfil the criteria of a good indicator organism (Resnick and Levin, 1981a). Criteria for a good indicator include that they do not multiply in the environment outside the host (Carrillo et al., 1985), they are exclusively of faecal origin; they are universally present in warm blooded animals or humans, are easily detectible by simple methods, present when pathogens are present and absent when they are not and exhibit similar survival to target pathogens (Scott et al., 2002; Savichtcheva and Okabe, 2006; Ahmed et al., 2008; Paruch and Maehlum, 2012).

Bifidobacterium spp. are present consistently in warm-blooded animals and humans (Mara and Horan, 2003; Wilson, 2005). Bifidobacteria have not been isolated via culture methods from dog faeces (Resnick and Levin, 1981a; Lamendella et al., 2008). Bifidobacteria is not present in most dog faeces or only tiny quantities have been isolated with PCR, their impact on the counts would be negligible (Greetham et al., 2002; Lamendella et al., 2008). *Bifidobacterium adolescentis* one of the main sorbitol utilising bacteria have been isolated from poultry and cattle wastewater, however this is rare (Bonjoch et al., 2004). They are unlikely to multiply in the environment due to their nutritional requirements and optimum growth temperature of 30°C (Carrillo et al., 1985; Sinton et al., 1998; Nebra et al., 2002). South African river water temperature seldom exceed 30°C (Tandlich et al., 2010b). The advantage here is the simplicity of culturing and minimal equipment needed in the laboratory and no additional training of personnel is required (Luyt et al., 2010).

Based on the relevant nutritional characteristics, bifidobacteria form two distinct groups (Resnick and Levin, 1981a; Genthe and Franck, 1999). The first group is sorbitol utilising (SUB) and is usually of human origin, while the other is the total bifidobacteria (TB) (Resnick and Levin, 1981a; Mara and Oragui, 1983; Beerens, 1998; Matsuki et al., 1999; Bonjoch et al., 2005). The ratio changes between human and animal sources (Tandlich and Muller, 2008).

$$\textbf{Equation 1: Tracking Ratio} = \frac{[\textbf{Sorbitol Utilising bifidobacteria (SUB)}]}{[\textbf{Total Bifidobacteria (TB)}]} \quad \textbf{(Bonjoch et al., 2005)}$$

The SUB/TB ratio, also called the tracking ratio, was greater than 4, while warm blooded animals produced a ratio of less than 0.7, similar to the reports by Jagals and Grabow (1995). The problem is that the threshold ratio changes between countries, e.g. Bonjoch et al (2005) proposed 0.2 in Spain and Blanch et al (2006) proposed 3.2 as a 95% certainty in Europe, making it necessary for each geographic region to have its own calibration. There is also uncertainty as to the survival rate of bifidobacteria in contaminated water resources (Carrillo et al., 1985), which provides addition problems with estimating ratios after a pollution event. Jagals and Grabow (1996b) showed a large decrease in bifidobacteria in comparison to faecal streptococci (FS) downstream, while FS often over grow the media despite its specificity (Bonjoch et al., 2005; Tandlich and Muller, 2008). No survival times are presently available under South African conditions. Many studies have proposed survival time is short (Gyllenberg et al., 1960; Resnick and Levin, 1981a; Ottoson, 2009). Bifidobacteria die off 26 times faster than *E. coli* (Resnick and Levin, 1981a). Thus bifidobacteria indicate recent pollution (Jagals et al., 1995).

Bifidobacteria are chlorine sensitive and they die within 15 minutes, even at a low concentration of 0.2 ppm. Different survival rates have been documented, with it reported to survive 5 hours in fresh water and 10 hours in marine water at 20°C (Gyllenberg et al., 1960; Resnick and Levin, 1981a). Oxygen stress (Nebra et al., 2002), temperature fluctuations and chemical composition may help identify their die off rate. The survival rate of the two different groups could prevent the tracking ratio from working. If one group dies off faster or is in a viable non culturable (VNBC) state, the ratio would change and identify the incorrect source. Another reason the tracking ratios may not work is the lack of continuous input, thus the sampling may not reflect the initial concentrations excreted.

One of the first media developed for the enumeration of bifidobacteria from animal and human faeces was YN-6 medium (Resnick and Levin, 1981a; 1981b). They tested their media using environmental samples and wastewater from raw, primary, secondary and chlorinated sewage (Resnick and Levin, 1981b; 1981a). But the media was overgrown by presumptive faecal streptococci, necessitating the development of another medium (Mara and Oragui, 1983).

Multiple authors have modified the medium, leading to the creation of YN-17 medium and human bifido sorbitol agar for bifidobacteria enumeration (Mara and Oragui, 1983; Bonjoch et al., 2005). Bonjoch et al. (2005) noted the sorbitol fermenting bifidobacteria concentrations were always higher than 10^6 CFUs/100 mL in human -polluted wastewaters and lower in animal-polluted water. The total bifidobacteria did not vary significantly between animal and human polluted water, with values between 10^6 to 10^7 CFUs/100 mL (Bonjoch et al., 2005). This led to the applicability of the tracking ratio to identify the pollution source being investigated (Bonjoch et al., 2005). The tracking ratio here was lower than 0.05 in animal-polluted waters and higher than 0.2 in human wastewaters (Bonjoch et al., 2005) Thus a 0.2 cut-off value was considered to identify the source (Bonjoch et al., 2005).

However in a larger geographic environment, such as the European study by Blanch et al. (Blanch et al., 2006), a ratio of 3.2 was identified to distinguish pollution sources with a 95% certainty. Survival rates at different locations and of the different groups may cause this effect (Bonjoch et al., 2009; Ottoson, 2009).

The high antibiotic levels of YN-17 used for total bifidobacteria enumeration may stress bacteria and prevent growth, leading to lower growth levels compared to the sorbitol utilising bacteria (Mara and Oragui, 1983). A tracking ratio would thus need to be calibrated under South African conditions before being used by the NMMP (Luyt et al., 2012). In South Africa, the sorbitol-fermenting bifidobacteria were enumerated with faecal streptococci and faecal coliforms on the banks of the Modder River in the Free State, near an informal settlement using, mainly, pit and bucket latrines for sanitation (Jagals and Grabow, 1996b). In a tropical study, in Dar es Salaam in Tanzania, sorbitol utilising bacteria alone was used as indicator of recent human faecal pollution (Mushi et al., 2010). Studies in Zimbabwe and Nigeria reported bifidobacteria *adolescentis* as a more reliable indicator of recent faecal pollution in tropical environments compared to *E. coil* (Carrillo et al., 1985). *E. coli* can multiply in such warm climates while bifidobacteria have not been reported to do so.

1.9 CHEMICAL SOURCE TRACKING

Chemical methods' results are generally available faster than culture-based microbial methods due to the biological constraints posed by grow times and environmental conditions (Seurinck et al., 2005; Hagedorn and Weisberg, 2009). Review articles are numerous (Scott et al., 2002; Meays et al., 2004; Seurinck et al., 2005; Field and Samadpour, 2007; Stoeckel and Harwood, 2007; Hagedorn and Weisberg, 2009). The cost of equipment and sample preparation, due to extremely low concentrations, is often higher (Hagedorn and Weisberg, 2009). In South Africa, another barrier would be training staff, especially due to the current shortage of technical staff (Momba et al., 2006b; Momba et al., 2009a; Luyt et al., 2012), as these processes would be new and equipment would be different (Hagedorn and Weisberg, 2009). Another problem is detection at low levels due to dilution. Pathogens may be present when chemical levels are low.

Three studies have been done in multiple laboratories to compare methods (Griffith et al., 2003; Stoeckel et al., 2004; Blanch et al., 2006). Although caffeine and optical brighteners are found frequently in American rivers, would these compounds be good markers in rural South Africa? In South Africa, rural people wash clothes in rivers or have grey water run into rivers which may not have high bacterial or pathogen counts compared to the anionic surfactant concentrations.

1.9.1 Optical Brighteners

Optical brighteners (OB) are found in washing powders to help clothing appear whiter. They absorb ultraviolet light (UV) at 365nm and reemit it at 415-435nm in the blue spectrum (Hagedorn and Weisberg, 2009). Clothing being washed will absorb between 25-95% (Hagedorn and Weisberg, 2009) of the OB and the rest will be discharged into wastewater if clothes are washed in the river. No environmental source has been identified yet (Hagedorn and Weisberg, 2009). The use of optical brighter or fluorescent whitening agents, found in detergents is quick (with the use of a hand-held fluorometer) and cheap. after initially purchasing an expensive fluorometer (Hagedorn and Weisberg, 2009). Other methods are available, but there is a lack of sensitivity, equipment and the technical skills needed (Hagedorn and Weisberg, 2009). However, the dilution factor may pose a problem, as well as the degradation rates which are unknown for highly sunny South Africa, affecting sensitivity which is often worse than microbial methods (Hagedorn and Weisberg, 2009). It would be

impractical with current technology and skills shortages to adopt this method, in its current state, in South Africa.

1.9.2 Caffeine

Caffeine is an alkaloid (1,3,7-trimethylxanthine) found in more than 60 different plants (Heckman et al., 2010). South Africa is a low consumer of caffeine, with an average daily consumption of 40 mg/day as opposed to the global average of 70 mg/day and the USA (United states of America) at 168 mg/day and Denmark at 390 mg/day (Buerge et al., 2003; Hagedorn and Weisberg, 2009; Heckman et al., 2010). It is available as tea, coffee, soda, energy drinks and chocolates, contributing to the global average caffeine consumption of 70 mg per day (Buerge et al., 2003). Caffeine has been proposed as a good indicator of human contamination (Standley et al., 2000; Chen et al., 2002; Gardinali Piero R and Xu, 2002; Scott et al., 2002; Sankararamakrishnan and Guo, 2005; Hagedorn and Weisberg, 2009). Correlation between caffeine and enterococci or faecal coliforms has been poor (Buerge, 2003). Over dilution has been a problem in at least one study (Peeler et al., 2006).

Caffeine can be detected accurately with good reproducibility and has good detection level sensitivity (Hagedorn and Weisberg, 2009). Possible dilution problems and a need for training and equipment are once again problematic in the South African context.
There is also the possibility of plant species secreting caffeine in the water body and interfering with the results, but this may be unlikely in South Africa with no coffee plantations There are no degradation rates available for different environments and studies showing correlation with faecal indicator bacteria (FIB) are scarce (Hagedorn and Weisberg, 2009). One report stated that the half-life of caffeine in water when exposed to sunlight is approximately 12 days (Hagedorn et al., 2011). Thus the persistence in the environment will not correlate with bacterial survival rates, which are dependent on nutrients, predation sunlight and other factors.

1.9.3 Faecal Sterols and Stanols

Faecal sterols and stanols can be used to identify human and animal contamination, making them unique to the chemical group of tracking agents. Sterol tracking has been conducted in many different geographic regions around the world (Leeming et al., 1996; Leeming and Nichols, 1996; Leeming et al., 1998; Bull et al., 2002; Isobe et al., 2002; Isobe et al., 2004; Noblet et al., 2004; Hagedorn and Weisberg, 2009). Numerous reviews have been published (Scott et al., 2002; Hughes and Thompson, 2004; Savichtcheva and Okabe, 2006; Cimenti et al., 2007; Field and Samadpour, 2007; Hagedorn and Weisberg, 2009).

Faecal stanols are formed by metabolism of sterols and steroid compounds in the intestines of animals and humans (Leeming et al., 1996). Concentrations of stanols depend on the diet and intestinal flora of the animal, for example a lack of *Bifidobacterium spp*. may cause the cholesterol concentration in dog and bird faeces (Leeming et al., 1996). Cholesterol is metabolised by bacteria such as *Clostridium spp., Bacteroides spp. Bifidobacterium spp.* and *Eubacterium spp*. (Kay, 1981; Macdonald et al., 1983; Leeming et al., 1996; Nichols et al., 1996; Elhmmali et al., 1997; Veiga et al., 2005; Gérard et al., 2007). Coprostanol (COP, 5β-stanol) is produced in humans by biohydrogenation of the sterol at carbon 5 and 6 on ring B (Rosenfeld and Gallagher, 1964; Leeming et al., 1996; Sundram et al., 2000). COP represents a high proportion of the faecally excreted stanols (Hagedorn and Weisberg, 2009). COP (5β-cholestan-3β-ol) is a non-ionic, non-polar, organic molecule found in surface water sediments (Leeming et al., 1996; Scott et al., 2002; Hughes and Thompson, 2004; Field and Samadpour, 2007; Shah et al., 2007a; Shah et al., 2007b). Faeces also contain sitosterol, campestanol and 5-β-stanol, 24-ethylcoprostanol (Leeming et al., 1996), while COP and 24-ethylcoprostanol are used as the main source tracking stanols (Leeming et al., 1996; Sinton et al., 1998). These compounds chemical structures are shown in Figure 1. Production of 5β-campestanol/5β-stigmastanol is not limited to humans and herbivores, but preponderate compared to others, making it a potentially suitable marker (Leeming et al., 1996).

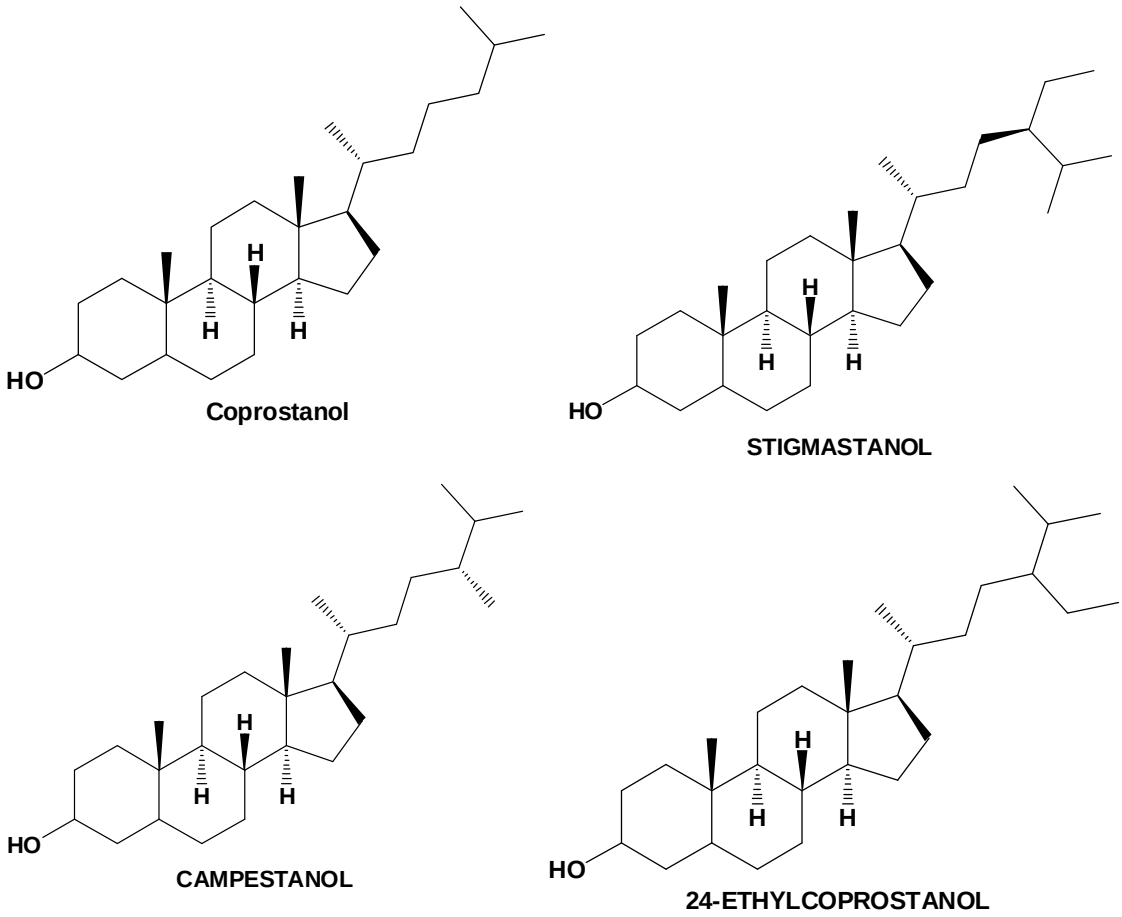


Figure 1: The chemical structures of coprostanol (cholestanol), stigmastanol, 24-ethylcoprostanol and camperstanol.

Animals excrete significantly lower quantities of COP (Bull et al., 2002), while herbivores (cattle, horses, sheep) excrete more 5β-campestanol and 5β-stigmastanol (24-ethylcoprostanol), indicating animal contamination (Leeming et al., 1996; Scott et al., 2002; Hughes and Thompson, 2004; Savichtcheva and Okabe, 2006). Carnivores and birds excrete primarily cholesterol and sitosterol (Leeming et al., 1997; Hughes and Thompson, 2004), possibly due to different intestinal bacteria.

The COP concentration in comparison to the total sterol levels is used as a possible biomarker of human faecal contamination (Leeming et al., 1996; Leeming et al., 1998; Scott et al., 2002; Hughes and Thompson, 2004; Savichtcheva and Okabe, 2006; Shah et al., 2007a; Edwards et al., 2008).

Environmental microbes degrade cholesterol to cholestanol (Hagedorn and Weisberg, 2009). COP undergoes biodegradation in aerobic conditions at 20°C within 10 days, rendering this method only effective in identifying recent contamination (Isobe et al., 2004; Savichtcheva and Okabe, 2006). However, the sorption to soils and river sediments can extend the detection period (Isobe et al., 2004; Seurinck et al., 2005; Field and Samadpour, 2007).

Several different ratios have been used to identify faecal sources (Hagedorn and Weisberg, 2009). Grimalt et al. (1990) proposed the use of a ratio of 0.7 for human pollution, using Equation 1.

Equation 2: $\frac{\text{5β-stanol}}{\text{5α-stanol + 5β-stanol}}$. **(Grimalt et al., 1990)**

Equation 3: $\frac{\text{coprostanol}}{\text{5β-stigmastanol}}$ **(Evershed Richard and Bethell Philip, 1996)**

A ratio greater than 1.5 using the Equation 2 was proposed by Evershed & Bethell (1996) to eliminate ruminant contamination (Hagedorn and Weisberg, 2009). Leeming et al (1997) proposed a mathematical equation to identify the source, as shown in Equation 3.

Equation 4: $\boldsymbol{Y = 100 \times \frac{\text{Coprostanol concentration}}{\text{Coprostanol concentration+24-ethylcoprostanol concentration}}}$

(Leeming et al., 1997)

If the Y value is higher than 73% only contamination by humans has occurred, while below 38% indicates only animal inputs, with middle values showing mixed contamination (Leeming et al., 1997). The amount which herbivores contribute was calculated using Equation 5 (Leeming et al., 1997).

Equation 5: % herbivore contribution=2.86 × (73-*Y*) **(Leeming et al., 1997)**

While no ratio has yet been accepted as standard yet, this method could prove useful in rural South African areas, where streams have livestock grazing nearby and laundry being washed in them. The South African study was done by Sundram (2006) and was performed using gas

chromatography (GC) and sterols were derivatised using N,O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) forming trimethylsilyl esters.

Most of the methods use GC, however, there are high-performance liquid chromatography using an ultraviolet light detector (HPLC-UV) methods available (Parris, 1977; Shaikh et al., 1978; Mingrone et al., 1980; Armstrong and Carey, 1982; Roda et al., 1993). South Africa could source this equipment from universities, decreasing equipment costs and enabling studies where no microbiology laboratories have been established.

The limitations to the use of sterols include the cost of equipment, the shortage of trained people, and sampling procedures. There is no standard analysis method. Considerable skill is required to separate the similar peaks from stanols and sterols (Reeves and Patton, 2001).

Sample volumes of 4L or greater are required. Anaerobic sediment bacteria can generate small quantities of COP (Mudge and Lintern, 1999). Correlations between FIB and high FC with COP have been noted, but no direct relationship has been established (Leeming et al., 1997). While *E. coli* was correlated with COP by Isobe et al (2004). Blanch et al (2006) found multiple samples misclassified, preventing recommendation for regular use.

CHAPTER 2: SCREENING FOR IDENTIFICATION OF CALIBRATION SAMPLING SITES AND THE H_2S STRIP TEST MODIFICATION.

2.1 BACKGROUND

The Millennium Development Goals for water access have been achieved, but it has been noted that the water is not necessarily safe (Clasen, 2012). South Africa has poor water quality in rural areas (Genthe and Jagals, 2003). The cost of water testing is a major factor restricting its frequency, and access to accredited laboratories affects the ability to test sites. There are provinces and areas within provinces where water testing laboratories are less inaccessible, for example the Eastern Cape, Northern Cape, Limpopo and Mpumalanga (Balfour et al., 2011). A test which can be done without a laboratory or incubator would thus be useful.

The South African National Monitoring Programme for surface water (NMMP) monitors river water quality in areas highly prone to faecal contamination using *Escherichia coli* (*E. coli*), pH and turbidity as indicators (Murray et al., 2004).

Currently up to 30% of surface water resources are not monitored regularly (Rivett et al., 2009). Other indicator bacteria used to identify water quality are total coliforms, faecal coliforms and heterotrophic bacteria. The heterotrophic plate count (HPC) is used as an indicator of treatment efficiency in treated water or distribution systems, and can be used as an indicator of the total heterotrophic bacteria present in rivers (Wright, 1978; Chowdhury, 2012). Indicator bacteria have been used for a while to assess the risk of water for human consumption (APHA, 1992; Genthe and Franck, 1999; Payment and Locas, 2011). Thus, large database have been developed correlations between indicator bacteria and waterborne outbreaks. Future tests need to prove their superiority or to correlate with indicator bacteria for calibration purposes.

2.2 RIVER WATER QUALITY IN SOUTH AFRICA

Previous studies in the Western Cape in South Africa, by Paulse et al (2009), reported that FC and *E. coli* concentrations ranged between 1.00×10^1 and 3.5×10^6 cells/100 mL in the Plankenburg River. While the Berg River had FC concentrations between 1.7×10^2 and 3.5×10^7 CFUs/100 mL with *E. coli* concentrations being higher, ranging between 3.6×10^1 to 1.7×10^7 CFUs/100 mL (Paulse et al., 2007). In the Limpopo province, the Chume River had FC concentrations between 6 and 40.5 CFUs/100 mL (Germs et al., 2004). Jagals and Grabow (1996b) isolated between 0 and 8.4×10^5CFUs/100 mL 60 km westward of Bloemfontein near an informal settlement, in the Modder River. The Jukskei River near Johannesburg, running past the Alexandra peri-urban settlement, had *E. coli* concentrations of 3.7×10^5 cells/100 mL at the settlement, while above and below were 1.5×10^3 cells/100 mL and 1.3×10^5 cells/100 mL respectively (de Wet et al., 2000-2010). The Bloukrans River, in the Eastern Cape, one of the screened rivers of this study had FC concentrations between 2×10^6 and 1.1×10^7CFUs/100 mL, while *E. coli* concentrations ranged between 2×10^6 and 1.3×10^7 CFUs/100 mL (Wutor et al., 2009). This data is shown in Table 1.

Escherichia coli has only been reported to grow in tropical river water (Solo-Gabriele et al., 2000) or to originate from non-faecal sources (Tallon et al., 2005), while some strains may be capable of growing in soil environments (Topp et al., 2003; Ishii and Sadowsky, 2008). *E. coli* is considered an independent, as it is less likely from non-faecal sources, as 95% of thermotolerant coliforms excreted in animal faeces are accounted for by *E. coli* (Mara and Horan, 2003).

The FC concentrations are used to identify public health risk posed by the rivers, according to the DWAF (1996c) guidelines in South Africa. It is used and is familiar to many field healthcare professionals and regulatory people (Genthe and Jagals, 2003). However, FC and *E. coli* are normally excreted by healthy humans and warm blooded animals, including cattle and donkeys (APHA, 1998). There are other thermotolerant FC species excreted, but due to their different survival rates and inter-species competition, these can significantly change the composition in the rivers (Foppen and Schijven, 2006).

Table 1: Previous studies in South Africa and their faecal coliform (FC) and *Escherichia coli* (*E. coli*) concentrations ranges reported for rivers.

Province	River	FC (CFUs/ 100 mL)	*E. coli* (CFUs/ 100 mL)	Reference
Western Cape	Plankenburg River	1.00×10^1 to 3.5×10^6		(Paulse et al., 2009)
Western Cape	Berg River	1.7×10^2 to 3.5×10^7	3.6×10^1 to 1.7×10^7	(Paulse et al., 2007)
Eastern Cape, Grahamstown	Bloukrans River	2×10^6 to 1.1×10^7	2×10^6 to 1.3×10^7	(Wutor et al., 2009)
Limpopo	Chune River	6 to 40.5		(Germs et al., 2004)
Bloemfontein	Modder River	0 to 8.4×10^5		(Jagals and Grabow, 1996b)
Johannesburg	Jukskei River		1.5×10^3 3.7×10^5 1.3×10^5	(de Wet et al., 2000-2010)

2.2 H_2S STRIP TEST

The hydrogen sulphide strip test (H_2S strip test) is a screening technique using precipitation of iron due to hydrogen sulphide production for a positive result (Venkobachar et al., 1994; Genthe and Franck, 1999; Sobsey and Pfaender, 2002; Hirulkar and Tambekar, 2006; McMahan et al., 2012). It is a low cost method with an easy-to-read colour change and can be used by minimally trained people (Sobsey and Pfaender, 2002; Genthe and Jagals, 2003; Hirulkar and Tambekar, 2006; Luyt et al., 2011a).

A meta-analysis of the H_2S test versus indicator bacteria confirming it to be cheaper than alternative methods was performed (Wright et al., 2012b). The H_2S test has been used in many different countries (Manja et al., 1982; Sobsey and Pfaender, 2002; Gupta et al., 2008; Luyt et al., 2011a). Since its development, many alterations to the media composition have occurred to improve its sensitivity and selectivity (Sobsey and Pfaender, 2002). After a literature search, the Venkobachar *et al.*'s (1994) H_2S strip test was modified by the addition

of 0.5% deoxycholate (mH_2S strip test) to improve its sensitivity (Venkobachar et al., 1994; Sobsey and Pfaender, 2002; Tandlich et al., 2010a; Luyt et al., 2011a).
The test takes 72 hours before a result can be confirmed. The H_2S test has also been used by the South African Department of Water Affairs (DWA) where alternative methods are not feasible, e.g. in rural areas, but positive results still need to be confirmed by a more specific test (DWAF, 2009). Genthe and Jagals (2003) proposed the H_2S test be used as an early warning system in monitoring South Africa's, for water quality. Their study with environmental health officers showed the test was easy to use and embraced as a potential monitoring tool (Genthe and Jagals, 2003). There is a need to test its suitability with volunteers from different social groups and in different climatic conditions before promoting its use (Genthe and Jagals, 2003); see chapter 5 for more information.

The H_2S test has many limitations which need to be overcome. Its limitations include the fluctuation of temperatures during transport and enumeration, as incubators are not used the lack of sensitivity to low faecal coliform or *E. coli* concentrations and the proposed interference of sulphates and nitrates.

2.3 AIM

This study aimed to identify continuously polluted sites in order to identify the hydrogen sulphide test correlation with the faecal coliform (FC) and heterotrophic plate count (HPC) concentrations in the environment. The sites were also screened to identify sites suitable for testing source tracking methods in the Eastern Cape, South Africa.

Identification of the limitations of the H_2S strip test and their influence minimised or disproved. The sensitivity of the H_2S strip test needs to be increased and the influence of temperature fluctuation and possible chemical interference such as sulphates, nitrates and nitrites investigated. Thus the survival of HPC and FC needs to be comparison to identify their influence on the H_2S strip test. The suitability of the test for use by the population and its ability to be used as an early warning system also need to be identified, however this will be performed in Chapter 4.

2.4 MATERIALS AND METHOD

2.4.1 Materials

All chemicals purchased were 99% pure or higher (unless indicated otherwise) and used as received from the supplier.

The following chemicals and consumables were purchased from the suppliers stated below. Merck Ltd. (Pty; Johannesburg/Cape Town, South Africa) supplied: anaerobic jars; $BaCl_2.2H_2O$; $CaCO_3$; HCl (32 % aqueous solution); H_2SO_4 (95-98 % aqueous solution); KH_2PO_4; KNO_3; Kovacs reagent for indoles; Na_2HPO_4;NaCl; $Na_2S_2O_3.5H_2O$; phenol; 500 mL polyethylene sampling bottles; the chloride kit (catalogue number 1.14897.0001); the ammonium kit (catalogue number 1.14752.0001); the phosphate kit (catalogue number 1.14848.0001); m-FC agar; tryptic soy broth; peptone from casein and peptone from meat.

The main supplier was Sigma-Aldrich (Johannesburg, South Africa) which provided the following chemicals: bacteriological agar; bromocresol green; bromocresol purple; cysteine hydrochloride; ferric ammonium citrate; $FeSO_4.7H_2O$; $ZnSO_4.7H_2O$; sodium propionate; $MgSO_4.7H_2O$; $MnSO_4.4H_2O$; NaN_3; cycloheximide; kanamycin sulphate; nalidixic acid; polymyxin B (1 mg/mL aqueous solution); lactose; tryptone powder; salicin; Triton X-100; L-cysteine; deoxycholate; sorbitol; yeast extract; glucose; bovine serine albumin; Bradford's reagent; the Kovacs reagent for indoles; and toluene. Glycerol; acetone and absolute ethanol were purchased from Chemstores (Rhodes University, Grahamstown, South Africa).

Membrane filtration (MF) filters (nylon, size 0.45 μm) for microbial enumeration; Oxoid gas generating kits for anaerobic enumeration for use with the anaerobic gas jars; sterile petri dishes (90 mm) were procured from Spellbound Labs (Port Elizabeth, South Africa).

Microsep (Port Elizabeth, South Africa) supplied the 11 mm glass fibre prefilters for total suspended solid (TSS) determination and the glass fibre filters (pore diameter 1.2 μm) for the measurement of chlorophyll a (chla).

The instrument grade Ar, N_2 and CO_2 were purchased from Afrox-Linde (Port Elizabeth, South Africa), while casamino acids were obtained from BD (Pretoria, South Africa).

2.4.2 Enumeration of Bacteria

The different agars were prepared in 1 L schott bottles (Labotec, Midrand, South Africa). All the media autoclaving was performed in a Model RAU-53Bd REX MED autoclave (Hirayama Manufacturing, Tokyo, Japan).

Samples were inoculated onto plates in a LA1200 BII laminar-flow hood. All incubations took place in one of the following incubators: the Labcon incubator Model FSIM B (Labmark, Johannesburg, South Africa), the TS 606/3-I incubator (WTW, Weilheim, Germany), the Labcon low temperature incubator LTIE 10 (Labmark, Johannesburg, South Africa) and/or the Heraeus Model FT 420 (***Heraeus*** Kulzer GmbH, Dormagen, Germany).

Bacteria samples were either spread plated or membrane filtrated. Samples were analysed in duplicates or triplicates. The average of the replicates was recorded as the concentration.

2.4.2.1 Coliforms

Faecal coliforms were enumerated on m-FC agar (Merck (Pty.) Ltd., Cape Town/ Johannesburg, South Africa), at 44.5°C for 24 hours. The mFc agar was prepared according to the manufacturer's instructions. The mFc agar (52 g) was dissolved in 1000 mL of deionised water by boiling for 1 minute, while being stirred. The media was allowed to cool before pouring into petri-dishes, under sterile laminar flow conditions. The media was not autoclaved.

For the screening part of the project, A1 media was used to identify FC. Positive results were identified by the characteristic production of gas with characteristic bubbles and the media going cloudy with growth (APHA, 1998).

The total coliforms were inoculated onto MacConkey agar and incubated at 35°C for 24 hours. The MacConkey agar was prepared according to the manufacturer's instructions. The MacConkey agar was prepared by weighing 50 grams of the powder into a schott bottle and dissolved by boiling for 1 minute, while being continuously stirred, and then autoclave at 121°C for 15 minutes. The agar was allowed to cool to approximately 45°C before being poured into petri-dishes under sterile laminar flow conditions.

The A1 media for faecal coliform enumeration was prepared according to the standard methods for the examination of water and wastewater (APHA, 1992). The single strength media has the following ingredients weighed into a 1 L schott bottle: lactose (5 g); tryptone (20 g); sodium chloride (5 g), salicin (0.5 g); Polyethylene glycol (1 mL) and deionised water (1 L). The double strength version just doubled the quantities. The solution was autoclaved for 10 minutes at 121°C and stored in foil in the fridge until used. The media was not stored longer than 2 weeks.

Samples were inoculated into single or double strength media according to the dilution required. Five replicates of each of the three MPN dilutions were made. The three dilutions were 10 mL:10 mL with double strength; 1 mL:5 mL single strength and 0.1 mL and 5 mL single strength (APHA, 1992). The results were read from the MPN table in the standard methods for the examination of water and wastewater (APHA, 1992).

Screening of faecal coliforms was conducted using A1 medium and the most probable number (MPN) technique with Durham tubes (APHA, 1992; APHA, 1998), at 44.5 °C for 24 hours. Positive results were tested for indole production by a 500 uL aliquot being inoculated in sterile 5 mL of 0.1% tryptone water (Tryptone weighed into deionised water and autoclaved at 121°C for 15 min) for 24 hours and then tested with Kovac's reagent (Sigma Aldrich, Johannesburg, South Africa). After 24 hours Toluene (1 mL) and Kovac's reagent (500 µL) was added to the 5 mL sample. A positive indole reaction, indicated by a pink colour, denoted the presence of *E. coli* (Fluka Analytical; Niemela et al., 2003).

2.4.2.2 Particle Attachment

The attachment of bacteria onto particulate matter was tested using a collection of two or three rocks with particular matter attached onto them (Tandlich et al., 2010b). These rocks were collected from the river using sterile nitrile gloves and placed into a plastic ADDIS® container before being transported to the laboratory on ice. The selected rocks with sediment were vortexed in an MP-19 Deluxe Vortex Mixer (Chiltern Scientific, Pretoria, South Africa) for 2 minutes in 75 mL of sterile quarter-strength Ringer's solution (Tandlich and Muller, 2008) to detach and re-suspend the sediment.

A quarter-strength Ringer's was prepared by diluting Ringer's Solution. Ringer's solution was prepared by mixing sodium chloride (8.6 g); potassium chloride (0.3 g); calcium chloride hydrate (0.33 g) and deionised water (1000 mL) (Sweetman, 2011). The solution was autoclaved for 20 minutes.

The quarter-strength Ringer's solution was then inoculated onto the respective medium and incubated according to the microbe being identified. Results were presented as CFUs (Colony Forming Units) /1 g of the dry weight particulate matter. The dry weight was obtained by drying the sediment and rock in a UFE 700 oven (Memmert, Schwabach, Germany) at 60°C for at least one day. The dry sediment was weighed on a 4 decimal place balance. The original clean container weight was subtracted from the dry sediment and container weight.

2.4.3 Chemical Analysis for Water Sampling and Survival Experiments

Chemical parameters were analysed to identify the water quality and then to develop model water for survival experiments. The nitrates, chlorides, protein, phosphates, ammonium and sulphates were measured in the laboratory after bacterial inoculation occurred. The following Merck kits were used: the chloride kit (catalogue number 1.14897.0001); the ammonium kit (catalogue number 1.14752.0001) and the phosphate kit (catalogue number 1.14848.0001). Nitrates were measured using the Winsona State University method (Miertschin, 2011). The sulphates were analysed using Environmental Protection Agency (EPA) Method 375.4 (EPA, 1978). A microplate reader (manufactured and supplied by Biotek) was used for chemical quantification. The microplates (PLATE F/B CLR PS 96WELL) were supplied by Lasec (Port Elizabeth, South Africa).

The dissolved oxygen was measure using a Cyberscan Waterproof Dissolved oxygen/C/F DO 300 data meter (Eutech Instruments, Singapore). The turbidity was measured using an Orbeco Hellige Model 966 Portable Turbidimeter (Lasec, Port Elizabeth, South Africa). The pH and EC of the water samples were measured using the HANNA pH and conductivity/ TDS (total dissolved solids) tester probe and a mercury thermometer (Sigma Aldrich, Johannesburg, South Africa). The model numbers were HI 98129 (low range) and HI 98130 (High range) (HANNA, South Africa). The hardness was tested using a Salifert KH/Alk Profi test (Holland, (Salifert, 2012)) or Blue 52 hardness test kit (Blue 52^{TM}, Midrand, South Africa).

Concentration of total carbohydrates was determined using the phenol-H_2SO_4 method (DuBois et al., 1956), while the protein concentrations were determined using the Bradford's method distributed by Sigma-Aldrich with the reagent (Bradford, 1976)(Sigma-Aldrich, Johannesburg, RSA).

The chlorophyll (chla) was measured by on-site filtering of 120 mL of the water sample through a glass fibre filter (pore size 1.2 μm) with aid of a hand held pump (Geotech, Denver, CO, USA). The filtrate and extractions were all performed in the dark shielding the chlorophyll to prevent degradation. The filtrated with the filter paper was placed into a test tube with 8 mL of acetone (90% aqueous) and refrigerated in the dark at 4°C for 12-24 hours to allow for diffusion. The extractant was centrifuges at 3000 rpm on the Roto-Uni 752 centrifuge (Hettich Instruments, Beverly, MA, USA).The chla concentration was measured using a 10-AU Fluorometer (Turner Designs, Sunnyville, CA, USA) via florescence spectroscopy (Bukin et al., 2008).

Light intensity was measured using the LX101 LUX light meter (DM Agencies, Cape Town, South Africa). All samples were stored at 4°C until analyses.

2.4.4 Model Water

The survival experiments were performed to identify the discrepancies between the FC and *E. coli* concentrations and the mH_2S strip test (modified H_2S strip test). The term model water is used to describe the water developed for survival experiments.

The composition of the survival experiment water was prepared by adding glucose, sodium sulphate, bovine serum albumin, potassium phosphate, ammonium chloride, sodium nitrate, calcium carbonate and algae to MilliQ water (800 mL). The MilliQ water is prepared using a MilliQ RO® purification system (Millipore Co., Massachusetts, United States of America) and filtered through a Super C® carbon cartridge, two ion-X® exchange cartridges and an Organex-Q® cartridge before being filtered through a 0.22 μm Millipak® filter (Millipore Co., Milford, Massachusetts, United States of America).

The quantities of the chemicals differed according to the two different model waters and are show in Table 2. The nutrient restricted model water did not have bovine serum albumin

(protein), phosphates, ammonium or nitrates added, while it was to the nutrient rich model water. The total suspended solids (TSS) were set at 0 mg/L to increase photosynthesis, while the nutrient depleted water had a TSS of 50 mg/L to decrease photosynthesis and increase light scattering. The pH was adjusted with NaOH or HCl to 7.71, while the nutrient depleted water was higher due to the addition of calcium carbonate (100 mg) resulting in a pH of 7.87. The particulate matter was represented by course pool filter sand (100 g) (Sparrow Pools, Grahamstown, South Africa) which was washed twice in water and dried before being added to the model water. The model water was then autoclave for 15 minutes at 121°C. The water was left to cool, in the laminar flow, to room temperature before the bacterial were inoculated into the water. The inoculum of the bacteria and algae is explained below in section 2.4.7. The model water was stored in the laminar flow at 21.5 ± 1°C and out of direct sunlight, but allowing for diurnal variation in light. The schott bottles were opened for once a day for 2 hours under laminar flow to allow aeration.

The bacteria were enumerated, in triplicate, on the appropriate media. An aliquot was diluted in saline and spread plate before being incubated at the appropriate temperature.

Table 2: The composition of the nutrient restricted and nutrient rich model water for survival experiments for HPC and faecal coliforms. Concentrations are based on selected site concentrations.

Parameter	Nutrient rich model water	Nutrient restricted model water
pH	7.71	7.87
Turbidity (NTU)	23	87
Salinity (mS/m)	82	82
DO (mg/L)	6.01	6.01
Cl^- (mg/L)	77	77
T (ºC)	21.5	21.5
Light Intensity (lx)	386-1760	386-1760
HPC (CFUs/mL)	48000	48000
SO_4^{2-} (mg/L)	43	43
Saccharides (mg GE/L)	5.7	5.7
Proteins (mg BSAE/L)	5.7	0
PO_4^{3-} (mg/L)	1.49	0
NH_4^+ (mg/L)	1.08	0
NO_3^- (mg/L)	11.7	0
TSS (mg/L)	0	50
TA (mg $CaCO_3$ /L)	60	100
Chla (μg/L)	2.9	2.9

BSAE - Bovine Serum Albumin Equivalent

TA - Total Alkalinity

2.4.5 Inoculum for Model Water

The trypitc soy broth used to grow the bacteria was prepared according to the manufacturer's instruction. Thus 7.5 g was placed into 250 mL deionised water in a conical flask (500 mL). The broth was autoclaved at 121°C for 15 minutes. The inoculations of the broth were performed under sterile conditions and laminar flow.

The heterotrophic bacteria (HPC) were isolated from R2A agar (Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa) and grown in tryptic soy broth (Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa) overnight and then diluted with sterile saline solution (0.9% saline BP (Sweetman, 2011)) to the correct dilution before they were inoculated into the model water.

The faecal coliforms (FC) were picked off mFc agar plates ((Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa). They were grown in tryptic soy broth (Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa) overnight at 37°C. An aliquot of tryptic soy broth containing bacteria was diluted with sterile saline solution (0.9% saline BP (Sweetman, 2011)) to the correct dilution before they were inoculated into the model water. The dilution factor required was calculated from the number of colonies inoculated and the grow rate from previous experiments

The algal inoculum was obtained from the Institute for Water Research at Rhodes University as a seven-strain mixed culture which had been isolated from the rivers in the Eastern Cape and Limpopo.

2.4.6. Preparation of the H_2S Sampling Kits

A modified version of the Venkobachar *et al.* (1994) H_2S strip test medium was used. This contained peptone (40 g), dipotassium hydrogen phosphate (3 g), ferric ammonium citrate (1.5 g); Sodium thiosulphate anhydrous (2 g); Teepol (2 mL), L-cysteine (25 mg); Distilled water (100 mL) (Venkobachar et al., 1994). It was modified by the addition of deoxycholate (0.5% w/v) (Sobsey and Pfaender, 2002; Luyt et al., 2011a). Deoxycholate has been suggested to inhibit non-faecal bacterial growth, decreasing false positive result occurrence (Sobsey and Pfaender, 2002).

The kit's preparation method was modified from Genthe and Franck's method (1999). The medium was mixed with a magnetic stirrer for 30 minutes and then left to stand for 15 minutes forming a coffee brown colour. A one millilitre aliquot was absorbed into a 5 by 10 cm piece of filter paper which had been folded and placed into a sterile urine jar (Spellboud Labs, Port Elizabeth, South Africa). The jars with the filter paper were placed into the UFE 700 oven (Memmert, Schwabach, Germany) overnight at 54°C and was then either placed into new sterile urine jars and stored until used or autoclaved (Model RAU-53Bd REX MED autoclave (Hirayama Manufacturing, Tokyo, Japan)) at 121kPa for 20 minutes, in a 2 L aluminium foil covered beaker sealed with autoclave tape. The beaker of anhydrous sodium sulphate (150 g) was placed in the 2 L beaker to prevent moisture absorbance during autoclaving. It was used to hold the sodium sulphate to prevent it from touching the paper strips. The filter paper was placed in to pre-sterilised urine jars. The urine jars were sterilised using 70% ethanol and were then exposed to ultraviolet light (UV) irradiation for 30 minutes, in an LA1200 BII laminar flow hood (Laboratory and Air Purification Services, Midrand, South Africa). The filter paper was then placed aseptically into sterile urine jars and then closed immediately.

The sterility of the sterilised kits was checked periodically with 20 mL of sterile deionised water and incubation to identify positive H_2S result due to contamination. Autoclaving increased the sterile period by 1 week, making it reliable for up to 6 weeks. Thus an autoclave, which only extends the sterility period by 1 week, is not needed for the preparation as long as a 50°C regulated oven is available. This method could therefore be used in isolated areas as a low cost water monitoring tool. All kits were stored out of direct sunlight in a light proof box at room temperature.

2.4.6.1 Use of H_2S Strip Test Kit

The H_2S strip tests had 20 mL of sampling water placed aseptically into the bottles. These were sealed and hand shaken to suspend the media, before being incubated at room temperature (18 - 25°C) for 72 hours. Samples were checked for the presence of a black precipitate, which was recorded as a positive result, every 12 hours. Tests remaining the initial yellow colour or which turned grey without going black within the 72 hour time limit were recorded as negative.

The 72 hour time limit arises from a suggestion by Genthe and Jagals (2003). This was based on the length of time taken for low bacterial concentrations to produce positive results at 22°C (Genthe and Franck, 1999). Higher concentrations tend to produce positive results within 48 hours (Genthe and Franck, 1999).

Where samples were not placed directly into the kit bottle, the bottle used to transfer the water was sterilised through lab inoculations.

2.4.6.2 Testing Chemical Interferences and Temperature Effects on the H_2S Strip Test Kit

The H_2S strip tests were tested for delays of positive results due to differing transportation temperatures. Three replicates were used for each sampling site. Site 10 and 11 were selected for this experiment. The control was incubated between 18 and 25°C for 72 hours. One of the other 2 replicates was incubated at 10°C for 5 hours before being placed with the control, while the other was incubated at 32°C for 5 hours before being placed with the control.

The effect of sulphates, in the water, on the production of positive results in the H_2S strip test was tested using a sterile solution of sodium sulphate within the concentration range of 14 to 4240 mg/L.

2.5 SCREENING

Identifying if a test works under all conditions requires the identification of sites with continuous faecal pollution at high and low concentrations. Thus sampling sites with continued presence of microbial pollution were identified in order to identify the source of pollution. This allowed the calibration of tracking techniques.

Sites were tested over the period between April and September 2008 for indicator bacteria. Concentrations of phosphates, nitrates, sulphates and others were measured. These chemical concentrations were used later, as elaborated on in Chapter 5, for source tracking bacteria survival experiments.

2.6 RESULTS AND DISCUSSION

E. coli and FC concentrations in South African rivers vary significantly. The mH_2S strip test was used to identify faecal pollution in a different river site in the Buffalo and Makana municipalities, in conjunction with faecal coliform (FC) and *E. coli* tests.

Sampling sites screened were selected according to data gathered from other Rhodes University research projects on water quality in the Eastern Cape. Fifteen sites were chosen from the research literature and screened for faecal coliforms, *E. coli* and H_2S strip test. Final sampling sites were selected from these 12 sites, which were screened for continuous faecal pollution.

The sites were considered to have continuous faecal pollution if three independent tests identified faecal pollution on three randomly selected occasions (Tandlich et al., 2010a). The mH_2S strip test and the faecal coliform (FC) test were used as independent tests, but *E. coli* was included as it was more specific for faecal contamination than FC. If selection criteria were met, there was a 99% probability the site had continuous faecal pollution and less than 0.5% chance of it being due to non-faecal sources (Tandlich et al., 2010b). Three tests that were chosen as faecal coliforms have been reported in literature to be heavily influenced by environmental factors and non-faecal pollution sources e.g. regrowth in water, retention in soil and others (Caplenas and Kanarek, 1984; Gauthier and Archibald, 2001; Tallon et al., 2005; Sinigalliano et al., 2010).

Identifying FC alone is less reliable than three independent tests to identify faecally polluted sites (Tandlich et al., 2010b). The FC group contains the *Klebsiella spp.* which is selected for with *E. coli* as thermotolerant coliforms in test media (Hoadley and Dutka, Bacterial Indicators/Health Hazards Associated with Water, 1977). Paper mills effluents and waste samples had *Klebsiella spp.* concentration accounting for 46 to 97% of the total FC concentrations (Caplenas et al., 1981). *Klebsiella Pneumoniae* is found in a high proportion of dairy cattle (Munoz et al., 2006) and 5% of health adult faeces (Degener et al., 1983). But *Klebsiella pneumoniae* is found on soil and plant surfaces, leading to environmental inputs contributing to water concentrations (Abbott, 1997).

The H_2S test is also not completely independent as the enumerated bacteria are closely related to FCs and overlap with FC members as is shown by the list below. H_2S producing bacteria are members of the Enterobacteriaceae group and include: *Aeromonas spp. , Citrobacter spp.* (Ratto et al., 1989)*; Clostridium spp.* (Castillo et al., 1994; Sobsey and Pfaender, 2002)*; Enterobacter spp.* (Castillo et al., 1994)*; Escherichia spp.* (Sobsey and Pfaender, 2002)*; Klebsiella spp.* (Manja et al., 1982)*; Proteus spp.* (Manja et al., 1982; Nagaraju and Sastri, 1999) and *Salmonella spp*. (Manja et al., 1982; Castillo et al., 1994; Mosley and Sharp, 2005). The FC group contains mainly *E. coli*, but non-faecal bacteria such as *Klebsiella, Enterobacter* and *Citrobacter* have been isolated from faecal coliform media (Schraft and Watterworth, 2005).

Site 8 was excluded due to the zero concentration found on the 4th of August 2008. Sites 3 and 5 were excluded due to zero FC concentrations found on the 15th of April 2008 and 8th of May 2008 respectively. Site 7 had a negative mH_2S strip test on the 5th of June 2008, and was excluded as both tests were not positive.

The highest FC concentrations were observed at Site 11 (11th of September 2008) and at Site 12 on all occasions (1st of August 2008; on the 22nd of August 2008; and on the 12th of September 2008). The MPN table showed that the concentration was higher than 16000 cells/100 mL and this was not analysed further due to its use as an indicator of continuous FC pollution only. The FC concentrations are comparable to those of Jagals and Grabow (1996b), but they span a narrower interval than the data of Wutor et al. (2009) and Paulse et al. (2007; 2009). The values by Germs et al. (2004) varied more than the data in this study.

The minimum and non-zero concentration of *E. coli* was equal to 2 cells/100 mL at Site 10 on the 4th of August 2008 and at Site 15 on the 5th of August and the 2nd of September 2008. The maximum concentration of *E. coli* was higher than 1.6×10^4 cells/100 mL and it was measured at Site 11 and 12 on the 12th of September 2008.

Data span a narrower interval than reported by Wutor et al. (2009) and Paulse et al. (2007; 2009). The time to detect a positive mH_2S strip test result range between 14 hours (seen at Site 12 from the 1st of August 2008 sampling) and 60 hours (seen on the following sampling dates at the respective sites: at Site 8 on the 5th of June 2008; 11th of June 2008; 4th of August

2008 and at Site 9 on the 4th of August 2008 and the25th ofAugust 2008, as well as at Site 15 on the 4th of August 2008).

There was only 1 negative H_2S result from sampling on the 5th of June 2008. The results are shown in Table 3 below.

Table 3: Three tests used in combination to identify sites with continuous faecal pollution. These sites were not selected as all three tests were not positive at all the randomly selected sampling times. These Sites were excluded from further screening. The 'less than' sign in the H_2S strip test implies that positive results occurred before the next 12-hour time period, indicating qualitative data.

Site	Sampling date	FC (MPN cells/100 mL)	*E. coli* (MPN cells/100 mL)	mH_2S strip test[a]	Site selected
1	15/04/2008	260	0	<36 hours	No
1	30/05/2008	800		24 hours	No
1	24/09/2008	30		24 hours	No
2	15/04/2008	130	0	<36 hours	No
2	30/05/2008	130		<36 hours	No
2	24/09/2008	230		24 hours	No
3	15/04/2008	0	0	<36 hours	No
4	15/04/2008	3000	0	<36 hours	No
4	08/05/2008	1100		<24 hours	No
4	30/05/2008	2200		24 hours	No
5	15/04/2008	170	0	<24 hours	No
5	08/05/2008	0		<24 hours	No
6	15/04/2008	80	0	24 hours	No
6	08/05/2008	20		<24 hours	No
6	30/05/2008	80		24 hours	No
7	05/06/2008	2	0	Negative	No
8	05/06/2008	8	8	60 hours	No
8	11/06/2008	2	2	60 hours	No
8	04/08/2008	0	0	60 hours	No

Based on the data in Table 3 and Table 4 only six sites meet the criterion to identify continuous faecal contamination. Rivers which had high faecal pollution, and a couple with low faecal pollution, were chosen for the rest of the study. From six possible sites, none of them were from the Buffalo River, while only some Bloukrans River sites were chosen. Sampling Sites 3, 5, 7 and 8 were excluded. Sites 1, 2, 4, 6, 9 and 10 were possible for

selection and, due to the low number of sampling sites, potential sites in the Kwandwe Game Reserve (Sites 13-15) and two local dams (Sites 11 and 12) were screened. The use of local dams and a game reserve were added to these potential sites as selected sites. The proximity to the sampling lab was also taken into consideration, and the final sites selected were Sites 9 to 15. These 7 sites will be used throughout the rest of the study.

Table 4: The selected Sites for screening and calibration. Further tests were not considered due to the screening nature of the experiment. All three tests were positive at all the randomly selected sampling times. The 'less than' sign in the H_2S strip test implies that positive results occurred before the next 12-hour time period, indicating qualitative data.

Site	Sampling date	FC (MPN cells/100 mL)	*E. coli* (MPN cells/100 mL)	mH_2S strip test[a]	Site selected
9	11/06/2008	30	30	48 hours	Yes
9	04/08/2008	8	4	60 hours	Yes
9	25/08/2008	8	8	60 hours	Yes
10	29/05/2008	20	20	24 hours	Yes
10	11/06/2008	13	4	24 hours	Yes
10	04/08/2008	2	2	48 hours	Yes
11	01/08/2008	1700	700	<36 hours	Yes
11	22/08/2008	5000	1300	24 hours	Yes
11	12/09/2008	> 16000	> 16000	24 hours	Yes
12	01/08/2008	> 16000	7600	<24 hours	Yes
12	22/08/2008	> 16000	110	24 hours	Yes
12	12/09/2008	> 16000	> 16000	24 hours	Yes
13	05/08/2008	23	23	48 hours	Yes
13	02/09/2008	300	300	<48 hours	Yes
13	16/09/2008	110	110	<36 hours	Yes
14	05/08/2008	13	13	48 hours	Yes
14	02/09/2008	30	30	>48 hours	Yes
14	16/09/2008	170	110	<36 hours	Yes
15	05/08/2008	2	2	60 hours	Yes
15	02/09/2008	2	2	<48 hours	Yes
15	16/09/2008	70	50	<36 hours	Yes

[a]The time for the black precipitate to form using the mH_2S strip test was recorded for each sample.

The mH_2S strip test did not correlate with the FC concentrations on the following sampling dates: at Site 3 on the 15th of April 2008, Site 5 on the 8th of May 2008 and Site 8 on the 4th of August 2008. There were result differences between the three tests which may be due to

chemical interferences, environmental stress or the ability to grow on the specific media (Tandlich et al., 2010b). Discrepancies are thus further investigated below.

2.6.1 Particle Attachment

One sampling occasion was used to determine whether the particular matter (sediment) on the rocks had high faecal coliform concentration. This was done to identify how river concentrations would change due to storms or re-suspension of this matter. Table 5 shows the FC concentration in the water column and the sediment on the representative stones collected. It can be noted that the water column has far higher microbial concentrations.
The highest percentage suspendable faecal coliform (PSFC) value was 2.6%, with the 1.83% and 0.21% the only values above 0.02%, which occurs 3 times (Tandlich et al., 2010b).
The game park sites had no PSFC. The median PSFC value was 0.02%, recorded for Sites 1, 6 and 10 (Tandlich et al., 2010b). Highest value is so low it would be negligible and below the precision value of the standard methods thus the attached particle sediment does contribute, but not significantly enough to affect the results at these sites (Tandlich et al., 2010b). The PSFC value is calculated by Equation 6, and represents the possible change in FC concentration on the suspension of particles attached to stones or sediment.

Equation 6: $$\mathbf{PSFC = 100 \times \frac{0.1 \times TSS \times FC_{Sediment}}{FC_{Water}}.}$$

The FC_{Water} is the concentration in the water without suspending a particular matter from the surrounding area. The TSS is the total suspended solids

Equation 7: $$\mathbf{FC_{Sediment} = \frac{0.75 \times FC_{Extracted}}{1000 \times \text{Stone Sediment Dry weight}}.}$$

Equation 7 was used to determine the FC concentration of the biofilm/sediment particles attached to the stones collected. This was recorded as MPN cells per milligram of sediment dry weight. The $FC_{Extract}$ is the MPN cells/100 mL from the Ringer's solution from the sediment extract. The value 0.75 is used to convert the MPN value to the 75 mL of Ringer's solution used to extract the FC. The final component is the dry weight of the sediment attached to the stone in grams (g), thus it is multiplied by 1000 to convert it to milligrams (mg).

Table 5: Evaluation of the particular attached bacteria on the results of the faecal coliforms and H_2S strip test at the selected sampling sites

Sampling site	Date of sampling in 2008	FC_{Water} (MPN cells /100 mL)	Sediment dry weight (g)	$FC_{Sediment}$ (MPN cells/mg sediment)	TSS (mg/L)	PSFC[a] (%)	H_2S strip test (in hours (h))
1	24/09	30	23.57	0.003	70.0	0.02	Water column: 28 h Sediment: 21 h
2	24/09	80	28.51	0.020	57.0	0.01	Water column: 28 h Sediment: 21 h
4	24/09	50	1.85	0.003	52.5	0.21	Water column: 28 h Sediment: 21 h
6	24/09	1600	22.81	1.22	60.0	0.02	Water column: 28 h Sediment: 37 h
9	10/12	540	0.98	0.18	340	2.60	Water column: 22 h Sediment: 22 h
10	10/12	79	6.78	26.1	57.5	0.02	Water column: 22 h Sediment: 22 h
11	15/12	> 16000	0.46	0.12	113	1.83	Water column: 16 h Sediment: 16 h
12	20/12	> 16000	ND[b]	ND	ND	ND	Water column: 12 h and no sediment sample
13	22/11	240	0.44	2.44	0	0.00	Water column: 22 h Sediment: 22 h
14	22/11	23	0.04	0.39	0	0.00	Water column: 22 h Sediment: 22 h
15	22/11	4	0.06	0.003	0	0.00	Water column: 18 h Sediment: 12 h

[a] The PSFC is the percentage suspendable faecal coliforms
[b] ND is not determined

2.6.2 Chemical Composition of Selected Sampling Sites

The chemical composition of the different sites may influence the survival of indicator and tracking bacteria. Thus the following parameters were analysed: alkalinity, conductivity, pH, temperature, ammonium, chloride, nitrate and phosphate. On one occasion, all the sites were analysed for an extended range to identify if these parameters were significant. Saccharides and proteins are commonly included in media used to enumerate indicator bacteria and were thus quantified in the water column. HPC concentrations indicate the total bacteria in the water which may compete for nutrients. Salinity was used instead of the total hardness. This provided the total ion concentration of magnesium and calcium and other anions or cations. The chlorophyll (chla) concentration was measured to estimate the algal biomass, as algae may potentially excrete antimicrobial compounds, changing the oxygen concentration of the water column.

The chemical composition varied between sites and can be seen in Table 6. DWAF (1996d) guidelines indicate that, for recreational purposes, pH should range between 6.5 and 8.5, while the aquatic guidelines state that most South African rivers are within the pH range of 6 to 8 (DWAF, 1996). The pH ranged between 6.21 (Site 10) and 8.62 (Site 15). Zuma (2010) reported a range of 5.30 to 8.00 for Sites 1, 2, 4 and 6. A pH range of 5.30 to 8.10 has also been reported for different sites along the Buffalo River (Palmer et al., 1994).This data is thus similar to other literature data. Sites 6, 9 and 15 however are slightly higher.

The turbidity values went up to 225 NTU (Site 1). Values for Sites 1, 2, 4 and 6 are slightly higher than the values determined by Zuma (2010), which had a maximum of 80 NTU.

Salinity concentrations ranged between 27mS/m (Site 10) and 202mS/m (Site 11), which was lower than those reported by Zuma (2010). It was a larger range than Palmer et al. (1994) established, with a range of 3.3 to 57.9 mS/m for separate Buffalo River sites. Salinity is high in the Buffalo River due to agricultural runoff, industrial effluents and anthropogenic activities (Palmer and O'Keeffe, 1990; Fatoki et al., 2001).

The total dissolved solids (TDS) were calculated by multiplying the salinity value by 6.5, for inland rivers, shown in Equation 8.

Equation 8: TDS = 6.5 × Salinity (DWAF, 1996)

DWAF guidelines use TDS as a measure of salinity. The electrical conductivity is a result of ions such as: calcium, carbonate, chloride, nitrate, sulphate, sodium, magnesium and potassium (DWAF, 1996). Values usually range between 5.5 and 7.5 (DWAF, 1996). Here, the values ranged between 177 (Site 10) and 1315 mg/L (Site 11). Guidelines indicate values above 65 mg/L are in contact with Precambrian shield areas, and a range of 200 - 1100 mg/L is in contact with Palaeozoic and Mesozoic sedimentary rock (DWAF, 1996). All these sites comply with these requirements (DWAF, 1996). Site 11, however, undergoes a low amount of evaporation, as the value is greater than 1100 mg/mL (DWAF, 1996).

The chlorides levels range between 24 mg/L (Site 1) and 410 mg/L (Site 12). Panno et al (2002) reported animal waste chloride levels between 9.3 and 980 mg/L, while septic tank waste ranged from 91 to 5620 mg/L. It has been a suggestion the river's salinity is increasing (Kefford et al., 2005).

The dissolved oxygen (DO) ranged between 2.50 mg/L (Site 12) and 15.8 mg/L (Site 6). These values were higher than Zuma's (2010). The usual value is 9.09 at 20 °C for unpolluted waters, with TDS values below 3000 mg/L (DWAF, 1996).Eutrophication is affected by the DO level of the site.

The river water column temperature ranged from 18.5 (Site 13) to 24.0°C (Site 12) between spring and early summer. This range does not have sufficient seasonal data to be compared to other literature effectively. These values are similar or lower than Zuma (2010) range of 9 to 26°C. Other literature for Buffalo River temperatures are between 8.8 and 22.8°C (Palmer et al., 1994), while a 1990 study reported a range of 6-22°C for winter and 14-35°C for summer (Palmer and O'Keeffe, 1990).

The light intensity in a shaded area ranged from 412 lx (Site 10) to 75086 lx (Site 15) on a very sunny day. It must be noted that the time of day affected these readings. Sunlight is reported to increase indicator die-off in river (Fujioka and Narikawa, 1982; Rhodes and Kator, 1990; Reed, 1997; Sinton et al., 2002; Noble et al., 2004; Sinton L et al., 2007; Schultz-Fademrecht et al., 2008; Boehm et al., 2009; Maïga et al., 2009; Sassoubre et al., 2011) and photodegradation of some pharmaceutical and aromatic chemical compounds

(Huang and Mabury, 2000; Andreozzi et al., 2003; Lin and Reinhard, 2005; Matamoros et al., 2009). Sensitivity of bacteria to solar inactivation may slightly depend on other environmental factors (Bolton et al., 2010). The faecal sterols would probably be photodegraded too, and the bacterial degradation may also differ.

Saccharide concentrations ranged between 4.2 mg/L (Site 13) to 39.4 mg/L (Site 9). There is very little data on the saccharide concentration of rivers and streams, as it is not often measured. Lake concentrations were mostly less than 10 μg/L (Meon and Jüttner, 1999). Bacteria use saccharides as a rapid energy source and thus it would be consumed quickly (Wetzel, 2001). It is not clear why this study's concentrations would be so high.

Protein was only detected in very low concentrations in the rivers, thus the method use was not sensitive enough to detect protein except at Site 9 which had 5.7 mg/L. The aqueous environment usually only has protein in association with humic substances from dead plant material, highly resistant to biodegradation (Wetzel, 2001). The other possibility is the release of protein and monosaccharides during the transition to the VBNC state (Arana et al., 2004). Grey water from eThekwini Municipality (Kwazulu- Natal, South Africa) had protein concentrations of 57 ± 4 mg/L (Arjun et al., 2006). The protein possibly originates from sloughed skin, which may also be the reason for Site 9's higher protein concentration, as it's a bathing dam.

Phosphate and nitrate concentrations will affect how much eutrophication the river has undergone. The phosphate concentrations ranged between below the detection level of 0.10 mg/L (Sites 6, 9, 10) to 7.00 mg/L (Site 12). Zuma (2010) showed a range of 0.02 to 9.5 mg/L for Sites 1, 2, 4 and 6. Thus his range was slightly wider than the present range. Phosphorus and chla concentrations were highest at Site 12, but no inorganic nitrogen was present. This high phosphorus level indicates the site's high eutrophic state, implying it has degraded significantly.

The ammonium (NH_4^+) concentration ranged from below the detection level of 0.01 mg/L (Sites 6, 9, 10, 12, 13, 14 and 15) to 4.95 mg/L (Site 11). Zuma (2010) had a range from below detection level to 4.12 mg/L. These results are very similar.

The nitrate (NO_3^-) concentrations range between below detection limit (BDL) of 1.0 mg/l (Sites 6, 10, 12, 13, 14 and15) to 22.0 mg/L (Site 4). Zuma (2010) had ranges from below the detection level to 27.86 mg/L, which were slightly higher than those of this study.

Sulphate (SO_4^{2-}) concentrations ranged between 5 - 20 mg/L at the level of detection (Sites 1, 2, 6) and 75 mg/L (Site 9). Zuma (2010) reported concentrations between 5.45 and 55.00 mg/L. These results were comparable for Sites 1, 2, 4 and 6. The highest value in this study was found at Site 9, was not included in Zuma's sampling sites (Zuma, 2010). Sulphate concentrations have been linked to diarrhoea when consumed in quantities by humans (DWAF, 1996c).

The total suspended solids (TSS) concentrations had a maximum of 340 mg/L (Site 9). The TSS is important as it affects the sunlight penetration of the water and the settling of bacteria attached to particulate matter.

The total alkalinity ranged up to 160 mg $CaCO_3$/L (Site 12). The chlorophyll a (chla) concentrations were between 0.5 and 1511 μg/L.

Heterotrophic bacteria concentration (HPC) ranged from 6×10^2 (Site 13) to 5.45×10^6 CFUs/L (Site 12). No literature was available on the Buffalo River for comparison purposes. The HPC concentration in the Western Cape rivers ranged between 3.00×10^3 and 3.6×10^5 CFUs/mL (Paulse et al., 2007), but a direct comparison is not possible due to environmental and anthropogenic input differences.

Table 6: The chemical composition of the water column at selected sites on a specific occasion of sampling in 2009.

Sites	pH	Turbidity	Salinity	TDS	DO	Cl^-	T	Light Intensity	HPC	SO_4^{2-}	Saccharides	Proteins	PO_4^{3-}	NH_4^+	NO_3^-	TSS	TA	Chla
		NTU	mS/m	mg/L	mg/L	mg/L	ºC	Lux	CFUs/ mL	mg/ L	mg GE/L	mg BSAE/L	mg/L	mg/L	mg/L	mg/L	mg $CaCO_3$/ L	µg/L
1	6.91	225	63	412	5.49	24	21.5	46100	2.40×10^4	5-20	6.1	< 2	1.49	1.43	11.7	70	0	1.3
2	7.25	139	67	438	4.33	31	23.0	54600	5.34×10^5	5-20	6.3	< 2	0.82	1.01	9.4	57	20	1.6
4	7.98	110	66	432	8.90	73	19.5	69000	8.70×10^4	30	5.7	< 2	1.05	1.08	22.0	53	10	0.1
6	8.21	153	82	530	15.8	77	22.7	34900	1.43×10^5	5-20	4.3	< 2	< 0.1	< 0.10	< 1.0	60	60	3.8
9	8.32	186	38	246	8.88	64	21.0	15400	6.49×10^5	75	5.3	5.7	< 0.1	< 0.10	4.28	340	0	5.6
10	6.21	0.0	27	177	7.49	40	19.9	412-1760	1.64×10^4	25	39.4	< 2	< 0.1	< 0.10	< 1.0	58	0	5.7
11	7.37	5.8	202	1315	5.58	207	22.0	3500	4.25×10^6	34	6.0	< 2	0.78	4.95	19.5	113	80	104
12	7.43	18.5	160	1042	2.50	410	24.0	5287	5.45×10^6	58	23.0	< 2	7.00	< 0.10	< 1.0	130	160	183
13	7.74	14.8	111	722	5.89	145	18.5	16000	6.00×10^2	53	4.2	< 2	2.07	< 0.10	< 1.0	0	100	2.6
14	7.71	23.0	114	740	6.01	154	20.0	14389	3.00×10^3	54	5.4	< 2	1.70	< 0.10	< 1.0	0	120	2.8
15	8.62	3.8	122	793	7.48	186	22.7	75086	3.00×10^3	28	5.0	< 2	0.56	< 0.10	< 1.0	0	70	2.9

TA – Total Alkalinity

On the whole, the chemical values compared well with previous literature and studies. This information will now be used to identify if the survival rates of bacteria are affected by the chemical composition of the water and to what extent these concentrations correlate to the indicator bacteria present in the water. The chemical parameters identified were used to identify any possible interference with the mH_2S strip test and conduct survival experiments with the HPC and faecal coliforms. Sulphate concentrations were not sufficiently high to cause a false positive reaction in the sulphate test, nor were the nitrate or nitrite levels sufficiently high to cause toxicity. But there may be a cause for concern, as some of the rivers are becoming eutrophic.

2.6.3 H_2S Test

The discrepancies between the H_2S strip test and the FC and *E. coli* tests have been reported by literature (Genthe and Jagals, 2003; McMahan et al., 2012). Possible inconsistencies between the total coliforms (TC) and FC results compared to those of the H_2S test were also reported (Genthe and Jagals, 2003). From a total of 40 collected samples, the mH_2S strip test was positive on almost every occasion the FC concentrations were 2 MPN cells/100 mL or larger. The only exception was at Site 7 on the 5th of June 2008, where it was negative.

The mH_2S strip test was negative when the FC and *E. coli* concentrations were zero per 100 mL on the 15th of April 2008 at Site 4. The higher the bacterial concentration, the faster the mH_2S strip test produced a positive result. This was also noticed by Genthe and Franck (1999). As this test is qualitative in nature, using time as a quantitative indicator is unreliable (Coulbert, 2005). The mH_2S strip test produced reliable results in water with both high and low bacterial concentrations.

Of the 40 samples collected, only 3 mH_2S strip test results did not correspond with the FC concentrations, producing a correspondence value of approximately 90%. This is higher than Genthe and Franck's (1999) value of 86%. It should be noted that at the detection limit of 2 cells/100 mL, half of the samples had a discrepancy between the two test results, which is far lower than the 90% total agreement. This was also noticed with tap water samples, where positive mH_2S strip tests occurred when the FC was below 0 cells/100 mL, but the heterotrophic plate count (HPC) was high e.g. 3200 CFUs/mL (Luyt et al., 2009; Tandlich et al., 2010a). This was also observed with rainwater samples (shown later in Chapter 5).

The mH_2S strip test results may have differed due to bacteria entering a viable-non-culturable state, caused by environmental stress and predation or pesticides (Rozen and Belkin, 2001; Winfield and Groisman, 2003; Na et al., 2006). Other possible interferences in the H_2S test results, identified by literature, were the concentration of sulphates, nitrites and nitrates. Sobsey and Pfaender (2002) proposed sulphate concentration could yield positive results despite the absence of microorganisms. Genthe and Jagals' (2003) environmental health professionals study questioned the interference of nitrate (NO_3^{--}) and nitrite (NO_2^{-}).

2.6.3.1 The Influence of Sulphate on the H_2S Test Results

The interference of sulphate (SO_4^{2-}) was tested at several different concentrations of sterile sodium sulphate (Na_2SO_4), ranging between 14 and 4240 mg/L. incubated in mH_2S strip tests for 72 hours. The mH_2S strip tests were checked every 12 hours for a black precipitate, but, as no black colour occurred, no false positives were seen. The negative results at both concentrations in triplicate are shown in Figure 2. The range of sulphates used was higher than the ranges identified in the rivers analysed in this study. The river ranges of this study are shown below in Table 6. Results can therefore be generalised for South African river conditions which have sulphate concentrations below 4240 mg/L.

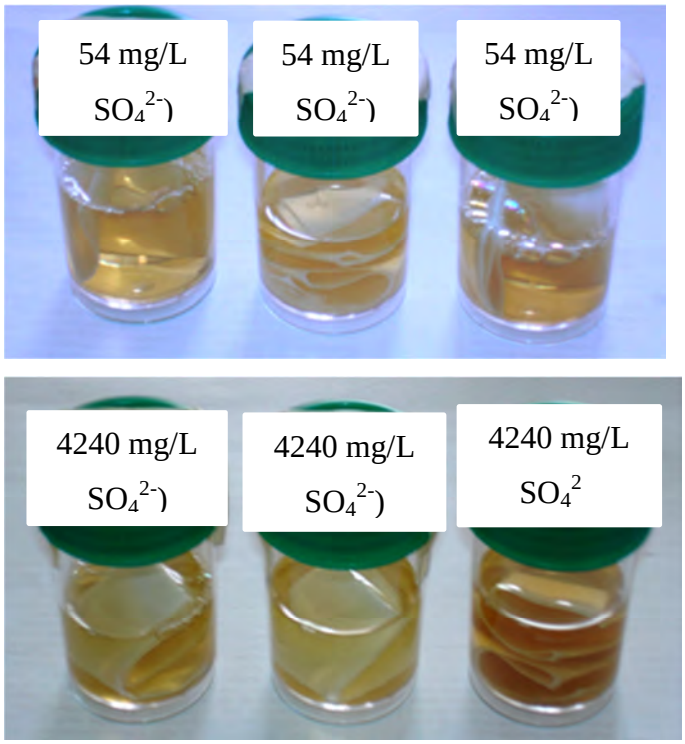


Figure 2: The potential sulphate (SO_4^{2-}) interference with the results of the H_2S strip test at sulphate concentrations of 54 mg/L (above) and 4240 mg/L (below).

2.6.3.2 The Influence of Nitrates and Nitrites on the H_2S Test Results

Genthe and Jagals et al (2003) proposed that high nitrate and nitrite concentrations could interfere with positive results. River nitrate and nitrite levels were usually below 1 mg/L, although the highest recorded was 47 mg/L (see below). Streams are low in nitrogen, as shown by a study of 20 of South Africa's largest rivers, with ranges of 20 to 3085 μg N $L^{-1}$and a median of 158 of the medians, conducted between 1970 and 2005 (De Villiers and Thiart, 2007). The cut off value of 400 μg N L^{-1} is designed to protect aquatic animals, while values below 50 μg N L^{-1} would be considered pristine water (De Villiers and Thiart, 2007). The median values of nitrate, nitrites and ammonium exceed 400 μg N L^{-1} in the Swartkops, Phongola, Upper Orange and Great Fish rivers annually (De Villiers and Thiart, 2007).

Nitrate levels are decreased in water by the conversion of nitrates to nitrites by enteric bacteria (Mara and Horan, 2003; Madigan and Martinko, 2006). Nitrites are usually quickly converted to nitrate and ammonium (NH_4^+) due to denitrification and nitrification processes, so it is highly unlikely that nitrate and nitrite toxicity cause interferences in the results of the mH_2S strip test.

Pseudomonas aeruginosa and *Acinetobacter spp*., members of the hydrogen sulphide producing bacteria, could possibly be inhibited by high nitrate or nitrite concentrations. Rowe et al (1979) stated that glucose uptake in *Pseudomonas aeruginosa* was affected by 25mM concentrations (155 mg/L NO_3^-), far higher than the rivers' concentrations. The phosphate-accumulating strain of *Acinetobacter spp*. is inhibited by 20% with nitrite levels of 604 mg/L (Weon et al., 2002). *Acinetobacter junii* can be inhibited by very low levels of nitrites, but as this is not a significant faecal bacteria, it will have a minimal effect on the results (Hrenović et al., 2007).

The impact of temperature fluctuations during sample collection, transport and incubation are unknown (Genthe and Jagals, 2003). The mH_2S strip test was incubated at room temperature which ranged between 18 and 25°C. Environmental temperature outside ranged between 10 and 35°C during sample collection. All samples were analysed within 5 hours of collection. Sampling Site 10 and 11 were used for this experiment, as Site 10 usually has low faecal coliform concentrations, while Site 11 has very high faecal coliform concentrations up to 16000 cells/100 mL (see Table 4, site descriptions Sampling Site 10 and 11).

2.6.3.3 Effect of Transportation Temperature on the H_2S Test Results

The two extreme temperatures of 32°C and 18°C stimulated the fluctuation experienced by the samples. The sample incubated at 32°C was positive approximately 12 hours earlier than the control, while the sample incubated at 10°C had a 5 hour delay for a positive result. Thus temperature fluctuation did not have a significant impact on colour development. The arithmetic average from duplicates showed that faecal coliform concentration was 36 CFUs/100 mL at Site 10 and 10^4 CFUs/100 mL at Site 11.

Temperature influences the time taken for a positive reaction to occur, but not significantly enough for positive reactions to occur outside cut-off times. Sulphates and nitrates do not cause false positives within the range found in rivers. The most likely explanation for discrepancies is the loss of culturability due to the bacteria entering of viable non-culturable (VBNC) states (Barer and Harwood, 1999; Rowan, 2004; Servais et al., 2009). Some of the FC, *E. coli* and the H_2S strip test bacteria may enter the VBNC state. Thus survival studies were used to identify this aspect. Figure 3 and Figure 4 show the development of a positive result and the time difference which occurs due to 5 hour exposure to 32°C or 10°C. Figure 3 shows a positive result from the sample incubated at 32°C at 16 hours while the control and sample incubated at 10°C are still negative. At 28 hours only the 10°C sample is still negative, while at 21 hours all the tests are positive. Thus the sample incubated at 10°C still produced a positive result within the 72 hour time limit. Grey dam (Site 10) usually had lower faecal pollution than Gowie dam (Site 9) highly polluted. Figure 4 shows that only the 10°C sample was negative at 16 hours and all the samples were positive at 21 hours. The control was positive in Figure 4 than in Figure 3 probably due to the higher faecal contamination and thus larger bacterial concentration in the dam water. Once again this demonstrates that the results were positive within the 72 hour time limit despite the delay caused by incubation at 10°C for 5 hours. The laboratory control never produced a positive result as it had sterile water added to it. Thus it indicates that the positive results were not due to bacterial contamination of the test.

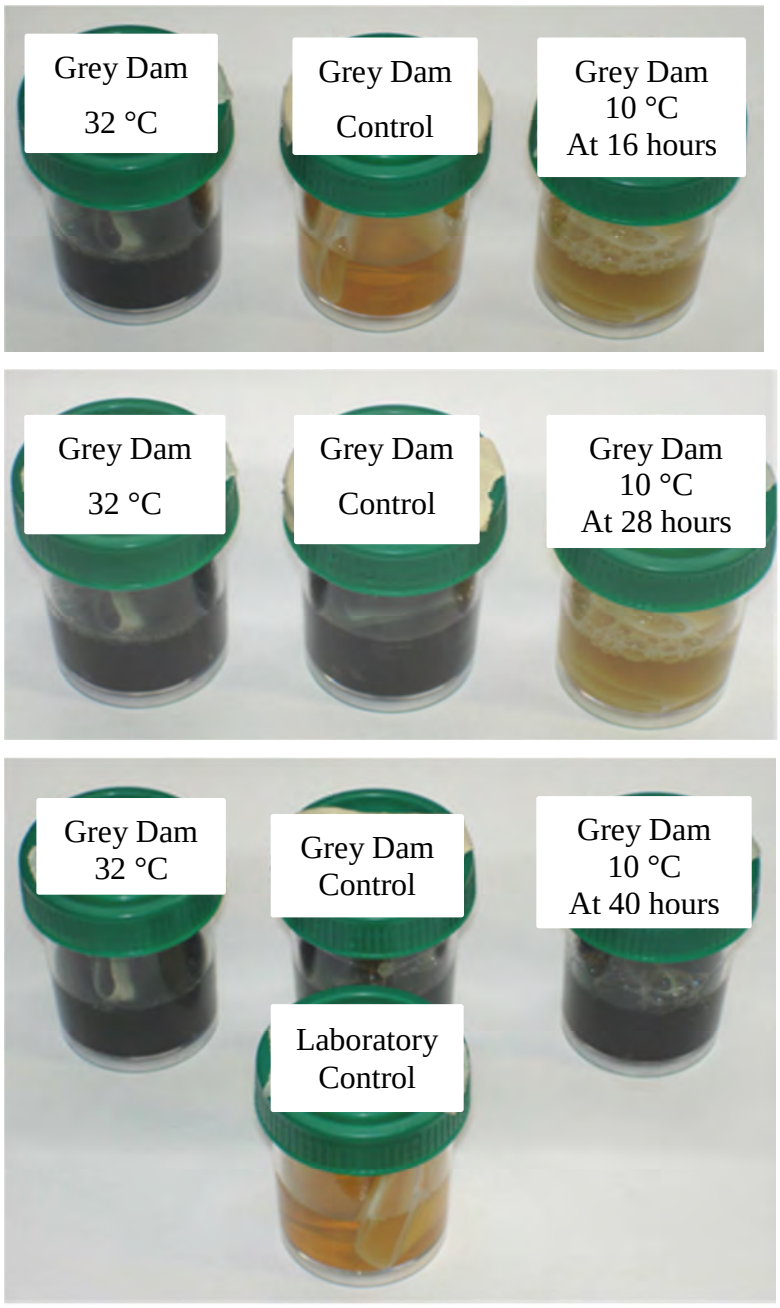


Figure 3: Influence of temperature fluctuations on the results of the H_2S strip test during the twelve-hour sampling trip at Site 10 after 16 hours (a), 28 hours (b) and 40 hours (c) of incubation.

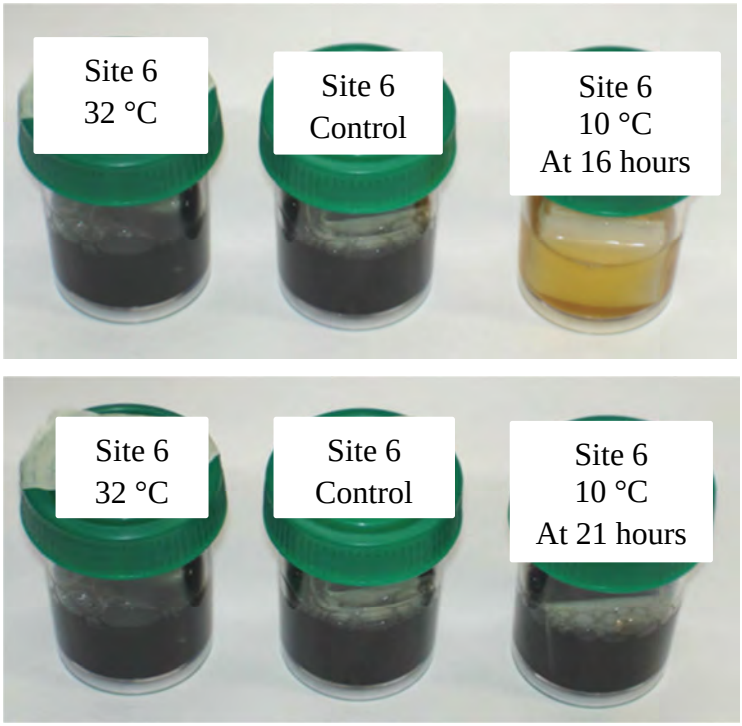


Figure 4: The influence of temperature fluctuations during sampling and transport on the results of the H_2S strip test during the five-hour sampling trip in water samples from Site 11 after 16 hours (a) and 21 hours (b).

2.6.4 Survival Experiments for Faecal Coliforms

Faecal coliforms were used in order to compare this data to published data. Model water was developed from the chemical concentrations seen in literature and site concentrations shown in Table 6. The model water composition is shown in Table 2. Survival experiments were started with 4.60×10^5 CFUs/100 mL faecal coliforms and 4.80×10^4 CFUs/mL of heterotrophic bacteria. After 15 hours of incubation, the FC concentration increased to 9.00×10^6 CFUs/100 mL in nutrient restricted model water, while in nutrient rich model water with saccharides and proteins, it increased to 2.21×10^9 CFUs/100 mL. This increase could be due to growth resulting from the availability of nutrients. More growth was seen in nutrient rich versus nutrient restricted water. The heterotrophic bacteria concentration increased from 4.80×10^4 CFUs/mL to 8.80×10^7 CFUs/mL in the nutrient restricted model water (Tandlich et al., 2010b), while the nutrient rich model water increased from 4.80×10^4 CFUs/mL to 4.12×10^6 CFUs/mL (Tandlich et al., 2010b). Both model waters had 0 CFUs/mL of HPC at 696 hours. The H_2S strip test showed positive results until 528 hours of the survival incubation, but were negative after 696 hours. The FC concentration was at 1.96×10^5 CFUs/100 mL in the nutrient restricted water while the nutrient rich water had

0 CFUs/100 mL at 696 hours. The bacteria may have entered a VBNC state, thus they are undetectable using culture methods. Thus there may be a discrepancy between the H_2S test and FC enumeration at 22 and 29 days. This may be a possible cause of the discrepancies observed during screening and shown in Table 3 and Table 4. These preliminary studies are further discussed in Chapter 3, along with the proposal that protein affected survival.

2.7 CONCLUSION

The selected sampling site had positive results for all three tests on at least two separate random occasions. The sites which did not have continuous faecal pollution were not studied further. The chemical conditions of the water were determined for each site. This provided the maximum concentration of sulphate and nitrate to be used for the nitrate test and later for survival experiments.

The mH_2S strip test has approximately a 90% correspondence with FC in rivers, while it corresponds more closely to heterotrophic bacteria in distribution water. The concentrations of nitrates and sulphates do not cause false positives or negatives within concentrations found in South African rivers. The transportation of the mH_2S strip test on ice causes delays in positive results, however they don't fall outside the specified time period. The HPC and FC survival shows that discrepancies between the FC enumeration and the mH_2S strip test would be between 22 and 29 days.

CHAPTER 3: FAECAL SOURCE TRACKING, SURVIVAL RATES AND OTHER PROBLEMS

3.1 INTRODUCTION

Piped water is still unavailable to 10.5% of the South African population, so many rural South Africans rely on alternative water sources (Statistics South Africa, 2011d; Statistics South Africa, 2012d). Numerous pipe breaks (PMG, 2009a) also affect access. These water sources are not always of adequate drinking water quality (Duse et al., 2003; Momba et al., 2006a; Tandlich and Muller, 2008). The IRIN (Integrated Regional Information Networks) study of 2009 estimated 5 million South Africans lack access to any safe drinking water source (IRIN News, 2009). South Africa had approximately 78% of the rural population with improved water in 2008, while 99% of the urban population had access to improved water sources (UNICEF and WHO, 2010).

Only 74,8% of households in the Easter Cape have access to piped water (Statistics South Africa, 2012d). Of 22 % of the rural population in South Africa without access to tap water, approximately 13 % used river water as their water source (WHO and UNICEF, 2010c). Rivers are often polluted by wastewater plants not being operated efficiently (Momba et al., 2006a), while pastures and roaming cattle also pose a problem (Spooner et al., 1992; Crowther et al., 2002; Collins and Rutherford, 2004).

Water quality is monitored using indicator bacteria to identify potential problems (Cunliffe et al., 2003; WHO, 2003). They are used as they are easy to culture and have similar survival rates as pathogens, but are often not pathogenic themselves (José Figueras and Borrego, 2010; Luyt et al., 2011a; Chowdhury, 2012). Water quality is determined by a result comparison against South African National Standards (SANS 241 of 2005)(Viljoen, 2006). But problems of lack of water treatment and pathogens of parasites entering the system cannot all be solved at this point in the chain. A safety plan is needed to prevent poor quality water entering water treatment plants (Viljoen, 2006). The process of eliminating water diseases requires steps outlined by the World Health Organisation (WHO), such as: prevention of source water pollution preventing of contamination at storage; treatment of water and prevention of contamination during distribution and household storage, or treatment at the point of use (Davison et al., 2005). Water therefore needs to be monitored at

the catchment level. Catchment management agencies and water management areas must be defined as stated in the Act.

The South African National Microbial Monitoring Programmes (NMMP) is designed to monitor surface water and the microbial water quality in areas highly prone to faecal contamination (Murray et al., 2004). *Escherichia coli* (*E. coli*) is monitored using culture based methods, and preferably, chromogenic media, namely Colilert -18 (Murray et al., 2004). Laboratories are therefore geared for culture based methods. The NMMP in South Africa uses *E. coli* as its main faecal indicator (Murray et al., 2004). But *E. coli* is excreted from both humans and animals and so cannot identify the source of faecal pollution (Viljoen, 2006). Identifying the source of faecal pollution using tracking bacteria or source tracking methods helps identify the potential risk posed by the water to humans (Scott et al., 2002; Field and Samadpour, 2007; Roslev and Bukh, 2011). Microbial source tracking is a collection of methodologies aimed at identifying the source of faecal contamination and possibly quantifying the extent of such contamination (Stoeckel and Harwood, 2007). Source tracking is currently not performed in South Africa.

Any new method needs to be compared to the older, well-established methods of *E. coli* or indicator bacteria monitoring. This means a comparison of survival rates between *E. coli*, bifidobacteria and enterococci is essential.

Multiple source tracking indicators have been suggested over the years and multiple reviews published (Scott et al., 2002; Simpson et al., 2002; Meays et al., 2004; Seurinck et al., 2005; Blanch et al., 2006; Savichtcheva and Okabe, 2006; Field and Samadpour, 2007; Stoeckel and Harwood, 2007; Hagedorn and Weisberg, 2009; Hagedorn et al., 2011; Roslev and Bukh, 2011). There are two main groups of indicators used for source tracking. Library dependent methods require a database or library of information from different animal and human sources to which phenotypes, genotypes or other traits can be matched (Field and Samadpour, 2007; Stoeckel and Harwood, 2007). They are mainly culture based methods (Stoeckel and Harwood, 2007), while the library independent methods are a mixture of culture and chemical based techniques. Library independent methods rely on isolating bacteria which are specific to certain populations e.g. animals only or chemicals such as sterols, optical brighteners which are present due to human or animal presence and ratios of different bacteria (Field and Samadpour, 2007; Stoeckel and Harwood, 2007).

Bifidobacteria has been proposed as a library independent method for human source tracking (Mara and Oragui, 1983; Carrillo et al., 1985; Jagals and Grabow, 1996a; Blanch et al., 2006; Bonjoch et al., 2009). Bifidobacteria are Gram positive anaerobic bacteria naturally found in human and warm blooded animal digestive tracts (Mara and Oragui, 1983; Mitsuoka, 1990; Bonjoch et al., 2005; Madigan and Martinko, 2006). They are part of the actinomycetes family and are their own genus and not part of *Lactobacillus*, as previously thought (Ballongue, 2004). Of the 32 recognised bifidobacteria found in human digestive tracts, the following are the most common *Bifidobacterium* strains: *B. pseudocatenulatum, B. longum, B. angulatum, B. adolescentis, B. catenulatum, B. bifidum, B. gallicum, B. infantis*, and B. *breve* (Wilson, 2005). Two different groups have been proposed as sorbitol utilising bifidobacteria (SUB), found almost exclusively in humans, and the total bifidobacteria (TB) (Resnick and Levin, 1981a; Mara and Oragui, 1983; Beerens, 1998; Matsuki et al., 1999; Bonjoch et al., 2005). Bifidobacteria are suggested as good indicators as they are excreted in adult faeces, at concentration ranges of 7.9×10^4 to 2.5×10^{13} CFUs (colony forming units)/g dry weight of faeces (Mara and Horan, 2003; Wilson, 2005), while Tannock (1997) reported 10^9-10^{10} CFUs /g. Bifidobacteria are used as probiotics (Borriello et al., 2003; Collado and Sanz, 2006) and, as such, are often found in higher concentrations than other faecal coliforms. Ottoson (2009) also found higher concentrations of bifidobacteria in comparison to faecal coliforms (FC).

They are unlikely to multiply in water, as their optimal growth temperature is between 37 and 38 °C in humans and between 41 and 43°C in animals, although growth has been reported between 20 and 49.5°C (Ballongue, 2004; Wilson, 2005). Surface water (river) temperatures seldom reach 30°C (Carrillo et al., 1985; Sinton et al., 1998; Nebra et al., 2002). Bifidobacteria are reported to die above 60°C (Ballongue, 2004), which is far higher than river temperatures. Their growth in river or oligotrophic environments is further limited by their nutritional requirements of ammonium salts or organic nitrogen. *B. bifidum* requires magnesium, manganese and iron (Ballongue, 2004). Bifidobacteria have been shown to produce bacteriocins and prevent enteropathogen attachment or prevent their growth such as *Vibrio cholerae, Shigella sonnei, Campylobacter spp., Salmonella spp. and E. coli spp*. (Wilson, 2005).

In comparison to the bifidobacteria levels excreted, the *E. coli* concentrations range from 7.9×10^3 to 2×10^{12} CFUs /g dry weight of faeces and streptococci concentrations fall within approximately the same range. Streptococci are considered important pollution monitoring bacteria. However, enterococci and *E. coli* cannot be used as library independent methods. Faeces of adult humans also contain *Bacteroides spp.*, *Clostridium spp.*, *Streptococcus spp.*, anaerobic cocci and *Lactobacillus spp*., with infants' faeces containing slightly different strains and concentrations (Wilson, 2005). Thus *Bacteroides* which range from 1.5×10^9 to 3.2×10^{13} CFUs /g dry weight of faeces (Wilson, 2005) have also been proposed as tracking indicators.

The source tracking method uses these two groups to identify the pollution source with a tracking ratio (Jagals and Grabow, 1996a; Ballesté and Blanch, 2011). The tracking ratio is the ratio of sorbitol utilising bifidobacteria (SUB) to the total bifidobacteria (TB) concentrations (see Equation 1, Chapter 1). The cut-off value is 3.2 or 0.5 $\log_{10}$, so values above this indicate human pollution, while indicating animal sources (Blanch et al., 2006). Previously, Bonjoch et al. (2005) used a cut off of 0.2 for human pollution in a much smaller study. But the survival and enumeration of bifidobacteria has not yet been fully elucidated. The cut-off value varies geographically (Blanch et al., 2006), and thus needs to be calibrated for South Africa.

There is uncertainty about the bacteria's ability to survive in the environment and its activity outside the host (Carrillo et al., 1985). Survival experiments for bifidobacteria have been performed in sterilised ground water, surface water and in phosphate buffered saline. Not only have these different survival experiments been conducted under different environmental conditions, but they have also used on different agars and are thus not necessarily comparable. Resnick and Levin (1981a) showed that bifidobacteria die off as much as 26 times faster than *E. coli*, while they reported their rates to be similar to those of Gyllenberg et al. (Gyllenberg et al., 1960; Resnick and Levin, 1981a). Bifidobacteria were reported to survive 5 hours in fresh and 10 hours in marine water, at 20°C, and the ratio of bifidobacteria to *E. coli* fell off rapidly with increased distance from the point source (Resnick and Levin, 1981a). They also found that the survival rate was dependent on temperature in fresh surface water, while marine water did not seem to follow this trend (Resnick and Levin, 1981a).

Ottoson (2009) found the die-off rate of 3.9 CFUs/ mL per day in surface water at 22°C higher than the die-off rate of *B. adolescentis* reported by Resnick and Levin (1981a). Survival rate at 4°C was 1.4 CFUs/ mL a day (Ottoson, 2009). This was faster than FC in the same medium. But the effects of changing the chemical constituents or their concentrations in river water have not been established. Some data can nevertheless be extrapolated as to bifidobacteria' s sensitivity to different environmental parameters from the extensive dairy survival experiments (Shah et al., 1995; Shah and Lankaputhra, 1997; Hansen et al., 2002; Wang et al., 2002; Bolduc et al., 2006; Jayamanne and Adams, 2006; Jayamanne and Adams, 2009; Saarela et al., 2011). From these studies, it can be noted that bifidobacteria are sensitive to pH (Shah et al., 1995; Jayamanne and Adams, 2006; Jayamanne and Adams, 2009), redox potential (Jayamanne and Adams, 2006; Jayamanne and Adams, 2009) and the difference between species in relation to oxygen sensitivity (Jayamanne and Adams, 2009). Oxygen toxicity occurs due to the absence of superoxide dismutase and catalase, which scavenges reactive oxygen species (Jayamanne and Adams, 2009). Bifidobacteria species have different concentrations of NADH-peroxidase, an enzyme which can convert H_2O_2 to water (Jayamanne and Adams, 2009). This means a decrease in redox potential increases their survival rate.

The pH tolerance of different species depends on H^+-ATPase (ATP-Adenosine triphosphate enzyme) activity, which act as a pH maintenance pump (Matsumoto et al., 2004). Co-culturing with *Lactobacillus*, which acts as an oxygen scavenger in dairy products, increases survival rates (Lourens-Hattingh and Viljoen, 2001). Bolduc et al. (2006) showed electro-reduction increased bifidobacteria survival in deaerated yoghurt. Supplementing soymilk with glucose, lactose, galactose, yeast extract, peptone, tryptone or casitone increased the growth of bifidobacteria (Chou and Hou, 2000). The addition of cysteine, whey protein, acid casein hydrolysates or tryptone increased bifidobacteria viability (Dave and Shah, 1998). Higher survival rates in buffalo curd milk, which has a high protein and fat content compared to skimmed milk, allude to an increased survival due to protein (Jayamanne and Adams, 2006). Heat and oxygen tolerance change across species, while storage above 8°C seems to be associated with increased die-off rates (Simpson et al., 2005). Drops in pH or the increase in acidity and redox potential depended on the added ingredients. The addition of cysteine, whey protein concentrate, acid casein hydrolysates or tryptone improved the viability of bifidobacteria to a variable extent, which whey powder failed to do. Survival rates of bifidobacteria in dairy products aid in identifying their sensitivity to different environmental

conditions, but it must be emphasised that dairy products are buffered and contain far more nutrients than water environments, and so only general trends can be extrapolated when predicting survival rates in water.

The enumeration of bifidobacteria on media is dependent on their being sufficient nutrients to resuscitate them and for growth. VBNC state bacteria cannot be identified by culturing, thus PCR techniques have shown longer survival rates. Enumeration media do not affect the survival of their bifidobacteria content, but may affect the ability to enumeration them and possibly increase reports of accelerated die-off rates. Over the years, different media have been developed to improve selectivity, sensitivity and cultivability on agar. The most appropriate enumeration method is still the subject of debate (Ballongue, 2004). Initially Bifidobacteria media used sodium azide and ascorbic acid as selective agents (Ballongue, 2004). Next, Beck et al. added N-acetyl-D-glucosamine and a relatively low pH of 5.8 and an anaerobic environment to eliminate most enterobacteria (Ballongue, 2004). Chang et al in 1983 modified MRS agar by adding cysteine and china ink (Ballongue, 2004). The addition of antibiotics was tried to increase the media's selectivity, however some inhibition of certain *Bifidobacterium spp.* was a concern as antibiotic resistance differed among species.

Resnick and Levin (1981b)developed YN-6 agar, which was modified by Mara and Oragui (1983) to YN-17 agar in 1981, the same time human bifido (bifidobacteria) sorbitol agar (HBSA) was developed. Development of HBSA for the enumeration of bifidobacteria specifically associated with humans was done by Mara and Oragui (1983) to try eliminate overgrowth of Gram positive cocci, faecal streptococci in particular. YN-6 differed from YN-17 in the antibiotic. YN-6 used Neomycin (2.5 mg/mL), while YN-17 uses kanamycin (50μg/mL) and polymyxin B (50 IU/mL) (Resnick and Levin, 1981b; Mara and Oragui, 1983) Matteuzzi et al (1983) added kanamycin and polymyxin B, as most bifidobacteria are resistant to these antibiotics. This has also been shown by others (Mayrhofer et al., 2011). In order to increase selectivity, Bifidobacterium iodoacetate medium 25 (BIM-25), which is based on reinforced clostridial medium (RCM) with antibiotics such as nalidixic acid, polymyxin B, kanamycin added, was developed (Muñoa and Pares, 1988). Use of a low pH media reduced by propionic acid, has been shown to stimulate bifidobacteria growth with organic acids (Salminen et al., 2004). Thus Beerens media, with a Columbia agar base, was developed and commonly used (Beerens, 1991; Ballongue, 2004). The reduced pH helped limit the number of bacteria able to grow (Salminen et al., 2004). Addition of iodoacetic acid

inhibited glyceraldehyde-3-phosphate dehydrogenase and decreased growth of other bacterial species (Muñoa and Pares, 1988; Ballongue, 2004). The dye 2, 3, 5-triphenyltetrazolium chloride (TTC) is colourless, relatively soluble in the oxidised form and reduced enzymatically to a red, insoluble triphenyl formazan (TF) precipitate in cells by actively respiring and growing bacteria (Tian et al., 2006; Sigma Aldrich, 2012; Sun et al., 2012). The dye acts as a terminal acceptor in biochemical reactions (Sun et al., 2012). Red salt forms due to a reaction between the TTC iron and hydrogen in the cell (Sun et al., 2012). The dye is sensitive to oxidation and light (Sun et al., 2012). It has been beneficial in food microbiology as it only identifies living bacteria and distinguishes between food debris (Beloti et al., 1999; Ballongue, 2004).It also helps identify bifidobacteria specifically, as they form white colonies (Ballongue, 2004). In order to change the media, the following vitamin requirements for human strains are required: thiamine, pyridoxine, folic acid, cyanocobalamine and nicotinic acid for growth, while *B. adolescentis* and *B longum* can grow in unsupplemented media (Ballongue, 2004). Optimal pH range is between 6.5 and 7, while they can survive in a range between 4 to 8.5 (Wilson, 2005). No growth occurs below pH 5 or above 8 (Ballongue, 2004). They are acid tolerant and produce a mixture of lactic and acetic acid (Wilson, 2005; Adams and Moss, 2008).

Table 7 summarises the antibiotic resistances and sensitivities of bifidobacteria and enterococci. The information is also presented below in the text. Bifidobacteria is resistant to nalidixic acid, gentamicin, kanamycin, metronidazole, neomycin, polymyxin B and streptomycin, while erythromycins and penicillin inhibit the bacteria (Ballongue, 2004).

Enterococci are resistant to ciprofloxacin, tetracycline, rifampicin, gentamicin, streptomycin and sometimes ampicillin and penicillins, cefotaxime, lincomycin and aminoglycosides (Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012), susceptible to vancomycin, nitrofurantoin (Pangallo et al., 2008; Patel et al., 2011), benzyl penicillin, ampicillin, linezolid, tigecycline, daptomycin (Kuch et al., 2012) and teicoplanin (Foulquié Moreno et al., 2003).

Bifidobacteria are resistant to nalidixic acid, polymixin B, gentamicin, streptomycin mupirocin (Thitaram et al., 2005), metronidazole tetracycline, erythromycin and clindamycin antibiotics (Delgado et al., 2005; Moubareck et al., 2005a; Moubareck et al., 2005b; Masco et al., 2006; Ammor et al., 2007). Only selected strains are resistant to vancomycin and

erythromycin (Charteris et al., 1998), others to clindamycin, cefoxitin and, possibly, tetracycline (Delgado et al., 2005; Moubareck et al., 2005b). Animal bifidobacterial strains, such as *B. animalis*, *B. pseudolongum,* and *B. thermophilum,* are resistant to ampicillin, clindamycin, erythromycin, gentamicin, kanamycin, neomycin, streptomycin, tetracycline and vancomycin (Mättö et al., 2006; Mayrhofer et al., 2011).

Many animal bifidobacteria species are also susceptible to linezolid, quinupristin/dalfopristin and chloramphenicol (Delgado et al., 2005; Ammor et al., 2007; D'Aimmo et al., 2007; Delgado et al., 2008; Ouoba et al., 2008). But Kheadr et al.(2004) have demonstrated slight resistance to chloramphenicol in some strains. Increased resistance to trimethoprim has been identified (Ouoba et al., 2008; Mayrhofer et al., 2011).

Bifidobacteria are susceptible to Gram positive spectrum antibiotics e.g. macrolides, erythromycin, vancomycin, rifampicin, chloramphenicol, penicillin and beta lactams (Delgado et al., 2005; Moubareck et al., 2005b; Masco et al., 2006; Ammor et al., 2007). The problem with enterococci cannot be overcome by higher antibiotic concentrations, as enterococci are resistant to many of the same antibiotics as bifidobacteria. Vancomycin is one exception, although it would be inhibitory of too many bifidobacteria to be useful for increasing selectivity.

DNA typing or the use of a digoxigenin (DIG)-labelled oligonucleotide probe was suggested by Lynch et al. (2002). This would increase the cost as well as the amount of equipment needed to analyse water. In South Africa, this would also limit the amount of laboratories capable of performing such analysis. South African laboratories are equipped for bacteria culturing and, possibly, viral enumeration, but no PCR or DNA work is conducted in them. Using molecular methods would require expensive equipment and highly qualified technicians, prohibitive in a country where cost and staff shortages are already cause for concern.

Table 7: The antibiotics resistances (R) and sensitivities (S) of *Bifidobacterium spp.* and *Enterococcus spp.* compared.

Antibiotics	***Enterococcus***		***Bifidobacterium***	
Ciprofloxacin	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	Some S	(Masco et al., 2006)
Tetracycline	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	R Some sensitive	(Delgado et al., 2005; Moubareck et al., 2005b)
Rifampicin	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	S	(Masco et al., 2006)
Gentamicin	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	R , animal strains sensitive	(Thitaram et al., 2005; Mättö et al., 2006; Mayrhofer et al., 2011)
Streptomycin	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	R Animal strains S	(Thitaram et al., 2005; Mättö et al., 2006; Mayrhofer et al., 2011)
Penicillins: Ampicillin, Benzyl Penicillin,	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	R Animal strains S	(Delgado et al., 2005; Moubareck et al., 2005b; Masco et al., 2006; Mättö et al., 2006; Ammor et al., 2007; Mayrhofer et al., 2011; Kuch et al., 2012)
Cefotaxime,	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	S	(Auras et al., 2012)
Lincomycin,	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)		
Aminoglycosides	R	(Pangallo et al., 2008; Patel et al., 2011; Kuch et al., 2012)	R	(Mayrhofer et al., 2011)
Vancomycin	S	(Pangallo et al., 2008; Patel et al., 2011)	R – some strains S - Animals strains	(Charteris et al., 1998) (Mättö et al., 2006; Mayrhofer et al., 2011)

Table 7: The continuation of the comparison of the antibiotic resistance (R) and sensitivities of *Bifidobacterium spp.* and *Enterococcus spp.*.

Nitrofurantoin	S	(Pangallo et al., 2008; Patel et al., 2011)	S	(Auras et al., 2012)
Linezolid, Tigecycline Daptomycin	S, but some R	(Palmer et al., 2011)	R	(Kuch et al., 2012)
Teicoplanin	S, some R	(Gagnon et al., 2011; Lai et al., 2011)	R	(Foulquié Moreno et al., 2003)
Nalidixic Acid, Polymixin B, Mupirocin Cefoxitin	R at low concentrations	(Marothi et al., 2005)	R	(Thitaram et al., 2005) (Delgado et al., 2005; Moubareck et al., 2005b)
Metronidazole Clindamycin	Some R	(Marothi et al., 2005)	R Animal strains S	(Delgado et al., 2005; Moubareck et al., 2005a; Moubareck et al., 2005b; Masco et al., 2006; Mättö et al., 2006; Ammor et al., 2007; Mayrhofer et al., 2011)
Erythromycin	R - some	(Zou et al., 2011)	R – Some strains. Animal strains S	(Charteris et al., 1998; Mättö et al., 2006; Mayrhofer et al., 2011)
Linezolid, Quinupristin/Dalfopristin, Rifampicin,			S	(Delgado et al., 2005; Ammor et al., 2007; D'Aimmo et al., 2007; Delgado et al., 2008; Ouoba et al., 2008)
Chloramphenicol	Some R	(Wood and Murray, 2000; Gould et al., 2004)	R –some, others S	Kheadr et al.(2004; Delgado et al., 2005; Moubareck et al., 2005b; Masco et al., 2006; Ammor et al., 2007).
Trimethoprim	Some R	(Cattoir et al., 2009)	R	(Ouoba et al., 2008; Mayrhofer et al., 2011)

R= Resistance and S= Sensitivity

Enterococci which overgrow the bifidobacteria media are Gram positive cocci, catalase negative, non-spore forming, aero-tolerant anaerobe bacteria which are natural inhabitants of human and animal gastrointestinal tracts (Salminen et al., 2004; Wilson, 2005; Madigan and Martinko, 2006). There are seventeen species of which only *Enterococcus faecalis* and *Enterococcus faecium* are regularly found in the human gastrointestinal tract and dominate the human bowel and faeces, with concentrations between 10^4 and 10^7 CFUs/g (Chenoweth and Schaberg, 1990; Salminen et al., 2004; Wilson, 2005). Some enterococci have been used as probiotics (Salminen et al., 2004). But the concentrations of *Enterococcus faecalis* and *Enterococcus faecium* change across geographical regions due to diets (Chenoweth and Schaberg, 1990). *Enterococcus faecalis* and *Enterococcus faecium* are far less dominant in livestock, such as cattle and sheep (Vancanneyt et al., 2002; Mara and Horan, 2003).

Survival data from milk-modelled experiments proposed that the supplementation of soymilk with glucose, lactose, galactose, yeast extract, peptone, tryptone or casitone increased the growth of bifidobacteria (Chou and Hou, 2000). The addition of cysteine, whey protein, acid casein hydrolysates or tryptone increased bifidobacteria viability (Dave and Shah, 1998). A higher survival rate in Buffalo curd milk, with high protein and fat content when compared to skimmed milk, may allude to an increased survival due to protein (Jayamanne and Adams, 2006). Heat and oxygen tolerances change across species, while storage above 8°C seems to be associated with increased die-off rates (Salminen et al., 2004; Simpson et al., 2005; Wilson, 2005; Madigan and Martinko, 2006).

Enterobacter is found in the large intestine in a pH 7 environment, like bifidobacteria, as well as in the small intestines (Salminen et al., 2004; Wilson, 2005). The faecal streptococci group has been divided into *Streptococcus, Lactococcus and Enterobacter*, according to DNA, rRNA (ribosomal ribonucleic acid) hybridisation studies (Salminen et al., 2004). The group includes streptococci from faecal origins (Madigan and Martinko, 2006), with the organisms associated with the intestinal tract classified as the *Enterobacter* genus (Salminen et al., 2004). Identification often requires phenotypic, genotypic and phylogenetic information (Salminen et al., 2004). Enterococci are often found in soil, surface waters, wastewater treatment plants and in the gastrointestinal tract of warm blooded animals (Salminen et al., 2004). *Enterococcus faecalis* has a decimal decrease after 13 days, while *Enterococcus faecium* survives even longer, with a decimal decrease only occurring after 20 days in river water at 20°C (Wilson, 2005).

Enterococci are very resistant to antibacterial agents, detergents, desiccation and temperatures and it is thus difficult to inhibit them (Wilson, 2005; Madigan and Martinko, 2006). *Enterococcus faecalis* is also an opportunistic pathogen with increasing bacterial resistance (Hartke et al., 1998), especially to vancomycin, and is increasingly the cause of nosocomial infections. So they are becoming a public health concern (Chenoweth and Schaberg, 1990; Salminen et al., 2004; Amyes, 2007), particularly as they can cause hard-to-treat infections in susceptible individuals (Chenoweth and Schaberg, 1990; Salminen et al., 2004; Wilson, 2005). Certain enterococci have thus been used as probiotics (Salminen et al., 2004).

Use of antibiotic-resistant indicators for monitoring may not be appropriate, as survival rates may differ (Pettibone et al., 1987), although no differences in survival rates have been reported.

Temperature and pH have been closely associated with survival rates in milk products, which are considered ideal growth media for streptococci and bifidobacteria (Gardini et al., 2001). Enterococci are resistant to a wide range of pHs, heating and salt conditions (Rince et al., 2000). They have been reported to survive pHs between 4.5 and 10 (Fisher and Phillips, 2009) and temperatures ranging up to 60°C for 30 minutes (Fisher and Phillips, 2009). Their resistance to heat depends on their growth phase, as the stationary phase provides more resistance (Fisher and Phillips, 2009).

But their growth media before exposure to stress also affects their resilience with a lack of stress leading to lower resistances (Fisher and Phillips, 2009). A low pH decreases survival initially, especially under starvation conditions, but the bacteria develop an increased resistance over time (Trainor et al., 1999). Bacteria can enter a stationary phase or dormant state as a result of stress or nutrient limitations (Trainor et al., 1999). Bacteria can also enter a viable non-culturable (VBNC) state often induced by temperature, salinity and light, but occasionally due to the lack of needed nutrients or required conditions in the cultivation medium (Trainor et al., 1999).

VBNC states exist with bacteria having different metabolic activity, thus plating efficiency decreases over time, but resistance to pH, UV and sodium hypochlorite (NaClO) increases (Hartke et al., 1998). While *E. coli* and *Vibrio spp.* are resistant to limited nutrients and survive long periods under these conditions (Trainor et al., 1999).

Enterococcus faecalis is able to survive longer periods in tap water than faecal coliforms (Hartke et al., 1998), due to their high resistance to environmental changes (Pettibone et al., 1987; Morandi et al., 2005). *Enterococcus faecium* is most resistant to environmental changes out of the Enterococci group (Pettibone et al., 1987). Enterococci can adapt to extreme environments cultured under these conditions (Belli and Marquis, 1991). Low temperatures tend to prolong survival of bacteria, possibly due to lower metabolic activity (Trainor et al., 1999). The limitation of phosphate and glucose and, possibly, carbon starvation increase the survival time (Trainor et al., 1999).

Enterococci produce lactic acid as their main product, and are therefore acid tolerant to some extent (Wilson, 2005). Enterococci rarely tolerate a pH below 5, although they have an H^+-ATPase system to help compensate for their acid production (Salminen et al., 2004).

Survival due to starvation can lead to changes in colony morphology in some bacteria groups. Bacteria can enter a stationary phase or dormant state as a result of stress or nutrient limitations (Trainor et al., 1999). The bacteria can also enter a viable non-culturable (VBNC) state which is often induced by temperature, salinity and light, but occasionally due to lack of the required nutrients or conditions in the cultivation medium (Trainor et al., 1999).VBNC states exist with bacteria having different metabolic activity and thus plating efficiency decreases over time, although resistance to pH, UV and sodium hypochlorite (NaClO) increases over time (Hartke et al., 1998; Trainor et al., 1999) due to their high resistance to environmental changes (Pettibone et al., 1987; Morandi et al., 2005). Low temperatures tend to prolong the survival of bacteria, possibly due to lowered metabolic activity (Trainor et al., 1999).

Limitation of phosphate and glucose as well as carbon starvation could increase survival time (Trainor et al., 1999). But, limitation of nitrogen and amino acids seems to shorten survival time, which may be due to either a protein synthesis or energy requirement, as these can be used as a source of energy under carbohydrate starvation (Trainor et al., 1999). Blocking protein synthesis by antibiotics decreases survival time (Trainor et al., 1999). The synthesis of protein may be a vital adaptation to acidic pH tolerance (Rince et al., 2000). While bifidobacteria and *Lactobacillus* have intrinsic resistance to acidic conditions, enterococci can adapt and use the F_1F_0-ATPase proton pump for some of their adaptation (Belli and Marquis, 1991).

Enterococci produce lactic acid as their main product, and are thus, to some extent, acid tolerant (Wilson, 2005). Enterococci rarely tolerate a pH below 5, although they have a H^+-ATPase system to help them compensate for their acid production (Trainor et al., 1999; Salminen et al., 2004). This has been shown by the coccoid shape and shrunk size of *E. coli*, while Gram positive bacteria do not seem to shrink, but their membrane fatty acid composition changes (Hartke et al., 1998; Cotter and Hill, 2003).

Bifidobacteria and enterococci survival rates are to be compared to presently used indicator bacteria. The Enterobacteriaceae group has previously been used as monitoring indicators of faecal pollution. This family contains over 150 species, all natural human gastrointestinal tract (GIT) inhabitants (Wilson, 2005). They include, amongst others, *Bacillus*, *E. coli*, *Citrobacter* and *Proteus* (Wilson, 2005).

Of these faecal coliforms and, more recently, *Escherichia coli* have been used extensively for monitoring faecal pollution. It is presently the main South African monitoring bacteria (Murray et al., 2004). *E. coli* ferment many sugars, producing acetic acid, lactic acid, carbon dioxide and other products (Wilson, 2005). This could well cause a drop in dissolved oxygen (DO) in closed environments. *E. coli* has many serotypes with differing infection-causing abilities (Griffin and Tauxe, 1991; Nataro and Kaper, 1998b; Nataro and Kaper, 1998a). Its survival has been extensively studied. Numerous kinetic models have been performed for *E. coli* (Henis et al., 1989; Buchanan and Bagi, 1994; Sutherland et al., 1995; Presser et al., 1997; Sutherland et al., 1997; Presser et al., 1998; Ross et al., 2003).

These studies have shown different survival results, depending on the survival conditions and media, as well as different agars. There is a correlation between pH, temperature, salinity, predation, sediment as well as numerous other factors and survival. The design of these experiments could be adapted for survival experiments for bifidobacteria.

3.2 AIM

The classification of Sampling Sites according to the source of the faecal pollution, e.g. human or animal.

The use of *Rhodococcus* to identify animal pollution at Sites.

The identification of a tracking ratio of SUB:TB in South Africa, as different geographical reports have used different cut-off values.

Identifying the survival of bifidobacteria in river water conditions in South Africa aids to discern if this causes the tracking ratio to not always identify the faecal source correctly.

Identifying the survival rate of enterococci and faecal coliforms for comparison purposes.

The modification of bifidobacteria media to prevent streptococci overgrowth.

The investigation of *Lactobacillus* in the rivers and its potential use for identifying faecal pollution.

3.3 METHODS AND MATERIALS

3.3.1 Chemical Consumables

All consumables and chemicals were purchased from Chemstores at Rhodes University (Grahamstown, South Africa); Spellbound Labs (Port Elizabeth, South Africa); Merck (Pty.) Ltd. (Cape Town/Johannesburg, South Africa) and Sigma-Aldrich (Johannesburg, South Africa). All chemicals were 99% pure or higher (unless stated otherwise) and were used as received by supplier.

The following chemicals and consumables were purchased from the suppliers stated below. Merck Ltd. (Pty; Johannesburg/Cape Town, South Africa) supplied: anaerobic jars; $BaCl_2.2H_2O$; $CaCO_3$; HCl (32 % aqueous solution); H_2SO_4 (95-98 % aqueous solution); KH_2PO_4; KNO_3; Kovacs reagent for indoles; Na_2HPO_4; NaCl; $Na_2S_2O_3.5H_2O$; phenol; 500 mL polyethylene sampling bottles; the chloride kit (catalogue number 1.14897.0001); the ammonium kit (catalogue number 1.14752.0001); the phosphate kit (catalogue number 1.14848.0001); m-FC agar; tryptic soy broth; peptone from casein and peptone from meat. Sigma-Aldrich (Johannesburg, South Africa) was the main supplier, providing the following chemicals: bacteriological agar; bromocresol green; bromocresol purple; cysteine hydrochloride; ferric ammonium citrate; $FeSO_4.7H_2O$; $ZnSO_4.7H_2O$; sodium propionate; $MgSO_4.7H_2O$; $MnSO_4.4H_2O$; NaN_3; cycloheximide; kanamycin sulphate; nalidixic acid; polymyxin B (1 mg/mL aqueous solution); lactose; tryptone powder; salicin; Triton X-100;

L-cysteine; deoxycholate; sorbitol; yeast extract; glucose; bovine serine albumin; Bradford's reagent; the Kovacs reagent for indoles; and toluene.

Glycerol; acetone and absolute ethanol were purchased from Chemstores (Rhodes University, Grahamstown, South Africa).

Membrane filtration (MF) filters (nylon, size 0.45 μm) for microbial enumeration; Oxoid gas generating kits for anaerobic enumeration for use with the anaerobic gas jars; and sterile petri dishes (90 mm) were procured from Spellbound Labs (Port Elizabeth, South Africa).

Microsep (Port Elizabeth, South Africa) supplied the 11 mm glass fibre prefilters for total suspended solid (TSS) determination and the glass fibre filters (pore diameter 1.2 μm) for the measurement of chlorophyll a (chla).

Instrument grade Ar, N_2 and CO_2 were purchased from Afrox-Linde (Port Elizabeth, South Africa), while casamino acids were obtained from BD (Pretoria, South Africa).

3.3.2 Methods

Selected Sites 9 to 14 (from Chapter 2) were used for the identification of bifidobacteria and river nutrient concentrations.

3.3.2.1 Microbial Parameters

Samples were collected and transported to the laboratory as described by Wutor et al. (2009), using sterile 500 mL polyethylene bottles. They were protected from sunlight and placed on ice until they reached the laboratory and were processed. Different indicator bacteria were enumerated using the membrane filtration or spread plating methods, in appropriate dilutions. The samples were inoculated onto plates in an LA1200 BII laminar-flow hood. All incubations were done in one of the following incubators: the Labcon incubator Model FSIM B (Labmark, Johannesburg, South Africa), the TS 606/3-I incubator (WTW, Weilheim, Germany), the Labcon low temperature incubator LTIE 10 (Labmark, Johannesburg, South Africa); or the Heraeus Model FT 420 (*Heraeus* Kulzer GmbH, Dormagen, Germany). All the media were autoclaved in a Model RAU-53Bd REX MED autoclave (Hirayama

Manufacturing, Tokyo, Japan). The oven used for the H_2S strip tests was a UFE 700 oven (Memmert, Schwabach, Germany).

3.3.2.2 Media Preparation

All media was prepared according to the manufacturer's instructions

The m-FC agar was prepared according to the manufacturer's instructions. It was not autoclaved but boiled for 1 minute while being continuously stirred, before being cooled to pour. The plates were poured under a laminar flow in an LA1200 BII laminar-flow hood. The working surface was pre-swabbed with 70% ethanol. The MacConkey agar, R2A agar and m-Enterococcus agar were prepared according to the manufacturer's instructions. The m-Enterococcus agar was sterilised in a steamer. The YN-17 agar was prepared according Mara and Oragui (1983), which follows. The yeast extract, peptone from meat (5g) and peptone from casein (5g), lactose, casamino acids, NaCl, bromocresol green, and deionized water were boiled together for 5 to 10 minutes. Cysteine hydrochloride and nalidixic acid were added once the media had sufficiently cooled to adjust the pH to 6.9 ± 0.1. The agar was added and the media was autoclaved for 15 minutes at 121°C. The media was then cooled and filter sterilised polymyxin B and kanamycin sulphate were added. The media was mixed and then poured under laminar flow. The agar plates were stored in the fridge until used.

The Beerens media was prepared according to Beerens (1990), described next. The Columbia agar (Fluka, Merck, Johannesburg/Cape Town, South Africa) was prepared according to the manufacturer's instructions. The media then had 5g agar (Bacteriological agar, Fluka, Merck, Johannesburg/Cape Town, South Africa) added, with cysteine hydrochloride (5g/L) and glucose (5g/L). The media was boiled for 1 minute and then cooled to approximately 70°C. The pH was adjusted with NaOH after propionic acid (5 mL) was added. Human bifido sorbitol agar was prepared according to Mara and Oragui (1983) and described subsequently. The sorbitol, yeast extract, polypeptone (peptone from meat (5g) and peptone from casein (5g)), lactose, casamino acids, NaCl, bromocresol purple and deionized water were boiled together for 5 to 10 minutes. Cysteine hydrochloride and nalidixic acid and were added and the pH adjusted to 6.9 ± 0.1. The rest was completed as per YN-17 agar above.
Faecal coliforms were isolated on m-FC agar and incubated at 44.5 °C for 24 hours. Blue colonies were counted as faecal coliforms. The bifidobacteria were enumerated as per

Blanch et al's (2006) method. The total bifidobacteria concentrations were enumerated on YN-17 (Mara and Oragui, 1983) and, later, on modified Beerens media (MBM) (pH increased to 5.7 by decreasing the NaOH concentration) (Beerens, 1990) while the human bifidobacteria were enumerated on Human Bifidobacteria Sorbitol-fermenting Agar (HBSA) (Mara and Oragui, 1983). The bifidobacteria was incubated at 37°C for 48 hours under anaerobic conditions using a CO_2 generating kit (Oxoid gas generating kits Spellbound labs, Port Elizabeth, South Africa) in gas jars (Merck, Johannesburg/Cape Town, South Africa).

Enterococci were enumerated on m-Enterococcus Selective Agar (Fluka, Merck, Johannesburg/Cape Town, South Africa) at 35°C for 48 hours, aerobically (Slanetz and Bartley, 1957). The yellow, dome shaped mucoid colonies were counted as sorbitol utilising bacteria on HBSA. White colonies were counted as positive total bifidobacteria colonies on Beerens agar.

Lactobacilli media used was MRS agar (Merck, Johannesburg/Cape Town, South Africa) which is a standard media and prepared by weighing out 68.2 g per litre and autoclaved for 15 minutes (Dave and Shah, 1996; Merck, 2012). The smaller white colonies were read as *Lactobacillus,* however differentiation from *Bifidobacterium* was not always possible.

Gram staining was done using crystal violet (Sigma Aldrich, South Africa); Safranin (Rhodes University chemistry stores) on Knittel Glaser brand glass microscope slides (Merck Ltd., South Africa). The stained slides were visualised using an Olympus UCMAD3 microscope mounted with an Olympus ultra 20 soft imaging system UTVX-2 at magnification of X900.

3.3.2.3 *Rhodococcus coprophilus*

Water samples containing *Rhodococcus* bacteria were spread plated (200 uL) onto MM3 agar using Mara and Oragui's method (Mara and Oragui, 1981; Oragui and Mara, 1983):requiring preparation of MM3 agar which contains: potassium hydrogen phosphate (0.466 g); sodium hydrogen phosphate (0.732 g); potassium nitrate (0.1 g); sodium chloride (0.29 g); magnesium sulphate hydrate (0.1 g); calcium carbonate (0.02 g); Sodium propionate (0.2 g); ferric sulphate hydrate (200 μg); zinc sulphate hydrate (180 μg); manganese sulphate hydrate (20 μg), sodium azide (3.5 mg); Nalidixic acid (5 mg); bacterial agar (18 g); Distilled water (1000 mL). The ingredients were mixed and autoclaved for 15 minutes before previously

membrane filter sterilised cycloheximide (10 mL of 0.5% w/v) and thiamine (1 mL of 0.4% (w/v) were added. The agar was poured into 90 mm petri dishes and left to solidify.

The water samples were diluted using quarter-strength Ringer's solution and 200 µL was spread onto plates. The plates were incubated at 30°C for 12 days before being exposed to sunlight for 4 days on a bench at room temperature. Stellate bright orange central papillae with an aesteroidal colony appearance under a microscope were counted as *R. coprophilus* (Mara and Oragui, 1981).

3.3.2.4 Chemical Parameters

Chemical parameters were analysed to identify the water quality and then to develop model water for survival experiments. The pH, electrical conductivity (EC) and temperature were measured on site, using a HANNA pH and conductivity/ TDS (total dissolved solids) tester probe and a mercury thermometer (Sigma Aldrich, Johannesburg, South Africa). The model numbers were HI 98129 (low range) and HI 98130 (High range) (HANNA, South Africa). pH and conductivity were checked in the laboratory, using the HANNA pH and conductivity/ TDS tester probes, and the hardness was tested using a Salifert KH/Alk Profi test (Holland, (Salifert, 2012)) or Blue 52 hardness test kit (Blue 52^{TM}, Midrand, South Africa). The nitrates, chlorides, protein, phosphates, ammonium and sulphates were measured in the laboratory after bacterial inoculation occurred. Protein concentrations were measured using Bradford reagent (Sigma-Aldrich, South Africa). The following Merck kits were used: the chloride kit (catalogue number 1.14897.0001); the ammonium kit (catalogue number 1.14752.0001) and the phosphate kit (catalogue number 1.14848.0001). Nitrates were measured using the Winsona State University method (Miertschin, 2011). The sulphates were analysed using Environmental Protection Agency (EPA) Method 375.4 (EPA, 1978). A microplate reader (manufactured and supplied by Biotek) was used for chemical quantification. The microplates (PLATE F/B CLR PS 96WELL) were supplied by Lasec (Port Elizabeth, South Africa).

3.3.2.5 Model Water

The survival experiments were based on factorial design and thus the model water was adapted to represent all the experiments proposed by the DOE++ program.

The basic model water contained glucose (5.7 mg GE/L), sodium sulphate (43 mg/L sulphate), ammonium chloride (1.08 mg/L ammonium), and then protein (Bovine serum albumin), sodium nitrate, potassium dihydrogen phosphate sodium chlorides and sediments (Pool filter sand) was added and the pH modified and temperature at which it was incubated set according to experiment design tables (Table 10, Table 11, Table 12, Table 14 and Table 16). The basic model water is shown in Table 8. Inclusion of glucose was to provide a sugar source for the bacteria. Sorbitol was also added to the model water. The sediment (particulate matter) was represented by course pool filter sand (100 g) (Sparrow Pools, Grahamstown, South Africa) which was washed twice in water and dried before being added to the model water. Calcium carbonate was added to some of the model waters especially for the enterococci and bifidobacteria survival experiments.

Table 8: Initial concentrations of nutrients did not change during the different experiment runs.

Parameter	Basic Model water
Turbidity (NTU)	23
Salinity (mS/m)	82
DO (mg/L)	6.01
Sulphates (mg/L)	43
Glucose (mg GE/L)	5.7
Sorbitol (mg/L)	4
NH_4^+ (mg/L)	1.08
TSS (mg/L)	0

Model water was prepared using solutions of the salts and then water added to MilliQ water (800 mL). The MilliQ water is prepared using a MilliQ RO® purification system (Millipore Co., Massachusetts, United States of America) and filtered through a Super C® carbon cartridge, two ion-X® exchange cartridges and an Organex-Q® cartridge before being filtered through a 0.22 µm Millipak® filter (Millipore Co., Milford, Massachusetts, United States of America).

The model water was then autoclaved for 15 minutes at 121°C (Model RAU-53Bd REX MED autoclave (Hirayama Manufacturing, Tokyo, Japan)). The model water was cooled to room temperature in the laminar flow before each experiment began. The pH was modified with sterile sodium hydroxide or hydrochloric acid before the bacteria were inoculated into the model water. The water was then mixed and sampled immediately (at time - 0 hours) to identify the initial enumerable concentration of bacteria in each model. Model water was then incubated at the appropriate temperature, and covered with foil to prevent light or ultraviolet light radiation entering and affecting the results. The bottles were covered with foil to prevent

The model water was swirled twice a day and the lid opened once a day, under laminar flow to prevent contamination, to allow oxygen replacement and under laminar flow to prevent contamination. Bottles were protected from light most times. The model water was either stored at 8°C in the fridge or at 32°C in a water bath incubator (Labcon shaking water bath, Laboesign engineering Pty, Maraisburg, South Africa).

The bacteria were enumerated, in triplicate, on the appropriate media. An aliquot was diluted in saline and spread plate before being incubated at the appropriate temperature.

Model water was developed using design of experiment techniques to minimise the number of experiments and to maximise the information obtained from them. The chemical constituents of the model water were based on analysis of the water quality at selected sampling sites. The parameters being tested were based on the river quality and the chemical correlation to the bacterial concentration at the selected sampling sites. The final composition was also based on literature's evidence of factors affecting bacterial survival in water bodies.

3.3.2.6 Inoculum for Model Water

The inoculation media and inoculum was prepared and transferred using aseptic techniques (Madigan and Martinko, 2006). The trypitc soy broth used to grow the bacteria was prepared according to the manufacturer's instruction. Thus 7.5 g was placed into 250 mL deionised water in a conical flask (500 mL). The broth was autoclaved at 121°C for 15 minutes. The inoculations of the broth were performed under sterile conditions and laminar flow.

The heterotrophic bacteria (HPC) were isolated from R2A agar (Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa) and grown in tryptic soy broth (Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa) overnight and then diluted with sterile saline solution (0.9% saline BP (Sweetman, 2011)) to the correct dilution before they were inoculated into the model water.
The faecal coliforms (FC) were picked off mFc agar plates ((Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa). They were grown in tryptic soy broth (Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa) overnight at 37°C. An aliquot of tryptic soy broth containing bacteria was diluted with sterile saline solution (0.9% saline BP (Sweetman, 2011)) to the correct dilution before they were inoculated into the model water. The dilution factor required was calculated from the number of colonies inoculated and the grow rate from previous experiments

The bifidobacteria colonies were picked of HBSA agar and Beerens media from Grahamstown river water samples. Thus using bifidobacteria found in this area for the experiments. The colonies were streak plated onto HBSA and Beerens media. Colonies on the streak plate with the most bifidobacteria morphology characteristics, were isolated and placed into degassed (with Nitrogen) tryptic soy broth with sorbitol (1 g/L) added. This was done to prevent bifidobacteria from growing without sorbitol sensitivity or selectivity. The degassing was designed to minimise the oxygen levels in the broth. The broth prepared in 250 mL schott bottles to minimise oxygen presence and filled to the 250 mL make to decrease air space. The inoculated broth was shaken at 37° C for 56 hours before a sample was enumerated onto HBSA agar and the colonies were Gram stained to confirm their morphology. The dilution factor was calculated from the growth rate and colony counts in trial experiments. The isolated colonies from the survival experiment were also Gram stained to help identify their morphology and ensure that they are bifidobacteria.

3.3.2.7 Statistics and Factorial / Fractional Factorial Design

Statistical tests were performed using Statistica version 10 (StatSoft Inc., 2011) or PRIMER 6+ PERMANOVA (Plymouth Marine laboratory, 2009). Statistica was used to analyse correlations using t-tests for parametric parameters and Mann- Whitney U tests as a non-parametric method. PRIMER was used to analyse the correlation between multiple variables

simultaneously, using DISTLM (Distance-based linear models) with Bray Curtis and Akaike Information Criterion (AIC) as measurement parameters.

DISTLM is a multivariate data analysis model using a resemblance matrix for analysing relationships between multiple variables (Anderson et al., 2008). It uses one or two predictor variables to which other variables are compared, using ANOVA (Anderson et al., 2008). DISTLM uses a distance based redundancy analysis (dbRNA), which is a multivariate multiple regression of principle coordinate analysis, using a resemblance matrix to create an ordinance in a cloud and is a linear analysis. It predicts which variables are more closely correlated to predictor variables.

Experimental design uses different models to identify the interaction or effect of one thing on another (Berger and Maurer, 2002). Factorial design is a design with each factor containing two levels (Lewis et al., 2005). Fractional factorial design is using a subset (fraction) of the factorial experiments to identify the trends of two or more factors at two levels (Berger and Maurer, 2002; Lewis et al., 2005). Design of factorial or fractional factorial models was performed using DOE++ version 1.0.7. (ReliaSoft Corparation, 2011). It was used to design survival experiments with multiple parameters and to identify the significance of each parameter.

The statistical significance or insignificance of the results is shown in the Pareto Graphs. The length of the bar in the graph is proportional to the estimate effect of the factor. The vertical lines indicate the values and a significant effect on Y (survival rate) is denote by the bar crossing the vertical (critical line).

3.3.2.7.1 Factorial Design of Bifidobacteria Survival Experiments

The effect of the following factors on the survival rate of bifidobacteria was determined in a factorial design (5 × 2 × 1). The factorial design used two storage temperatures (8°C, 32°C), pH (5.3, 9.85), protein (0, 140mg/L), nitrate (0, 6.8mg/L) and chlorides (0, 302 mg/L). These are shown in Table 10. The lower numbers in the brackets were the used where -1 is shown in Table 9, while the higher numbers in the brackets where it is written 1 and the code is in Table 10. This data is shown in Table 10. The ranges were chosen from physical chemical water quality data in the surrounding rivers and literature (Zuma, 2010; Tandlich et al., 2012).

All experiments were done in duplicate and the average recorded. The designed experiments are shown in the experiment number while the run number is the order in which the experiments should have been run. The bifidobacteria were enumerated at 0; 1; 3; 24; 72 and 152 hours. The pH of the model water was also analysed at each enumeration time.

Table 9: The factorial design of the survival experiments, where 1 indicated the maximum concentration and -1 the minimum concentration. The concentrations used are shown in Table 10.

Experiment Number	Run Number	pH	Temperature	Nitrates	Chloride	Protein
1	24	1	1	1	-1	1
2	22	1	-1	1	-1	1
3	21	-1	-1	1	-1	1
4	26	1	-1	-1	1	1
5	14	1	-1	1	1	-1
6	13	-1	-1	1	1	-1
7	20	1	1	-1	-1	1
8	1	-1	-1	-1	-1	-1
9	5	-1	-1	1	-1	-1
10	15	-1	1	1	1	-1
11	16	1	1	1	1	-1
12	8	1	1	1	-1	-1
13	17	-1	-1	-1	-1	1
14	28	1	1	-1	1	1
15	27	-1	1	-1	1	1
16	18	1	-1	-1	-1	1
17	23	-1	1	1	-1	1
18	32	1	1	1	1	1
19	12	1	1	-1	1	-1
20	6	1	-1	1	-1	-1

Table 10: The concentrations of the factors used for the survival experiment of bifidobacteria in a non-stabilised pH model water.

Factors	Level	
	-1	1
pH	5.3	9.8
Temperature incubated at in degrees Celsius	8 °C	32 °C
Nitrate concentrations of the model water	0 mg/L	6.8 mg/L
Chloride concentrations of the model water	0 mg/L	302 mg/L
Protein concentrations of the model water	0 mg/L	140 mg/L

3.3.2.7.2 Fractional Factorial Design of Bifidobacteria Survival Experiments

The fractional factorial design for the survival of enterococci is shown in Table 14. The X represents the factors which were used in the experiment and are explained in Table 15. The number 1 symbolised the higher concentration in the experiments and -1 the lower concentration shown in Table 15. The fractional factorial survival experiments for bifidobacteria were temperatures (8°C, 32°C), pH (5.3, 9.85), protein (0, 140mg/L), nitrate (0, 6.8mg/L) and chlorides (0, 302 mg/L). The media were prepared according to the table and then the bacteria enumerated at the following time periods: 0; 12; 24; 48; 96; 300 hours. The data was collated and the survival rate calculated. This was then placed into the DOE++ program and analysed.

Table 11: The fractional factorial design for bifidobacteria survival experiments with pH stabilisation of calcium carbonate.

Experiment number	**Run Order**	pH	Temperature	Nitrate	Chloride	Phosphate	Protein	Sediment
1	3	-1	-1	-1	1	1	1	-1
2	2	-1	-1	1	1	-1	-1	1
3	8	-1	1	-1	-1	1	-1	1
4	5	-1	1	-1	1	-1	1	-1
5	6	1	-1	-1	-1	-1	1	1
6	7	1	-1	-1	1	1	-1	-1
7	1	1	1	1	-1	-1	-1	-1
8	4	1	1	1	1	1	1	1

Table 12: The fractional factorial design for the bifidobacteria survival experiments with calcium carbonate addition for pH stabilisation and minimised factors to identify main effects. X represents the factors used in the experiment.

Experiment number	Run Order	X_1	X_2	X_3	X_4	X_5
1	5	-1	-1	1	1	-1
2	3	-1	-1	1	-1	1
3	8	-1	1	-1	1	1
4	1	-1	1	-1	1	-1
5	6	1	-1	-1	-1	1
6	7	1	-1	-1	1	-1
7	4	1	1	1	-1	-1
8	2	1	1	1	1	1

Table 13: The coding and real values of the factorial factors for the pH stabilised bifidobacteria survival experiment. The coding applies to Table 12.

Factors	Levels	
	-1	+1
X_1 A: pH of the solution adjusted with NaOH or HCl	5.3	9.8
X_2 B: Temperature incubated at in degrees Celsius	8 °C	32 °C
X_3 C: Nitrates concentrations of the model water	0 mg/L	6.8 mg/L
X_4 D: Phosphate concentrations of the model water	0 mg/L	10 mg/L
X_5 E: Sediment as washed and sterilised pool sand per litre	0 g	5 g

3.3.2.7.3 Fractional Factorial Design of Enterococci Survival Experiments

The fractional factorial design for the survival of enterococci is shown in Table 14. The X represents the factors which were used in the experiment and are explained in Table 15. The number 1 symbolised the higher concentration in the experiments and -1 the lower concentration shown in Table 15. The media were prepared according to the table and then the bacteria enumerated at the following time periods: 0; 12; 24; 48; 96; 300 hours. The data was collated and the survival rate calculated. This was then placed into the DOE++ program and analysed.

Table 14: The fractional factorial design for the enterococci. X represents the factors used in the experiment and are explained in Table 15 below.

Experiment number	Run Order	X_1	X_2	X_3	X_4	X_5	X_6
1	5	1	1	1	1	1	1
2	3	1	1	1	1	1	1
3	8	1	-1	-1	-1	-1	1
4	1	-1	-1	1	1	-1	-1
5	6	1	1	-1	1	-1	-1
6	7	-1	1	1	-1	-1	1
7	4	-1	1	-1	-1	1	-1
8	2	1	-1	1	-1	1	-1

Table 15: The coding and real values of the factorial factors for the pH stabilised survival experiment enterococci. The coding applies to Table 14.

Factors	Levels	
	-1	+1
X_1 A: Temperature incubated at in degrees Celsius	8 °C	32 °C
X_2 B: Nitrates concentrations of the model water	0 mg/L	6.8 mg/L
X_3 C: Chlorides concentrations of the model water	0 mg/L	302 mg/L
X_4 D: Phosphate concentrations of the model water	0 mg/L	10 mg/L
X_5 E: Protein concentrations of the model water	0 mg/L	140 mg/L
X_6 F: Sediment as washed and sterilised pool sand	0 g/L	5 g/L

3.3.2.7.4 Fractional Factorial Design of FC Survival Experiments

The fractional factorial design for the FC experiments was performed using DOE++ version 1.0.7. (ReliaSoft Corparation, 2011). The fractional factorial method was used to minimise experiments and identify the key chemicals which affect the FC survival in rivers despite their varying chemical water quality.

The fractional factorial design for the survival of faecal coliforms is shown inTable 16. The X represents the factors which were used in the experiment and are explained inTable 17. The number 1 symbolised the higher concentration in the experiments and -1 the lower concentration shown in Table 15. The media were prepared according to the table and then the bacteria enumerated at the following time periods: 0; 1; 6; 12; 24; 48; hours and days 7; 9; 14; 22; 29; 36; 42. The data was collated and the survival rate calculated. This was then placed into the DOE++ program and analysed.

Table 16: The fractional factorial design for the survival experiments for faecal coliforms. X represents the factors used in the experiment.

Experiment number	Run Order	X_1	X_2	X_3	X_4	X_5
1	5	-1	-1	1	1	-1
2	3	-1	-1	1	-1	1
3	8	-1	1	-1	1	1
4	1	-1	1	-1	1	-1
5	6	1	-1	-1	-1	1
6	7	1	-1	-1	1	-1
7	4	1	1	1	-1	-1
8	2	1	1	1	1	1

Table 17: The coding and real values of the factorial factors for the faecal coliform survival experiment. The coding applies toTable 16.

Factors	Levels	
	-1	+1
X_1 A: pH of the solution adjusted with NaOH or HCl	5.3	9.8
X_2 B: Temperature incubated at in degrees Celsius	8 °C	32 °C
X_3 C: Nitrates concentrations of the model water	0 mg/L	6.8 mg/L
X_4 D: Phosphate concentrations of the model water	0 mg/L	10 mg/L
X_5 E: Sediment as washed and sterilised pool sand per litre	0 g	5 g

3.3.2.7.5 Bacterial Survival Curves

Survival curves were analysed with GInaFIT for fit to the different survival models (Geeraerd, 2012), using Microsoft Excel 2010. GInaFIT (Geeraerd and Van Impe Inactivation Model Fitting Tool) is a freeware program used by people in the food industry (Geeraerd et al., 2005; Geeraerd et al., 2006). It tests experimental data of bacteria concentrations verses time against nine different survival models (Geeraerd et al., 2005; Geeraerd et al., 2006).

More precisely, the tool is useful for testing nine different types of microbial survival models on user-specific experimental data relating to the evolution of the microbial population over time. The nine different survival models used are: classical log-linear curves; curves

displaying a shoulder before a log-linear decrease; curves with a tail after log-linear decreases; curves demonstrating both shouldering and tailing; concave curves; convex curves; convex/concave curves with tailing; biphasic inactivation kinetics; and biphasic inactivation kinetics before a shoulder (Geeraerd et al., 2005; Geeraerd et al., 2006).

3.3.2.8 Site Description

Figure 5 shows the selected sampling sites in Grahamstown as the East London sites were either not polluted enough or too far for bifidobacteria analysis which required analysis within 2 hours of sampling.

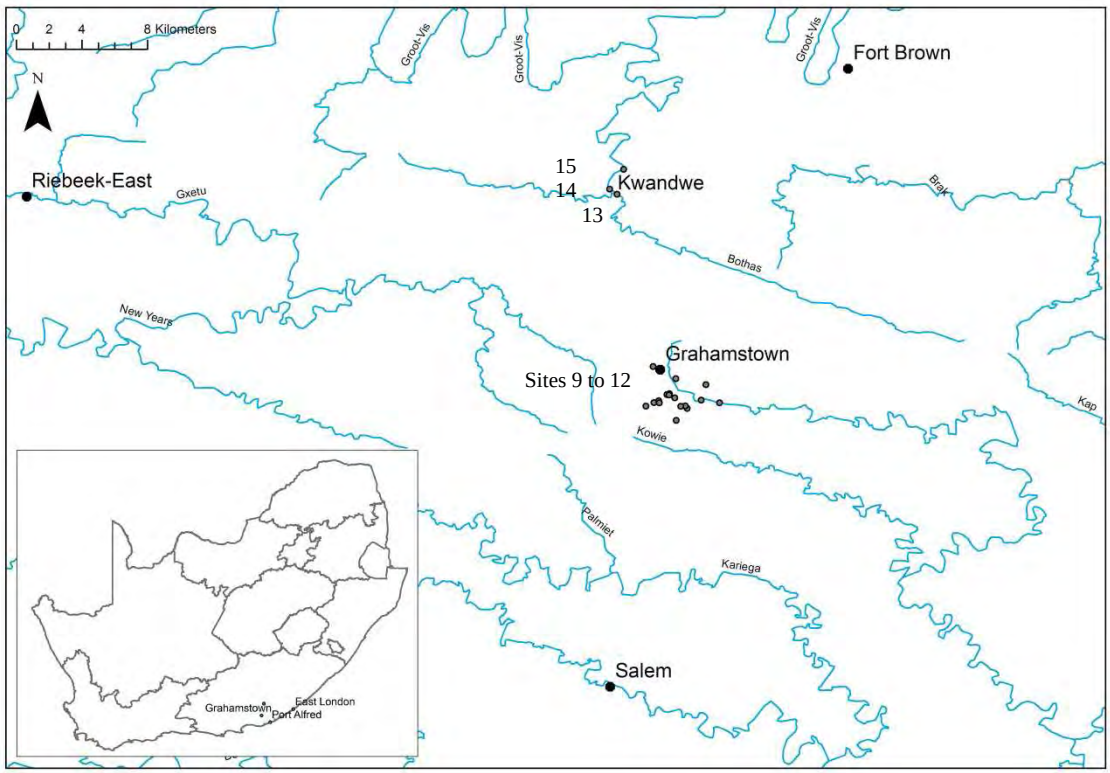


Figure 5: Selected sampling sites identified for faecal source tracking experiments are shown by the light coloured circles. Sites 1 to 8 are in East London and are not included on this map. Sites 9 to 12 are in the Grahamstown area. Sites 13 to 15 are around Kwandwe.

Sampling Site 1: R2Buff-Kwabo: Buffalo River at Zwelitsha (32° 54’ 51.2” S, 27° 24’ 34.3” E).

Sampling Site 1 is a Department of Water Affairs and Forestry (DWAF) ecological assessment monitoring point on the Buffalo River. It receives industrial and municipal sewage, (Scherman et al., 2004) as it is downstream of King William’s Town wastewater treatment works (GPS coordinates: 32°53'59.28"E, 27°24'11.016"S, (DWA, 2012b). This wastewater treatment works (WWTW) facility has been reported to insufficiently treat sewage before releasing it into the river (Morrison et al., 2001). There were no green drop scores before 2011, when it scored 82.9% compliance (DWA, 2012a). There is an illegal dumping site approximately 50 m away from the right river bank. There is plenty of human and animal activity in the area, leading to a stench in the area.

Biofilm formation on the rocks is extensive and elevated nitrate and phosphate levels were observed. Due to the fast flowing nature of the river, it is turbid. This project has classified it as a mixed input of faecal pollution e.g. from human and cattle.

Sampling Site 2: R2Buff-Kwami: Buffalo River downstream of Zwelitsha Township (32° 56.7”S, 27° 26’ 58.1.3”E).

This site is situated downstream of Sampling Site 1 and the Zwelitsha Township (informal settlement). It receives runoff from the settlement, wastewater discharge and industrial effluent and thus has an unpleasant odour.

Sampling Site 3 (GPS: 32° 57’ 29.8”S, 27° 31’ 32.8”E)

Sampling Site 4: R2Buff-Umtiz: Buffalo River at Buffalo Pass (33° 00’ 21.3”S, 27° 49’ 31.7”E).

Located within the Umtiza Coastal Nature Reserve area, this site is located near a river estuary and should have had low human impact. But there is an illegal dumping site nearby (Zuma, 2010). No visible excrement was observed in the area. The source of faecal pollution was not able to be determined, and no animals were seen within the area.

Sampling Site 5 (GPS: 33° 00’ 21.3”S, 27° 49’ 31.7”E)

Sampling Site 6: Yellowwoods River at Londsale Bridge at Bisho Town (32° 48’ 26.0”S, 27° 28’ 11.1”E).

This site is located in the middle reaches of the Yellowwoods River near Bisho. It is approximately 1000 m from informal settlements, thus limited human faecal pollution is predicted. The bridge may however increase the possibility of human influences. Cattle and goats were observed on the river banks, with cow dung visible.

This site was classified as a mixed input site from primarily cattle and humans. There was a high level of phosphate and nitrate with substantial biofilm formation on stones and algal biomass noted.

Sampling Site 7: (GPS: 33° 19’ 13.9”S, 26° 31’ 14.8”E)

Sampling Site 8: (GPS: 33° 19’ 29.4”S, 26° 25’ 29.0”E)

Sampling Site 9: Gowie dam in Grahamstown. (GPS: 33° 17’ 40.0”S, 26° 30’ 54.2”E)

This site is located in Grahamstown, on the north west side, in a residential area called Somerset Heights. It is a small dam used for recreational activities, such as swimming. Cattle dung as well as large quantities of litter has been observed on the banks. Due to human and animal uses, this site was classified as having mixed input of faecal pollution from human and livestock (mainly cattle).

Figure 6: Gowie dam is sampling Site 9.

Sampling Site 10: Grey Dam in Grahamstown. (GPS: 33° 19’ 26.7”S, 26° 31’ 39.9”E)

Grey Dam is situated on the outskirts on the south side of Grahamstown, near the N2 highway to Port Elizabeth. Site 10 is shown in Figure 7. The dam was built in 1860 and is one of the oldest water reservoirs in Grahamstown (Mullins, 2011). The Site is currently used for walking dogs and, frequently, for family recreation and swimming. Dog excrement is often observed in several locations around the area, which could impact the dam by runoff. Humans swimming in the dam could increase the potential human faecal inputs.

The site has been classified as mixed source of faecal input from humans and dogs, although only humans should excrete sorbitol-utilizing bifidobacteria and total bifidobacteria, with negligible input from dogs (Greetham et al., 2002; Lamendella et al., 2008).

Figure 7: Grey Dam is sampling Site 10.

Sampling Site 11 Bloukranz River in the vicinity of the sewage treatment works. (GPS: 33° 18’ 51.9”S, 26° 33’ 05.5”E)

Site 11 is located just above the Grahamstown sewage treatment works, on the outskirts of the Grahamstown Township on Belmont valley. Site 11 is shown in . The river banks are steep, leading to runoff from cattle dung, which has been observed on many occasions in the area. Dogs also roam this area. This site has been classified as a mixed site with faecal pollution from humans and grazing animals.

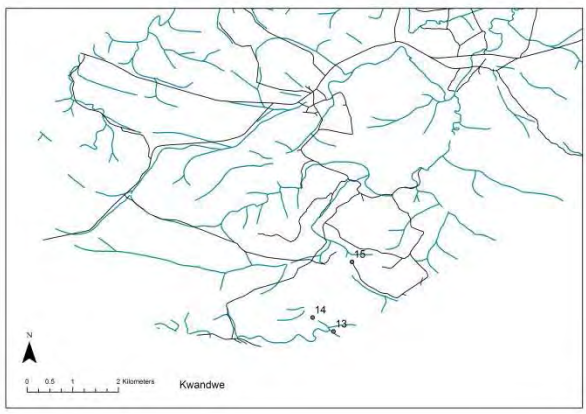
Figure 8: Bloukranz River in the vicinity of the sewage treatment works, named Site 11.

Sampling Site 12 Bloukranz River at the sewage outflow pipe near Fingo Village. (GPS: 33° 18’ 46.7”S, 26° 32’ 29.3”E)

Site 12 is at the junction between Matthew and Siegfield Street. Figure 9 shows Site 12. The river banks are steep, but it is a good grazing area with cattle and goats (and their dung) often observed on them. This will allow surface runoff. Faecal input from these grazers after heavy rains is evident. This site was classified as a mixed input site with faecal pollution from both cattle and humans.

Figure 9: Bloukranz River at the sewage outflow pipe near Fingo Village, name Site 12.

Sampling Site 13 to 15 were in a Kwandwe Game Reserve



Sampling Site 13: Botha’s River in the Kwandwe Game Reserve. (GPS: 33° 11’ 59.4”S, 26° 29’ 42.8”E)

Site 13 is located on the southern side of the Kwandwe Game Reserve near the Botha’s River. The site is depicted in . The Grahamstown military base is located upstream of this site. It is an animal drinking site. Animal and human impact on this site is possible.
Septic tank leakage from the military base may also be a contributor to the faecal pollution of this river. This is classified as mixed faecal pollution with human and animal input. It is unlikely but still possible for cattle to contribute to this pollution.

Figure 10: The Botha's River in the Kwandwe Game Reserve.

Sampling Site 14: A junction point between Botha's River and Brak Rivers and a retention dam at Kwandwe Game Reserve. (GPS: 33° 11' 49.5"S, 26 29' 28.0"E)

Site 14 is located downstream of Site 13, where the ephemeral Brak River flows into the Botha's River and a retention dam. It is used as a drinking point by Kwandwe Game Reserve animals. These include antelope, different carnivores, rhinos and elephants.

Figure 11: Sampling Site 14 at the junction point between Botha's River and Brak Rivers and a retention dam at Kwandwe Game Reserve.

Sampling Site 15: Dam at Kwandwe Game Reserve. (GPS: 33° 11’ 10.1”S, 26° 29’ 55.9”E)

The water is used for kayaking and as an animal drinking point. It often has ducks and elephants wallowing in it. Both the Brak and Botha rivers flow into the dam. The Brak River often dries up during dry periods, flowing again after the rain.

Figure 12: Dam at Kwandwe Game Reserve.

3.4 RESULTS AND DISCUSSION

The selected sampling sites were chosen from the screened sites in Chapter 2. The screening for *Rhodococcus* and bifidobacteria was also performed on all sites before the site selection was completed. Bifidobacteria was selected as a source tracking method based on the ability to enumerate it with limited extra equipment in a microbiology laboratory. The use of viruses was excluded on this basis. The use of PCR was excluded as it could not be done cheaply and under South African conditions cost is a major consideration. The use of *Rhodococcus* was to identify faecal pollution form cattle or animals in the area. *Rhodococcus* is reported to only be excrete by animals and thus helped distinguish animal faecal pollution at sites.

3.4.1. Enumeration of *Rhodococcus coprophilus*

Available literature about the selected sites along with public domain information and visible evidence on site were used to identify potential faecal pollution inputs (Tandlich et al., 2010b).The use of *Rhodococcus coprophilus* was also used to identify faecal pollution inputs from animals at the selected sites. The *Rhodococcus* was enumerated in order to help identify the source of the faecal pollution present in the water. This test was only performed on the selected sites and not during the screening stage. This allowed sites to be divided into human, animal and mixed faecal pollution input sites.

Having identified the continuously faecal polluted waters, the use of a bacterium specific to only animal or only human pollution would help identify the source of pollution. Thus *Rhodococcus coprophilus* was used, as it is found in herbivorous animal sources, and not human faeces (Mara and Oragui, 1981; Seurinck et al., 2005; Hagedorn et al., 2011). *Rhodococcus coprophilus* was enumerated from water samples at all of the selected sites. Concentrations of *Rhodococcus coprophilus* at the selected sites are shown in . On-site observation of faeces and information from literature or local people was used to identify if the pollution was from a mixed source or animals only. But what was seen and information given was occasionally misleading. *Rhodococcus coprophilus* can survive longer than other indicator bacteria in water (Mara and Oragui, 1983).

No *Rhodococcus* was detected at Site 10 water samples on the 10^{th} of December 2008 nor on the 25^{th} of June 2009. Nor was there any at Site 12 on the 20^{th} of December 2008. The maximum concentration was equal to 1.45×10^{7}CFUs/100 mL at Site 11 on the 15^{th} of December 2008.

This study's results are comparable to those of Jagals et al. (1995). *R. coprophilus* concentrations from water samples higher than 5×10^{3} CFUs/100 mL indicates faecal contamination from herbivorous animals (Long et al., 2002). The *R. coprophilus* concentrations above as well as the site descriptions has led to the classification of Sites 9 and 10 as human contamination, while Sites 11 and 12 were a mixture of human and animal contaminants, but primarily cattle. Sites 13 through 15 are situated in a game reserve and are prone mainly to wild animal faecal contamination, although some human pollution is possible. Sites 1 and 2 were classified as mixed sources, while Sites 4 and 6 are mainly cattle or livestock. Sites 1 to 9 are mainly below 5000 CFUs/100 mL, except for Site 6 which ranges to 10000 CFUs/100 mL. The concentrations are arithmetic means of the replicates. Ranges are due to samples being taken at different points of the same sampling site. Cattle dung was situated on one side of the river, possibly causing higher concentrations on one side, as it was washed into the river. However this was not found to be significant for indicator bacteria (Zuma, 2010). *Rhodococcus* is said to survive between 5 and 30°C for up to 35 days (Oragui and Mara, 1983).

Sites 1, 2, 4 and 6 were not selected due to their faecal input being mostly from mixed sources and livestock/cattle origin. Selected sites included Sites 9, 10 and 12, as they contain human faecal pollution, and Sites 11 and 13 to 15, as they have mixed sources which could help calibrated the tracking ratio. Site 11 was also chosen as it is downstream of Site 10 and this may help identify dilution over a distance.

Table 18: The *Rhodococcus coprophilus* concentrations in water samples from selected Sites with continuous faecal pollution for faecal source identification

Sampling site	Date of sampling	*Rhodococcus coprophilus* (CFUs/100 mL)	Source of faecal contamination
1	24/09/2008	5000	Mixed origin
2	24/09/2008	1500-5000	Mixed origin
4	24/09/2008	2500	Livestock/cattle
6	24/09/2008	500-10000	Livestock/cattle
9	10/12/2008	3500	Mixed
9	20/05/2009	4500	Human
10	10/12/2008	BDL	Human and dog
10	25/06/2009	0	Human
11	15/12/2008	1850000-14500000	Livestock/cattle
11	20/05/2009	150000	Mixed
12	20/12/2008	BDL	Human
12	25/06/2009	7500	Mixed
13	22/11/2008	3000-49500	Possibly wild animals
13	15/07/2009	3300	Unknown
14	22/11/2008	4000-30000	Possibly wild animals
14	15/07/2009	5000	Wild game
15	22/11/2008	13000	Possibly wild animals
15	15/07/2009	15000	Wild game

BDL Below detection limit

The faecal coliforms (FC) and bifidobacteria at the 7 selected sites from the Eastern Cape were enumerated in Chapter 2 (Section 7, Table 2). These sites were selected as they had permanent faecal pollution. This was important to ensure the bacteria would be present

continuously. Three Sites (13-15) were in a game park (Kwandwe) and were thus expected to have more animal pollution. Sampling sites containing continuous faecal pollution were used to identify the cut-off value for the bifidobacteria tracking ratio (see Chapter 1, Equation 1). Two different media were used to identify two separate Bifidobacteria groups. Results were then calculated to identify the tracking ratios according to Blanch et al. (2006).

The tracking ratio is the ratio of sorbitol utilising bifidobacteria (SUB) to the total bifidobacteria (TB). The cut-off value is 3.2 or 0.5 for logged values (Blanch et al., 2006). The total bifidobacteria were first enumerated on YN-17 agar, but the media was often overgrown by streptococci. This has been previously reported in literature (Mara and Oragui, 1983; Bonjoch et al., 2005). The problem of streptococci overgrowing the YN-17 agar was resolved by the use of Beerens media with an adjusted pH of 5.7 (modified Beerens medium, MBM), as growth was very poor or non-existent at pH 5.5. This may be due to the pH being close to pH 5, where no growth has been reported (Ballongue, 2004).

To test if the MBM media was suitable for substitution, a subculture of SUB was grown in a deoxygenated tryptic soy broth at 37°C under anaerobic conditions. The broth was diluted in deoxygenated physiological saline and shaken – not - vortexed to prevent aeration. Dilutions were enumerated on HBSA agar and MBM to identify whether media was suitable and would provide similar results using SUB. Results are shown in Table 19. When enumerating bifidobacteria using dilutions the potential for variation between the replicates is high, as the shaking in a media can exposed the bacteria to oxygen. Thus replication is essential for more reliable results. The SUB average concentrations on HBSA were $(1.53 \pm 1.44) \times 10^6$ CFUs/100 mL, while MBM gave $(1.33 \pm 0.95) \times 10^6$ cells/100 mL. T-tests using Statistica (StatSoft Inc., 2011) provided *p*-values equal to 0.214 at a five per cent level of significance. At the 1×10^4 mL dilution level, concentrations were equal to $(2.08 \pm 1.06) \times 10^6$ CFUs/100 mL on HBSA and $(1.53 \pm 1.56) \times 10^6$ CFUs/100 mL. The Mann- Whitney U test gave a p-value of 1. The t-test produced a *p*-value of 0.581, where the counts were deemed most reliable, at a five-per cent level of significance. As the *p*-value is greater than the α value of 0.05, the H_0 is not rejected and the two media do not produce significantly different results. So the MBM was deemed a suitable replacement for the YN-17 for the measurement of TB. All sites were then re-sampled and the respective data are summarised in Table 20. The tracking ratio at Site 15 was not determined as the total bifidobacteria concentration was zero

and thus it is not possible to identify the result. SUB concentrations ranged from 0 CFUs/100 mL at Site 10 and 12 to 2.30×10^4 CFUs/100 mL at Site 11, while the TB values ranged from 0 CFUs/100 mL at Site 15 to 8.00×10^3 CFUs/100 mL at Site 11. No faecal streptococci (FS) were enumerated from these sites on this date.

Table 19: The average concentrations of bifidobacteria grown in tryptic soy broth enumerated on Beerens media and HBSA in CFUs/mL at 1×10^4 dilution in replicate for all four broth solutions.

Beerens Media		HBSA	
Broth 1 **Broth 1 replicate**	**Broth 2** **Broth 2 replicate**	**Broth 1** **Broth 1 replicate**	**Broth 2** **Broth 2 replicate**
2.6×10^6 1×10^6	1.4×10^6 3.30×10^6	3.7×10^6 3.00×10^5	1.60×10^6 5.00×10^5

Table 20: Concentrations of bifidobacteria, faecal streptococci and the tracking ratio at Sites 9 to 15 on the 27th of March 2010.

Sites	**Sampling date**	**Sorbitol Utilising bifidobacteria (SUB) (CFUs/100 mL)**	**Total bifidobacteria (TB) (CFUs/100 mL)**	**Tracking Ratio**	**Faecal streptococci (FS) (CFUs/100 mL)**
Site 9	27/03/2010	55	36	1.5	0
Site 10	27/03/2010	0	7	0	0
Site 11	27/03/2010	23000	8000	2.9	0
Site 12	27/03/2010	0	2500	0	0
Site 13	31/03/2010	123	81	1.5	0
Site 14	31/03/2010	24	39	0.6	0
Site 15	31/03/2010	95	0	ND	0

ND – Not determined

Despite the MBM agar repressing faecal streptococci, the tracking ratio was unable to discriminate between faecal contamination sources. The survival rate of bifidobacteria in modelled river water needed to be identified. The enterococci and streptococci do not usually tolerate pHs below 4.5 (Salminen et al., 2004), however lactobacilli can survive lower pHs. Lactobacilli are less likely to grow on this medium, due to its selectivity.

HBSA agar was also sometimes overgrown by Gram positive cocci bacteria, according to Gram staining, when bacterial concentrations in the rivers were extremely high. But, it is not known if this is *Enterococcus*, as it did not significantly reduce the pH. Some enterococci do not produce large quantities of acid under certain conditions (Salminen et al., 2004). This was shown by the purple colour on the agar. The dye in the HBSA agar is bromocresol, which changes colour with a decrease in pH from 6.8.(purple) to 5.2 (yellow) (Sigma-Aldrich Co, 2012).

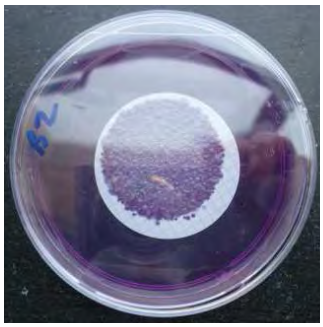


Figure 13: The human sorbitol agar plate overgrown by bacterial colonies.

Presumptive bifidobacteria bacterial colonies on HBSA and MBM were reinoculating onto m-Enterococcus agar. The square root of the total colonies on MBM and HBSA agar was chosen and screened on the m-Enterococcus agar. Approximately 81% grew as enterococci from the HBSA agar and 77% from mBeerens agar. But it was postulated the enterococci grew with the bifidobacteria and was not necessarily the only bacteria in the colony.

Some of the plates reisolated had no growth on the m-Enterococci agar. In highly polluted rivers, it would decrease the likelihood of the bifidobacteria tracking ratio working. The number of enterococci positive bacteria on both MBM and HBSA agar were similar. As the growth was similar on both the TB and SUB media, the tracking ratio should not be affected.

Lactobacilli are excreted by humans and animals and do not grow in the environment. They were enumerated to identify their potential as markers for faecal pollution. It was also used to identify whether some of the growth on the bifidobacteria media was *Lactobacillus* and whether they could be good indicators of faecal pollution, as they are unlikely to grow in the water environment due to their anaerobic nature. In January 2012, the rivers were sampled to identify the correlation between enterococci and bifidobacteria concentrations. The use of MRS agar for *Lactobacillus* enumeration was not a success as *Bifidobacterium*, *Enterobacter*

and other bacteria could grow on the media. Table 21 shows that the bifidobacteria were far lower than the enterococci in the rivers and *Lactobacillus* agar usually grew more bacteria than bifidobacteria and enterococci, possibly due to the growth of other bacteria on the media. The enterococci concentrations seemed to be closer to the faecal coliform concentrations, while the *Lactobacillus* did not correlated well. *Lactobacillus* is incubated for 56 hours, which is longer than the incubation periods for enterococci and bifidobacteria. Vancomycin has been suggested to increase selectivity (Hartemink et al., 1997), but it was not taken further, as *Lactobacillus* is excrete by both animals and humans.

Table 21: The concentrations of faecal coliforms (FC), bifidobacteria *(Bifido), Lactobacillus* and *Enterococcus* in selected sites.

	pH	EC (ms)	Temperature (°C)	Alkalinity (mg/L $CaCO_3$)	*Enterococcus* (CFUs/ 100 mL)	*Bifido* (CFUs/ 100 mL)	*Lactobacillus* (CFUs/ 100 mL)	FC (CFUs/ 100 mL)
Site 9	7.32	0.18	18	21.428	120	0	273	122
Site 10	6.87	0.88	18	135.714	196	0	108	6
Site 11	7.22	1.28	18	250	11000	6	161	13000
Site 12	7.32	1.23	18	217.8571	3000	1382	136	4000

3.4.2 River Water Physico-chemical Parameters

The next step was to identify why the tracking ratio was not creating a clear cut-off value to distinguish between animal and human faecal pollution. This could be an effect of the chemical composition of the water on bifidobacteria survival at the calibration sampling sites. Model water was developed by measuring the physico-chemical parameters of the selected sites. The chemical composition of the model water composition is shown in Table 8. It will be used later to develop survival experiments. The river water was sampled approximately once a month between September 2010 and November 2011.

Table 22: The average concentration of different chemicals and bacteria at seven continuously polluted selected site over a 6 month period.

Site number	9		10		11		12	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Bifidobacteria on HBSA agar	33	20 - 43	18.38	0 - 47	7354.38	0 - 15900	9680	0 - 26512
Bifidobacteria on Beerens Modified agar	14	2 - 21	15.00	0 - 44	1716.50	4 - 3600	434	0 - 1700
Chlorides (mg/L)	111.00	8.66 - 197.75	53.90	2.03 - 151.50	119.04	6.00 - 235.13	145.56	7.34 - 283.19
Ammonia (mg/L)	4.63	0 - 17.93	5.09	0.02 - 20.21	4.67	0.7 - 12.17	4.75	0.2 - 12.11
Phosphate (mg/L)	4.38	0 - 12.14	2.62	0 - 7.36	2.75	0 - 6.12	4.30	0.54 - - .85
Nitrates (mg/L)	11.87	0 - 45.85	11.83	0 - - 7.3	11.73	0.02 - 41.5	12.28	0 - 45.82
Sulphates (mg/L)	31.11	0.56 - 63.8	21.80	2.96 - 43.57	48.63	4.9 - 90.01	50.39	28.81 - 82.55
pH	7.69	7.32 - 8	8.20	7.06 - 9.27	7.85	7.39 - 8.27	7.73	7.24 - 8.04
EC (mS/m)	50	34 - 90	45	20 - 99	75	9 - 138	137	66.8 - 238
Hardness (mg $CaCO_3$/L)	56.07	30 - 80	27.94	10 - 80	171.47	0 - 240	171.47	0 - 240
Temperature	22.3	18.9 - 19	20.58	18 - 26.5	19.85	13.5 - 28.6	20.43	17.3 - 27.2
***E. coli* CFUs/100 mL**	1.5	0 - 4	0.50	0 - 2	27.00	0 - 80	6.5	0 - 14
Faecal coliforms CFUs/100 mL	34	22 - 34	10.50	0 - 30	1287.50	350 - 1600	1425	900 - 1600
H_2S time to positive	27.5	22 - 40	22.75	19 - 24	13.50	12 - 18	13.5	12 - 18

Table 22 **(continued)**

Site number	13		14		15		All sites
	Mean	Range	Mean	Range	Mean	Range	Range
Bifidobacteria on HBSA agar (CFUs/ 100 mL)	28.63	0 - 50.5	36.5	14 - 76	19.13	0 – 38	0 - 26512
Bifidobacteria on Beerens Modified agar (CFUs/ 100 mL)	41.63	0 - 94	33.625	9 - 64	27	0 – 80	0 - 3600
Chlorides (mg/L)	173.76	57.40 - 225.06	241.33	202.81 - 276.44	198.38	178.75 - 227.25	2.03 - 283.19
Ammonia (mg/L)	0.20	0.08 - .31	0.0875	0 - 0.2	0.18	0.11 - 0.22	0 - 20.21
Phosphate (mg/L)	6.15	4.65 - 7.4	2.3325	1.76 - 2.73	5.69	4.32 - 7.62	0 - 12.14
Nitrates (mg/L)	0.97	0 - 3.87	0	0	0.53	0 - 2.11	0 - 47.3
Sulphates (mg/L)	42.46	21.75 - 55.86	32.1625	16.84 - 48.63	34.55	27.55 - 47.6	0.56 - 90.01
pH	8.45	8.01 - 8.78	8.975	8.39 - 9.66	9.29	8.33 - 9.85	7.06 - 9.85
EC (mS/m)	126	99 – 153.9	125.875	101 - 149	150	126.2 – 181	9 - 238
Hardness (mg/L $CaCO_3$)	284.12	160 - 403.52	249.22	16 - 343.68	289.52	160 - 400.96	0 - 403.52
Temperature	20.8	19 - 22.2	21.05	20.4 - 22	24.4	20 – 26	13.5 - 28.6
***E. coli* CFUs/ 100 mL**	5.25	0 - 17	3.5	0 - 8	1.5	0 – 4	0 - 80
Faecal coliforms CFUs/ 100 mL	275	0 - 900	112.25	50 - 240	33	0 – 79	0 - 1600
H_2S time to positive	16.5	12 - 24	22.5	12 - 48	22.5	12 – 48	12 - 48

Temperatures ranged between 13.5 and 28.6 °C, with both minimum and maximum values occurring at Site 11. Optimal temperature for bacterial growth is 37°C, as faecal coliforms, bifidobacteria and enterococci normally grow in the human body. Enterococci can survive temperatures from 5 to 65°C (Fisher and Phillips, 2009). However, enterococci are not reported to grow or replicate below 20°C (Bergley, 1989). Bifidobacteria do not tend to grow below 30°C. *E. coli* can grow at 22°C and survives well between 15 and 45°C (Bergley, 1989). Water temperature is thus only really conducive to *E. coli* and, possibly, *Enterococcus* growth.

Ammonium (0.05 mg/L NH_4-N - EPA 350.1,), nitrate (0.4 mg/L NO_3^- - DIN 38405 D9) and phosphate (0.2 mg/L PO_4^{3-} - EPA 365.2+3) concentrations are close to the limits of detection shown in the brackets (Environmental Protection Agency, 1971; Environmental Protection Agency, 1978; Environmental Protection Agency, 1993). The protein level was below quantifiable concentrations of 0.1–1.4 mg/mL, the range detectible by Bradford's Reagent (Sigma). The hardness of water is mainly affected by the calcium concentration, while magnesium also contributes (Lehloesa and Muyima, 2000). Hardness in surface waters rarely exceeds 100 mg $CaCO_3$/L in natural water, although it does depend on the geology of the area and inland waters can reach 1000 mg $CaCO_3$/L (DWAF, 1996d). So it varies from very soft to hard (403.52 mg $CaCO_3$/L) at Site 13, however Sites 14 and 15 also had values above 400 mg $CaCO_3$/L. The alkalinity of the water also indicated the carbon available for plant growth (Egan et al., 2004). When hardness increases beyond natural variations, it will change the fauna and flora of the river (Patrick, 1962). Calcium carbonate does not have antibacterial properties and can be used as a buffer (Farhad and Mohammadi, 2005).

The pH ranged between 7.24 and 8.04. Department of Water Affairs and Forestry (DWAF) guidelines state that natural water ranges between 4 and 12, but needs to be within the range of 6.5 and 9 for fish to survive (DWAF, 1996a). These rivers are very neutral. Some of these rivers and dams are used for recreational purposes and the recommended pH for this is between 6.5 and 8.5 (DWAF, 1996d). So this water is well-suited for recreational use according to this parameter. The pH of water or any medium affects the survival rate of bacteria. Some bifidobacteria have been shown to survive for 90 minutes at pH 2 (Noriega et al., 2004), but their optimal pH ranges between 6.5 and 7.0 (Salminen et al., 2004). But they cannot grow below pH 5 or above pH 8 (Salminen et al., 2004). Most of the river water in this study is close to the optimal pH for bifidobacteria. While bifidobacteria thrive in the human intestines with a pH range of 5.9 to 8.6 (Wilson, 2005), *Escherichia coli* grow at pHs between 5 and 9 (Bergley, 1989). Enterococci are more sensitive to pH than to temperature, but can still grow between pH 4.5 and 10 (Fisher and Phillips, 2009). These river pHs should not significantly affect the survival of *E. coli*, enterococci or bifidobacteria on its own.

Chloride concentrations ranged from 2.0 to 283.2 mg/L. Chlorides are usually part of salts and are attached to alkaline metals and alkaline earth metals such as sodium, potassium, calcium and magnesium, which are highly soluble in water (DWAF, 1996a). These often leach from the rocks and depend on the geology of the area (DWAF, 1996a).

Usually, freshwater chloride concentrations range from almost zero to several hundred mg/L (DWAF, 1996a). Enterococci have been known to survive in 6.5% NaCl, far higher than these concentrations. Bifidobacteria have been found in river water with a NaCl level of 3.3% (Morrison et al., 2008). Sorbitol fermenting bifidobacteria have been found in rivers with 850 mg/L chloride and salinity of 30 mg/L (Mushi et al., 2010). Bifidobacteria can survive up to 6.0×10^4 mg/L NaCl (Gomes et al., 1998). Very low salinity rates should not affect the survival of bifidobacteria, *E. coli* or enterococci.

The total dissolved solids (TDS) are directly proportional to the electrical conductivity (EC). Organic content of water does not usually contribute to the EC, making it a measure of inorganic ion content, such as carbonate, bicarbonate, calcium, chloride, magnesium, nitrate, potassium, sodium and sulphate, which are charged (DWAF, 1996c). EC is almost totally influenced by the geological rock formations of an area, although industrial waste and grey water would could affect it too (DWAF, 1996c). The EC ranged from 9 - 238 mS/m (0.585 - 15.47 mg TDS/L). The conversion factor for EC to TDS is 6.5, the average value for freshwater (DWAF, 1996c). The EC was indicative of Precambrian shelf areas with values below 65 mg TDS/L (DWAF, 1996c). These ions could provide possible nutrients to the bacteria in the rivers. An extremely high EC could potentially cause osmotic pressure for bacteria, but the values in this study are too low for this effect.

The rivers' water temperature ranged between 13.5 and 28.6°C, over this spring and summer period. 13.5°C is lower than the minimum temperatures found during the 2009 screening. With temperatures not exceeding 30°C, the likelihood of bifidobacteria multiplying in the water is very small.

The river water chemical parameters were shown in Figure 14. The river water was sampled once a month in the second week. The data is shown in tabulated form above. The high standard deviation or variance is due to the large difference seen between sampling times. This could be due to seasonal variation or contamination by humans and litter. Large variations have been reported before by Zuma (2010).

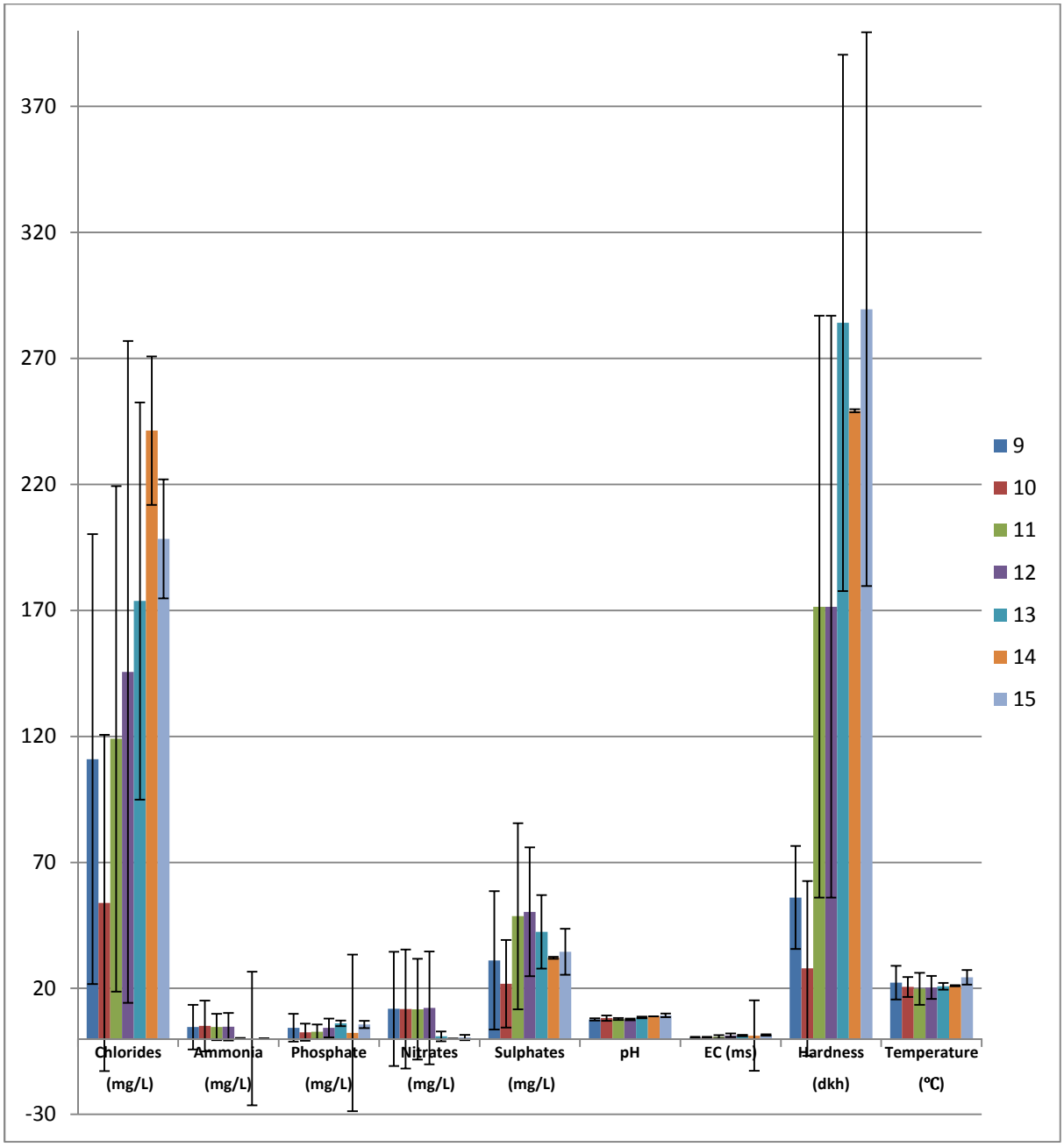


Figure 14: The average concentrations from Table 22, with variance over the sampling period from September 2010 to January 2011.

Figure 14 shows how the condition of the river changed over the last sampling period after a large rainfall in June 2010, which seemed to wash out some of the refuse and cleared a large percentage of the bacterial pollution from within the river. After the rain, concentrations of faecal coliforms initially increased and this was soon followed by a drop, while the Bifidobacteria concentration has drastically decreased after the washout. Sites 13 to 15 also changed in bacterial concentration related to the rain. After each rainfall, the faecal coliform level increases and then decreases as the water level subsides, possible due to die-off.

This is probably due to wash off of faecal matter from the banks and surrounding areas, into the rivers (LaLiberte and Grimes, 1982, Mallin et al., 2009).

3.4.3 Multivariate Analysis

Data from Table 22 showing how river water changed over time was used to develop model water for survival experiments. The chemical data was analysed against the bacteria concentrations to identify the possible contributing factors to bacteria survival. The results were analysed using PRIMER 6 + with the PERMANOVA add on, using DISTLM, Bray Curtis and AIC, being the least reliant on linearity (Plymouth Marine laboratory, 2009). Results are shown in Table 23 and Figure 14. pH was shown to be most closely related to the concentration, while the other closest related factors were the sulphates, temperature and chlorides, in descending order. The Bray Curtis analysis using AIC and the Euclidian distance yielded similar results.

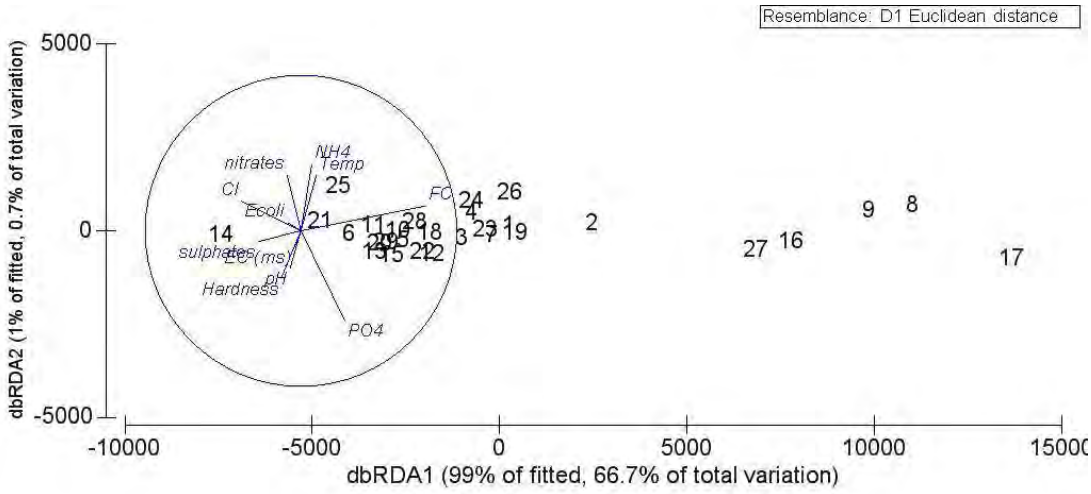


Figure 15: Correlation between river water chemical parameters and the concentration of bifidobacteria, using PRIMER 6+ PERMANOVA DISTLM.

Table 23: The correlation between faecal coliforms and the environmental factors of the sampled river water data from between September 2010 to June 2011, using DISTLM in PRIMER 6 + PERMANOVA add on. The Bray Curtis AIC states that the concentration of faecal coliforms is actually best represented by a combination of all the different parameters.

MARGINAL TESTS with residual degrees of freedom of 24 (res.df:24)				
Variable	**SS (trace)**	**Pseudo-F**	**P**	**Prop.**
pH	4548.2	3.823	0.03	0.13741
Conductivity	458.73	0.33728	0.938	1.3859E-2
Chloride	2020.7	1.5604	0.201	6.1048E-2
Ammonia	26.022	1.8883E-2	0.995	7.8616E-4
Phosphate	499.4	0.36764	0.648	1.5087E-2
Nitrates	570.44	0.42086	0.639	1.7234E-2
Sulphates	2210.3	1.7173	0.186	6.6776E-2
Hardness	599.06	0.44236	0.584	1.8098E-2
Temperature	2160.8	1.6761	0.188	6.5278E-2

SS is the sum of squares. This is the square of the sum of differences between the measure and average values.

P is the probability. It states how significant the value is. Thus the lower the value the more significant it is.

Prop is the proportion of the data explained by this result

Pseudo-F is the same as the ANOVA F statistic which shows the variance between the means

df is the degrees of freedom

The information on the chemical river data was used to generate model water for survival experiments. Model water was developed, using those chemical parameters which could be adjusted and kept constant over time. The influence of Dissolved Oxygen (DO) on aerobic bacteria is well known to increase die-off rates, while a decreasing DO with facultative and obligate anaerobes should not significantly influence their die-off (Rolfe et al., 1977; Talwalkar and Kailasapathy, 2004). Fast flowing rivers with turbulence may have more oxygenation (Guasch et al., 1998), but this could not be reliably modelled and was excluded. Die-off due to light exposure may be significant, as UV is detrimental to bacteria (Fujioka and Narikawa, 1982; Davies-Colley et al., 1994; Sinton et al., 2002; Curtis and Cairncross, 2003; Sinton L et al., 2007). UV exposure is also dependent on water turbidity and the depth of the bacteria present. Survival experiment bottles were never exposed to direct sunlight and sunlight exposure was minimised.

Some parameters are known to affect the survival of bacteria, such as temperature, pH and chloride (Patel et al., 1995; Sutherland et al., 1995; Thomas et al., 1999). Thought that bacteria need a nitrogen source was the reason for inclusion. Faecal coliforms, which are part of the Enterobacteria, tend to reduce nitrate to nitrite (Bergley, 1989). Thus, as nitrate is often broken down to nitrite (Jia and Cole, 2005), it is a more likely used by bacteria.

Protein was chosen as it was unsure if this was a need or not. Experiments with enterococci showed that nitrogen and amino acid starvation caused a rapid decrease in the survival rate (Trainor et al., 1999), and this was thus considered here. The only thing which may be required is an organic source of nutrients, which was not included in the model water.

3.4.4 Model Water

Model water was used to identify the survival rate of bacteria. Survival of faecal coliforms was tested as a comparison to that of bifidobacteria, as it is currently being used in current monitoring methods and is well established. The survival of enterococci was monitored, as it is often found in rivers in warmer climates and could possibly affect the survival of other bacteria. Survival of bifidobacteria was performed to understand the instability of the tracking ratio. The tracking ratio may not work due to the fast die-off rates of one of the Bifidobacteria groups, or due to the lack of continuous faecal input. Survival rates will help identify how recently faecal pollution occurred.

This could aid public health risk identification. The public health risk will also be affected by the faecal pollution source, distinguishing between human and animal faecal pollution, which will aid risk assessment and remediation programmes.

The chemical composition of the model water was designed from the chemical concentrations found in the Grahamstown rivers over a period of 6 months.

3.4.5 Survival of Bifidobacteria

Bifidobacteria survival is due to environmental parameters. Temperatures chosen were based on dairy studies and possible river water temperatures. The lowest temperature was 8°C, as no growth is found below 4°C and rivers in South Africa are unlikely to reach such a low temperature, although survival has been identified in dairy studies (Dave and Shah, 1997). The combined effects of pH, acidity and temperature have been studied in yoghurt (Dave and Shah, 1997; Jayamanne and Adams, 2009). They showed that pH significantly affected the survival rate. The use of MBM agar showed the bacteria die-off much faster than on HBSA agar. HBSA agar was thus used for the remaining survival experiments. This could be due to the low pH and the bifidobacteria being less resistant after stress or some other factor of the media, as RCM or tryptic soy agar did not present the same die-off problem. The bacteria did not survive beyond 152 hours except in model water 5, which cannot be explained, as the inoculated cultures were the same for all the experiments and ran simultaneously. There is the possibility of enterococci being present and growing at this low temperature. The experiments were stopped at this point, and there is insufficient information to compare the nutrients with survival rates.

The bifidobacteria in the model water were enumerated and selected colonies were Gram stained. A change in shape was notice over the survival period. This could possibly be due to the lack of nutrients. The composition and nutrients change the shaped between V, Y or X in *Bifidobacterium* (Ballongue, 2004).

The inactivation rate is equal to the regression value for the $\log_{10}$ CFUs/20 mL per hour.

Table 24: The average bifidobacteria concentration (CFUs/ 20 mL) on HBSA agar from the different factorial design model water according to the number of hours they survived. The inactivation rate is calculated by the log of the CFUs/20 mL per hour.

Time Design number	0 hours	1 hours	3 hours	24 hours	72 hours	152 hours	Initial pH	Final pH	Inactivation rate
5	552	222	226	220	210	>500	9.8	3.01	0.8853
6	471	100	60	59	50	3	5.3	3.56	0.0776
7	368	233	230	51	36	0	9.8	6.4	0.9974
8	726	526	230	81	150	0	5.3	2.93	0.7982
9	298	195	100	24	10	0	5.3	3.33	0.5957
10	295	270	133	75	0	0	5.3	4.59	0.8318
11	296	283	176	134	0	0	9.8	6.73	0.7631

OG – overgrown membrane filter

Table 25: The average concentration of total bifidobacteria (CFUs/20 mL) on modified Beerens Media from the factorial design model water, according to their survival time. The inactivation rate is calculated by the log of the CFUs/20 mL per hour.

Time (hours) Design number	0	1	3	24	72	Inactivation rate	Initial pH	Final pH
5	258	140	86.5	200	0	0.5458	9.8	3.01
6	264	140	136	217	0	0.7532	5.3	3.56
7	424	420	400	299	0	0.8216	9.8	6.4
8	400	138	313.33	155	42	0.5981	5.3	2.93
9	456	366	286	250	0	0.7586	5.3	3.33
10	457	407	349	276	0	0.799	5.3	4.59
11	300	289	200	154	0	0.7532	9.8	6.73

Initial experiments produced a problem, as the pH decreased significantly over the time period, as the bifidobacteria produced lactic and acetic acid, lowering the pH. This would not naturally occur in the river, as fresh river water would always be available and the acid would be greatly diluted. This could contribute to a faster die-off rate.

Calcium carbonate was used to identify its suitability as a buffer for the model water. The survival experiments were run with one model water with varying concentrations of calcium carbonate to ensure the calcium carbonate did not injure or kill the bacteria alone. Results are shown in Table 26. The pH changed as nutrients were used and the acid increased overtime. The pH change was not substantial and calcium carbonate was used as a buffer at 1g/L concentration. Increasing the calcium carbonate also increased the turbidity of the solution at the beginning of the experiment, while at the end of the experiment, calcium carbonate had formed lumps and was no longer free-flowing or in suspension, even after swirling. The model water was never shaken as this would have caused a huge difference to DO. Thus the bottle with model water was swirled well before samples were taken.

Table 26: Different concentrations of calcium carbonate and pH changes while the bifidobacteria survived.

Concentration of Calcium Carbonate	**0 Days**	**1 Day**	**2 Days**	**4 Days**	**7 Days**	**11 Days**	**Final change in pH**
0 g/L – no bacteria	7	6.4	6.3	6.3	6.4	6.2	0.63
0.1 g/L	7.15	6.69	7.07	7.09	6.81	7.27	0.46
0.2 g/L	8.18	7.63	7.45	7.64	7.54	7.7	0.55
1 g/L	6.56	7.2	7.37	7.86	7.98	8.08	-0.64
2 g/L	7.87	8.02	7.44	7.91	8.08	8.2	-0.15

New survival experiments were done in model water with an initial 1g/L calcium carbonate concentration. Results were also examined on MRS agar, as this media was reported to be less of an inhibitor to stressed bifidobacteria.

Survival rates were lower at 32°C verses those at 8°C. This is something previously shown by Ottoson (2009). The effect of sediment was added to the equation, as FC coliforms were reported in literature to be highly influenced by sediment (LaLiberte and Grimes, 1982; Burton et al., 1987; Sherer et al., 1992). The sediment used was pool sand which had been washed and autoclaved. However, Sediment did not seem to significantly affect the survival of bifidobacteria.

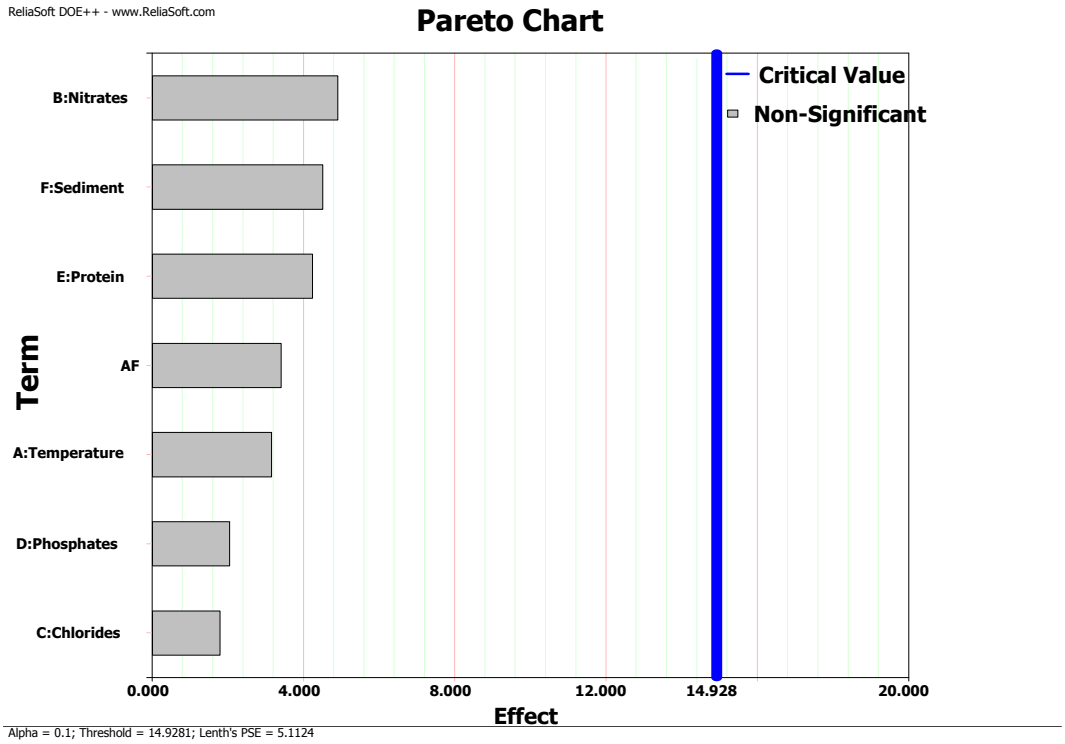


Figure 16: The Pareto Chart of bifidobacteria survival rates verse the different environmental parameters using DOE^{++} version 1.0.7.

The study indicates that all the variable were not significant at the concentrations tested and under the set conditions. The under these concentrations and conditions thus have no significant effect on the inactivation rate of the bifidobacteria.

The Pareto Chart depicted in Figure 19 enables the identification of the statistically significant effects. The vertical line represents the critical value (6.514) indicates that none of the variables or combinations thereof were significant at a 95% significance level.

A commonly method for evaluating the model is Analysis of variance (ANOVA) as shown in Table 28. It provides some statistical parameters such as the coefficient of determination, R^2 and the F-value, which compare regression and residual variance (Box and William, 2005; Pontes et al., 2011). The F-value for the model is higher than the tabulated one, indicating model reliability at the 95% level of confidence. Moreover the R^2 (0.9561) approaches one which reinforces the good fit of the model. Consequently, 1 is therefore able to navigate the design space in order to determine the response Y. These parameters are shown in Table 27.

Table 27: The statistical ANOVA results for the survival of bifidobacteria with buffered pH using calcium carbonate.

ANOVA Table					
Source of Variation	**Degrees of Freedom**	**Sum of Squares [Sequential]**	**Mean Squares [Sequential]**	**F Ratio**	**P Value**
Model	5	4.3532	0.8706	8.7073	0.1062
Main Effects	5	4.3532	0.8706	8.7073	0.1062
Residual	2	0.2	0.1		
Lack of Fit	2	0.2	0.1		
Total	7	4.5532			
R^2 = 0.9561 and adjusted R^2 is 0.8463					

Regression Information							
Term	**Effect**	**Coefficient**	**Standard Error**	**Low CI**	**High CI**	**T Value**	**P Value**
Intercept		3.1465	0.1168	2.6441	3.6489	26.9464	0.0014
A:pH	0.1033	0.0517	0.1168	-0.4507	0.5541	0.4425	0.7014
B: Temperature	-0.4862	-0.2431	0.1168	-0.7455	0.2593	-2.0818	0.1728
C:Nitrate	**1.1499**	**0.575**	**0.1168**	**0.0726**	**1.0774**	**4.924**	**0.0389**
D:Phosphate	0.6742	0.3371	0.1348	-0.243	0.9173	2.5003	0.1296
E:Sediment	**1.0606**	**0.5303**	**0.1168**	**0.0279**	**1.0327**	**4.5417**	**0.0452**

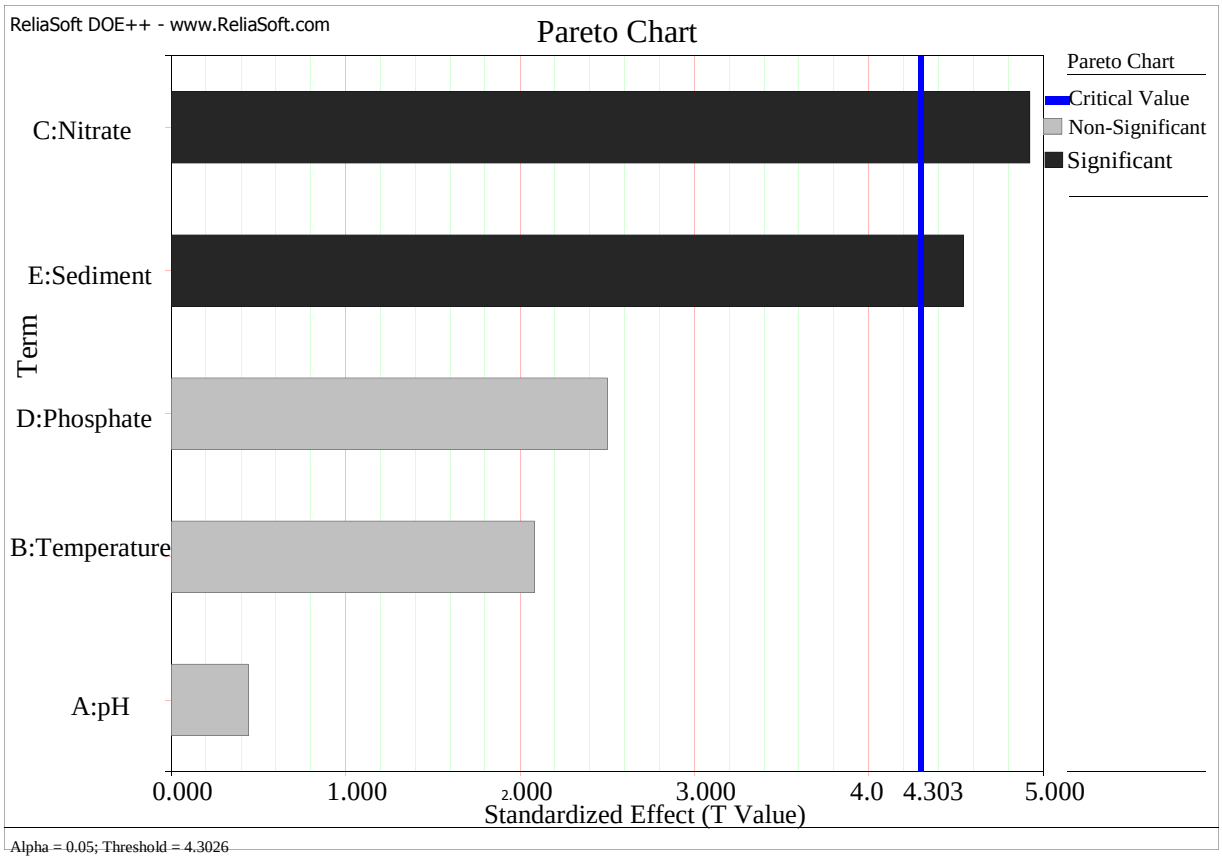


Figure 17: The Pareto Chart shows the main effects of the environmental parameters on the survival rates of bifidobacteria using DOE^{++} version 1.0.7.

The graph in Figure 16 shows that none of the different factors are significant on their own. The combination of sediment and temperature had the most effect. The data did not fit any of the GInaFit survival models either (Geeraerd, 2012).

The tracking ratio not working is not necessarily attributable to the survival rate, but perhaps could be due to their survival rate being so low. There did not seem to be a difference in the die-off of the total bifidobacteria verses the sorbitol utilising bacteria. This was also found by Ottoson (2009). Bifidobacteria did not grow well under stressed conditions on modified Beerens media.

The survival of bifidobacteria was not significantly different in the presence of faecal coliforms. The enterococci and faecal coliforms survived far longer than bifidobacteria, also noted by Ottoson (2009).The inclusion of enterococci survival with bifidobacteria is important as enterococci produce a range of different bacteriocins which could inhibit the

growth of other bacteria (Salminen et al., 2004). But they did not seem to affect the survival rates of bifidobacteria.

3.4.6 Enterococci Survival

The survival of presumptive enterococci was measured at the same time as the bifidobacteria.

Bacterial concentrations tended to increase in some model waters. Their resilience and tolerance to many different environmental conditions, and the ability of some enterococci to grow at 10°C, 45°C and pH 9.6 may explain this result (Salminen et al., 2004). Some enterococci are bile tolerant to 40% (Salminen et al., 2004). Some species such as *Enterococcus faecium* can hydrolyse proteins (Wilson, 2005). Their ability to grow within the model water may be explained by their resistance to low nutrient levels and extreme environments. They are highly resistant to extreme environment conditions (Fisher and Phillips, 2009), despite their normally considerable need for many specific vitamins and amino acids for growth (Salminen et al., 2004). Enterococci produce a range of different bacteriocins which could inhibit the growth of other bacteria (Salminen et al., 2004).

Enterococci can be differentiated from streptococci as streptococci only grows at 45°C (Salminen et al., 2004) and incubating it at 35°C is thus more likely to be enterococci.

The study indicates that X_1, X_2, X_3, X_4, X_5 are not significant under these concentrations and conditions thus have no significant effect on the inactivation rate of the faecal coliforms.

The Pareto Chart shows the statistically significant factors or lack thereof. The bar graph lengths are proportional to the absolute values of the estimated effects. A bar graph which crosses the vertical (critical line, blue line) can be interpreted as having a significant effect on Y (inactivation rate).

The Pareto Chart depicted in Figure 19 enables the identification of the statistically significant effects. The vertical line represents the critical value (697.26) indicates that none of the variables or combinations thereof were significant at a 95% significance level.

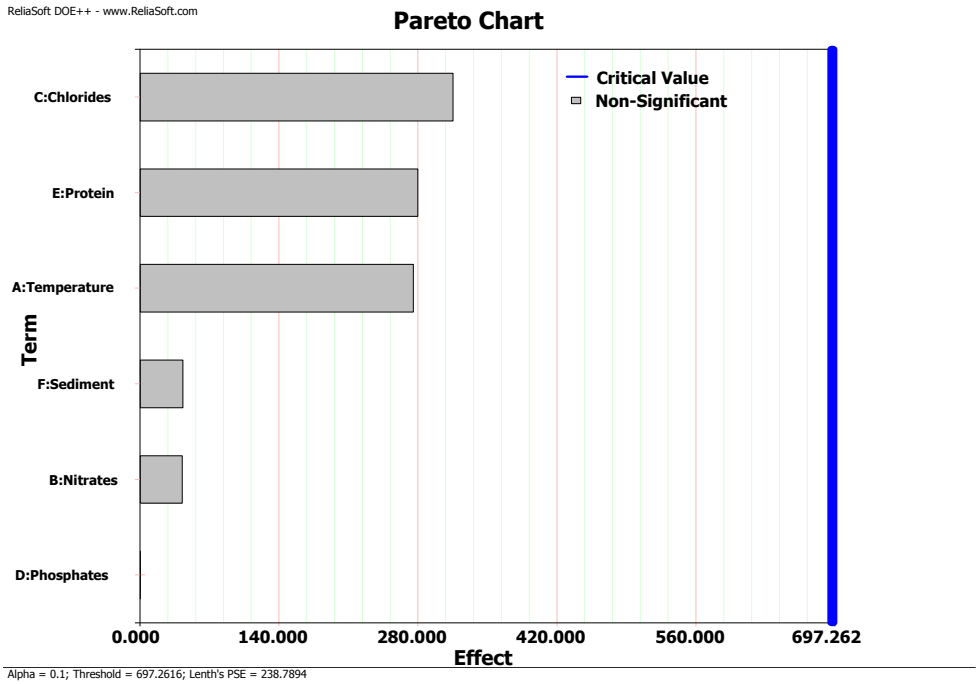


Figure 18: The survival rate of Enterococci versus the environmental parameters of the model water.

A commonly method for evaluating the model is Analysis of variance (ANOVA) as shown in Table 28. It provides some statistical parameters such as the coefficient of determination, R^2 and the F-value, which compare regression and residual variance (Box and William, 2005; Pontes et al., 2011). The F-value for the model is higher than the tabulated one, indicating model reliability at the 95% level of confidence. Moreover the R^2 (0.556) is far from 1 which shows that this is not a good fit of the model and thus other things are influencing the survival of the enterococci. Consequently, the determination of Y is not possible from this data. These parameters are shown in Table 28and Table 29.

3.4.7 Faecal Coliform Survival

Factors which affect survival include predation, UV radiation, temperature, pH, DO, salinity, suspended matter, distance from the source (Krolska et al., 1997; Pachepsky and Shelton, 2011), toxic compounds, osmotic pressure, nutrient starvation, plankton, dilution and possibly others (Krolska et al., 1997; Darakas, 2001).

Survival of *Escherichia coli* has been studied by many different groups. Each study has its own limitations, as laboratory experiments are not representative of predators in the rivers, but river experiments have their own problems. Additionally, the aquatic exposure depends

on the experimental design, and unless similar methods are used, experiments are not easily comparable (Mcfeters and Terzieva, 1991). Experiments done in temperate environments often differ from tropical experiment results due to differing predators, and other environmental conditions (Carrillo et al., 1985; Krolska et al., 1997).

Their survival is well correlated with irradiation from UV, as on a bright, sunny day, the number drop by 10% in 20 minutes in the water column (Krolska et al., 1997). *E. coli* is particularly susceptible to irradiation at 250-270nm, but is less susceptible at temperatures below 10°C (Krolska et al., 1997). McCambridge and McMeekin (1981) also found predation and irradiation were very significant. The effect of salinity depends on the concentration at low salinities, such as 0-10 psu (practical salinity scale unit) (below approximately 8822 mg/L), however above 15 psu (approximately 12935 mg/L) accelerated the die-off rate (Faust et al., 1975; Krolska et al., 1997). Other studies have shown an increased die-off rate at 5 mS/cm (approximately 31 mg/L chloride) (Anderson et al., 2005; Pachepsky and Shelton, 2011). Plants in the water provide protection and thus increase the concentrations of bacteria (Pachepsky and Shelton, 2011). Pachepsky and Shelton (2011) stated the distance from the river bank may also influence the bacterial concentration.

The effect of salinity depends on the concentration at low salinities, such as 0-10 psu (practical salinity scale unit) (below approximately 8822 mg/L), however above 15 psu (approximately 12935 mg/L) accelerate the die-off rate (Faust et al., 1975; Krolska et al., 1997). Other studies have shown an increased die-off rate at 5 mS/cm (approximately 31 mg/L chloride) (Anderson et al., 2005; Pachepsky and Shelton, 2011). Plants in the water provide protection and thus increase the bacteria concentrations (Pachepsky and Shelton, 2011). Pachepsky and Shelton (2011) stated that the distance from the river bank may also influence the bacterial concentration.

Using specific bacterial strains gives a good idea of those strains' survival, but bacteria tend to become resistant to their environmental factors (Krolska et al., 1997). Bacteria used from the stream or river may give more reliable results for that environment's survival.

The Dissolved Oxygen (DO) level has a significant effect on *E. coli* survival, with deoxygenation detrimentally increasing with temperature (Faust et al., 1975; Krolska et al., 1997).

Resuspension elevates the FC concentration in water (Pachepsky and Shelton, 2011). Surface samples are less likely to represent the whole load (Pachepsky and Shelton, 2011). Diurnal oscillations were up to 1.5 magnitude difference, while seasonal trends also occur (Pachepsky and Shelton, 2011).

High bacterial content in course sediments protect it from light and predators and often has high organic content (Pachepsky and Shelton, 2011). The type of sediment also influences survival rate (Pachepsky and Shelton, 2011). Survival in lake sediment is significantly longer than in the water column, which may be due to the lack of UV penetration and protection from predators (Darakas, 2001). In a German study using radiation equivalent to sunlight at midday in summer, with the mean radiation in brackets, gave a faecal coliform inactivation rate of 21.4 day^{-1} (12.7 day^{-1}) and for enterococci 20.0 day^{-1} (9.3 day^{-1})(Schultz-Fademrecht et al., 2008). Biofilms increased the survival time due to protection they afforded (Schultz-Fademrecht et al., 2008).

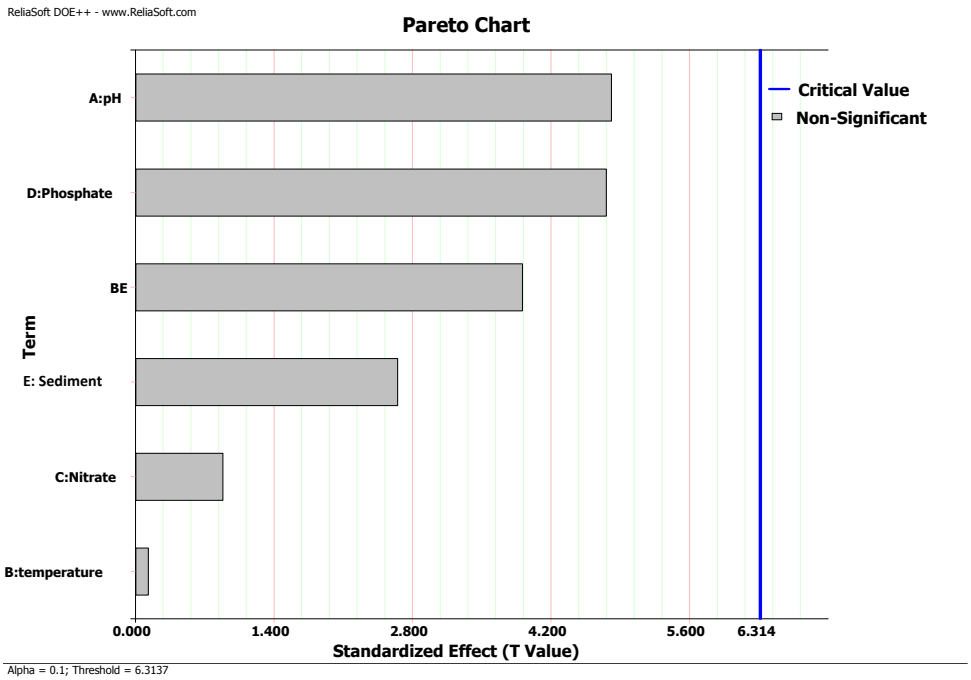


Figure 19: The Pareto Chart for the survival of FC. None of the environmental factors alone seem to be significant, but the pH of the environment and sediment and temperature interact together with a high effect.

The study indicates that X_1, X_2, X_3, X_4, X_5 are not significant under these concentrations and conditions thus have no significant effect on the inactivation rate of the faecal coliforms.

The Pareto Chart shows the statistically significant factors or lack thereof. The bar graph lengths are proportional to the absolute values of the estimated effects. A bar graph which crosses the vertical (critical line, blue line) can be interpreted as having a significant effect on Y (inactivation rate).

The Pareto Chart depicted in Figure 19 enables the identification of the statistically significant effects. The vertical line represents the critical value (6.514) indicates that none of the variables or combinations thereof were significant at a 95% significance level.

A commonly method for evaluating the model is Analysis of variance (ANOVA) as shown in Table 28. It provides some statistical parameters such as the coefficient of determination, R^2 and the F-value, which compare regression and residual variance (Box and William, 2005; Pontes et al., 2011). The F-value for the model is higher than the tabulated one, indicating model reliability at the 90% level of confidence. Moreover the R^2 (0.984) approaches one which reinforces the good fit of the model. Consequently, 1 is therefore able to navigate the design space in order to determine the response Y. These parameters are shown in Table 28 and Table 29.

Table 28: ANOVA table of the effects of the inactivation rate of faecal coliforms due to the conditions established.

ANOVA Table					
Source of Variation	**Degrees of Freedom**	**Sum of Squares [Partial]**	**Mean Squares [Partial]**	**F Ratio**	**P Value**
Model	6	328.534	54.7557	10.346	0.2336
Main Effects	5	324.0148	64.803	12.2445	0.2135
2-Way Interaction	1	80.9406	80.9406	15.2937	0.1594
Residual	1	5.2924	5.2924		
Lack of Fit	1	5.2924	5.2924		
Total	7	333.8264			
R^2 = 98.41%		Adjusted to 88.90%			

Table 29: The regression information for the different parameters in the designed faecal coliform survival experiments.

Regression Information							
Term	**Effect**	**Coefficient**	**Standard Error**	**Low CI**	**High CI**	**T Value**	**P Value**
Intercept		12.2006	0.9962	5.9111	18.4901	12.2476	0.0519
A:pH	-9.5842	-4.7921	0.9962	-11.0816	1.4974	-4.8106	0.1305
B: Temperature	-0.2578	-0.1289	0.9962	-6.4184	6.1606	-0.1294	0.9181
C:Nitrate	1.7569	0.8785	0.9962	-5.411	7.1679	0.8818	0.5399
D:Phosphate	-21.8967	-10.9483	2.3005	-25.4733	3.5766	-4.7591	0.1319
E:Sediment	-5.2779	-2.6389	0.9962	-8.9284	3.6505	-2.6491	0.2298
BE	14.9193	7.4597	1.9075	-4.5838	19.5031	3.9107	0.1594

3.5 CONCLUSION

The cut-off value for the tracking ratio SUB: TB should not have been significantly affected by the bifidobacteria survival rates. But the quicker lack of culturability on the mBeerens media may affect the results slightly. The tracking ratio could not be determined due to the media being overgrown by streptococci, the survival rates of the bifidobacteria and other interfering factors.

Enterococci were significantly correlated with faecal coliforms and are good monitoring bacteria in the temperate/ tropical climate. The minimisation of enterococci from the total bifidobacteria media was improved with mBeerens media compared to YN-17, although molecular methods seem the only way to improve this further, as bifidobacteria and enterococci show similar antibiotic resistances and sensitivities.

The use of antibiotics could prevent specific strains of bifidobacteria from growing. HBSA is a well-suited media to identify human bifidobacteria and could possibly be used as an indicator of potential human contamination without the tracking ratio. The problem of Gram positive cocci overgrowing this media should not be significant as it was only found to occur under extreme pollution periods and improved by spread plating these samples as it decreased the concentration of Gram positive bacteria on the media. Fluorescent probes could help

identify bifidobacteria, but any enzymatic method would have to be carefully evaluated, as cost is a problem in South Africa.

CHAPTER 4: USE OF GIS, COMMUNITY BASED MONITORING AND COMMUNITY ENGAGEMENT

4.1 CONTEXT

South Africa has a shortage of people to monitor water, and there is thus little data available to make public health decisions (Luyt et al., 2011a). Citizens are taking municipalities to court over issues of sanitation and water (Raymer and Buckle, 2010). Not only is South Africa a water stressed country (Seckler et al., 1999; Otieno and Ochieng, 2004; Quinn et al., 2011), but Grahamstown is also a water scarce region (DWAF, 2004; DWAF, 2006). It is estimated that 89.3% of the South Africa population have access to piped water (Statistics South Africa, 2011a). Thus there are still many South Africans without access to sources of water of adequate microbial quality (Momba et al., 2006b; Tandlich and Muller, 2008).

The Eastern Cape has infrastructural problems, with less than 73.9% of the population with access to piped water. It is also not known for good service delivery (PMG, 2009a). The main problem is the increase in dissatisfaction of water quality (Statistics South Africa, 2011a), which brings water safety into consideration (Duse et al., 2003).

Water supply interruptions and system failures occurred in 47.6% of South African households in 2010. 59.9% of the Eastern Cape population experienced interruption in water supply due to poor maintenance, insufficient water, pump failure, water leakage and pipe breaks (Statistics South Africa, 2011d), with 40.5% of households experiencing interruptions for longer than 15 days (Statistics South Africa, 2011d). Pipe breaks and maintenance leading to drops in water pressure often lead to increased risks of gastrointestinal disease, due to microbes entering the distribution system (Nygård et al., 2007; Besner et al., 2011).

The municipal water supply is not necessarily safe from a microbial point of view; and this has been reiterated in many developing countries (Lee and Schwab, 2005). South Africa has problems with pipe breaks (PMG, 2009a). Like many other municipalities, Makana has old infrastructure, as Grahamstown has existed for 200 years. The pipes and infrastructure are old, leading to frequent pipe breaks and subsequent water outages (Snowball et al., 2008; Makana Municipality, 2011; Makana Tourism, 2012).

Pipe breaks lead to soil entering the system and to possible sewage contamination if sewage pipes have also been broken/leaking in the same area (PMG, 2009a).

This causes taps to produce soily or coloured water, which decreases satisfaction with water quality (Dietrich, 2006). Newspaper reports do not help this situation as they overstress problems (Mini, 2010). A compounding problem is the poor operation of water treatment facilities in some areas (Momba et al., 2009a). The use of unskilled personnel has led to malfunctions in water treatment works and thus to increased microbial concentrations (Momba et al., 2006b; Momba et al., 2009a). Community taps lack maintenance (Jagals et al., 2004; Haarhoff et al., 2009). The government introduced the Blue Drop system to help identify how well different municipalities conduct water monitoring, treatment and to increase compliance.

This system has highlighted the poor water quality in rural areas (Wilson-Jones and Rivett, 2012). A press release in 2012 stated that at least two Eastern Cape municipalities lacked access to safe drinking water (DWA, 2012c). There is a need to monitor water in the community to ensure that safe drinking water is available (Wilson-Jones and Rivett, 2012).

The Blue Drop Score is calculated on a weighted system. Grahamstown had a municipal Blue Drop score of 28.4% and 96.43% microbial drinking water quality (DWQ) compliance in 2010 (DWA, 2010), increasing in 2011 to a 55.07% and 89.89% for microbial and 99.54% for chemical compliance (DWA, 2011a).

According to the Department of Water Affairs' (DWA) website, Makana Municipality is currently ranked 7th in the province, but their monitoring compliance on the site is very low (DWA, 2011b). Compliance is determined according to SANS 241:2006 and government requirements (DWA, 2011a). Compliance with the Department of Water Affairs and Forestry (DWAF) drinking water guidelines require a heterotrophic plate count (HPC) of less than 100 Colony forming units (CFUs)/mL; total coliforms of 5 CFUs/100 mL; faecal coliforms 0/100 mL (DWAF, 1996c). Other regulations used in South Africa include those composed by the World Health Organisation (WHO) and South African National Standard 241 (SANS).

4.2 AIMS

To compare and correlate HPC concentrations to the modified H_2S strip test (Venkobachar et al., 1994; Sobsey and Pfaender, 2002; Luyt et al., 2011a).

To test and evaluated the usability of the modified hydrogen sulphide strip test (mH_2S strip test) by volunteers and their reliability to return samples.

To evaluate the correlation between the H_2S strip test results and the HPC concentrations in diagnosing infrastructural problems and evaluating water quality.

4.3 BACKGROUND TO COMMUNITY MONITORING

The Millennium Development Goal (MDG) 7 C aims to halve the proportion of the population without access to safe drinking water and sanitation before 2015 (WHO and UNICEF, 2012). While half of the MDG 7 C has been reached before 2015 (WHO and UNICEF, 2012) and the JMP 2011 report states that South Africa has improved its water access by 22% since 1995 (UNICEF and World Health Organization, 2012), there is still a need for safe drinking water access, especially in rural areas (UNICEF and World Health Organization, 2012).

The “Aquatest” being designed by Bristol University(University of Bristol, 2012) and its collaborates as a low cost onsite H_2S test is being used with Android open source mobile technology, designed by the University of Cape Town (UCT), to increase communication of water quality results in rural areas in the Eastern Cape (Wilson-Jones and Rivett, 2012). This is designed to decrease transport time and costs due to bad road conditions in rural areas and vast distances, and to increase the data available for public health decisions (Wilson-Jones and Rivett, 2012). The use of mobile phones as communication devices is well thought-out, as most people own a cellphone, and are familiar with the technology, even if they have no access to computers or the internet in rural areas(Statistics South Africa, 2007b; Wilson-Jones and Rivett, 2012).

Using Geographic Information Systems (GIS) to identify problem areas helps with decisions and with increasing ease of use (Wilson-Jones and Rivett, 2012).

The use of GIS in monitoring tap water systems helps identify areas of concern or possible distribution problems, e.g. residual bacteria after pipe breaks.
Community water monitoring groups in developed countries have been using GIS maps for data entry (Masucci, 1996; Savan et al., 2003; Gouveia et al., 2004). Although the databases are online, a paper copy of the results is needed to verify that no typographical errors have taken place (National Water Program, 2012).Data maps, systems allowing data to be viewed, are important to identify trends and to motivate volunteers. The secchi dips map in the USA is a good example (Secchi Dipin, 2009) or the "York University Centre for Applied Sustainability GIS-based website (Savan et al., 2003), while the Alabama Water Watch also uses maps to display data (AWW, 2009).

Community monitoring has been used to track changes in river pollution and extend the monitoring power of the government, even though the data is not used for legal purposes, in many developed countries to great success. (Savan et al., 2003). Governments are often constrained both financially and by manpower. The use of trained community members allows people to get involved and to make a difference by decreasing pollution (Savan et al., 2003; Overdevest et al., 2004). Although this provides manpower, motivation dwindles if volunteers do not perceive they are making a difference, or feel abused (Measham and Barnett, 2008).

Depending on the objectives of the monitoring program, different tests will be required (DWA, 2009). Monitoring programs designed solely to educate need less funding and commitment, while programs designed to give information to the government need sampling, careful planning, lots of commitment and financial resources (DWA, 2009). Sampling needs to be done weekly, monthly or seasonally and requires formal training, with quality control procedures and detailed records (DWA, 2009). At present, South African data is stored by the DWA, unless the project is only used for education purposes, when results are often not stored at all.

The question is: Are these groups' data reliable? The Water Watch Group in America has shown that volunteers can do many simple tests as reliably as experts if they are properly trained (Fore et al., 2001; Savan et al., 2003; Gouveia et al., 2004). Some field-based tests may not be as reliable as laboratory tests, but using standard error correction to raw data can make it more accurate for use (Loperfido et al., 2010).

Volunteer programs need well-written protocols and quality assurance guidelines to reduce training differences and methods (National Water Program, 2012). The use of certification and possibly exams may help standardise training, making data more reliable. This would be required if the data was going to be used by the DWA (DWA, 2009).

The use of standards helps identify the tests accuracy and identifies problems quickly. The use of the DWAF Quality of Domestic Water Supplies guidelines could help with the interpretation of and standardising training material (Moolman and Winter, 2010). Guides have also been of economic benefit, since their development results in increased equipment sales and in alleviating skills shortages, while improving water quality. This leads to decreased waterborne disease events, which would have decreased worker productivity (Moolman and Winter, 2010). Another set is the Alabama Water Watch Group procedures and manuals for training, which could be adapted (Deutsch et al., 2007). One factor which would have to be taken into account in water monitoring in rural areas of South Africa, though, is the population's low literacy level (French, 1992; McKay, 2007). Manuals would also need to be available in all eleven official languages, which could be costly.

Volunteers provide man power which is often in short supply. Although their skills and equipment constraints limit the variety of tests they can do, they can access a large number of sites, providing more data for decisions(Nicholson et al., 2002). Water monitoring groups largely examine macro-invertebrates and chemical parameters, as they are easy to do on site and are very educational (Firehock and West, 1995; Savan et al., 2003). Volunteers retrieve samples for microbiological testing and use simple tests or , in some projects, deliver them to designated labs (Citizens Monitoring Bacteria, 2004). In fact, Rand Water offers a service for people to bring home water samples to be tested and returns the results, called the Tap Analysis Program (TAP) to them (Rossouw et al., 2006; Rand Water, 2012). Using volunteer groups in South Africa could provide more regular testing to wider rural areas while providing a base line alert system to identify problem areas. It could also be cost effective, as it decreases transport and laboratory costs (Savan et al., 2003). Problems of transport remuneration and equipment distribution may prove costly (Rossouw et al., 2006).

The H_2S strip test was designed and works well as an alert test for highly polluted areas (Genthe and Franck, 1999; Genthe and Jagals, 2003; Gupta et al., 2008; Wilson-Jones and Rivett, 2012). It is a presence-absence test which can be read easily by volunteers (Genthe and Jagals, 2003). There is a need for good quality control protocols and training, as well as databases and education programs. The use of community monitoring programmes may increase the community's perception of service delivery and water quality, as it has with the Wilson- Jones project (2012).

One problem with volunteers monitoring is sustaining interest and reliability (Byron and Curtis, 2002). This was even seen during the Wilson-Jones project (2012), conducted with government employees. They need passion for volunteering, e.g. people keen on benefiting the community or making the world better, as well as a perceived benefit to stay involved and to feel valued (Measham and Barnett, 2008).Rewards, magazines and newsletters are important to provide feedback (Savan et al., 2003; Gouveia et al., 2004; Deutsch et al., 2007).

One advantage of the community monitoring water is the use of invaluable indigenous knowledge about the water resource from local leaders, within communities (Rossouw et al., 2006). Education of communities could help prevent further deterioration of rivers and offer safer water use, as stewardship is created (Conrad and Hilchey, 2011). Projects need buy-in from the community to be sustainable (Mathabatha and Naidoo, 2004).

4.4 LAWS FOR COMMUNITY MONITORING IN SOUTH AFRICA

A review of the legislation governing volunteer monitoring and its implementation has not been performed in South Africa. One study reported legislation requirements as being communicated by government officials (Department of Water Affairs (DWA), 2009). The Oxford dictionary defines "volunteer", as someone who voluntarily offers their services, in any capacity, or agrees to take part in an activity or works for an organisation, without remuneration (Oxford University Press, 2012). The Labour Law does not provide for volunteers *per se* (in itself). The Basic Conditions of Employment Act (No. 75 of 1997 as amended 2002) specifically excludes unpaid volunteers working for charitable organisations There is thus no provision nor protection for volunteers, which has also been stated in DWA monitoring guidelines (DWA, 2009).

It is important to inform volunteers about potential risks before they agree to participate in monitoring, and a consent and indemnity form will be needed to waiver any responsibility of the monitoring group. The Occupational Health and Safety Act (Act 85 of the South African Goverment, 1993,as amended) states that employers are required to provide education on protective methods and equipment to prevent health related problems.

The National Water Act (NWA, Act No. 36 of 1998) in Chapter 14, recognises water quality monitoring to be a fundamental part of water resource management. The Act also compels the DWA to establish and maintain national water monitoring programmes. The DWAF excluded volunteers when designing a strategic framework plan for water quality monitoring. Therefore a mandate would be needed to establish a volunteer program. The National Water Act (NWA Act No. 36 of 1998, Chapter 13, Part 1, Article 124) provides for the appointment of volunteers by the DWA, but requires an appointment certificate signed by the Regional Director. The appointment letter would stipulate the volunteer's responsibilities, duties, and power, which are slightly outlined in the NWA (Act No. 36 of 1998, Chapter 13, Part 1, Article 125). This is required before volunteers can be used for sampling.

4.5 SOUTH AFRICAN MONITORING GROUPS

The Minister of Water Affairs and Forestry is obligated by the National Water Act No.36 of 1998 to form national monitoring systems and to publish the records (South African Goverment, 1998; Rossouw et al., 2006; DWA, 2009). Chapter 3 of the National Water Resources Strategy developed by the DWAF has initiated several monitoring programmes, including the National Microbial Monitoring Programme and the Adopt-a-River Programme (Rossouw et al., 2006). These initiatives are used for planning and policy making, and are governed by strict quality control procedures (DWA, 2009).

The National Microbial Monitoring Programme (NMMP) is only used for surface and not ground or tap water (DWA, 2009). These programmes are expensive to run and do not utilise volunteers (DWA, 2009). It was estimated that the DWAF funds the River Health programme, with a budget greater than R1.5 million per annum estimated in 2006 (Rossouw et al., 2006). The Adopt-A-River campaign in South Africa has a manual for volunteer monitoring, but it is based on education and not true water monitoring (DWA, 2009).

4.6 DIFFERENT MONITORING GROUPS AROUND THE WORLD

There are numerous international volunteer monitoring programs. Programmes range from having educational purposes to monitoring the environment. These programs monitor streams, rivers, lakes, reservoirs and other aquatic environments. Programs differ in the chemical, biological and microbial data they collect. The Secchi-dip-in group in the USA collects transparency (visibility) data from visual means, such as the transparency tube or secchi disc (Secchi Dipin, 2009). Global Water Watch (GWW) provides assistance to the US Environmental Protection Agency using registered volunteer community-based monitoring groups (Auburn University and Global Water Watch, 2012). It provides help for establishing groups to monitoring physical, chemical and macro-invertebrate indicators in order to educate people and remediate rivers (Rossouw et al., 2006; DWA, 2009). The United States Environmental Protection Agency (USEPA) supports monitoring groups (DWA, 2009; USEPA, 2012). In Canada, the Canada's Stewardship Agenda and the Voluntary Sector Initiative (VSI) funds and supports volunteer monitoring groups, while Volunteer Canada advocates for volunteers (DWA, 2009). Australia has the Waterwatch Groups, part of an initiative to get people involved with national resource management programs (DWACommonwealth of Australia, 2008; 2009). World Water Watch (WWW) helps to assist NGOs set-up water monitoring, protection or restoration of environment groups (DWA, 2009). One important consideration before starting a volunteer group is that the group must operate within the confines of the availability of resources, technical and financial support, or whether it would be more viable for a few key people to run the organisation and possibly burn out over time (Byron and Curtis, 2002). The capacity and time committed by volunteers need to be realistic to the situation (Byron and Curtis, 2002). Good leadership and financial support are critical for things to be organised and to run without the risk of burn out (Byron and Curtis, 2002). Well organised projects often get better support.

4.7 COMMUNITY MONITORING

Water resource management will only succeed if skills development and capacity building take place in all relevant organisations (Grobler and Ntsaba 2003). Priority issues need to be targeted and partnerships encouraged to build local knowledge and capacity. This will reduce the amount of polluted domestic and industrial water being returned to pollute the environment.

4.8 GRAHAMSTOWN WATER SUPPLY AND COMMUNITY WATER MONITORING

Grahamstown's main water supply comes from the Gariep River (Orange), which diverts approximately 25% of its water through the Orange-Fish Tunnel to the Little Fish River via a diversion at Hermanuskraal weir and transported through a tunnel to Glen Melville Dam on the Ecca River (DWAF, 2004). Glen Melville Dam water is treated at a nearby plant. and pumped to Grahamstown (DWAF, 2004). Another main supply comes from the Kariega River System, providing water to the Settlers and Howieson Poort Dams. Water is pumped from Settlers to Howieson Poort, on to Jameson and then to Waainek Water Treatment works, supplying water to west Grahamstown (DWAF, 2004). East Grahamstown is supplied by Milner Dams via the James Kleynhans Water Treatment Works on the hill above Grahamstown (DWAF, 2004). These rivers are of unacceptable quality according to the DWAF due to their salinity and mineral contents (DWAF, 2004). The catchments in this area are the Great Fish River, the Sundays River, and the Indian Ocean (DWAF, 2004).

The highest rainfall generally occurs in December and January for the Albany area, within which Grahamstown falls. The highest rainfall months for Grahamstown, collated from the Grocotts Mail newspaper's weekly reports from the last 20 years, are between October and December. Highest temperatures usually occur in January and February (DWAF, 2004). The area's mean annual precipitation is 635 mm and evaporation is 1500 mm (DWAF, 2004). Water requirements for the Makana municipality and its surrounding areas are not met by the Great Fish River and Sundays River catchments alone. Extra water is transferred from the Orange River- Fish River transfer system via a tunnel 82.9 km long and 5.4 m in diameter that discharges into the Teebus Spruit (DWAF, 2004). Water then flows into the Grassridge Dam on the Great Brak River, which is a Great Fish River tributary, and reaches the Elandsdrift Weir (DWAF, 2004). Water is then channelled by an aqueduct, which includes the Cookhouse Tunnel, into the Little Fish River (DWAF, 2004). The Hermanuskraal Weir diverts water into the Fish–Ecca Tunnel, which discharges into the Glen Melville Dam on the Ecca River (DWAF, 2004). The Glen Melville and Settlers Dams are situated such that they can also be used by Port Alfred (DWAF, 2004).

Water from the dams is treated in the treatment plants and then released into the pipe distribution system. But the condition of the water at the tap depends on breaks in and the condition of the pipes. Brown coloured water from soil in the distribution system, due to pipe breaks, has heightened an already sceptical Grahamstown population's mistrust of the safety of its water (Richards and Daniel, 2008). Grahamstown's Community is very water conscious due to newspaper reports (Maher, 2006; Naketi, 2009; MacGregor, 2010; Mini, 2010; Mmango, 2010; Mngcambe, 2010) about unsafe drinking water and other reports around town. So many people are left wondering, if the water is indeed safe. Thus the inception of this project, to identify the microbial condition of tap water in Grahamstown.

4.9 MATERIALS AND METHODS

Laboratory A (Lab A) is the laboratory on Rhodes University Campus, in which the rest of this thesis' experiments were performed. The agars from Rhodes University Laboratory (Lab A) were purchased from Merck (Pty.) Ltd. (Johannesburg/Cape Town, South Africa): m-FC agar, R2A agar, MacConkey agar. Polyethylene sample bottles (500 mL) for microbiological sample collection, Kovacs indole reagent and other chemicals were purchased from Sigma-Aldrich (Johannesburg, South Africa). Ethanol was purchased from Chemstore (Rhodes University, Grahamstown). The nitrile gloves were purchased from Lasec (Port Elizabeth, South Africa). Membrane filters (cellulose acetate, pore size 0.45µm, 45mm); sterile plastic Petri dishes (90 mm) and sterile urine jars were purchased from Spellbound Labs (Port Elizabeth, South Africa).

Sterile urine jars or polyethylene sample bottles (500 mL), sterilised using an autoclave (Model RAU-53Bd REX MED [Hirayama Manufacturing, Tokyo, Japan]), were used for sample collection. Incubation took place in one of the following incubators: the Labcon incubator Model FSIM B (Labmark, Johannesburg, South Africa), the TS 606/3-I incubator (WTW, Weilheim, Germany), the Labcon low temperature incubator LTIE 10 (Labmark, Johannesburg, South Africa); and/or the Heraeus Model FT 420 (HeraeusKulzer GmbH, Dormagen, Germany).

4.9.1 Training

All the students and staff used in the various projects were shown a short 15 minute presentation on how to collect samples, sterilise the tap with ethanol and store samples before bringing them to the lab. Due to the academic nature of the volunteers and their laboratory background, training was done over a short time period. Students were asked to run taps for at least 30 seconds before taking the sample and were trained in sample collection methods. This was done to prevent stagnant water and, thus, possible increased bacterial concentrations, from confounding the results (Pepper et al., 2004). Pepper et al. (2004) found that this decreased the HPC bacterial count by 1 order of magnitude, in Arizona (USA).

4.9.2 Community Tap Water Monitoring Using the H_2S Strip Test in 2009 and 2010

Water samples were collected by volunteer Rhodes University students from the GRASS society (Gaian Revolutions and Social Solutions) and the Pharmacy faculty. The GRASS students emailed their H_2S results, while in 2010 the pharmacy students brought water samples to the lab. The samples were analysed on delivery or stored between 4 and 8° C for no longer than 1 hour before analysis.

4.9.3 Tap Water Monitoring

Tap water was monitored on the first Tuesday of the month, or the nearest feasible date to that, between May and September 2011. Samples were collected by two different labs simultaneously from the Albany History Museum (lower area distribution), 2A Cathcart Street (medium level distribution) and the Grahamstown Veterinary Laboratory (high area distribution). Microbial analysis was done separately in the respective labs, within an hour of collection. Samples were not transported on ice, but were returned to the lab within 20 minutes of collection. As the samples were returned within 1 hour of collection, it falls within the acceptable WHO time period of 2 hours which does not require transportation on ice to the facility, but that samples be kept in the dark and cool (1996). Sampling bottles (autoclave sterilised), nitrile gloves worn and the taps were sterilised chemically, on site, with 70% ethanol. Taps were run for a minimum of 30 seconds before sampling. Laboratory A (Lab A) was a DAFF (Department of Agriculture, Fishery and Forestry) certified lab.

This was used to verify results. Lab A (Grahamstown Veterinary Laboratory) analysed samples using Chromocult media for *Escherichia coli* (*E. coli*) at 44°C and total coliforms at 37°C using membrane filtration. The HPC and enterococci were analysed on Kf media, according to Dir 80/778/EEC – ANNEX III No L.229/28. Laboratory B (Rhodes University Pharmacy Research Laboratory) used R2A media for HPC, MacConkey agar (Merck) at 35°C for total coliforms and m-FC agar (Merck) for faecal coliforms at 44.5°C. The modified Hydrogen Sulphide test (mH_2S strip test)(Luyt et al., 2011a) was analysed at room temperature for 72 hours. A black precipitate during this time was recorded as a positive result.

4.9.4 Community Monitoring of Tap Water in 2011

Samples provided by volunteers (lectures and technical staff of Rhodes University) were analysed by Laboratory B. These samples were collected on the same day as the tap water monitoring by Lab A. All volunteers were shown how to sterilise the taps with ethanol and samples were brought in and placed in the fridge before analysis. Microbial analysis of samples was done within 2 hours of sample delivery. No volunteers were younger than 21 and no remuneration was provided. The sites were chosen to include a large range of area. This can be seen in the GIS map presented later.

4.9 RESULTS AND DISCUSSION

Using GIS maps to pin-point where drinking water is not of satisfactory microbial quality helps identify the extent of the problem as well as the size of the area affected. The GIS maps were drawn for all Grahamstown sampling points, but two points in Fingo Village were not included and community members did not want to reveal their residences. Figure 20 shows the sampling points according to GPS coordinates on the street layout of Grahamstown.

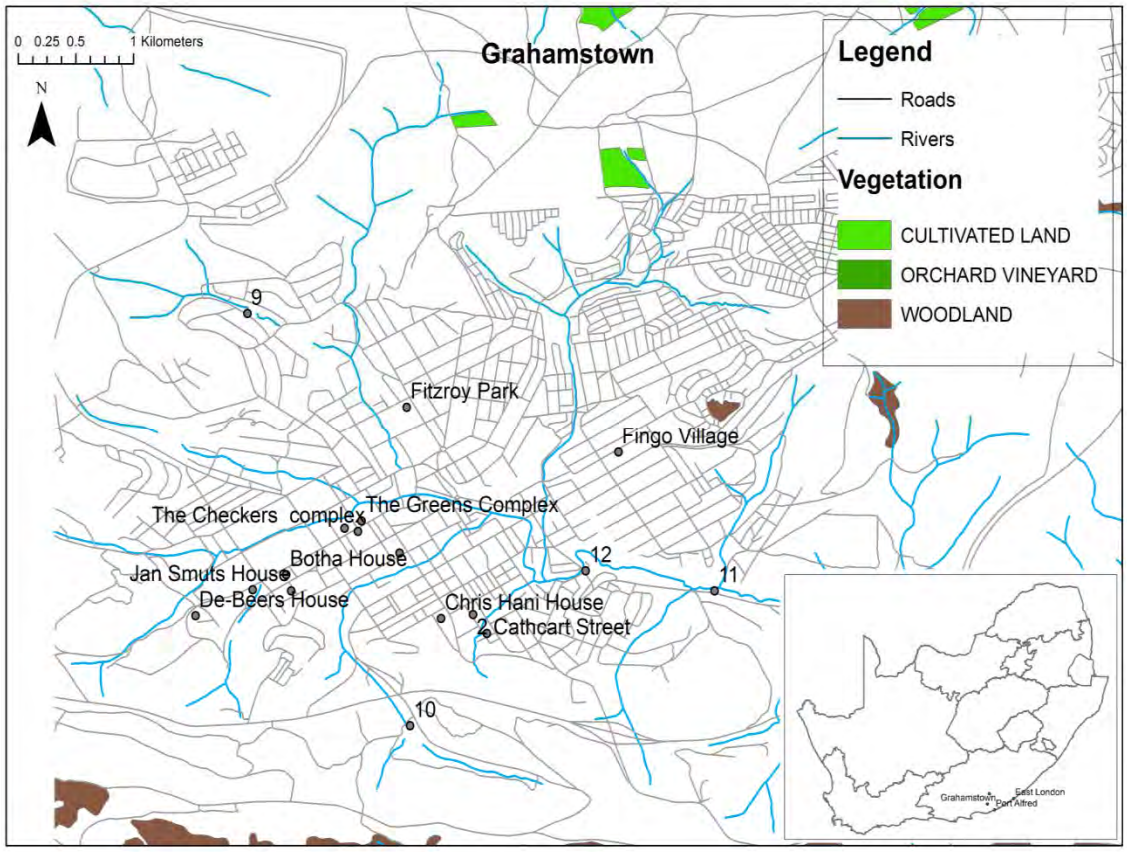


Figure 20: This GIS map shows the sampling sites around Grahamstown. GPS coordinates for two of the township sites are not included due to privacy concerns.

Due to the different dams and water entry points, water is piped to different parts of Grahamstown depending on the water pressure, dam levels and water mains situation. There are three different levels, depending on the height of the buildings in the town, which are defined as low, medium and high distribution release points. Valves are used to regulate pressure in the distribution system. Water testing was done at sites from all three levels. The use of GIS also allows the identification of the different areas and levels which may be affected. Pipe grid maps from the municipality could isolate the exact distribution release point and possible affected areas when used in conjunction with GIS.

Results from the different sampling rounds varied due to changing conditions of the water quality due to treatment efficiency and the number of pipe breaks within the area sampled.

The pilot programme for community tap water monitoring using mH_2S in 2009 used Rhodes University students from the GRASS society (GRASS Society, 2011). They were eager and willing to monitor water quality in Grahamstown. This initial motivation dwindled over time, with the last sampling date only having one sample being returned from a possible ten. This lack of volunteer interest has been demonstrated previously (Conrad and Hilchey, 2011). The use of SMSes (Short Message Services – known as "texts" in the United Kingdom) to remind students did not increase the number of samples taken on the correct day. The return of information via email took a long time, with students apologising because they forgot. Multiple reminders were required, and the validity of information after long time delays is questionable. Not using delayed information is a safe guard against unreliable volunteer data, which is needed to maintain reputability (Conrad and Hilchey, 2011). Some forgot to sample on the correct day. This unreliability has been identified in other volunteer programs (Leslie et al., 2004). The unreliability of students has been noticed in service learning (Blouin and Perry, 2009). The lack of response may have been due to the fact that results were negative, and thus not considered important. Another possible reason for low return rates in the last sampling could be the start of the exam period starting and students prioritising their studies. This is similar to using life guards on beaches to collect data when they do not have time to devote to the task and are then unreliable (Williamson, 2006). Results in Table 30 below show a good bacterial quality of the tap water in Grahamstown over this period, as only two out of ten samples showed positive results. Table 30 also shows the rate of sample return success. Where samples were not returned, the number of reminders is recorded in Table 30 as these results were never communicated. Of the twelve students volunteering, two never returned results, and the only volunteer to sample correctly and return results was one of the leaders of the GRASS society. Enthusiasm for sampling seemed to dwindle over time, as it became more difficult to have the results returned. None of the volunteer groups reported the mH_2S strip test as being difficult to use. Genthe and Jagals (2003) found the same thing using health workers in different provinces.

Table 30: Results from tap water sampling by GRASS volunteers from June to September 2009 using the mH_2S strip test. If no results were not returned then the number of times the students were contacted and how is shown.

Street address/ Residence	Results for the mH_2S strip test		
	Thursday 28/05/2009	**17/06 or 18/06/ 2009**	**1/09/2009**
Bartholomew Street	Negative	Negative	Positive
Beaufort Street	Negative	Negative	1 SMS & 2 emails
Cross Street	Negative	3 emails	1 SMS & 2 emails
Jan Smuts House	Negative	Negative	1 SMS & 2 emails
Milner House	Negative	Negative	1 SMS & 2 emails
Milner House	Negative	Negative	1 SMS & 2 emails
Milner Street	Negative	Dark brown to black at 72 hours	1 SMS & 2 emails
Walker House	Negative	Negative	1 SMS & 2 emails
Worcester Mews	Negative	3 emails	1 SMS & 2 emails
Worcester Street	Negative	Negative	1 SMS & 2 emails

There was concern about brown water due to a pipe break at one of the students' houses in New Street before he started conducting the test at his home. These results returned positive, and the tap water was resampled the following day. The results were again positive, leading to an investigation of the extent of the problem and analysis of HPC concentrations to identify the public health risk posed.

The HPC concentrations were measured, as they have been correlated with treatment efficiency, and were regrown (Chowdhury, 2012). The use of HPC was also considered, as the bacteria could be grown at 25°C, similar to room temperature, for a week. The H_2S strip test is incubated at room temperature, thus the HPC may be more likely to produce similar results and have similar bacterial growth due to the environmental temperature. Ingredients included for selectivity in the H_2S strip test will however inhibit some of the bacterial growth within its media. HPC could theoretically be enumerated without an incubator like the H_2S strip test in an area without a laboratory.

Table 31 shows the results from sampling between the 13th and the 24th of August 2009. Only one sample on the 18th of August at Rhodes' apartments was analysed for faecal coliform presence and no cells were found in a 100 mL sample, using the most probably number (MPN) method (APHA, 1992). There was an apparent pipe break in the area around the 10th of August 2009.

The municipality was notified and a report was written on the 5th of September 2009. Sampling points were decided by allowing the water flow direction to identify the area affected. The Rat and Parrot is above the Rhodes apartments, while the Greens and Checkers apartment complexes are below. The higher HPC values were only seen below the pipe break, which makes sense due to the flow direction.

Table 31: New Street sampling results from 13 to 24 August 2009, after a pipe burst around 10 August 2009. Samples were analysed for faecal coliforms, HPC and the H_2S strip test. HPC values are reported as the geometric mean of four replicates and Colony Forming Units (CFUs).

Sampling site description	Sampling Date in 2009	H_2S strip test	HPC (CFUs/mL)	Domestic use guideline evaluation(DWAF, 1996c)
1)Rhodes apartments in 43 New Street	13/08	Positive at 56 h	ND[a]	Potential faecal contamination
1)Rhodes apartments in 43 New Street	18/08	Replicates: 1) Positive at 39h 2) Positive at 48h	4315	High public health risk due to concentrations well above 1000 CFUs/ mL. Treatment is insufficient or regrowth has occurred. Disinfection is required as this could form biofilms in the pipes
2) Rat and Parrot	20/08	Negative at 72h	35	Within DWAF drinking water guideline limits, as less than 100 CFUs/ mL
3) Scotts Avenue	21/08	Negative at 72h	10	Within DWAF drinking water guideline limits

[a] Not determined

Table 31 (continued)

1)Rhodes apartments in 43 New Street	22/08	Replicates: 1) Positive at 48h 2) Negative at 72h	5	Within DWAF drinking water guideline limits
4) The Greens Complex at 21A New Street	24/08	Positive at 48h	2950	High public health risk due to concentrations above 1000 CFUs/ mL. Treatment is insufficient or regrowth has occurred. Disinfection is required.
5) The complex of townhouses next to the Checkers parking lot	24/08	Replicates: 1) Positive at 48h 2) Negative at 72h	135	Negligible health risk, except in long term use. Inadequate treatment or regrowth in the distribution system. Pipe chlorination may be required

[a] Not determined

Water quality deteriorated significantly after pipe breaks or treatment failure. This has also been reported in other countries (Allen et al., 2004). Pipe breaks can lead to an increased likelihood of diarrhoeal diseases (Nygård et al., 2007). Using HPC as an indicator of treatment failure and distribution network issues is well established (Carter et al., 2000; Sartory, 2004; Chowdhury, 2012). A good correlation between the modified H_2S strip test and HPC values shows that it is a good alert system even under chlorinated water conditions. This is not surprising as the original H_2S strip test was used for tap water tests amongst others (Genthe and Jagals, 2003). The tap water in Durban was tested on one opportunistic occasion on 2 December 2009. The mH_2S strip was negative and the HPC average values were 10 and 90 CFUs/ mL respectively. This was not unexpected as Durban's Blue Drop score was 96.5% in 2009 (DWAF, 2009), demonstrating that the water was safe to drink and of good quality. Other opportunistic samplings were run in middle income houses in Matlosana City (North-West province), Fochville (North-West province) and eThekwini Municipality (KwaZulu-Natal province) and produced negative results for the mH_2S strip test (Luyt et al., 2011a). Ten samples from one house in Matlosana City were analysed between 17 and 28 December 2008 (Luyt et al., 2011a). Ten samples from two houses in Fochville were analysed between the 14th of August and the 7th of September 2009 (Luyt et al., 2011a). Samples taken from a hotel

and the University of Limpopo (Northern Province) were negative on the 15th of September 2010.

A study by Obi et al (2007) showed that HPC concentrations were between 1.6 and 15 CFUs/mL in Limpopo and Mpumalanga from point-of-use sampling. The mH_2S strip test is expected to be negative. The mH_2S test also never tested positive when investigating East London tap water. The testing in Durban and other places allowed the evaluation of the test under different climatic conditions.

In 2010, Rhodes University Pharmacy students were recruited from different residences across campus to test their residences' microbial water quality using the mH_2S strip test. A total of twenty students were recruited, while only five were identified to bring water for HPC analysis. Not all the students were reliable and not all samples were received on the correct day. This is shown in Table 32. The unreliability of students collecting from different pools across campus led to the recruitment of Rhodes University lecturers and technical staff in 2012, in the hopes they would be more reliable.

Results from 2010 are shown in Table 32, which demonstrates the poor water quality on Rhodes University's campus at that time. This was due to a breakdown of the chlorinator plant at the James Kleynhans treatment plant (Lourenço, 2010). This led to the high number of positive mH_2S strip tests and high HPC concentrations. Students were issued with a boil alert.

Table 32: The results from water samples collected from residence bathroom taps by students on the 12th and the 18th of February and the 5th of March 2010. Samples were analysed in duplicate, while some residences had more than one volunteer sampling from them to increase the replication number. Only specific volunteers' samples were analysed for HPC. Ninety two per cent of the samples and ninety per cent of the replicates were.

Dates in 2010	Residence	mH$_2$S tests in duplicate	Residence Replicates	HPC (Average CFUs/ mL)
12 February	Adamson House	40 hours	1	
12 February	Allan Gray House	40 hours	2	
12 February/	Botha House	24 hours		>5040 CFUs/ mL
12 February	Chris Hani House	Negative		
12 February	Chris Hani House	40 hours		4350 CFUs/ mL
12 February	De-Beers House	24 hours		> 900 CFUs/ mL
12 February	Dingemans House	40 hours	3	
12 February	Graham House	40 hours		
12 February	Hobson House	24 hours		
15 February	Hobson House	24 hours		
12 February	Jan Smuts House	24 hours	2	7085 CFUs/ mL
12 February	John Kotze House	24 hours	3	6605 CFUs/ mL
12 February	Phelps House	24 hours	2	
12 February	Stanley Kidd Annex	24 hours		
15 February	John Kotze House	40 hours	2	
17 February	John Kotze House	40 hours		
18 February	Phelps House	24 hours		
18 February	Jan Smuts House	24 hours		Overgrown
18 February	Chris Hani House	1 positive - 24 hours	2	Overgrown
18 February	Botha House	24 hours		Overgrown
18 February	John Kotze House	24 hours		Overgrown
18 February	De-Beers House	Negative		1038 CFUs/ mL
18February	Hobson House	40 hours		
05 March	Botha House	48 hours		2283 CFUs/ mL
05 March	Chris Hani House	Negative		2529 CFUs/ mL

05 March	Allan Gray House	48 hours		2637 CFUs/ mL
05 March	Jan Smuts House	48 hours		-

This situation had changed by 2011, when this project was expanded. Microbial water quality had improved significantly. Pipe breaks are a problem in Grahamstown due to its old infrastructure (Makana Municipality, 2011). When water is restored, it is often brown in colour due to soil intrusion. This and newspaper reports (Mini, 2010) have led to a mistrust of tap water quality in Grahamstown. The mH_2S strip tests show positive results when pipe breaks have occurred, making it a good alert/early warning test to allay fears of continuous microbial problems. Pipe breaks have been correlated with high HPC values and positive mH_2S tests. But it is difficult to track pipe breaks, as the municipality does not keep good records of breaks and repairs, or are unwilling to share them due to fears of publication. This would be understandable, as they have had really bad press in the last few years (Mini, 2010).

The use of Rhodes University lecturers and technical staff to collect tap water samples was the most successful of all the projects. Sample bottles, ethanol and gloves were distributed a day or two in advance and samples were collected from the staff in the morning. Although there were times when staff members forgot or were unavailable to sample the water, on the whole, samples were brought in timeously and on the correct day for a far longer period than in other projects. The exam period was still not ideal as many staff members were not on campus over this time.

The H_2S strip test results once again correlated well with the heterotrophic bacteria count and worked well as a screening test.

Table 33: Tap water sampling results for different sampling sites between May and August 2011.

Sampling site	H_2S strip test				HPC (CFUs/ mL) at 35°C				
DATE	MAY	JUNE	JULY	AUGUST	MAY	JUNE	JULY	AUGUST	
								(2nd)	(11th)
Extension 6	Negative	Negative	Negative	Negative	0	100	0	30	0
Fingo Village	Negative	Negative	Negative	Positive at 72 hours	0	0	0	200	0
Hillsview Road	Negative	Negative	Negative	Negative	83	120	83	-	110
Oatlands[a]	Negative	Positive at 20 hours	Negative	Negative	10	500	10	-	0
Wilshire Crescent	Negative	Negative	Negative	Negative	0	280	0	30	30
Cradock Heights	Positive at 48 hours	Negative	Negative	Negative	333	0	0	-	0
Near Fort England	Negative	Negative	Negative	Negative	0	125	10	20	40
Rhodes University	Negative	Negative	Negative	Negative	0	30	90	-	0
Cathcart Street		Negative	Negative	Negative	0	70	-	600	125

Table 33 (continued)

Sampling site	Total coliforms (CFUs/100 mL)			
DATE	MAY	JUNE	JULY	AUGUST
Extension 6	0	0		0
Fingo Village	0	0		0
Hillsview Road	0	100	0	0
Oatlands[a]	0	18000	0	0
Wilshire Crescent	0	0	0	0
Cradock Heights	17600	2000	0	0
Near Fort England	0	1400	0	0
Rhodes University	0	0	0	0
Cathcart Street	0	1000		3000

The most surprising results were for samples collected in the township, where water quality seemed to be better than in town, despite the township-based volunteers and people at

township schools where rainwater was collected complaining that they received second rate water. The township is left without water more frequently and for longer periods of time than the rest of Grahamstown. Positive mH_2S strip test results were correlated with pipe breaks in the area and, on resampling, were negative. This is a distinct contrast to the situation arising from the broken chlorinator in 2010.

The motivation to use two different laboratories for tap water monitoring was to validate results. The Grahamstown Veterinary Laboratory was DAFF certified and the only accredited laboratory in Grahamstown. Water samples for microbial analysis are sent to Amatole water or other municipal laboratories for analysis. Verification of results was required to ensure results from the community monitoring program were reliable. The Grahamstown Veterinary Laboratory will be referred to as laboratory A (Lab A) in the rest of the text. The tap water samples were collected at 3 different sites in town, concurrently with the veterinary unit. Results were analysed in separate labs using different media. There were minor differences between results from Laboratory A and B, although differences in media may be the cause to some extent. Few samples exceeded the DWAF (1996c) microbial guidelines for domestic use. DWAF (1996c) limits were only exceeded in cases when samples were collected shortly after a pipe break in the area. On resampling these sites a day or two later, bacterial concentrations had drastically decreased. This was also shown in a previous study (Luyt et al., 2011a). Literature has shown pipe breaks can introduce bacteria into the distribution system (LeChevallier et al., 2003; Lee and Schwab, 2005; Nygård et al., 2007). It explains the correlation between pipe breaks and the higher bacterial concentrations. Tap water was sampled during the first week of the month, as this was the time the municipality claimed to test. This was done to identify any differences between municipal results and those from the community testing. Problems arose as the municipal results are often not published on their website. The last results published on the Grahamstown municipal website were for March 2011, and these results fell within the SANS 241 and DWAF guidelines (DWAF, 1996c; South African Bureau of Standards, 2006).

One interesting fact was that the community often complained about pipe breaks in the second week of the month; however this information could not be confirmed.

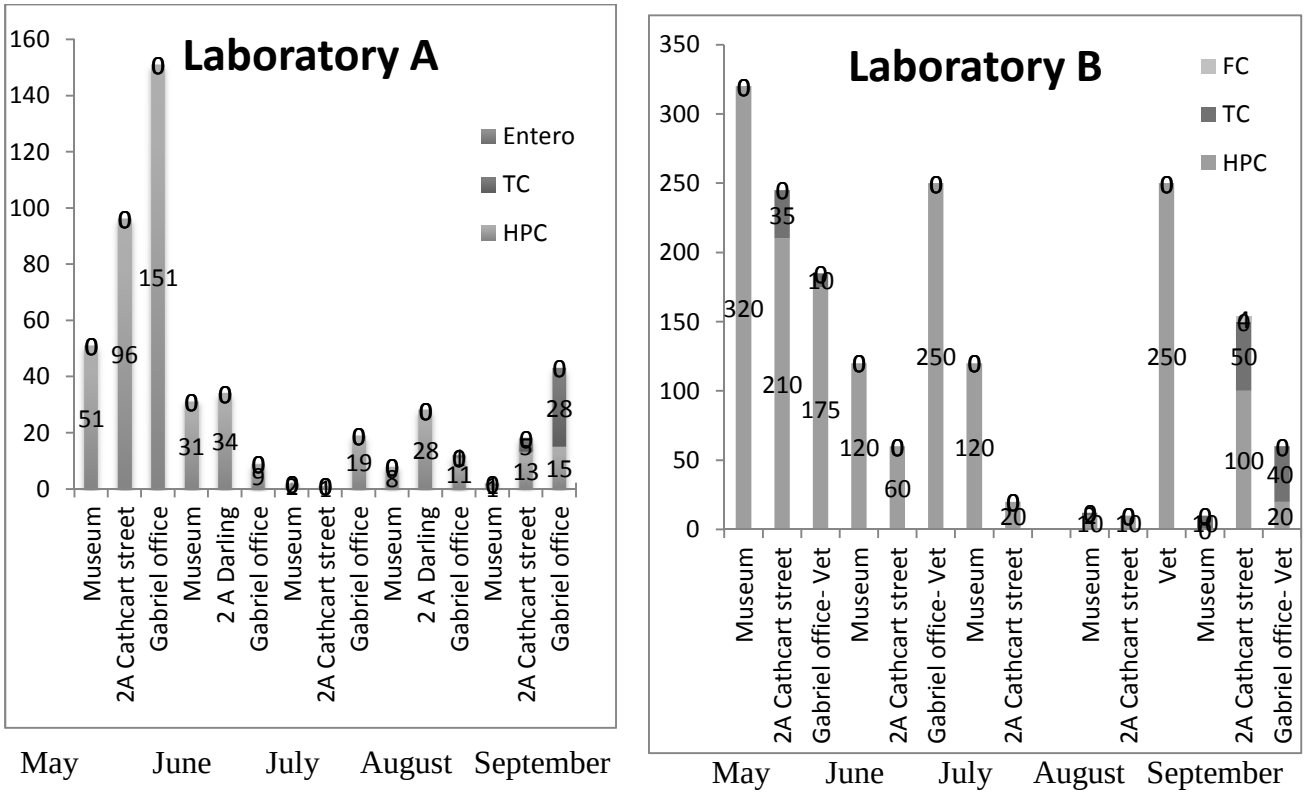


Figure 21: Data recorded by Laboratory A and B, from 3 sampling sites, during May and September 2011. The numbers denote the actual number of Colony Forming Units present. The FC (Faecal coliforms) and *E. coli* were below 0 CFUs/ 100 mL. The highest concentration for faecal enterococci was 1 CFUs/ 100 mL in Lab A on the 11th of August 2012. The HPC (Heterotrophic Plate Count) ranged between 1 and 151 CFUs/100 mL in Lab A and between 0 and 500 CFUs/ 100 mL in lab B. TC (total coliforms) ranged between 0 and 180 CFUs/ 100 mL in Lab B, while Lab A was between 0 and 28 CFUs/ 100 mL.

The data shows that there were no faecal coliforms nor enterococci present except for on the l1th of August 2011, where one enterococci was enumerated. The *E. coli* data was not added to the graph as no coliforms were ever detected in the 100 mL sample. There was not expected to be a difference between the two labs in this regard, as Chromocult agar and m-FC agar are reported to have no statistical difference between bacterial counts (Manafi, 2000). Total Coliforms ranged from 0 to 28 CFUs/ 100 mL in Lab A which was lower than Lab B, with a range of 0 to 180 CFUs/100 mL. One possible reason for the difference was the use of MacConkey agar vs. Chromocult agar.

The Chromocult media seemed to grow fewer colonies than the MacConkey agar, which is strange, as MacConkey agar is reported to have lower growth rates than Chromocult media (Finney et al., 2003). It also claims not to be affected by high HPC concentrations, which MacConkey agar is susceptible to due to its lactose base (WHO, 2003). The MacConkey agar does have a report of higher false positive results, which may have been a contributing factor (Finney et al., 2003). But MacConkey agar is far cheaper than Chromocult agar and was therefore used in this project to minimise cost. The DWAF guidelines use m-Endo agar as their choice media, which is not currently being used and has its own set of problems (Grabow and du Preez, 1979). The Amatole Water Board purports to be using Chromocult agar for their analysis (personal communication). They are presently testing the municipal water in Grahamstown.

Heterotrophic bacteria ranges were 1 to 151 CFUs/ mL in Laboratory A and 0 to 500 CFUs/ mL in Laboratory B. Thus Laboratory A only had one value above DWAF guidelines (DWAF, 1996c) on the 10th of May, while laboratory B had many above the guidelines for the same water. The heterotrophic bacteria were analysed on R2A by Lab B, as it was shown to be more sensitive (Uhl and Schaule, 2004). Other media may have slightly different results (DWAF, 1996c), which could contribute to the differences between Lab A and B's HPC results. The data showed a vast improvement from the previous studies in 2009 and 2010, which had heterotrophic bacteria counts often greater than 1500 colonies per millilitre and mH_2S strip tests that were almost always positive (Luyt et al., 2011a).

Results are better than those of a previous study in 2010, with its data shown in Table 31 above, when a pipe break was investigated and on another occasion were treatment breakdown shown in Table 32 (Luyt et al., 2011a).

Experiences with volunteers showed that projects only work well when volunteers perceive a benefit in doing the work, are continuously motivated to continue with the project and when they have time available. Students and lecturers do not tend to collect water regularly during exam or test periods of the year. Here, technical staff performed better, as they do not have the same work pressures during these periods. But this project has not negated the concerns voiced by Rand Water and Umgeni Water that volunteers will not take samples reliably and regularly according to a time schedule (Rossouw et al., 2006).

The issues of equipment were less of a problem here as volunteers were close to the laboratory, and so dropping off and collecting samples were neither an inconvenience nor expensive in terms of transport costs. Health and safety was not considered a major concern as collecting tap water, was no more hazardous to volunteers than using it in their daily lives. Timing may be a problem. Volunteers did not adhere to a strict time schedule, meaning samples had to be analysed at multiple times during the day instead of at once, which would have been more efficient. The use of the mH_2S strip test does not required lots of equipment or storage space and can be distributed 2 months before samples are taken. Also, the lack of need of an incubator makes it easily usable in rural areas.

4.10 CONCLUSIONS

The mH_2S strip test can be used as an early warning system to identify potential public health events. The mH_2S strip test correlated well with HPC concentrations in tap water. Correlation with high HPC concentrations indicates insufficient treatment or bacterial entrance into the system via pipe breaks. The correlation with HPC allows the test to identify increases in microbial concentrations, providing a warning system before any outbreaks occur. The mH_2S strip test was easily used by student volunteers for water monitoring in this study, and has previously been used by health workers successfully (Genthe and Jagals, 2003). It can be used by the general population with little training.

Tap water monitoring highlighted the need for comparable media to be used for better result correlation. However, different media do not produce extremely different results. The search for an ideal agar which is sensitive, selective and cost effective, continues. More laboratories are using chromogenic media for *E. coli* monitoring verses faecal coliform monitoring.

Microbial water quality will continue to change with pipe breaks and treatment failures, but there has been an increase in the Blue Drop score and an improvement in water quality shown by the 2012 tap water data in Table 33 and Figure 21. Meanwhile, citizens are still concerned the water may not be safe due to its taste and colour, which are influenced by pipe breaks. Community monitoring conducted independently of the municipality may be more credible in the public's opinion and may help ease anxiety over the water quality, especially in rural areas.

As the mH_2S strip test can be used easily by people with little formal education (with low literacy), it could be used to check point-of-use water, e.g. the water stored in buckets from taps or rivers to see how safe their drinking water source is staying.

CHAPTER 5: RAINWATER HARVESTING AND NGO INVOLVEMENT

5.1 INTRODUCTION

Grahamstown is a water-stressed region in South Africa (DWAF, 2004; DWAF, 2006). People access water from rivers, rainwater tanks or from the piped water distribution system. Rainwater is used as a drinking water source when there is no access to piped water, such as in rural areas, where infrastructure is lacking (Mamba, 2008) or when pipe water is unavailable due to pipe breaks or infrastructure failure. Problems of infrastructure failure were explained in Chapter 4 (Statistics South Africa, 2012d). Piped water access is still not available for approximately 9% of the South African population, while about 22.2% of the Eastern Cape population lack access to this service (Statistics South Africa, 2012c).

Dissatisfaction with water quality is increasing, as approximately 74.4 % of the Eastern Cape did not rate their water as being good quality (Statistics South Africa, 2012d). Grahamstown rural residents complain about the condition of their water and about the lack of it (Maher, 2006; Mngcambe, 2010). The Eastern Cape experienced many interruptions in water supply (Macleod, 2011; Statistics South Africa, 2012d), leading to many rural schools requiring alternative water sources, like rainwater tanks. Community taps are also not well maintained (Jagals et al., 2004; Haarhoff et al., 2009). People sometimes have to fetch water from great distances or from inadequate microbial sources (Klasen, 2000; Steyn et al., 2004; Momba et al., 2006b; Hemson, 2007; Mwenge Kahinda et al., 2007; Tandlich and Muller, 2008). Access to safe tap water from a fair distance away does not necessarily decrease the risk of diarrhoeal diseases, as contamination may also arise from poor storage or transport, or due to contaminated containers (Mintz et al., 1995; Sobsey, 2002; Jagals et al., 2003; Wright et al., 2004).

Satellite clinics in South Africa used water from improved and unimproved sources like rivers, rainwater and tank water (Duse et al., 2003). It was reported that 295953 households in the Eastern Cape use river water as their main water source (Statistics South Africa, 2012a). This river water is not from an improved source (WHO and UNICEF, 2010c).

Rainwater harvesting could provide safer potable water in these area (Tandlich et al., 2011) and prevent the need for long distance travel and the health problems associated with it (Hemson, 2007).

In South Africa, 0.98 % of the population use rainwater as their source for drinking water (Statistics South Africa, 2007a; Statistics South Africa, 2011a; Statistics South Africa, 2012a). The Eastern Cape is the province with the highest use of rainwater in South Africa, with 4.78% of the population using it as their drinking water source (Statistics South Africa, 2012a). The next highest province is KwaZulu Natal, with only 0.96% of their population using it (Statistics South Africa, 2012a). These percentages equate to approximately 80773 homes in the Eastern Cape and 141475 households in South Africa (Statistics South Africa, 2012a). The high rate of rainwater use in the Eastern Cape is disproportionately high compared to other provinces, possibly due to the province's high amount of rural settlements.

Rainwater is an underutilized water source in South Africa, considering the climate changes and water scarcity (Mwenge Kahinda and Taigbenu, 2011). Water scarcity is predicted to increase by 2025 (Otieno and Ochieng, 2004), but the pollution of rivers will only aggravate the problem (McDermott, 2012a; McDermott, 2012b). South Africa lags behind other countries on its utilization of rainwater (Houston and Still, 2002), due to political pressures for water reticulation systems and a lack of a cap on the cost of installation (Mwenge Kahinda and Taigbenu, 2011). Legislation in South Africa also hinders the use of rainwater, as it states that the service provider's permission for rainwater use is needed, while it also cannot be used for commercial purposes (Water Service Act (WSA) 108 of 1997) (Kahinda et al., 2008; Mwenge Kahinda and Taigbenu, 2011). Neither the National Water Act (Act 36 of 1998) nor the National Water Services Act (Act 108 of 1997) refer to RWH (Kahinda et al., 2008). There are thus no monitoring requirements prescribed for rainwater and using strictly DRWH would be illegal under the current legislation, while clear policy guidelines may encourage installation (Mwenge Kahinda et al., 2007; Mwenge Kahinda et al., 2010).

Despite the government's push for piped water due to political pressure, Non-Governmental Organisations (NGOs) have promoted the use of rainwater tanks in rural areas across South Africa (Mwenge Kahinda and Taigbenu, 2011).

Galela Amanzi, an NGO in Grahamstown run by students from Rhodes University, is one of the groups promoting rainwater use and providing rainwater tanks for rural populations where water is less accessible (Galela Amanzi, 2012). But while NGOs install tanks, they often leave the maintenance and aftercare to the owners. The problem is that maintenance does not occur, as people perceive tanks should maintain themselves, or they do not understand what is required to maintain them. Other reasons for a lack of maintenance could also be present. This lack of maintenance could lead to deterioration of the water quality and of the tap itself.

Installation of rainwater tanks is as important as maintenance to prevent contamination of rainwater (Abbott et al., 2007a). The correct gutter installation can prevent midges and mosquitoes from growing in the rainwater tanks (Mwenge Kahinda et al., 2007; Frans, 2011). The correct installation of gutters will also prevent water loss (Worm and van Hattum, 2006). Many countries have plumbing laws which need to be adhered to (Chapman et al., 2008a). Other laws may also be applicable (Ward, 2010).The size of the tank needed depends on the rainfall within the area. In Australia, guidelines and information on rainfall patterns and tank sizes required in each region is readily available (Chapman et al., 2008b). Equations can be used to identify the necessary tank size needed to meet the household's demand for water (Worm and van Hattum, 2006). This was not considered in the installation of the rainwater tanks currently under discussion, as only one size of tank was installed. Its installation in households further than 500 m away from clean water sources is advised to improve the quantity of water available (Thomas and Martinson, 2007).

The location of the rain tank on the site can prevent problems such as tree debris entering the tank or tanks breaking due to subsidence of the area (Chapman et al., 2008b). Preventing light from entering the tank may prevent algal growth, and thus placement under eaves may be beneficial (Chapman et al., 2008b). This was also not taken into account in most rainwater tank installations in this study. Installing first flush devices and filters should be considered carefully, as the amount of maintenance required depends on the accessibility and the tendency of tanks to block due to debris (Chapman et al., 2008b; Egodawatta et al., 2009; Abbasi and Abbasi, 2011; Mendez et al., 2011; Lee et al., 2012). Use a filtration device would greatly improve the water quality.

Rainwater harvesting (RWH) involves the collection of storage from rooftops and land catchments and subsequent use of rainwater for potable and non-potable purposes (Mwenge Kahinda et al., 2007; Sharma, 2007). Domestic rainwater harvesting refers to collection from rooftops, yards and hard surfaces for storage in onsite tanks, with water being used for domestic purposes such as garden watering and for drinking and irrigation during municipal interruptions and drought periods (SOPAC; Mwenge Kahinda et al., 2007; Sharma, 2007). Buildings often used for Domestic Rainwater harvesting (DRWH) include houses, community centres and schools (Salukazana et al., 2005). Rainwater harvesting is considered an improved water source by the World Health Organisation (WHO) (WHO and UNICEF, 2010c) and thus a potable water source. Rainwater usage has improved water supply and, with usage instructions, can significantly increase safe water use (Baguma et al., 2010). In South Africa, it is considered a potable water source for rural areas, although the high cost of installation hinders widespread use (Amin and Han, 2011).

There are questions as to whether rainwater meets the country's drinking water guidelines (Gould, 1999; Abdulla and Al-Shareef, 2009; Ahmed et al., 2011; Amin and Han, 2011). International studies have shown that it is often not suitable for drinking without treatment (Abdulla and Al-Shareef, 2009; Amin and Han, 2011; Otieno and Adeyemo, 2012). This raw water should, at a minimum be sand filtered, boiled or have bleach added before ingestion (Murray et al., 2004) in order to minimise health risks. But household level treatment is expensive and time consuming, and is thus not often practised (Monyai, 2004). It is expensive as, historically, rainwater tanks have been used on farms, teaching institutions and in rural areas and are seen as being solutions "for the poor" (Mwenge Kahinda and Taigbenu, 2011).

Rainwater quality is affected by the microbes and pathogens entering rainwater tanks (Evans et al., 2006). These tanks are found in many different environments and are exposed to different air pollutants (Evans et al., 2006; Evans et al., 2007). Roofing materials and environmental positions make a difference to the water quality (Evans et al., 2007; Egodawatta et al., 2009; Abbasi and Abbasi, 2011). Galvanised iron roofs tend to neutralise acidic rain to an extent (Mendez et al., 2011) as well as provide a harsh environment for bacteria as they reach high temperatures during the day (Lee et al., 2012). Clay roofs tend to neutralise acid more than galvanised iron roofs (Lee et al., 2012). These tend to collect more dirt in the crevices between tiles (Sharma, 2007).

Asbestos roofs tend to produce poor rainwater quality (Efe, 2006). The danger of developing cancer from drinking water with asbestos dust is not considered significant (Australian Government, 2004; Mosley, 2005). Inhaling asbestos dust is, however, strongly linked to cancer or asbestosis (Hueper, 1965). Asbestos roofs should not be cleaned with high pressure washers, and should be replaced with a substitute when worn (Australian Government, 2004).

Removing lichens with fungicides (Van de Voorde et al., 2009) could cause health risks, as this could be washed into the rainwater tank. Lichens degrade ceramic tiles (Kiurski et al., 2005) and increase the release of tile ions and carbon into the water (Adeniyi and Olabanji, 2005). Lichens tend to increase organic matter, ammonium and phosphorus concentrations, adversely affecting the microbial and chemical quality of rainwater (Farreny et al., 2011; Lee et al., 2012). Tank and roof maintenance are both important to prevent lichen growth and water quality deterioration (Mosley, 2005). Water quality monitoring is essential to prevent waterborne disease outbreaks and should be part of the public health policy in South Africa.

5.2 AIM

The aim of this project was to identify potential microbial contaminants and their sources, where possible. The microbial water quality of rainwater would be compared to Department of Water Affairs and Forestry (DWAF) 1996 guidelines.

Rainwater is considered a potable water source in the rural areas of developing countries, although its actual water quality has not been tested (Amin and Han, 2011). The quality of the available rainwater needs to be established before it is drunk (Tandlich et al., 2011).

Galela Amanzi was formed in 2007 by Rhodes University students (Grahamstown) and North Eastern University in the United States of America (USA) through the ‘GLOBAL PACT Program’ (Galela Amanzi, 2010). Its focus is to supply potable water for historically disadvantaged communities in Grahamstown, Eastern Cape, South Africa (Galela Amanzi, 2010). It now wishes to improve water access in Grahamstown (Chirenda, 2011; Rhodes University, 2011a). They use sponsorship and donations to fund rainwater tanks which are donated to selected sites (Galela Amanzi, 2010).

Galela Amanzi partnered with the Kowie Catchment Campaign, Umthathi Training Centre and the Catchment Research Group and the EHB Group, amongst others, for these projects (Galela Amanzi, 2010). By 2011 they had installed 20 tanks in the Grahamstown area (Galela Amanzi, 2011; Rhodes University, 2011b). This project was started to inform Galela Amanzi about possible contaminants and to identify suitable means to prevent future contamination. No maintenance has been done on the tanks since their installation by Galela Amanzi (Chirenda, 2011). The community has become reliant on rainwater tanks to supply dependable potable water in their rural settings, due to pipe breaks and water shortages (Mmango, 2010).

5.3 MATERIALS AND METHODS

MacConkey agar, R2A agar and mFc agars were purchased from Merck Pty. Ltd. Johannesburg/Cape Town, South Africa. The Kovacs indole reagent and other chemicals were purchased from Sigma- Aldrich (Johannesburg, South Africa). Ethanol was purchased from Chemstore (Rhodes University, Grahamstown). Nitrile gloves were purchased from Lasec (Port Elizabeth, South Africa). The membrane filters, sterile plastic Petri dishes (90 mm) and sterile urine jars were purchased from Spellbound Labs (Port Elizabeth, South Africa).

GPS coordinates were recorded using a GARMIN eTrex® Vista Cx or Garmin GPS 76 (Garmin International, Kansas City, USA). Rainwater was sampled from collection tanks at different schools and organizations around Grahamstown. Most of the monitored tanks were installed by Galela Amanzi and some by Rhodes University. Random once-a-month sampling dates were chosen, although school term dates did interfere with monthly sampling due to tanks only being accessible during term times. Samples were collected in sterile urine jars (40 mL, Spellbound Labs, Port Elizabeth, South Africa) and sterile polyethylene bottles (500 mL, Spellbound Labs, Port Elizabeth, South Africa). Sample bottles were sterilised using the Model RAU-53Bd REX MED autoclave (Hirayama Manufacturing, Tokyo, Japan). Plastic bottles were used for sampling water from rainwater tanks, as microbiological and nitrogen, chloride hardness and turbidity are not affected provided that the samples are analysed quickly (APHA, 1992). They were analysed within one hour of collection.

The rainwater tank taps were sterilized using 70 % ethanol. Sterilizing with a flame was not practical due to them being plastic (Mosley and Sharp, 2005). The water was allowed to run from the taps for 30 seconds before samples were taken. Samples were transported in a cooler box. Samples in 2011 were not transported on ice (returned within 1 hour of collection). This was acceptable as the WHO states that samples analysed within 2 hours without ice during transportation would be valid, as long as they were kept in the dark and cool (1996). Samples collected in 2012 were transported on ice and were then allowed temperate before analysis. Using ice did not influence microbial concentrations due to the speed of transport and analysis. Water samples were transported in a cooler box at the same temperature as its source or colder. Rainwater samples for 2012 were kept in the fridge if a delay of more than 1 hour was going to occur before analysis.

The concentrations of the faecal coliforms (FC), total coliforms (TC) and the heterotrophic plate count (HPC) were enumerated using the membrane filtration technique (cellulose acetate membrane filters; pore size 0.45 μm) or spread plating on m-FC agar (Merck) according to DWAF (DWAF, 1996c), MacConkey agar (Merck)(Au et al., 2000) and R_2A agar (Merck) (Uhl and Schaule, 2004). Plates were incubated at 44.5 and 35°C respectively for 24 hours. Incubation took place in a Labcon incubator, Model FSIM B (Labmark, Johannesburg, South Africa), the TS 606/3-I incubator (WTW, Weilheim, Germany), the Labcon low temperature incubator LTIE 10 (Labmark, Johannesburg, South Africa); and/or the Heraeus Model FT 420 (Heraeus Kulzer GmbH, Dormagen, Germany). An average value from the two replicates was used. The mH_2S strip test (Luyt et al., 2010) was analysed at room temperature for 72 hours, in the dark. Results were compared to the DWAF Standards of Freshwater for Domestic Use (DWAF, 1996). Nitrate (Merck Catalogue No. 1.09713.0002) levels and Ammonium (Merck, Catalogue No. 1.14752.0002) levels were analysed using the Merck kits.

Representative colonies were isolated from the plates and identified using the API® 20 E™ kit (|REF|20 1 0 0 / 20 160 5 0€1SPI®2O E ™) (BioMérieux). The three colonies from each plate, forming a total of 12 representative colonies, were isolated twice, using streak plating, to help purify the colonies before being identified. One colony was isolated with a sterile pipette tip and resuspended in sterile saline (0.9%) for identification. The API strips were read and the results analysed using API LAB PLUS computer software (BioMérieux).

Statistical analysis was performed using Statistica 10 (StatSoft Inc., 2011). Spreadsheets were created in Excel 2010 (Microsoft).

5.3.1 Rationale for Monitoring Organisms Used

The total coliforms were monitored as they provide an indication of coliform regrowth of high organic levels in rainwater tanks. The high total coliforms may indicate more risk of pathogens being present in rainwater.

Monitoring faecal coliforms meant results could be compared to those of other studies. It was also selected due to the ease of culturing and potential links to pathogens. It is known some faecal coliforms may come from environmental sources rather than animals or humans.

The heterotrophic plate count (HPC) was used to identify the number of bacteria present and the potential cleanliness of the tank. It may also indicate the maintenance of the tank, as cleaning should decrease bacterial loads.

The mH_2S strip test was used to identify contamination in the tank and the relation to other bacterial culturing methods, as well as its viability as a cheap method for the community to test tanks.

5.4 MAP

Using Geographic Information System (GIS) maps allows rainfall data to be correlated with rainwater quality. Rainfall varies across Grahamstown. Figure 22 shows the location of the rainwater tanks in Grahamstown.

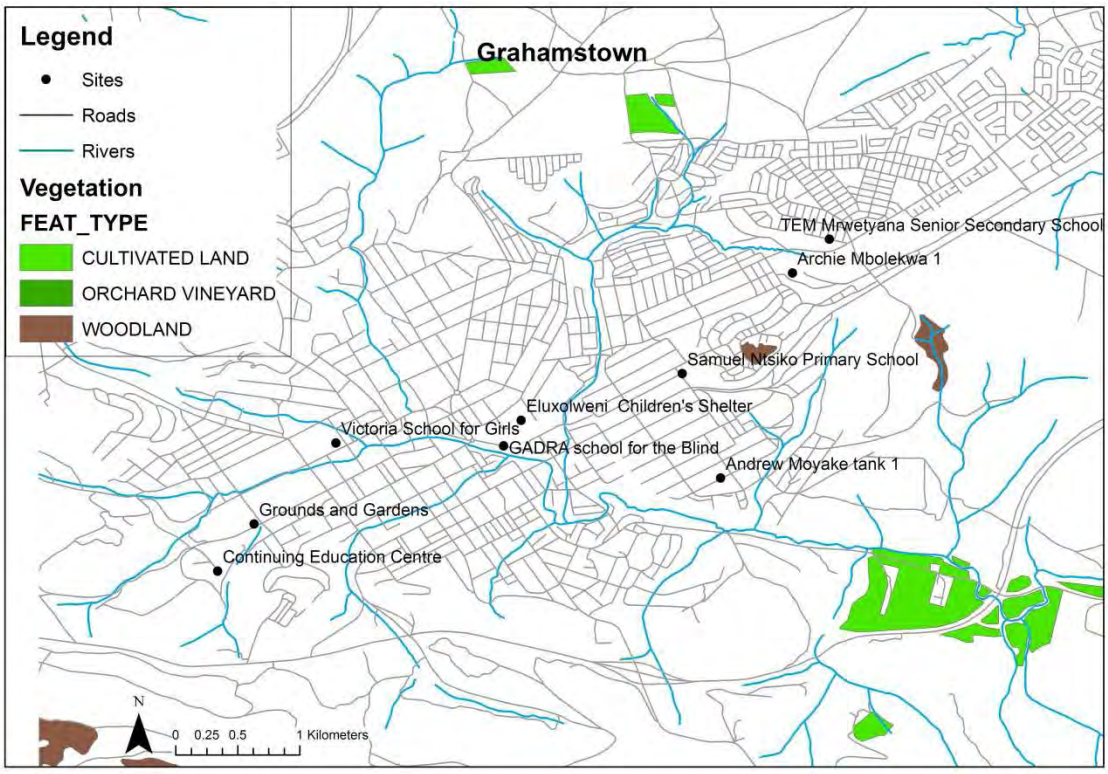


Figure 22: A Geographic Information System (GIS) map showing the position of rainwater tank sites in Grahamstown

5.5 SITE DESCRIPTIONS

All of the downpipes lead directly to the rainwater tanks. There are neither filters nor first flush devices. All tanks are on a raised platform made of brick with a cement slab on top. All green plastic (polyethylene) rainwater tanks in this study had a 5000L capacity.

Andrew Moyake Tank 1 and Tank 2

GPS: S33°18’37.6” E26°32’58.1”

Andrew Moyake Primary School is a township school in B Street, just off Lady Grey Street. The school has two water tanks, used for drinking and watering the gardens. The school presently has piped tap water. The area around the tanks, is always kept clean and tidy. The roof is made of galvanized steel, with asbestos gutters and plastic downpipes.

Figure 23: Andrew Moyake Tank 1

Archie Mbolekwa 1 GPS: S33°17’44.0” E26°33’16.9”

Archie Mbolekwa Primary School had two tanks at the start of the project. Water from the tap did not drain well from the area and tended to pool, causing unhappiness amongst the teachers. They were not happy with water pooling outside one of the tanks, so it has been removed. They currently only use 1 tank. The removed tank was used for drinking and cooking.

The roof and gutters are made of Asbestos.

Figure 24: Archie Mbolekwa Tank 1

Continuing Education Centre (Rhodes University (RU) GPS: S33°19’01.9” E26°30’46.7”

The Continuing Education Centre on the Rhodes University campus has a cement tiled roof with plastic guttering and was the only tank with a metal tap. The roof gutters are often filled with pine needles from pine trees across the road. Bird droppings were only noted once during the sampling period.

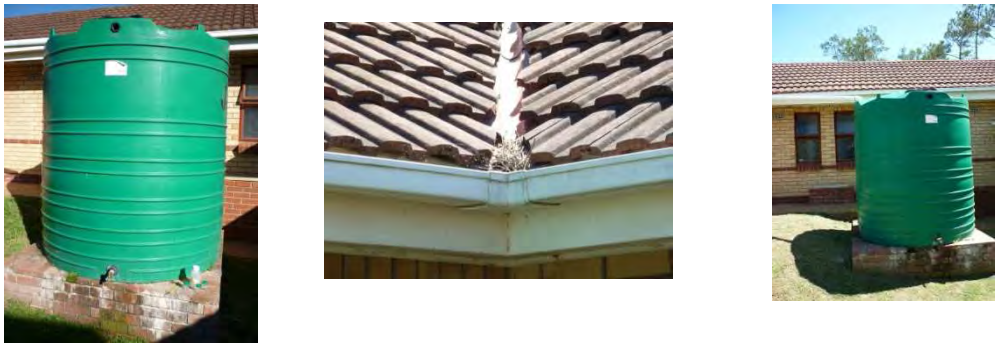


Figure 25: The rainwater tank at the Continuing Education Centre on Rhodes University’s campus. The middle photo shows pine needles collected in the gutters.

Eluxolweni Children's Shelter GPS: S33°18’22.5” E26°32’006”

The roof is galvanised iron with plastic gutters. It only has one tree close by which is unlikely to cause accumulated debris on the roof. Bird droppings are often visible on the tanks, although none could be observed on the roof. One of the tanks did have a slight leak at the connection to the tap at one point, but this has been resolved. The area around the tap and tanks was always kept clean. The tanks are used for watering the garden, although children often also use them for drinking when they walk past.

Figure 26: The rainwater tanks and tap at the Eluxolweni Children’s Shelter.

GADRA School for the Blind GPS: S33°18’29.2” E26°32’01.4”

The area around the tanks is soily and often leaf littered due to the big *Jacaranda mimosifolia* trees nearby.

The water is only used for gardening. It sometimes has a smell to it and is always an orange-brown colour. The roofing material is asbestos with Polyvinyl chloride (PVC) plastic gutters. Leaf litter and debris, along with lichen, were visible on the roof.

Figure 27: The rainwater tank and roof of the GADRA School for the Blind. This is situated behind one of the Grahamstown clinics.

Grounds and Gardens Department on Rhodes University Campus GPS: S33°18'49.6" E26°30'56.2"

The roof is made of galvanised iron and has plastic guttering. The area around the tank is kept clean and no birds dropping were ever visible. The plastic tap was changed in April to a more sophisticated one, to be used for the garden. This has helped to further improve the water quality.

Figure 28: The rainwater tank at the Rhodes University Grounds and Gardens building.

Samuel Ntsiko Primary School GPS: S33°18’10.3” E26°32’48.1”

Samuel Ntsiko Primary School had two tanks in 2011 but only one in 2012. The tank installed by Galela Amanzi has been removed. This tank is used for garden watering, although students have been observed drinking from it and washing their lunch bowls there. The tank has run dry due to the tap being left open, leaving gaps in the sampling data at various times. The sampling dates reflecting this are the 17th of August 2011; the 8th of March 2012 and the 13th of June 2012. The roof is galvanised iron with plastic pipes, guttering and tap.

Figure 29: The rainwater tank at Samuel Ntsiko Primary School.

TEM Mrwetyana Senior Secondary School GPS: S33°17’35.2” E26°33’26.5”

TEM Mrwetyana Senior Secondary School has two tanks, but only one of them was included in the sampling in 2012 due to logistical problems. The roof is asbestos with plastic gutters. The roof has lichens growing on it, but there are no trees in the area to cause leaf accumulation. Bird droppings were never visible.

Figure 30: The rainwater tank at the TEM Mrwetyana Senior Secondary School.

Victoria Girls' High School (VG) GPS: S33°17'42.8", E26°33'17.6"

Most of the galvanised iron roof looks dirty and soily with lichens growing on it, but not on the area draining directly into the tank. The down pipes are plastic, but the pipes to the tap are made of copper. The tanks are used for gardening and not drinking. This decision was made as the tanks could not support the number of learners which may use them. There are trees overhanging the tanks, but this should not affect the tank water quality, as the tanks are sealed. The area around them is kept very clean.

Figure 31: Rainwater tanks and tap at the Victoria Girls' High School

5.6 RESULTS AND DISCUSSION

Rainwater was monitored from the 25th of March to the 17th of August 2011 and again from the 1st of March 2012 to the 2nd of August 2012. The different bacterial concentrations are summarised in the Table 35 and 25 below. Site conditions were explained above. From the pilot sampling in 2010, it can be seen that Andrew Moyake Primary School, VG and CEC had no detectible indicator organisms in a 100 mL sample on the 1st of November 2010.

Department of Water Affairs and Forestry (DWAF) domestic water guidelines state that faecal coliform concentrations below 10 Colony Forming Units per one hundred millilitres (CFUs/100 mL) have a slight risk of microbial infection with continual use, but this is negligible if it is short term or intermittent (DWAF, 1996c). But as faecal coliform concentrations above 20 Colony Forming Units (CFUs)/100 mL have a significant risk related to their use, proportional to the volumes consumed and the frequency of their use (DWAF, 1996c). Total coliform concentrations below 5 CFUs/100 mL are not considered a public health risk, while those above 100 CFUs/100 mL have a significant risk of waterborne disease transmission (DWAF, 1996c).

Thus only VG Tank 1 and Andrew Moyake Primary School's rainwater would meet domestic use guidelines, although neither of these tanks are being used for more than irrigation.

DWAF irrigation guidelines only stipulate that faecal coliforms be below 1 CFUs/100 mL for any irrigation method, while below 1000 CFUs/100 mL for crops not eaten raw, provided the pastures of these crops are allowed to dry before harvesting (DWAF, 1996b). Rainwater from VG Tank 1 and Andrew Moyake Primary School can be used for any form of irrigation. As rainwater is not treated, it is not known if high concentrations are due to environmental input or growth inside the rainwater tanks. The Sun City Community Centre is no longer using its rainwater tank.

The VG Tank 1 and Andrew Moyake Primary School had very clean gutters, which may be the reason for no bacterial coliforms being enumerated (shown in Table 34). Many reports recommend that gutters be cleaned (Dillaha Iii and Zolan, 1985; Australian Government, 2004; Abbott et al., 2007b; Amin and Han, 2008) to prevent organic material building up in the tank, as it could decrease the pH and result in metals becoming soluble. A lack of maintenance seems related to poor microbial water quality (Abbott et al., 2007b). Debris trapped between the tiles and in the gutters could have contributed to the high total coliform counts at the CEC on the 14th of September 2010. This water was then used as a family's only source of drinking water, due to lack of municipal water supply (verbally confirmed by the municipality). The two children developed diarrhoea over this period. Diarrhoea is defined as three or more loose stool per day (Baqui et al., 1991). This possibly links higher total coliform counts to diarrhoea (Tandlich et al., 2011). Cleaning the gutters seemed to decrease the total and faecal coliform counts to below detectible levels again, and no more diarrhoea cases were reported (Tandlich et al., 2011). This is more evident in 2011 (shown below). Due to the unreliability of municipal water, many township schools rely on rainwater. This is reiterated regularly by teachers. The CEC asked for the site to be continuously monitored (Tandlich et al., 2011). The mH_2S strip test (Luyt et al., 2009; Tandlich et al., 2010b) was used by the inhabitants of the CEC after receiving training. Inhabitants have however changed and a new manager took over the CEC in September 2011. No more diarrhoea cases have been reported despite the presence of higher total coliform and faecal coliform counts. Residents, however, have not been using it as their sole drinking source. The gutters are only occasionally cleaned, but each cleaning does improve the water quality.

The DWAF (1996b) allows 1 CFUs/100 mL for irrigation purposes on any crops. Faecal coliform concentrations up to 1000 CFUs/100 mL are permitted in irrigating fruit trees, as long as the crops are not wetted and crops are not consumed raw and are allowed to dry before harvesting or grazing takes place (DWAF, 1996b).Thus tank water from all the sites could be used for the irrigation of crops not eaten raw, while only Samuel Ntsiko Primary, VG Tank 1 and the CEC tank water could be used for irrigation of crops eaten raw. If a subsurface drip irrigation system was used, then all could be safely used for irrigation (Salukazana et al., 2005).

5.6.1 Rainwater Results from 2010

Table 34: Concentrations of rainwater during opportunistic sampling of rain tanks installed around Grahamstown by Galela Amanzi in 2010 (Tandlich et al., 2011).

Rainwater source	GPS coordinates	Sampling Date	FC (CFUs/100 mL)	TC (CFUs/100 mL)
Sun City Community Centre	S 33º 17’ 20.4’’ E 26º 31’ 51.2’’	16/04/2010	6	44
Eluxolweni Children’s Shelter	S 33º 18’ 23.3’’ E 26º 32’ 00.6’’	16/04/2010	26	144
Andrew Moyake Primary School	S 33º 18’ 38.0’’ E 26º 32’ 58.2’’	16/04/2010	0	0
St Augustine’s Church	S 33º 17’ 21.4’’ E 26º 33’ 32.7’’	16/04/2010	0	10
Samuel Ntsiko Primary School	S 33º 18’ 10.8’’ E 26º 32’ 48.1’’	16/04/2010	1	17
Victoria Girls’ High School Tank 1	S 33º 18’ 55.4’’ E 26º 31’ 28.4’’	05/08/2010	0	0
Victoria Girls’ High School Tank 2		05/08/2010	98	150
Victoria Girls’ High School Tank 3		05/08/2010	54	200
Continuing Education Centre (RU)	S 33º 19’ 01.9’’ E 26º 30’ 46.6’’	14/09/2010	0	22
Continuing Education Centre (RU)		15/10/2010	0	2
Continuing Education Centre (RU)		01/11/2010	0	0

The Sun City Community Centre had a maximum total coliform concentration of 200 CFUs/100 mL, while faecal coliforms only reached 98 CFUs/100 mL. These maximum values were observed in the VG rainwater tanks. No South African literature was found for

comparison purposes. An Australian study reported maximums of 118 CFUs/100 mL and 743 CFUs/100 mL for total and faecal coliform respectively (Coombes et al., 2000).

The reported results are from rain water sampling between the 25th of March and the 17th of August 2011. The bacterial faecal coliform median in 2011 was 13 times higher than in 2010, while 2012 was only 1.3 times higher. This cannot be explained as the environmental conditions, sampling methods and media used in the laboratory have not changed. The age of the tanks has changed, and so, possibly, their internal cleanliness would be the only explanation.

Literature data from international studies have a wide range of microbial concentrations influenced by the filtration or first flush techniques used. An Australian study showed that within the first flush tank, total coliform range depended on the roof type, with the highest being 131 CFUs/100 mL and the highest *Escherichia coli* (*E. coli*) level of 18 CFUs/100 mL (Lee et al., 2012), while an earlier study had a total coliform range of 0 to 320 CFUs/100 mL and *E. coli* range of 0 to 60 CFUs/ 100 mL Ahmed et al. had 5% of the samples with *E. coli* concentrations above 1000 CFUs/100 mL , whereas approximately 32% of the samples were below 1 CFUs/ 100 mL (Ahmed et al., 2009; Lee et al., 2010). A similar environmental study in Newcastle, New South Wales, Australia, had FC concentrations up to 743 CFUs /100 mL and TC concentrations up to 1118 CFUs/ 100 mL (Coombes et al., 2000).

Two New Zealand studies showed differences. Simmons et al. (2001) showed HPC concentrations up to 130000 CFUs/mL and total coliforms of up to 19000 CFUs/ 100 mL, while FC had a maximum of 840 CFUs/ 100 mL. On the other hand, only 30% of Abbott et al.'s (2007a) samples contained total coliform concentrations above 200 CFUs/ 100 mL. An Ireland study had total coliforms up to 1203.3 CFUs/100 mL and FC up to 22 CFUs/ 100 mL with HPC up to 7041/mL at 37°C (O'Hogain et al., 2011).

Other South African studies have also showed wide variations in microbial concentrations as well, with total coliforms up to 20000 CFUs/100 mL in screened tanks. (Amin and Han, 2011).
A KwaZulu-Natal study at schools showed much higher total coliform concentrations ranging up to 2000 CFUs/ 100 mL and faecal coliforms up to 900/100 mL while HPC ranged to 15400 CFUs/100 mL (Otieno and Adeyemo, 2012). From this study, only 2 school tanks

complied with the South African Bureau of Standards (SABS) and DWAF domestic water guidelines (Otieno and Adeyemo, 2012)

Microbial concentrations from this study in 2010 were lower than literature values, while they were higher than most studies in 2011. This may be due to lack of maintenance, as this has been proposed as a cause for water quality deterioration.

Table 35: Microbiological results of rainwater monitoring in the Makana Municipality are from March 2011 to August 2011, showing the concentration of indicator organisms (faecal coliforms, total coliforms, heterotrophic bacteria) and mH_2S strip test results.

Location/Tank site	GPS Coordinates	Date in 2011	Heterotrophic plate count (CFUs/mL)	Faecal coliforms (CFUs/100 mL)	Total coliforms (CFUs/100 mL)	mH_2S strip test
Grounds and Gardens (RU)	S33°18'49.6" E26°30'56.2"	25-Mar	4880	8	7900	Positive at 48 hours
Continuing Education Centre (RU)	S33°19’01.9” E26°30’46.7”	25-Mar	1000	16	500	Positive at 48 hours
Victoria Girls’ High School	S33°17’42.8” E26°33’17.6”	19-Apr	4660	224	7400	Positive at 48 hours
Continuing Education Centre (RU)	S33°19’01.9” E26°30’46.7”	19-Apr	150	138	600	Positive at 48 hours
Eluxolweni Children's Shelter	S33°18’22.5” E26°32’006”	19-Apr	800	1648	700	Positive at 48 hours
Samuel Ntsiko Primary School	S33°18’10.3” E26°32’48.1”	06-May	70	0	200	Positive at 48 hours
GADRA School for the Blind	S33°18’29.2” E26°32’01.4”	06-May	5620	66	700	Positive at 48 hours
Andrew Moyake Tank 1	S33°18’37.6” E26°32’58.1”	06-May	7870	0	4100	Negative
Andrew Moyake Tank 2	S33°18’37.6” E26°32’58.1”	06-May	1010	4	BDL	Positive at 48 hours

Table 35 (continued)

Location/Tank site	GPS Coordinates	Date in 2011	Heterotrophic plate count (CFUs/mL)	Faecal coliforms (CFUs/100 mL)	Total coliforms (CFUs/100 mL)	mH_2S strip test
Eluxolweni Children's Shelter	S33°18’22.5” E26°32’006”	13-May	3520	254	4000	Positive at 24 hours
Grounds and Gardens (RU)	S33°18'49.6" E26°30'56.2"	13-May	2630	554	BDL	Positive at 24 hours
Continuing Education Centre (RU)	S33°19’01.9” E26°30’46.7”	13-May	870	40	500	Positive at 24
Victoria Girls’ High School	S33°17’42.8” E26°33’17.6”	13-May	2360	408	400	Positive at 24 hours
Andrew Moyake Tank 1	S33°18’37.6” E26°32’58.1”	26-May	4050	180	2000	Positive at 48 hours
Andrew Moyake Tank 2	S33°18’37.6” E26°32’58.1”	26-May	4320	208	2100	Positive at 48 hours
Samuel Ntsiko Primary School	S33°18’10.3” E26°32’48.1”	26-May	3930	644	30700	Positive at 48 hours
GADRA School for the Blind	S33°18’29.2” E26°32’01.4”	27-May	6580	> 500	13700	Positive at 48 hours
Archie Mbolekwa 1	S33°17’44.0” E26°33’16.9”	27-May	2810	> 500	16200	Positive at 48 hours
Archie Mbolekwa 2	S33°17’44.0” E26°33’16.9”	27-May	650	> 500	4100	Positive at 48 hours
Victoria Girls’ High School	S33°17’42.8” E26°33’17.6”	27-May	1270	> 500	13500	Positive at 48 hours
TEM Mrwetyana Senior Secondary School	S33°17’35.2” E26°33’26.5”	27-May	890	> 500	4900	Positive at 48 hours
GADRA School for the Blind	S33°18’29.2” E26°32’01.4”	17-Aug	290	< 4	1100	Negative

Table 35 (continued)

Location/Tank site	GPS Coordinates	Date in 2011	Heterotrophic plate count (CFUs/mL)	Faecal coliforms (CFUs/100 mL)	Total coliforms (CFUs/100 mL)	mH_2S strip test
TEM Mrwetyana Senior Secondary School	S33°17'35.2" E26°33'26.5"	17-Aug	1770	< 4	500	Negative
Andrew Moyake Tank 1	S33°18'37.6" E26°32'58.1"	17-Aug	80	0	0	Negative
Andrew Moyake Tank 2	S33°18'37.6" E26°32'58.1"	17-Aug	270		600	Negative
Eluxolweni Children's Shelter	S33°18'22.5" E26°32'006"	17-Aug	1190		6200	Negative
Grounds and Gardens (RU)	S33°18'49.6" E26°30'56.2"	17-Aug	10		3900	Negative
Archie Mbolekwa 2	S33°17'44.0" E26°33'16.9"	17-Aug	1250		1300	Negative

TTC is too many to count, thus the plate was overgrown

GADRA stands for Grahamstown Area Distress Relief Organisation

BDL is below the detection limit

5.6.2 Rainwater Results from 2011

Bacterial concentrations found in 2011 did not comply with the DWAF drinking water guidelines (DWAF, 1996c). The mH_2S strip test was negative on the 6th of May, at Samuel Ntsiko Primary School and Andrew Moyake Tank 1, and at all sites in August. The Andrew Moyake tanks differed in microbial concentrations despite draining from the same roof. Tank 2 always had a lower microbial concentration than Tank 1. This may be due to the condition of the gutters, as Tank 1 was closer to the end of the roof than Tank 2, which drained from the middle of the roof.

The roofing material is said to have an influence on rainwater quality. Galvanised iron roofs have relatively smooth surfaces and are unlikely to have a build-up of dust or debris, while the high temperatures are said to sterilise (Lee et al., 2012) any dust or droppings on the roof (Mosley, 2005). Cement roofs are a lot rougher and leaves and animal droppings can collect

on these roofs and in the joints (Sharma, 2007). Cement tiles have a similar water quality to galvanised iron roofs (Mendez et al., 2011). Table 36 shows the maxima and minima according to the roofing material. The data supports theories stating that galvanised iron roofs have better water quality than asbestos roofs (Adeniyi and Olabanji, 2005; Mendez et al., 2011; Lee et al., 2012). Cement tile roofs had the lowest bacterial concentrations of the entire sample collection analysed, which has not been noted in other studies. The CEC roof did not have any visible bird droppings or dirt on it, except the collection of pine needles in the gutters. The bacterial concentration was higher when gutters were dirty. For example, on the 19th of April 2011, the FC concentration was 138 CFUs/100 mL. This concentration dropped drastically after the gutters were cleaned. This is again demonstrated on the 25th of March and the 17th of August 2011, with FC concentrations of 16 and 40 CFUs/100 mL respectively. This was observed previously in 2010 (Tandlich et al., 2011).

An incident of faecal material being thrown onto the roof at the Eluxolweni Children's Shelter by a child on the 19th of April 2011 produced outliers in the faecal coliform concentrations for galvanised iron roofs. On the whole, the heterotrophic bacteria and total coliform ranges, from the galvanised iron roof sampling points were lower than the asbestos roofed sites. The total coliforms had a maximum concentration of 30700 CFUs/100 mL for galvanised iron roofs, while Asbestos roofs had values up to 41750 CFUs/100 mL, while the cement tile roof only had a maximum of 3050 CFUs/100 mL.

Bacterial concentrations only met the DWAF (1996c) domestic water standards once at Andrew Moyake Primary School Tank1 on the 17th of August 2011. Concentrations of FC and TC were 0 CFUs/ 100 mL. The rainwater was otherwise within DWAF (1996c) irrigation guidelines for irrigating vegetables not eaten raw at all times except on the 19th of April 2011 at Eluxolweni Children's Shelter, described above. Using the water for growing vegetables that are going to be peeled and cooked is safe. The water should be treated before drinking or watering of vegetables going to be eaten raw, as stated by Amin and Han (2009).

Public health implications of using the rainwater include the risk of diarrhoeal diseases. As the water is only used occasionally and less water is drunk, the likelihood of the infectious dose of pathogens consumed is lower. When it is the primary drinking water source, the quantity of water consumed is higher and thus the chance of reaching the infectious dose is too.

Continuous exposure to bacteria does not result in continuous disease, as humans acquire immunity against these bacteria (Mara and Horan, 2003). People could therefore become immune to bacteria in the rainwater tanks, illuminating the diseases associated with its consumption.

Bacteria such as faecal /thermotolerant coliforms in the rainwater tanks have been contributed from the environment - for example from the soil or small animals and birds - thus the potential for it to contain human pathogens is reduced (Cunliffe, 1998). Their significance in water is not fully understood, despite their common presence (Cunliffe, 1998; Lye, 2002). The rate of birds carrying human pathogens is not known (Cunliffe, 1998),while they may carry *Campylobacter. Campylobacter* has caused infection in immunocompromised people who use it solely as a drinking water source (Cunliffe, 1998). The problem was rectified by boiling the water prior to its use (Cunliffe, 1998).

Isolation tests of pathogens in rainwater in Australia showing the presence of *Mycobacterium spp.*(Lye, 2002) highlight the presence of pathogens in rainwater tanks. Australian studies up to 1998 did not shown the presence of pathogens such as *Salmonella, Shigella, Cryptosporidium* and *Giardia* (Cunliffe, 1998). But other studies have shown the presence of *Salmonella arechevalata; Campylobacter spp.; Legionella pneumophila; Clostridium botulinum; Echinococcus granulosus; Giardia lamblia* and *Cryptosporidium parvum* (Lye, 2002). Pathogens isolated in developed countries include the *Campylobacter spp., Salmonella spp., Cryptosporidium spp.* and *Giardia spp.* although these are not necessarily human pathogens (Fewtrell and Kay, 2007). The correlation between indicator bacteria and pathogens is not well established in rainwater (Ahmed et al., 2008), thus the use of indicators may not be sufficient to prevent diarrhoeal outbreaks. The public health risk is therefore not certain.

Table 36: The concentration maxima and minima for the faecal coliforms, heterotrophic bacteria and total coliforms at the different sites collated according to their roof type over the sampling period of March to August 2011.

Roofing Type:	**Galvanised iron roof**				
Location/ Tank Site	**Andrew Moyake Tank 1**	**Victoria Girls' High School**	**Eluxolweni Children's Shelter**	**Grounds and Gardens (RU)**	**Samuel Ntsiko Primary School**
Maximum HPC	7870	4660	3520	4880	75750
Maximum FC	208	600	1648	554	644
Maximum TC	11000	27000	6200	7900	30700
Minimum HPC	80	1270	304	10	70
Minimum FC	0	224	1	0	0
Minimum TC	0	400	456	0	35

Table 36 (continued)

Roofing Type:	**Asbestos roof**			**Cement tile roof**
Location/ Tank Site	**TEM Mrwetyana Senior Secondary School**	**GADRA School for the Blind**	**Archie Mbolekwa**	**Continuing Education Centre (RU)**
Maximum HPC	1770	6580	2810	1000
Maximum FC	600	500	500	138
Maximum TC	38000	41750	37500	3050
Minimum HPC	418	290	650	103
Minimum FC	0	3	0	5
Minimum TC	500	700	1300	500

TC – Total Coliforms

FC - Faecal Coliforms

HPC – Heterotrophic Plate Count

5.6.3 Rainwater Results from 2012

The rainwater had a total coliform (TC) range of 0 to 41750 CFUs/ 100 mL. Faecal coliforms (FCs) ranged between 0 and 1648 CFUs/100 mL. The HPC ranged between 10 and 75750 CFUs/100 mL. The Eluxolweni Children's Shelter reported worms in their tank in June, and there was still a problem in August. Although no worms were found in the water sampled, the shelter stated that no one was drinking the water since the worms were seen. The children also stated when we were sampling that we should not use the water, indicating

that they are aware of the problem. The water is not suitable for drinking or for garden watering under this condition. The worms were probably red worms caused by midges laying their eggs in stagnant water. The stagnant water was probably in the gutters due to soil or debris, as the gutter pipes lead straight into the tank. The tanks should be checked to ensure that they are sealed, preventing midges from entering the tanks. The position of a flood light just above the rainwater tanks may have contributed to the problem by attracting midges, leading to a higher chance of infestation (Frans, 2011). The best way to eliminate the problem is to empty the tanks and clean them. Chlorine does not kill the worms (Frans, 2011). Such emptying and cleaning need to take place before the water can be used for drinking or gardening again.

This brings highlights another point of concern for rainwater use in South Africa. Mosquitos breed in stagnant water too, so tanks need to be sealed or have a filter mesh size of less than 1mm to prevent their entry and that of other insects (Mwenge Kahinda et al., 2007). Water in gutter need to be free flowing to prevent stagnant water occurring, and using first-flush techniques may help prevent larvae in the gutters entering the tanks (Mwenge Kahinda et al., 2007).

The problem of maintenance was also demonstrated by the tap at Andrew Moyake Tank 1 which was found broken at the June sampling run, and has not been fixed since. The tank was still empty when sampling was discontinued, despite the substantial amount of rain during the last month of sampling. Samuel Ntsiko Primary School did not have water in their tank in March and August 2011, nor in March and June in 2012. This could have been due to the taps being left open or a shortage of rain compared to the tank usage.

Table 37: Average concentrations of indicator bacteria (faecal coliforms, heterotrophic bacteria and total coliforms) for the sampling days between March and August 2012.

	March 2012					April 2012				
	Date	**FC (CFUs/ 100 mL)**	**TC (CFUs/ 100 mL)**	**HPC (CFUs/ mL)**	**mH_2S strip test**	**Date**	**FC (CFUs/ 100 mL)**	**TC (CFUs/ 100 mL)**	**HPC (CFUs/ mL)**	**mH_2S strip test**
Eluxolweni Children's Shelter	1 March	34	1240	>500	48 hours	24 April	276	21000	1160	48 hours
Victoria Girls' High School	1 March	0	12	4	Negative	19 April	0	OG	1020	Negative
Continuing Education Centre (RU)	1 March	44	>500	840	72 hours	24 April	79	1548	310	Positive
Grounds and Gardens (RU)	1 March	0	4	4	Negative	24 April	52	1536	250	Negative
Andrew Moyake Tank 1	8 March	0	1000	0	Negative	19 April	215	17	3000	60 hours
Andrew Moyake Tank 2	8 March	1	47500	40	72 hours	19 April	57	74500	4030	48 hours
Samuel Ntsiko Primary School	8 March	No water	-	-	-	19 April	72	1900	13000	48 hours
Archie Mbolekwa 1	8 March	4	500	1255	72 hours	24 April	762	89000	3670	48 hours
GADRA School for the Blind	8 March	22	4500	1085	48 hours	24 April	364	27000	6740	48 hours
TEM Mrwetyana Senior Secondary School	8 March	14	>500	1635	48 hours	24 April	147	>400	>4000	48 hours

OG – Over grown

Table 37: (continued). The average concentrations of indicator bacteria (the faecal coliforms, heterotrophic bacteria and total coliforms) in the rainwater between March and August.

	May 2012					13 June 2012			
	Date	**FC (CFUs/ 100 mL)**	**TC (CFUs/ 100 mL)**	**HPC (CFUs/ mL)**	**mH_2S strip test**	**FC (CFUs/ 100 mL)**	**TC (CFUs/ 100 mL)**	**HPC (CFUs/ mL)**	**mH_2S strip test**
Eluxolweni Children's Shelter	10 May	5	2063	355	48 hours	26	477000	2543	Negative
Victoria Girls' High School	3 May	> 600	10000	235	56 hours	No water	-	-	-
Continuing Education Centre (RU)	3 May	33	4075	38	48 hours	95	750	>2000	56 hours
Grounds and Gardens (RU)	3 May	24	563	125	48 hours	45.5	2738	73	Negative
Andrew Moyake Tank 1	10 May	29	1963	26	Negative	No water	-	-	-
Andrew Moyake Tank 2	10 May	0	1100	7	48 hours	1	10250	3200	Negative
Samuel Ntsiko Primary School	10 May	51	1430	8840	36 hours	No water	-	--	-
Archie Mbolekwa 1	3 May	762	89000	3670	48 hours	No water	-	-	-
GADRA School for the Blind	10 May	4	5500	2425	36 hours	22.5	19000	1905	72 hours (2 replicates negative)
TEM Mrwetyana Senior Secondary School	10 May	16	23000	1660	36 hours	9	55750	1115	Negative

Table 37: (continued). Average concentrations of indicator bacteria in the rainwater in August.

	August 2012				
	Date	**FC (CFUs/ 100 mL)**	**TC (CFUs/ 100 mL)**	**HPC (CFUs/mL)**	**mH_2S strip test**
Eluxolweni Children's Shelter	2 August	1	456	304	Negative
Victoria Girls' High School	2 August	484	27000	1128	Negative
Continuing Education Centre (RU)	2 August	5	3050	103	Negative
Grounds and Gardens (RU)	2 August	0	2788	2025	Negative
Andrew Moyake Tank 1	2 August	-	-	-	-
Andrew Moyake Tank 2	2 August	0	11000	403	Negative
Samuel Ntsiko Primary School	2 August	0	35	75750	72 hours
Archie Mbolekwa 1	2 August	17	37500	1055	72 hours
GADRA School for the Blind	2 August	9	41750	2000	72 hours
TEM Mrwetyana Senior Secondary School	2 August	0	38000	418	Negative

The pH of the rainwater was evaluated in 2012. It ranged between 5.41 and 8.33. DWAF guidelines state that the pH should fall between 6 and 9 for drinking water, to prevent metal ion solubility or taste disturbances (DWAF, 1996c). This was generated from the raw water pH ranging between 6.5and 8.5 (DWAF, 1996c).Aluminium solubility increases at pH 6 (DWAF, 1996c), which would be problematic for galvanised roofs. The bitter taste at pH 9 and increased ammonium toxicity (DWAF, 1996c) would not be a problem at this range. The pH is occasionally a bit lower than DWAF guidelines probably due to acidic rain and organic fermentation.

Rainwater temperatures ranged between 9 and 18°C, cool enough for bacterial survival, but should be below the optimum temperature for bacterial growth. Bacterial growth within the water cannot be precluded.

5.6.4 Study Parameter Comparison

Overall, rainwater quality varied greatly and was influenced by cleanliness of the roofs, the amount of rain and, most probably, other environmental factors.

The difference between the rainwater quality in 2011 and 2012 was analysed using Statistica 10.

The total and faecal coliform concentrations were significantly higher in 2011 verses 2012 with *p*-values of 0.020113744 for FC, 0.028904556 for TC. The HPC concentrations were not statistically different, with a *p*-value of 0.338316477. Quality seemed especially poor in April and then improved in May, both in 2011 and 2012. But the water quality decreases again, slightly, in June 2012.

From the data above, it can be observed that the Samuel Ntsiko Primary School tank did not have water twice during 2011 and twice during 2012. They had an extra tank installed during 2011 but still seem to be running out of water. The teacher in charge of the tanks says they use the tanks frequently due to a lack of municipal supply, but there is no evidence to substantiate this claim. Another problem was the tap being left often, reported by one teacher at the school. The cleanliness of the gutters is not visible, but, if they are full of dust and soil, this may decrease the amount of water flowing into the tank, which could help explain why, even after rains, there was no water in the tank.

Water quality results from 2010 (Tandlich et al., 2011) were significantly lower than those in 2011 and 2012. The *p*-value for the FC (TC) between 2010 and 2011 was 0.008243 (0.001636), while between 2010 and 2012 was 0.00626 (0.012286).

Bacterial concentration in 2010 had the following ranges: the FC ranged between 0 and 98 CFUs/100 mL while the TC ranged between 0 and 200 CFUs/100 mL. In 2011, the sampling between March and May, as well as August, had FC concentration ranging between 0 and 3900 CFUs/100 mL; the TC ranged between 0 and 30700 CFUs/100 mL and the HPC ranged between 70 and 7870 CFUs/100 mL. No sampling took place during the school holidays of June and July due to inaccessibility of the tanks. Most of the rain fell during this period. In 2012, the 6 month sampling period showed that the FC concentration ranged

between 0 and 1648 CFUs/100 mL; the TC concentration ranged between 0 and 41750 CFUs/100 mL; the HPC concentration ranged between 10 and 75750 CFUs/mL.

The quality fell within the agricultural DWAF guidelines (DWAF, 1996b) for all tanks, except the Eluxolweni Children's Shelter tanks on the 19th of April 2011, as discussed previously.

Samuel Ntsiko Primary School ran out of water in August 2011, March 2012 and June 2012.The tank has been emptied on numerous occasions. The water quality in August 2012 was very good, possibly aided by the emptying of the tank. But this was not the case in April, which had a high bacterial concentration, suggesting the water quality is largely related to the environmental or roof condition at the time. The school used the water for drinking, and although the children would only use it once or twice a day, the FC concentration between 0 and 644 CFUs/100 mL is worrying. The use of this water for gardening would be fine.

Table 38: A summary of the concentration ranges of indicator bacteria (faecal coliforms, heterotrophic bacteria and total coliforms) in 2010 (April, October, September to December), 2011 (March to August) and 2012 (March to August).

	2010	**2011**	**2012**
Sampling months	May, August to December	March to May and August	March to August excluding July
Total coliforms (CFUs/ 100 mL)	0 - 200	0 - 30700	0 - 41750
Faecal coliforms (CFUs/ 100 mL)	0 - 98	0 – 3900	0 - 1648
Heterotrophic bacteria (CFUs/ mL)	Not determined	70 - 7870	10 - 75750

The amount of soil and contamination on the roofs was difficult to establish as not all were visible due to their height. The Eluxolweni Children's Shelter roof never had visible bird droppings on it, although there were plenty of droppings on the tank on the 1st of March 2012. Victoria Girls School had a relatively clean rain runoff area despite the rest of the roof having plenty of lichens and a soily appearance.

In 2012 the Continuing Education Centre roof had twigs and pine needles in its junctions and gutters at all sampling times, except on the 2nd of August 2012. Debris extent differed but was always present. This was demonstrated in Figure 25, under the site description. The Grounds and Gardens roof is flat and not visible from the ground. The roofs on the Andrew Moyake and Samuel Ntsiko Primary Schools look fairly clean, with no trees around to shed leaves. Many lichens are growing on Archie Mbolekwa School's roof.

The roof at the GADRA School for the Blind has lichens growing on it, with leaf litter, and is very soily. Thus, on numerous occasions, the water was actually brown. As this water is only used for watering the garden, the soil and colour of the water are not problems. The roof on TEM Mrwetyana Senior Secondary School has lichens growing on it; otherwise there is no noticeable dirt. The gutters were full of soil and dust on the 2nd of August and the 10th of May 2012. Cleaning these gutters would improve the water quality. The CEC showed a good correlation between the amount of leaf debris and water quality. After the roof and gutters were cleaned, the water quality improved.

The rainwater in Grahamstown varies a lot depending on the weather and the amount of debris on the roofs. With the increased mistrust of tap water and the lack of access to it, people are increasingly turning to rain water for drinking water. Amazingly, one of the reasons proposed by South African and Australian researchers as to why rainwater is not used more is the perception that rainwater is inferior to tap water (Evans et al., 2007; Mwenge Kahinda et al., 2010). Many in Grahamstown prefer using rainwater, as they are concerned about heavy metals in the tap water.

All sites have neither first flush nor filtration systems to improve water quality conditions. First flush systems have been shown to greatly decrease bacterial and dust contamination in rainwater tanks (Egodawatta et al., 2009; Abbasi and Abbasi, 2011; Mendez et al., 2011; Lee et al., 2012). This is something which will need to be addressed after this study. Easy modifications could be undertaken to address this issue.

The educational institution or NGO (Galela Amanzi) which installed the rainwater tanks initiated the microbial rainwater monitoring (Tandlich et al., 2011). Although the NGO has mostly communicated well with the project, they have not initiated any of the suggestions put

forward and are still discussing which filtration or first flush devices to install, despite indicating that this would be done by the beginning of 2012.

The installation of rainwater tanks is as important as their maintenance to prevent contamination of rainwater (Abbott et al., 2007a). Installing the correct gutters can prevent midges and mosquitoes from growing in the tanks (Mwenge Kahinda et al., 2007; Frans, 2011). Many countries also have plumbing laws which need to be adhered to (Chapman et al., 2008a). Necessary tank sizes depend on the rainfall within the area.

In Australia, guidelines and information on rainfall patterns and the tanks sizes required in each region are readily available (Chapman et al., 2008b). The location of the rain tank on the site can prevent problems such as those posed by tree debris entering the tank or by tanks breaking due to subsidence of the area (Chapman et al., 2008b). Preventing light from entering the tank may prevent algal growth, thus placement under eaves may be beneficial (Chapman et al., 2008b). But this was not taken into account in most of the rainwater tank installations in this study. The installation of first flush devices and filters should be considered carefully, as the amount of maintenance required depends on the accessibility and the tendency to block due to debris (Chapman et al., 2008b; Egodawatta et al., 2009; Abbasi and Abbasi, 2011; Mendez et al., 2011; Lee et al., 2012). Using a filtration device would greatly improve the water's quality.

Water from a positive mH_2S test was reinoculated onto agar plates. Isolated colonies from HPC plates and from MacConkey agar plates were identified using API 20E strips. The bacteria identified using API 20E software were: *Enterobacter; Klebsiella oxytoca; Klebsiella pneumoniae spp.; Serratia marcescens.* For more specific identification, additional analysis methods or molecular typing would be needed.

It should also be noted that Gram positive bacteria were not identifiable using this kit. Certain of the microbial colonies could not be identified. *Klebsiella spp.* and *Enterobacte*r *spp.* have previously been found in rainwater in Pretoria (Nevondo and Cloete, 1999) and Singapore (Kaushik and Balasubramanian, 2012). *Serratia spp*. has been isolated in multiple studies in Australia (Coombes et al., 2005; Coombes et al., 2006; Evans, 2007). These bacteria have also been isolated from positive H_2S bottles in Australia (Nair et al., 2000).

The isolated organisms are all Gram negative and part of the Enterobacteriaceae family (Madigan and Martinko, 2006).*Serratia marcescens*is often found in soil, water and insect digestive systems and occasionally in humans (Madigan and Martinko, 2006). It is a human pathogen involved in nosocomial infections, urinary tract infections and wound infections (Acar, 1986; Hejazi and Falkiner, 1997). *Klebsiella* is a Gram negative bacillus associated with opportunistic infections, but not when isolated from water (Allen et al., 2004), and certain strains with pneumonia (Madigan and Martinko, 2006). *Klebsiella* survives and multiplies in polysaccharide rich environments (Huntley et al., 1976) and in many water environments (Allen et al., 2004). *Klebsiella* has been isolated from rainwater in Singapore.

5.7 LIMITATIONS TO THE STUDY

The problem of bacteria overgrowing the plate due to such a high concentration in 2011 was rectified by increasing the dilution of water samples. Membrane filtration (100 mL) and spread plating (100μL) was performed for every sample in 2012.

The rainwater tanks used for the study were not owned or maintained by the researchers. There were thus times when they were inaccessible due to school holidays, as they were in secure (locked) grounds. Occasionally, tanks were emptied, however there was no communication about the maintenance from the caretakers. No evidence of maintenance was observed. Communication between the NGO, tank owners and researchers was not very effective and better communication would drastically improve the maintenance and water quality research. Monitoring bacterial concentrations without knowledge of maintenance is less beneficial than monitoring before and after cleaning has taken place. This would elucidate if cleaning procedures were effective, thereby optimising maintenance.

Gutter cleaning and water treatment was not within the researcher's control. Only the CEC occasionally cleaned their gutters, although the timing was not always communicated. Environmental factors were beyond the researcher's control. The histories of the tanks were limited by the caretaker's knowledge. There was no maintenance plan or records. A maintenance plan and record could provide guidance as to when the tank needed to be cleaned again, and would prevent taps from being left broken for long periods.

Local expertise should be built and strengthened to improve the water quality and user satisfaction from the tanks. Increased knowledge would help the community use the water more efficiently and prevent water shortfalls. The education of students will encourage water conservation and improve water use and sanitation in the wider community. The value of rainwater tanks and satisfaction from them could help alleviate water shortfalls within the Grahamstown area, which increasingly suffers water outages due to pump failures, pipe breaks and other maintenance problems. The use of rainwater to develop gardens would also improve food security for the unemployed within the area.

No first flush systems or filters are currently being used. First flush systems have been reported to increase tank water quality, as they prevent a large proportion of atmospheric dust accumulated on the roof from reaching the tank (Lee et al., 2012).This was not considered by the NGOs installing the tanks, and although the NGOs proposed installing filters, nothing has been done to date.

Communication with Galela Amanzi was not forthcoming due to the communication member. Information about the tanks was limited. Feedback from the researchers to the community had to be undertaken by researchers, as the NGOs were not maintaining the tanks. This brings the information and communication with the schools by the NGOs about their responsibility for maintenance and how to implement it into question.

5.8 CONCLUSIONS

Rainwater tanks are essential in Grahamstown due to water shortages and infrastructural problems. Rural communities benefit from NGO rainwater projects, but there needs to be maintenance and guidance for a while after installation in order to prevent such high bacterial counts. The use of filters and a first-flush device is required to increase water quality. Filters should be installed by the NGO when rain tanks are installed, to prevent the requirement of structural changes to the plumbing, after installation. The lack of first-flush devices or of filters being installed, coupled with maintenance not occurring has led to deterioration of the water quality. This is a cause of concern, as many schools used the water for drinking purposes in rural areas where infrastructure is not available. First-flush devices or filters would make the water more suitable for drinking and make it more likely to meet WHO and DWAF guidelines.

Many rainwater tanks have high concentrations of coliforms. Rainwater tanks in Makana Municipality could only be used for irrigation of crops not eaten raw, preferably as part of a drip irrigation system. Microbial water quality is affected by the tanks' proximity to trees and gutter debris. There is a correlation between the presence of debris and the concentration of indicator bacteria in the rainwater tanks. Regular cleaning of gutters and maintenance is highly advisable if the water is to be used for drinking purposes.

Some of the tanks need to be treated with chlorine or another disinfectant to eliminate the high bacteria concentrations presently in the tanks. Emptying tanks once in a while would be advisable, if the water is not being used continuously. Rainwater needs to be treated through boiling or disinfection before it can be drunk safely. More collaboration between NGOs and local government is required to ensure the safety of rainwater being used for drinking and irrigation, in order to meet the community's needs, especially in rural areas.

Increased education on the use and maintenance of rainwater tanks is required to facilitate willingness to maintain the tanks. An installation certificate to indicate the ownership and the responsibilities of the owners may help to encourage rainwater tank maintenance and care of the tanks.

CHAPTER 6: PUBLIC HEALTH CONCERNS AND DISASTER MANAGEMENT CONTEXT

6.1 Background

Water quality is affected by many different environmental factors, as well as anthropogenic activities (Mallin et al., 2000; Yillia et al., 2007; Ertel et al., 2012). Many of these activities can introduce pathogens and microbes into the water. Pathogens and microbes can cause a public health risk, while the severity of this risk is influenced by the health of the community. Malnutrition, associated with poverty, has been associated with more severe diarrhoea during infection (Connolly, 2005). Poverty can increase the community's vulnerability to disease (El-Masri and Tipple, 2002; Ahern et al., 2005). Immunocompromised people, the elderly and children are more prone to infections than healthy adults (Gerba et al., 1996; Laurent, 2005). Immunocompromised people include people with the Human Immunodeficiency Virus (HIV), who are more susceptible to skin infections and waterborne diseases (Laurent, 2005; Momba et al., 2010). South Africa has a high HIV prevalence (Momba et al., 2010; Chikulo, 2011). The Eastern Cape, KwaZulu-Natal and Gauteng provinces, in South Africa, have high rates of infection (NDOH, 2008; NDOH, 2011). HIV positive patients, in Limpopo, experiencing diarrhoea were reported to excrete one of the following bacteria: *Campylobacter jejuni*; *Campylobacter coli*; *Plesiomonas shigelloides*; *Aeromonas species*; *Shigella spp.*; *Salmonella spp.*; *Escherichia coli*; *Yersinia enterocolitica* and *Vibrio cholerae* (Obi and Bessong, 2002).

The problem of diarrhoea in rural informal settlements is compounded by the lack of proper sanitation facilities (Sanders and Chopra, 2006; Lewin et al., 2007; Richards et al., 2007). High influxes of people within a small area with minimal sanitation, due to refugee status and flooding, can often compromise water sources, leading to public health problems (Waring and Brown, 2005). Runoff of greywater, sewage or storm water from urban and rural areas can increase the bacterial concentrations in rivers. The introduction of *Vibrio cholerae* into rivers can cause a major outbreak, as many rural people do not have alternate water supplies (Archer et al., 2009). Sustainable solutions are thus required to prevent outbreaks (Archer et al., 2009).

6.2 Disaster Management

The Eastern Cape is a poor province in South Africa (Momba et al., 2006b). It is also a "homeland" area for many of the black population (Tempelhoff et al., 2009). Informal settlements are found on the outskirts of towns and cities due to increased urbanisation caused by historical and employment factors (Tempelhoff et al., 2009; PCCOGTA, 2011a). Shacks in these informal settlements are not designed to withstand storms or floods and can be built vulnerable places (Tempelhoff et al., 2009). Informal settlements are not planned and thus are often in low lying areas, which increases the risk of disaster and provides a disaster management challenge (Vermaak and van Niekerk, 2004).

The Cacadu district within the Eastern Cape is prone to droughts, which often renders water quality of boreholes and other supplies undrinkable (IDP, 2008). Makana is a municipal area within the Cacadu district, which has a low level of water quality staffing compared to the levels recommended by the Department of Water Affairs and Forestry (DWAF)(IDP, 2008). Access to water and sanitation within the area has increased since 2004, but the ever increasing demand for water is concerning, as it could lead to more frequent drought conditions (IDP, 2008). But the Eastern Cape (PGE, 2009) is not the only area to experience these problems, as droughts and floods have also been reported in areas such as the Western Cape (Tempelhoff et al., 2009), KwaZulu Natal, Gauteng and the North West provinces (Chikulo, 2011; Zuma et al., 2012). Droughts are due to insufficient rain or an increase in the population of an area with insufficient infrastructure (PGE, 2009; Tempelhoff et al., 2009). Flooding can be caused by small or major rivers swelling, bursting their banks and catchment areas being filled with water without adequate drainage (Douglas et al., 2008). Poor storm water infrastructure (Tempelhoff et al., 2009) and insufficient drainage or solid waste management could also cause flooding (Satterthwaite et al., 2007). Flooding could possibly be a problem in the Makana area as there is a backlog of storm water infrastructure (IDP, 2008).

Drainage systems in South Africa were not designed for continuous or frequent floods (Chikulo, 2011). Climate change could lead to more disasters occurring and increase the risk of people's homes being flooded (Chikulo, 2011). Floods often disrupt infrastructure, which can compromise water quality and lead to poor sanitation habits, as water becomes less available (Euripidou and Murray, 2004; Waring and Brown, 2005). Water washed into storm

water drains from settlement areas and urban areas could potentially carry many pathogens. Other possible problems are household contamination and chemical contamination of rivers from fertilisers (Euripidou and Murray, 2004). Flooding in the house or surrounding areas has been linked to increased diarrhoea infections (Ahern et al., 2005).

Poor infrastructure can lead to stagnant water, and where inadequate sanitation and greywater disposal occurs, there is an increased risk of waterborne outbreaks (Douglas et al., 2008; Chikulo, 2011). Flood water can carry increased pathogen concentrations as well as insect borne infections (Few, 2003). The leading causes of diarrhoea are due to *Vibrio Cholerae*, *Escherichia coli* (*E. coli*), *Shigella* (Waring and Brown, 2005), *Listeria spp.*, *Campylobacter jejuni* and *Klebsiella pneumonia*. Flooding in Bangladesh has shown an increase in infections of enterotoxigenic *E. coli, Shigella*, *Salmonella* species but mostly cholera (Mitchell and Gu, 2010). *Vibrio cholerae* is a global problem under disaster situations, especially in endemic areas where contaminated water is used (Waring and Brown, 2005; WHO, 2005; Luyt et al., 2011b). There are many different pathogens and opportunistic bacteria in water bodies (Louw, 2007).

Being a water-stressed region, South Africa has experienced water shortages and occasional droughts (Jury and Levey, 1993; Otieno and Ochieng, 2004; Reason et al., 2005). Disaster management policies need to be in place to prevent outbreaks of cholera and other pathogens. A major concern is the development of a cholera outbreak, especially in the Limpopo, Mpumalanga and KwaZulu Natal areas.

Disasters affect the water quality, quantity and its availability. Flooding is a major natural disaster in the world (McMichael et al., 2006). It can in turn lead to public health problems other than those expected by the disaster itself (Luyt et al., 2011b). The Republic of South Africa's Constitution (Act 108 of 1996) and Bill of Rights (Section 24) advocates that the environment should not harm the citizens' health and should be preserved for future generations, by prevention of pollution and degradation using legislation, while promoting conservation (Tempelhoff et al., 2009; Chikulo, 2011).

Disaster management in South Africa is governed by the White Paper on Disaster Management (WPDM, 1999). This has led to risk reduction programmes and an improvement in coordination and management of disaster efforts.

The Disaster Management Act No 57 of 2002 (DMA, 2002) was established with an Intergovernmental Committee on Disaster Management (IGCDM) (DMA, 2002). This group includes the national ministers of water and environmental affairs, health, defence, finance, the presidency, justice and constitutional development, education, police, members of the executive council in charge at each province and the South African Local Government Association (SALGA) (Zuma et al., 2012).

The disaster management system in South Africa is a combination of bottom-up and top-down approaches (Zuma et al., 2012). Provinces need to develop an integrated development plan and a disaster management advisory form (DAF) to help with disaster management and prevent duplication (Tempelhoff et al., 2009). The South African Government gazetted (Government Gazette Notice No. 1689 of 2005) (DMR, 2005) guidelines for development of volunteer units to aid with disaster management and alleviate existing skills shortages (Zuma et al., 2012). Volunteers need to be over 16 years of age and have filled out and submitted the necessary forms (DMR, 2005). The municipality is responsible for developing a data base with the relevant information and issuing identification documents, protective clothing, any other equipment needed for the task and training (DMR, 2005). The Eastern Cape had 8000 volunteers registered in 2004 (Vermaak and van Niekerk, 2004).

The South African Disaster Management Act no. 57 of 2002 does not estimate the level of environmental degradation or how using continuous assessments would reduce risk (PCCOGTA, 2011b; Zuma et al., 2012). People are building settlements below flood lines or in unsafe areas. This poses a risk and should be solved by forced eviction, using the Prevention of Illegal Eviction From and Unlawful Occupation of Land Act No. 19 of 1998 (DHS, 1998). Although there are currently municipal campaigns in certain areas, more national intervention is required to decrease risks of household flooding and shack-building on flood plains (Vermaak and van Niekerk, 2004; Zuma et al., 2012). The priority should be disaster risk reduction and not merely management once a disaster occurs (Vermaak and van Niekerk, 2004). One application of this disaster management plan in South Africa was the 2000-2001 cholera outbreak in KwaZulu-Natal (Hemson and Dube, 2004).

Climate change will affect the rainfall and increase temperatures within regions. This could possibly lead to higher multiplication rates of pathogens in rivers (Louw, 2007), making the multiplication of faecal coliforms in water cause for concern. Higher temperatures can also

lead to increased diarrhoeal infections (McMichael et al., 2006). It is therefore helpful to understand the replication rates in rivers under different climatic conditions. Bacterial survival and growth rates differ at various temperatures (Hazen and Toranzos, 1990). *E. coli,* for instance, is the best faecal indicator bacteria, although some questions have been raised in tropical environments, where *E. coli* occur without faecal inputs (Phillip et al., 2009), implying that global warming may necessitate identifying a different indicator.
The rainfall around and temperatures of rivers have been associated with increased bacterial counts (Bezuidenhout et al., 2002). It should also be noted that as water temperature increases, residual chlorine levels decrease, increasing the risk of bacterial growth and resultant gastrointestinal infections (Sisti et al., 1998).

Bacteria used for tracking or indicating faecal pollution are excreted from human and animal faeces (Gerba, 2000). The indicator bacteria used to identify water quality are total coliforms, faecal coliforms, *Escherichia coli (E. coli)* and heterotrophic bacteria (DWAF, 1996c). But the enterococci *spp*. have also been used and are also closely related to health risks (Barrell et al., 2000). Indicator bacteria do not identify the source of the bacterial contamination.

Identifying the source of faecal pollution assists in characterizing the health risks associated with contact with or drinking the water (Jagals and Grabow, 1996b; Scott et al., 2002; Field and Samadpour, 2007; Santo Domingo et al., 2007; Soller et al., 2010).

Human faecal pollution is considered more likely to carry human pathogens than animal pollution (Curtis et al., 2000). Bifidobacteria, especially the human sorbitol utilising bifidobacteria, are associated with human faecal pollution (Mitsuoka, 1990; Germond et al., 2002; Lynch et al., 2002; Ballesté and Blanch, 2011). Bifidobacteria have short survival times in water environments (Ravenscroft, 2000; Lamendella et al., 2008; Bonjoch et al., 2009; Ottoson, 2009) and are thus associated with recent faecal inputs. Understanding the survival rate in water in South Africa will help identify how recently the faecal pollution occurred. When bifidobacteria concentrations are high, water should be considered unsafe, as it may contain human pathogens. Bifidobacteria themselves are not pathogens, and are beneficial to humans (Borriello et al., 2003; Wilson, 2005).

When diarrhoea cases emerge, prompt action must be taken to prevent an epidemic from breaking out. Diarrhoea is one of the major causes of morbidity and mortality (Waring and Brown, 2005). Diarrhoeal diseases are transmitted via multiple routes, with hygiene and sanitation often being direct causes (Louw, 2007). The impact of flooding on health is difficult to evaluate (Few, 2003). Disruption of safe water supplies may also indirectly affect health (Few, 2003). This could result in people collecting water from various sources other than tap water distribution systems. The safety of the tap water distribution system is regulated by municipalities, water boards and government and should be safe.
But if disruptions to the drinking water distribution systems occur, healthcare workers and disaster management personnel should advise people of alternative safe water sources (Connolly, 2005). Correct extraction methods need to be explained to avoid contamination of these sources (Connolly, 2005; WHO, 2005; Louw, 2007). Consultation with the community is important, as discrete groups of people would preferentially use specific water sources for different activities, e.g. drinking water verses washing water (Louw, 2007).

A multidisciplinary approach is needed to prevent health problems (Waring and Brown, 2005). Public health teams need an epidemiologist and environmental health specialist as members (Connolly, 2005). The water supplied or used needs to be assessed for quality and adequate treatment needs to be identified (Connolly, 2005). Water needs to be distributed through the most appropriate source, ensuring the availability of sufficiently good quality water (Connolly, 2005). In emergency situations, the microbial/biological quality of water is more important than the chemical quality (Connolly, 2005). The World Health Organisation (WHO) recommends faecal coliform (FC) concentrations should be below 10 colony forming units (CFUs)/100 mL, while it should ideally be below 0 CFUs/100 mL (DWAF, 1996c; Connolly, 2005). pH should be between 6.5 and 8.5, with the total dissolved solids (TDS) being below 500ppm and turbidity below 5 NTU, with no taste or odour associated with it (Connolly, 2005). Water needs to be tested daily, preferably three times a day, when waterborne outbreaks occur. Free chlorine levels are important to prevent microbial re-growth (Connolly, 2005). Chlorine is the most effective disinfectant in emergency conditions, with desired levels of 0.2 to 0.5 mg/L (Connolly, 2005). Water quality is improved if water is stored in a closed container for at least two days, as the pathogens die and settle to the bottom (Connolly, 2005). High turbidity requires aluminium sulphate as a flocculent, as settling will not occur rapidly enough on its own (Connolly, 2005). Aluminium levels should not exceed WHO guidelines (Connolly, 2005). Collection of water from ground and other water sources

should not occur within 30 m of defecation points and preferably 50 meters away (Connolly, 2005). But as the distance from settlements increases, so the likelihood of people using the area or water source decreases (Connolly, 2005). There should not be sanitation points up stream of camps or defecation taking place near rivers and streams (Connolly, 2005). Hygiene education is important to prevent waterborne disease outbreaks (Connolly, 2005). Simple processes like regularly washing hands before preparing food and using safe water to rinse it can drastically decrease diarrhoeal diseases (Ligon, 2006). Water sources must also be protected from animal access.

The UNHCR (United Nations High Commission for Refugees) states that a tap should be available for every 200-250 people, otherwise people will use other sources rather than waiting (Connolly, 2005). It is all very well to provide safe drinking water, but if the buckets and containers used to transport and store the water are unsafe, the advantages are nullified (Connolly, 2005). This was noticed in other studies during normal use periods (Momba and Kaleni, 2002; Wright et al., 2004; Gundry et al., 2006). It is important to prevent stagnant water near homes, as this can be a vector for disease (Connolly, 2005). Animal faeces are less likely to carry human pathogens than human faeces, which are more dangerous (Connolly, 2005). Source tracking is thus of the essence.

Seasons and environmental factors affect the risk of diarrhoeal pathogens being problematic (Louw, 2007). Rainfall can affect microbial concentrations in drinking water (Louw, 2007). The Milwaukee, Wisconsin, 1993 incident of *Cryptosporidium* contamination led to many people falling ill and immunocompromised people died from this problem which was attributed to heavy rainfall and runoff, as well as to treatment system malfunction (Mac Kenzie et al., 1994). Kirtemann et al (2002) found microbial concentrations of *E. coli*, coliforms, faecal streptococci and *Clostridium perfringens* increase significantly during runoff events after heavy rain. The pH, turbidity and nitrate concentration differed significantly to normal conditions, while cryptosporidium and Gardia contamination increased (Kistemann et al., 2002). Increased turbidity of raw water requires more treatment as suspended particles protect microbes from disinfection (LeChevallier et al., 1988; Thompson et al., 2003; Momba et al., 2004). Studies have shown an increase in rotavirus (Fun et al., 1991), typhoid and paratyphoid fever (Vollaard Am and et al., 2004) and other non-specific diarrhoeas after heavy rainfalls (Kouadio et al., 2011).

Droughts can also lead to increases in microbial concentrations due to reduced amounts of water, while water scarcity increases the use of this poorer-quality river water (Louw, 2007). Flooding can overwhelm wastewater systems and increase the risk of disease (Louw, 2007).

So while water is needed for hygiene practices, it can also spread disease (Louw, 2007). The Cape Winelands can be used as an illustration of poor water quality causing diarrhoeal diseases (Louw, 2007). *E. coli* is often used as an indicator of water pollution by faecal sources or resulting from inadequate treatment (Barnes, 2003). The Berg River shows high pollution levels and is often unsuitable for drinking purposes (Louw, 2007; Paulse et al., 2007). The problem of sewage treatment works lacking capacity is only further exacerbated by the continual influx of squatters.

Tap and distribution water are often monitored using heterotrophic bacteria. The heterotrophic bacteria group (HPC) includes *Aeromonas spp., Klebsiella spp., Pseudomonas spp.,* which are opportunistic pathogens (Allen et al., 2004; Edberg and Allen, 2004). Opportunistic pathogens are not clinically correlated with diseases, but have potential to cause disease in immunocompromised individuals or in those suffering from infection (Allen et al., 2004). HPC concentrations above 100 CFUs/100 mL can inhibit coliform enumeration of lactose media, and thus are often used as a guideline level (Allen et al., 2004). The infectious dose of *Pseudomonas aeruginosa* is above 10^8 CFUs, while that of *Aeromonas hydrophilia* is greater than 10^{10} CFUs (Allen et al., 2004). The HPC in tap water is not explicitly correlated with disease, but is still a useful method to monitor water quality (Allen et al., 2004). Studies dosing immunocompromised mice have not shown a link between HPC concentrations and disease (Edberg and Allen, 2004).

Using alternate, non-improved water sources, such as rivers, may be detrimental and the use of improved sources should thus be encouraged. Survival rates of bacteria help identify the potential duration of outbreaks, as long as the bacteria are not continuously reintroduced. Bifidobacteria are good indicators of recent faecal pollution and may be helpful, in conjunction with other indicators, to identify whether faecal pollution is continuing. The die-off rates of faecal coliforms more closely relate to enteric pathogen survival than to the survival rates of bifidobacteria. Die- off rates for *Salmonella typhimurium* in the water column have been reported as 2.21 CFUs per day (Karim et al., 2004). Reported survival rates of faecal coliforms and *E. coli* vary depending on the environment (Carrillo et al., 1985;

Barcina et al., 1986; Anderson et al., 2005). This study has shown an average die-off of 1.3 CFUs per day. The average survival rate of bifidobacteria in this study is 2.39 CFUs per day. Ottoson (2009) found a survival rate of 3.9 CFUs/day at 22°C and 1.4 CFUs/day at 4°C, higher than that of *Salmonella.*

The concentration of HPC in tap water is related to its treatment and the ability of the environmental bacteria population to grow in low nutrient media (Edberg and Allen, 2004). Just as bottled water will have a higher HPC level than tap water (Edberg and Allen, 2004), rainwater will also display fairly high levels. The South African guidelines state that there is a negligible risk of infection below 100 CFUs/mL, while there is a major risk at above 1000, as there is a major treatment or re-growth in the distribution (DWAF, 1996c).

The highest HPC value seen in tap water during the sampling in Grahamstown was 500 CFUs/mL, which would not indicate acceptable treatment, although it would not be a disease risk, either. HPC values of rainwater are more concerning, as they are higher than 1000 CFUs/mL, with maximum concentrations reaching 7870 in 2011 and, in one isolated case, 75750 CFUs/mL (showing a high chance of pathogens being present). Food HPC concentrations can be as high as 10000 CFUs/mL without any disease being associated with its consumption (Allen et al., 2004). But HPC concentrations are not used as indicators for environmental water use such as agriculture.

6.3 Public Health Concerns

Public health concerns about drinking rainwater range from non-existent, when shown that the water meets drinking water guidelines in their country, to high, when gastrointestinal diseases have been reported (Evans et al., 2006; Lye, 2009). Many countries use rainwater for non-potable uses such as agricultural irrigation, toilet flushing and clothes washing (Lye, 2009). Rooftop rainwater quality is rarely acceptable for potable use (Lye, 2009). Pathogens detected include *Pseudomonas, Salmonella, Aeromonas, Cryptosporidium, Gardia spp. Legionella pneumophilia* and *E. coli* O157:H7 (Fewtrell and Kay, 2007). A correlation between Aeromonas and gastrointestinal symptoms exists (Lye, 2009). Lye reported in a Danish study by Albrechtsen H-J. (2002) that rainwater microbes are different from those in the distribution system (Lye, 2002; Lye, 2009). There have been at least 9 documented disease outbreaks due to rainwater until 2009 (Heyworth et al., 2006; Lye, 2009).

Epidemiological studies to correlate the bacteria in rainwater with disease are needed (Lye, 2009).

This study identified the following opportunistic pathogens: *Enterobacter; Klebsiella oxytoca; Klebsiella pneumoniae spp.; Serratia marcescens.* The minimum number of bacteria needed to cause disease is not known for these opportunistic pathogens, but it is believed to be high. *Klebsiella* has only been associated with disease in hospital settings.

Pathogenic infectious doses range depending on the bacteria. Values vary slightly, depending on the dose-response model used to identify the infectious doses. *Campylobacter* is reported to be at 800 CFUs for a 50% infection rate, while at 10^7 there seemed to be a 100% infection and disease rate (Teunis et al., 1996). *Escherichia coli* needed 10^6 CFUs for a high percentage risk of infection, although the number suffering from disease was low (Teunis et al., 1996).
Shigella needs approximately 10 CFUs, while *Vibrio Cholerae* requires approximately 10^6 bacteria (Mara and Horan, 2003). It should be noted that increases in pH due to antacids decrease the number of pathogens needed for infection. There is no data on the infective dose of faecal coliforms or total coliforms, but they are still used due to their ease of culturing and indication of treatment (Quiroz, 1999).

Monitoring river and drinking water is important to prevent disease outbreaks and minimise the risk of pathogen ingestion. Microbe concentrations and the quantity of water ingested affect the risk of outbreaks or diseases occurring.

The total coliforms were used to evaluate the hygienic quality of water and the efficiency of treatment, as their presence is indicative of re-growth or contamination (DWAF, 1996c). The total coliform group includes: *Escherichia, Citrobacter, Enterobacter, Klebsiella, Serratia* and *Rahnella* (DWAF, 1996c) and potential pathogens such as *Salmonella spp., Shigella spp., Vibrio cholerae, Campylobacter jejuni, Campylobacter coli, Yersinia enterocolitica* and pathogenic *E. coli* (DWAF, 1996c). It could potentially indicate the presence of pathogens. Not all the bacterial groups are associated with faecal origins, though, (DWAF, 1996c) and soil bacteria could contribute significantly to these concentrations. Their enumeration with faecal coliforms is more useful. DWAF guidelines state that there is insignificant risk of

microbial infection below 5 CFUs/100 mL, and significant risks are associated with concentrations greater than 100 CFUs/100 mL (DWAF, 1996c).
Tap water samples occasionally exceeded 5 CFUs/100 mL, but never exceeded 100 CFUs/ 100 mL. It was thus not likely to cause any microbial infection or disease, according to this indicator. Rainwater tanks were below 100 CFUs/100 mL only twice in 2011 and 2012. This water would therefore not be suitable for drinking water requirements without treatment. Total coliforms are not usually assessed in fresh water samples, as soil bacteria favour bacterial growth and are not indicative of the actual risk.

The faecal coliforms (FC) are not well correlated with disease outbreaks, but their concentration and the quantity of water ingested play a role (DWAF, 1996c). A United States meta-analysis study states that *E. coli* is the best predictor of gastrointestinal illness risk in freshwater environments, while enterococci is better suited to marine environments (Wade et al., 2003). It is stated that, in temperate environments, an increase in *E. coli* concentration by $\log_{10}$ causes a relative risk of gastrointestinal infection increase of 2.12 (Wade et al., 2003). A study of the FC is used to evaluate rivers, drinking water, treated water, irrigation, recreational and other water bodies (DWAF, 1996c). *E. coli* is used as an indicator of warm blooded animal pollution or human pollution (DWAF, 1996c). Some FC tests enumerate *Klebsiella spp.,* which are often from sources other than faecal pollution (DWAF, 1996c).

The faecal coliform group includes *E. coli, Klebsiella spp., Enterobacter spp.* and *Citrobacter spp.* (DWAF, 1996c). It is used as an indicator of the possible presence of *Salmonella spp., Shigella spp. Vibrio cholerae, Campylobacter spp.* and pathogenic *E. coli* (DWAF, 1996c). The main coliform in human faeces is *E. coli* (DWAF, 1996c). DWAF guidelines for domestic use state that 0 CFUs should be present in 100 mL of water. Concentrations greater than 20 CFUs/mL produce significant risks of infectious diseases occurring.

Rainwater FC concentrations, which were mostly above 20 CFUs/100 mL, posed a significant risk for diarrhoeal diseases, especially if used as a continuous source of drinking water. Rainwater tank FC concentrations were only below 20 CFUs/100 mL 29 % of the time in 2011 and 41 % of the time in 2012. Rainwater thus had a lower public health risk in 2012 than 2011. Tap water was only above 0 CFUs/ 100 mL on one occasion and thus, from this point of view, does not pose a public health risk. The river water, however, falls under the

recreational guidelines, where readings below 130 CFUs/ 100 mL of *E. coli* are considered safe; greater than 400 *E. coli* is very risky. 25% of the samples analysed had concentrations far higher than 400 CFUs/100mL. Many of the concentrations in these rivers rendered the water unsuitable for recreation purposes, despite the fact that children play in them.

Survival data can be used to identify the length of time required before bacterial levels reach acceptable levels from the time of a faecal pollution incident. This would help reduce the risk of microbial infections from recreational activities or river or beach contact after a run-off event. Survival data can be transformed into mortality data and the time required can then be calculated using Equation 9 (Harhcegani and Cornish, 2003).

Equation 9: $\log_{10}\left(\frac{N_t}{N_0}\right) = -kT$ **(Harhcegani and Cornish, 2003)**

The FC survival data transforms to a mortality rate of 0.291. The time taken from a FC concentration of 16000 CFUs/100 mL to reach 150 CFUs/ 100 mL, a safe level specified by the guidelines for recreational water use, would be 7 days. The ability to predict the time for microbial levels to subside help with risk evaluation and decrease the number of samples required over that period to determine when the water is safe for recreational use. The number of days could serve as a guideline, as differences in temperature or chemical composition, as well as bacterial predators, may reduce or increase the survival rate of bacteria.

When a storm or flood occurs, the water can be sampled and the timeframe to avoid use of the river water estimated and published, informing the community of potential risks. EPA guidelines for recreation has a statistical threshold value of 410 CFUs/ 100 mL for *E. coli* and 30 CFUs/ 100 mL for enterococci (Environmental Protection Agency, 2012). These values are used for closing beaches in the United States of America, but South Africa neither monitors beach water regularly nor closes beaches due to high bacterial concentrations. The ability to monitor and prevent public health risks could decrease the number of work days lost due to diarrhoeal diseases.

Low cost tools for bacteria presence identification can help communities identify if the water is safe to drink during drought periods or after water interruptions. This may help alleviate

fears caused by the brown colour of the water or the smell of chlorine. It could also be used to identify when water should be boiled and when rainwater tanks need to be cleaned. The H_2S strip test can be used to indicate high bacterial concentrations and as a training tool. The H_2S strip test allows people to visually see the result of microorganisms and thus understand why sanitation is important. This would help them develop better sanitation habits.

6.4 Conclusions

Using rainwater harvesting during water distribution disruptions is better than using river water and is an improved source (WHO and UNICEF, 2010b). It prevents people from having to walk long distances for water. But this water probably needs some form of treatment such as chlorination or boiling before ingestion.

The bacterial concentrations in this study seem to fall within prescribed agricultural use ranges and not in acceptable drinking water quality ranges, without treatment, thus it would be most helpful for irrigation during drought period.

Using river water is not satisfactory for drinking purposes, although many rural people drinking it seem immune to many of the bacteria present, as they rarely report gastrointestinal disease.

6.5 Suggestion for Future Work

Future studies could include the development of a bifidobacteria media which could differentiate between and identify the different groups while eliminating Gram positive bacteria.

The development of an agent to identify *E. coli* in the H_2S strip test would increase its specificity and, in so doing its usefulness. Developing a low cost method to identify faecal pollution in tropical or subtropical environments which does not grow like *E. coli* could help improve risk assessment.

Developing skills in a resource-stressed environment and development of a community monitoring programme would help decrease skills shortages and prevent rivers from not being monitored. The development of a standardised media used to analyse bifidobacteria

with help with comparison between different geographic region studies. The use of multiple laboratories in studies may help identify the robustness of the method and validity under different climatic conditions.

REFERENCES

(EPA), U. S. E. P. A. 1978. Sulfate by Turbidity. Official Name: Sulfate (Turbidimetric). EPA.

(PCCOGTA), P. C. O. C. G. A. T. A. 2011a. Flood Disaster Management; Local Government Municipal Systems Amendment Bill: Departmental Briefing. Minutes from the Committee Meeting held on 25 January 2011. *In:* AFRICA, P. O. S. (ed.). Cape Town, South Africa.

(PCCOGTA), P. C. O. C. G. A. T. A. 2011b. Flooding Disaster Management: December 2010/ January 2011: Update by Deputy Minister of Cooperative Governance. Minutes from the Committee Meeting held on 28 February 2011. *In:* AFRICA, P. O. S. (ed.). Cape Town, South Africa.

ABBASI, T. & ABBASI, S. A. 2011. Sources of Pollution in Rooftop Rainwater Harvesting Systems and Their Control. *Critical Reviews in Environmental Science and Technology,* 41**,** 2097-2167.

ABBOTT, S. 1997. *Klebsiella, Enterobacter, Citrobacter*, and *Serratia*. *In:* MURRAY, P., BARON, E. J., PFALLER, M., TENOVER, F. & YOLKEN, R. (eds.) *Manual of Clinical Microbiology.* Washington, DC, USA: American Society for Microbiology.

ABBOTT, S., CAUGHLEY, B. & DOUWES, J. The Microbiological Quality of Roof-Collected Rainwater of Private Dwellings in New Zealand. Rainwater and Urban Design Conference, 2007a Sydney, Australia. 23.

ABBOTT, S. E., CAUGHLEY, B. P., WARD, A., GOWAN, G. & ASHWORTH, J. An Evaluation of Measures for Improving the Quality of Roof-Collected Rainwater. 13th International Rainwater Catchment Systems Conference, 23rd August 2007 2007b Sheraton on the Park, Sydney, Australia.

ABDULLA, F. A. & AL-SHAREEF, A. W. 2009. Roof Rainwater Harvesting Systems for Household Water Supply in Jordan. *Desalination,* 243**,** 195-207.

ACAR, J. F. 1986. *Serratia marcescens* Infections. *Infection Control,* 7**,** 273-278.

ADAMS, M. R. & MOSS, M. O. 2008. Food Microbiology. 577.

ADENIYI, I. F. & OLABANJI, I. O. 2005. The Physico-Chemical and Bacteriological Quality of Rainwater Collected over Different Roofing Materials in Ile-Ife, Southwestern Nigeria. *Chemistry and Ecology,* 21**,** 149-166.

AHERN, M., KOVATS, R. S., WILKINSON, P., FEW, R. & MATTHIES, F. 2005. Global Health Impacts of Floods: Epidemiologic Evidence. *Epidemiologic Reviews,* 27**,** 36-46.

AHMED, W. 2007. Limitations of Library-Dependent Microbial Source Tracking Methods. *Water: Journal of the Australian Water Association,* 34**,** 39-43.

AHMED, W., GOONETILLELCE, A. & GARDNER, T. 2008. Alternative Indicators for Detection and Quantification of Faecal Pollution. *Water,* 35**,** 59-65.

AHMED, W., HODGERS, L., MASTERS, N., SIDHU, J. P. S., KATOULI, M. & TOZE, S. 2011. Occurrence of Intestinal and Extraintestinal Virulence Genes in *Escherichia coli* Isolates from Rainwater Tanks in Southeast Queensland, Australia. *Applied and Environmental Microbiology,* 77**,** 7394-7400.

AHMED, W., STEWART, J., GARDNER, T., POWELL, D., BROOKS, P., SULLIVAN, D. & TINDALE, N. 2007. Sourcing Faecal Pollution: A Combination of Library-Dependent and Library-Independent Methods to Identify Human Faecal Pollution in Non-Sewered Catchments. *Water Research,* 41**,** 3771-3779.

AHMED, W., VIERITZ, A., GARDNER, T. & GOONETILLEKE, A. 2009. Microbial Risks from Rainwater Tanks in South East Queensland. *Water,* 36**,** 80.

ALLEN, M. J., EDBERG, S. C. & REASONER, D. J. 2004. Heterotrophic Plate Count Bacteria--What is Their Significance in Drinking Water? *International Journal of Food Microbiology,* 92**,** 265-274.

ALLSOP, K. & STICKLER, D. J. 1984. The Enumeration of Bacteroides fragilis Group Organisms from Sewage and Natural Waters. *Journal of Applied Bacteriology,* 56**,** 15-24.

ALLSOP, K. & STICKLER, D. J. 1985. An Assessment of Bacteroides fragilis Group Organisms as Indicators of Human Faecal Pollution. *Journal of Applied Bacteriology,* 58**,** 95-99.

ALLWOOD, P., MALIK, Y., MAHERCHANDANI, S., HEDBERG, C. & GOYAL, S. 2005. Effect of Temperature on the Survival of F-Specific RNA Coliphage, Feline Calicivirus, and *Escherichia coli* in Chlorinated Water. *International Journal of Environmental Research and Public Health,* 2**,** 442-446.

ALLWOOD, P. B., MALIK, Y. S., HEDBERG, C. W. & GOYAL, S. M. 2003. Survival of F-Specific RNA Coliphage, Feline Calicivirus, and *Escherichia coli* in Water: a Comparative Study. *Applied and Environmental Microbiology,* 69**,** 5707-5710.

AMIN, M. T. & HAN, M. Probable Sources of Microbial Contamination of Stored Rainwater and Its Remediation. 3rd RWHM Workshop, IWA World Water Congress, 8 Nov 2008 Vienna. IWA (International Water Association).

AMIN, M. T. & HAN, M. Y. 2009. Roof-harvested Rainwater for Potable Purposes: Application of Solar Collector Disinfection (SOCO-DIS). *Water Research,* 43**,** 5225-5235.

AMIN, T. & HAN, M. 2011. Domestic Rainwater Harvesting as an Adaptation Measure to Climate Change in South Africa. *Australian Journal of Basic and Applied Sciences,* 5**,** 1804-1813.

AMMOR, M. S., BELÉN FLÓREZ, A. & MAYO, B. 2007. Antibiotic Resistance in Non-enterococcal Lactic Acid Bacteria and Bifidobacteria. *Food Microbiology,* 24**,** 559-570.

AMYES, S. G. B. 2007. Enterococci and Streptococci. *International Journal of Antimicrobial Agents,* 29, Supplement 3**,** S43-S52.

ANDERSON, B. D., GORLEY, R. N. & CLARKE, K. R. 2008. *PERMANOVA + for PRIMER: Guide to Software and Statistical Methods,* Plymouth, United Kingdom, PRIMER-E Ltd.

ANDERSON, K. L., WHITLOCK, J. E. & HARWOOD, V. J. 2005. Persistence and Differential Survival of Fecal Indicator Bacteria in Subtropical Waters and Sediments. *Applied and Environmental Microbiology,* 71**,** 3041-3048.

ANDERSON, M. A., WHITLOCK, J. E. & HARWOOD, V. J. 2006. Diversity and Distribution of *Escherichia coli* Genotypes and Antibiotic Resistance Phenotypes in Feces of Humans, Cattle and Horses. *Applied and Environmental Microbiology,* 72**,** 6914-6922.

ANDREOZZI, R., RAFFAELE, M. & NICKLAS, P. 2003. Pharmaceuticals in STP Effluents and Their Solar Photodegradation in Aquatic Environment. *Chemosphere,* 50**,** 1319-1330.

APHA 1992. Standard Methods for the Examination of Water and Wastewater. *In:* GREENBERG, A. E., CLESCERT, L. S. & EATON, A. D. (eds.) *Standard Methods for the Examination of Water and Wastewater.* 18th ed. Maryland Baltimore: Victor Graphics Inc.

APHA, A. P. H. A., AMERICAN WATER WORKS ASSOCIATION, WATER ENVIRONMENTAL FEDERATION 1998. *Standard Methods for the Examination of Water and Wastewater,* APHA Washington (DC).

ARANA, I., SECO, C., EPELDE, K., MUELA, A., FERNÃ¡NDEZ-ASTORGA, A. & BARCINA, I. 2004. Relationships Between *Escherichia coli* Cells and the Surrounding Medium During Survival Processes. *Antonie van Leeuwenhoek,* 86**,** 189-199.

ARCHER, B. N., CENGIMBO, A., DE JONG, G. M., KEDDY, K. H., SMITH, A. M., SOOKA, A., NTSHOE, G. & BLUMBERG, L. 2009. Cholera Outbreak in South Africa: Preliminary Descriptive Epidemiology on Laboratory-Confirmed Cases, 15 November 2008 To 30 April 2009. *Communicable Diseases Surveillance Bulletin,* 7**,** 3-8.

ARJUN, N., FOXON, K., RODDA, R., SMITH, M. & BUCKLEY, C. Characterisation of Wastewater Produced by Developing Communities. WISA 2006 Biennial Conference and Exhibition May 21st - 25th 2006 Durban, South Africa.

ARMSTRONG, M. & CAREY, M. 1982. The Hydrophobic-Hydrophilic Balance of Bile Salts. Inverse Correlation between Reverse-Phase High Performance Liquid Chromatographic Mobilities and Micellar Cholesterol-Solubilizing Capacities. *Journal of Lipid Research,* 23**,** 70.

ATLAS, R. M. 2010. *Handbook of Microbiological Media,* Washington, D.C., CRC Press/Taylor and Francis,.

AU, J., BAGCHI, P., CHEN, B., MARTINEZ, R., DUDLEY, S. A. & SORGER, G. J. 2000. Methodology for Public Monitoring of Total Coliforms, *Escherichia coli* and Toxicity in Waterways by Canadian High School Students. *Journal of Environmental Management,* 58**,** 213-230.

AUBURN UNIVERSITY AND GLOBAL WATER WATCH. 2012. *Welcome to Global Water Watch* [Online]. Alabama, United States of America: Auburn University and Global Water Watch,. Available: http://www.globalwaterwatch.org/ [Accessed 10 July 2012].

AURAS, B. H., MILOUD, H., BETTACHE, G., NOUREDDINE, S. & MEBROUK, K. 2012. Characterization and Technological Properties of *Bifidobacterium* Strains Isolated from Breast-fed Infants. *Journal of Food Science and Engineering,* 2**,** 576-582.

AUSTRALIAN GOVERNMENT 2004. Guidance on Use of Rainwater Tanks. *In:* DEPARTMENT OF HEALTH AND AGEING (ed.). Australia: Production Unit (Corpotate Support Branch). Australian Government Department of Health and Ageing.

AWW, A. U. A. 2009. *Alabama Water Watch* [Online]. Alabama, USA: Auburn University. Available: https://fp.auburn.edu/icaae/index.aspx [Accessed 10 July 2012].

Bacterial Indicators/Health Hazards Associated with Water. 1977. *In:* HOADLEY, A. W. & DUTKA, B. J. (eds.). America.

BAGUMA, D., LOISKANDL, W. & JUNG, H. 2010. Water Management, Rainwater Harvesting and Predictive Variables in Rural Households. *Water Resources Management,* 24**,** 3333-3348.

BALFOUR, F., BADENHORST, H. & TROLLIP, D. 2011. A Gap Analysis of Water Testing Laboratories in South Africa. Pretoria, South Africa: Water Research Commission.

BALLESTÉ, E. & BLANCH, A. R. 2011. Bifidobacterial Diversity and the Development of New Microbial Source Tracking Indicators. *Applied and Environmental Microbiology,* 77**,** 3518-3525.

BALLONGUE, J. 2004. Bifidobacteria and Probiotic Action. *In:* SALMINEN, S., WRIGHT, A. V. & OUWEHAND, A. (eds.) *Lactic Acid Bacteria: Microbiological and*

Functional Aspects, Third Edition, Revised and Expanded. Third Edition, Revised and Expanded ed. USA: Marcel Dekker, Inc.
BAQUI, A. H., BLACK, R. E., YUNUS, M. D., HOQUE, A. R. A., CHOWDHURY, H. R. & SACK, R. B. 1991. Methodological Issues in Diarrhoeal Diseases Epidemiology: Definition of Diarrhoeal Episodes. *International Journal of Epidemiology,* 20**,** 1057-1063.
BARCINA, I., ARANA, I., IRIBERRI, J. & EGEA, L. 1986. Influence of Light and Natural Microbiota of the Butrón River on *E. coli* Survival. *Antonie van Leeuwenhoek,* 52**,** 555-566.
BARER, M. R. & HARWOOD, C. R. 1999. Bacterial Viability and Culturability. *In:* POOLE, R. K. (ed.) *Advances in Microbial Physiology.* Academic Press.
BARNES, J. 2003. *The Impact of Water Pollution from Formal and Informal Urban Developments along the Plankenburg River on Water Quality and Health Risk.* PhD dissertation, University of Stellenbosch.
BARRELL, R. A., HUNTER, P. R. & NICHOLS, G. 2000. Microbiological Standards for Water and Their Relationship to Health Risk. *Communicable Disease and Public Health / PHLS,* 3**,** 8-13.
BARTRAM, J. & CAIRNCROSS, S. 2010. Hygiene, Sanitation, and Water: Forgotten Foundations of Health. *PLoS Med,* 7**,** e1000367.
BEERENS, H. 1990. An Elective and Selective Isolation Medium for *Bifidobacterium spp. Letters in Applied Microbiology,* 11**,** 155-157.
BEERENS, H. 1991. Detection of Bifidobacteria by Using Propionic Acid as a Selective Agent. *Applied and Environmental Microbiology,* 57**,** 2418.
BEERENS, H. 1998. Bifidobacteria as Indicators of Faecal Contamination in Meat and Meat Products: Detection, Determination of Origin and Comparison with *Escherichia coli*. *International Journal of Food Microbiology,* 40**,** 203-207.
BELLI, W. A. & MARQUIS, R. E. 1991. Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to Acid Stress in Continuous Culture. *Applied and Environmental Microbiology,* 57**,** 1134-1138.
BELOTI, V., BARROS, M. A. F., DE FREITAS, J. C., NERO, L. A., DE SOUZA, J. A., SANTANA, E. H. W. & FRANCO, B. D. G. M. 1999. Frequency of 2,3,5-triphenyltetrazolium Chloride (TTC) Non-reducing Bacteria in Pasteurized Milk. *Revista de Microbiologia,* 30**,** 137-140.
BERGER, P. D. & MAURER, R. E. 2002. *Experimental Design : With Applications in Management, Engineering and the Sciences,* United States, Duxbury Press.
BERGLEY, B. H. (ed.) 1989. *Bergey's Manual of Bacteriology,* Baltimore: Williams and Wilkins Co.
BESNER, M.-C., PRÉVOST, M. & REGLI, S. 2011. Assessing the Public Health Risk of Microbial Intrusion Events in Distribution Systems: Conceptual Model, Available Data, and Challenges. *Water Research,* 45**,** 961-979.
BETTAREL, Y., BOUVIER, T. & BOUVY, M. 2009. Viral Persistence in Water as Evaluated from a Tropical/Temperate Cross-Incubation. *Journal of Plankton Research,* 31**,** 909-916.
BEZUIDENHOUT, C. C., MTHEMBU, N., PUCKREE, T. & LIN, J. 2002. Microbiological Evaluation of the Mhlathuze River, KwaZulu-Natal (RSA). *Water SA,* 28**,** 281--286.
BLANCH, A. R., BELANCHE-MUNOZ, L., BONJOCH, X., EBDON, J., GANTZER, C., LUCENA, F., OTTOSON, J., KOURTIS, C., IVERSEN, A., KUHN, I., MOCE, L., MUNIESA, M., SCHWARTZBROD, J., SKRABER, S., PAPAGEORGIOU, G., TAYLOR, H. D., WALLIS, J. & JOFRE, J. 2004. Tracking the Origin of Faecal

Pollution in Surface Water: an Ongoing Project Within the European Union Research Programme. *Journal of Water and Health,* 2**,** 249-260.
BLANCH, A. R., BELANCHE-MUNOZ, L., BONJOCH, X., EBDON, J., GANTZER, C., LUCENA, F., OTTOSON, J., KOURTIS, C., IVERSEN, A., KUHN, I., MOCE, L., MUNIESA, M., SCHWARTZBROD, J., SKRABER, S., PAPAGEORGIOU, G. T., TAYLOR, H., WALLIS, J. & JOFRE, J. 2006. Integrated Analysis of Established and Novel Microbial and Chemical Methods for Microbial Source Tracking. *Applied and Environmental Microbiology,* 72**,** 5915-5926.
BLOUIN, D. D. & PERRY, E. M. 2009. Whom Does Service Learning Really Serve? Community-Based Organizations' Perspectives on Service Learning. *Teaching Sociology,* 37**,** 120-135.
BOEHM, A. B., YAMAHARA, K. M., LOVE, D. C., PETERSON, B. M., MCNEILL, K. & NELSON, K. L. 2009. Covariation and Photoinactivation of Traditional and Novel Indicator Organisms and Human Viruses at a Sewage-Impacted Marine Beach. *Environmental Science and Technology,* 43**,** 8046-8052.
BOLDUC, M.-P., RAYMOND, Y., FUSTIER, P., CHAMPAGNE, C. P. & VUILLEMARD, J.-C. 2006. Sensitivity of Bifidobacteria to Oxygen and Redox Potential in Non-Fermented Pasteurized Milk. *International Dairy Journal,* 16**,** 1038-1048.
BOLTON, N., CROMAR, N., HALLSWORTH, P. & FALLOWFIELD, H. 2010. A Review of the Factors Affecting Sunlight Inactivation of Micro-Organisms in Waste Stabilisation Ponds: Preliminary Results for Enterococci. *Water Science and Technology,* 61**,** 885-890.
BONJOCH, X., BALLESTE, E. & BLANCH, A. R. 2004. Multiplex PCR with 16S rRNA Gene-Targeted Primers of *Bifidobacterium spp.* to Identify Sources of Fecal Pollution. *Applied and Environmental Microbiology,* 70**,** 3171-3175.
BONJOCH, X., BALLESTÉ, E. & BLANCH, A. R. 2005. Enumeration of Bifidobacterial Populations with Selective Media to Determine the Source of Waterborne Fecal Pollution. *Water Research,* 39**,** 1621-1627.
BONJOCH, X., LUCENA, F. & BLANCH, A. R. 2009. The Persistence of Bifidobacteria Populations in a River Measured by Molecular and Culture Techniques. *Journal of Applied Microbiology,* 107**,** 1178-1185.
BORRIELLO, S. P., HAMMES, W. P., HOLZAPFEL, W., MARTEAU, P., SCHREZENMEIR, J., VAARA, M. & VALTONEN, V. 2003. Safety of Probiotics that Contain Lactobacilli or Bifidobacteria. *Clinical Infectious Diseases,* 36**,** 775-80.
BOSCH, A., GUIX, S., SANO, D. & PINTÓ, R. M. 2008. New Tools for the Study and Direct Surveillance of Viral Pathogens in Water. *Current Opinion in Biotechnology,* 19**,** 295-301.
BOX, G. E. P. & WILLIAM, J. S. H. 2005. *Statistics for Experimenters : Design, Innovation, and Discovery* New York, John Wiley and Sons.
BRADFORD, M. M. 1976. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding *Analytical Biochemistry,* 72**,** 248 - 254.
BRENNER, K. P., RANKIN, C. C., ROYBAL, Y. R., STELMA, G. N., SCARPINO, P. V. & DUFOUR, A. P. 1993. New Medium for the Simultaneous Detection of Total Coliforms and *Escherichia coli* in Water. *Applied and Environmental Microbiology,* 59**,** 3534-3544.
BRION, G. M., MESCHKE, J. S. & SOBSEY, M. D. 2002. F-specific RNA Coliphages: Occurrence, Types, and Survival in Natural Waters. *Water Research,* 36**,** 2419-2425.

BUCHANAN, R. L. & BAGI, L. K. 1994. Expansion of Response Surface Models for the Growth of *Escherichia coli* O157 : H7 to Include Sodium Nitrite as a Variable. *International Journal of Food Microbiology,* 23**,** 317-332.

BUERGE, I. J., POIGER, T., MÜLLER, M. D. & BUSER, H.-R. 2003. Caffeine, an Anthropogenic Marker for Wastewater Contamination of Surface Waters. *Environmental Science and Technology,* 37**,** 691-700.

BUKIN, O., GOLIK, S., SALYUK, P., BAULO, E. & LASTOVSKAYA, I. 2008. Efficiency of Fluorescence Excitation in Chlorophyll A by the Second and Third Harmonics of Emission from an Nd:YAG Laser. *Journal of Applied Spectroscopy,* 75**,** 231-235.

BULL, I. D., LOCKHEART, M. J., ELHMMALI, M. M., ROBERTS, D. J. & EVERSHED, R. P. 2002. The Origin of Faeces by Means of Biomarker Detection. *Environment International,* 27**,** 647-654.

BURTON, G. A., GUNNISON, D. & LANZA, G. R. 1987. Survival of Pathogenic Bacteria in Various Freshwater Sediments. *Applied and Environmental Microbiology,* 53**,** 633-638.

BYAMUKAMA, D., KANSIIME, F., MACH, R. L. & FARNLEITNER, A. H. 2000. Determination of *Escherichia coli* Contamination with Chromocult Coliform Agar Showed a High Level of Discrimination Efficiency for Differing Fecal Pollution Levels in Tropical Waters of Kampala, Uganda. *Applied and Environmental Microbiology,* 66**,** 864-868.

BYRON, I. & CURTIS, A. 2002. Maintaining Volunteer Commitment to Local Watershed Initiatives. *Environmental Management,* 30**,** 59-67.

CABRAL, J. P. 2010. Review: Water Microbiology. Bacterial Pathogens and Water. *International Journal of Environmental Research and Public Health,* 7**,** 3657-3703.

CAMPOY, S., HERVÀS, A., BUSQUETS, N., ERILL, I., TEIXIDÓ, L. & BARBÉ, J. 2006. Induction of the SOS Response by Bacteriophage Lytic Development in *Salmonella enterica*. *Virology,* 351**,** 360-367.

CAPLENAS, N. R. & KANAREK, M. S. 1984. Thermotolerant Non-Fecal Source *Klebsiella pneumoniae*: Validity of the Fecal Coliform Test in Recreational Waters. *American Journal of Public Health,* 74**,** 1273-1275.

CAPLENAS, N. R., KANAREK, M. S. & DUFOUR, A. P. 1981. Source and Extent of *Klebsiella pneumoniae* in the Paper Industry. *Applied and Environmental Microbiology,* 42**,** 779-785.

CARRILLO, M., ESTRADA, E. & HAZEN, T. C. 1985. Survival and Enumeration of the Fecal Indicators *Bifidobacterium adolescentis* and *Escherichia coli* in a Tropical Rain Forest Watershed. *Applied and Environmental Microbiology,* 50**,** 468-476.

CARTER, J. T., RICE, E. W., BUCHBERGER, S. G. & LEE, Y. 2000. Relationships between Levels of Heterotrophic Bacteria and Water Quality Parameters in a Drinking Water Distribution System. *Water Research,* 34**,** 1495-1502.

CASTILLO, G., DUARTE, R., RUIZ, Z., MARUCIC, M. T., HONORATO, B., MERCADO, R., COLOMA, V., LORCA, V., MARTINS, M. T. & DUTKA, B. J. 1994. Evaluation of Disinfected and Untreated Drinking Water Supplies in Chile by the H_2S Paper Strip Test. *Water Research,* 28**,** 1765-1770.

CATTOIR, V., HUYNH, T. M., BOURDON, N., AUZOU, M. & LECLERCQ, R. 2009. Trimethoprim Resistance Genes in Vancomycin-Resistant *Enterococcus faecium* Clinical Isolates from France. *International Journal of Antimicrobial Agents,* 34**,** 390-392.

CHAO, K.-K., CHAO, C.-C. & CHAO, W.-L. 2004. Evaluation of Colilert-18 for Detection of Coliforms and *Eschericha coli* in Subtropical Freshwater. *Applied and Environmental Microbiology,* 70**,** 1242-1244.

CHAO, W. L. 2006. Evaluation of Colilert-18 for the Detection of Coliforms and *Escherichia coli* in Tropical Fresh Water. *Letters in Applied Microbiology,* 42**,** 115-120.
CHAPMAN, H., CARTWRIGHT, T., HUSTON, R. & O'TOOLE, J. 2008a. Water Quality and Health Risks from Urban Rainwater Tanks. Sydney, Australia: Cooperative Research Centre (CRC) for Water Quality and Treatment.
CHAPMAN, H., CARTWRIGHT, T. & TRIPODI, N. 2008b. Guidance Manual for the Design and Installation of Urban Roofwater Harvesting Systems in Australia (Edition 1). Salisbury, Australia: Cooperative Research Centre for Water Quality and Treatment.
CHARTERIS, KELLY, MORELLI & COLLINS 1998. Antibiotic Susceptibility of Potentially Probiotic *Bifidobacterium* Isolates from the Human Gastrointestinal Tract. *Letters in Applied Microbiology,* 26**,** 333-337.
CHEN, Z., PAVELIC, P., DILLON, P. & NAIDU, R. 2002. Determination of Caffeine as a Tracer of Sewage Effluent in Natural Waters by On-Line Solid-Phase Extraction and Liquid Chromatography with Diode-Array Detection. *Water Research,* 36**,** 4830-4838.
CHENOWETH, C. & SCHABERG, D. 1990. The Epidemiology of Enterococci. *European Journal of Clinical Microbiology and Infectious Diseases,* 9**,** 80-89.
CHIKULO, B. C. 2011. Local Government and Climate Change in South Africa Prospects and Challenges. *CLG Research Colloquium on Sustainable Local Governance for Prosperous Communities: Commonwealth Local Government Conference on Energising Local Economies: Partnerships for Prosperous Communities* City Hall, Cardiff UK.
CHIRENDA, T. 2011. *RE: Microbial Water Quality of Selected Rainwater Tanks Installed by Galela Amanzi*
CHO, K. H., HAN, D., PARK, Y., LEE, S. W., CHA, S. M., KANG, J.-H. & KIM, J. H. 2010. Evaluation of the Relationship between Two Different Methods for Enumeration Fecal Indicator Bacteria: Colony-Forming Unit and Most Probable Number. *Journal of Environmental Sciences,* 22**,** 846-850.
CHOU, C.-C. & HOU, J.-W. 2000. Growth of Bifidobacteria in Soymilk and Their Survival in the Fermented Soymilk Drink During Storage. *International Journal of Food Microbiology,* 56**,** 113-121.
CHOWDHURY, S. 2012. Heterotrophic Bacteria in Drinking Water Distribution System: a Review. *Environmental Monitoring and Assessment,* 184**,** 6087-6137.
CIMENTI, M., HUBBERSTEY, A., BEWTRA, J. K. & BISWAS, N. 2007. Alternative Methods in Tracking Sources of Microbial Contamination in Waters. *Water SA,* 33**,** 183-194.
CITIZENS MONITORING BACTERIA. 2004. *Building Capacity of E. coli Monitoring By Volunteers: A Multi-State Effort* [Online]. USA: Cooperative State Research, Education, and Extension Service, U.S. Department of Agriculture, National Integrated Water Quality Program, under Agreement No. 51130-01787. . Available: http://www.usawaterquality.org/volunteer/EColi/index.html [Accessed 10 July 2012].
CLASEN, T. F. 2012. Millennium Development Goals Water Target Claim Exaggerates Achievement. *Tropical Medicine and International Health* [Online]. Available: http://dx.doi.org/10.1111/j.1365-3156.2012.03052.x.
CLASEN, T. F., ROBERTS, I., TABER, R., WOLF-PETER, S. & SANDY, C. 2006. Interventions to Improve Water Quality for Preventing Diarrhoea. *Cochrane Database of Systematic Reviews: Reviews 2006, Issue 3* Chichester: John Wiley and Sons, Ltd

COLLADO, M. C. & SANZ, Y. 2006. Method for Direct Selection of Potentially Probiotic *Bifidobacterium* Strains from Human Feces Based on their Acid-adaptation Ability. *Journal of Microbiological Methods,* 66**,** 560-563.
COLLINS, R. & RUTHERFORD, K. 2004. Modelling Bacterial Water Quality in Streams Draining Pastoral Land. *Water Research,* 38**,** 700-712.
COMMONWEALTH OF AUSTRALIA. 2008. *Waterwatch Australia* [Online]. Canberra, Australia: Australian Government Department of Sustainability, Environment, Water, Population and Communities
Waterwatch Australia. Available: http://www.waterwatch.org.au/ [Accessed 10 July 2012].
CONNOLLY, M. A. E. 2005. Communicable Disease Control in Emergencies: A Field Manual. Available: http://www.who.int/hac/techguidance/pht/comdisease/en/ [Accessed 11 September 2012].
CONRAD, C. C. & HILCHEY, K. G. 2011. A Review of Citizen Science and Community-Based Environmental Monitoring: Issues and Opportunities. *Environmental Monititoring and Assessment,* 176**,** 273-291.
COOMBES, P. J., DUNSTAN, H., SPINKS, A., EVANS, C. & HARRISON, T. Key Messages from a Decade of Water quality Research into Roof Collected Rainwater Supplies. 1st National HYDROPOLIS Conference 2006, 2006 Burswood Convention Centre, Perth, Western Australia.
COOMBES, P. J., DUNSTAN, R., SPINKS, A., EVAN, C. & HARRISON, T. An Overview of a Decade of Research into the Quality of Rainwater Supplies Collected from Roofs. 2005.
COOMBES, P. J., KUCZERA, G., KALMA, J. D. & DUNSTAN, R. H. Rainwater Quality from Roofs, Tanks and Hot Water Systems at Figtree Place. The Hydro 2000 - Third International Hydrology and Water Resources Symposium of The Institution of Engineers, 20 to 23 November 2000 2000 Perth, Australia. 1042-1047.
COTTER, P. D. & HILL, C. 2003. Surviving the Acid Test: Responses of Gram-Positive Bacteria to Low pH. *Microbiology and Molecular Biology Reviews,* 67**,** 429-453.
COULBERT, B. 2005. *An Evaluation of Household Drinking Water Treatment Systems in Peru: the Table Filter and the Safe Water System.* Master of Engineering, Massachusetts Institute of Technology.
CROWTHER, J., KAY, D. & WYER, M. D. 2002. Faecal-Indicator Concentrations in Waters Draining Lowland Pastoral Catchments in the UK: Relationships with Land Use and Farming Practices. *Water Research,* 36**,** 1725-1734.
CUNLIFFE, D. 1998. Guidance on the Use of Rainwater Tanks. *In:* SERVICE, P. A. E. H. (ed.) *Internet and print.* Australia: National Environmental Health Forum Monographs.
CUNLIFFE, D., ASHBOLT, N. & STEVENS, M. 2003. Review of Coliforms as Microbial Indicators of Drinking Water Quality. *Recommendations to Change the Use of Coliforms as Microbial Indicators of Drinking Water Quality.* Australia: Australian Goverment, National Health and Medical Research Council.
CURTIS, V. & CAIRNCROSS, S. 2003. Effect of Washing Hands with Soap on Diarrhoea Risk in the Community: a Systematic Review. *The Lancet Infectious Diseases,* 3**,** 275-281.
CURTIS, V., CAIRNCROSS, S. & YONLI, R. 2000. Review: Domestic Hygiene and Diarrhoea – Pinpointing the Problem. *Tropical Medicine and International Health,* 5**,** 22-32.
D'AIMMO, M. R., MODESTO, M. & BIAVATI, B. 2007. Antibiotic Resistance of Lactic Acid Bacteria and *Bifidobacterium spp*. Isolated from Dairy and Pharmaceutical Products. *International Journal of Food Microbiology,* 115**,** 35-42.

DALSGAARD, A., FORSLUND, A., SANDVANG, D., ARNTZEN, L. & KEDDY, K. 2001. Vibrio cholerae O1 Outbreak Isolates in Mozambique and South Africa in 1998 are Multiple-Drug Resistant, Contain the SXT Element and the aadA2 Gene Located on Class 1 Integrons. *Journal of Antimicrobial Chemotherapy,* 48**,** 827-838.

DARAKAS, E. 2001. A Simple Mathematical Formula Describing the Survival Kinetics of *E.coli* in Natural Waters. *International Journal of Environmental Studies,* 58**,** 365-372.

DAVE, R. I. & SHAH, N. P. 1996. Evaluation of Media for Selective Enumeration of *Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus, Lactobacillus acidophilus,* and Bifidobacteria. *Journal of Dairy Science,* 79**,** 1529-1536.

DAVE, R. I. & SHAH, N. P. 1997. Viability of Yoghurt and Probiotic Bacteria in Yoghurts Made from Commercial Starter Cultures. *International Dairy Journal,* 7**,** 31-41.

DAVE, R. I. & SHAH, N. P. 1998. Ingredient Supplementation Effects on Viability of Probiotic Bacteria in Yogurt. *Journal of Dairy Science,* 81**,** 2804-2816.

DAVIES-COLLEY, R. J., BELL, R. G. & DONNISON, A. M. 1994. Sunlight Inactivation of Enterococci and Fecal Coliforms in Sewage Effluent Diluted in Seawater. *Applied and Environmental Microbiology,* 60**,** 2049-2058.

DAVISON, A., DEERE, D., STEVENS, M., CALLAN, P., FEWTRELL, L., DEERE, D. & BARTRAM, J. 2005. Water Safety Plans: Managing Drinking-Water Quality from Catchment to Consumer. Geneva: World Health Organization.

DE BOER, E. & BEUMER, R. R. 1999. Methodology for Detection and Typing of Foodborne Microorganisms. *International Journal of Food Microbiology,* 50**,** 119-130.

DE VILLIERS, S. & THIART, C. 2007. The Nutrient Status of South African Rivers: Concentrations, Trends and Fluxes from the 1970s to 2005. *South African Journal of Science,* 103**,** 343-349.

DE WET, T., MATHEE, A. & BARNES, B. 2000-2010. *Anthropology and Epidemiology: a Case Study of Health and Environment in Alexandra, Johannesburg* [Online]. Cape Town (RSA). Available: http://www.mrc.ac.za/healthdevelop/casestudy.pdf. [Accessed 30 March 2010].

DEGENER, J. E., SMIT, A. C. W., MICHEL, M. F., VALKENBURG, H. A. & MULLER, L. 1983. Faecal Carriage of Aerobic Gram-Negative Bacilli and Drug Resistance of *Escherichia coli* in Different Age-Groups in Dutch Urban Communities. *Journal of Medical Microbiology,* 16**,** 139-145.

DELGADO, S., FLÓREZ, A. B. & MAYO, B. 2005. Antibiotic Susceptibility of *Lactobacillus* and *Bifidobacterium* Species from the Human Gastrointestinal Tract. *Current Microbiology,* 50**,** 202-207.

DELGADO, S., O'SULLIVAN, E., FITZGERALD, G. & MAYO, B. 2008. In Vitro Evaluation of the Probiotic Properties of Human Intestinal *Bifidobacterium* Species and Selection of New Probiotic Candidates. *Journal of Applied Microbiology,* 104**,** 1119-1127.

DEPARTMENT OF HUMAN SETTLEMENTS (DHS) 1998. Prevention of Illegal Eviction From and Unlawful Occupation of Land Act No. 19 of 1998. Cape Town, South Africa.

DEPARTMENT OF WATER AFFAIRS 2010. NMMP Details of Implementation. Pretoria, South Africa: Department of Water Affairs.

DEPARTMENT OF WATER AFFAIRS. 2012a. *NMMP Bimonthly Reports 2012.* [Online]. South Africa: Department of Water Affairs. Available: http://www.dwa.gov.za/iwqs/microbio/report/NMMPkey_2012.htm [Accessed 8 August 2012].

DEPARTMENT OF WATER AFFAIRS. 2012b. *Water Service Institutions* [Online]. Johannesburg, South Africa: Department of Water Affairs. Available: http://www.dwaf.gov.za/IO/wsi.aspx [Accessed 15 September 2012].
DEPARTMENT OF WATER AFFAIRS (DWA) 2009. Adopt-A- River Programme Phase II: Development of an Implementation Plan Monitoring Models. *In:* ROSSOUW, L. (ed.). Pretoria, South Africa: Department of Water Affairs.
DEPARTMENT OF WATER AFFAIRS AND FORESTRY 2009. The Blue Drop Report 2009: Version 1, South African Drinking Water Quality Management Performance. Pretoria, South Africa: Department of Water Affairs and Forestry.
DEPARTMENT OF WATER AFFAIRS AND FORESTRY, D. 1996. South African Water Quality Guidelines Volume 7: Aquatic Ecosystems. Pretoria, South Africa: Department of Water Affairs and Forestry.
DESMARAIS, T. R., SOLO-GABRIELE, H. M. & PALMER, C. J. 2002. Influence of Soil on Fecal Indicator Organisms in a Tidally Influenced Subtropical Environment. *Applied and Environmental Microbiology,* 68**,** 1165-1172.
DEUTSCH, W., REUTEBUCH, E. & RUIZ-CORDOVA, S. 2007. Validity and Applications of Citizen Volunteer Water Quality Data: a Case from Alabama. *Water Resource IMPACT,* 9**,** 16-20.
DIETRICH, A. M. 2006. Aesthetic Issues for Drinking Water. *Journal of Water and Health,* 4**,** 11.
DILLAHA III, T. A. & ZOLAN, W. J. 1985. Rainwater Catchment Water Quality in Micronesia. *Water Research,* 19**,** 741-746.
DMA 2002. Disaster Management Act No. 57 of 2002. South Africa.
DMR 2005. The Disaster Management Regulations: Disaster Management Act 57 of 2002. *In:* GOVERNMENT, D. O. L. A. P. (ed.). Pretoria, South Africa.
DOUGHARI, H. J., NDAKIDEMI, P. A., HUMAN, I. S. & BENADE, S. 2011. Virulence Factors and Antibiotic Susceptibility Among Verotoxic Non O157: H7 *Escherichia coli* Isolates Obtained from Water and Wastewater Samples in Cape Town, South Africa. *African Journal of Biotechnology,* 10**,** 14160-14168.
DOUGLAS, I., ALAM, K., MAGHENDA, M., MCDONNELL, Y., MCLEAN, L. & CAMPBELL, J. 2008. Unjust Waters: Climate Change, Flooding and the Urban Poor in Africa. *Environment and Urbanization,* 20**,** 187-205.
DU PREEZ, M. & GENTHE, B. 1996. Evaluation of Rapid Methods for the Detection of Indicator Organisms in Drinking Water. *WISA 1996 Biennial Conference and Exhibition* Port Elizabeth, South Africa: WISA Online.
DU PREEZ, M., MURRAY, K. & VAN NIEKERK, H. 2001. A Pilot Study to Demonstrate Implementation of the National Microbial Monitoring Programme. Pretoria, South Africa: Water Research Commission: .
DU PREEZ, M., MURRAY, K. & VAN NIEKERK, H. 2002. A pilot Study to Demonstrate Implementation of the National Microbial Monitoring Programme, Progress Report. Pretoria, South Africa: Water Research Commission.
DU PREEZ, M., VENTER, S. N., VAN GINKEL, C., HARRIS, J., KUHN, A., ZINGITWA, L. & SILBERBAUER, M. 1999. Research on the Selection of Procedures for Faecal Pollution Monitoring to Describe Health Risks. Pretoria, South Africa: Water Research Commission.
DUBOIS, M., GILLES, K. A., HAMILTON, J. K., REBERS, P. A. & SMITH, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry,* 28**,** 350-356.

DUSE, A., DA SILVA, M. & ZIETSMAN, I. 2003. Coping with Hygiene in South Africa, a Water Scarce Country. *International Journal of Environmental Health Research,* 13**,** S95-S105.
DWA 2010. Blue Drop Report 2010 : South African Drinking Water Quality Management Performance. Department of Water Affairs.
DWA 2011a. Blue Drop Report 2011. Department of Water Affairs.
DWA. 2011b. *Blue Drop System: Makana Local Municipality* [Online]. Department of Water Affairs. Available: http://www.dwa.gov.za/dir_ws/DWQR/Default.asp?Pageid=42&WQDate=2011/12/15&Provid=2&WSACode=EC104 [Accessed 15 February 2012].
DWA 2012a. Chapter 5 – Eastern Cape Province. *Green Drop 2012 Progress Report.* Pretoria, South Africa: Department of Water Affairs.
DWA. 2012b. *Resource Water Quality Data for Mzimvubu to Keiskamma WMA* [Online]. Pretoria, South Africa: DWA. Available: http://www.dwaf.gov.za/iwqs/wms/data/WMA12_reg_WMS_nobor.htm [Accessed 26 June 2012].
DWA. 2012c. Statement by, Minister of Water and Environmental Affairs, Edna Molewa at The Release of the National Blue Drop Water Quality Assessment Report May 7 2012. 7 May 2012.
DWAF 1996a. South African Water Quality Guidelines *In:* FORESTRY, D. O. W. A. A. (ed.) 2 ed. Pretoria, South Africa: Department of Water Affairs and Forestry.
DWAF 1996b. South African Water Quality Guidelines Agricultural Use: Irrigation. *In:* DEPARTMENT OF WATER AFFAIRS AND FORESTRY (ed.) 2 ed. Pretoria, South Africa: Department of Water Affairs and Forestry,.
DWAF 1996c. South African Water Quality Guidelines, Vol. 1 (Domestic use) *In:* DEPARTMENT OF WATER AFFAIRS AND FORESTRY (ed.). Pretoria (RSA) Department of Water Affairs and Forestry,.
DWAF 1996d. South African Water Quality Guidelines: Recreational use. *In:* DEPARTMENT OF WATER AFFAIRS AND FORESTRY, D. (ed.). Pretoria, South Africa.
DWAF. 2000. *Prioritisation of Faecally Contaminated Areas* [Online]. Pretoria, South Africa: Department of Water Affairs and Forestry,. Available: http://www.dwaf.gov.za/iwqs/microbio/Document/prioritisefin2.htm.
DWAF 2004. Albany Coast Situation Assessment Study. *Department of Water Affairs and Forestry Directorate: National Water Resource Planning Albany Coast Situation Assessment Study* Albany District: DWAF.
DWAF 2006. Water Available for Allocation Per Water Management Area (WMA). *In:* PLANNING, D. O. T. N. W. R. (ed.). DWA.
DWAF 2009. A Drinking Water Quality Framework for South Africa: Minimum Requirements for Blue Drop Certification *Blue Drop Certification Requirements.* Pretoria, South Africa: Department of Water Affairs and Forestry,.
DWAF. 2011. Blue Drop Handbook. 1. Available: http://www.dwaf.gov.za/dir_ws/DWQR/subscr/ViewComDoc.asp?Docid=243
http://www.dwaf.gov.za/dir_ws/DWQR/subscr/ViewComDoc.asp?Docid=244
http://www.dwaf.gov.za/dir_ws/DWQR/Default.asp?Pageid=6&SearchString=Handbook [Accessed 15 September 2012].
EBDON, J. E. & TAYLOR, H. D. 2006. Geographical Stability of Enterococcal Antibiotic Resistance Profiles in Europe and Its Implications for the Identification of Fecal Sources. *Environmental Science and Technology,* 40**,** 5327-5332.

EDBERG, S. C. & ALLEN, M. J. 2004. Virulence and Risk from Drinking Water of Heterotrophic Plate Count Bacteria in Human Population Groups. *International Journal of Food Microbiology,* 92**,** 255-263.
EDWARDS, A., KAY, D., MCDONALD, A. T., FRANCIS, C., WATKINS, J., WILKINSON, J. R. & WYER, M. D. 2008. Farmyards, an Overlooked Source for Highly Contaminated Runoff. *Journal of Environmental Management,* 20**,** 1-9.
EFE, S. I. 2006. Quality of Rainwater Harvesting for Rural Communities of Delta State, Nigeria. *Environmentalist,* 26**,** 175-181.
EGAN, K. J., HERRIGES, J. A., KLING, C. L. & DOWNING, J. A. 2004. Recreation Demand Using Physical Measures of Water Quality. Ames, Iowa, USA: Center for Agricultural and Rural Development Iowa State University.
EGODAWATTA, P., THOMAS, E. & GOONETILLEKE, A. 2009. Understanding the Physical Processes of Pollutant Build-Up and Wash-Off on Roof Surfaces. *Science of the Total Environment,* 407**,** 1834-1841.
EHLERS, M. M., VAN ZYL, W. B., PAVLOV, D. N. & MÜLLER, E. E. 2004. Random Survey of the Microbial Quality of Bottled Water in South Africa. *Water SA,* 30**,** 202-210.
EL-MASRI, S. & TIPPLE, G. 2002. Natural Disaster, Mitigation and Sustainability: The Case of Developing Countries. *International Planning Studies,* 7**,** 157-175.
ELHMMALI, M. M., ROBERTS, D. J. & EVERSHED, R. P. 1997. Bile Acids as a New Class of Sewage Pollution Indicator. *Environmental Science and Technology,* 31**,** 3663-3668.
ENVIRONMENTAL PROTECTION AGENCY 1971. Methods for the Chemical Analysis of Water and Wastes (MCAWW) (EPA/600/4-79/020): Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Single Reagent). *METHOD #: 365.2 Approved for NPDES (Issued 1971).* EPA.
ENVIRONMENTAL PROTECTION AGENCY 1978. Methods for the Chemical Analysis of Water and Wastes (MCAWW) (EPA/600/4-79/020): Phosphorous, All Forms (Colorimetric, Ascorbic Acid, Single Reagent). *METHOD #: 365.3 Approved for NPDES (Issued 1978).* EPA.
ENVIRONMENTAL PROTECTION AGENCY 1993. Method 350.1: Determination of Ammonia Nitrogen by Semi-Automated Colorimetry. *In:* O'DELL, J. W. (ed.) *Methods for the Determination of Inorganic Substances in Environmental Samples (EPA/600/R-93/100).* Environmental Protection Agency.
ENVIRONMENTAL PROTECTION AGENCY 2012. Recreational Water Quality Criteria. Environmental Protection Agency.
ERTEL, A. M., LUPO, A., SCHEIFHACKEN, N., BODNARCHUK, T., MANTUROVA, O., BERENDONK, T. U. & PETZOLDT, T. 2012. Heavy Load and High Potential: Anthropogenic Pressures and Their Impacts on the Water Quality along a Lowland River (Western Bug, Ukraine). *Environmental Earth Sciences,* 65**,** 1459-1473.
ESREY, S., POTASH, J., ROBERTS, L. & SHIFF, C. 1991. Effects of Improved Water Supply and Sanitation on *Ascariasis*, Diarrhoea, *Dracunculiasis*, Hookworm Infection, Schistosomiasis, and Trachoma. *Bulletin of the World Health Organization,* 69**,** 609.
EURIPIDOU, E. & MURRAY, V. 2004. Public Health Impacts of Floods and Chemical Contamination. *Journal of Public Health,* 26**,** 376-383.
EVANS, C. A. Coliforms, Biofilms, Microbial Diversity and the Quality of Roof-Harvested Rainwater. American Rainwater Catchment System Association Conference, 2007 Kilauea Military Camp, Hawaii, USA.

EVANS, C. A., COOMBES, P. J. & DUNSTAN, R. H. 2006. Wind, Rain and Bacteria: The Effect of Weather on the Microbial Composition of Roof-Harvested Rainwater. *Water Research,* 40**,** 37-44.
EVANS, C. A., COOMBES, P. J., DUNSTAN, R. H. & HARRISON, T. 2007. Identifying the Major Influences on the Microbial Composition of Roof Harvested Rainwater and the Implications for Water Quality. *Water Science and Technology,* 55**,** 245-253.
EVERSHED RICHARD, P. & BETHELL PHILIP, H. 1996. Application of Multimolecular Biomarker Techniques to the Identification of Fecal Material in Archaeological Soils and Sediments. *Archaeological Chemistry.* American Chemical Society.
FARHAD, A. & MOHAMMADI, Z. 2005. Calcium Hydroxide: a Review. *International Dental Journal,* 55**,** 293-301.
FARNLEITNER, A. H., HOCKE, L., BEIWL, C., KAVKA, G. C., ZECHMEISTER, T., KIRSCHNER, A. K. T. & MACH, R. L. 2001. Rapid Enzymatic Detection of *Escherichia coli* Contamination in Polluted River Water. *Letters in Applied Microbiology,* 33**,** 246-250.
FARRENY, R., MORALES-PINZÓN, T., GUISASOLA, A., TAYÀ, C., RIERADEVALL, J. & GABARRELL, X. 2011. Roof Selection for Rainwater Harvesting: Quantity and Quality Assessments in Spain. *Water Research,* 45**,** 3245-3254.
FATOKI, O. S., MUYIMA, N. Y. O. & LUJIZA, N. 2001. Situation Analysis of Water Quality in the Umtata River Catchment. *Water SA,* 27**,** 467-473.
FAUST, M. A., AOTAKY, A. E. & HARGADON, M. T. 1975. Effect of Physical Parameters on the In Situ Survival of *Escherichia coli* MC-6 in an Estuarine Environment. *Applied Microbiology,* 30**,** 800-806.
FEW, R. 2003. Flooding, Vulnerability and Coping Strategies: Local Responses to a Global Threat. *Progress in Development Studies,* 3**,** 43-58.
FEWTRELL, L., KAUFMANN, R. B., KAY, D., ENANORIA, W., HALLER, L. & COLFORD, J. M. 2005. Water, Sanitation, and Hygiene Interventions to Reduce Diarrhoea in Less Developed Countries: A Systematic Review and Meta-Analysis. *The Lancet Infectious Diseases,* 5**,** 42-52.
FEWTRELL, L. & KAY, D. 2007. Microbial Quality of Rainwater Supplies in Developed Countries: A Review. *Urban Water Journal,* 4**,** 253-260.
FIELD, K. G. & SAMADPOUR, M. 2007. Fecal Source Tracking, the Indicator Paradigm, and Managing Water Quality. *Water Research,* 41**,** 3517-3538.
FINNEY, M., SMULLEN, J., FOSTER, H. A., BROKX, S. & STOREY, D. M. 2003. Evaluation of Chromocult Coliform Agar for the Detection and Enumeration of Enterobacteriaceae from Faecal Samples from Healthy Subjects. *Journal of Microbiological Methods,* 54**,** 353-358.
FIREHOCK, K. & WEST, J. 1995. A Brief History of Volunteer Biological Water Monitoring Using Macroinvertebrates. *Journal of the North American Benthological Society,* 14**,** 197-202.
FISHER, K. & PHILLIPS, C. 2009. The Ecology, Epidemiology and Virulence of *Enterococcus*. *Microbiology,* 155**,** 1749-1757.
FLEISHER, J. M. 1990. Conducting Recreational Water Quality Surveys: Some Problems and Suggested Remedies. *Marine Pollution Bulletin,* 21**,** 562-567.
FLUKA ANALYTICAL 60983 Kovac's Reagent for Indoles (Indole Reagent according to Kovac). Sigma Aldrich.
FOPPEN, J. W. A. & SCHIJVEN, J. F. 2006. Evaluation of Data from the Literature on the Transport and Survival of *Escherichia coli* and Thermotolerant Coliforms in Aquifers under Saturated Conditions. *Water Research,* 40**,** 401-426.

FORE, L. S., PAULSEN, K. & O'LAUGHLIN, K. 2001. Assessing the Performance of Volunteers in Monitoring Streams. *Freshwater Biology,* 46**,** 109-123.
FOULQUIÉ MORENO, M. R., CALLEWAERT, R., DEVREESE, B., VAN BEEUMEN, J. & DE VUYST, L. 2003. Isolation and biochemical characterisation of enterocins produced by enterococci from different sources. *Journal of Applied Microbiology,* 94**,** 214-229.
FRANS. 2011. *Red Worms in your Water Tank* [Online]. Water Rhapsody Eastern Cape. Available: http://www.watersafe.co.za/2011/01/14/red-worms-in-your-water-tank/ [Accessed 7 August 2012].
FRENCH, E. 1992. Adult Literacy in South Africa: Past to Present. *In:* HUTTON, B. (ed.) *Adult Basic Education in South Africa: Literacy, English as a Second Language, and Numeracy.* Cape Town: Oxford University Press.
FUJIOKA, R., SIAN-DENTON, C., BORJA, M., CASTRO, J. & MORPHEW, K. 1998. Soil: The Environmental Source of *Escherichia coli* and Enterococci in Guam's Streams. *Journal of Applied Microbiology,* 85**,** 83S-89S.
FUJIOKA, R. S. & NARIKAWA, O. T. 1982. Effect of Sunlight on Enumeration of Indicator Bacteria Under Field Conditions. *Applied and Environmental Microbiology,* 44**,** 395-401.
FUN, B. N., UNICOMB, L., RAHIM, Z., BANU, N. N., PODDER, G., CLEMENS, J., VAN LOON, F. P., RAO, M. R., MALEK, A. & TZIPORI, S. 1991. Rotavirus-Associated Diarrhea in Rural Bangladesh: Two-Year Study of Incidence and Serotype Distribution. *Journal of Clinical Microbiology,* 29**,** 1359-1363.
GAGNON, S., LÉVESQUE, S., LEFEBVRE, B., BOURGAULT, A. M., LABBÉ, A. C. & ROGER, M. 2011. VanA-Containing *Enterococcus faecium* Susceptible to Vancomycin and Teicoplanin Because of Major Nucleotide Deletions in Tn1546. *Journal of Antimicrobial Chemotherapy,* 66**,** 2758-2762.
GALELA AMANZI. 2010. *Galela Amanzi "Pour the Water" About Us* [Online]. Grahasmtown, South Africa: Rhodes University. Available: http://amanzi.rucus.ru.ac.za/aboutUs.html [Accessed 5 June 2011].
GALELA AMANZI. 2011. Grahamstown, South Africa: Rhodes University. Available: http://www.ru.ac.za/ceprogrammes/galelaamanzi/ [Accessed 10 February 2012].
GALELA AMANZI. 2012. *Galela Amanzi* [Online]. Grahamstown, South Africa: Rhodes University. Available: http://www.ru.ac.za/environment/action/studentaction/galelaamanzi/ [Accessed 20 February 2012].
GANTZER, C., HENNY, J. & SCHWARTZBROD, L. 2002. *Bacteroides fragilis* and *Escherichia coli* Bacteriophages in Human Faeces. *International Journal of Hygiene and Environmental Health,* 205**,** 325-328.
GARDINALI PIERO R & XU, Z. 2002. Trace Determination of Caffeine in Surface Water Samples by Liquid Chromatography-Atmospheric Pressure Chemical Ionization-Mass Spectrometry (LC-APCI-MS). *Environment International,* 28**,** 521-528.
GARDINI, F., MARTUSCELLI, M., CARUSO, M. C., GALGANO, F., CRUDELE, M. A., FAVATI, F., GUERZONI, M. E. & SUZZI, G. 2001. Effects of pH, Temperature and NaCl Concentration on the Growth Kinetics, Proteolytic Activity and Biogenic Amine Production of *Enterococcus faecalis*. *International Journal of Food Microbiology,* 64**,** 105-117.
GAUTHIER, F. & ARCHIBALD, F. 2001. The Ecology of “Fecal Indicator” Bacteria Commonly found in Pulp and Paper Mill Water Systems. *Water Research,* 35**,** 2207-2218.

GEERAERD, A. 2012. GInaFiT: Geeraerd and Van Impe Inactivation Model Fitting Tool. 1.6 ed. Belgium: Katholieke Universiteit Leuven (Belgium).
GEERAERD, A. H., VALDRAMIDIS, V. P. & VAN IMPE, J. F. 2005. GInaFiT, a Freeware Tool to Assess Non-Log-Linear Microbial Survivor Curves. *International Journal of Food Microbiology,* 102**,** 95-105.
GEERAERD, A. H., VALDRAMIDIS, V. P. & VAN IMPE, J. F. 2006. Erratum to "GInaFiT, a Freeware Tool to Assess Non-Log-Linear Microbial Survivor Curves" [Int. J. Food Microbiol. 102 (2005) 95–105]. *International Journal of Food Microbiology,* 110**,** 297.
GEERE, J.-A., HUNTER, P. & JAGALS, P. 2010a. Domestic Water Carrying and Its Implications for Health: A Review and Mixed Methods Pilot Study in Limpopo Province, South Africa. *Environmental Health,* 9**,** 52.
GEERE, J. L., MOKOENA, M. M., JAGALS, P., POLAND, F. & HARTLEY, S. 2010b. How Do Children Perceive Health to Be Affected by Domestic Water Carrying? Qualitative Findings from a Mixed Methods Study in Rural South Africa. *Child: Care, Health and Development,* 36**,** 818-826.
GEISSLER, K., MANAFI, M., AMORÓS, I. & ALONSO, J. L. 2000. Quantitative Determination of Total Coliforms and *Escherichia coli* in Marine Waters with Chromogenic and Fluorogenic Media. *Journal of Applied Microbiology,* 88**,** 280-285.
GENTHE, B. & FRANCK, M. 1999. A tool for Assessing Microbial Quality in Small Community Water Supplies: an H_2S Strip Test. *WRC Report***,** 33.
GENTHE, B. & JAGALS, P. 2003. Application of an H_2S Strip as a Field Test Kit for Microbial Water Quality Assessment. Division of Water, Environment and Forestry Technology, CSIR, Stellenbosch, RSA; Centre for Health and Environment Research and Development, Free State Technikon, Bloemfontein, RSA.: Water Research Commission.
GÉRARD, P., LEPERCQ, P., LECLERC, M., GAVINI, F., RAIBAUD, P. & JUSTE, C. 2007. *Bacteroides sp.* Strain D8, the First Cholesterol-Reducing Bacterium Isolated from Human Feces. *Applied and Environmental Microbiology,* 73**,** 5742-5749.
GERBA, C. P. 2000. Indicator Microorganisms. *In:* MAIER, R. M., PEPPER, I. L. & GERBA, C. P. (eds.) *Environmental Microbiology.* Arizona, Canada: Academic Press.
GERBA, C. P., ROSE, J. B. & HAAS, C. N. 1996. Sensitive Populations: Who Is at the Greatest Risk? *Risk Analysis and Production of Safe Food,* 30**,** 113-123.
GERMOND, J.-E., MAMIN, O. & MOLLET, B. 2002. Species Specific Identification of Nine Human *Bifidobacterium spp*. in Feces. *Systematic and Applied Microbiology,* 25**,** 536-543.
GERMS, W., COETZEE, M., VAN RENSBURG, L. & MABOETA, M. 2004. A Preliminary Assessment of the Chemical and Microbial Quality of the Chunies River - Limpopo. *Water SA,* 30**,** 267-272.
GLOBAL ENVIRONMENT, U., UNEP, SOPAC 2007. Sustainable Integrated Water Resources and Wastewater Management for Tuvalu: Draft SOPAC Miscellaneous. Suva, Fiji Islands.: Pacific Islands Applied GeoScience Commission, .
GOMES, A. M. P., TEIXEIRA, M. G. M. & MALCATA, F. X. 1998. Viability of *Bifidobacterium lactis* and *Lactobacillus acidophilus* in Milk: Sodium Chloride Concentration and Storage Temperature. *Journal of Food Processing and Preservation,* 22**,** 221-240.
GORDON, D. 2001. Geographical Structure and Host Specificity in Bacteria and the Implications for Tracing the Source of Coliform Contamination. *Microbiology,* 147**,** 1079-1085.

GOULD, C. V., FISHMAN, N. O., NACHAMKIN, I. & LAUTENBACH, E. 2004. Chloramphenicol Resistance in Vancomycin-Resistant Enterococcal Bacteremia: Impact of Prior Fluoroquinolone Use? *Infection Control and Hospital Epidemiology,* 25**,** 138-145.

GOULD, J. Is Rainwater Safe to Drink? A Review of Recent Findings. 9th International Rainwater Catchment Systems Conference, 1999 Petrolina, Brazil.

GOUVEIA, C., FONSECA, A., CÂMARA, A. & FERREIRA, F. 2004. Promoting the Use of Environmental Data Collected By Concerned Citizens through Information and Communication Technologies. *Journal of Environmental Management,* 71**,** 135-154.

GRABOW, W. O. K. 1996. Waterborne Diseases: Update on Water Quality Assessment and Control. *Water SA,* 22**,** 193-201.

GRABOW, W. O. K. 2001. Bacteriophages: Update on Application as Models for Viruses in Water. *Water SA,* 27**,** 251-268.

GRABOW, W. O. K. & DU PREEZ, M. 1979. Comparison of m-Endo LES, MacConkey, and Teepol Media for Membrane Filtration Counting of Total Coliform Bacteria in Water. *Applied and Environmental Microbiology,* 38**,** 351-358.

GRABOW, W. O. K., FAVOROV, M. O., KHUDYAKOVA, N. S., TAYLOR, M. B. & FIELDS, H. A. 1994. Hepatitis E Seroprevalence in Selected Individuals in South Africa. *Journal of Medical Virology,* 44**,** 384-388.

GRABOW, W. O. K., HILNER, C. A. & COUBROUGH, P. 1981. Evaluation of Standard and Modified M-FC, MacConkey, and Teepol Media for Membrane Filtration Counting of Fecal Coliforms in Water. *Applied and Environmental Microbiology,* 42**,** 192-199.

GRABOW, W. O. K., NEUBRECH, T. E., HOLTZHAUSEN, C. S. & JOFRE, J. 1995. *Bacteroides fragilis* and *Escherichia coli bacteriophages*: Excretion by Humans and Animals. *Water Science and Technology,* 31**,** 223-230.

GRABOW, W. O. K., VREY, A., UYS, M. & DE VILLIERS, J. C. 1998. Evaluation of the Application of Bacteriophages as Indicators of Water Quality. Pretoria, South Africa: Water Research Commission.

GRASS SOCIETY, R. U. 2011. *Gaian Revolutions and Social Solutions* [Online]. Grahamstown, South Africa: Rhodes Universuty. Available: http://www.ru.ac.za/environment/action/studentaction/grass/ [Accessed 10 July 2012].

GREEN, J., BRUCE, K., CONNER, C. & MUTESI, R. 2000. The APHA Standard Method for the Enumeration of Somatic Coliphages in Water Has Low Efficiency of Plating. *Water Research,* 34**,** 759-762.

GREETHAM, H. L., GIFFARD, C., HUTSON, R. A., COLLINS, M. D. & GIBSON, G. R. 2002. Bacteriology of the Labrador Dog Gut: A Cultural and Genotypic Approach. *Journal of Applied Microbiology,* 93**,** 640-646.

GRIFFIN, P. M. & TAUXE, R. V. 1991. The Epidemiology of Infections Caused by *Escherichia coli* O157: H7, Other *Enterohemorrhagic E. coli,* and the Associated Hemolytic Uremic Syndrome. *Epidemiologic Reviews,* 13**,** 60-98.

GRIFFITH, J. F., WEISBERG, S. B. & MCGEE, C. D. 2003. Evaluation of Microbial Source Tracking Methods Using Mixed Fecal Sources in Aqueous Test Sample. *Journal of Water and Health,* 1**,** 141-152.

GRIMALT, J. O., FERNANDEZ, P., BAYONA, J. M. & ALBAIGES, J. 1990. Assessment of Fecal Sterols and Ketones as Indicators of Urban Sewage Inputs To Coastal Waters. *Environmental science and technology,* 24**,** 357-363.

GUASCH, H., ARMENGOL, J., MARTí, E. & SABATER, S. 1998. Diurnal Variation in Dissolved Oxygen and Carbon Dioxide in Two Low-Order Streams. *Water Research,* 32**,** 1067-1074.

GUERRANT, R. L., KOSEK, M., LIMA, A. A. M., LORNTZ, B. & GUYATT, H. L. 2002. Updating the DALYs for Diarrhoeal Disease. *Trends in Parasitology,* 18**,** 191-193.
GUNDRY, S. W., WRIGHT, J. A., CONROY, R., DU PREEZ, M., GENTHE, B., MOYO, S., MUTISI, C., NDAMBA, J. & POTGIETER, N. 2006. Contamination of Drinking Water between Source and Point-Of-Use in Rural Households of South Africa and Zimbabwe: Implications for Monitoring the Millennium Development Goal for Water. *Water Practice and Technology,* 1.
GUPTA, S. K., SHEIKH, M. A., ISLAM, M. S., RAHMAN, K. S., JAHAN, N., RAHMAN, M. M., HOEKSTRA, R. M., JOHNSTON, R., RAM, P. K. & LUBY, S. 2008. Usefulness of the Hydrogen Sulfide Test for Assessment of Water Quality in Bangladesh. *Journal of Applied Microbiology,* 104**,** 388-395.
GYLLENBERG, H., NIEMELA, S. & SORMUNEN, T. 1960. Survival of Bifidobacteria in Water as Compared with that of Coliform Bacteria and Enterococci. *Applied Microbiology,* 8**,** 20-22.
HAARHOFF, J., C, R. L. & P, J. 2009. Rapid Technical Assessment and Troubleshooting of Rural Water Supply Systems. *Proceedings of the 10th Annual Water Distribution Systems Analysis Conference.* Kruger National Park, South Africa: WSDA 2008.
HAGEDORN, C., BLANCH, A. R. & HARWOOD, V. J. (eds.) 2011. *Microbial Source Tracking: Methods, Applications, and Case Studies*: Springer (Springer Science+Business Media, LLC 2011).
HAGEDORN, C. & WEISBERG, S. 2009. Chemical-Based Fecal Source Tracking Methods: Current Status and Guidelines for Evaluation. *Reviews in Environmental Science and Biotechnology,* 8**,** 275-287.
HANSEN, L. T., ALLAN-WOJTAS, P. M., JIN, Y. L. & PAULSON, A. T. 2002. Survival of Ca-alginate Microencapsulated *Bifidobacterium spp*. in Milk and Simulated Gastrointestinal Conditions. *Food Microbiology,* 19**,** 35-45.
HARDINA, C. M. & FUJIOKA, R. S. 1991. Soil - the Environmental Source of *Escherichia coli* and *Enterococci* in Hawaii Streams. *Environmental Toxicology and Water Contamination,* 6**,** 185-195.
HARHCEGANI, H. B. & CORNISH, P. S. 2003. A Catchment Approach to Understanding the Sources and Fate of Indicator Bacteria in an Intensive Agricultural Area. *In:* BRUEN, M. (ed.) *Diffuse Pollution and Basin Management.* Dublin, Ireland: Proceedings of the 7th International Specialised IWA Conference.
HARPE, J. D. L., FERRIERA, J.-A. & POTTER, A. 2006. Water Management Institutions: Overview. *In:* FORESTRY, D. O. W. A. A. (ed.). Pretoria, South Africa: Department of Water Affairs and Forestry.
HARTEL, P. G., SUMMER, J. D., HILL, J. L., COLLINS, J. V., ENTRY, J. A. & SEGARS, W. I. 2002. Geographic Variability of *Escherichia coli* Ribotypes from Animals in Idaho and Georgia. *Journal of Environmental Quality,* 31**,** 1273-1278.
HARTEMINK, R., DOMENECH, V. R. & ROMBOUTS, F. M. 1997. LAMVAB—A New Selective Medium for the Isolation of Lactobacilli from Faeces. *Journal of Microbiological Methods,* 29**,** 77-84.
HARTKE, A., GIARD, J.-C., LAPLACE, J.-M. & AUFFRAY, Y. 1998. Survival of *Enterococcus faecalis* in an Oligotrophic Microcosm: Changes in Morphology, Development of General Stress Resistance, and Analysis of Protein Synthesis. *Applied and Environmental Microbiology,* 64**,** 4238-4245.
HARWOOD, V. J., LEVINE, A. D., SCOTT, T. M., CHIVUKULA, V., LUKASIK, J., FARRAH, S. R. & ROSE, J. B. 2005. Validity of the Indicator Organism Paradigm for Pathogen Reduction in Reclaimed Water and Public Health Protection. *Applied and Environmental Microbiology,* 71**,** 3163-3170.

HARWOOD, V. J., WIGGINS, B., HAGEDORN, C., ELLENDER, R. D., GOOCH, J., KERN, J., SAMADPOUR, M., CHAPMAN, A. C., ROBINSON, B. J. & THOMPSON, B. C. 2003. Phenotypic Library-Based Microbial Source Tracking Methods: Efficacy in the California Collaborative Study. *Journal of Water and Health,* 1**,** 153-166.

HAZEN, T. C. & TORANZOS, G. A. 1990. Drinking Water Microbiology: Progress and Recent Developments. *In:* MCFETERS, G. A. (ed.). New York, United States of Ameria: Springer- Verlag.

HEALTH, N. D. O. 2008. The National HIV and Syphilis Prevalence Survey South Africa. Pretoria, South Africa Department of Health,.

HEALTH SYSTEMS TRUST. 2011. Diarrhoea Incidence in under 5 Year Old Children in South Africa, 2011. . Available: http://www.hst.org.za/recently-updated-indicators [Accessed 12 January 2012].

HEALTH SYSTEMS TRUST 2012. District Health Barometer 2010/11. *In:* DAY, C., BARRON, P., MASSYN, N., PADARATH, A. & ENGLISH, R. (eds.) Durban, South Africa: Health Systems Trust.

HECKMAN, M. A., WEIL, J. & DE MEJIA, E. G. 2010. Caffeine (1, 3, 7-trimethylxanthine) in Foods: A Comprehensive Review on Consumption, Functionality, Safety, and Regulatory Matters. *Journal of Food Science,* 75**,** R77-R87.

HEJAZI, A. & FALKINER, F. R. 1997. *Serratia marcescens*. *Journal of Medical Microbiology,* 46**,** 903-912.

HEMSON, D. 2007. 'The Toughest of Chores': Policy and Practice in Children Collecting Water in South Africa. *Policy Futures in Education,* 5**,** 315-326.

HEMSON, D. & DUBE, B. 2004. Water Services and Public Health: The 2000-01 Cholera Outbreak in KwaZulu-Natal, South Africa. *8th World Congress on Environmental Health 22-27 February 2004.* Durban, South Africa.

HENIS, Y., GURIJALA, K. R. & ALEXANDER, M. 1989. Factors Involved in Multiplication and Survival of *Escherichia coli* in Lake Water. *Microbial Ecology,* 17**,** 171-180.

HEYWORTH, J. S., GLONEK, G., MAYNARD, E. J., BAGHURST, P. A. & FINLAY-JONES, J. 2006. Consumption of Untreated Tank Rainwater and Gastroenteritis Among Young Children in South Australia. *International Journal of Epidemiology,* 35**,** 1051-1058.

HIGASHITANI, N., HIGASHITANI, A., ROTH, A. & HORIUCHI, K. 1992. SOS Induction in *Escherichia coli* by Infection with Mutant Filamentous Phage that are Defective in Initiation of Complementary-Strand DNA Synthesis. *Journal of Bacteriology,* 174**,** 1612-1618.

HIRULKAR, N. B. & TAMBEKAR, D. H. 2006. Suitability of the H_2S Test for Detection of Fecal Contamination in Drinking Water. *African Journal of Biotechnology,* 5**,** 1025-1028.

HOUSTON, P. C. & STILL, D. A. Rain Water Harvesting: a Neglected Rural Water Supply Option. Biennial Conference of the Water Institute of Southern Africa (WISA) 19-23 May 2002 2002.

HRENOVIĆ, J., ORHAN, Y., BÜYÜKGÜNGÖR, H. & HORVATIČEK, M. 2007. Influence of Ammonium, Nitrate and Nitrite on the Performance of the Pure Culture of *Acinetobacter Junii*. *Biologia,* 62**,** 517-522.

HSU, F. C., SHIEH, Y. S., VAN DUIN, J., BEEKWILDER, M. J. & SOBSEY, M. D. 1995. Genotyping Male-Specific RNA Coliphages by Hybridization with Oligonucleotide Probes. *Applied and Environmental Microbiology,* 61**,** 3960-3966.

HUANG, J. & MABURY, S. A. 2000. The Role of Carbonate Radical in Limiting the Persistence of Sulfur-Containing Chemicals in Sunlit Natural Waters. *Chemosphere,* 41**,** 1775-1782.
HUEPER, W. C. 1965. Occupational and Nonoccupational Exposures to Asbestos. *Annals of the New York Academy of Sciences,* 132**,** 184-195.
HUGHES, K. A. & THOMPSON, A. 2004. Distribution of Sewage Pollution Around a Maritime Antarctic Research Station Indicated by Faecal Coliforms, *Clostridium perfringens* and Faecal Sterol Markers. *Environmental Pollution,* 127 315-321.
HUNTER, P. R. 2003. Climate Change and Waterborne and Vector-Borne Disease. *Journal of Applied Microbiology,* 94**,** 37-46.
HUNTLEY, B. E., JONES, A. C. & CABELLI, V. J. 1976. *Klebsiella* Densities in Waters Receiving Wood Pulp Effluents. *Journal (Water Pollution Control Federation),* 48**,** 1766-1771.
IDEXX LABORATORIES INC. 2001. Colilert® An Easy 24-Hour Test for Coliforms and *E. coli*. *In:* IDEXX LABORATORIES, I. (ed.) *Online.* United States of America.
IDP 2008. Cacadu District Municipality: Integrated Development Plan (IDP) 2007 – 2012 (Review 2008). *In:* IDP (ed.). Cacadu, Eastern Cape, South Africa.
INTERNATIONAL STANDARDISATION ORGANISATION 1995. Water Quality - Detection and Enumeration of Bacteriophages, Enumeration of F-specific RNA Bacteriophages. Geneva, Switzerland: International Standardisation Organisation.
IRIN NEWS. 2009. *IRIN: South Africa the Quiet Water Crisis* [Online]. Johannesburg (RSA): IRIN News. Available: http://www.irinnews.org/Report.aspx?ReportId=82750 [Accessed 29 March 2009].
ISHII, S. & SADOWSKY, M. J. 2008. *Escherichia coli* in the Environment: Implications for Water Quality and Human Health. *Microbes and Environments,* 23**,** 101-108.
ISOBE, K. O., TARAO, M., CHIEM, N. H., MINH, L. Y. & TAKADA, H. 2004. Effect of Environmental Factors on the Relationship between Concentrations of Coprostanol and Fecal Indicator Bacteria in Tropical (Mekong Delta) and Temperate (Tokyo) Freshwaters. *Applied and Environment Microbiology,* 70**,** 814-821.
ISOBE, K. O., TARAO, M., ZAKARIA, M. P., CHIEM, N. H., MINH, L. Y. & TAKADA, H. 2002. Quantitative Application of Fecal Sterols Using Gas Chromatography−Mass Spectrometry To Investigate Fecal Pollution in Tropical Waters: Western Malaysia and Mekong Delta, Vietnam. *Environmental Science and Technology,* 36**,** 4497-4507.
JACKSON, S. A. F., BAILEY, I., BRITZ, R., DEWET, C., FORTUNE, A., JOHNSTON_ROBERTSON, A. J., NAIDOO, K. M. & SUNDRAM, A. Report on the South African Colilert™ Ring Trial. Biennial Conference of the Water Institute of Southern Africa (WISA), 19 - 23 May 2002 2002 Durban, South Africa. Water Research Commission (WRC).
JAGALS, P. & GRABOW, W. 1996a. An Evaluation of Sorbitol-Fermenting Bifidobacteria as Specific Indicators of Human Faecal Pollution of Environmental Water. *Water SA,* 22**,** 235-238.
JAGALS, P. & GRABOW, W. O. K. 1996b. An Evaluation of Sorbitol-Fermenting Bifidobacteria as Specific Indicators of Human Faecal Pollution of Environmental Water. *Water SA,* 22**,** 235-238.
JAGALS, P., GRABOW, W. O. K. & DE VILLIERS, J. C. 1995. Evaluation of Indicators for Assessment of Human and Animal Faecal Pollution of Surface Run-Off. *Water Science and Technology,* 31**,** 235-241.
JAGALS, P., JAGALS, C. & BOKAKO, T. C. 2003. The Effect of Container-Biofilm on the Microbiological Quality of Water Used from Plastic Household Containers. *Journal of Water and Health,* 1**,** 101-108.

JAGALS, P., NALA, N., TSUBANE, T., MOABI, M. & MOTAUNG, K. 2004. Measuring Changes in Water-Related Health and Hygiene Practices by Developing-Community Households. *Water Science and Technology,* 50**,** 91-97.

JANION, C. 2001. Some Aspects of the SOS Response System - A Critical Survey. *Acta Biochimica Polonica,* 48**,** 599-610.

JAYAMANNE, V. S. & ADAMS, M. R. 2006. Determination of Survival, Identity and Stress Resistance of Probiotic Bifidobacteria in Bio-Yoghurts. *Letters in Applied Microbiology,* 42**,** 189-194.

JAYAMANNE, V. S. & ADAMS, M. R. 2009. Modelling the Effects of pH, Storage Temperature and Redox Potential (Eh) on the Survival of Bifidobacteria in Fermented Milk. *International Journal of Food Science and Technology,* 44**,** 1131-1138.

JIA, W. & COLE, J. A. 2005. Nitrate and Nitrite Transport in *Escherichia coli*. *Biochemical Society Transactions,* 33**,** 159-161.

JOSÉ FIGUERAS, M. & BORREGO, J. J. 2010. New Perspectives in Monitoring Drinking Water Microbial Quality. *International Journal of Environmental Research and Public Health,* 7**,** 4179-4202.

JURY, M. R. & LEVEY, K. 1993. The Climatology and Characteristics of Drought in the Eastern Cape of South Africa. *International Journal of Climatology,* 13**,** 629-641.

KAHINDA, J.-M. M., TAIGBENU, A. E. & BOROTO, J. R. 2007. Domestic Rainwater Harvesting to Improve Water Supply in Rural South Africa. *Physics and Chemistry of the Earth,* 32**,** 1050-1057.

KAHINDA, J. M., SEJAMOHOLO, B. B. P., TAIGBENU, A. E., BOROTO, J. R., LILLIE, E. S. B., TAUTE, M. & COUSINS, T. 2008. Water Resource Management/IWRM - Catchment Management, Agricultural Water - Rainwater Harvesting. Johannesburg, South Africa.

KAI, S., WATANABE, S., FURUSE, K. & OSAWA, A. 1985. Bacteroides Bacteriophages Isolated from Human Faeces. *Microbiology and Immunology,* 29**,** 895-899.

KAMEL, M. M. 2006. Thermotolerant Coliform and *Escherichia coli* Detection and Enumeration Through Multiple Tube Fermentation. *Journal of Medical Sciences,* 6**,** 125-130.

KARIM, M. R., MANSHADI, F. D., KARPISCAK, M. M. & GERBA, C. P. 2004. The Persistence and Removal of Enteric Pathogens in Constructed Wetlands. *Water Research,* 38**,** 1831-1837.

KARIM, S. S. A., CHURCHYARD, G. J., KARIM, Q. A. & LAWN, S. D. 2009. HIV Infection and Tuberculosis in South Africa: An Urgent Need to Escalate the Public Health Response. *The Lancet,* 374**,** 921-933.

KAUSHIK, R. & BALASUBRAMANIAN, R. 2012. Assessment of Bacterial Pathogens in Fresh Rainwater and Airborne Particulate Matter Using Real-Time PCR. *Atmospheric Environment,* 46**,** 131-139.

KAY, R. M. 1981. Effects of Diet on the Fecal Excretion and Bacterial Modification of Acidic and Neutral Steroids, and Implications for Colon Carcinogenesis. *Cancer Research,* 41**,** 3774-3777.

KEFFORD, B. J., PALMER, C. G. & NUGEGODA, D. 2005. Relative Salinity Tolerance of Freshwater Macroinvertebrates from the South-East Eastern Cape, South Africa Compared With the Barwon Catchment, Victoria, Australia. *Marine and Freshwater Research,* 56**,** 163-171.

KHEADR, E., BERNOUSSI, N., LACROIX, C. & FLISS, I. 2004. Comparison of the Sensitivity of Commercial Strains and Infant Isolates of Bifidobacteria to Antibiotics and Bacteriocins. *International Dairy Journal,* 14**,** 1041-1053.

KISTEMANN, T., CLAßEN, T., KOCH, C., DANGENDORF, F., FISCHEDER, R., GEBEL, J., VACATA, V. & EXNER, M. 2002. Microbial Load of Drinking Water Reservoir Tributaries during Extreme Rainfall and Runoff. *Applied and Environmental Microbiology,* 68**,** 2188-2197.

KIURSKI, J. S., RANOGAJEC, J. G., UJHELJI, A. L., RADEKA, M. M. & BOKOROV, M. T. 2005. Evaluation of the Effect of Lichens on Ceramic Roofing Tiles by Scanning Electron Microscopy and Energy-Dispersive Spectroscopy Analyses. *Scanning,* 27**,** 113-119.

KLASEN, S. 2000. Measuring Poverty and Deprivation in South Africa. *Review of Income and Wealth,* 46**,** 33-58.

KOUADIO, I. K., ALJUNID, S., KAMIGAKI, T., HAMMAD, K. & OSHITANI, H. 2011. Infectious Diseases Following Natural Disasters: Prevention and Control Measures. *Expert Review of Anti-infective Therapy,* 10**,** 95-104.

KROLSKA, M., LATALA, A., MICHALSKA, M., NOWACKI, J. & SOBOL, Z. 1997. *Escherichia coli* Survival in the Water from the Gulf of Gdansk Under Laboratory Conditions. *Oceanological Studies,* 26**,** 133-143.

KUCH, A., WILLEMS, R. J. L., WERNER, G., COQUE, T. M., HAMMERUM, A. M., SUNDSFJORD, A., KLARE, I., RUIZ-GARBAJOSA, P., SIMONSEN, G. S., VAN LUIT-ASBROEK, M., HRYNIEWICZ, W. & SADOWY, E. 2012. Insight into Antimicrobial Susceptibility and Population Structure of Contemporary Human *Enterococcus faecalis* Isolates from Europe. *Journal of Antimicrobial Chemotherapy,* 67**,** 551-558.

LAI, C., WANG, C., CHU, C., TAN, C., LU, C., LEE, Y., HUANG, Y., LEE, P. & HSUEH, P. 2011. Correlation between Antimicrobial Consumption and Resistance Among *Staphylococcus aureus* And Enterococci Causing Healthcare-Associated Infections At a University Hospital in Taiwan from 2000 to 2009. *European Journal of Clinical Microbiology and Infectious Diseases,* 30**,** 265-271.

LALIBERTE, P. & GRIMES, D. J. 1982. Survival of *Escherichia coli* in Lake Bottom Sediment. *Applied and Environmental Microbiology,* 43**,** 623-628.

LAMENDELLA, R., DOMINGO, J. W. S., KELTY, C. & OERTHER, D. B. 2008. Bifidobacteria in Feces and Environmental Waters. *Applied and Environmental Microbiology,* 74**,** 575-584.

LANTAGNE, D. S., QUICK, R. & MINTZ, E. D. 2006. Household Water Treatment and Safe: Storage Options in Developing Countires: A Review of Current Implementation Practices. *In:* PARKER, M., WILLIAMS, A. & YOUNGBLOOD, C. (eds.) *Water Stories: Expanding Opportunities in Small-Scale Water and Sanitation Projects.* Washington DC, USA: Woodrow Wilson International Center for Scholars.

LAURENT, P. 2005. Household Drinking Water Systems and Their Impact on People with Weakened Immunity. *In:* DEPARTMENT, P. H. (ed.). Geneva: Medicins Sans Frontier, MSF-Holland

World Health Organisation.

LAZCKA, O., CAMPO, F. J. D. & MUÑOZ, F. X. 2007. Pathogen Detection: A Perspective of Traditional Methods and Biosensors. *Biosensors and Bioelectronics,* 22**,** 1205-1217.

LECHEVALLIER, M. W., BABCOCK, T. M. & LEE, R. G. 1987. Examination and Characterization of Distribution System Biofilms. *Applied and Environmental Microbiology,* 53**,** 2714-2724.

LECHEVALLIER, M. W., CAWTHON, C. D. & LEE, R. G. 1988. Factors Promoting Survival of Bacteria in Chlorinated Water Supplies. *Applied and Environment Microbiology,* 54**,** 649-654.

LECHEVALLIER, M. W., GULLICK, R. W., KARIM, M. R., FRIEDMAN, M. & FUNK, J. E. 2003. The Potential for Health Risks from Intrusion of Contaminants into the Distribution System from Pressure Transients. *Journal of Water and Health,* 1**,** 3-14.
LECHEVALLIER, M. W. & MCFETERS, G. A. 1985. Interactions Between Heterotrophic Plate Count Bacteria and Coliform Organisms. *Applied and Environmental Microbiology,* 49**,** 1338-1341.
LECHEVALLIER, M. W., WELCH, N. J. & SMITH, D. B. 1996. Full-scale Studies of Factors Related to Coliform Regrowth in Drinking Water. *Applied and Environment Microbiology,* 62**,** 2201-2211.
LECLERC, H., SCHWARTZBROD, L. & DEI-CAS, E. 2002. Microbial Agents Associated with Waterborne Diseases. *Critical Reviews in Microbiology,* 28**,** 371-409.
LEE, E. J. & SCHWAB, K. J. 2005. Deficiencies in Drinking Water Distribution Systems in Developing Countries. *Journal of Water and Health,* 3**,** 109-127.
LEE, J. E., LEE, S., SUNG, J. & KO, G. 2011. Analysis of Human and Animal Fecal Microbiota for Microbial Source Tracking. *ISME Journal,* 5**,** 362-365.
LEE, J. Y., BAK, G. & HAN, M. 2012. Quality of Roof-Harvested Rainwater – Comparison of different Roofing Materials. *Environmental Pollution,* 162**,** 422-429.
LEE, J. Y., YANG, J.-S., HAN, M. & CHOI, J. 2010. Comparison of the Microbiological and Chemical Characterization of Harvested Rainwater and Reservoir Water as Alternative Water Resources. *Science of the Total Environment,* 408**,** 896-905.
LEEMING, R., BALL, A., ASHBOLT, N. & NICHOLS, P. 1996. Using Faecal Sterols from Humans and Animals to Distinguish Faecal Pollution in Receiving Waters. *Water Research,* 30**,** 2893-2900.
LEEMING, R., LATHAM, V., RAYNER, M. & NICHOLS, P. 1997. Detecting and Distinguishing Sources of Sewage Pollution in Australian Inland and Coastal Waters and Sediments. *ACS Symposium Series,* 671**,** 306-319.
LEEMING, R. & NICHOLS, P. D. 1996. Concentrations of Coprostanol that Correspond to Existing Bacterial Indicator Guideline Limits. *Water Research,* 30**,** 2997-3006.
LEEMING, R., NICHOLS, P. D. & ASHBOLT, N. J. 1998. DistinguishingSources of Faecal Pollution in Australian Inland and Coastal Waters Using Sterol Biomarkers and Microbial Faecal Indicators. Water Services Association of Australia.
LEHLOESA, L. J. & MUYIMA, N. Y. O. 2000. Evaluation of the Impact of Household Treatment Procedures on the Quality of Groundwater Supplies in the Rural Community of the Victoria District, Eastern Cape. *Water SA,* 26**,** 285-290.
LEIBBRANDT, M. & WOOLARD, I. 1999. A Comparison of Poverty in South Africa's Nine Provinces. *Development Southern Africa,* 16**,** 37-54.
LESLIE, L. L., VELEZ, C. E. & BONAR, S. A. 2004. Utilizing Volunteers on Fisheries Projects. *Fisheries,* 29**,** 10-14.
LEWIN, S., NORMAN, R., NANNAN, N., THOMAS, E., BRADSHAW, D. & COLLABORATION, S. A. C. R. A. 2007. Estimating the Burden of Disease Attributable To Unsafe Water and Lack of Sanitation and Hygiene in South Africa in 2000. *South African Medical Journal,* 97**,** 755-762.
LEWIS, G. A., MATHIEU, D. & PHAN-TAN-LUU, R. 2005. *Pharmaceutical Experimental Design,* New York, USA, Marcel Dekker Inc.
LIGON, B. L. 2006. Infectious Diseases that Pose Specific Challenges After Natural Disasters: A Review. *Seminars in Pediatric Infectious Diseases,* 17**,** 36-45.
LIN, A. Y.-C. & REINHARD, M. 2005. Photodegradation of Common Environmental Pharmaceuticals and Estrogens in River Water. *Environmental Toxicology and Chemistry,* 24**,** 1303-1309.

LIN, J., BIYELA, P. T. & PUCKREE, T. 2004. Antibiotic Resistance Profiles of Environmental Isolates from Mhlathuze River, Kwazulu-Natal (RSA). *Water SA,* 30**,** 23-28.

LONG, S. C., ARANGO P, C. & PLUMMER, J. D. 2005. An Optimized Enumeration Method for Sorbitol-Fermenting Bifidobacteria in Water Samples. *Canadian Journal of Microbiology,* 51**,** 413-422.

LOPERFIDO, J. V., BEYER, P., JUST, C. L. & SCHNOOR, J. L. 2010. Uses and Biases of Volunteer Water Quality Data. *Environmental science and technology,* 44**,** 7193-7199.

LOURENÇO, M. 2010. Water Worries Not Yet Resolved. *Grocotts Mail,* 5 March 2010.

LOURENS-HATTINGH, A. & VILJOEN, B. C. 2001. Yogurt as Probiotic Carrier Food. *International Dairy Journal,* 11**,** 1-17.

LOUW, E. J. M. 2007. *Climate Change in the Western Cape: A Disaster Risk Assessment of the Impact on Human Health.* PhD, University of Stellenbosch.

LUYT, C. D., MULLER, W. J. & TANDLICH, R. Low Cost Tool for Monitoring Drinking Water and Environmental Water Quality in Selected Parts of South Africa. 5th Public Health Association of South Africa Conference, 2009 Durban, South Africa. PHASA, 88.

LUYT, C. D., MULLER, W. J. & TANDLICH, R. 2011a. Low Cost Tools for Microbial Quality Assessment of Drinking Water in South Africa. *Special Issue of the IOMC 2011 Conference Abstracts.* Conference e-Proceedings: HealthMed.

LUYT, C. D., MULLER, W. J., WILHELMI, B. S. & TANDLICH, R. 2010. Faecal Contamination of Potable Water Resources in South Africa: Sources, Monitoring and Challenges for the Future. *Environmental Monitoring and Assessment,* (Submitted on 5th May 2010).

LUYT, C. D., MULLER, W. J., WILHELMI, B. S. & TANDLICH, R. Health Implications of Flood Disaster Management in South Africa. 18th Annual Conference of the International Emergency Management of Society, 7 - 10 June 2011 2011b Bucharest, Romania. 376-385.

LUYT, C. D., TANDLICH, R., MULLER, W. J. & WILHELMI, B. S. 2012. Review: Microbial Monitoring of Surface Water in South Africa: An Overview. *International Journal of Environmental Research and Public Health* 9**,** 2669-2693.

LYE, D. 2002. Health Risks Associated with Consumption of Untreated Water from Household Roof Catchment Systems. *Journal of the American Water Resources Association* 38**,** 1301-1306.

LYE, D. J. 2009. Rooftop Runoff as a Source of Contamination: A Review. *Science of the Total Environment,* 407**,** 5429-5434.

LYNCH, P. A., GILPIN, B. J., SINTON, L. W. & SAVILL, M. G. 2002. The Detection of *Bifidobacterium adolescentis* by Colony Hybridization as an Indicator of Human Faecal Pollution. *Journal of Applied Microbiology,* 92**,** 526-533.

MAC KENZIE, W. R., HOXIE, N. J., PROCTOR, M. E., GRADUS, M. S., BLAIR, K. A., PETERSON, D. E., KAZMIERCZAK, J. J., ADDISS, D. G., FOX, K. R., ROSE, J. B. & DAVIS, J. P. 1994. A Massive Outbreak in Milwaukee of Cryptosporidium Infection Transmitted Through the Public Water Supply. *The New England journal of medicine,* 331**,** 161-167.

MACDONALD, I. A., BOKKENHEUSER, V. D., WINTER, J., MCLERNON, A. M. & MOSBACH, E. H. 1983. Degradation of Steroids in the Human Gut. *Journal of Lipid Research,* 24**,** 675-700.

MACGREGOR, D. 2010. Grahamstown Water Debate Continues. *Daily Dispatch,* 10 March 2010.

MACLEOD, I. 2011. Problematic Pipes Leave Businesses Dry. *Grocotts Mail*, 22 August 2011.
MADIGAN, M. T. & MARTINKO, J. M. 2006. *Brock Biology of Microorganisms* Upper Saddle River, NJ Pearson Prentice Hall.
MAHER, V. 2006. Water Update: No Threat to Humans. *Grocotts Mail*, 2 November 2006.
MAHEUX, A. F., HUPPÉ, V., BOISSINOT, M., PICARD, F. J., BISSONNETTE, L., BERNIER, J.-L. T. & BERGERON, M. G. 2008. Analytical Limits of Four β-Glucuronidase and β-Galactosidase-Based Commercial Culture Methods Used to Detect *Escherichia coli* and Total Coliforms. *Journal of Microbiological Methods,* 75**,** 506-514.
MAIER, R. M., PEPPER, I. L. & GERBA, C. P. 2000. *Environmental Microbiology,* Canada, Academic Press.
MAÏGA, Y., WETHE, J., DENYIGBA, K. & OUATTARA, A. S. 2009. The Impact of Pond Depth and Environmental Conditions on Sunlight Inactivation of *Escherichia coli* and Enterococci in Wastewater in a Warm Climate. *Canadian Journal of Microbiology,* 55**,** 1364-1374.
MAKANA MUNICIPALITY. 2011. *Notice: Water Supply Interruptions* [Online]. Makana, Grahamstown. Available: http://www.makana.gov.za/index.php?option=com_content&view=article&id=562:notice-water-supply-interruptions&catid=77:news&Itemid=29 10/11/2011].
MAKANA TOURISM. 2012. *Makana Celebrates Grahamstown's 200 Years with Fun-Filled Day* [Online]. Grahamstown, RSA: Makana Tourism. Available: http://www.grahamstown.co.za/news.php?nid=23 [Accessed 5 March 2012].
MALLIN, M. A., WILLIAMS, K. E., ESHAM, E. C. & LOWE, R. P. 2000. Effect of Human Development on Bacteriological Water Quality in Coastal Watersheds. *Ecological Applications,* 10**,** 1047-1056.
MAMBA, B. 2008. *An Appraisal of Basic Infrastructural Service Delivery and Community Participation at the Local Level: A Case Study of Three Municipalities in the Eastern Cape.* Master of Commerce (Financial Markets), Rhodes University.
MANAFI, M. 2000. New developments in chromogenic and fluorogenic culture media. *International Journal of Food Microbiology,* 60**,** 205-218
MANJA, K. S., MAURYA, M. S. & RAO, K. M. 1982. A Simple Field Test for the Detection of Fecal Pollution in Drinking Water. *Bulletin of the World Health Organization,* 60**,** 797-801.
MARA, D. & HORAN, N. J. 2003. *Handbook of Water and Wastewater Microbiology,* Great Britain, Academic Press.
MARA, D. D. & ORAGUI, J. I. 1981. Occurrence of *Rhodococcus coprophilus* and Associated Actinomycetes in Feces, Sewage, and Freshwater. *Applied and Environmental Microbiology,* 42**,** 1037-1042.
MARA, D. D. & ORAGUI, J. I. 1983. Sorbitol-Fermenting Bifidobacteria as Specific Indicators of Human Faecal Pollution. *Journal of Applied Microbiology,* 55**,** 349-357.
MARAJ, S., RODDA, N., JACKSON, S., BUCKLEY, C. & MACLEOD, N. 2006. Microbial Deterioration of Stored Water for Users Supplied by Stand-Pipes and Ground-Tanks in a Peri-Urban Community. *Water SA,* 32**,** 693-699.
MAROTHI, Y., AGNIHOTRI, H. & DUBEY, D. 2005. Enterococcal Resistance-an Overview. *Indian Journal of Medical Microbiology,* 23**,** 214.
MARTINS, M. T., RIVERA, I. G., CLARK, D. L., STEWART, M. H., WOLFE, R. L. & OLSON, B. H. 1993. Distribution of uidA Gene Sequences in *Escherichia coli* Isolates in Water Sources and Comparison with the Expression of Beta-Glucuronidase

Activity in 4-Methylumbelliferyl-Beta-D-Glucuronide Media. *Applied and Environmental Microbiology,* 59**,** 2271-2276.
MASCO, L., VAN HOORDE, K., DE BRANDT, E., SWINGS, J. & HUYS, G. 2006. Antimicrobial Susceptibility of *Bifidobacterium* Strains from Humans, Animals and Probiotic Products. *Journal of Antimicrobial Chemotherapy,* 58**,** 85-94.
MASUCCI, M. Developing GIS for Citizen Environmental Monitoring and Hazards Mitigation in Alabama and Sao Paulo, Brazil. GIS/LIS `96 Proceedings, 1996 Bethesda: ACSM/ASPRS, AAG, URISA, AM/FM. 250-265.
MATAMOROS, V., DUHEC, A., ALBAIGÉS, J. & BAYONA, J. 2009. Photodegradation of Carbamazepine, Ibuprofen, Ketoprofen and 17α-Ethinylestradiol in Fresh and Seawater. *Water, Air, and Soil Pollution,* 196**,** 161-168.
MATHABATHA, S. & NAIDOO, D. 2004. A Review of Public Participation in the Rural Water and Sanitation Setting. *1381/1/04.* South Africa: WRC Report 1381/1/04.
MATSUKI, T., WATANABE, K., TANAKA, R., FUKUDA, M. & OYAIZU, H. 1999. Distribution of Bifidobacterial Species in Human Intestinal Microflora Examined with 16S rRNA-Gene-Targeted Species-Specific Primers. *Applied and Environment Microbiology,* 65**,** 4506-4512.
MATSUMOTO, M., OHISHI, H. & BENNO, Y. 2004. H^+-ATPase activity in *Bifidobacterium* with Special Reference to Acid Tolerance. *International Journal of Food Microbiology,* 93**,** 109-113.
MATTEUZZI, D., CROCIANI, F. & BRIGIDI, P. 1983. Antimicrobial Susceptibility of *Bifidobacterium*. *Annales de l'Institut Pasteur / Microbiologie,* 134**,** 339-349.
MÄTTÖ, J., SUIHKO, M. L. & SAARELA, M. 2006. Comparison of Three Test Media for Antimicrobial Susceptibility Testing Of Bifidobacteria Using the E test Method. *International Journal of Antimicrobial Agents,* 28**,** 42-48.
MAYRHOFER, S., MAIR, C., KNEIFEL, W. & DOMIG, K. J. 2011. Susceptibility of Bifidobacteria of Animal Origin to Selected Antimicrobial Agents. *Chemotherapy Research and Practice,* 2011.
MCCAMBRIDGE, J. & MCMEEKIN, T. A. 1981. Effect of Solar Radiation and Predacious Microorganisms on Survival of Fecal and Other Bacteria. *Applied and Environmental Microbiology,* 41**,** 1083-1087.
MCDERMOTT, S. K. 2012a. Carolina: Living in a Town With Acid Water. *Mail and Guardian Online,* 2 March 2012.
MCDERMOTT, S. K. 2012b. Water Watch Increasingly Urgent. *Mail and Guardian,* 20 March 2012.
MCFETERS, G. A., CAMERON, S. C. & LECHEVALLIER, M. W. 1982. Influence of Diluents, Media, and Membrane Filters on Detection fo Injured Waterborne Coliform Bacteria. *Applied and Environmental Microbiology,* 43**,** 97-103.
MCFETERS, G. A. & TERZIEVA, S. I. 1991. Survival of *Escherichia coli* and *Yersinla enterocolitica* in Stream Water: Comparison of Field and Laboratory Exposure. *Microbial Ecology,* 22**,** 65-74.
MCKAY, V. 2007. Adult Basic Education and Training in South Africa. *In:* COMINGS, J., GARNER, B. & SMITH, C. (eds.) *Review of Adult learning and literacy: Connecting Research, Policy and Practice.* United states: Lawrence Erlbaum.
MCMAHAN, L., GRUNDEN, A. M., DEVINE, A. A. & SOBSEY, M. D. 2012. Evaluation of a Quantitative H_2S MPN Test for Fecal Microbes Analysis of Water Using Biochemical and Molecular Identification. *Water Research,* 46**,** 1693-1704.
MCMICHAEL, A. J., WOODRUFF, R. E. & HALES, S. 2006. Climate Change and Human Health: Present and Future Risks. *The Lancet,* 367**,** 859-869.

MEASHAM, T. G. & BARNETT, G. B. 2008. Environmental Volunteering: Motivations, Modes and Outcomes. *Australian Geographer,* 39**,** 537-552.
MEAYS, C. L., BROERSMA, K., NORDIN, R. & MAZUMDER, A. 2004. Source Tracking Fecal Bacteria in Water: A Critical Review of Current Methods. *Journal of Environmental Management,* 73**,** 71-79.
MEDEMA, G., PAYMENT, P., DUFOUR, A., ROBERTSON, W., WAITE, M., HUNTER, P., KIRBY, R. & ANDERSSON, Y. 2003. Safe Drinking Water: an Ongoing Challenge. In: Assessing Microbial Safety of Drinking Water - Improving Approaches and Methods. *In:* MEDEMA, G. J., PAYMENT, P., DUFOUR, A., ROBERTSON, W., WAITE, M., HUNTER, P., KIRBY, R. & ANDERSSON, Y. (eds.). Geneva, Switzerland: WHO Press: .
MENDEZ, C. B., KLENZENDORF, J. B., AFSHAR, B. R., SIMMONS, M. T., BARRETT, M. E., KINNEY, K. A. & KIRISITS, M. J. 2011. The Effect of Roofing Material on the Quality of Harvested Rainwater. *Water Research,* 45**,** 2049-2059.
MEON, B. & JÜTTNER, F. 1999. Concentrations and Dynamics of Free Mono-And Oligosaccharides in a Shallow Eutrophic Lake Measured by Thermospray Mass Spectrometry. *Aquatic Microbial Ecology,* 16**,** 281-293.
MERCK. 2012. *Merck Microbiology Manual 12th Edition: MRS Agar (Lactobacillus Agar acc. to De Man, Rogosa and Sharpe)* [Online]. Johannesburg, South Africa: Merck. Available: http://www.merckmillipore.com/chemicals/en_US/Merck-ZA-Site/USD/ViewProductDocuments-File?ProductSKU=MDA_CHEM-110660&DocumentType=APPL&DocumentId=200512.132.Appl&DocumentSource=GINAPPL [Accessed 23 September 2011].
MIERTSCHIN. 2011. *Water* [Online]. USA: Winona State University. Available: http://course1.winona.edu/cmiertschin/213/Inquiry_lab/Exp7_Water.pdf [Accessed 15 September 2012].
MINGRONE, G., GRECO, A. V. & PASSI, S. 1980. Reversed-Phase High-Performance Liquid Chromatographic Separation and Quantification of Individual Human Bile Acids. *Journal of Chromatography: Biomedical Applications,* 183**,** 277-286.
MINI, P. 2010. Experts Question Water Quality Claims. *Grocotts Mail*, 24 August 2010.
MINTZ, E. D., REIFF, F. M. & TAUXE, R. V. 1995. Safe Water Treatment and Water-Storage in the Home: A Practical New Strategy to Prevent Waterborne Disease. *JAMA-Journal of the American Medical Association,* 273 948-953.
MITCHELL, R. & GU, J.-D. (eds.) 2010. *Environmental Microbiology* USA: Wiley-Blackwell.
MITSUOKA, T. 1990. Bifidobacteria and Their Role in Human Health. *Journal of Industrial Microbiology and Biotechnology,* 6**,** 263-267.
MMANGO, M. 2010. Water Crisis Continues in the Townships. *Grocotts Mail,* 7 Spetember 2010.
MNGCAMBE, N. 2010. Locals Say Why They Don't Drink Tap Water *Grocotts,* 29 June 2010.
MOMBA, M. N. B. & KALENI, P. 2002. Regrowth and Survival of Indicator Microorganisms on the Surfaces of Household Containers Used For the Storage of Drinking Water in Rural Communities of South Africa. *Water Research,* 36**,** 3023-3028.
MOMBA, M. N. B., MADOROBA, E. & OBI, C. L. 2010. Apparent impact of enteric pathogens in drinking water and implications for the relentless saga of HIV/AIDS in South Africa. *Current Research, Technology and Education Topics in Applied Microbiology and Microbiological Technology* [Online]. Available: http://www.formatex.info/microbiology2/615-625.pdf.

MOMBA, M. N. B., OBI, C. L. & THOMPSON, P. 2009a. Survey of Disinfection Efficiency of Small Drinking Water Treatment Plants: Challenges Facing Small Water Treatment Plants in South Africa. *Water SA,* 35**,** 485-493.

MOMBA, M. N. B., OSODE, A. N. & SIBEWU, M. 2006a. The Impact of Inadequate Wastewater Treatment on the Receiving Water Bodies Case Study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. *2006 Water Institute of South Africa (WISA) Biennial Conference.* Durban, South Africa: Water SA.

MOMBA, M. N. B., SIBEWU, M. & MANDEYA, A. 2009b. Survival of Somatic and F-RNA Coliphages in Treated Wastewater Effluents and Their Impact on Viral Quality of the Receiving Water Bodies in the Eastern Cape Province-South Africa. *Journal of Biological Sciences,* 9**,** 648-654.

MOMBA, M. N. B., TYAFA, Z. & MAKALA, N. 2004. Rural Water Treatment Plants Fail to Provide Potable Water to Their Consumers: The Alice Water Treatment Plant in the Eastern Cape Province of South Africa: Research Letter. *South African Journal of Science,* 100**,** 307-310.

MOMBA, M. N. B., TYAFA, Z., MAKALA, N., BROUCKAERT, B. M. & OBI, C. L. 2006b. Safe Drinking Water Still a Dream in Rural Areas of South Africa. Case Study: The Eastern Cape Province. *Water SA,* 32**,** 715-720.

MONTGOMERY, M. A. & ELIMELECH, M. 2007. Water And Sanitation in Developing Countries: Including Health in the Equation. *Environmental Science and Technology,* 41**,** 17-24.

MONYAI, P. B. 2004. Health - Related Water Quality and Surveillance Model for the Peddie District in the Eastern Cape. Pretoria (RSA): : Water Research Commission.

MOOIJMAN, K. A., BAHAR, M., MUNIESA, M. & HAVELAAR, A. H. 2002. Optimisation of ISO 10705-1 on Enumeration of F-Specific Bacteriophages. *Journal of Virological Methods,* 103**,** 129-136.

MOOLMAN, A. & WINTER, D. 2010. Quality Of Domestic Water Supplies Guidelines: 10years Of Relevance To The Sector. . *WISA 2010 Biennial Conference and Exhibition.* Durban, South Africa: Water SA.

MOORE, D. F., HARWOOD, V. J., FERGUSON, D. M., LUKASIK, J., HANNAH, P., GETRICH, M. & BROWNELL, M. 2005. Evaluation of Antibiotic Resistance Analysis and Ribotyping for Identification of Faecal Pollution Sources in an Urban Watershed. *Journal of Applied Microbiology,* 99**,** 618-628.

MORANDI, S., BRASCA, M., ALFIERI, P., LODI, R. & TAMBURINI, A. 2005. Influence of pH and Temperature on the Growth of *Enterococcus faecium* and *Enterococcus faecalis*. *Le Lait,* 85**,** 181-192.

MORRISON, C. R., BACHOON, D. S. & GATES, K. W. 2008. Quantification of Enterococci and Bifidobacteria in Georgia Estuaries Using Conventional and Molecular Methods. *Water Research,* 42**,** 4001-4009.

MORRISON, G., FATOKI, O., ZINN, E. & JACOBSSON, D. 2001. Sustainable Development Indicators for Urban Water Systems: A Case Study Evaluation of King William's Town, South Africa, and the Applied Indicators. *Water SA,* 27**,** 219-232.

MOSLEY, L. 2005. Water Quality of Rainwater Harvesting Systems. South Pacific Applied Geoscience Commission.

MOSLEY, L. M. & SHARP, D. S. 2005. The Hydrogen Sulphide Paper Strip Test.

MOTT, J. & SMITH, A. 2011. Chapter 3: Library-Dependent Source Tracking Methods *In:* HAGEDORN, C., BLANCH, A. R. & HARWOOD, V. J. (eds.) *Microbial Source Tracking: Methods, Applications, and Case Studies.* Springer.

MOUBARECK, C., GAVINI, F., VAUGIEN, L., BUTEL, M. J. & DOUCET-POPULAIRE, F. 2005a. Antimicrobial susceptibility of bifidobacteria. *Journal of Antimicrobial Chemotherapy,* 55**,** 1057.

MOUBARECK, C., GAVINI, F., VAUGIEN, L., BUTEL, M. J. & DOUCET-POPULAIRE, F. 2005b. Antimicrobial Susceptibility of Bifidobacteria. *Journal of Antimicrobial Chemotherapy,* 55**,** 38-44.

MUDGE, S. M. & LINTERN, D. G. 1999. Comparison of Sterol Biomarkers for Sewage with other Measures in Victoria Harbour, B.C., Canada. *Estuarine, Coastal and Shelf Science,* 48**,** 27-38.

MULLINS, R. L. G. 2011. Grahamstown's Water Supply: a brief history from 1812 to 2008 *Annals of the Eastern Cape Museums,* 7**,** 1−64.

MUNNIK, V., MOLOSE, V., MOORE, B., TEMPELHOFF, J., GOUWS, I., MOTLOUNG, S., SIBIYA, Z., VAN ZYL, A., MALAPELA, P., BUANG, B., MBAMBO, B., KHOADI, J., MHLAMBI, L., MOROTOLO, M., MAZIBUKO, R., MLAMBO, M., MOEKETSI, M., QAMAKWANE, N., N, K. & TSOTETSI, A. 2011. Final Consolidated Report: The Potential of Civil Society Organisations in Monitoring and Improving Water Quality. Pretoria, South Africa: Water Research Commission.

MUÑOA, F. J. & PARES, R. 1988. Selective Medium for Isolation and Enumeration of *Bifidobacterium spp. Applied and Environmental Microbiology,* 54**,** 1715-1718.

MUNOZ, M. A., AHLSTROM, C., RAUCH, B. J. & ZADOKS, R. N. 2006. Fecal Shedding of *Klebsiella pneumoniae* by Dairy Cows. *Journal of Dairy Science,* 89**,** 3425-3430.

MURRAY, K., DU PREEZ, M., KUHN, A. & VAN NIEKER, H. 2004. A Pilot Study to Demonstrate Implementation of the National Microbial Monitoring Programme. Pretoria, South Africa: Report to the Water Research Commission.

MUSHI, D., BYAMUKAMA, D., KIVAISI, A. K., MACH, R. L. & FARNLEITNER, A. H. 2010. Sorbitol-Fermenting Bifidobacteria are Indicators of Very Recent Human Faecal Pollution in Streams and Groundwater Habitats in Urban Tropical Lowlands. *Journal of Water and Health,* 8**,** 466-478.

MWENGE KAHINDA, J.-M., TAIGBENU, A. E. & BOROTO, J. R. 2007. Domestic Rainwater Harvesting to Improve Water Supply in Rural South Africa. *Physics and Chemistry of the Earth, Parts A/B/C,* 32**,** 1050-1057.

MWENGE KAHINDA, J. & TAIGBENU, A. E. 2011. Rainwater Harvesting in South Africa: Challenges and Opportunities. *Physics and Chemistry of the Earth, Parts A/B/C,* 36**,** 968-976.

MWENGE KAHINDA, J., TAIGBENU, A. E. & BOROTO, R. J. 2010. Domestic Rainwater Harvesting as an Adaptation Measure to Climate Change in South Africa. *Physics and Chemistry of the Earth, Parts A/B/C,* 35**,** 742-751.

NA, S., MIYANAGA, K., UNNO, H. & TANJI, Y. 2006. The Survival Response of *Escherichia coli* K12 in a Natural Environment *Applied Microbiology and Biotechnology,* 72**,** 386-392.

NAGARAJU, D. & SASTRI, J. C. V. 1999. Confirmed Faecal Pollution to Bore Well Waters of Mysore City. *Environmental Geology,* 38**,** 322-326.

NAIR, J., GIBBS, R., MATHEW, K. & HO, G. 2000. Suitability of the H_2S Method for Testing Untreated and Chlorinated Water Supplies. *Water Science and Technology,* 44**,** 119-126.

NAKETI, A. 2009. High Levels of *E. coli* in Water Under Question. *Grocotts Mail*, 20 November 2009.

NATARO, J. P. & KAPER, J. B. 1998a. Diarrheagenic *Escherichia coli*. *Clinical Microbiology Reviews,* 11**,** 403.

NATARO, J. P. & KAPER, J. B. 1998b. Diarrheagenic Escherichia coli. *Clinical Microbiology Reviews,* 11**,** 142-201.
NATH, K. J., BLOOMFIELD, S. F. & JONES, M. 2006. Household Water Storage, Handling and Point-Of-Use Treatment. . A Review Commissioned by the International Scientific Forum on Home Hygiene.
NATIONAL DEPARTMENT OF HEALTH 2011. Epidemiological Comments. Progress on HIV Incidence Estimation Methods in South Africa. Pretoria, South Africa: Department of Health.
NATIONAL WATER PROGRAM. 2012. *The National Water Program* [Online]. National Water Program,. Available: http://www.usawaterquality.org/volunteer/ [Accessed 5 June 2012].
NEBRA, Y., BONJOCH, X. & BLANCH, A. R. 2003. Use of *Bifidobacterium dentium* as an Indicator of the Origin of Fecal Water Pollution. *Applied and Environmental Microbiology,* 69**,** 2651-2656.
NEBRA, Y., JOFRE, J. & BLANCH, A. R. 2002. The Effect of Reducing Agents on the Recovery of Injured *Bifidobacterium* Cells. *Journal of Microbiological Methods,* 49**,** 247-254.
NEVONDO, T. S. & CLOETE, T. E. 1999. Bacterial and Chemical Quality of Water Supply in the Dertig Village Settlement. *Water SA,* 25**,** 215-220.
NHA 2008. National Heath Act No.61 of 2003 as Amended 2008 South Africa.
NICHOLS, P. D., LEEMING, R., RAYNER, M. S. & LATHAM, V. 1996. Use of Capillary Gas Chromatography for Measuring Fecal-Derived Sterols Application to Stormwater, the Sea-Surface Microlayer, Beach Greases, Regional Studies, and Distinguishing Algal Blooms and Human and Non-Human Sources of Sewage Pollution. *Journal of Chromatography A,* 733**,** 497-509.
NICHOLSON, E., RYAN, J. & HODGKINS, D. 2002. Community Data-Where Does the Value Lie? Assessing Confidence Limits of Community Collected Water Quality Data. *Water Science and Technology,* 45**,** 193-200.
NIEMELA, S. I., LEE, J. V. & FRICKER, C. R. 2003. A Comparison of the International Standards Organisation Reference Method for the Detection of Coliforms and *Escherichia coli* in Water with a Defined Substrate Procedure. *Journal of Applied Microbiology,* 95**,** 1285-1292.
NKONDO, M. N., VAN ZYL, F. C., KEURIS, H. & SCHREINER, B. 2012. Proposed National Water Resource Strategy 2 (NWRS 2): Summary South Africa: Department of Water Affairs (DWA).
NOBLE, R. T., ALLEN, S. M., BLACKWOOD, A. D., CHU, W., JIANG, S. C., GREG L. LOVELACE, SOBSEY, M. D., STEWART, J. R. & WAIT, D. A. 2003. Use of Viral Pathogens and Indicators to Differentiate Between Human and Non-Human Fecal Contamination in a Microbial Source Tracking Comparison Study. *Journal of Water and Health,* 01**,** 195-207.
NOBLE, R. T., LEE, I. M. & SCHIFF, K. C. 2004. Inactivation of Indicator Micro-Organisms from Various Sources of Faecal Contamination in Seawater and Freshwater. *Journal of Applied Microbiology,* 96**,** 464-472.
NOBLET, J. A., YOUNG, D. L., ZENG, E. Y. & ENSARI, S. 2004. Use of Fecal Steroids To Infer the Sources of Fecal Indicator Bacteria in the Lower Santa Ana River Watershed, California: Sewage Is Unlikely a Significant Source. *Environmental Science and Technology,* 38**,** 6002-6008.
NORIEGA, L., GUEIMONDE, M., SÁNCHEZ, B., MARGOLLES, A. & DE LOS REYES-GAVILÁN, C. G. 2004. Effect of the Adaptation to High Bile Salts Concentrations on

Glycosidic Activity, Survival at Low pH and Cross-Resistance to Bile Salts in *Bifidobacterium*. *International Journal of Food Microbiology,* 94**,** 79-86.
NYGÅRD, K., WAHL, E., KROGH, T., TVEIT, O. A., BØHLENG, E., TVERDAL, A. & AAVITSLAND, P. 2007. Breaks and Maintenance Work in the Water Distribution Systems and Gastrointestinal Illness: A Cohort Study. *International Journal of Epidemiology,* 36**,** 873-880.
O'HOGAIN, S., MCCARTON, L., MCINTYRE, N., PENDER, J. & REID, A. 2011. Physicochemical and Microbiological Quality of Water from a Pilot Domestic Rainwater Harvesting Facility in Ireland. *Water and Environment Journal,* 25**,** 489-494.
OBERHOLSTER, P. J. & ASHTON, P. J. 2008. State of the Nation Report: An Overview of the Current Status of Water Quality and Eutrophication in South African Rivers and Reservoirs. *Parliamentary Grant Deliverable – March 2008.* South Africa: Goverment.
OBI, C. L. & BESSONG, P. O. 2002. Diarrhoeagenic Bacterial Pathogens in HIV-Positive Patients with Diarrhoea in Rural Communities of Limpopo Province, South Africa. *Journal of Health Population and Nutrition,* 20**,** 230-234.
OBI, C. L., ONABOLU, B., MOMBA, M. N. B., IGUMBOR, J. O., RAMALIVAHNA, J., BESSONG, P. O., VAN RENSBURG, E. J., LUKOTO, M., GREEN, E. & MULAUDZI, T. B. 2006. The Interesting Cross-Paths of HIV/AIDS and Water in Southern Africa with Special Reference to South Africa. *Water SA,* 32**,** 323-343.
OBI, C. L., POTGIETER, N., BESSONG, P. O. & MATSAUNG, G. 2003. Scope of Potential Bacterial Agents of Diarrhoea and Microbial Assessment of Quality of River Water Sources in Rural Venda Communities in South Africa. *Water Science and Technology,* 47**,** 59-64.
OBI, C. L., RAMALIVHANA, J., MOMBA, M. N. B., ONABOLU, B., IGUMBOR, J. O., LUKOTO, M., MULAUDZI, T. B., BESSONG, P. O., JANSE VAN RENSBURG, E. L., GREEN, E. & NDOU, S. 2007. Antibiotic Resistance Profiles and Relatedness of Enteric Bacterial Pathogens Isolated From HIV/AIDS Patients With and Without Diarrhoea and Their Household Drinking Water in Rural Communities in Limpopo Province South Africa. *African Journal of Biotechnology,* 6**,** 1035-1047.
ORAGUI, J. I. & MARA, D. D. 1983. Investigation of the Survival Characteristics of *Rhodococcus coprophilus* and Certain Fecal Indicator Bacteria. *Applied and Environmental Microbiology,* 46**,** 356-360.
OTIENO, F. & ADEYEMO, J. Drinking Water Quality at Some Schools in the Rural Areas of Kwazulu-Natal Province of South Africa. WISA 2012 Biennial Conference and Exhibition, 2012 Cape Town. WISA.
OTIENO, F. & OCHIENG, G. 2004. Water Management Tools as a Means of Averting a Possible Water Scarcity in South Africa by the Year 2025. *2004 Water Institute of South Africa (WISA) Biennial Conference.* Cape Town, South Africa: Water SA.
OTTOSON, J. R. 2009. Bifidobacterial Survival in Surface Water and Implications for Microbial Source Tracking. *Canadian Journal of Microbiology,* 55**,** 642-647.
OUOBA, L. I. I., LEI, V. & JENSEN, L. B. 2008. Resistance of Potential Probiotic Lactic Acid Bacteria and Bifidobacteria of African and European Origin to Antimicrobials: Determination and Transferability of the Resistance Genes to Other Bacteria. *International Journal of Food Microbiology,* 121**,** 217-224.
OVERDEVEST, C., ORR, C. H. & STEPENUCK, K. 2004. Volunteer Stream Monitoring and Local Participation in Natural Resource Issues. *Human Ecology Review,* 11**,** 177-185.

OXFORD UNIVERSITY PRESS 2012. Oxford Dictionary. *In:* MCCRACKEN, J. (ed.) *Oxford English Dictionary.* Online ed. London, United Kingdom: Oxford University Press.

PACHEPSKY, Y. A. & SHELTON, D. R. 2011. *Escherichia coli* and Fecal Coliforms in Freshwater and Estuarine Sediments. *Critical Reviews in Environmental Science and Technology,* 41**,** 1067-1110.

PALMER, C., PALMER, A., O'KEEFE, J. & PALMER, R. 1994. Macroinvertebrate Community Structure and Altitudinal Changes in the Upper Reaches of a Warm Temperate Southern African River. *Freshwater Biology,* 32**,** 337-348.

PALMER, K. L., DANIEL, A., HARDY, C., SILVERMAN, J. & GILMORE, M. S. 2011. Genetic Basis for Daptomycin Resistance in Enterococci. *Antimicrobial Agents and Chemotherapy,* 55**,** 3345-3356.

PALMER, R. W. & O'KEEFFE, J. H. 1990. Downstream Effects of Impoundments on the Water Chemistry of the Buffalo River (Eastern Cape), South Africa. *Hydrobiologia,* 202**,** 71-83.

PANGALLO, D., DRAHOVSKÁ, H., HARICHOVÁ, J., KARELOVÁ, E., CHOVANOVÁ, K., FERIANC, P., TURNA, J. & TIMKO, J. 2008. Assessment of Environmental Enterococci: Bacterial Antagonism, Pathogenic Capacity and Antibiotic Resistance. *Antonie van Leeuwenhoek (2008),* 94**,** 555-562.

PANNO, S., HACKLEY, K., HWANG, H., GREENBERG, S., KRAPAC, I., LANDSBERGER, S. & O'KELLY, D. Source Identification of Sodium and Chloride Contamination in Natural Waters: Preliminary Results. 12th Annual Conference of the Illinois Groundwater Consortium, 22 April 2002 2002 Illinois, USA.

PARRIS, N. A. 1977. Liquid Chromatographic Separation of Bile Acids. *Journal of Chromatography A,* 133**,** 273-279.

PARUCH, A. M. & MAEHLUM, T. 2012. Specific Features of *Escherichia coli* That Distinguish It from Coliform and Thermotolerant Coliform Bacteria and Define It as the Most Accurate Indicator of Faecal Contamination in the Environment. *Ecological Indicators,* 23**,** 140-142.

PATEL, H., SHAH, A., MISTRY, M. & CHANDA, S. 2011. In vitro antimicrobial susceptibility study in clinical isolates of Streptococci and Enterococci. *AFRICAN Journal of Microbiology Research* 5**,** 1374-1378.

PATEL, M., ISAÄCSON, M. & GOUWS, E. 1995. Effect of Iron and pH on the Survival of *Vibrio cholerae* in Water. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 89**,** 175-177.

PATRICK, R. 1962. Effects of River Physical and Chemical Characteristics on Aquatic Life. *Journal (American Water Works Association),* 54**,** 544-550.

PAULSE, A. N., JACKSON, V. & KHAN, W. 2007. Comparison of Enumeration Techniques for the Investigation of Bacterial Pollution in the Berg River, Western Cape, South Africa. *Water SA,* 33**,** 165-174.

PAULSE, A. N., JACKSON, V. A. & KHAN, W. 2009. Comparison of Microbial Contamination at Various Sites along the Plankenburg and Diep Rivers, Western Cape, South Africa. *Water SA,* 35**,** 469-478

PAYMENT, P. & LOCAS, A. 2011. Pathogens in Water: Value and Limits of Correlation with Microbial Indicators. *Ground Water,* 49**,** 4-11.

PEELER, K. A., OPSAHL, S. P. & CHANTON, J. P. 2006. Tracking Anthropogenic Inputs Using Caffeine, Indicator Bacteria, and Nutrients in Rural Freshwater and Urban Marine Systems. *Environmental Science and Technology,* 40**,** 7616-7622.

PEPPER, I. L., RUSIN, P., QUINTANAR, D. R., HANEY, C., JOSEPHSON, K. L. & GERBA, C. P. 2004. Tracking the Concentration of Heterotrophic Plate Count

Bacteria from the Source to the Consumer's Tap. *International Journal of Food Microbiology,* 92**,** 289-295.
PETTIBONE, G. W., SULLIVAN, S. A. & SHIARIS, M. P. 1987. Comparative Survival of Antibiotic-Resistant and -Sensitive Fecal Indicator Bacteria in Estuarine Water. *Applied and Environmental Microbiology,* 53**,** 1241-1245.
PHILLIP, D. A. T., ANTOINE, P., COOPER, V., FRANCIS, L., MANGAL, E., SEEPERSAD, N., RAGOO, R., RAMSARAN, S., SINGH, I. & RAMSUBHAG, A. 2009. Impact of Recreation on Recreational Water Quality of a Small Tropical Stream. *Journal of Environmental Monitoring,* 11**,** 1192-1198.
PISCIOTTA, J. M., RATH, D. F., STANEK, P. A., FLANERY, D. M. & HARWOOD, V. J. 2002. Marine Bacteria Cause False-Positive Results in the Colilert-18 Rapid Identification Test for *Escherichia coli* in Florida Waters. *Applied and Environmental Microbiology,* 68**,** 539-544.
PLETSCHKE, B. I., TOGO, C. A. & WUTOR, V. C. 2006. On-Line Real-Time Enzyme Diagnostic System for the Detection and Monitoring of Faecal Contamination of Water Intended for Drinking Purposes. South Africa: Water Research Commission.
PLUMMER, J. D. & LONG, S. C. 2007. Monitoring Source Water for Microbial Contamination: Evaluation of Water Quality Measures. *Water Research,* 41**,** 3716-3728.
PLYMOUTH MARINE LABORATORY 2009. PRIMER 6 and PERMANOVA +. 6.1.13 and 1.03 ed. United Kingdom: Primer - E.
PMG, P. M. G. 2009a. Regional Departments of Water Affairs in Eastern Cape, Western Cape and Limpopo on their Support to Local Municipalities
PMG, P. M. G. 2009b. Water Cuts and Cholera Situation in City of Cape Town: City, Departmental and Civil Community Responses. . Available: http://www.pmg.org.za/report/20090204-department-health-department-water-affairs-forestry-responses-cholera.
PONTES, K. V., WOLF MACIEL, M. R., MACIEL, R. & EMBIRUÇU, M. 2011. Process Analysis and Optimization Mapping Through Design of Experiments and Its Application to a Polymerization Process. *Brazilian Journal of Chemical Engineering,* 28**,** 137-150.
PORTFOLIO COMMITTEE ON HUMAN SETTLEMENTS 2010. Sanitation Programme Progress and Beneficiary List Management Update: Meeting Minutes held on 10 August 2010. *In:* SETTLEMENTS, D. O. H. (ed.). Cape Town, South Africa: Parliament of South Africa.
PRESSER, K. A., RATKOWSKY, D. A. & ROSS, T. 1997. Modelling the Growth Rate of *Escherichia coli* as a Function of pH and Lactic Acid Concentration. *Applied and Environmental Microbiology,* 63**,** 2355-60.
PRESSER, K. A., ROSS, T. & RATKOWSKY, D. A. 1998. Modelling the Growth Limits (Growth/No Growth Interface) of *Escherichia coli* as a Function of Temperature, pH, Lactic Acid Concentration, and Water Activity. *Applied and Environmental Microbiology,* 64**,** 1773-1779.
PROVINCIAL GAZETTE EXTRAORDINARY, P., 2009) 2009. Premier's Notice: Declaration of the Provincial State of Drought. Bhisho: Eastern Cape Provincial Government.
PRÜSS, A. 1998. Review of Epidemiological Studies on Health Effects from Exposure to Recreational Water. *International Journal of Epidemiology,* 27**,** 1-9.
PUIG, A., JOFRE, J. & ARAUJO, R. 1998. Use of Oxyrase Enzyme (Oxyrase®) for the Detection of Bacteriophages of *Bacteroides fragilis* in Aerobic Incubating Conditions. *Journal of Microbiological Methods,* 31**,** 205-207.

PUIG, A., QUERALT, N., JOFRE, J. & ARAUJO, R. 1999. Diversity of *Bacteroides fragilis* Strains in Their Capacity To Recover Phages from Human and Animal Wastes and from Fecally Polluted Wastewater. *Applied and Environmental Microbiology,* 65**,** 1772-1776.
QUINN, C. H., ZIERVOGEL, G., TAYLOR, A., TAKAMA, T. & THOMALLA, F. 2011. Coping with Multiple Stresses in Rural South Africa. *Ecology and Society,* 16**,** 2.
QUIROZ, C. C. 1999. *Risk assessment of Selected Opportunistic Pathogens in Drinking Water.* PhD, The University of Arizona.
RAND WATER. 2012. *Water Quality* [Online]. Johannesburg, South Africa: Rand Water. Available: http://www.randwater.co.za/WaterAndInfastructureManagement/Pages/WaterQuality.aspx [Accessed 10 July 2012].
RATTO, A., DUTKA, B. J., VEGA, C., LOPEZ, C. & EL-SHAARAWI, A. 1989. Potable Water Safety Assessed By Coliphage and Bacterial Tests. *Water Research,* 23**,** 253-255.
RAVENSCROFT, J. 2000. *Evaluation of Survival and Recovery Characteristics of Bifidobacteria as Indicators of Fecal Pollution of Water.* Master of Science, College of Agriculture, Forestry, and Consumer Sciences at West Virginia University.
RAYMER, D. & BUCKLE, J. 2010. We are Facing a Water and Sanitation Crisis in South Africa. *WISA 2010 Biennial Conference and Exhibition.* Durban, South Africa: Water SA.
REASON, C. J. C., HACHIGONTA, S. & PHALADI, R. F. 2005. Interannual Variability in Rainy Season Characteristics over the Limpopo Region of Southern Africa. *International Journal of Climatology,* 25**,** 1835-1853.
REED, R. H. 1997. Solar Inactivation of Faecal Bacteria in Water: The Critical Role of Oxygen. *Letters in Applied Microbiology,* 24**,** 276-280.
REEVES, A. D. & PATTON, D. 2001. Measuring Change in Sterol Input to Estuarine Sediments. *Physics and Chemistry of the Earth, Part B: Hydrology, Oceans and Atmosphere,* 26**,** 753-757.
RELIASOFT CORPARATION 2011. DOE ++. 1.0.7 ed.: ReliaSoft Corparation,.
RESNICK, I. G. & LEVIN, M. A. 1981a. Assessment of Bifidobacteria as Indicators of Human Fecal Pollution. *Applied and Environment Microbiology,* 42**,** 433-438.
RESNICK, I. G. & LEVIN, M. A. 1981b. Quantitative Procedure for Enumeration of Bifidobacteria. *Applied and Environmental Microbiology,* 42**,** 433-438.
RHODES, M. W. & KATOR, H. I. 1990. Effects of Sunlight and Autochthonous Microbiota on *Escherichia coli* Survival in an Estuarine Environment. *Current Microbiology,* 21**,** 65-73.
RHODES UNIVERSITY. 2011a. *Community Exerience Programmes* [Online]. Grahamstown, South Africa: Rhodes University. Available: http://www.ru.ac.za/ceprogrammes/galelaamanzi/ [Accessed 26 August 2011].
RHODES UNIVERSITY. 2011b. *Five Years, 20 Tanks Later* [Online]. Grahamstown, South Africa: Rhodes University. Available: http://www.ru.ac.za/latestnews/name,41558,en.html [Accessed 10 September 2011].
RICHARDS, E. & DANIEL, P. 2008. Grahamstown's Other Drinking Problem. *Activate,* 3 March 2008.
RICHARDS, R., O'LEARY, B. & MUTSONZIWA, K. 2007. Measuring Quality of Life in Informal Settlements in South Africa. *Social Indicators Research,* 81**,** 375-388.
RIJSBERMAN, F. R. 2006. Water scarcity: Fact or fiction? *Agricultural Water Management,* 80**,** 5-22.

RINCE, A., FLAHAUT, S. & AUFFRAY, Y. 2000. Identification of General Stress Genes in *Enterococcus faecalis*. *International Journal of Food Microbiology,* 55**,** 87-91.
RIVETT, U., LOUDON, M. & WRIGHT, J. 2009. Introduction the Aquatest Project: Increasing the Ability to Monitor Water Quality at Supply Level. Available: http://spatialdatamanagement.uct.ac.za/assets/RivettEtAl2009.pdf [Accessed 21 February 2010].
RODA, A., GATTI, R., CAVRINI, V., CERRÈ, C. & SIMONI, P. 1993. HPLC Study of the Impurities Present In Different Ursodeoxycholic Acid Preparations: Comparative Evaluation of Four Detectors. *Journal of Pharmaceutical and Biomedical Analysis,* 11**,** 751-760.
ROLFE, R. D., HENTGES, D. J., BARRETT, J. T. & CAMPBELL, B. J. 1977. Oxygen Tolerance of Human Intestinal Anaerobes. *The American Journal of Clinical Nutrition,* 30**,** 1762-9.
ROMPRÉ, A., SERVAIS, P., BAUDART, J., DE-ROUBIN, M.-R. & LAURENT, P. 2002. Detection and Enumeration of Coliforms in Drinking Water: Current Methods and Emerging Approaches. *Journal of Microbiological Methods,* 49**,** 31-54.
ROSENFELD, R. S. & GALLAGHER, T. F. 1964. Further Studies of the Biotransformation of Cholesterol to Coprostanol. *Steroids,* 4**,** 515-520.
ROSLEV, P. & BUKH, A. 2011. State of the Art Molecular Markers for Fecal Pollution Source Tracking in Water. *Applied Microbiology and Biotechnology,* 89**,** 1341-1355.
ROSS, T., RATKOWSKY, D. A., MELLEFONT, L. A. & MCMEEKIN, T. A. 2003. Modelling the Effects of Temperature, Water Activity, pH and Lactic Acid Concentration on the Growth Rate of *Escherichia coli*. *International Journal of Food Microbiology,* 82**,** 33-43.
ROSSOUW, L., FEBRUARIE, D. A. & WATER RESEARCH COMMISSION 2006. *Guidelines for Implementing Volunteer Water Quality Monitoring in South Africa,* Gezina, South Africa, Water Research Commission.
ROWAN, N. J. 2004. Viable But Non-Culturable Forms of Food and Waterborne Bacteria: Quo Vadis? *Trends in Food Science and Technology,* 15**,** 462-467.
ROWE, J., YARBROUGH, J., RAKE, J. & EAGON, R. 1979. Nitrite Inhibition of Aerobic Bacteria. *Current Microbiology,* 2**,** 51-54.
ROZEN, Y. & BELKIN, S. 2001. Survival of Enteric Bacteria in Seawater. *FEMS Microbiology Reviews,* 25**,** 513-529.
RUSHBY, J. F. & HANSON, K. 2001. Calculating and Presenting Disability Adjusted Life Years (DALYs) in Cost-Effectiveness Analysis. *Health Policy and Planning,* 16**,** 326-331.
SAARELA, M., ALAKOMI, H. L., MÄTTÖ, J., AHONEN, A. M., PUHAKKA, A. & TYNKKYNEN, S. 2011. Improving the Storage Stability of *Bifidobacterium breve* in Low pH Fruit Juice. *3rd International Symposium on Propionibacteria and Bifidobacteria: Dairy and Probiotic Applications.* Oviedo, Spain: International Journal of Food Microbiology, Elsevier.
SABS 2006. South African Standard Specifications for Water for Domestic Supplies. . Pretoria (RSA): South African Bureau of Standards.
SAID, M., LE ROUX, W., BURKE, L., SAID, H., PAULSEN, L., VENTER, S., POTGIETER, N., MASOABI, D. & DE WET, C. 2005. Origin, Fate and Clinical Relevance of Water-Borne Pathogens Present In Surface Waters. Pretoria, South Africa: Water Research Commission.
SAKYI, P. A., ASARE, R., ANANI, C. & DAMPARE, S. B. 2012. Nutrient-Induced Growth of Coliform and HPC Bacteria in Drinking-Water Pipes. *Journal of Environmental Protection,* 3.

SALIFERT. 2012. *Salifert Alkalinity (KH/Alk) Test Kit* [Online]. Available: http://www.f3images.com/IMD/UserManuals/SF1123.pdf http://www.marinedepot.com/Salifert_KH_Alkalinity_Test_Kit_Alkalinity_%28KH%29_Test_Kits_for_Saltwater_Aquariums-Salifert-SF1123-FITKAL-vi.html#Manuals [Accessed 15 September 2012].

SALMINEN, S., VON WRIGHT, A. & OUWEHAND, A. 2004. *Lactic Acid Bacteria: Microbiological and Functional Aspects*, Marcel Dekker, Inc.

SALUKAZANA, L., JACKSON, S., RODDA, N., SMITH, M., GOUNDEN, T., MCLEOD, N. & BUCKLEY, C. Plant Growth and Microbiological Safety of Plants Irrigated With Greywater. 3rd International Conference on Ecological Sanitation, 23rd until 26th May 2005 2005 Durban, South Africa. peer-reviewed proceedings.

SANDERS, D. & CHOPRA, M. 2006. Key Challenges to Achieving Health for All in an Inequitable Society: The Case of South Africa. *American Journal of Public Health,* 96**,** 73-78.

SANKARARAMAKRISHNAN, N. & GUO, Q. 2005. Chemical Tracers as Indicator of Human Fecal Coliforms at Storm Water Outfalls. *Environment International,* 31**,** 1133-1140.

SANTO DOMINGO, J. W., BAMBIC, D. G., EDGE, T. A. & WUERTZ, S. 2007. Quo Vadis Source Tracking? Towards A Strategic Framework for Environmental Monitoring of Fecal Pollution. *Water Research,* 41**,** 3539-3552.

SARTORY, D. P. 2004. Heterotrophic Plate Count Monitoring of Treated Drinking Water in the UK: A Useful Operational Tool. *International Journal of Food Microbiology,* 92**,** 297-306.

SARTORY, D. P. & WATKINS, J. 1998. Conventional Culture for Water Quality Assessment: Is There a Future? *Journal of Applied Microbiology,* 85**,** 225S-233S.

SASSOUBRE, L. M., WALTERS, S. P., RUSSELL, T. L. & BOEHM, A. B. 2011. Sources and Fate of *Salmonella* and Fecal Indicator Bacteria in an Urban Creek. *Journal of Environmental Monitoring,* 13**,** 2206-2212.

SATTERTHWAITE, D., HUQ, S., PELLING, M., REID, H. & LANKAO, P. R. 2007. Adapting to Climate Change in Urban Areas: The Possibilities and Constraints in Low- and Middle-Income Nations. *Human Settlements Discussion Paper Series.* London, United Kingdom: International Institute for Environment and Development.

SAVAN, B., MORGAN, A. J. & GORE, C. 2003. Volunteer Environmental Monitoring and the Role of the Universities: The Case of Citizens' Environment Watch. *Environmental Management,* 31**,** 561-568.

SAVICHTCHEVA, O. & OKABE, S. 2006. Alternative Indicators of Fecal Pollution: Relations with Pathogens and Conventional Indicators, Current Methodologies for Direct Pathogen Monitoring and Future Application Perspectives. *Water Research,* 40**,** 2463-2476.

SCHAPER, M., DURÁN, A. E. & JOFRE, J. 2002a. Comparative Resistance of Phage Isolates of Four Genotypes of F-Specific RNA Bacteriophages to Various Inactivation Processes. *Applied and Environmental Microbiology,* 68**,** 3702-3707.

SCHAPER, M., JOFRE, J., UYS, M. & GRABOW, W. O. K. 2002b. Distribution of Genotypes of F-Specific RNA Bacteriophages in Human and Non-Human Sources of Faecal Pollution in South Africa and Spain. *Journal of Applied Microbiology,* 92**,** 657-667.

SCHERMAN, P.-A., MULLER, W., GORDON, A., REYNHARDT, D., DU PREEZ, L. & CHALMERS, R. 2004. Eastern Cape River Health Programme Draft Technical Report: Buffalo River Monitoring, 2002 - 2003. *In:* COASTAL AND ENVIRONMENTAL SERVICES (ed.). Grahamstown, South Africa: for DWAF.

SCHRAFT, H. & WATTERWORTH, L. A. 2005. Enumeration of Heterotrophs, Fecal Coliforms and *Escherichia coli* in Water: Comparison of 3M™ Petrifilm™ Plates With Standard Plating Procedures. *Journal of Microbiological Methods,* 60**,** 335-342.
SCHREINER, B. & VAN ZYL, F. Water Services: Yesterday, Today and Tomorrow-A Strategic Perspective. WISA Biennial Conference and Exhibition 2006, 2006 Durban, South Africa.
SCHULTZ-FADEMRECHT, C., WICHERN, M. & HORN, H. 2008. The Impact of Sunlight on Inactivation of Indicator Microorganisms both in River Water and Benthic Biofilms. *Water Research,* 42**,** 4771-4779.
SCOARIS, D. D. O., BIZERRA, F. C. & YAMADA-OGATTA, S. F. 2008. The Occurrence of *Aeromonas spp.* in the Bottled Mineral Water , Well Water and Tap Water from the Municipal Supplies. *Brazilian Archives of Biology and Technology,* 51**,** 1049-1055.
SCOTT, T. M., ROSE, J. B., JENKINS, T. M., FARRAH, S. R. & LUKASIK, J. 2002. Microbial Source Tracking: Current Methodology and Future Directions. *Applied and Environment Microbiology,* 68**,** 5796-5803.
SECCHI DIPIN. 2009. *Secchi Dip-In Results* [Online]. United States of America. Available: http://www.secchidipin.org/view.htm [Accessed 10 July 2012].
SECKLER, D., BARKER, R. & AMARASINGHE, U. 1999. Water Scarcity in the Twenty-first Century. *International Journal of Water Resources Development,* 15**,** 29 - 42.
SERVAIS, P., PRATS, J., PASSERAT, J. & GARCIA-ARMISEN, T. 2009. Abundance of Culturable Versus Viable *Escherichia coli* in Freshwater. *Canadian Journal of Microbiology,* 55**,** 905-909.
SEURINCK, S., VERSTRAETE, W. & SICILIANO, S. 2005. Microbial Source Tracking for Identification of Fecal Pollution. *Reviews in Environmental Science and Biotechnology,* 4**,** 19-37.
SHAH, N. P. & LANKAPUTHRA, W. E. V. 1997. Improving Viability of *Lactobacillus acidophilus* and *Bifidobacterium spp*. in Yogurt. *International Dairy Journal,* 7**,** 349-356.
SHAH, N. P., LANKAPUTHRA, W. E. V., BRITZ, M. L. & KYLE, W. S. A. 1995. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in Commercial Yoghurt During Refrigerated Storage. *International Dairy Journal,* 5**,** 515-521.
SHAH, V. G., DUNSTAN, R. H., GEARY, P. M., COOMBES, P., ROBERTS, T. K. & VON NAGY-FELSOBUKI, E. 2007a. Evaluating Potential Applications of Faecal Sterols in Distinguishing Sources of Faecal Contamination from Mixed Faecal Samples. *Water Research,* 41**,** 3691-3700.
SHAH, V. G., HUGH DUNSTAN, R., GEARY, P. M., COOMBES, P., ROBERTS, T. K. & ROTHKIRCH, T. 2007b. Bacterial Source Tracking from Diverse Land Use Catchments by Sterol Ratios. *Water Research,* 41**,** 3667-3674.
SHAIKH, B., PONTZER, N. J., MOLINA, J. E. & KELSEY, M. I. 1978. Separation and Detection of UV-Absorbing Derivatives of Fecal Bile Acid Metabolites by High-Performance Liquid Chromatography. *Analytical Biochemistry,* 85**,** 47-55.
SHARMA, R. & SINGH, R. 2012. Determination of Safe Carrying Load Limit for Women Carrying Water. *Journal of Ergonomics,* 2**,** 1-7.
SHARMA, S. (ed.) 2007. *Rainwater Harvesting,* Delft, The Netherlands: UNESCO-IHE Institute for Water Education.
SHERER, B. M., MINER, J. R., MOORE, J. A. & BUCKHOUSE, J. C. 1992. Indicator Bacterial Survival in Stream Sediments. *Journal of Environmental Quality,* 21**,** 591-595.

SIGMA-ALDRICH CO. 2012. *Bromocresol Purple* [Online]. Sigma-Aldrich Co. Available: http://www.sigmaaldrich.com/catalog/product/SIGMA/17490?lang=en&region=ZA [Accessed 12 September 2012].

SIGMA. *Bradford Reagent: Technical Bulletin* [Online]. South Africa: Sigma Aldrich. Available: http://www.sigmaaldrich.com/etc/medialib/docs/Sigma/Bulletin/b6916bul.Par.0001.File.tmp/b6916bul.pdf [Accessed 15 September 2012].

SIGMA ALDRICH. 2012. *45183 Enterococcus Selective Agar* [Online]. Sigma Aldrich. Available: http://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Fluka/Usage/45183_enterococcus_selective_agar.pdf [Accessed 15 September 2012].

SIMMONS, G., HOPE, V., LEWIS, G., WHITMORE, J. & GAO, W. 2001. Contamination of Potable Roof-Collected Rainwater in Auckland, New Zealand. *Water Research,* 35**,** 1518-1524.

SIMPSON, J. M., SANTO DOMINGO, J. W. & REASONER, D. J. 2002. Microbial Source Tracking: State of the Science. *Environmental Science and Technology,* 36**,** 5279-5288.

SIMPSON, P. J., STANTON, C., FITZGERALD, G. F. & ROSS, R. P. 2005. Intrinsic Tolerance of *Bifidobacterium* Species to Heat and Oxygen and Survival Following Spray Drying and Storage. *Journal of Applied Microbiology,* 99**,** 493-501.

SINIGALLIANO, C. D., FLEISHER, J. M., GIDLEY, M. L., SOLO-GABRIELE, H. M., SHIBATA, T., PLANO, L. R. W., ELMIR, S. M., WANLESS, D., BARTKOWIAK, J., BOITEAU, R., WITHUM, K., ABDELZAHER, A. M., HE, G., ORTEGA, C., ZHU, X., WRIGHT, M. E., KISH, J., HOLLENBECK, J., SCOTT, T., BACKER, L. C. & FLEMING, L. E. 2010. Traditional and Molecular Analyses for Fecal Indicator Bacteria in Non-Point Source Subtropical Recreational Marine Waters. *Water Research,* 44**,** 3763-3772.

SINTON L, HALL C & BRAITHWAITE, R. 2007. Sunlight Inactivation of *Campylobacter jejuni* and *Salmonella enterica*, Compared With *Escherichia coli*, in Seawater and River Water. *Journal of Water and Health,* 5**,** 357-365.

SINTON, L. W., FINLAY, R. K. & HANNAH, D. J. 1998. Distinguishing Human from Animal Faecal Contamination in Water: A Review. *New Zealand Journal of Marine and Freshwater Research,* 32**,** 323 - 348.

SINTON, L. W., HALL, C. H., LYNCH, P. A. & DAVIES-COLLEY, R. J. 2002. Sunlight Inactivation of Fecal Indicator Bacteria and Bacteriophages from Waste Stabilization Pond Effluent in Fresh and Saline Waters. *Applied and Environmental Microbiology,* 68**,** 1122-1131.

SISTI, ALBANO & BRANDI 1998. Bactericidal Effect of Chlorine on Motile *Aeromonas spp.* in Drinking Water Supplies and Influence of Temperature on Disinfection Efficacy. *Letters in Applied Microbiology,* 26**,** 347-351.

SLANETZ, L. W. & BARTLEY, C. H. 1957. Numbers of Enterococci in Water, Sewage, and Feces Determined by the Membrane Filter Technique with an Improved Medium. *Journal of Bacteriology,* 74**,** 591-595.

SMITH, J. L. & ROCKLIFF, S. 1982. Rapid Single-Tube Confirmatory Test for *Escherichia coli*. *Journal of Hygiene,* 89**,** 149-154.

SNOWBALL, J., WILLIS, K. & JEURISSEN, C. 2008. Willingness to Pay for Water Service Improvements in Middle-Income Urban Households in South Africa: A Stated Choice Analysis. *South African Journal of Economics,* 76 705-720.

SOBSEY, M. D. 2002. Managing Water in the Home: Accelerated Health Gains from Improved Water Supply. Geneva: Water, Sanitation and Health Department of Protection of the Human Environment, World Health Organization.
SOBSEY, M. D. & PFAENDER, F. K. 2002. Evaluation of the H_2S Method for Detection of Fecal Contamination of Drinking Water. Geneva: University of North Carolina.
SOBSEY, M. D., STAUBER, C. E., CASANOVA, L. M., BROWN, J. M. & ELLIOTT, M. A. 2008. Point of Use Household Drinking Water Filtration: A Practical, Effective Solution for Providing Sustained Access to Safe Drinking Water in the Developing World. *Environmental Science and Technology,* 42**,** 4261-4267.
SOLLER, J. A., SCHOEN, M. E., BARTRAND, T., RAVENSCROFT, J. E. & ASHBOLT, N. J. 2010. Estimated Human Health Risks from Exposure to Recreational Waters Impacted by Human and Non-Human Sources of Faecal Contamination. *Water Research,* 44**,** 4674-4691.
SOLO-GABRIELE, H. M., WOLFERT, M. A., DESMARAIS, T. R. & PALMER, C. J. 2000. Sources of *Escherichia coli* in a Coastal Subtropical Environment. *Applied and Environmental Microbiology,* 66**,** 230-237.
SOUTH AFRICAN BUREAU OF STANDARDS 2006. South African Bureau of Standards South African Standard Specifications for Water for Domestic Supplies. Pretoria, South Africa.
SOUTH AFRICAN GOVERMENT 1993. Amended Occupational Health and Safety Act. *Act 85 of 1993.* Pretoria, South Africa: South African Goverment Gazette.
SOUTH AFRICAN GOVERMENT 1998. National Water Act. Cape Town, Republic of South Africa: Goverment Gazette.
SPOONER, J., HUFFMAN, R. L., LINE, D. E., GALE, J. A., JENNINGS, G. D., COFFEY, S. W., JON, A. A., WYATT, L., OSMOND, D. L. & HASEEB, S. K. 1992. Nonpoint Sources. *Water Environment Research,* 64**,** 503-514.
STANDLEY, L. J., KAPLAN, L. A. & SMITH, D. 2000. Molecular Tracers of Organic Matter Sources to Surface Water Resources. *Environmental Science and Technology,* 34**,** 3124-3130.
STATISTICS SOUTH AFRICA 2007a. Community Survey 2007 Basic Results. *In:* AFRICA, S. S. (ed.). Pretoria.
STATISTICS SOUTH AFRICA 2007b. Community Survey, 2007
Basic Results: Eastern Cape. *Community Survey, 2007.*
STATISTICS SOUTH AFRICA 2011a. General Household Survey 2010 Revised version ed. Pretoria, South Africa: Statistics South Africa.
STATISTICS SOUTH AFRICA 2011b. Mid-Year Population Estimates 2011. Pretoria, South Africa: Statistics South Africa.
STATISTICS SOUTH AFRICA 2011c. Selected Development Indicators: General Household Survey, 2011. Pretoria, South Africa: Statistics South Africa.
STATISTICS SOUTH AFRICA 2011d. Water and Sanitation 2002–2010: In-Depth Analysis of the General Household Survey data. *GHS Series.* Pretoria, South Africa: Statistics South Africa.
STATISTICS SOUTH AFRICA 2012a. Census 2011: Census in brief. Pretoria, South Africa: Statistics South AFrica.
STATISTICS SOUTH AFRICA 2012b. Census 2011: Key Results. Pretoria, South Africa: Statistics South Africa.
STATISTICS SOUTH AFRICA 2012c. Census 2011: Statistical Release. Pretoria, South Africa: Statistics South Africa.
STATISTICS SOUTH AFRICA 2012d. General Household Survey - July 2011. Pretoria, South Africa: Statistics South Africa.

STATSOFT INC. 2011. STATISTICA (Data Analysis Software System), Version 10. 10 ed.: StatSoft Inc.
STEWART-PULLARO, J., DAUGOMAH, J. W., CHESTNUT, D. E., GRAVES, D. A., SOBSEY, M. D. & SCOTT, G. I. 2006. F+RNA Coliphage Typing for Microbial Source Tracking in Surface Waters. *Journal of Applied Microbiology,* 101**,** 1015-1026.
STEYN, M., JAGALS, P. & GENTHE, B. 2004. Assessment of Microbial Infection Risks Posed by Ingestion of Water during Domestic Water Use and Full-Contact Recreation in a Mid-Southern African Region. *Water Science and Technology,* 50**,** 301-308.
STOECKEL, D., MATHES, M., HYER, K., HAGEDORN, C., KATOR, H., LUKASIK, J., O'BRIEN TL, FENGER, T., SAMADPOUR, M., STRICKLER, K. & WIGGINS, B. 2004. Comparison of Seven Protocols to Identify Fecal Contamination Sources Using *Escherichia coli. Environmental Science and Technology,* 38**,** 6109-6117.
STOECKEL, D. M. & HARWOOD, V. J. 2007. Performance, Design, and Analysis in Microbial Source Tracking Studies. *Applied and Environmental Microbiology,* 73**,** 2405-2415.
SUN, S., GUO, Z., YANG, R., SHENG, Z. & CAO, P. 2012. Study on the Triphenyl Tetrazolium Chloride-Dehydrogenase Activity (TTC-DHA) Method in Determination of Bioactivity for Treating Tomato Paste Wastewater. *African Journal of Biotechnology,* 11**,** 7055-7062.
SUNDRAM, A., BAILEY, I. & GREEN, E. The Application of New Technology Used for Identifying Bacteria from Water Samples. WISA 2000 Biennial Conference, 28 May - 1 June 2000 Sun City, South Africa.
SUNDRAM, A., JUMANLAL, N., JOHN, W. & EHLERS, M. 2006. Studies to Distinguish between Human and Animal Faecal Pollution Using F-RNA Coliphages and Faecal Sterols. *WISA 2006 Biennial Conference and Exhibition.* Durban, South Africa: Water SA.
SUTHERLAND, J. P., BAYLISS, A. J. & BRAXTON, D. S. 1995. Predictive Modelling of Growth of *Escherichia coli* O157:H7: the Effects of Temperature, pH and Sodium Chloride. *International Journal of Food Microbiology,* 25**,** 29-49.
SUTHERLAND, J. P., BAYLISS, A. J., BRAXTON, D. S. & BEAUMONT, A. L. 1997. Predictive Modelling of *Escherichia coli* O157:H7: Inclusion of Carbon Dioxide as a Fourth Factor in a Pre-existing Model. *International Journal of Food Microbiology,* 37**,** 113-120.
SWEETMAN, S. C. (ed.) 2011. *Martindale: The Complete Drug Reference,* London: Pharmaceutical Press.
TAGUCHI, T., TAKEYAMA, H. & MATSUNAGA, T. 2005. Immuno-capture of *Cryptosporidium parvum* using micro-well array. *Biosensors and Bioelectronics,* 20**,** 2276-2282.
TALLON, P., MAGAJNA, B., LOFRANCO, C. & LEUNG, K. T. 2005. Microbial Indicators of Faecal Contamination in Water: A Current Perspective. *Water, Air, and Soil Pollution,* 166**,** 139-166.
TALWALKAR, A. & KAILASAPATHY, K. 2004. A Review of Oxygen Toxicity in Probiotic Yogurts: Influence on the Survival of Probiotic Bacteria and Protective Techniques. *Comprehensive Reviews in Food Science and Food Safety,* 3**,** 117-124.
TANDLICH, R., LUYT, C. & MULLER, W. 2012. Faecal Contamination Source Identification Using a Combination of Chemical and Microbial Biomarkers. Pretoria: Division of Pharmaceutical Chemistry, Faculty of Pharmacy, Rhodes University; Unilever Centre for Environmental Water Quality Institute for Water Research Rhodes University.

TANDLICH, R., LUYT, C. D., GORDON, A. K. & SRINIVAS, S. C. 2011. Concentrations of Indicator Organisms in the Stored Rainwater in the Makana Municipality, South Africa. *Air and Water Components of the Environment Conference (Aerul şi Apa : Componente ale Mediului).* Cluj, Romania.

TANDLICH, R., LUYT, C. D. & MULLER, W. J. 2010a. Project No. K8/806: Progress report as 2nd deliverable. Pretoria, RSA: Project report submitted as a deliverable to the Water Research Commission in April 2010, .

TANDLICH, R., LUYT, C. D. & MULLER, W. J. 2010b. Project No. K8/806: Progress Report as Second Deliverable. *Project Report Submitted as a Deliverable to the Water Research Commission in April 2010.* Pretoria: RSA.

TANDLICH, R. & MULLER, W. J. 2008. Methods for Detection of Faecal Pollution and Identifying Its Origins: Linking the South African and the International Context. Pretoria, South Africa: Water Research Comission.

TANNOCK, G. W. 1997. Probiotic Properties of Lactic-Acid Bacteria: Plenty of Scope for Fundamental R and D. *Trends in Biotechnology,* 15**,** 270-274.

TARTERA, C., BOSCH, A. & JOFRE, J. 1988. The Inactivation of Bacteriophages Infecting *Bacteroides fragilis* by Chlorine Treatment and UV-Irradiation. *FEMS Microbiology Letters,* 56**,** 313-316.

TARTERA, C. & JOFRE, J. 1987. Bacteriophages Active Against *Bacteroides fragilis* in Sewage-Polluted Waters. *Applied and Environmental Microbiology,* 53**,** 1632-1637.

TARTERA, C., LUCENA, F. & JOFRE, J. 1989. Human Origin of *Bacteroides fragilis* Bacteriophages Present in the Environment. *Applied and Environmental Microbiology,* 55**,** 2696-2701.

TEMPELHOFF, J., VAN NIEKERK, D., VAN EEDEN, E., GOUWS, I., BOTHA, K. & WURIGE, R. 2009. The December 2004-January 2005 Floods in the Garden Route Region of the Southern Cape, South Africa. *Journal of Disaster Risk Studies (JAMBA),* 2**,** 93-112.

TEUNIS, P. F. M., VAN DER HEIJDEN, O. G., VAN DER GIESSEN, J. W. B. & HAVELAAR, A. H. 1996. The Dose Respose Relation in Human Volunteers for Gastrointestinal Pathogens. Rijksinstituut voor Volksgezondheid en Milieu RIVM .

THITARAM, S. N., SIRAGUSA, G. R. & HINTON, A. 2005. Bifidobacterium-Selective Isolation and Enumeration from Chicken Caeca by a Modified Oligosaccharide Antibiotic-Selective Agar Medium. *Letters in Applied Microbiology,* 41**,** 355-360.

THOMAS, C., HILL, D. J. & MABEY, M. 1999. Evaluation of the Effect of Temperature and Nutrients on the Survival of *Campylobacter spp.* in Water Microcosms. *Journal of Applied Microbiology,* 86**,** 1024-1032.

THOMAS, T. H. & MARTINSON, D. B. 2007. Roofwater Harvesting: A Handbook for Practitioners. Delft, The Netherlands: IRC International Water and Sanitation Centre.

THOMPSON, T., SOBSEY, M. & BARTRAM, J. 2003. Providing Clean Water, Keeping Water Clean: An Integrated Approach. *International Journal of Environmental Health Research,* 13**,** S89-S94.

TIAN, Q., CHEN, J., ZHANG, H. & XIAO, Y. 2006. Study on the Modified Triphenyl Tetrazolium Chloride - Dehydrogenase Activity (TTC-DHA) Method in Determination of Bioactivity in the Up-Flow Aerated Bio-Activated Carbon Filter. *African Journal of Biotechnology,* 5**,** 181-188.

TOPP, E., WELSH, M., TIEN, Y.-C., DANG, A., LAZAROVITS, G., CONN, K. & ZHU, H. 2003. Strain-Dependent Variability in Growth and Survival of *Escherichia coli* in Agricultural Soil. *FEMS Microbiology Ecology,* 44**,** 303-308.

TRAINOR, V. C., UDY, R. K., BREMER, P. J. & COOK, G. M. 1999. Survival of *Streptococcus pyogenes* Under Stress and Starvation. *FEMS Microbiology Letters,* 176**,** 421-428.
UHL, W. & SCHAULE, G. 2004. Establishment of HPC (R2A) for Regrowth Control in Non-Chlorinated Distribution Systems. *International Journal of Food Microbiology,* 92**,** 317-325.
UNESCO, WHO & UNEP 1996. Water Quality Monitoring: A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. *In:* BARTRAM, J. & BALLANCE, R. (eds.). UNESCO, WHO and UNEP using E and FN Spon.
UNICEF & WHO 2010. Progress on Sanitation and Drinking-Water: 2010 Update. 2010 ed. France: UNICEF and WHO.
UNICEF & WORLD HEALTH ORGANIZATION 2012. Progress on Drinking Water and Sanitation. 2012 Update. United States of America: WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation.
UNITED NATIONS 2012. The Millennium Development Goals Report 2012. New York, United States of America: United Nations.
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY. 2012. *Volunteer Monitoring* [Online]. USA: United States Environmental Protection Agency. Available: http://water.epa.gov/type/watersheds/monitoring/vol.cfm [Accessed 10 July 2012].
UNIVERSITY OF BRISTOL. 2012. *Aquatest Research Programme* [Online]. Bristol: University of Bristol, Water and Health Research Centre, Department of Civil Engineering. Available: http://www.bristol.ac.uk/aquatest/documents/brochure.pdf http://www.bristol.ac.uk/aquatest/ [Accessed 10 July 2012].
VAN DE VOORDE, A., CHEBBO, G. & GROMAIRE, M.-C. Rainwater Harvesting: What are the Potential Effects of Roof Maintenance on Runoff Quality? France as an Example. 9th World Wide Workshop for Young Environmental Scientists WWW-YES-Brazil-2009: Urban Waters: Resource or Risks?, 26-30 October 2009 Belo Horizonte, MG : Brazil.
VAN POUCKE, S. O. & NELIS, H. J. 2000. Rapid Detection of Fluorescent and Chemiluminescent Total Coliforms and *Escherichia coli* on Membrane Filters. *Journal of Microbiological Methods,* 42**,** 233-244.
VANCANNEYT, M., LOMBARDI, A., ANDRIGHETTO, C., KNIJFF, E., TORRIANI, S., BJÖRKROTH, K. J., FRANZ, C. M. A. P., FOULQUIÉ MORENO, M. R., REVETS, H., DE VUYST, L., SWINGS, J., KERSTERS, K., DELLAGLIO, F. & HOLZAPFEL, W. H. 2002. Intraspecies Genomic Groups in *Enterococcus faecium* and Their Correlation With Origin and Pathogenicity. *Applied and Environmental Microbiology,* 68**,** 1381-1391.
VANTARAKIS, A., VENIERI, D., KOMNINOU, G. & PAPAPETROPOULOU, M. 2006. Differentiation of Faecal *Escherichia coli* from Humans and Animals by Multiple Antibiotic Resistance Analysis. *Letters in Applied Microbiology,* 42**,** 71-77.
VEIGA, P., JUSTE, C., LEPERCQ, P., SAUNIER, K., BÉGUET, F. & GÉRARD, P. 2005. Correlation between Faecal Microbial Community Structure and Cholesterol-to-Coprostanol Conversion in the Human Gut. *FEMS Microbiology Letters,* 242**,** 81-86.
VENKOBACHAR, C., KUMAR, D., TALREJA, K., KUMAR, A. & LYENGAR, L. J. 1994. Assessment of Bacteriological Water Quality Using Modified H_2S Strip Test. *Water SRT Aqua,* 43**,** 311-314.
VENTER, S. N. 2000. Rapid Microbiological Monitoring Methods: The Status Quo. Available:

http://iwahq.org/contentsuite/upload/iwa/Document/RAPID_MICROBIOLOGICAL_MONITORING_METHODS_2000.pdf.
VENTER, S. N., KUHN, A. L. & HARRIS, J. 1998. Method for the Prioritization of Areas Experiencing Microbial Pollution of Surface Water. *Water Science and Technology,* 38**,** 23-27.
VERMAAK, J. & VAN NIEKERK, D. 2004. Disaster Risk Reduction Initiatives in South Africa. *Development Southern Africa,* 21**,** 555-574.
VILJOEN, F. C. A Critical Overview of Water Quality Management into the Future – A Holistic Approach is Essential. WISA 2006 Biennial Conference and Exhibition, 2006 Durban, South Africa.
VOLLAARD AM, A. S. V. A. H. H. & ET AL. 2004. Risk Factors for Typhoid and Paratyphoid Fever in Jakarta, Indonesia. *JAMA: The Journal of the American Medical Association,* 291**,** 2607-2615.
WADE, T. J., PAI, N., EISENBERG, J. N. S. & COLFORD JR, J. M. 2003. Do U.S. Environmental Protection Agency Water Quality Guidelines for Recreational Waters Prevent Gastrointestinal Illness? A Systematic Review and Meta-Analysis. *Environmental Health Perspectives,* 111**,** 1102-1109.
WANG, Y.-C., YU, R.-C. & CHOU, C.-C. 2002. Growth and Survival of Bifidobacteria and Lactic Acid Bacteria During the Fermentation and Storage of Cultured Soymilk Drinks. *Food Microbiology,* 19**,** 501-508.
WARD, S. L. 2010. *Rainwater Harvesting in the UK : A Strategic Framework to Enable Transition from Novel to Mainstream.* Doctor of Philosophy in Engineering, University of Exeter.
WARING, S. C. & BROWN, B. J. 2005. The Threat of Communicable Diseases Following Natural Disasters: A Public Health Response. *Disaster Management and Response,* 3**,** 41-47.
WEON, S.-Y., LEE, C.-W., LEE, S.-I. & KOOPMAN, B. 2002. Nitrite Inhibition of Aerobic Growth of *Acinetobacter sp. Water Research,* 36**,** 4471-4476.
WETZEL, R. 2001. Bacterioplankton. *In:* ANONYMOUS (ed.) *Limnology: Lake and River Ecosystems* 3rd ed. San Diego, CA, USA: Academic Press.
WHO 2003. Heterotrophic Plate Counts and Drinking-Water Safety: The Significance of HPCs for Water Quality and Human Health. *In:* BARTAM, J., COTRUVO, J., EXNER, M., FRICKER, C. & GLASMACHER, A. (eds.). IWA Publishing.
WHO. 2005. Flooding and Communicable Diseases Fact Sheet: Risk Assessment and Preventative Measures. Available: http://www.who.int/diseasecontrol_emergencies/guidelines/CDs%20and%20flooding%20fact%20sheet_2005.pdf [Accessed 11 Septeber 2012].
WHO. 2012. *Burden of Disease and Cost-Effectiveness Estimates* [Online]. WHO. Available: http://www.who.int/water_sanitation_health/diseases/burden/en/index.html [Accessed 7 August 2012].
WHO & UNICEF 2004. Meeting the MDG Drinking Water and Sanitation Target: A Mid-Term Assessment of Progress. *WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation.* Geneva, Switzerland: World Health Organization
United Nations Children's Fund.
WHO & UNICEF 2005. Water for Life : Making it Happen. *WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation.* France: WHO
UNICEF.
WHO & UNICEF 2010a. Progress on Sanitation and Drinking Water 2010 Update. *Joint Monitoring Programme for Water Supply and Sanitation.* France.

WHO & UNICEF. 2010b. *Types of Drinking-Water Sources and Sanitation* [Online]. JMP. Available: http://www.wssinfo.org/definitions-methods/watsan-categories/ [Accessed 15 September 2012].
WHO & UNICEF. 2010c. WHO / UNICEF Joint Monitoring Programme for Water Supply and Sanitation : Estimates for the Use of Improved Drinking-Water Sources. Available: http://www.doh.gov.za/docs/stats/2010/drinkingwater2010.pdf.
WHO & UNICEF. 2012. Millennium Development Goal Drinking Water Target Met, Sanitation Target Still Lagging Far Behind. 6 March 2012.
WIGGINS, B. A., CASH, P. W., CREAMER, W. S., DART, S. E., GARCIA, P. P., GERECKE, T. M., HAN, J., HENRY, B. L., HOOVER, K. B., JOHNSON, E. L., JONES, K. C., MCCARTHY, J. G., MCDONOUGH, J. A., MERCER, S. A., NOTO, M. J., PARK, H., PHILLIPS, M. S., PURNER, S. M., SMITH, B. M., STEVENS, E. N. & VARNER, A. K. 2003. Use of Antibiotic Resistance Analysis for Representativeness Testing of Multiwatershed Libraries. *Applied and Environmental Microbiology,* 69**,** 3399-3405.
WILKINSON, M., DU TOIT, A., MASHIMBYE, D. & COLLIGNON, S. 2012. Assessment of Handwashing and Hand Hygiene Behaviour. Pretoria, South Africa: Water Research Commission (WRC).
WILLIAMSON, A. 2006. Feasibility Study of a Water Safety Data Collection for Beaches. *Journal of Science and Medicine in Sport,* 9**,** 243-248.
WILSON-JONES, T. & RIVETT, U. 2012. Using Mobile Phones to Monitor and Manage Water Supply Quality in Rural Environments. *WISA 2012 Biennial Conference and Exhibition.* Cape Town.
WILSON, M. 2005. *Microbial Inhabitants of Humans: Their Ecology and Role in Health and Disease*, Cambridge, United Kingdom, Cambridge University Press.
WINFIELD, M. D. & GROISMAN, E. A. 2003. Role of Nonhost Environments in the Lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology,* 69**,** 3687-3694.
WONGA, K., FONG, T.-T., BIBBY, K. & MOLINA, M. 2012. Application of Enteric Viruses for Fecal Pollution Source Tracking in Environmental Waters. *Environment International,* 45**,** 151-164.
WOOD, A. J. J. & MURRAY, B. E. 2000. Vancomycin-Resistant Enterococcal Infections. *New England Journal of Medicine,* 342**,** 710-721.
WORLD HEALTH ORGANISATION 2006. Guidelines for Drinking-water Quality:First Addendum to Third Edition 2006: Volume 1 Recommendations. Geneva, Switzerland: World Health Organisation.
WORLD HEALTH ORGANIZATION 2011. Guidelines for Drinking-Water Quality. 4th ed. Malta: Gutenberg.
WORM, J. & VAN HATTUM, T. 2006. Rainwater Harvesting for Domestic Use. Wageningen: Agromisa Foundation and CTA.
WPDM 1999. White Paper on Disaster Management. Pretoria, South Africa.
WRIGHT, J., GUNDRY, S. & CONROY, R. 2004. Household Drinking Water in Developing Countries: A Systematic Review of Microbiological Contamination between Source and Point-Of-Use. *Tropical Medicine and International Health,* 9**,** 106-117.
WRIGHT, J. A., YANG, H., RIVETT, U. & GUNDRY, S. W. 2012a. Public Perception of Drinking Water Safety in South Africa 2002-2009: A Repeated Cross-Sectional Study. *BMC Public Health,* 12**,** 556.
WRIGHT, J. A., YANG, H., WALKER, K., PEDLEY, S., ELLIOTT, J. & GUNDRY, S. W. 2012b. The H_2S Test Versus Standard Indicator Bacteria Tests for Faecal

Contamination of Water: Systematic Review and Meta-Analysis. *Tropical Medicine and International Health,* 17**,** 94-105.
WRIGHT, R. T. 1978. Measurement and Significance of Specific Activity in the Heterotrophic Bacteria of Natural Waters. *Applied and Environmental Microbiology,* 36**,** 297-305.
WUERTZ, S., WANG, D., REISCHER, G. H. & FARNLEITNER, A. H. 2011. Chapter 4: Library-Independent Bacterial Source Tracking Methods. *In:* HAGEDORN, C., BLANCH, A. R. & HARWOOD, V. J. (eds.) *Microbial Source Tracking: Methods, Applications, and Case Studies.* Springer.
WUTOR, V. C., TOGO, C. A. & PLETSCHKE, B. I. 2009. Suitability of Total Coliform β-Galactosidase Activity and CFUs Counts in Monitoring Faecal Contamination of Environmental Water Samples. *Water SA,* 35**,** 85-88.
YILLIA, P. T., KREUZINGER, N. & MATHOOKO, J. M. In-Stream Activities Influence Microbial Water Quality of a Shallow Mountain Stream in Rural Kenya. 8th WaterNet/WARFSA/GWP-SA IWRM Symposium, 31 October – 2 November 2007 2007 Lusaka, Zambia.
ZAMBERLAN DA SILVA, M. E., SANTANA, R. G., GUILHERMETTI, M., FILHO, I. C., ENDO, E. H., UEDA-NAKAMURA, T., NAKAMURA, C. V. & DIAS FILHO, B. P. 2008. Comparison of the Bacteriological Quality of Tap Water and Bottled Mineral Water. *International Journal of Hygiene and Environmental Health,* 211**,** 504-509.
ZOU, L. K., WANG, H. N., ZENG, B., LI, J. N., LI, X. T., ZHANG, A. Y., ZHOU, Y. S., YANG, X., XU, C. W. & XIA, Q. Q. 2011. Erythromycin Resistance and Virulence Genes in *Enterococcus faecalis* from Swine in China. *New Microbiologica,* 34**,** 73-80.
ZUMA, B. M. 2010. *Microbial Ecology of the Buffalo River in Response to Water Quality Changes.* Msc, Rhodes University.
ZUMA, B. M., LUYT, C. D., CHIRENDA, T. G. & TANDLICH, R. 2012. Flood Disaster Management in South Africa: Legislative Framework and Current Challenges. *International Conference on Applied Life Sciences (ICALS2012)* Konya, Turkey