A Large-Scale Study of Microbial and Physico-Chemical Quality of Selected Groundwaters and Surface Waters in the North West Province, South Africa

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by

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EXECUTIVE SUMMARY

BACKGROUND AND RATIONALE

Water from the North West Province (NWP) catchment areas supports prosperous gold and platinum mining, manufacturing industries, agricultural sector as well as a growing urban and rural population. This land-locked province thus contributes generously to the Gross Domestic Product of South Africa. However, water allocation for the province has almost reached the quota available, based on surface water estimates. Furthermore, there are reports that the source water within the catchment may be exposed to pollution from various sources but particularly from economic activities. These reports have demonstrated that several surface waters and groundwaters are faecally contaminated and some with opportunistic pathogenic bacteria.

Faecal pollution of surface waters has been blamed on service delivery problems in several peri-urban areas and small towns. There are also reports of poorly treated and in some cases untreated effluent being discharged into rivers and other receiving waters. This practice has health, economic as well as environmental implications. The growth in the agricultural and mining sectors has resulted in increased water requirements as well as pollution. These developments have also played a role in human population distribution dynamics. Such economic activities were responsible for the development of major growth points in the province.

Mining activities are mainly centred in the eastern part of the province and the agricultural development across the province. Mining operations were (in some cases still are) responsible for decant of heavy metal-containing water of low pH (acid mine drainage: AMD) into surface water. This has devastating ecological and economics effects. The real impacts of the AMD on groundwater in the North West Province are undetermined. Water pollution from agricultural runoff from livestock farming, including poultry farming and feedlots as well as several other practices in the province is also undetermined.

Rainfall in the eastern and central parts of the province is higher than the western part. The latter borders on and is part of the Kalahari Desert. Further social and economic developments as well as climate change will impact on the anticipated water availability, requirements and quality of water. Baseline data in these categories will be important for long-term planning. Water requirements are national priorities and processes such as National Water Resource Strategy are in place. However, detailed large water quality studies have not been conducted. The present study was an effort to address this gap. It extended the WRC-funded work done by Bezuidenhout (2011).

AIM

1. To determine the water quality of surface and groundwater in the North West Province from chemico-physical and microbiological perspectives.

2. To determine the profile of selected gastroenteritis-associated pathogens in the contaminated water sources.

3. To investigate the usefulness of molecular fingerprinting data in microbial water quality determination.

4. To determine the cytotoxic effects of associated with consumption of untreated water as well as such effects exerted by some of the indicator mycological and bacterial species isolated from the water sources.

5. To investigate potential risk of consuming such water without any prior treatment.

6. To provide an overview of previous large-scale water quality studies conducted nationally and internationally.

7. To generate a report on whether human health risks could be associated with current trends of water provision in the North West Province and if necessary suggest improvement strategies.

The following describes the scope of the work in each chapter:

Chapter 2: The purpose of this work was to generate data on microbiological and physico-chemical quality of surface water sources in the North West Province. A secondary goal was to establish skills with respect to several research methodologies. These technologies include determining levels of (a) culturable indicator bacteria (faecal coliforms, enterococci), (b) yeasts, (c) pathogenic *E. coli* (d) bacteria by culture independent microbial detection methods (Denaturing Gradient Gel Electrophoresis and High Throughput Sequencing) and (e) enteroviruses and bacteriophages.

Chapter 3: The purpose was to generate data on microbiological and physico-chemical quality of groundwater using some of the methodologies and methods referred to in Chapter 2.

Chapter 4: The purpose was to evaluate faecal sterol determination in water quality applications in the North West Province.

Chapter 5: The purpose was to evaluate the various bacteria and fungi isolated during this study for potential human health effects and to develop a cost an effective method to determine the cytotoxicity effects of polluted water on intestinal cells.

Chapter 6: The purpose was to conduct a social and water management study in order to identify the most important social aspects for future research and interventions in the province.

METHODOLOGY

Water samples were collected at specific time periods from selected groundwater and surface water sources in the North West Province using standard procedures. An effort was made to include dry winter as well as wet summer sampling periods for each of the sites. The groundwater sampling sites were grouped into sampling areas so groundwater locations in this report refer to an area, not specific boreholes.

Standard methodologies were used to collect and analyse the water samples. Global positioning data (GPS waypoints) were recorded and used to generate the sampling maps. Certain physical and chemical (temperature, pH, electrical conductivity) parameters were measured on site using multi-probe equipment. Chemical parameters such as chemical oxygen demand, nitrates, phosphates etc. were measured in the laboratory using Hach kits and equipment. The levels of faecal indicators and other microbial organisms were determined using standard or previously-published methods. Standard biochemical and DNA sequencing methods were used for identification of microbes. The presence of *E. coli* and pathogenic *E. coli* were determined in water using direct DNA isolation and PCR. Faecal sterol levels were determined by the Szűcs method which involves liquid-liquid extraction and resolution by Gas Chromatography-Mass Spectrometry. Levels of bacteriophages were determined by standard methods and enterovirus by qPCR based methodology.

A sequential exploratory mixed-methods design was used for the social water management study. A mixed-methods approach includes the use of both qualitative and quantitative research methods to collect, analyse and interpret the research data. Interviews, focus group discussions and observations (n = 25 participants) were used as the main data gathering methods during the qualitative phase. This was followed by the development of a research questionnaire and a community survey (n = 1 000 participants) during the quantitative phase of the study.

RESULTS AND DISCUSSION

Microbiological and physico-chemical quality of surface water sources

Selected river systems covering the upper middle and lower Vaal river management areas were targeted. Average physico-chemical and microbial levels measured at some sites during the 2010 and 2011 seasons were elevated. Some exceeded the Target Water Quality Range (TWQR) for full and intermediate recreational contact, livestock watering and irrigation. Elevated electrical conductivity when compared to TWQR for drinking water was not at levels likely to cause health impacts. However, this elevated EC could be indicative of increasing salinisation occurring. This may have long-term effects on agriculture activities but also on drinking water production. Electrical conductivity should be flagged as a parameter that is critical to monitor.

Enterococcus spp. as well as *Escherichia coli* were isolated from various surface water sources. Isolation of enterococci such as *E. faecium* and *E. faecalis* from surface waters illustrates that any full contact activity in the water is risky and could result in infectious disease. Bacteriophage data supported the general findings of the bacterial analysis, namely that many of the water sources in the North West Province have some form of faecal pollution. The following yeast genera were also isolated and identified in surface waters: *Candida*, *Clavispora, Cryptococcus, Cystofilobasidium Hanseniaspora*, *Meyerozyma*, *Pichia*, *Rhodotorula, Saccharomyces*, *Sporidiobolis*, and *Wickerhamomyces*. Amongst these are several opportunistic pathogenic species that could cause invasive infections when individuals come into contact with contaminated water.

Baseline culture-independent molecular profiling (Polymerase Chain Reaction Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and Next Generation Sequencing (NGS)) data of the dominant bacterial communities in the Vaal River are also provided. Although DGGE profiling has some limitations it allowed for the identification of the bacterial community composition and dynamics in the planktonic component in this river system. Next generation sequencing or high through-put sequencing (HTS) analysis presented a better resolution of the bacterial diversity and dynamics in the Vaal River. This technology was not suitable for detecting faecal indicator bacteria in surface water samples that contained known high levels of *E. coli* and enterococci.

Results presented here demonstrated that some of the wastewater treatment plants (WWTPs) were decanting huge concentrations of enterovirus particles into surface water sources. This was probably due to the operational challenges at the plants. Some other plants in the province were operating in such a manner that up to 99.99% of enteroviruses were removed. Impacts of municipal WWTPs as well as other forms of faecal pollution on virus diversity and dynamics in water sources should be further investigated. Such a study should focus on the percentage of viable viruses that are reaching the water bodies and the epidemiology thereof. In such a virology study the presence of other relevant viruses should also be targeted. Furthermore, detection of bacteriophages in the surface waters that are associated with faecal pollution supports the finding that some of the targeted surface water sources in the North West Province are faecally polluted.

Microbiological and physico-chemical quality of groundwater

In the North West Province more than 80% of the rural community depend on groundwater for all their water needs. The approach was to mainly target boreholes that provided water for domestic use. This included urban, peri-urban and rural areas. A total of 114 boreholes were sampled. Physico-chemical analyses demonstrated that the pH and EC levels were at acceptable ranges for domestic use. However, it was found that only 28% of the boreholes tested complied with the South African TWQR for nitrate (<6 mg/ℓ), and 43% of the boreholes had nitrate levels greater than 20 mg/ℓ. This study also demonstrated that groundwater from the North West Province is vulnerable to nitrate contamination.

In 2009 49% of the 76 boreholes tested were positive for faecal coliforms and 67% for faecal streptococci. Forty seven percent of these boreholes were also positive for presumptive *P. aeruginosa* and 7% for *S. aureus*. 33% of the boreholes had heterotrophic plate bacteria exceeding 1000 cfu/ml. In 2010, 38 boreholes were sampled. Of these 55% were positive for faecal coliforms and 63% for faecal streptococci. During this sampling period 55% of the boreholes were positive for *P. aeruginosa*. Detection of faecal indicators was higher in the warm wet seasons than the cold dry season. This was observed in both the number of positive results as well as levels detected. Members of the *Enterobacteriaceae* family identified included *E. coli* and *K. pneumonia*.

Based on MLGA results, 34% of the boreholes sampled in 2010 were positive for *E. coli.* However, using multiplex PCR on DNA directly isolated from water samples it was found that

E. coli was present in 47% of boreholes. This indicates that the membrane filtration approach underestimated the presence of *E. coli* in borehole water in the NWP. Using FC/FS (faecal coliform/faecal streptococci) ratio determination, it was estimated that at least 14% of the boreholes tested had FC/FS ratio >2, indicating potential human faecal contamination.

Next generation sequencing (or high through-put sequencing) results provided data on the microbial diversity in borehole water of the NWP. This method is rapid and provided information about the bacterial community structure in these water samples. It was demonstrated that the winter and summer bacterial compositions were different. In the summer samples there were Gamma and Beta proteobacteria as well as Actinobacteria present. *Enterobacter* sp., *Proteus* sp., *Stenotrophomonas maltophilia* are species that were always detected among the Gamma proteobacteria. Sequences from *E. coli* as well as *Pseudomonas* sp. and *S. aureus* were also detected in the summer samples. These results are consistent with the results from the culture dependent method. Next generation sequencing (or HTS) thus holds promise as a method for monitoring bacterial indicator species in groundwater. This, however, needs to be further tested.

The results presented here indicated that microbiological quality of groundwater in the North West Province may be of concern as more than 75% of boreholes were positive for faecal pollution. This indicates that groundwater should be tested and treated before being supplied to communities. It was, however, demonstrated that 23% of the boreholes tested negative for both faecal coliforms and faecal streptococci. This result indicates that there are boreholes where no faecal pollution has occurred. This could be due to protection of the borehole and thus the aquifer. Management practices should be put in place to prevent pollution of aquifers.

Faecal sterol determination in water quality applications

In this study the Szűcs method, an established method for detecting faecal sterols in water, was used to detect six target sterols (coprostanol, cholesterol, dehydrocholesterol, stigmasterol, β-sitosterol, and stigmastanol) in water samples from the NWP. Wastewater treatment plant influent and effluent samples were collected and analysed for human faecal sterol biomarkers. Environmental water samples were spiked with faeces from cattle, chickens, horses, pigs, and sheep. All the samples were subjected to liquid-liquid extraction, silylation and derivatisation. Derivatised samples were analysed by GC-MS. The method was evaluated for quantitation and differences between the water samples from each species. Standard curve assays were linear up to 160 ng/ℓ and the limit for quantification was 20 ng/ℓ. Coprostanol was the human faecal sterol biomarker, while herbivore profiles were dominated by terrestrial sterol biomarkers (stigmasterol and stigmastanol). Differences in sterol fingerprints and concentrations between various animals and humans provide the opportunity to determine the origin of faecal pollution.

Water samples collected from boreholes and rivers were analysed for faecal sterols, physico-chemical properties and bacteriological quality. Coprostanol and cholesterol were detected in one groundwater sample. These results were in some cases supported by bacteriological data and in other cases not. Sterols may become bound to soil particles. It is therefore not expected that sterols will be detected in groundwater. Their detection in groundwater in this study should therefore be further explored.

Surface water samples were also analysed for faecal sterols using the Szűcs method. Cholesterol was detected in some samples and was the only faecal sterol detected. This indicates that the faecal pollution at these sites were likely to be from animals, not humans. Faecal sterol analysis is a powerful method and has potential to distinguish between faecal pollution from various animal as well as human sources.

Potential human health effects

The main human health concern is the high level of nitrates in some of the groundwater sources, some exceeding the 20 mg/ℓ level that could cause methaemaglobinemia. This was not the case with surface water sources in associated areas. Signs of salinisation were observed for some of the surface water samples. However, at the moment this cannot be linked to human health concerns.

Faecal indicator and opportunistic bacteria were regularly detected in surface and groundwater sources of the NWP. It was shown that pathogenic *E. coli* may also be present among the environmental *E. coli* population. Various known opportunistic pathogenic enterococci were regularly detected in surface waters. *Pseudomonas* spp. were also regularly detected in groundwaters. Among the yeasts isolated several opportunistic pathogenic species were also regularly detected. These studies were conducted over two separate sampling periods, about one year apart. The results suggest that these bacteria (and in the case of surface waters also yeast) could be ubiquitous. This is cause for concern and regular monitoring of such sites is proposed. It could indicate that pathogenic microorganisms such as viruses (enterovirus, adenovirus and hepatitis A & B) and bacteria (*Vibrio cholerae, Shigella* spp. *Yersina* spp. and *Enterocolitica* spp.) may also be present in these water sources. This study has also detected bacteriophages associated with faecal pollution. The phages are surrogates for human viruses. What the study has further demonstrated is that entoviruses are being discharged into receiving surface waters. This study did not determine whether the viruses were viable. However, if one considers that faecal bacteria detected in the water sources were all viable there is a chance that the viruses may also be viable. The detection of virus genetic material in a water source is thus a cause for concern. All these findings demonstrate that people in the North West Province that directly use untreated water for household purposes or recreation may be exposed to several pathogenic or opportunistic pathogens. Such exposures imply that the health of these individuals may be compromised.

Amongst the enterococci isolates from surface waters, up to 20% displayed β-haemolysis, a character that demonstrates the production of an enzyme that is associated with virulence. Furthermore, dominant multiple antibiotic resistance patterns were observed for faecal streptococci isolates at most sites in both years. Between 40 and 55% of the isolates were resistant to selected β-lactam antibiotics including Penicillin G. Not all the isolates that were resistant to Penicillin G were resistant to Ampicillin or Amoxillin. A large proportion was also resistant to Vancomycin. Up to 68% was resistant to Neomycin and 56% to Ciprofloxacin. Most of the isolates (up to 99%) were susceptible to Streptomycin. All β-haemolysis positive isolates (potential pathogenic) were resistant to 3 and more antibiotics from various antibiotic groups. Similar results were observed for both study periods

Several of the faecal coliform isolates from surface water were resistant to multiple antibiotics, especially β-lactam antibiotics. Table 1 is a summary of the percentage of faecal coliforms that were resistant to antibiotics.

Table 1: Antibiotic resistance profiles (%) among faecal coliforms isolated in 2009 and 2010.

	2009	2010
Amoxyllin	54	21
Ampicillin	41	15
Cephalothin	40	42
Oxytetracyclin	30	22
Trimetroprim	11	8

Fewer isolates in 2010 were resistant to β-lactam antibiotics compared to those from 2009. However, resistance to Cephalothin, a cephem β-lactam, was similar for both sampling periods.

These results are of concern, particularly if one considers the potential of the isolates to cause invasive infections in sensitive individuals. In such cases antibiotics may not be effective. The cause of the resistance is unknown. A future investigation into the likely causes may be of value.

A colorimetric method which uses duodenum cells was developed to determine cytoxicity of polluted water. This method has the potential to provide direct answers on the cytotoxic potential of untreated water within 24 hours. It makes use of a cytotoxic index that was developed in this study in which the percentage cytotoxicity is determined and then compared to a negative control. In the present preliminary study the percentage cytotoxicity of tap water and water from a protected borehole was very low compared to untreated river and dam water, untreated and treated sewage. This method is still under development. The refined method will be tested in follow-up studies.

A social and water management study

Five different themes emerged during the qualitative phase of the study of the interactions of communities with water, namely: **perceptions about water**, with water quality, availability and management as three sub-themes; **beliefs or attitudes towards water**, with ‘water should be free’ and spiritual and cultural connections as two sub-themes; **sources of water**, where most participants produced a list of available sources; **uses of water**, with physical (physiological) needs, everyday household use, recreational purposes and religious/spiritual and cultural purposes as four sub-themes; and **water management**.

The quantified and verified results indicate that there are no meaningful differences between the two districts (Dr Keneth Kaunda and Bophirima) regarding their perceptions about the quality of their water or its availability. The majority of the participants perceive the quality of their water to be average (72%), while 25% thought that their water is good, 1% that their water is very good/ excellent and only 3% that it is of poor quality. In addition, the majority of the participants in each of the two districts perceives their water to be a limited resource (Dr Keneth Kaunda district = 85%; and Bophirima = 85%) but differed in their opinion regarding how their water is managed. In Bophirima, almost half of the participants (47%) said that they think their water is not managed correctly, while the minority of participants in the Dr Kenneth Kaunda district (28%) thought their water is managed incorrectly. Ninety percent of participants felt that they should not have to pay for water. They also felt that water must be used sparsely and be conserved.

With regard to their beliefs and attitudes towards water, almost half of the participants said that they have a spiritual connection with water (45%) and that they use water for example to cleanse themselves or others after a funeral ceremony (75%) or to make contact with their ancestors (45%).

The study also confirmed that the majority of households make use of municipal water (77%). Sources of water did not differ greatly between the two districts, except in regard to the harvesting of rain water. In the Bophirima district, communities make more use of rain water harvesting (32%), compared to the Dr Kenneth Kaunda district where only 11% harvest water. Other sources of water such as a borehole with a windmill (21%), or a borehole with an electrical pump (10%), seasonal pans (6%), fountains (5%), dams and rivers (4%) and wells (4%) are used to a lesser extent.

A number of uses differed statistically and practically between the two districts. In the Bophirima district, traditional uses such as using water to drive out evil spirits (cleansing themselves or members of their family or house) differed from the Dr Keneth Kaunda district (Table 2). It demonstrates that more individuals in the Bophirima district uses their water for the purposes listed compared to the Dr Kenneth Kaunda district participants.

Table 2: Recreational and traditional uses of water (%).

	Bophirima	Dr Kenneth Kaunda
Drive out spirits	75	50
Make traditional medicine	81	57
Recreational purposes	75	55
Harvest food	43	20
Livestock watering	70	28

Other popular uses of water that scored above 88% include the use of water for house building or other physical structures (for example in combination with soil) (94.2%), to cook their food (99%), to drink (98%), and to flush their toilet (96%). More than 90% indicated that water helps them when they are fasting (92%). Ninety seven percent indicated that water is used during religious ceremonies (e.g. washing their own or other’s feet before church) Almost all the participants indicated that they use water to wash their goods (cleaning of physical objects other than themselves) (99.3%). More than 19% indicated that they use water for gardening (domestic plants) (93.2). Eighty eight percent indicated that they use water to make traditional beer (*umqhombothi*).

The use of water for self-cleansing (enema) seemed less popular (68%). Seventy six percent indicated that they use water to initiate the traditional healer/s in their community. More than 60% indicated that they use water to wash themselves or others after a funeral service. Amongst all the participants 28% were farmers using water on a large scale to produce crops.

The majority of households still appear to make use of a tap in their yard. Fewer had access to piped water in their houses. A section of the communities was still using a communal tap for which they have to travel less than 50 meters or to a lesser extent more than 50 meters. Finally, most households appear to store water inside their homes (65%) where it is cooled down in most cases (83%). However, communities in the Bophirima district tend to store water more often in containers outside their house (56%) compared to those participants from the Dr Keneth Kaunda district (34%).

CONCLUSIONS

The purpose of the research was to conduct a large-scale study of microbial and physico-chemical quality of selected surface waters and groundwaters in the North West Province, South Africa. The sampling period was from 2009 to 2011. Results have shown various trends.

- Nitrates in groundwater and salts in surface water are the main physico-chemical hazards.
- A number of the groundwater and surface water sources in the North West Province are polluted with faecal matter. The faecal pollution was demonstrated by various methods including standard culture methods, direct DNA isolation and PCR, faecal sterols, bacteriophages and in the case of groundwater, also next generation sequencing.
- Bacteriophage and enterovirus data indicated that the sources may also contain viruses that could be human pathogens.
- Large numbers of faecal coliforms and enterococci isolated in this study were resistant to several antibiotic groups. This is cause for concern as it may eventually have human and animal health as well as plant pathology implications.
- A cytoxicity test to determine the impacts of microorganisms on human cell cultures was also developed as part of this study. It may be useful in future studies where water quality and suitability for human consumption is determined. The adaptations

proposed in the study make it more rapid and precise but also, at the moment, more costly to conduct.

- Baseline data on social aspects of source and drinking water management was also provided for 6 communities from 2 of the 4 districts of the North West Province. The study presented some data on how members from these communities interact with and manage water. It also demonstrated that certain perceptions, beliefs and behaviours are associated with these interactions. The results demonstrated that water has important social and cultural meaning and importantly, that future education programmes could build on knowledge existing with these communities.

RECOMMENDATIONS FOR FUTURE RESEARCH

- Sources of high EC values should be investigated as this could have serious implications on soil quality in the province and by implication also food security. It may eventually also impact on drinking water purification processes. Long-term studies are thus necessary to generate data that would be useful for predictive modelling studies. Such data would also be useful to provide advice to agricultural and water purification authorities.
- Boreholes should be identified that may be vulnerable to certain types of contamination. Data from the present study will be useful in identifying areas for further investigation. Once identified appropriate interventions or treatment options could then be put in place before such water is supplied as safe for human or animal consumption.
- Sources of faecal pollution in surface water should also be identified and programmes be put in place to ensure that this type of pollution is prevented. Data from the present study could be used to identify specific areas in the selected rivers that should be further investigated for faecal pollution sources.
- Results from this study have demonstrated that a full health risk assessment should be conducted on some of the water sources in the province. This is needed particularly within the context of the large section of the community of the NWP, particularly in rural and peri-urban centres, that are immuno-compromised. At present health effects of consuming the faecally-contaminated water could be under-reported and a detailed study is thus necessary
- The cause of multiple antibiotic resistance and the mechanisms involved should be further investigated. This was not established in the present study but should be determined. Broader understanding of antibiotic resistance may provide means to predict the spread of the resistance and in the process provide tools to curb the

spread. As more pathogens become resistant to available drugs the search for newer more effective drugs may drain resources. This could result in a vicious circle in the treatment of human, animal and plant infectious diseases.

- Future studies to determine the presence of *E. coli* and other indicators in the water should be conducted also using PCR (qPCR) methods instead of only culture based methods. Quantitative PCR (qPCR) is more accurate and rapid compared to plating methods. Analysis times will be decreased and larger numbers of samples could be analysed.
- In-depth HTS (and culture-based) analysis of the microbial diversity and water quality in the surface and groundwater is recommended with the focal point on the identification of specific species within the major key groups. This should be conducted for viruses, bacteria, yeasts, fungi and other eukaryotes that may have health implications. Culture dependent methods are time consuming and a limited number of samples are normally analysed. These methods also require specific media types for specific microorganisms and many important microorganisms may not be detected in samples. Methods based on analysis of genetic material (HTS, qPCR) are more efficient than culture dependent methods.
- More knowledge about the social context and management of water at a community level in the North West Province should also be obtained. In the present study data were collected from only 6 communities in 2 of the 4 districts of the province. These communities represent only a small section of the NWP and should thus be extended to include more communities but also communities from the other two districts. Data contained in these reports must also be responsibly translated into information that the affected communities could relate to and use.

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AMD	Acid mine drainage
β	Beta
CSIR	Council for Scientific and Industrial Research
DGGE	Denaturing Gradient Gel Electrophoresis
DoH	Department of Health
EAEC	Entero-Aggregative *E. coli*
EC	electrical conductivity
EHEC	Entero-Hemorrhagic *E. coli*
EIEC	Entero-Invasive *E. coli*
EPEC	Entero-Pathogenic *E. coli*
ETEC	Entero-Toxigenic *E. coli*
EU WFD	European Union Water Framework Directive
FC/FS	Faecal coliform/faecal streptococci ratio
GC-MS	Gas Chromatography-Mass Spectrometery
GDP	Gross Domestic Product
HIV	Human Immuno-deficiency Virus
HPC	Heterotrophic plate count
HTS	High Through-put Sequencing
IWRM	Integrated water resources management
mg/ℓ	milligram per litre
ng/mℓ	nanogram per millilitre
NGS	Next Generation Sequencing
NMMP	National Microbial Monitoring Programmes
NWDACE-SOER	North West Department of Agriculture, Conservation and Environment – State of the Environment Report
NWP	North West Province
NWRS	National Water Resource Strategy
PCR	Polymerase Chain Reaction
qPCR	quantitative Polymerase Chain Reaction
RDA	Redundancy analysis
TDS	Total dissolved solids
Temp	Temperature
TWQR	Target Water Quality Range
USA EPA	United States of America – Environmental Protection Agency

UN-WATER	United Nations – Water
VBNC	Viable but non-culturable
WHO	World Health Organisation
WMA	Water management area
WWTP	Waste water treatment plant
µg/mℓ	microgram per millilitre

CHAPTER 1

INTRODUCTION

1.1 Water and water quality

Water quality is used to describe the chemical, physical and biological characteristics of water. This is done usually in respect to its suitability for an intended purpose (DWAF, 2005). The standards of water quality vary for domestic, agricultural and industrial uses. Furthermore, water quality differs from continent to continent as well as from region to region. This is due to differences in climate, geomorphology, geology and biotic composition (Dallas and Day, 2004). The Department of Water Affairs (DWAF, 1996) defines the Target Water Quality Range (TWQR) for a particular constituent and water use as the range of concentrations or levels at which the presence of the constituent would have no known adverse effects on the fitness of the water assuming long-term continuous use. Thus, good quality drinking water may be consumed in any desired amount without adverse effect on health. It is free from harmful bacteria, viruses, minerals, and organic substances. It is also aesthetically acceptable (in respect of taste, colour, turbidity, and odours) (Haman & Bottcher, 1986). Both natural and human factors can influence the quality of a water source. It is therefore necessary to identify the factors involved that individually or jointly affect the quality of the water source. Once these factors that negatively impact on water quality are identified, correct remediation measures can be applied and regulations to monitor and maintain a required standard can be established (Reinert & Hroncich, 1990).

1.2 Large-scale studies of water quality

From a number of international and national peer-reviewed articles and other published resources it is evident that integrated management of quantity and quality of water is critical if a good ecological status is to be maintained (Hinsby et al., 2008; Janelidze et al., 2011; Loos et al., 2009; Nickel et al., 2005; Plummer and Long, 2007; Pybus, 2002; Roux et al., 1991; Sanchez et al., 2009; Verma et al., 2008). The European Union Water Framework Directive (EU WFD) aims at achieving good ecological status of all water bodies in the EU (Achleitner et al., 2005). Several groups used this directive as the basis to develop area specific water supply decision models (Hinsby et al., 2008; Loos et al., 2009; Moss, 2004; Nickel et al., 2005; Sanchez et al., 2009; Whiteman et al., 2010).

Ioris et al. (2008) developed and applied water management sustainability indicators in water catchment areas in Brazil and Scotland. They observed that this application was a challenging one since results of sustainability indicators needed organisation and presentation to the water managers in the two countries for it not to be plagued by biases. What made this study valuable is that the two countries have different needs and requirements in so far as sustainability indicators are concerned. It also demonstrated that for implementation of water quality frameworks it is critical to determine the exact needs of an area. The latter observation was also made by Nickel et al. (2005) who used a large-scale water resources management strategy within an existing research project to investigate setting up a model that would take various aspects of the dynamics of the system into account. Moss (2004) also argued this point and demonstrated that successful governance of land use in river basins is dependent on taking existing structures and practices into account.

To do justice to delineating groundwater bodies in accordance with EU WFD requirements Sanchez et al. (2009) developed a methodology and tested it on a Spanish river basin. They found that the WFD requirements presented some problems. An alternative delineating method was proposed to solve the complexity of the process. This application demonstrated that frameworks and directives may not always be static but may need some revision for practical application. In addition to this Whiteman et al. (2010) used the WFD to determine whether significant damage occurred to groundwater-dependent wetlands in England and Wales. This assessment was, however, only based on quantitative chemical parameters. What the researchers pointed out was that insufficient data was available, even for this developed, first world region.

Loos et al. (2009) used a large-scale water quality approach and global data sets to model impacts of nutrients on the Rhine basin as the river passes through the Netherlands. This study demonstrated the usefulness of such global data sets to develop nutrient flux models for specific applications. This model also provided the opportunity for predicting the effects of climate change on certain water sustainability issues.

Besides chemical and physical parameters, microbial faecal indicators could also be used in large-scale studies. Crowther et al. (2002) used faecal-indicator concentrations to demonstrate relationships between land use and farming practices in Britain. These authors also refer to models that were being developed that may assist with evaluating effectiveness of improvement strategies for bacteriological quality of surface water in particularly rural catchment. In Australia Toze et al. (2010) used quantitative microbial risk assessment to pathogen risks in an unconfined aquifer that is used for aquifer recharge. In this study it was demonstrated how the initial level of pathogens, aquifer retention times and conditions could influence the eventual acceptable health risk for water reclaimed for drinking purposes.

Nwachuku and Gerba (2004) argued that microbial risk assessment strategies should attempt to report on health and exposure data for adults and children separately. This is so because children may have greater risk when exposed to environmentally transmitted pathogens.

From the selected examples above it is evident that legislative and administrative frameworks for water quality management, the dynamic interaction of the environment, social and economics data are required for large-scale studies. Ioris et al. (2008) argued that it is evident that sustainable water resource management does not only imply establishing and maintaining physico-chemically and biologically stable water systems but also economic efficiency of water use, equitable distribution of cost and benefits as well as participatory systems to policy and decision making.

1.3 Water quality in South Africa

Water resources of the South Africa are in the form of rivers, dams, lakes, wetlands and subsurface aquifers (DWAF, 2004a). The country has an average rainfall of 480 mm/annum. This is below the world average of 860 mm/annum. Water is thus a scarce resource in South Africa and the amount of water available for human use depends on the availability and sustainability of the resource (Stats SA, 2010a).

South Africa shares water from four of its main rivers [Inkomati, Pongola (Maputo), Orange (Senqu) and Limpopo] with neighbouring countries. Surface water resources (river and dams) contribute 77% of the total exploited water supplies across the country (Stats SA, 2010a). Groundwater contributes 9% while 14% is water which has been made available for re-use from return flows (National Treasury, 2011).

Of the available water resources 63% is used by afforestation, agriculture (livestock watering, and irrigation). Municipal and domestic demands utilise 27%, which is divided into 24% for urban areas and 3% for rural areas. Industry, mining and power generation each contribute 2% of the total water demand (DWAF, 2010).

Water is a key resource that plays a central role in many cultural and religious ceremonies (Zenani and Mistri, 2005). In terms of Section 31 of the South African Constitution (108/1996) water from rivers, streams, dams and springs may be utilised for cultural and religious purposes such as baptism and initiation ceremonies (Zenani and Mistri, 2005).

In 2011 during a parliament briefing, the Acting Chief Director General for Water Resources Information Programmes at the Department of Water Affairs stated that the health status of South Africa's rivers is deteriorating (Parliamentary Monitoring Group, 2011). Faecal pollution (Anon, 2009b; Mguni, 2009; Thom, 2010), eutrophication high salinity and toxicity were among the major challenges mentioned (van Vuuren, 2009ab; DWAF, 2009). This is worrisome, particularly when one considers the link between the state of the environment and human health (CSIR, 2010). Measures regarding the protection and quality of South Africa’s water resources are imperative when considering the current state thereof.

The South African Department of Water Affairs has introduced two water certification schemes for municipalities to encourage provision of safe drinking water (Blue drop) and to ensure wastewater (Green drop) is sustainably managed (Swanepoel, 2008). Even so, and in the face of the possibility of being shamed by the authorities, several municipalities did not take part in these schemes (DWA, 2012a: Blue Drop report; DWA, 2012b: Green Drop Report). Since the inception of these certification programmes a general increase in the Blue and Green Drop score cards is observed. Although this is encouraging, concerns about inadequate facilities, skills shortages at all levels, overstretching of facilities, poor understanding of wastewater treatment as well as underfunding of facilities were raised (DWA, 2012b: Green Drop Report)

In South Africa several legislative processes governs water provision and wastewater management. This includes the National Water Act (No. 36 of 1998), Water Services Act (No 108 of 1997), Municipal Structures Act (No. 117 of 1998) and Municipal Systems Act (No. 32 of 2000). The legal framework is sometimes confusing and may result in parties not taking responsibility when there is a need to. Sometimes these inactions by management may cause severe pollution that may result in civil action (Anon, 2009b; Tempelhoff, 2009; Thom, 2010). As critical as it is to provide safe drinking water, there is also a responsibility to dispose of used water in such a condition that it is not harmful to the environment. To ensure that resource water is of appropriate quality the NWA (No. 36 of 1998) recognises that National Monitoring Programmes should be established. It is also recognised by the National Water Resource Strategy (NWRS, 2004) that a variety of monitoring programs is needed to provide an overview of the condition of various water sources. This includes the National Microbial Monitoring Programmes (NMMP) for surface and groundwater (Murray et al., 2004; 2007).

1.4 Water in the North West Province of South Africa

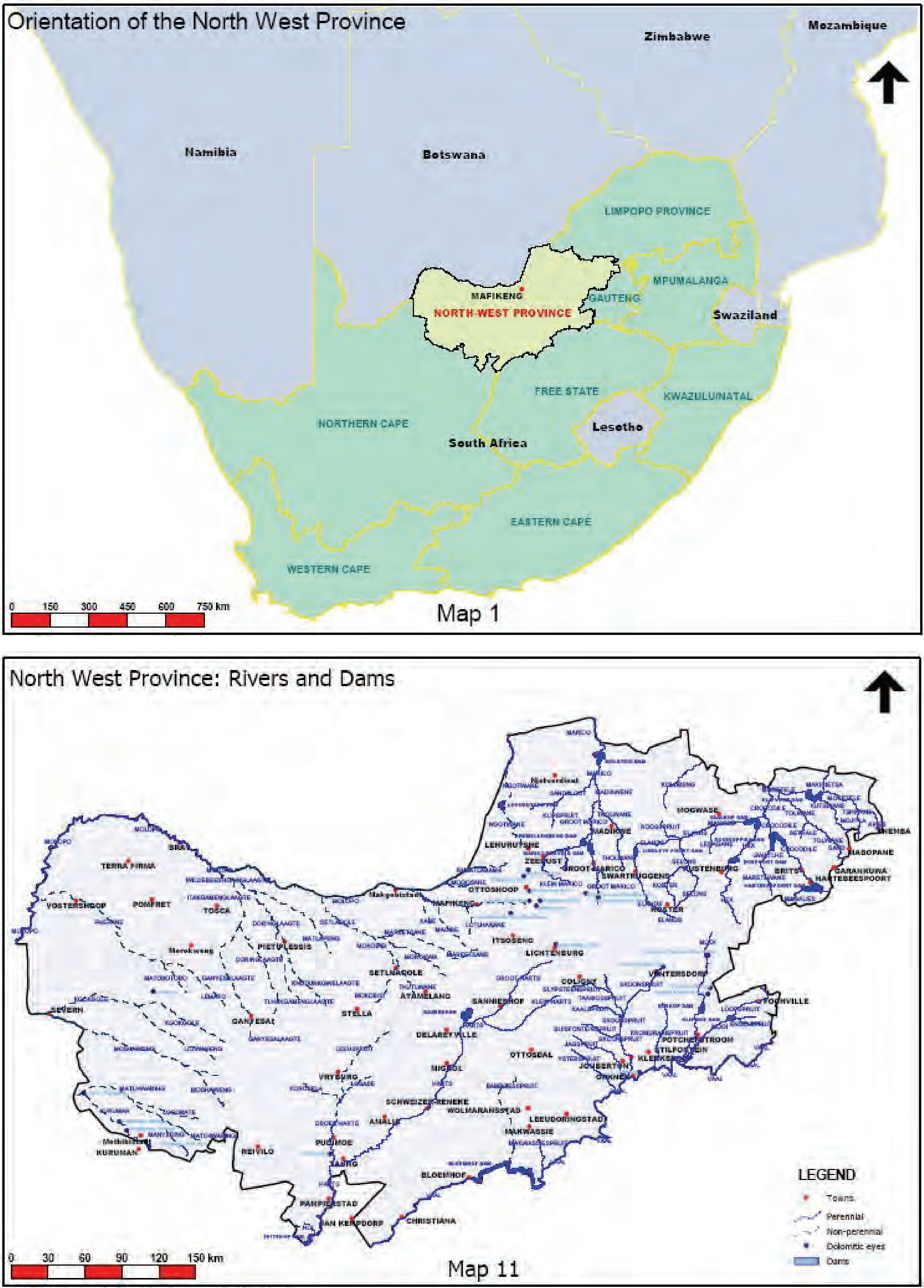
South Africa's water resources have been decentralised into 19 water management areas (WMAs) (DWAF, 2010). The North West Province shares some 4 these WMAs with neighbouring provinces as well as an independent country (Botswana). Figure 1 also provides an orientation of the North West Province (Map1) and location of the various rivers and dams (Map 11).

Many of the surface water sources in the North West Province (rivers, dams, pans and wetlands) are non-perennial. This is a key limiting factor for development in certain areas (NWDACE-SOER, 2008). Available and future water requirements for the three major Water Management Areas in which the North West Province lies are depicted in Table 3.

Table 3: Water yield and requirements in the three WMA in which the NWP lies. The 2000 values are the actual values in that year. The 2025 values are predicted for 2025 (at base; NWRS 2004).

	2000 actual		2025 at base	
Water management areas	Yield million m^3/annum	Requirements million m^3/annum	Yield million m^3/annum	Requirements million m^3/annum
Crocodile-West and Marico	716	1184	846	1438
Middle Vaal	126	643	127	641
Lower Vaal	50	369	55	381

According to table 1 the requirements and availability at base means that a considerable amount of water will have to be transferred into these WMAs, and this may include sections of the North West Province. Sections of all three WMAs are in the low rainfall region. From the present state of water requirements and future needs it make sense that resources are made available to investigate water quality related issues in these WMAs and develop models to predict impacts of future developments. Kalule-Sabiti and Heath (2008) indicated that several regions in the North West Province have been labelled as environmental pollution "hot spots" and recommended for surface- and groundwater monitoring.



Source: Environmental Potential Atlas 1999; Department of Water Affairs

Figure 1: The orientation of the North West Province (Map 1) and a map of rivers and dams (Map 11). The details of these can be found at (http://www.nwpg.gov.za/soer/FullReport/NWPSOERM.html).

1.4.1 Mooi River

The Mooi river catchment falls within the Upper Vaal Water Management Area. It is situated in the eastern region of North West Province and a section of the western region of Gauteng. It is divided into three sub-catchments: the Mooi River, Wonderfontein Spruit and the Loop Spruit. The catchment area is regulated by the Klerkskraal, Klipdrift, Boskop and Potchefstroom Dams (van der Walt *et al*., 2002; Wade *et al*., 2002). It has a total surface area of 1800 km^2. Only 44.2% of this surface area yields run-off. Most of the precipitation ends up as groundwater recharge (van der Walt et al., 2002).

Water management challenges are becoming increasingly complex in the Mooi River catchment. This is due to an increased water demand and pollution (le Roux, 2005). In 2001, Environmental problems experienced within the Mooi River are a result of the impact of gold mining, population growth and increased water utilisation (Currie, 2001). It has long been established that major gold mining activities carried out in this catchment have the potential of polluting surface and groundwater sources (DWAF, 1999). Water quality in the Mooi River catchment is heavily impacted on by water from the Wonderfontein Spruit tributary. This is mainly due to the regular pollution by gold mining industries and associated underground flooding of abandoned mines (le Roux, 2005; van der Walt et al., 2002). The mining pollution is a problem because the Tlokwe Local Municipality is currently reliant on the Mooi River as its sole source of domestic water and extracts a small portion of water (DWAF, 1999; le Roux, 2005). The Mooi River joins the Vaal River approximately 30 km downstream from Potchefstroom.

1.4.2 Harts River

The Harts River is situated in the Lower Vaal Water Management Area (DWAF, 2002b). It flows in a south-western direction and in the process into Barberspan, a Ramsar site (DWAF, 2009a). This catchment area is regulated by the Taung and Spitskop dams. The Little Harts River flows from near Coligny and the Great Harts River from Lichtenberg (DWAF, 2009c). The Great Harts River is one of the significant tributaries of the Vaal River (DWAF, 2009a). The Dry Harts River, on the other hand, is a seasonal river that passes Taung.

Large sections of the Harts River are located in a semi-arid to arid region. Rainfall in this catchment ranges from 100 mm to 500 mm per year. Evaporation rates reach 2 800 mm per year (DWAF, 2002b). One of the world’s largest irrigation schemes, the Vaalharts irrigation scheme, is located where the Vaal and Harts Rivers confluence (The Water Wheel, 2010). Land-use activities along the river and in the irrigation scheme include extensive livestock farming of beef, dairy, cattle, goats, sheep, pigs, ostriches, cotton, maize, wheat and groundnuts (DWAF, 2004a; The Water Wheel, 2010).

The water quality of the Harts River is impacted on by irrigation and municipal return flows. Water downstream from the irrigation scheme has been reported to be high in salinity due to saline leachates (DWAF, 2004c). The Vaalharts irrigation scheme contributes 50 000 t/a of fertiliser to the system while 130 000 t/a of salts are moving towards the Harts river in from the upper and middle Vaal WMA's (Ellington, 2003).

Baberspan is connected to the Harts River via a channel (DWAF, 2004a). The pan is situated ±17km north-east of Delareyville and is the largest pan and wetland in province (NWDACE-SoeR, 2002). It lies 9 m lower than the Harts River. Compared to other pans in the region, Barberspan is a perennial water body with a water depth of about 10 m (Swart and Cowan, 1994). It is recognised as an important sanctuary for birds in this relatively dry region (DWAF, 2002). The pan is state controlled and protected as a provincial nature reserve. It is used for bird watching and angling. Land use areas surrounding the pan include cattle and maize farming (Swart and Cowan, 1994).

1.4.3 Schoonspruit River

The Schoonspruit River is also a tributary of the Vaal River, has a catchment area of 325 km^2. It is located in the Middle Vaal WMA and is one of the major rivers in this WMA. The upper Schoonspruit, above Ventersdorp, is characterised by a –peat wetland habitat fed by a dolomitic eye. Below Ventersdorp, a narrow channel is found and the riparian zone is dominated by willows and white poplars. The lower Schoonspruit River is again an extensive wetland system (DWAF, 2007).

Dolomite springs in the upper regions feeds the Schoonspruit River (DWAF, 2004d). This river supplies most of Ventersdorp with its urban and irrigation water requirements. Diamond digging and agriculture are implicated as major sources of pollution in the Schoonspruit River (DWAF, 2004d). As a result of these mining operations, the Schoonspruit River also negatively impacts on water quality of the Vaal River (The Water Wheel, 2008). It is estimated that the Schoonspruit contributes to close to 26.7 million m^3/annum of water associated with salt loadings to the Vaal river (DWAF, 2004d).

1.4.4 Vaal River

The Vaal River is divided into three Water Management Areas (Upper, Middle and lower Vaal; DWAF, 2009a). It originates in the Drakensburg escarpment and drains majority of the

South African central Highveld. The catchment area stretches through five provinces (parts of Gauteng, Free State, Mpumalanga, North West and the Northern Cape). It is the largest tributary of the Orange River. Water flow in the Vaal River is regulated by the Grootdraai, Vaal and Bloemhof Dams as well as a number of weirs. The larger weirs include the Vaal, Vaal Harts, and Douglas Barrages (DWAF, 2009b). Water drawn from the Vaal River supports 12 million consumers in Gauteng as well as smaller towns in the NWP including Vryburg.

1.4.4.1 Upper Vaal Water Management Area

The upper Vaal Water Management Area (WMA) covers part of Gauteng, Free State, Mpumalanga and North West Province. Major rivers in this WMA include Wilge, Klip, Liebenbersvlei, Waterval, Suikerbosrand and the Mooi Rivers. Climate is characterised by seasonal (summer) rainfall. Peak rainfall is during December and January (DWAF, 2004b; DWAF, 2009b).

The Upper Vaal WMA is economically important because it contributes approximately 20% to South Africa’s Gross Domestic Products (GDP) making it the second largest contribution to the national wealth amongst all nineteen WMAs in the country (DWAF, 2004a). Water quality in this WMA is impacted by seepage from waste facilities, industries, waste water treatment plant discharges, mining and agriculture return flows (DWAF, 2004a; 2009b).

1.4.4.2 Middle Vaal Water Management Area

The middle Vaal WMA is located in a semi-arid region (DWAF, 2002b). A large section of this segment of the Vaal River forms the southern border of the North West Province and the Northern border of the and Free State Province. Other major rivers in this WMA include Schoonspruit (North West Province), Rhenoster, Vals and Vet Rivers (Free State province). The middle Vaal WMA is, however, dependent on water flowing from the upper Vaal WMA in order to meet water supply requirements for land use activities and urban sectors in Klerksdorp-Hartebeesfontein-Orkney-Stilfontein, Wolmaranstad as well as Welkom-Virginia areas (DWAF, 2009c).

Land use activities in the middle Vaal WMA contribute 4% of GDP of South Africa and include extensive dry land agriculture (wheat, maize, sorghum, sunflower), and gold mining (DWAF, 2002). Water quality is impacted on by return flows in the form of treated effluent from urban areas and mines as well as from agriculture. High nutrient washout from the agricultural lands results in periodic algal blooms which have aesthetic effects on the water in the WMA. Water is also subjected to high total dissolved solids (TDS) levels (DWAF, 2009a).

1.4.4.3 Lower Vaal Water Management Area

The Lower Vaal WMA is located in the north western region of South Africa and borders Botswana in the North (DWAF, 2002b). It originates downstream from Bloemhof Dam and flows to the confluence of the Vaal and Orange rivers. Major rivers found in this WMA include the Molopo, Harts, Dry Harts, Kuruman and Vaal Rivers (DWAF, 2004b).

In conclusion, water provision in the North West Province includes several WMAs. However, reports have indicated concerns about the quality of water in the province (Van Wyk et al. 2012; Van der Walt et al. 2002; Kalule-Sabiti and Heath, 2008; Mpenyana-Monyatsi and Momba, 2012; Ateba and Maribeng, 2011; Dikobe et al., 2011; Kwenamore and Bezuidenhout, 2008; 2009; Fereira and Bezuidenhout, 2011; Carstens and Bezuidenhout, 2012; Adeleke and Bezuidenhout, 2011ab; Ateba and Maribeng, 2011). These reports mainly dealt with physico-chemical but in many of the recent ones also with microbiological quality of the water. Concerns about indicator bacteria as well as other bacterial species are also discussed in these reports. Two of these studies (Van der Walt et al. 2002; Adeleke and Bezuidenhout, 2011a) also discussed the water in the North West Province from an integrated water resources management (IWRM) perspective.

1.5 Health and commercial implications of water and water quality

Direct links with consumption of water of poor quality and diarrhoea has long been established (Barrel et al., 2000; Pedley and Howard, 1997; Ashbolt, 2004; Ministry of health, 2005; WRC, 2003; Craun & Calderon, 1997) and demonstrated in South Africa (Grabow, 1996; Gaoganediwe, 2006; GCIS, 2005; Hemson and Dube, 2004). More than 3.4 million people die as a result of water related diseases every year, making it the leading cause of disease and death around the world (WHO, 2003). Primary waterborne transmission often goes unnoticed because a disease only manifests itself after secondary or tertiary transmission (Grabow, 1996). Table 4 summarises some of the most common waterborne pathogens, diseases, symptoms of the diseases as well as the bacteria, viruses or parasites causing the disease.

Table 4: Common waterborne diseases caused by the transmission of pathogens (WRC, 2003).

Pathogen	Disease	Symptoms
Enterovirus, Adenovirus, Rotavirus, *Salmonella enteritis*, *E. coli* 0157	Gastroenteritis	Vomiting, watery diarrhoea, moderate fever & stomach cramps
Hepatitis A virus	Hepatitis	Inflammation of liver, fatigue, loss of appetite, tender liver, white stool & jaundice
Vibrio Cholerae	Cholera	Profuse diarrhoea & vomiting – Fatal dehydration: if untreated death within 6 hours
Camphylobacter jejuni	Camphylobacteriosis	Slight to severe diarrhoea (might be bloody), abdominal cramps & fever. In severe cases vomiting & convulsions.
Cryptosporidium parvum	Cryptosporidiosis	Watery diarrhoea & stomach pains. Sometimes vomiting & slight fever. Life threatening to HIV patients.
Giardia lamblia	Giardiasis	Mild diarrhoea with flatulence, bloating, cramps and loose grease stools
Shigella dysentery	Shigellosis	Abdominal pain, cramps, diarrhoea. Mucus & blood in stools, fever and dehydration – kidney failure
Salmonella typhi	Typhoid fever	Headache, fever, abdominal pain. Initial constipation, bronchitis later.

Diarrhoea, abdominal pain, vomiting and slight fever are the most common symptoms associated with waterborne diseases (Table 4). Several of the pathogens listed in Table 2 are associated with faecal matter. However, infections from water sources are not limited to enteric diseases but may be extended to the skin, throat, ears, nose and eyes (Yoshpe-Pures & Golderman, 1987; van Wyk et al., 2012). This commonly-used list also exclude various other water borne enteric and other diseases that could have devastating effects on communities with high levels of immune-compromised individuals. Even so, waterborne diseases remain a cause for concern in both developing and developed countries (Duncker, 2000).

Water-borne diseases in South Africa are a major concern (National Treasury, 2011). South Africa's location is such that countries with greater environmental health challenges such as water-borne diseases border it (DoH, 2011). Population migration across borders is an influencing factor in the spread of diseases. This places the inhabitants of certain regions in South Africa at a vulnerable position especially with respect to water-borne diseases (DWAF, 2002a). In 2002 it was estimated that as many as 43 000 South Africans may die annually as a result of diarrhoeal diseases (DWAF, 2002a). An estimated 70% of diarrhoeal incidents in South Africa occur in children under the age of 5 years is linked to unacceptable basic level of service which include lack of sufficient potable water (DWAF, 2010; DoH, 2011). This is a global problem that is directly linked to a lack of potable water (WHO & UN-WATER, 2010).

To attain optimum human health gains an all-inclusive understanding of the interrelationship between water and human health is essential for the sustainable management of water quality (CSIR, 2010). According Statistics South Africa (Stats SA, 2010b), intestinal infectious diseases are among the leading underlying natural causes of death through all age groups. The leading cause of infant mortality (22.4%) in 2008 was attributed to intestinal infectious diseases. Many of the pathogens that could cause such diseases are listed in Table 1.

Health statistics for the North West Province indicates health burden increases in the economically marginalised group. For instance, in 1998 the incidence of diarrhoea among children in the North West Province that was younger than 5 years was the 5th highest among the nine provinces of South Africa. In 2000 these statistics (Figure 2) showed that diarrhoea among this age group has increased and was the highest in the country. The situation had improved since then but for the years 2001, 2003 and 2005 it was still the 3rd highest in South Africa (Figure 2).

The North West Province has also seen a general increase in the prevalence of HIV (Figure 3). It increased from less than 2% of the population in 1994 to over 10% by 2009. This figure is expected to stabilise (Figure 3). However, provision of good quality domestic water will be critical in establishing such a maturation rate for HIV prevalence.

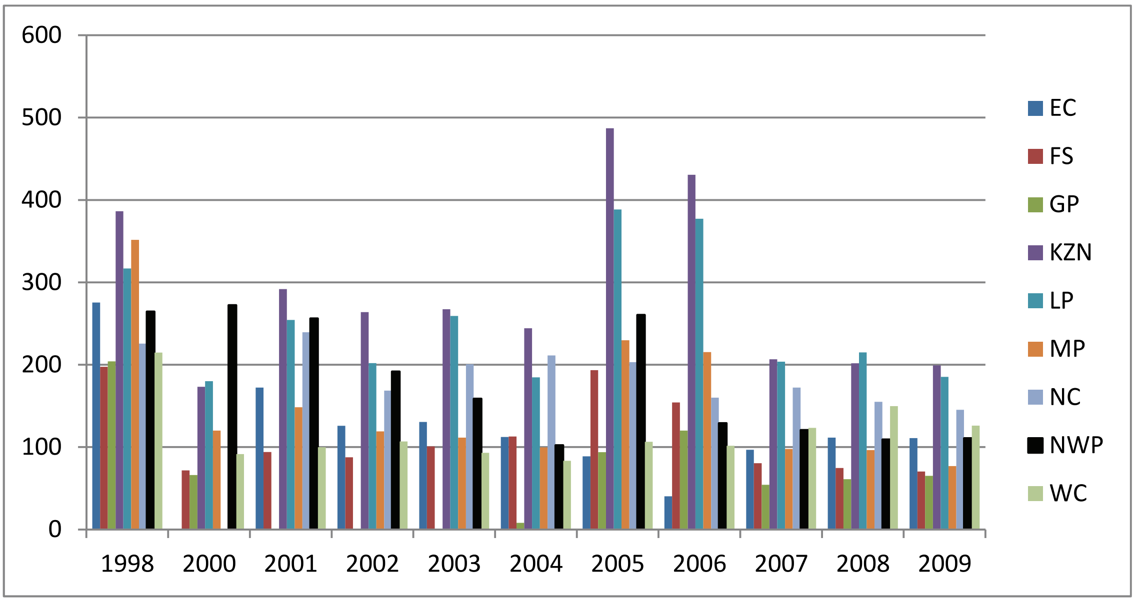


Figure 2: Incidence of diarrhoea among children under 5 years (per 1000); (http://www.healthlink.org.za/healthstats/132/data). EC: Eastern Cape FS: Free State GP: Gauteng KZN: KwaZulu-Natal LP: Limpopo MP: Mpumalanga NC: Northern Cape NW: North West WC: Western Cape.

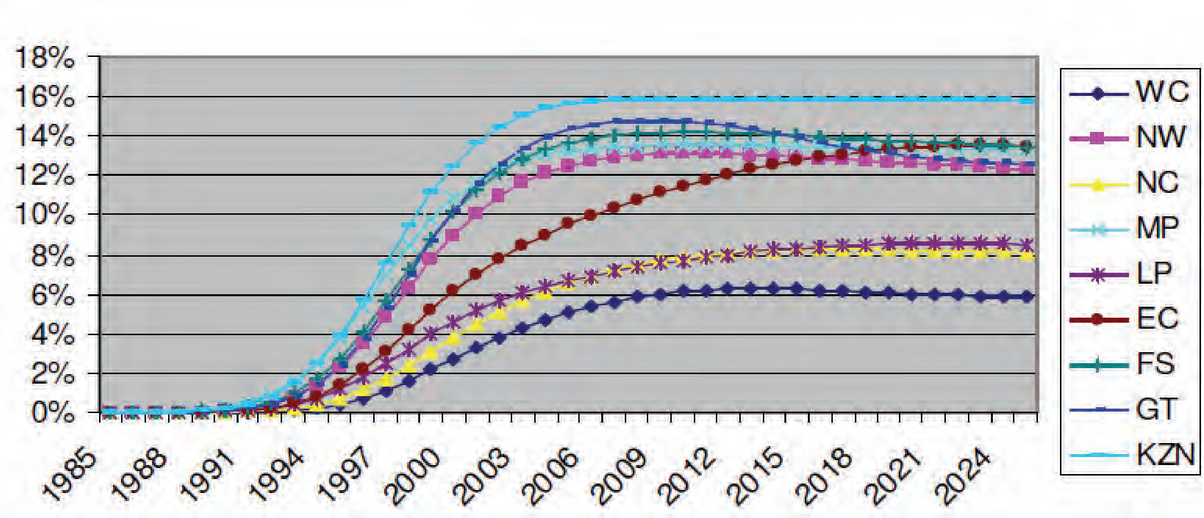


Figure 3: HIV prevalence and projected prevalence rate in South Africa (http://www.metam.co.za/documents_v2/File/RedRibbon_2009/Provincial%20HIV%20and%20AIDS%20statistics%20for%202008.pdf) EC: Eastern Cape FS: Free State GP: Gauteng KZN: KwaZulu-Natal LP: Limpopo MP: Mpumalanga NC: Northern Cape NW: North West WC: Western Cape.

Available (scattered) data on water quality in the North West Province demonstrated that a large number of these water sources are generally of a poor quality and that it may be deteriorating (Kalule-Sabiti and Heath, 2008; Kwenamore and Bezuidenhout, 2008; Mulamattathil et al., 2000; NWP-SOER, 2002; Van der Walt et al., 2002; Mpenyana-Monyatsi and Momba, 2012; Ateba and Maribeng, 2011; Dikobe et al., 2011; Kwenamore and Bezuidenhout, 2008; 2009; Fereira and Bezuidenhout, 2011; Carstens and

Bezuidenhout, 2012; Adeleke and Bezuidenhout, 2011ab; Ateba and Maribeng, 2011). Furthermore, available data includes mostly physical and chemical parameters that were measured but recently some microbiological data have also been generated (Mpenyana-Monyatsi and Momba, 2012; Ateba and Maribeng, 2011; Dikobe et al., 2011; Kwenamore and Bezuidenhout, 2008; 2009; Fereira and Bezuidenhout, 2011; Carstens and Bezuidenhout, 2012; Adeleke and Bezuidenhout, 2011ab; Ateba and Maribeng, 2011). Even though water of a poor microbiologically quality can have detrimental effects on individuals almost immediately, this aspect has until recently generally been neglected in ground water quality monitoring regimes. This is cause for concern. Adeleke and Bezuidenhout (2011a) argue for the inclusion of microbiological data in integrated water resources management (IWRM) schemes and the expansion of this, particularly in South Africa.

There is a need to establish water quality monitoring regimes in a coordinated manner and to use such data to influence policy towards protection of the water sources (Kalule-Sabiti and Heath, 2008). This resource, water, is the fibre of the socio-economics of society and must be managed appropriately. However, coordinated research efforts are necessary to demonstrate the impact of agriculture and the mining (as well as related) industries on water quality. Based on such data, these industries could be held accountable and required to assist in purifying and protection efforts. For this to be valid it would be essential that appropriate source tracking of the pollution is demonstrated (Nichols and Leeming, 1991). It is also important that the most appropriate parameters are measured. Conventional parameters may not always provide sufficient data about pollution and the sources thereof. Research programmes should thus also be established to investigate the appropriateness of newly developed technologies. In addition to this, it is critically important to generate data about water related social and water management issues in communities where sampling is conducted (Tempelhoff, 2009).

1.6 General structure of the report

This report is further divided into 4 research chapters, each one providing a focussed set of data. Chapter 2 provides data from the surface water study, Chapter 3 deals with the groundwater study, Chapter 4 with health aspects, antibiotic resistance, haemolysis and a cell culture based assay. Chapter 5 is an exploratory study of faecal sterol applications in water quality studies in the NWP. Chapter 6 presents the data from social and water management study. The social study (Chapter 6) format is different from the rest of the report (natural science format). In Chapter 7 conclusions and recommendations are provided.

CHAPTER 2

SURFACE WATER QUALITY IN THE NORTH WEST PROVINCE

2.1 Introduction

Water is generally a scarce resource in the North West Province particularly in the central and western regions. There is also an increasing demand on water for drinking, irrigation, mining and industrial purposes. Surface water is one of the main resources and is sometimes transferred into areas of the province (NWRS, 2004). This resource should thus be well managed and protected (Brettar and Höfle, 2008). Successful management should include regular monitoring data of the physico-chemical and bacteriological quality of water. Bacteria, viruses, inorganic, organic and water soluble substances are considered as the major water pollutants contributing to the deterioration of water quality and responsible for various public health problems (Azizullah et al., 2011).

2.1.1 Indicator bacteria

Within the context of safe drinking water provision it is impracticable and expensive to monitor water supplies for all potential human pathogens (Barrel et al., 2000; Zamxaka et al., 2004). Surrogates (indicators organisms) are used to indicate the possible faecal contamination. Faeces are regarded as the most frequent source of health-significant microbial contamination of water supplies (Barrel et al., 2000; Ministry of Health, 2005). As indicator organisms, the surrogates they should ideally fulfil several criteria (DWAF, 1996; Grabow, 1996). They should: 1) be suitable for all types of water; 2) be present in sewage and polluted waters whenever pathogens are present and in numbers higher than the pathogens; 3) not multiply in the aquatic environment, but must survive in the environment for at least as long as pathogens; 4) be absent from unpolluted water; 5) be detectable by practical and reliable methods; and 6) should not be pathogenic (safe to work with in the laboratory). There is no single indicator that meets all of these requirements. According to Jagals et al. (2006), cases where water samples tested positive for faecal indicator bacteria, does not necessarily mean that pathogens are also present. On the other hand, the absence of indicator bacteria in water sources would not necessarily imply the absence of pathogens (Jagals et al., 2006). There are thus gaps that need to be addressed when water quality is determined based on indicator organisms. Using a battery of indicators help to eliminate or predict better potentials of health risk.

The most commonly measured bacterial indicators are total coliforms, faecal coliforms and enterococci (Noble et al., 2003). Heterotrophic plate count (HPC) bacteria are also an indicator of the microbial quality of water sources (LeChevallier et al., 1980; WHO, 2008). The World Health Organisation (WHO, 2008) as well as the Department of Water Affairs (DWAF, 1996) has compliance guidelines for these organisms. According to these norms 100 mℓ of water should be absolutely free from faecal indicator species (DWAF, 1996; Fricker & Fricker, 1996; WRC, 2003). Limited levels of total coliforms and HPC are also allowed. There are debates about the health risk potential of several commonly occurring HPC in drinking water. Insufficient clinical and epidemiological evidence are available to conclude that HPC bacteria in drinking water pose a health risk (Allen *et al.,* 2004; Calderon & Mood, 1991; Selma *et al.*, 2004).

Due to the high prevalence of water-transmitted infectious diseases, regular screening for faecal indicator bacteria, like *E. coli*, is necessary (WHO & UN-WATER, 2010). Although *E. coli* is commensal in the human colonic micro-flora, it can be pathogenic after infection of mucosal surfaces (Caper and Nature 1998). Five groups of pathogenic *E. coli* are known to utilise environmental water as a reservoir after faecal contamination by sewage pollution (Kong et al*.* 2001) or open defecation (Ashbolt 2004). These groups of *E. coli* are; (I) Entero-Pathogenic *E. coli* (EPEC), (ii) Entero-Toxigenic *E. coli* (ETEC), (iii) Entero-Hemorrhagic *E. coli* (EHEC), (iv) Entero-Invasive *E. coli* (EIEC) and (v) Entero-Aggregative *E. coli* (EAEC) (Caper, 2005; Nature and Caper, 1998). However *E. coli* is used to confirm faecal contamination (DWAF, 1996), some restrictions are encountered using *E. coli* as indicator organism (Oliver et al., 2005; Leo et al., 2005, Kong et al., 2002). First, the treatment of sewage water by chlorine induces *E. coli* into a viable but non-culture able (VBNC) state (Oliver et al., 2005). The VBNC *E. coli* will still be able to cause infection, though it will not be detected by culture based methods (Lleo et al., 2005; Kong et al., 2002). Second, there are commensal and pathogenic species of *E. coli* (Kaper, 2005). To indicate the public health hazard of the polluted water, the pathogenicity of the detected *E. coli* strain must be determined and quantified.

2.1.2 Culture-independent microbial diversity

In aquatic microbial ecology the identification of bacterial assemblages in these ecosystems and their role in metabolic processes are major objectives. Traditionally this was accomplished through cultivation and subsequent characterisation of isolates (Cardenas and Tiedje, 2008). However, cultivation methods are insufficient to fully assess the bacterial diversity in environmental samples (Schäfer & Muyzer, 2001). Nutritional requirements and environmental parameters for every population of microorganisms existing within an aquatic community are unknown. Thus less than 1% of bacteria will grow on nutrient-rich media (Moyer, 2001; Hobbie & Ford, 1993). In addition, microscopic limitations such as the lack of conspicuous morphology and small cell size do not allow for the identification of most environmental bacteria (Schäfer & Muyzer, 2001). During the last decade, molecular methods, in particular the Polymerase Chain Reaction Denaturaing Gradient Gel Electrophoresis (PCR-DGGE) technology, have been developed and applied in several aquatic studies to determine microbial diversity without the need for cultivation (Xingqing et al., 2008; de Figueiredo et al., 2012; Zhang et al., 2011). Molecular techniques such as PCR-DGGE and other DNA profiling and detection methods have allowed insight into the vast diversity, interactions and metabolic processes of microorganisms present in complex environments (Gilbride et al., 2006). Although PCR-based molecular methods are fast and sensitive alternatives to culture-dependent techniques, biases and limitations have been identified (Dorigo et al. 2005). This called for new approaches to estimate total microbial diversity and functionality within natural environments, as well as to study specific groups that are unknown and uncharacterised.

Next generation sequencing (NGS) also known as high through-put sequencing (HTS) or metagenomic analysis of microbial ecology has lately been the focus of several environmental studies such as soil (Lemos et al., 2011), freshwater lakes (Marshall et al., 2008), planktonic marine assemblages (Breitbart et al., 2009) and deep sea microbiota (Sogin et al., 2006). Metagenomics is the study of microbial communities in natural environments based on direct sequencing of the community DNA (Committee on Metagenomics, 2007). It provides extensive information on community structure and composition (Kakirde et al., 2010). Phylogenetic and functional analyses of microorganisms can thus be determined at community level (Cowan et al., 2005).

2.1.3 Bacteriophages and viruses

It is essential to ensure that sewage effluents are properly treated at all times because they always find their way into water bodies either intentionally (after treatment) or through contamination. Municipal wastewater treatment systems, even in developed countries are capable of reducing the load of the above-mentioned microbes but in many cases cannot completely remove them from the effluent (Carducci et al., 2008). This is particularly of concern if the poor state of waste water treatment plants in South Africa is considered, particularly those decanting into the into the Vaal river and its tributaries (DWA, 2009). In many cases these systems are exceeding their design flow capacity, are not maintained, etc. (DWA, Green Drop report 2012). These problems lead to poorly treated (or untreated) sewage reaching the receiving waters. Within this matrix could be viruses that may have human health implications. Methods are available to test for these viruses but these are expensive. Although somatic phages are used as indicators of the presence of human enteric viruses, this indirect approach is not adequate (Carducci et al., 2008).

Bacteriophages closely resemble human enteric viruses in composition and structure and they are more persistent in aquatic environments than bacterial species (Snowdon Cliver, 1989). These phages occur abundantly in faecal polluted environments and are relatively resistant to inactivation by natural and treatment processes. They have therefore, been suggested as good indicators of human viral pollution as proposed by the USA EPA (Havelaar, 1993). Phages also have a potential application of being used to track or identify sources of faecal contamination as being either human or non-human in origin (Kott, 1984).

2.1.4 Yeasts

Domestic sewage usually contains, besides heavy protozoan, bacterial and viruses loads, also pathogenic and potential pathogenic yeasts and fungi. Treatment process should allow for the removal of the yeast and fungi. When WWTP systems do not work effectively these microorganism are deposited in receiving water bodies. The occurrence of yeasts in different types of natural aquatic environments has also been reported for lakes and ponds (Sláviková & Vadkertiová, 1997; Medeiros et al., 2008), estuaries, coasts and mangrove areas (van Uden & Fell, 1968) as well as oceans and the deep sea (Nagahama et al., 2003). Only a few studies have reported on the presence of yeasts in river water (Sláviková & Vadkertiová, 1997; Medeiros et al., 2008; Van Wyk et al., 2012).

More than 100 yeast species that have been identified as human pathogens, have been isolated from water (Fromtling et al., 2003). Most of these pathogens are classified in the genus *Candida*. Hurley et al. (1987) list these particular pathogenic yeasts that cause candidosis in probable descending order of virulence for man as: *C. albicans, C. tropicalis, C. stellatoidea, C. glabrata, C. krusei, C. parapsilosis, C. guilliermondii, C. viswanathii, Clavispora lusitaniae (Candida lusitaniae)* and *Rhodotorula mucilaginosa (Rh.rubra)*.

Awareness has been raised by recent studies on the presence of potentially pathogenic yeasts in both surface and ground water (Pereira et al., 2009). Several studies have focused on the occurrence of yeasts related to wastewater (Hagler & Mendonça-Hagler, 1981; More et al., 2010). These studies have demonstrated that yeast counts can be a potential monitoring indicator that may complement coliform counts reflecting the eutrophication potential of water (Hagler & Ahearn, 1997). High levels of yeasts in water sources could be an indication of either heavy pollution or low pollution, depending on the type of yeasts present in the specific water source (Woollett & Hendrick, 1970).

Nevertheless, compared to bacteria and viruses, yeasts are receiving little attention when the quality of water systems is at stake (Arvanitidou et al., 2002).

2.1.5 Aim and objective

The objective of this part of the study was to determine the physico-chemical and microbial quality of surface water in the North West Province, South-Africa using culture dependent as well as culture independent methods. Various methodologies were used and included direct PCR and sequencing.

2.2 Experimental procedures

2.2.1 Sampling

Water samples were collected from surface water sources in the North West Province. The maps for the sampling sites are provided in Figures 4 to 6. Surface water for bacteria and yeast analysis were collected from sites indicated on the map in Figure 4. Onsite and laboratory based methods were used to analyse the samples.

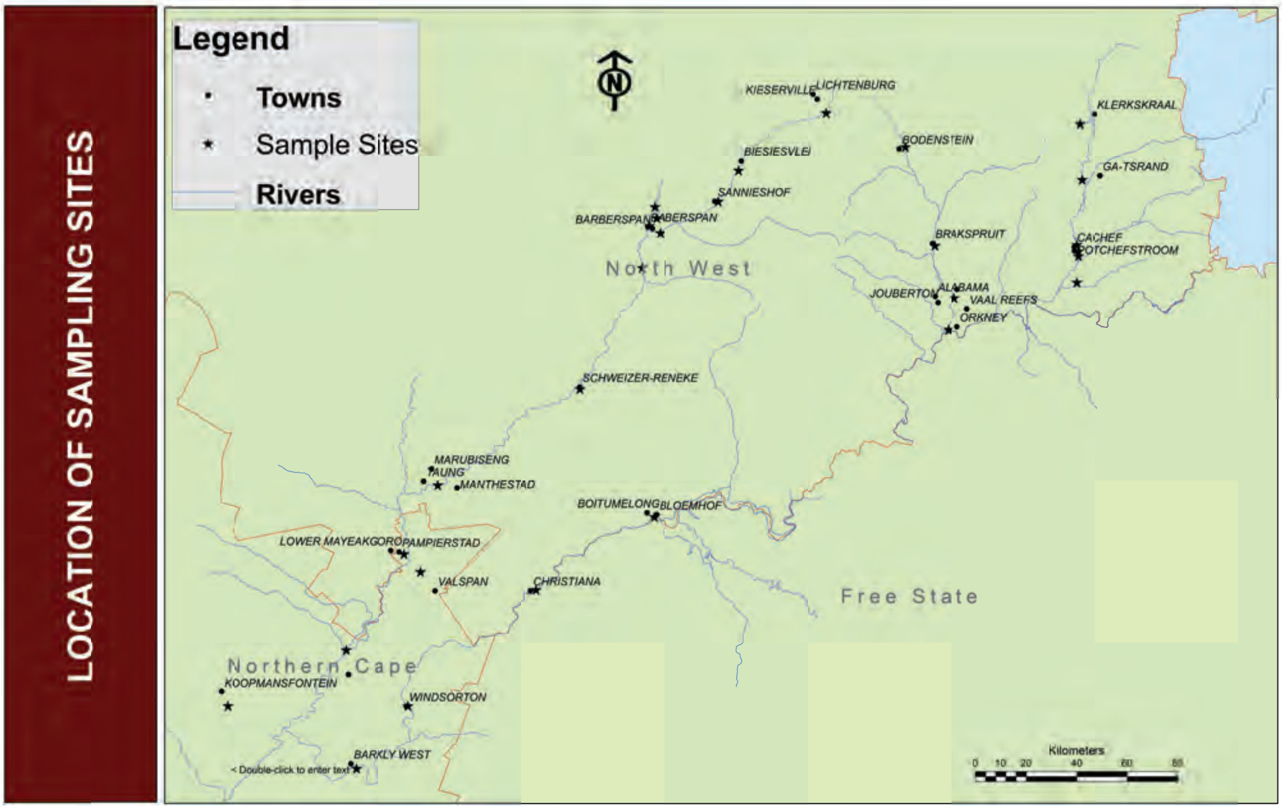


Figure 4: Map illustrating the spatial distribution and geographical location of each sampling site within the surface water systems of interest. Surface water samples were collected for indicator bacteria and yeast analysis on the same day.

2.2.2 Physico-chemical parameters

Water quality parameters such as temperature (°C), electrical conductivity (mS/m) and pH, were recorded on site using a multi-350 probe analyser (Merck, Germany) according to instructions from the manufacturer. Nitrate (NO_3-N), nitrite (NO_2-N) and phosphates levels were determined in the laboratory using methods, kits, reagents and a spectrophotometer from Hach (Hach, Germany).

2.2.3 Culturable indicator bacteria

One hundred millilitres (100 mℓ) of sample water was filtered through a 0.45 µm pore size Whatman filter, which were then placed on selective agar (m-Endo for total coliforms, m-Fc agar for heat tolerant faecal coliforms, and KF-enterococcus agar). Each of these plates were incubated for the recommended incubation period at appropriate temperatures (m-Endo agar: 24 h at 37°C; KF-streptococcus agar: 48 h at 37°C; and m-Fc agar: 24 h at 44.5°C). Following incubation, presumptive colonies (m-Endo agar plates – metallic sheen and pink-red colonies; m-Fc agar plates – blue colonies; KF-streptococcus agar plates – purple-pink colonies) observed on the surface of membranes were counted and recorded. The mean bacterial count for each sample point was then determined. The results were expressed as colony forming units per 100 mℓ.

Dilutions up to 10^{-6} were made for the enumeration of heterotrophic bacteria, of which 0.1 mℓ was used to make a spread plate on R2A selective agar (Merck, Germany). The incubation period was 5 days at room temperature. Results were expressed as colony forming units per mℓ.

For isolation and purification purposes, selected pink colonies on KF-Streptococcus were aseptically sub-cultured at least 3 times on nutrient agar using the streak plate technique and incubated for 24 hours at 37ºC. Gram staining was performed in order to confirm that all presumptive faecal streptococci isolates were Gram positive while ensuring they were pure and not mixed cultures. Various culture based tests were conducted including catalase activity (Rolfe *et al*., 1978), bile solubility (Health Protection Agency, 2010b), temperature growth capabilities and salt tolerance (Geldreich and Kenner, 1969). Identification of enterococci was confirmed using 16S rDNA PCR and sequencing. Amplified enterococci PCR products were subsequently purified and sequenced using a BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA) and Genetic Analyzer 3130 (Applied Biosystems, California, USA). Raw sequence data were transferred to Geospiza Finch TV (version 1.4) software which was used to view all chromatograms. All amplified

DNA sequences were identified using BLAST searches (http://www.ncbi.nlm.nih.gov/BLAST). Consensus sequences were imported into Mega 5.0 (www.megasoftware.net) (Tamura *et al*., 2011) within which neighbour-joining trees were constructed using the Jukes Cantor model and bootstrap values based on 1,000 replications were selected (Tamura *et al*., 2011).

Antibiotic susceptibility tests were performed on enterococci isolates using the Kirby-Bauer disk diffusion method according to Bauer et al. (1966). The overnight cultures were spread onto Mueller Hinton agar (Merck, Germany) and these were allowed to dry. Antibiotics used included: Ampicillin (10 µg), Amoxicillin (10 µg), Neomycin (30 µg), Streptomycin (300 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Oxy-tetracycline (30 µg), and Trimethoprim (2.5 µg), Penicillin G (10 µg) and Vancomycin (30 µg) and were obtained from Mast Diagnostics (UK). After incubation inhibition zones were measured in millimetres and compared to the NCCLS growth inhibition zone standards for Streptococci (NCCLS, 1999).

2.2.4 Yeast

Yeast-malt-extract agar (YM) (10 g/ℓ glucose, 3 g/ℓ malt extract, 3 g/ℓ yeast extract, 5 g/ℓ peptone and 15 g/ℓ agar) (Wickerham, 1951), supplemented with 100 ppm chloramphenicol pH 5.5 was used for isolation of yeast. This was done using the membrane filter method (APHA, 1988). Fifty millilitres of water was filtered through 0.45 µm HA membrane filters (Whatman®). Each sample was analysed in duplicate. These membranes were then placed onto YM agar plates, and incubated at 25°C. The formation of yeasts colonies were examined daily during a 5 day period. Isolates were purified by sub culturing by streaking onto YM agar (Wickerham, 1951).

The Diazonium blue B (Sigma-Aldrich, Germany) test was carried out to distinguish between ascomycetous and basidiomycetous yeasts (Kurtzman & Fell, 1998). Isolates were further identified using the ID 32C system (bioMerieux, France). The identities of the isolates were confirmed by generating 26S rRNA sequencing data. A similar approach and methodology to those used for the enterococci (Section 2.2.3) were used. Phylogenetic analysis was performed with the MEGA version 5 software (Tamura et al., 2011). Statistical analysis was based on the neighbour-joining method using bootstrap test of phylogeny (1000 replications) and maximum composite likelihood model.

2.2.5 Molecular studies of microbial diversity

In this part of the study DNA was directly isolated from water samples. Water was filtered through a 0.45 (and later through 0.22) µm filter and subsequently placed in a 1 mg/mℓ lysozyme solution and glass beads. The filter was incubated at 37°C in a shaking water bath for 10 min.

Proteinase K (1 mg/mℓ) was added and the solution still having the filter was incubated at 56°C for an additional 30 min. DNA was subsequently isolated from the crude lysate using the PeqGold Bacterial DNA Kit (PEQLAB Biotechnologie GmbH, Erlangen, Germany). The quality and quantity of the isolated nucleic acids were determined using the Nanodrop ND1000 (NanoDrop Technologies, Delaware, USA) and agarose electrophoresis.

In the culture independent method PCR was performed directly using the community genomic DNA. Denaturing gradient gel electrophoresis (DGGE) was conducted using methods described by Muyzer et al., (1993). DNA bands were excised from DGGE gels with a sterile scalpel blade, resuspended in twenty microlitres of nuclease-free water and incubated overnight at 4°C. The eluate was then amplified using corresponding primer pairs previously described (341F and 907R, Muyzer et al., 1993) without a GC-clamp. Amplified PCR products were subsequently purified with the NucleoSpin Extract II Kit (Macherey-Nagel GmbH, Dueren, Germany) and directly sequenced using a BigDye® Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems, California, USA) and Genetic Analyzer 3130 (Applied Biosystems, California, USA) as described in the manufacturer's instructions. Chromatograms were viewed in Geospiza Finch TV (version 1.4) software and BLASTN searches (http://www.ncbi.nlm.nih.gov/BLAST) were used to determine the identity of the amplified bands. The same sequencing approach was used in other Sections. DGGE sequences were subsequently submitted to GenBank with accession numbers JQ085826-JQ085849.

For NGS analysis the V1-V3 region of the 16S rRNA gene was amplified producing ~ 500 bp fragments. The pyrosequencing sequencing analysis was contracted to Inqaba Biotech, South Africa using the Roche 454 GS-FLXTM System. Sequences obtained from them were trimmed to remove GS tags and further analysed with the CLC Bio Genomics Workbench version 4.7.2 software (CLC Bio, Aarhus, Denmark). Sequences shorter than 200 bp in length were excluded from data sets. All remaining sequences were subjected to the National Center for Biotechnology Information (NCBI) database for BLAST analysis. Sequences were then submitted to Pintail version 1.0 to detect the presence of PCR artifacts. PCR products with chimeric properties were eliminated from data sets prior to phylogenetic analysis. The remaining sequences were submitted to GenBank with accession numbers JN865256-JN866178.

2.2.6 Enteroviruses and bacteriophages

a) Enteroviruses

Influent raw sewage (debris and grit already removed) and final effluent samples were collected from three different waste water treatment plants in South Africa, namely Potchefstroom (North West Province), Fochville and Carletonville (Gauteng province). Influent samples were labelled CSW, FSW and PSW representing Carletonville, Fochville and Potchefstroom respectively. Similarly, effluent samples were labelled CEF, FEF and PEF for Carletonville, Fochville and Potchefstroom respectively (randomly numbered 1 to and 3 not to identify the specific plant, results in Table 19 should not be associated with the order of plant names presented here). Samples were immediately transferred on ice to the laboratory for processing.

From November 2010 to September 2011, a total of 78 samples (13 influent and 13 effluent samples from each of the three sites) were collected. A total volume of 2 ℓ wastewater sample was collected at each instance.

Collected samples were processed immediately for virus recovery. The virus recovery method used has been described previously by Nadan et al. (2003) and Meley et al. (2008). Samples were replicated (n = 2) and named EF1 and EF2 for treated sewage and SW1 and SW2 for untreated sewage. The samples were clarified by centrifugation using centrifuge Harrier 18/8 for 30 min at 3,000 rpm. Each resultant pellet was resuspended in 10 mℓ of supernatant fluid and clarified by addition of 10% ($^{v}/_{v}$) of chloroform.

The supernatant from first and second clarification procedures were pooled. The combined supernatants were supplemented with 2.2% ($^{w}/_{v}$) NaCl and 7% ($^{w}/_{v}$) polyethylene glycol 6000. The mixture was stirred at 4°C overnight and then centrifuged for 2 h at 2,000 rpm at 4°C. The supernatant was discarded and the pellet was resuspended in lysis buffer of RNA purification kit.

A two-step qRT-PCR was carried out. In the first stage, extracted RNA samples were transcribed into cDNA using GoScript™ reverse transcription system. The resulting cDNA templates were later used in the second stage for real time PCR (primer and probe sequences are provided in Table 5). Standards were generated using known concentration of enterovirus culture. The concentrations of the target enterovirus were quantified by using the standard curves generated from known concentration of the enterovirus (Figure 16).

Table 5: Sequence details of the set of primers and probe used (Primers and Probe for amplification of enterovirus were synthesised by Bioresearch Technologies, Inc.).

Primer	Sequence	Reference
Ev1	5'-GATTGTCACCATAAGCAGC-3'	Fuhrman et al., 2005
Ev2	5'-CCCCTGAATGCGGCTAATC--3'	
Probe	5'-FAM-CGGAACCGACTACTTTGGGTGTCCGT-BHQ-Phosphor-3'	

b) Phage detection

i) Somatic coliphages

For isolation of somatic coliphages *Escherichia coli* strain C (ATCC 700078), also known as WG5, a nalidixic acid-resistant mutant of ATCC 13706 was used (Green et al., 1999; Ogorzaly and Ganzer, 2006). Into an microfuge tube 0.5 ml of the bacterial host and 1 ml of a test sample (or an appropriate dilution if needed) was pipetted, the eppendorf tube was then placed in an incubator at 37ºC for approximately 20-30 minutes for the phages to bind to their host bacteria. This phage-host mixture was then poured into the test tube containing the molten top agar (supplemented with naladixic acid) held at 50°C, mixed thoroughly and then rapidly poured on top of the bottom agar. The top agar was allowed to solidify and dry by opening the lids slightly. Once solidified and cooled, plates were incubated aerobically at 37°C for 18 hours. This procedure was carried out in duplicate.

ii) F-RNA phages

Salmonella enterica serovar Typhimurium WG 49 (ATCC 700730) was used as host in the isolation of F^{-}RNA phages. Processes were followed as described by Grabow, (2001) and Mooijman et al. (2002). The host culture must be in a logarithmic, or log, phase of growth to ensure the formation of the fertility-pili. To achieve this, an overnight culture is added to Tryptone Soy Broth. The flask with bacterial culture is then placed in a shaking incubator for 2 to 4 hours at 37°C (100-150 rpm) and at intervals the absorbance is measured at a wavelength 520 nm. At an absorbance approximately 0.5 the bacterial culture should be in the logarithmic growth phase and is ready for use.

To top agar (2.5 ml; supplemented with naladixic acid) held at 50°C in a water bath, 1 ml of premixed host and sample mixture was added, swirled to mix and poured onto the bottom agar layer in a 90 mm petri dish. This was allowed to solidify and dry before inverted and incubated overnight at 37°C.

2.2.7 Statistical analysis

The statistical analysis of all physico-chemical and microbiological parameters such as standard deviation, minimum and maximum values were conducted in Statistica version 10 (StatSoft, US) and Microsoft Office Excel 2007. This data was a representation of three replicates. Canoco (Canoco for Windows Version 4.0, GLW-CPRO ©) was used to create a Redundancy Analysis (RDA) multivariate ordination diagram (Ter Braak, 1990). The RDA was used to determine the relationship between dominant physico-chemical parameters and bacterial or yeast species isolated from the respective surface water systems in 2010 and 2011. Data was log-transformed and significance tests were carried out using the Monte Carlo permutation tests (499 permutations; $p \leq 0.05$). All statistical analyses relationships were considered to be statistically significant when the probability level was 0.05 or less (≤0.05).

2.3 Results and Discussion

2.3.1 Physico-chemical parameters

The physico-chemical parameters for surface water measured in 2010 and 2011 were generally within the Target Water Quality Ranges for domestic and recreational use. Electrical conductivity (EC) for some of the sites was quite high which indicates potentially some underlying quality challenges (Tables 7 and 8). The elevated EC levels were evident in the sample sites associated with the Harts River (including Baberspan). These elevated EC could be a sign of increasing salinity that could be a result of pollution.

Table 6: Physico-chemical parameters measured at each of the sampling sites at the respective surface water source during 2010 sampling period. Highlighted values exceeded TWQR for domestic use.

Water Source	Sampling site	pH	Temp (°C)	EC (mS/m)	NO_3-N (mg/ℓ)	NO_2-N (mg/ℓ)	PO_4^{2-} (mg/ℓ)
	TWQR	6-9	-	0-70	0-6 mg/ℓ	0-6 mg/ℓ	-
Mooi river	Klerkskraal Dam	8.36	23.6	39	0.5	2	1.42
	Muiskraal Dam	8.01	23.1	47	0.2	0	0.86
	Around The World Bridge	7.76	23.0	70	0.6	4	0.68
	Thabo Mbeki Drive	8.22	24.3	64	0.1	3	0.81
	Trimpark North Bridge	8.25	24.6	65	0.4	3	0.02
	Pedestrian Bridge	8.25	25.0	66	0.3	**9**	0.12
	Viljoenroad Bridge	8.11	26.0	61	0.1	**11**	1.80
Harts river	Dam 8	8.23	24.0	62	-	-	-
	Jan Kempdorp	7.90	26.1	**165**	-	**19**	-
	HRP Dam	7.10	24.1	**135**	3.5	2	1.70
Schoonspruit river	Orkney	7.61	21.7	52	1.7	3	2.44
	Klerksdorp Bridge	7.74	22.5	39	-	2	0.41
	Brakspruit	8.05	21.1	52	0.3	3	0.55
	Bodenstein	7.91	20.4	60	0	4	0.63
Lower Vaal river	Windsorton	8.23	21.1	36	-	1	0
	Barkley-Wes	8.47	19.3	40	-	-	0
	Schmidtsdrift	8.80	18.7	40	0.2	8	0
	Christiana	7.86	19.0	36	0.4	4	0
	Bloemhof	8.05	18.0	37	-	3	0
Barberspan	Harts River	8.62	13.7	**121**	3.0	0.7	1.68
	Inflow	8.58	15.2	**115**	4.0	-	1.02
	Hotel	**9.23**	14.7	**132**	-	-	0.11
	Outflow	8.61	12.0	**147**	4.0	1.2	0.19

TWQR – target water quality range; Temp = temperature; EC = electrical conductivity; mS/m = milli siemens per meter)

Table 7: Physico-chemical parameters measured at each of the sampling sites at the respective surface water sources during 2011. Highlighted values exceeded TWQR for domestic use.

Water Source	Sampling site	pH	Temp (°C)	EC (mS/m)	NO_3-N (mg/ℓ)	NO_2-N (mg/ℓ)	PO_4^{2-} (mg/ℓ)
	TWQR	**6-9**	**-**	**0-70 mS/m**	**0-6 mg/ℓ**	**0-6 mg/ℓ**	**-**
Harts river	Spitskop Dam	8.25	16.4	**84.3**	-	2	0.44
	Harts-Pampier Dam	8.28	15.4	**133.1**	1.0	4	0.33
	Taung	7.95	11.8	47.9	0.3	3	0.43
	Schweizer Reneke	8.33	13.7	**78.8**	0.1	4	0.34
	Delareyville	8.75	13.1	**109.6**	0.4	4	0.70
Schoonspruit river	Orkney	7.88	18.9	51.2	-	3	0.33
	Klerksdorp Bridge	7.85	18.9	43.2	0.1	2	0.86
	Brakspruit	8.01	18.0	54.1	0.3	4	0.58
	Bodenstein	7.92	17.0	64.4	0.8	3	1.03
Lower Vaal river	Windsorton	7.73	16.1	38.9	-	-	0.10
	Barkley-Wes	8.47	17.0	39.5	-	3	0.45
	Schmidtsdrift	**9.06**	15.6	60.0	-	2	0.16
	Christiana	**9.08**	11.2	40.1	-	-	-
	Bloemhof	8.58	11.2	36.0	0.3	4	0.25
Barberspan and the upper Harts river	Harts River	7.92	22.4	47.1	0.8	5	1.74
	Inflow	7.51	22.0	37.8	0.2	2	1.60
	Hotel	8.75	22.6	**99.8**	0.2	1	-
	Outflow	8.60	23.5	**101.6**	0.2	2	0.52
	Sannieshof	7.58	23.6	45.4	0.1	3	2.19
	Biesiesvlei	7.91	22.7	60.6	-	2	2.65
	Lichtenburg	8.02	23.3	**129.8**	0.4	4	2.97

TWQR – target water quality range; Temp = temperature; EC = electrical conductivity; mS/m = milli siemens per meter)

2.3.2 Levels of culturable indicator bacteria

Overall average total coliform, faecal coliform and *E. coli* levels were generally high at the Lower Harts and Schoonspruit rivers while Barberspan had the lowest levels (Table 8). Low levels of bacteria measured at Baberspan in 2010 may be due to the low water temperatures. These samples were collected during winter. Sampling in 2011 was during the warmer months and bacterial levels were considerably higher.

Total coliform, faecal coliform, *E. coli* and enterococci were present in all surface water systems sampled. From Table 8 it is evident that except for the Schoonspruit, overall average faecal coliform levels measured in surface water systems were generally within the permissible TWQR for full (150 cfu/100 mℓ) and intermediate contact recreational (0-1000 cfu/100 mℓ) activities (DWAF, 1996).

Table 8: The microbiological water quality analysis of each sampling site within the 5 respective surface water systems sampled in 2010 & 2011. TWQR for recreational use.

River system	**Sampling period**	**Total coliforms cfu/100 mℓ**	**Faecal coliforms cfu/100 mℓ**	***E. coli* cfu/100 mℓ**	**Enterococci cfu/100 mℓ**
TWQR		**N/A**	**0-150**	**0-130**	**0-30**
Mooi					
Min	2010	65.3	51.0	2.0	17.3
Max		154.3	60.0	>17.0	147.0
Ave±SD		**107.9±38.0**	**55.5±3.9**	**9.2±5.2**	**47.1±47.0**
Lower Harts					
Min	2010	70.0	20.6	22.0	44.0
Max		>300.0	242.0	76.0	287.3
Ave±SD		**192.5±173.2**	**131.0±126.5**	**49.0±38.2**	**165.8±117.8**
Baberspan					
Min	2010	4.0	6.0	0.0	36.0
Max		270.0	130.0	33.6	127.3
Ave±SD		**80.0±127.1**	**36.7±62.5**	**9.6±16.1**	**68.7±40.5**
Schoonspruit					
Min	2010	252.6	72.0	34.0	44.6
Max		>300.0	134.0	54.0	62.0
Ave±SD		**269.8±57.0**	**99.1±28.6**	**44.3±8.2**	**64.5±17.2**
Vaal					
Min	2010	74.6	42.6	17.3	64.6
Max		132.0	86.6	36.0	165.3
Ave±SD		**103.7±26.1**	**64.2±18.2**	**28.2±8.7**	**89.7±42.8**
Lower Harts					
Min					
Max	2011	26.0	36.0	2.0	2.0
Ave±SD		>300.0	139.0	128.6.0	252.0
		121.0±112.5	**62.14±44.0**	**47.8±49.1**	**70.8±102.6**
Upper Harts & Baberspan					
Min	2011	156.0	44.0	14.0	86.6
Max		>300.0	287.0	178.0	228.6
Ave±SD		**262.0±53.6**	**146.1±88.76**	**90.8±64.5**	**158.7±55.7**
Schoonspruit					
Min	2011	202.3	174.0	91.3	76.0
Max		>300.0	>300.0	>300.0	>300.0
Ave±SD		**286.8±56.4**	**279.8±70.5**	**259.1.0±111.9**	**250.3±116.6**
Vaal					
Min	2011	64.0	10.6	2.0	3.3
Max		146.0	44.6.0	18.7	50.6
Ave±SD		**96.4±33.7**	**24.64. ±12.7**	**13.2±9.5**	**16.0.±20.4**

TWQR – target water quality range; N/A – not applicable; cfu – colony forming unit; Min – minimum; Max – maximum; Ave – average; SD – standard deviation

During the 2010 sampling period results indicated that Barberspan and the Schoonspruit River had the highest overall average total coliform, faecal coliform, *E. coli* and enterococci levels, while the Vaal River had the lowest levels. Total coliform and particularly faecal coliform, *E. coli* and enterococci were also detected at all surface water systems during the 2011 sampling period. In this case Schoonspruit river was the only system with an overall average faecal coliform level exceeding the TWQR for full contact recreational activities (0-150 cfu/100 mℓ).

In 2010, enterococci levels measured at some of the sites in the Mooi River, Harts River, Barberspan, Schoonspruit and Vaal Rivers exceeded the TWQR for full contact recreational use. Enterococci levels exceeding the full contact recreational use TWQR were found at all Barberspan and Schoonspruit sampling sites as well as some sites in the Harts and Vaal Rivers. The acceptable TWQR for enterococci intermediate contact is 0-230 cfu/100 mℓ. Some of the sites in the Harts River and Schoonspruit also exceeded this TWQR range.

The presence of enterococci that exceeded the TWQR for full contact use in these river systems is of concern. This is because this water source is used for various water based recreational as well as religious activities such as full body baptism, where the body is fully submerged for brief periods. The lower body of the participants are in the water for extended periods during the ceremony.

The biplots in Figures 5 and 6 represent the joint effect of environmental variables on the levels of selected bacteria (Ter Braak, 1990). In these biplots the physico-chemical parameters are represented by the thick red arrows and the species (bacterial faecal indicators) by the thin blue arrows. The angle between the species (indicator bacteria) and environmental variables reflect the influence on each other. A small angle indicates that a strong correlation exists between data sets. An angle of 90° and larger indicates that there is no correlation.

A positive correlation existed between levels of faecal coliform and *E. coli*. This is demonstrated by the very small angles between these two variables in both Figures 5 and 6. This type of relationship is expected since *E. coli* is a subgroup of faecal coliforms. Both these figures further demonstrate that a positive correlation also existed between these groups of bacteria (faecal coliforms and *E. coli*) on the one hand, and temperature and phosphate levels on the other. A positive correlation also existed between enterococci and total coliform levels measured in 2010. The same correlation was not seen with the 2011 data (Figure 6). However, in this biplot (Figure 6) a positive correlation between enterococci on the one hand, and nitrate and TDS on the other hand is demonstrated. A positive correlation also exists between the enterococci and nitrate levels. The same correlation was, however, not observed in Figure 5.

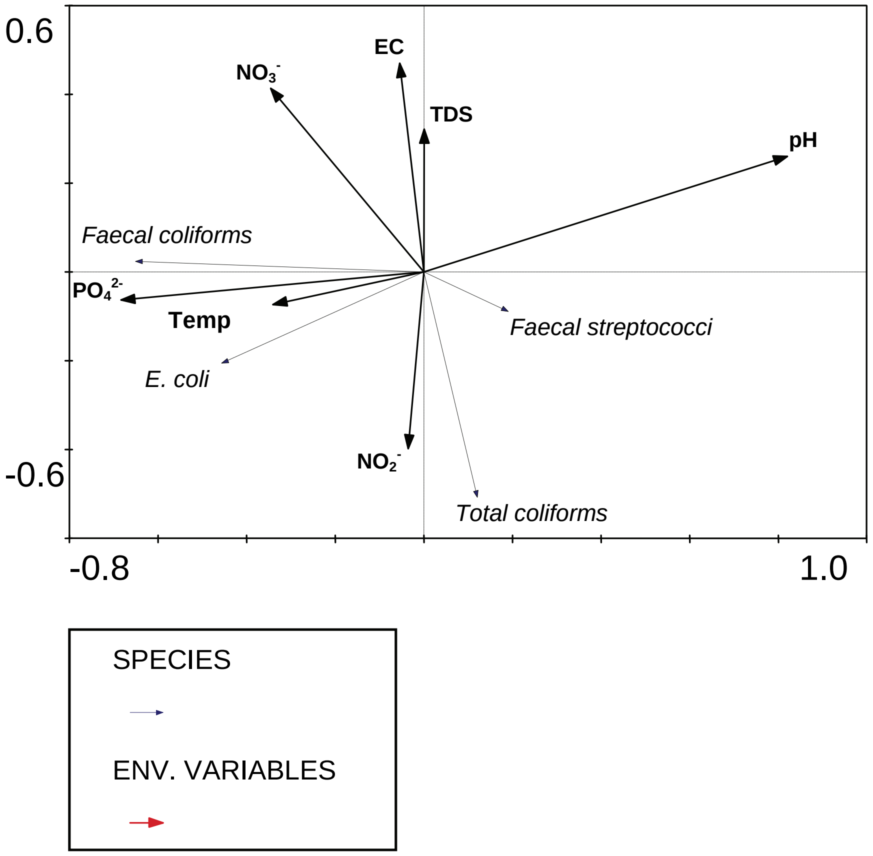


Figure 5: Correlation biplot of 2010 data showing the relationship between dominant physico-chemical parameters (pH, Temperature, TDS, EC, NO2-, NO3- and PO42-) and the levels of enterococci, total coliforms, faecal coliforms and E. coli in 5 surface water sources. The physico-chemical parameters are represented by red arrows, while the species are represented by blue arrows.

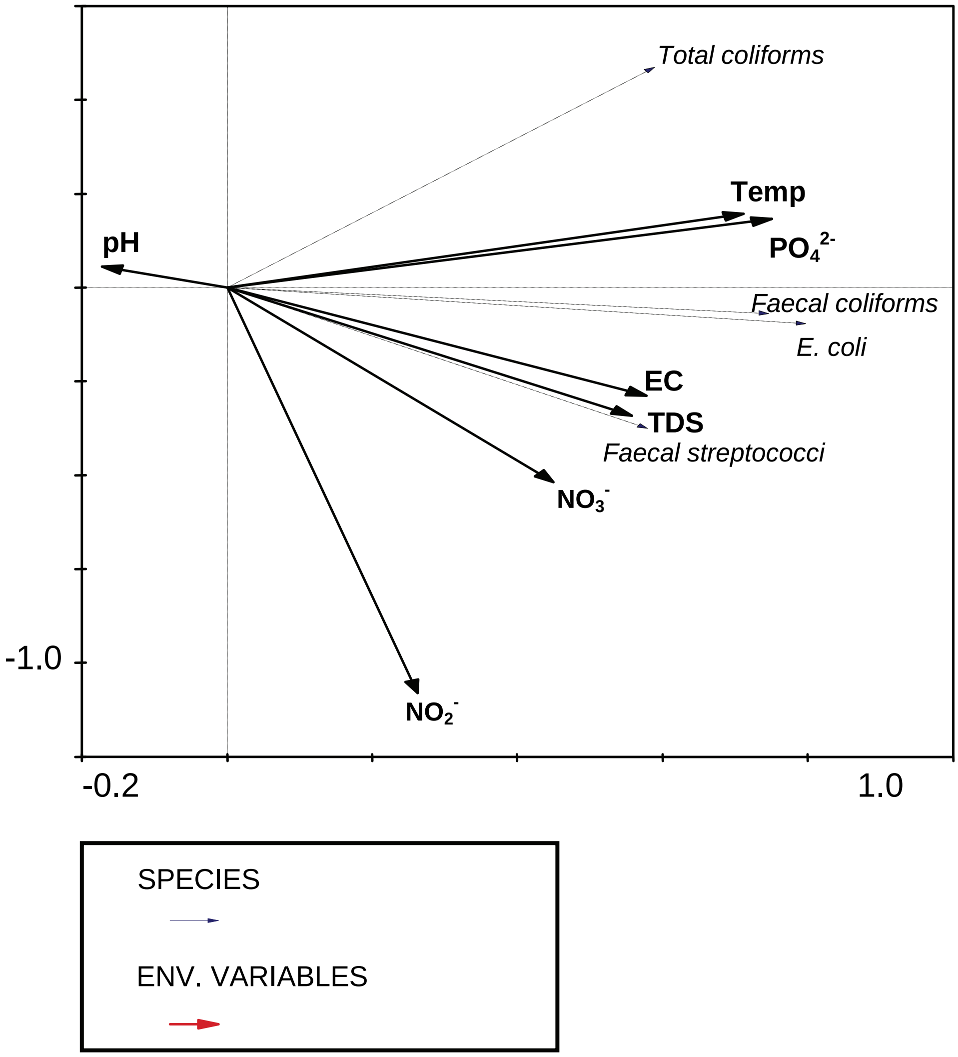


Figure 6: Correlation biplot of 2011 data illustrating the relationship between dominant physico-chemical parameters (pH, Temperature, TDS, EC, NO_2^-, NO_3^- and PO_4^{2-}) and the prevalence of enterococci, total coliforms, faecal coliforms and *E. coli* at 5 surface water sources. The physico-chemical parameters are represented by red arrows, while the species are represented by blue arrows.

2.3.3 Enterococcal species identification

Species identification was based on phenotypic and 16S rRNA sequence data. Table 9 below, presents a total of six species that were identified. These include *Enterococcus faecalis, E. faecium, E. mundtii, E. casseliflavus, E. gallinarum* and *E hirae.* The most frequently isolated species in 2010 were *E. faecium (*34%), *E. casseliflavus* (25%)*, and E. mundtii* (20%). Seventeen percent of the 2010 isolates were *Enterococcus gallinarum*.

The most frequently isolated species in 2011 were *E. mundtii* (37%), *E. faecalis* (33%) *and E. faecium* (20%) *E. casseliflavus, E. gallinarum and E hirae* were less frequently isolated in 2011. Prevalence levels were 7% and below (Table 7). *Enterococcus faecium* was isolated from all the water systems, for each sampling period in 2010 and 2011. *Enterococcus faecalis* was only isolated in 2011.

Table 9: Number of *Enterococci* species identified in the samples from the various sites. Results are provided for 2010 and 2011 sampling periods.

River System	**Year**	**Enterococci species**					
		E. faecalis	***E. faecium***	***E. mundtii***	***E. casseliflavus***	***E. gallinarum***	***E. hirae***
Mooi river	2010		8		1		1
Lower Harts River	2010		1			1	
	2011	1	1	10	1	1	
Barberspan & Upper Harts	2010		1	7			
	2011	8	3	8			
Schoonspruit	2010		1		2	3	
	2011	6	3		1		1
Vaal river	2010		1		6		
	2011	4	5	3	1		

A neighbour-joining phylogenetic tree of enterococci species isolated from water systems in 2010 in provided in Figure 7. The tree is divided into 2 main clusters (A and B). Cluster A is composed of 2 sub-clusters A1 and A2. Sub-cluster A1 is consists of *E. mundtii* and *E. faecium* species whereas sub-cluster A2 is consists of *E. faecium* species. Furthermore, in cluster B, *E. casseliflavus* and *E. gallinarum* species grouped into a single cluster. The *E. faecium* group in cluster A is further grouped into 2 sub-clusters differentiated by distinct branches. In this cluster grouping was according to species and strains but also according to spatial distribution.

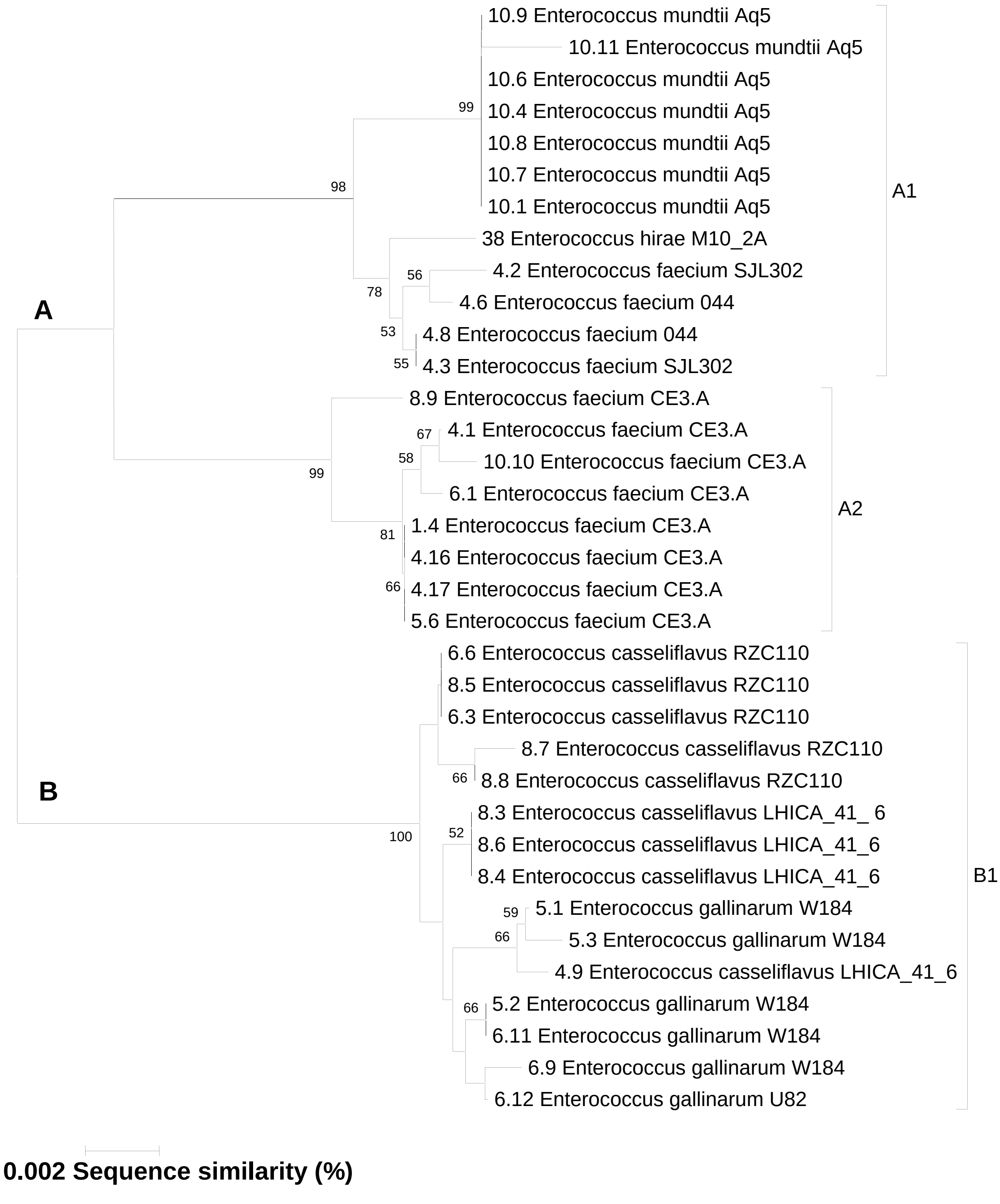


Figure 7: Neighbour-joining (N-J) tree showing the phylogenetic relationships of 35 enterococci species isolated from the 5 surface water systems in 2010. The Jukes Cantor model was used to generate the N-J tree. Bootstrap percentages are indicated at the branching points of the dendrogram.

The neighbour-joining phylogenetic tree of enterococci species isolated from 5 surface water systems during 2011 is depicted in Figure 8. This tree was similar to the 2010 tree depicted in Figure 7. In Figure 8 there are also 3 main clusters, A and B. Cluster A is composed of 3 sub-clusters A1, A2 and A3. These are species specific consisting of *Enterococcus mundtii* (A1), *E. faecium* (A2) and *E. casseliflavus* (A3). The A1 and A2 subclusters are supported by a 99% bootstrap. Cluster B is composed of the *E. faecalis* group. The individual isolates from the E*. mundtii* group is closely related to strain Aq5 sequences that were available on GenBank. These *E. mundtii* species were isolated from the Lower Harts River and Barberspan.

The *E. faecalis* species was first identified in the 2011 surface water samples. This group was predominantly isolated from the Upper Harts and Schoonspruit rivers. The *E. faecalis* group is divided into two subgroups at the interspecies level and the relatedness of these species is supported by an 88% bootstrap confidence. The sequences from two strains in GenBank (E12 and UGRA10) were identified in this species group.

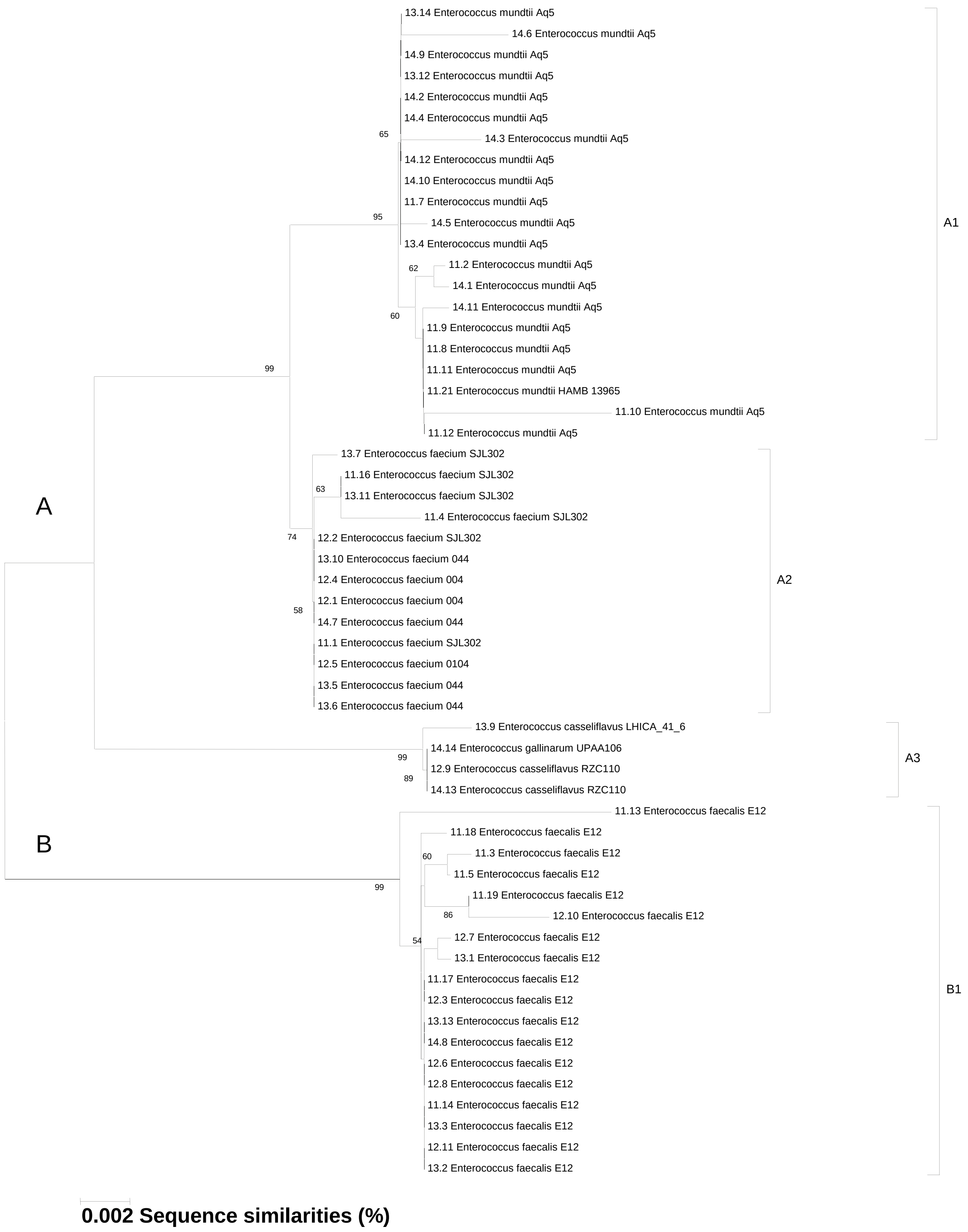


Figure 8: Neighbour-joining graph showing the phylogenetic relationships of 56 enterococci species isolated from 5 surface water systems in 2011 according to the Jukes Cantor model. Bootstrap percentages are indicated at the branching points of the dendrogram.

2.3.4 Levels and diversity of yeasts

The average yeast numbers, statistical differences in pigmented and non-pigmented yeasts between sites and their percentage detected at the sites are presented in Tables 8 and 9. The physico-chemical parameters listed in Tables 5 and 6 are also relevant for the levels of yeast. Sampling was done on the same days when sampling for enterococci was done. When the values of Tables 8 and 9 are compared to the physico-chemical data in Tables 5 and 6 it is evident that the non-pigmented yeast numbers were higher when the water temperature was elevated. This was expected as yeast generally preferred warmer temperatures.

Overall comparison of the total number of pigmented and non-pigmented yeasts shows that the latter were detected in a much higher levels. In 2010, the highest number of pigmented yeasts was found among the Schoonspruit river samples (e.g. Klerksdorp Bridge, 1066 cfu/L). Tukey's HSD statistics revealed significant differences in pigmented yeast numbers between different sites but no significant differences in non-pigmented yeasts (Tables 8 and 9). Pigmented yeast levels in the Schoonspruit River were significantly higher ($P< 0.05$) than in all the other water sources.

The results were analysed by Canoco software (Ter Braak, 1990). Figure 9 is a redundancy analysis diagram showing the correlations between the environmental parameters and levels of yeasts. Results obtained by RDA analysis indicated a positive correlation between the pigmented yeasts and phosphates (Figure 9). It also indicated that a positive correlation existed between Orkney, Klerkskraal Dam and Viljoen Road Bridge sites on the one hand, and phosphates on the other. The non-pigmented yeasts had a positive correlation with temperature. Furthermore, a positive correlation also existed between the yeast levels at Around the World bridge and Thabo Mbeki drive sites on the one hand, and temperature on the other. The other two environmental parameters (TDS and NO_2-N) however, did not have any correlation with the pigmented or non-pigmented yeast levels

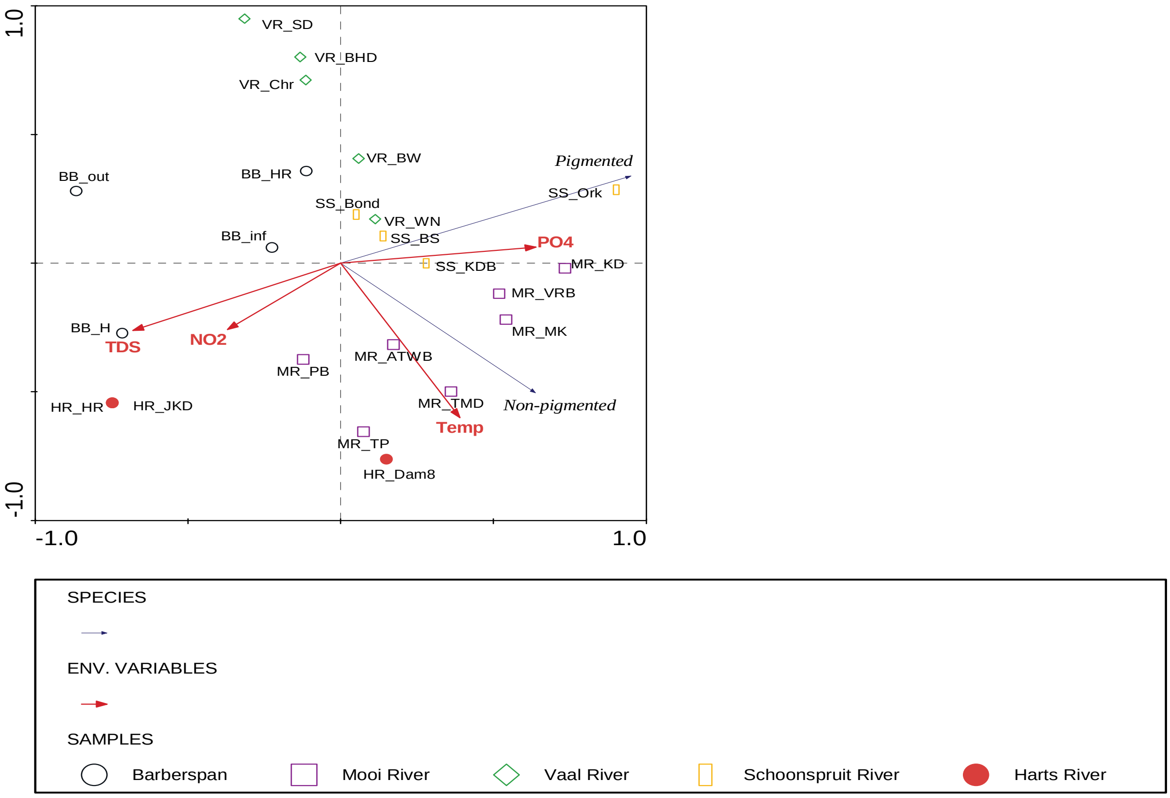


Figure 9: Redundancy analysis (RDA) diagram illustrating the correlation between environmental variables and species (pigmented and non-pigmented yeasts) measured in 2010. The red vectors represent the environmental parameters and blue vectors the species (pigmented and non-pigmented yeasts). Eigenvalues for the first two axes were 0.115 and 0.032 respectively.

Table 10: Average yeast numbers (cfu/ℓ) at various water sources of different sites indicating pigmented and non-pigmented yeasts distribution of 2010.

Water Source	Sites		Ave±SD (cfu/ℓ) ^{1,2}	Percentage (%)^{3}
Mooi river	Klerkskraal Dam	Pigmented	73.0±64.0$^{a, b}$	2.3
		Non-pigmented	3070.0±1930.0^{a}	97.7
	Muiskraal Bridge	Pigmented	210.0±93.0$^{a, b, c}$	42.5
		Non-pigmented	280.0±23.0^{a}	57.5
	Around The World Bridge	Pigmented	0.0^{a}	0
		Non-pigmented	8680.0±7810.0^{a}	100
	Thabo Mbeki drive	Pigmented	0.0^{a}	0
		Non-pigmented	473.0±154.0^{a}	100
	Trimpark North Bridge	Pigmented	0.0^{a}	4.4
		Non-pigmented	2270.0±1920.0^{a}	95.6
	Pedestrian Bridge	Pigmented	0.0^{a}	0
		Non-pigmented	0.0^{a}	0
	Viljoenroad Bridge	Pigmented	0.0^{a}	0
		Non-pigmented	1000.0±580.0^{a}	100
Lower Harts river	Dam 8	Pigmented	0.0^{a}	0
		Non-pigmented	16.0±4.0^{a}	100
	Jan Kempdorp	Pigmented	2.0±0.3^{a}	66.7
		Non-pigmented	1.0±0.6^{a}	33.3
	HRP Dam	Pigmented	0.0^{a}	0
		Non-pigmented	33.0±9.0^{a}	100
Schoonspruit	Orkney	Pigmented	646.0±26.6^{d}	58.0
		Non-pigmented	466.7±145.3^{a}	42.0
	Klerksdorp Bridge	Pigmented	1066.0±67.0^{e}	72.7
		Non-pigmented	400.0±28.9^{a}	27.3
	Brakspruit	Pigmented	430.0±190.0$^{c, d}$	73.8
		Non-pigmented	150.0±55.0^{a}	26.1
	Bodenstein	Pigmented	300.0±12.0$^{b, c}$	100
		Non-pigmented	0.0^{a}	0
Vaal river	Windsorton	Pigmented	0.0$^{a, b}$	0
		Non-pigmented	35.0±5.0^{a}	100
	Barkley-Wes	Pigmented	0.0$^{a, b}$	0

Water Source	Sites		Ave±SD (cfu/ℓ) ^{1,2}	Percentage (%)^{3}
Baberspan		Non-pigmented	10.0±5.0^{a}	100
	Schmidtsdrift	Pigmented	10.0±2.0$^{a, b}$	7.5
		Non-pigmented	120.0±2.5^{a}	92.5
	Christiana	Pigmented	0.0$^{a, b}$	0
		Non-pigmented	47.0±7.0^{a}	100
	Harts River (Town)	Pigmented	20.0±20.0$^{a, b}$	41.0
		Non-pigmented	10.0±10.0^{a}	59.0
	Inflow	Pigmented	45.0±15.0$^{a, b}$	52.9
		Non-pigmented	40.0±40.0^{a}	47.1
	Hotel	Pigmented	0.0$^{a, b}$	0
		Non-pigmented	5.0±5.0^{a}	100
	Outflow	Pigmented	10.0$^{a, b}$	40.0
		Non-pigmented	15.0±5.0^{a}	60.0

[1] Values given are average values at each sampled site (Ave) ± standard deviation (SD). [2] Sites with the same combination of superscript alphabetic letters ^{a,b,c,d} indicate no significant differences ($P > 0.05$) among sites, while those with different letters indicate significant differences at $P < 0.05$ (Tukey's HSD). [3] Percentage pigmented and non-pigmented yeasts

Table 11: Average yeast numbers (cfu/ℓ) at various water sources of different sites indicating pigmented and non-pigmented yeasts distribution of 2011.

Water Source	Sites		Ave±SD (cfu/ℓ) ^{1,2}	Percentage (%)^{3}
Upper Harts river	Spitskop Dam	Pigmented	0.0^{a}	0
		Non-pigmented	4120.0±200.0^{a}	100
	Harts-Pampier Dam	Pigmented	1310.0±270.0^{b}	51.6
		Non-pigmented	1230.0±50.0^{a}	48.4
	Taung	Pigmented	180.0±80.0^{a}	0.1
		Non-pigmented	17200.0±1760.0$^{c, d}$	99
	Schweizer Reineke	Pigmented	60.0^{a}	0.4
		Non-pigmented	16120.0±920.0^{c}	99.6
	Delareyville	Pigmented	30.0±10.0^{a}	0.6
		Non-pigmented	4590.0±190.0$^{a, b}$	99.4
Schoonspruit	Orkney	Pigmented	3760.0±400.0^{c}	13.9
		Non-pigmented	23240.0±1960.0$^{c, d}$	86.1

Water Source	Sites		Ave±SD (cfu/ℓ) ^{1,2}	Percentage (%)^{3}
Vaal river	Klerksdorp Bridge	Pigmented	100.0±20.0^{a}	0.4
		Non-pigmented	28280.0±520.0^{d}	99.6
	Brakspruit	Pigmented	130.0±110.0^{a}	0.8
		Non-pigmented	16040.0±8200.0$^{b, c}$	99.2
	Bodenstein	Pigmented	10.0±10.0^{a}	1.4
		Non-pigmented	730.0±310.0^{a}	98.6
	Windsorton	Pigmented	40.0±20.0^{a}	17.4
		Non-pigmented	190.0±50.0^{a}	82.6
	Barkley-Wes	Pigmented	0.0^{a}	0
		Non-pigmented	1830.0±1770.0^{a}	100
	Schmidtsdrift	Pigmented	0.0^{a}	0
		Non-pigmented	4180.0±2260.0^{a}	100
	Christiana	Pigmented	280.0±120.0^{a}	31.1
		Non-pigmented	620.0±140.0^{a}	68.9
	Bloemhof Dam	Pigmented	20.0±20.0^{a}	1.7
		Non-pigmented	1150.0±250.0^{a}	98.3
Upper Harts river and Baberspan	Harts River (Town)	Pigmented	230.0±150.0^{a}	33.8
		Non-pigmented	450.0±50.0^{a}	66.2
	Inflow	Pigmented	1180.0±160.0^{b}	43.4
		Non-pigmented	1540.0±120.0^{a}	56.6
	Hotel	Pigmented	50.0±10.0^{a}	18.5
		Non-pigmented	220.0±20.0^{a}	81.5
	Outflow	Pigmented	400.0±80.0^{a}	18.0
		Non-pigmented	1820.0±120.0^{a}	82.0
	Sannieshof	Pigmented	50.0±10.0^{a}	3.3
		Non-pigmented	1450.0±250.0^{a}	96.7
	Biesiesvlei	Pigmented	10.0±10.0^{a}	0.3
		Non-pigmented	2960.0±560.0^{a}	99.7
	Lichtenburg	Pigmented	110.0±90.0^{a}	5.8
		Non-pigmented	1800.0±400.0^{a}	94.2

[1]Values given are average values at each sampled site (Ave) ± standard deviation (SD). [2]Sites with the same combination of superscript alphabetic letters ^{a,b,c,d} indicate no significant differences (P> 0.05) among sites, while those with different letters indicate significant differences at P< 0.05 (Tukey's HSD). [3]Percentage pigmented and non-pigmented yeasts.

During the 2011 sampling significant differences were also found when pigmented and non-pigmented yeasts levels between sites were compared (Table 8). The only significant differences in pigmented yeast levels were observed between Harts Pampierstad Bridge, Baberspan Inflow and the Orkney sites. Pigmented yeast levels in the Schoonspruit River were again higher (P< 0.05) than in all the other water sources. In Section 2.3.2 it was also demonstrated that the enterococci levels at Schoonspruit was on average higher than the other sampling points.

The 2011 results were also analysed by Canoco software (Ter Braak, 1990). Figure 10 is a redundancy analysis diagram showing the correlations between the environmental parameters and levels of yeasts.

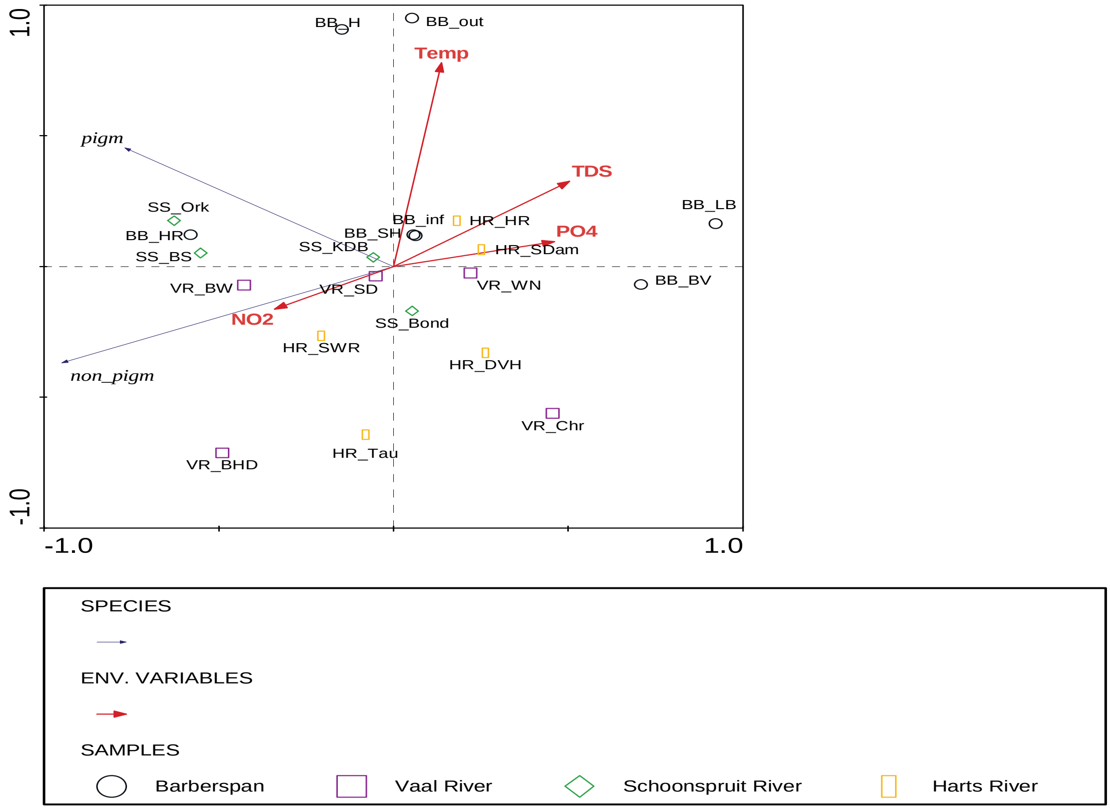


Figure 10: Redundancy analysis (RDA) diagram illustrating the correlation between environmental variables and species (pigmented and non-pigmented yeasts) measured in 2011. The red vectors represent the environmental parameters and blue vectors the species (pigmented and non-pigmented yeasts). Eigenvalues for the first two axes were 0.107 and 0.025 respectively**.**

RDA analysis indicated a positive correlation existed between the levels of non-pigmented yeasts and nitrate (Figure 10). It also indicated that yeast levels measured at Barkley-Wes and Schweizer Reineke were correlated to nitrate levels. The rest of the environmental parameters, however, did not have any correlation with the pigmented or non-pigmented yeasts or the levels of yeast at the specific sites.

Results in Table 10 are further summarised in Table 12 and results of Table 11 summarised in Table 13. These tables indicate that a large number of yeasts were isolated but also that the distribution of the various yeasts was not uniform.

Table 12: Percentage ascomycetous and basidiomycetous yeasts from the North West Province during 2010.

Surface water sources	% Ascomycetous yeasts	% Basidiomycetous Yeasts	% Pigmented	% non-Pigmented
Mooi river	85	-	-	100
(n=23)	-	15	5	-
Harts river	95	-	-	100
(n=17)	-	5	0	-
Schoonspruit	28	-	-	100
river (n=25)	-	72	56	44
Lower Vaal river	61	-	-	100
(n=25)	-	39	92	8
Barberspan	8	-	-	100
(n=13)	-	92	38	62

Table 13: Percentage ascomycetous and basidiomycetous yeasts from the North West Province during 2011.

Surface water sources	% Ascomycetous yeasts	% Basidiomycetous Yeasts	% Pigmented	% non-Pigmented
Harts river	50	-	-	100
(n=22)	-	50	32	18
Schoonspruit	64	-	-	100
River (n=22)	-	36	27	9
Lower Vaal river	83	-	-	100
(n=18)	-	17	17	6
Barberspan	50	-	-	100
(n=26)	-	50	38	12

All ascomycetous and basidiomycetous yeasts from the respective water sources were able to grow at 25°C (Table 14). This was expected since all these yeasts were isolated at this temperature. However, a large percentage of the isolates were able to grow at 37°C and higher temperatures. Determining the ability of yeasts to grow at 37°C may be important as this temperature is associated with warm-blooded animals. Human opportunistic pathogenic yeasts are known to thrive at this temperature. Typically such species include *Candida* *albicans* and *Candida tropicalis* and a number of other opportunistic pathogenic yeasts (Medeiros et al., 2008). Results also indicated that isolates from the respective surface water sources can grow at a minimum temperature of 4°C (psycrophylic) and at the maximum temperature used in this study (40°C; thermo-tolerant).

Table 14: Percentage ascomycetous and basidiomycetous yeasts growing at various temperatures.

Surface water sources		**% of isolates that grew at**				
		4°C	**25°C**	**30°C**	**37°C**	**40°C**
Harts river (n=22)	Ascomycetous	68	100	68	64	70
	Basidiomycetous	32	100	32	36	25
Schoonspruit river (n=22)	Ascomycetous	100	100	100	18	0
	Basidiomycetous	100	100	100	0	0
Lower Vaal river (n=18)	Ascomycetous	83	100	83	50	33
	Basidiomycetous	17	100	17	11	6
Barberspan (n=26)	Ascomycetous	42	100	42	27	38
	Basidiomycetous	50	100	50	19	38

2.3.5 Phenotypic and genetic identification of yeasts

The morphological characteristics were determined by microscopy. Figure 11 shows photographs of selected isolates indicating the morphological appearance. The cells were spheroidal or ovoidal. Yeast colonies were cream to tan or pink colour, with a smooth texture.

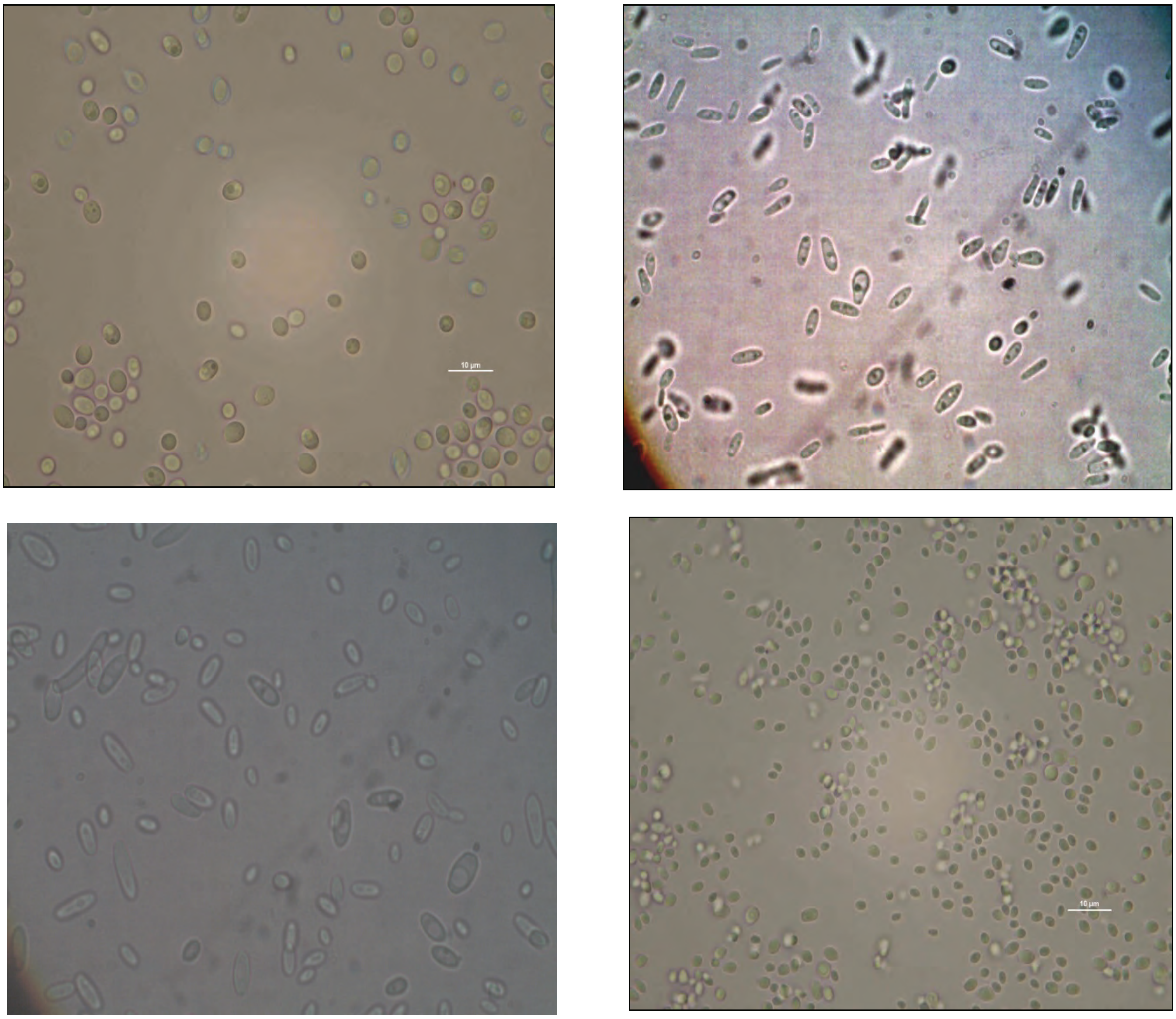


Figure 11: Photograph of some of the isolated yeasts (400X magnification)

Biochemical identification of the yeasts indicated that *Candida* spp. were the most prevalent, followed by *Rhodotorula* spp., *Wickerhamomyces* spp., *Pichia* spp., *Clavispora* spp., *Cryptococcus* spp., *Sporidiobolus* spp., *Hanseniaspora* spp., *Cystofilbasidium* spp., *Meyerozyma* spp., *Aureobasidium* spp. and *Saccharamyces* spp.. Identities of these species were confirmed using D1/D2 region of the 26S rRNA gene sequence data. These sequence data were analysed by MEGA 5 software and the resulting neighbour joining tree for the 2010 sampling period is provided in Figure 12 and those of the 2011 period in Figure 13.

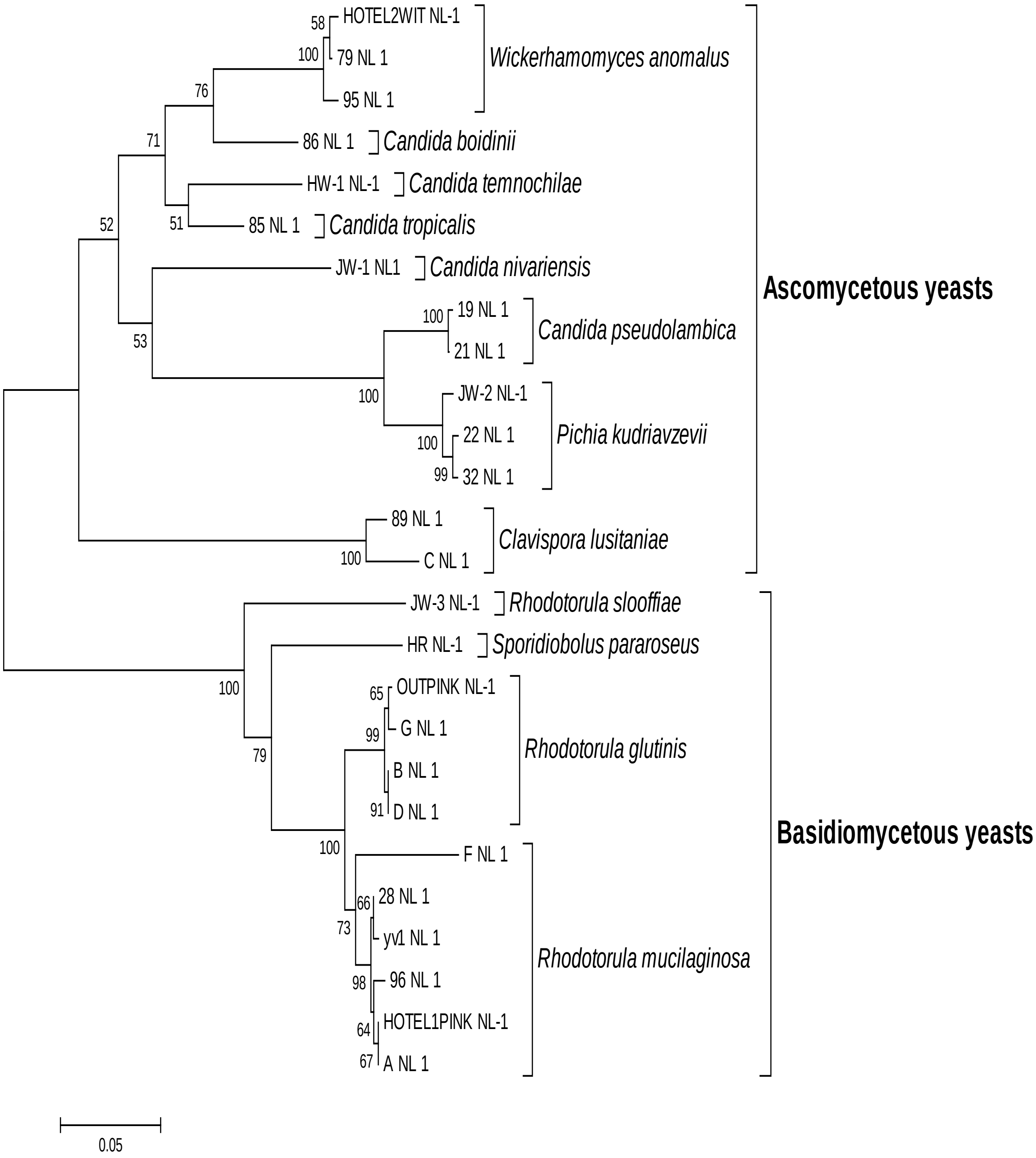


Figure 12: Neighbour-joining tree based on partial sequences of D1/D2 domain of 26S rDNA obtained in 2010. The tree was constructed using the Kimura two-parameter method. Local bootstrap probability values obtained by maximum likelihood analysis are indicated at the branche nodes.

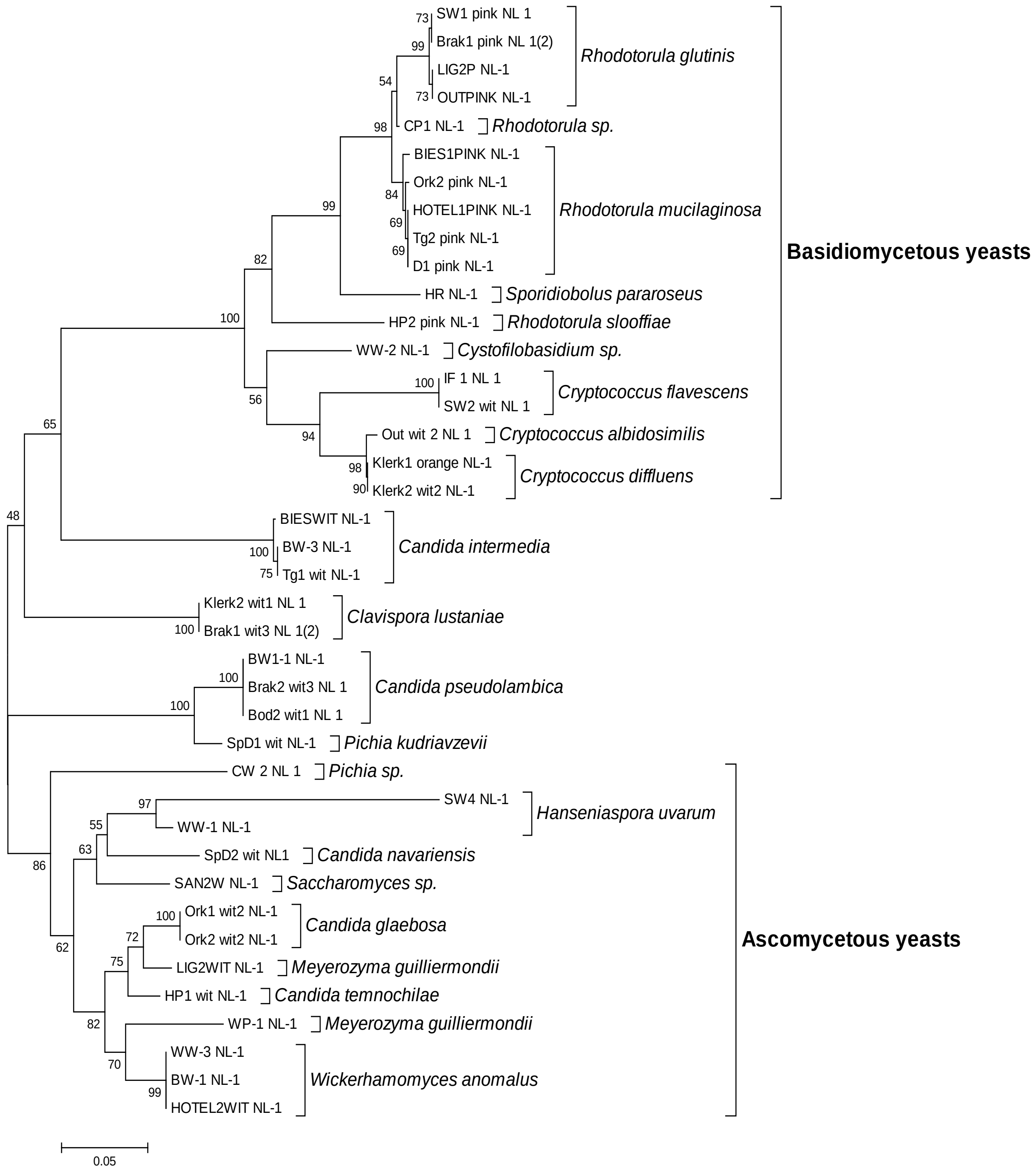


Figure 13: Neighbour-joining tree based on partial sequences of D1/D2 domain of 26S rDNA obtained in 2010. The tree was constructed using the Kimura two-parameter method. Local bootstrap probability values obtained by maximum likelihood analysis are indicated at the branche nodes.

The ascomycedious and basidiomycetous yeast formed separate clusters in both phyogenetic trees (Figures 12 and 13). Ascomycetous yeasts consisted mainly of *Wickerhamomyces* sp., *Candida* sp., *Pichia* sp. and *Clavispora* sp. The basidiomycetous yeast group was made up of *Rhodotorula* sp. and *Sporidiobolis* sp. In 2011 similar groupings were observed in the neighbour-joining tree. However, in this case there was a greater diversity of both basio- and ascomycetous yeasts. *Cryptococcus* sp. as well as *Cystofilobasidium* sp. were present among the basidiomycetous yeasts. Amongst the ascomycetous yeasts *Hanseniaspora* sp., *Saccharomyces* sp., and *Meyerozyma* sp. were also isolated (Figure 13).

2.3.6 Molecular studies of microbial diversity

Denaturing gradient gel electrophoresis (DGGE) as well as high throughput sequencing (HTS) or metagenomic sequencing was used to determine the diversity of bacterial species in some surface water sources. DGGE banding profiles of the bacterial diversity for the winter and summer season are depicted in Figure 14.

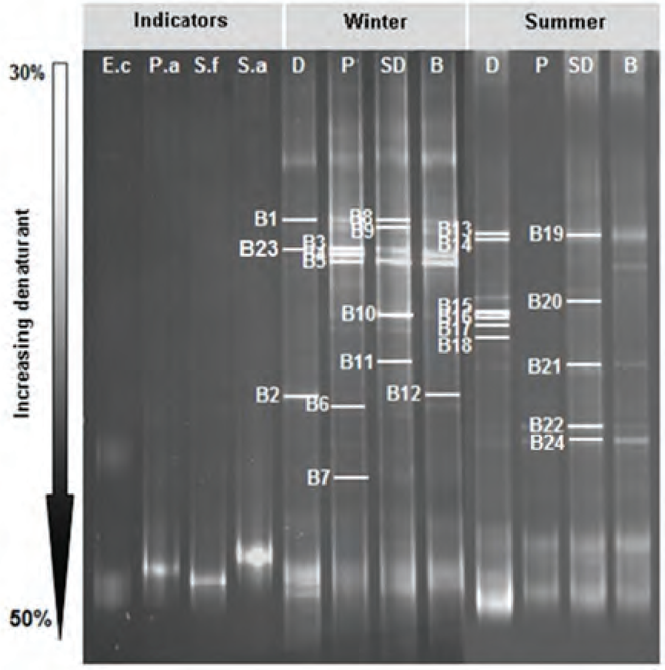


Figure 14: DGGE bacterial community analyses for surface water in winter and summer at the Vaal river sampling sites, Deneysville (D), Parys (P), Scandinawieë Drift (SD) and Barrage (B). Indicator species included were *E. coli* (E.c), *Pseudomonas aeruginosa* (P.a), *Streptococcus faecalis* (S.f) and *Staphylococcus aureus* (S.a).

DGGE profiles demonstrated high resolution and intensity at a denaturing gradient of 30–60%. Several bands were observed in the DGGE profiles. Four bacterial species, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus feacalis* and *Staphylococcus aureus*, were included in DGGE gels to determine the potential of using such an approach to establish the presence of these species in water samples. Corresponding bands for *Staphylococcus aureus* and *Pseudomonas aeruginosa* were observed for Parys, Scandinawieë Drift and Vaal Barrage. In addition, Parys had a band with similar migration patterns to *Escherichia coli*. The corresponding bands were excised and sequenced but produced poor quality sequences with indefinite identification. Since sequence data could not confirm accurate identification of the excised bands, results remain inconclusive. Bands with similar migration patterns for *Streptococcus feacalis* were not observed in DGGE profiles (Figure 14).

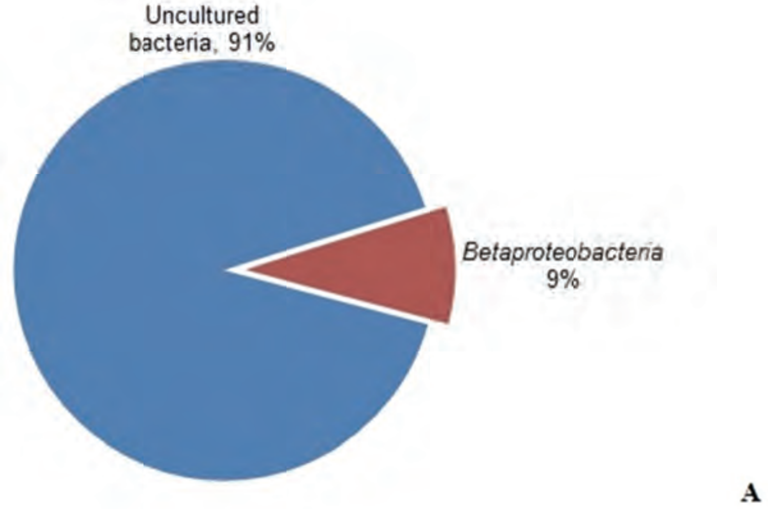
Spatial and temporal changes in the bacterial community composition among the four sampling sites were evident (Figure 14). DGGE profiles yielded several distinct bands representing the structure of the numerically dominant bacterial population at the time of sampling. There were a large number of faint bands that were visible in the gels that could potentially indicate community complexity. Dominant bands present at Vaal Barrage, Parys and Scandinawieë Drift displayed similar migration patterns that may indicate the distribution of potentially related and dominant bacterial species between the three sampling points. In contrast, Deneysville illustrated a distinctive bacterial diversity compared to the three other sites. In this case (Deneysville sample), a small number of dominant bands exhibited similar migration patterns to Parys, Scandinawieë Drift and Vaal Barrage. Similar banding patterns of dominant bands may suggest the growth and reproduction of a few dominant species at the Deneysville sampling site, compared to the downstream sampling sites (Parys, Scandinawieë Drift and Vaal Barrage)

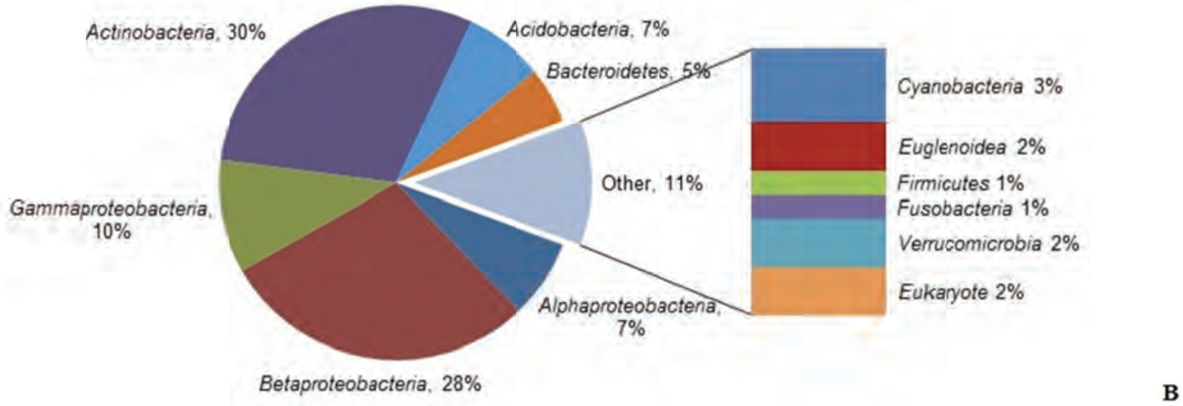
Dominant bands across all sampling sites were excised, re-amplified and sequenced. Sequence quality for the DGGE bands was generally sufficient for identification. Identification details are provided in Table 15. Phylogenetic groups present during the winter and summer season included *Cyanobacteria*, *Bacteroidetes* and *Betaproteobacteria*. However, bacterial community compositions for the four sampling stations shifted during seasonal changes. Community composition for Deneysville mainly consisted of *Betaproteobacteria* during the winter season, whereas *Cyanobacteria* dominated during the summer season. The high abundance of *Cyanobacteria* during summer may be associated with the green algal blooms observed at the time of sampling. Vaal Barrage, Parys and Scandinawieë Drift consisted primarily of *Bacteroidetes* and *Cyanobacteria* during winter, but shifted in community composition during summer so that *Bacteroidetes* and *Betaproteobacteria* were dominant at all three sites.

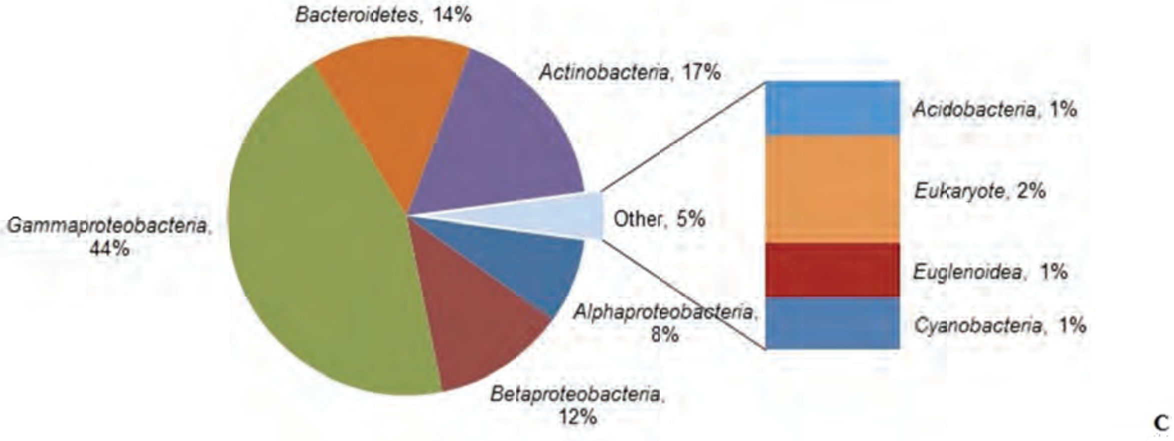
Table 15: Sequence identification of PCR-DGGE bands using reference sequences in the NCBI database.

DGGE band no.	NCBI accession no.	Closest relative (accession no.)	Phylogenetic affiliation	Percentage (%) similarity
B1	JQ085826	Uncultured bacterium clone XYHPA.0912.160 (HQ904787)	*Bacteria*	100
B2	JQ085827	Uncultured *Methylophilaceae* bacterium clone YL203 (HM856564)	*Beta proteobacteria*	100
B3	JQ085828	Uncultured bacterium clone SW-Oct-107 (HQ203812)	*Bacteria*	100
B4	JQ085829	Uncultured *Cyanobacterium* clone TH_g80 (EU980259)	*Cyanobacteria*	100
B5	JQ085830	Uncultured bacterium clone SINO976 (HM130028)	*Bacteria*	99
B6	JQ085831	Uncultured *Haliscomenobacter* sp. clone WR41 (HM208523)	*Bacteroidetes*	96
B7	JQ085832	Uncultured bacterium clone McSIPB07 (FJ604747)	*Bacteria*	98
B8	JQ085833	Uncultured bacterium clone ES3-64 (DQ463283)	*Bacteria*	99
B9	JQ085834	Uncultured bacterium clone ANT31 (HQ015263)	*Bacteria*	100
B10	JQ085835	Uncultured bacterium clone SING423 (HM129081)	*Bacteria*	99
B11	JQ085836	Uncultured *Bacteroidetes* sp. clone MA161E10 (FJ532864)	*Bacteroidetes*	100
B12	JQ085837	Uncultured *Nitrosomonadaceae* bacterium clone YL004 (HM856379)	*Beta proteobacteria*	92
B13	JQ085838	*Aphanizomenon gracile* ACCS 111 (HQ700836)	*Cyanobacteria*	91
B14	JQ085839	*Anabaena circinalis* LMECYA 123 (EU07859)	*Cyanobacteria*	97
B15	JQ085840	*Cymbella Helvetica* strain NJCH73 (JF277135)	*Cyanobacteria*	99
B16	JQ085841	Uncultured bacterium clone FrsFi208 (JF747973)	*Bacteria*	99
B17	JQ085842	Uncultured *Cyanobacterium* clone LiUU-11-80 (HQ386609)	*Cyanobacteria*	98
B18	JQ085843	Uncultured bacterium clone TG-FD-0.7-May-09-B061 (HQ532969)	*Bacteria*	99
B19	JQ085844	Uncultured bacterium clone C_J97 (EU735734)	*Bacteria*	89
B20	JQ085845	Uncultured bacterium clone Lc2yS22-ML-056 (FJ355035)	*Bacteria*	97
B21	JQ085846	Uncultured bacterium clone ncd240a07c1 (HM268907)	*Bacteria*	91
B22	JQ085847	Uncultured *Sphingobacterium* sp. HaLB8 (HM352374)	*Bacteroidetes*	100
B23	JQ085848	Uncultured *Cyanobacterium* isolate DGGE gel band B5 (JN377930)	*Cyanobacteria*	98
B24	JQ085849	Uncultured *Dechlorosoma* sp. clone MBfR-NSP-159 (JN125313)	*Beta proteobacteria*	86

The same DNA samples were subjected to high through put sequencing (HTS) analysis of 16S rRNA. A total of eighteen phylogenetic groups were identified among the four sampling sites. This data were analysed and are provided in Figure 15 (A-F).







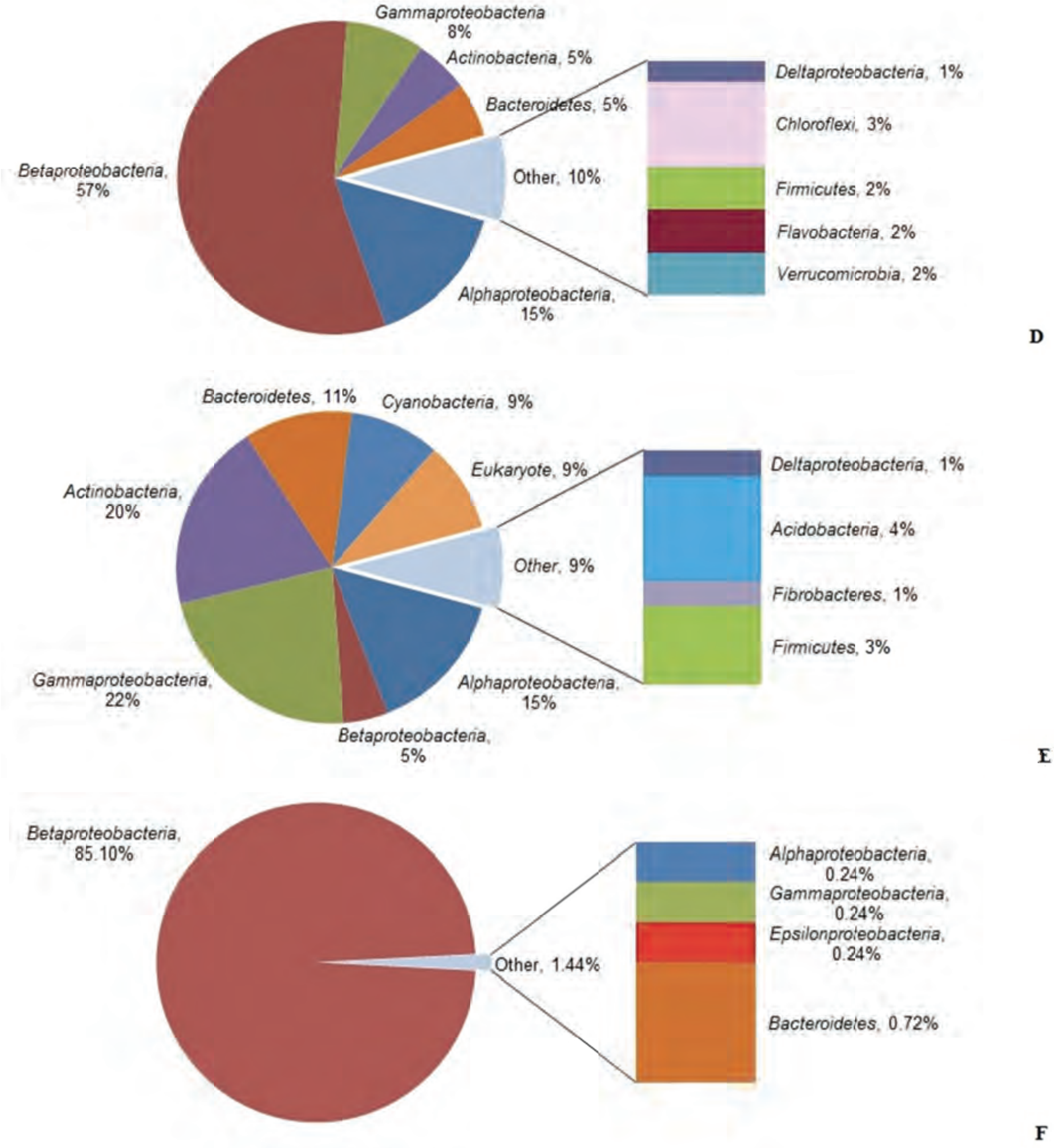


Figure 15: The relative abundance and composition of the dominant bacterial phylogenetic groups in the Vaal river obtained from high-throughput sequencing technology for the various sample sites [(A) Deneysville – summer, (B) Vaal Barrage – summer, (C) Parys – summer, (D) Parys – winter, (E) Scandinawieë Drift – summer and (F) Scandinawieë Drift – winter].

Betaproteobacteria was the only group identified at Deneysville (Figure 15 A). The remainder of the sequences affiliated to unidentified bacteria that could not be taxonomically classified. Relative abundance of *Proteobacteria* (13-17%), *Actinobacteria* (5-10%) and *Bacteroidetes* (2-4%) at Vaal Barrage, Parys and Scandinawieë Drift was, to some extent, similar. *Betaproteobacteria* dominated bacterial communities at Vaal Barrage, whereas

Gammaproteobacteria was the main bacterial class at Parys and Scandinawieë Drift. The remaining groups belonged to nine phyogenetic groups: *Acidobacteria*, *Chloroflexi*, *Cyanobacteria*, *Euglenoidea*, *Eukaryote*, *Fibrobacteres*, *Firmicutes*, *Fusobacteria*, and *Verrrucomicrobia*. The dominant groups *Betaproteobacteria*, *Gammaproteobacteria* and *Actinobacteria*, largely comprised of *Pandoraea* sp., *Pseudomonas* sp. and *Microbacterium* sp., respectively.

Identification of the four indicator organisms employed in DGGE profiling remains inconclusive. HTS analysis verified that the *Pseudomonadaceae* family and *Escherichia* species were present in some of the samples.

2.3.7 Enteroviruses and bacteriophages in water

a) Enteroviruses

Enterovirus cell cultures were obtained from Professor Maureen Taylor, Medical campus, University of Pretoria. RNA was extracted from these cultures and was subsequently used to set the standard that can be used for quantification. This enabled the preparation of a standard curve as shown in Figure 16.

The standard curve (Figure 16A) was used to estimate the amount of viruses in all the samples. The generated standard curve reflects the efficacy of the PCR assay as a quantification method, considering the values of linear correlation coefficient and slope, which were 0.984 and -3.319, respectively.

All the effluent samples from 3 wastewater treatment plants that decant into surface water sources had high viral counts, in some cases equal and greater than 10^3 (Table 16). Although the level of infectivity of the detected viruses could not be confirmed with the qPCR results, previous studies have established a link between qPCR results and data from cell culture methods. The frequent detection of viral genome in sewage effluents suggests that treated sewage may represent a source of environmental contamination with potentially infectious enteroviruses. From the results (Table 16), only site 3 had an average of +90% reduction in the concentration of enterovirus contained in the treated sewage. Even so, the fact that large quantities of viral particles are entering the surface water bodies is cause for concern. During this preliminary study two of the WWTPs did not effectively remove virus particles. On two occasions, plant 1 removed 31.45% and 39.17% of virus particles. Virus removal in plant 2 was also varying and on two occasions removed only 18.82% and 20.74% virus particles. On these occasions plant 3 remove between 90.05 and 99.97% of viruses.

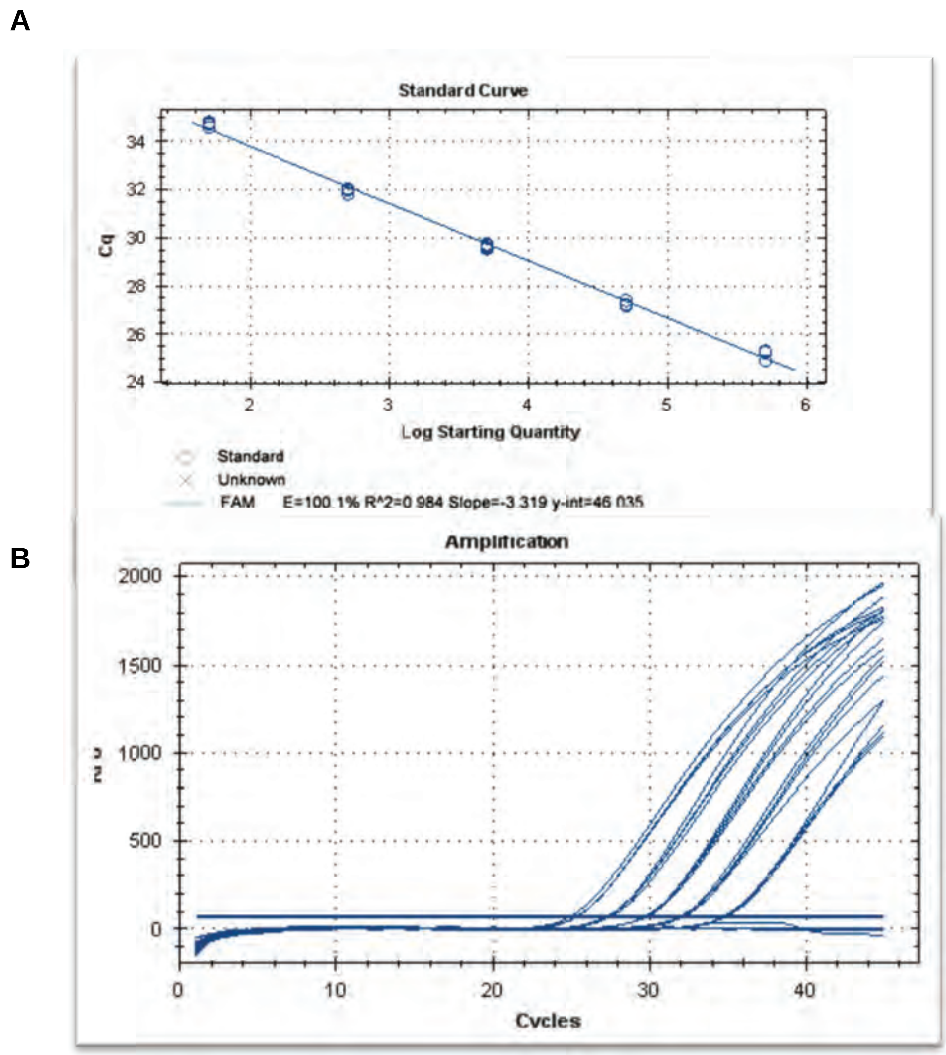


Figure 16: Standard curve (A) of the real time PCR assays for the detection of serially diluted cDNA templates of enterovirus 5^0 to 5^5(B).

Table 16: Preliminary enterovirus data indicating removal efficacy of 3 WWTPs and the counts of viruses entering the water bodies after tertiary treatment.

Date of sample collection	Sites	Viral count (Influent - enterovirus particles/mℓ)	Viral Count (Effluent– enterovirus particles/mℓ)	Percentage reduction (%)
May 10, 2011	1	7.54E+08	**2.54E+05**	99.97
	2	4.34E+06	**3.44E+06**	**20.74**
	3	8.38E+06	**2.10E+03**	99.97
May 23, 2011	1	1.20E+04	**7.30E+03**	**39.17**
	2	2.72E+04	**2.28E+03**	91.62
	3	2.16E+04	**2.14E+03**	90.09
June 08, 2011	1	3.18E+03	**2.18E+03**	**31.45**
	2	5.42E+01	**4.40E+01**	**18.82**
	3	2.02E+03	**1.20E+01**	99.41

b) Bacteriophages

Water samples were collected from different non-potable (rivers and dams) and potable (taps, boreholes, windmills) sources in the four districts of the North West Province. Some of the sampling sites were selected based on visual observation of water usage by the people living near the bank of the river or dam. Activities included fishing, washing of clothes, baptism and also animal watering. Figure 17 indicates typical plaques that were observed on the plates and counted.

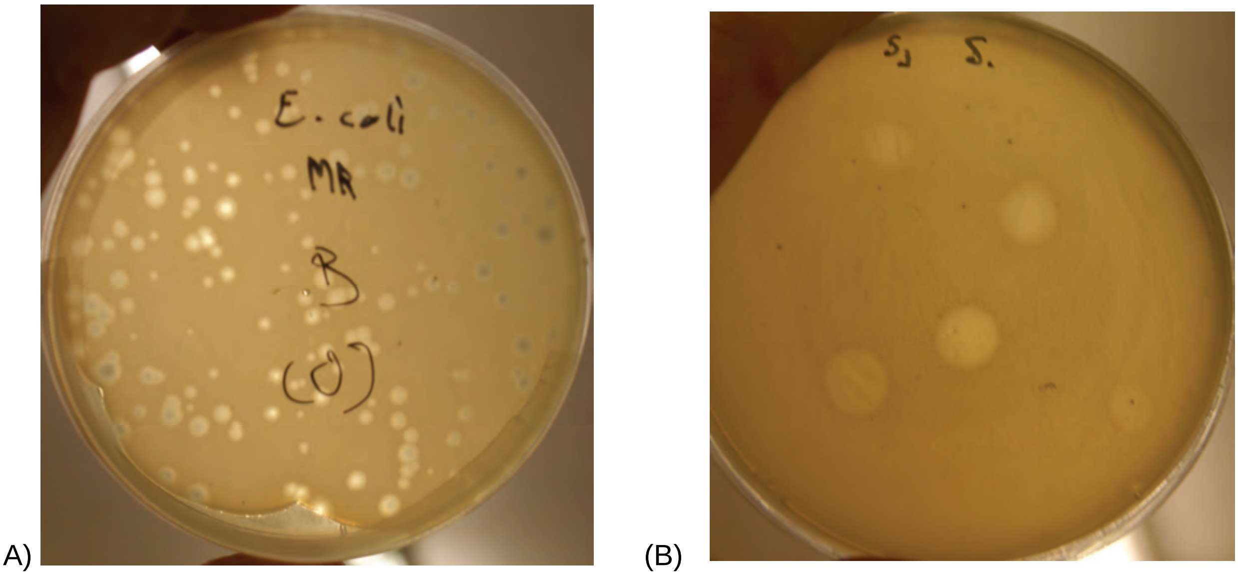


Figure 17: Typical somatic phages plaques (A) and F-specific RNA phages (B) observed.

Phage detection results are presented in Tables 20 and 21 as well as in Figure 18. Tables 20 to 22 have some values for physico-chemical parameters that were measured. Turbidity was quite high and exceeded the TWQR for full contact recreational use (0-3 NTU; Table 15) as well as domestic used (0-1 NTU; Table 14). Some of the pH levels were also outside these specific TWQRs. What is of concern is that several of the sources were positive for somatic and F-RNA phages indicating potential faecal pollution. A further concern is that these included potable water sources (Table 17). This is an aspect that needs further investigation.

Table 17: Physical parameters measured and levels of phages for potable water from selected sites in the North West Province. Highlited values exceeded TWQR for domestic use.

Sample Site	Temperature (°C)	pH	Turbidity (NTU)	Somatic phages pfu/mℓ	F-RNA phages pfu/mℓ
TWQR for domestic use		**6-9**	**0-1**	**0**	**0**
Disaneng Ground water	7.8	7.87	**13.9**	0	0
Madobigo Tap	12.0	7.4	**5.8**	0	0

Sample Site	**Temperature (˚C)**	**pH**	**Turbidity (NTU)**	**Somatic phages pfu/mℓ**	**F-RNA phages pfu/mℓ**
TWQR for domestic use		**6-9**	**0-1**	**0**	**0**
Delareyville Windmill	4.8	6.9	**8.5**	0	0
Ntsweletsoku Tank	7.6	7.5	**8.0**	**4**	0
Klerkskraal Tank	7.7	6.7	**7.9**	**2**	0
Potchefstroom Tap	8.5	**6.4**	**5.9**	**4**	0
Swartruggens Tap	5.6	6.8	**8.1**	0	**8**

TWQR – target water quality range; pfu/mℓ – **plaque forming units per millilitre.**

Table 18: Physical parameter measurements and levels of phages detected in the various water sources. Highlited values exceeded TWQR for full contact recreation use.

Sample Site	**Temperature (˚C)**	**pH**	**Turbidity (NTU)**	**Somatic phages pfu/mℓ**	**F-RNA phages pfu/mℓ**
TWQR – full contact recreation		**6.5-8.5**	**0-3**	**0-20**	**0-20**
Setumo Dam	26.6	7.7	**11.0**	0	0
Disaneng Dam	7.9	7.8	**5.9**	0	0
Moletsamongwe Dam	6.9	**4.8**	**8.1**	0	0
Letlamoreng river	17.3	8.4	**8.5**	0	0
Cooke's lake	11.0	**6.4**	**5.3**	0	0
Delareyville 1	10.6	7.8	**6.1**	0	0
Delareyville 2	6.9	**5.0**	**5.1**	0	0
Groot Marico river	12.8	9.1	**6.1**	0	**1**
Groot Marico Dam	14.9	9.0	**6.8**	0	0
Bloemhof Vaal River	16.4	6.9	**5.4**	0	0
Schoonspruit	12.8	7.0	**8.9**	**4**	0
Klerkskraal Dam	17.8	7.4	**7.0**	0	0
Potchefstroom (Mooi river)	11.6	**6.4**	**5.9**	**8**	0
Boskop Dam	8.2	6.8	**8.0**	**2**	0
Swartruggens Dam	6.9	7.0	**13.0**	0	0
Crocodile river	17.4	6.8	**11.5**	**7**	0
	12.8	**5.8**	**14.9**	**4**	0

Sample Site	Temperature (˚C)	pH	Turbidity (NTU)	Somatic phages pfu/mℓ	F-RNA phages pfu/mℓ
TWQR – full contact recreation		**6.5-8.5**	**0-3**	**0-20**	**0-20**
Lendlyspoort Dam					
Vaalkop Dam	13.9	6.6	**12.0**	**2**	0

TWQR – target water quality range; pfu/mℓ – **plaque forming units per millilitre.**

An expansion of the phage measurements in the Mooi river catchment is provided in Figure 18. Physico-chemical parameters from these sources were within TWQRs for various uses including recreation and livestock watering (Table 19). Results indicated that very high levels of phages are present in this river system, particularly in the borehole samples. The lower phage levels presented in Table 17 and 19 could be due to slight methodology differences.

Table 19: Physical parameter measurements detected in the various water sources in the Mooi River Catchments area in 2012.

Sample Site	Temp (˚C)	pH	Conductivity (mS/m)	DO mg/mℓ
TWQR – Recreational use		**6.0-9.0**	**0-70**	
Muiskraal	9.1	8.48	44.5	8.6
Around the world bridge	11.5	8.41	66.4	8.6
Potch dam	11.8	8.45	68.3	8.8
Viljoenskroon road bridge	73.7	8.47	10.0	8.0
Mooi river and Vaal river confluence	11.6	8.57	54.1	9.8
Borehole samples				
Trimpark	19.9	7.58	94.3	2.3
School	22.4	7.16	126.1	3.5
Cemetery	22	7.10	33.4	2.2

Temp = temperature; TWQR = target water quality range; DO = Dissolved oxygen; mS/m = micro Siemens per meter

More coliphages were detected in the borehole samples. Somatic coliphages averaging $4.3x10^4$ pfu/mℓ and F-specific coliphages at $5.3X10^3$ pfu/mℓ. Somatic coliphages from the surface water averages at $4.1x10^2$ pfu/mℓ and F-specific coliphages at $1.4x10^2$ pfu/mℓ.

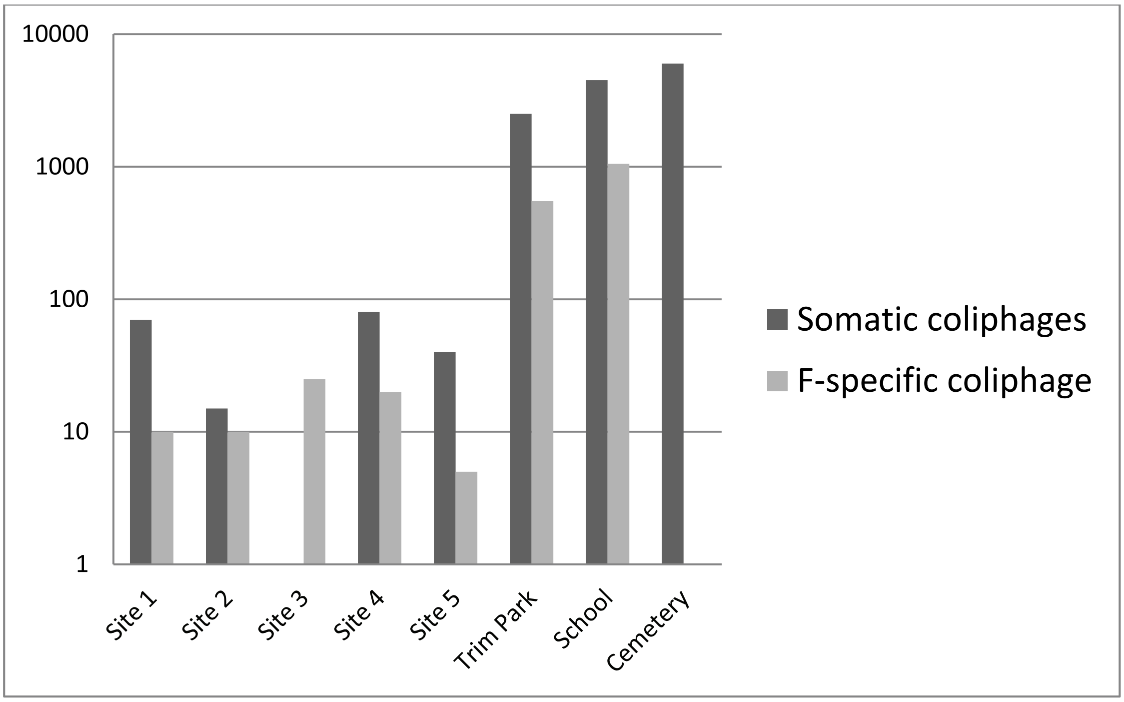


Figure 18: Comparison of coliphage counts isolated from surface water (Sites 1-5) and groundwater (Trim Park, School & Cemetery) samples.

2.4 Conclusions

- Physico-chemical results for surface water indicated that the water was generally suitable for several purposes such as full contact recreational as well as domestic use. This was reflected by TWQRs (DWAF, 1996). Although electrical conductivity (or TDS) for some of the sources was high, there were no cases of the levels being such that they would cause negative health implications if the water is consumed without treatment. This elevated EC could be indicative of increasing salinisation, one of the problems identified by DWAF (2009) for the Vaal River. Elevated EC was also observed at sites in the Harts River.
- *Enterococcus* sp. as well as *Escherichia coli*, were isolated from various surface water sources. This demonstrates that surface water of the North West Province is contaminated by various faecal indicator bacteria. Several of these are potentially pathogenic species if the correct conditions prevail.
- It was also demonstrated that several of the yeast species were found in the surface water sources of the North West Province. These included the following genera *Candida*, *Clavispora, Cryptococcus, Cystofilobasidium Hanseniaspora*, *Meyerozyma*, *Pichia*, *Rhodotorula, Saccharomyces*, *Sporidiobolis*, and *Wickerhamomyces*. Some the species of these genera could cause invasive infections as opportunistic pathogens if sensitive individuals are exposed to them.

- This study provides baseline data on the investigation of the dominant bacterial communities in the Vaal River using molecular methods including DGGE and High Throughput Sequencing. Although there are some limitations to DGGE analysis, this approach permitted the identification of the bacterial community compositions which may have not been detected with cultivation-based methods. Due to the limitations, HTS analysis was applied in this study that presented a better resolution of the bacterial diversity and dominant groups in the Vaal River. Data of the present study may be useful in future bacterial community studies that might be investigating the links between pollution and microbial community dynamics.
- Bacteriophages were detected in several potable and non-potable water sources. The results of the non-potable water sources support the general findings of the bacterial analysis i.e. that many of the water sources in the North West Province have some form of faecal pollution. The major concern is that several of the potable water sources were also contaminated as demonstrated by bacteriophage results. A further worrying aspect is that some of the wastewater treatment plants were decanting up to 10^6 enterovirus particles/mℓ into surface water sources. The qPCR method could not determine the viability of the virus particles. However, such high concentrations of enteroviruses flowing into surface water sources is of concern particularly if one considers that bacterial and yeast opportunistic pathogens were also detected in the surface water sources.

CHAPTER 3

GROUNDWATER QUALITY IN THE NORTH WEST PROVINCE

3.1 Introduction

Apart from the surface water resources, the NWP has a large reservoir of subterranean water in the form of fractured aquifers and dolomitic compartments (Kalule-Sabiti & Heath, 2008). Dolomitic aquifers are porous, facilitating fast and easy infiltration into this groundwater resource, therefore increasing the risk and probability of contamination. Anthropogenic activities exert strong pressures on groundwater resources (Murray *et al.,* 2004; Usher et al., 2004b). A study by Kwenamore (2006) showed relatively high bacteria counts of total and faecal coliforms in groundwater from the Disobotla and Molopo districts of the Province. It is known that elevated faecal coliforms in water may pose a public health risk (Bezuidenhout et al., 2002; Stevens et al., 2003).

In the NWP cultivated agriculture lands are either dryland or irrigation farming. In the latter case surface and/or groundwater is used. In many cases livestock receives water from groundwater sources (NWDACE, 2008). The rural communities of the NWP require about 70 million m^3 water per annum. Of this 25 million m^3 per annum is used for domestic consumption and the remainder is used for livestock-watering and irrigation agriculture (NWDACE, 2008). Studies demonstrated that solid waste dumps, grey water, animal rearing activities and pit latrines are major non-point sources related to shallow groundwater quality problems in informal settlements in South Africa, including the NWP (Dzwairo et al., 2006; Kulabako et al., 2007).

In most instances, commercial farmers have boreholes that are drilled much deeper into the underground aquifers that rural those of informal communities. The greater vertical distance of deeper boreholes and the absence of open pit latrines, leads to these boreholes being less-contaminated than shallow wells mostly found in informal settlements. The results of Gallegos et al. (1999) support this deduction. The authors detected higher coliform counts in shallow (<10 m) boreholes than deeper boreholes. Informal dwellers usually use these shallow wells (Gallegos *et al*, 1999), that are in many cases not properly sealed and which may be exposed to open faecal pollution for abstracting drinking water (Dzwairo et al., 2006; Griesel et al., 2006; Kulabako et al., 2007; Grönwall et al. 2010).

Research by the CSIR showed that groundwater in the NWP has high concentrations of fluoride and nitrate (Ashton, 2009). In many areas the concentrations of these substances are above the recommended maximum concentrations for human consumption (Ashton, 2009). A study monitoring the groundwater of the NWP from 2002 to 2005 demonstrated that the average nitrate concentration of 40% of the secondary drainage basins that were monitored exceeded the TWQR (6 mg/L) value for domestic use (NWDACE, 2008). It was also demonstrated that groundwater nitrate levels in the North West Province was higher surface water values. An increase in pit latrines in rural areas together with the rapid population growth of informal settlements may, amongst other factors, contribute to the nitrate loading of groundwaters (NWDACE, 2008).

In many of the rural populations, households receive groundwater by means of communal tanks and taps. Water for the households is mostly collected in buckets. In some of these settlements water is only pumped two to three times a week, resulting in water to be stored in tanks for an extended period before use. A study by Momba and Kaleni (2002) demonstrated that if contaminated water is stored in household containers, these containers will support the growth and survival of indicator and other bacteria. The microbiological quality of the stored drinking groundwater will deteriorate over time (Momba & Notshe, 2003). Those households that can afford it may have a private shallow-well borehole that is pumped by hand. The water from such boreholes may be of better quality, depending on the locality of the borehole.

In this part of the study, the goal was to determine the quality of selected bores in the North West Province. Specific objectives were to determine (i) physico-chemical chemical quality, (ii) microbiological quality based on (a) culture dependent and (b) culture independent methods. The measured values were compared to DWAF target water quality ranges for various uses.

3.2 Experimental procedures

3.2.1 Sampling

Water samples were collected from groundwater sources in the North West Province. The borehole positions are provided in Figure 19. Explanation of the borehole distribution is provided in Tables 21 and 22. The province was divided into sampling areas and each area consisted of several sampled boreholes. Thus for this chapter and specifically this section reference is made to sampling areas and not sampling sites. Triplicate samples were collected at each borehole. Onsite and laboratory based methods were used to determine physical and chemical parameters.

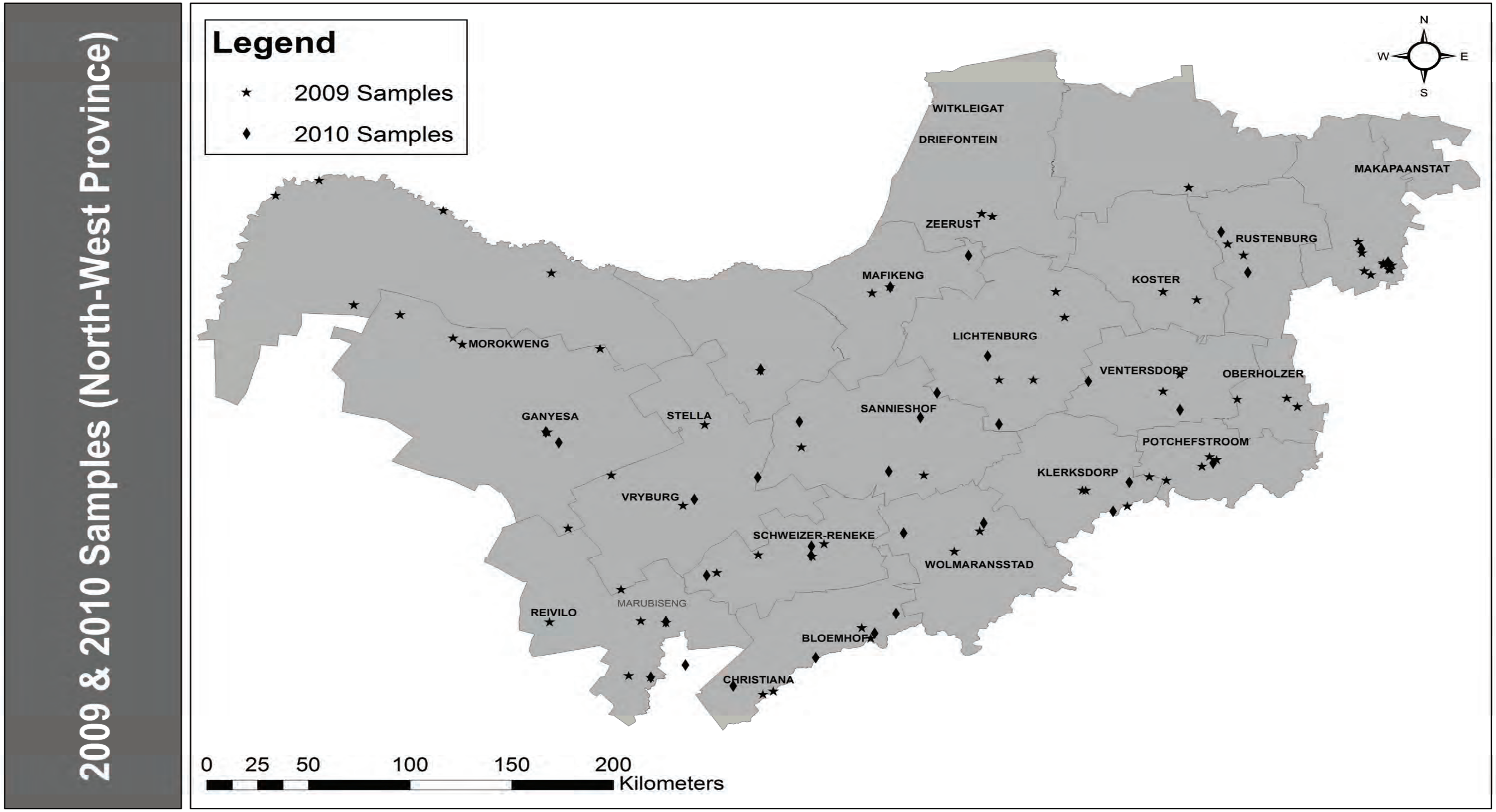


Figure 19: A map indicating the locations of the boreholes sampled in 2009 and 2010.

Table 20: Sample areas (A_{09}-J_{09}) number and distribution of boreholes sampled in 2009.

Sample date	**Sample Areas**	
Season	**n – number of boreholes sampled in area**	
16/2/2009	Klerksdorp; Stilfontein; Orkney; Mooibank; Potchefstroom	$\mathbf{A_{09}}$
Summer	n = 8	
10/3/2009	Carletonville-Ventersdorp Fochville-Welverdiend	$\mathbf{B_{09}}$
Summer	n = 5	
26/3/2009	Lichtenburg; Koster; Doornkop; Makokskraal; Derby; Coligny	$\mathbf{C_{09}}$
Summer	n = 7	
4/4/2009	Hartbeespoortdam; Brits; Matrooster; Rustenburg	$\mathbf{D_{09}}$
Autumn	n = 8	
17/4/2009	Hartbeespoortdam	$\mathbf{E_{09}}$
Autumn	n = 5	
9/5/2009	Delareyville; Zeerust; Mafikeng; Stella; Setlagole; Ottosdal	$\mathbf{F_{09}}$
Autumn	n = 8	
26/5/2009	Schweizer-Reneke; Bloemhof; Wolmaransstad; Christiana	$\mathbf{G_{09}}$
Autumn	n = 5	
10/6/2009	Vryburg-Ganyesa	$\mathbf{H_{09}}$
Winter	n = 4	
23/6/2009	Bray; Vostershoop; Piet-Plessis; Makopeng; Molopo;Tseoge	$\mathbf{I_{09}}$
Winter	n = 9	
26/7/2009	Taung; Salpeterspan; Sekhing; Geluk; Amalia; Reivilo	$\mathbf{J_{09}}$
Winter	n = 9	

Table 21: Sample areas (A_{10}-J_{10}) number and distribution of boreholes sampled in 2010.

Sample date	**Sample Areas**	
Season	**n – quantity boreholes sampled in area**	
12/04/2010	Klerksdorp; Stilfontein; Orkney; Potchefstroom	$\mathbf{A_{10}}$
Autumn	n = 5	
27/04/2010	Ventersdorp; Coligny; Lichtenburg; Biesiesvlei; Sannieshof	$\mathbf{B_{10} + C_{10}}$
Autumn	n = 6	
10/05/2010	Hartbeespoortdam; Brits; Derby; Rustenburg	$\mathbf{D_{10}}$
Autumn	n = 5	
18/05/2010	Delareyville-Ottosdal; Zeerust; Mafikeng; Geysdorp; Setlagole;	$\mathbf{F_{10}}$
Autumn	n = 5	
23/03/2010	Schweizer-Reneke; Bloemhof; Wolmaransstad	$\mathbf{G_{10}}$
Autumn	n = 7	

Sample date	**Sample Areas**	
Season	**n – quantity boreholes sampled in area**	
31/05/2010	Vryburg; Ganyesa; Broedersput	**H_{10}**
Autumn	n = 4	
24/05/2010	Taung; Hartswater; Sekhing; Amalia; Christiana	**J_{10}**
Autumn	n = 6	

3.2.2 Physico-chemical parameters

Details are provided in section 2.2.2

3.2.3 Culturable indicator bacteria

Details are provided in section 2.2.3.

In addition, mannitol salt agar was used for enumeration of *Staphylococcus aureus*. Incubation was at 37°C for 24 hours. On these plates – small to large yellow colonies surrounded by a yellow zone were counted.

Dilutions up to 10^{-6} were made for the enumeration of heterotrophic plate count bacteria, of which 0.1 mℓ was used to make a spread plate on R2A agar. The incubation period was 5 days at room temperature. Results were expressed as colony forming units per mℓ.

Presumptive *Pseudomonas aeruginosa* were isolated from m-FC agar plates (grey colonies). Representative grey colonies from each borehole (when present) were sub-cultured onto nutrient agar plates. If the nutrient agar plates changed colour to green after a 24 hour at 37°C incubation period it was noted as potentially *P. aeruginosa*. *E. coli* were identified by biochemical methods including API 20 E (bioMerieux, Inc. Hazelwood) according to the instruction of the manufacturer. Identities confirmed by PCR amplification of the *lac*Z and *mdh* genes.

For the isolation of *E. coli*, the same methods were followed as described in section 2.2.3. However, in 2010 a different selective agar was used to enumerate total coliforms and *E. coli*. Membrane lactose glucuronide agar (MLGA) (Oxoid, UK) was used to enumerate *E. coli* (green colonies) and total coliforms (green and yellow colonies). This agar substituted M-Endo agar for the enumeration of total coliforms, while also providing valuable *E. coli* counts. MLGA plates were incubated for 24 h at 37°C. The remainder of the selective agars for bacterial enumeration was the same as those used in 2009.

3.2.4 Detection and identification of *E. coli* directly from water samples

Multiplex PCR (mPCR) was used to detect the presence of *E. coli* without culturing in borehole water samples. DNA was isolated directly from borehole water samples using the Nexttec^{TM} Genomic DNA isolation kit for bacteria (Nexttec^{TM} Biotechnology GmbH, Germany). See section 2.2.5 for more details of the DNA isolation process. The mPCR was optimised to simultaneously amplify fragments two of the *E. coli* house-keeping genes [malate dehydrogenase gene (*mdh*) and of the lactose promoter (*lacZ*)]. Each reaction consisted of 1 x PCR Mastermix (2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.025 U/µl *Taq*-polymerase; Fermentas Life Sciences, US) forward and reverse primers (1µM; Table 22), additional (0.5 mM) $MgCl_2$ and (0.1 mM) betaine. RNase/DNase free PCR grade water (Fermentas Life Sciences, US) was used to make a final volume of 25 µl. One hundred nanogram of DNA was used as template in each reaction. Cycle conditions consisted of: initial denaturation at 95°C for 300 seconds, 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds. Final extension was at 72ºC for 300 seconds. PCR reactions were processed in an ICycler (Bio-Rad, UK). Agarose electrophoresis was used to visualise the PCR fragments.

Table 22: Primer sets used in the *E. coli* multiplex PCR.

Primer	**Specificity**	**Sequence (5' → 3')**	**Size** (bp)	**Reference**
mdh	*E. coli*	FW: GGTATGGATCGTTCCGACCT RV: GGCAGAATGGTAACACCAGAGT	301	Tarr *et al.*, 2004.
lacZ	*E. coli*	FW: CTGGCGTAATAGCGAAGAGG RV: GGATTGACCGTAATGGGATATG	228	Ram & Shanker, 2005

3.2.5 Bacterial diversity by High Through-put sequencing

Water from two of the boreholes were selected and analysed by HTS as described in section 2.2.5. One sample was from a rural borehole that is used for agricultural purposes (near Schweizer-Reineke). The other sample was from a rural town (Wolmaransstad) and in this case was used for drinking purposes. In each case a sample was collected in summer and one in winter.

3.2.6 Statistical analysis

See section 2.2.7

3.3 Results and Discussion

3.3.1 Physico-chemical quality

The sampling sites were grouped into sample areas (A to J). In 2009 a total of 76 boreholes were sampled and in 2010, 38. pH levels for groundwater were within the TWQR for domestic use (pH 6-9). What is of concern is the very high electrical conductivity and nitrates (Tables 24 and 25). The nitrate levels are further explored in Figures 20 and 21.

Table 23: Physico-chemical data of water from boreholes for various sampling areas (A_{09} – J_{09}) measured in 2009. The details of the area location are found in Table 17. Highlighted values exceeded TWQR for domestic use.

Sample Area		**pH**	**Temp** (°C)	**EC** (mS/m)	**Nitrate – nitrogen** (mg/ℓ NO_3-N)
TWQR		**6-9**		**0-70** mS/m	**0-6** mg/ℓ
	Min	6.8	20.4	34.7	1.8
A_{09}	Max	7.6	24.5	**140.0**	**209.8**
	Ave±SD	7.1±0.3	22.9±1.4	**88.4**±41.03	**33.1±71.4**
	Min	6.8	21.1	6.7	0.1
B_{09}	Max	7.9	22.3	70.0	3.0
	Ave±SD	7.4±0.4	21.6±0.5	44.7±23.7	0.9±1.2
	Min	6.9	20.6	12.7	4.5
C_{09}	Max	7.5	26.0	**135.0**	**120.9**
	Ave±SD	7.1±0.2	23.0±6.9	66.2±41.8	**58.7±59.2**
	Min	7.1	20.2	48.9	0.9
D_{09}	Max	8.1	24.1	**122.5**	**55.2**
	Ave±SD	7.3±0.4	23.0±1.8	**90.0**±26.5	14.1±6.9
	Min	6.4	19.2	31.2	0.3
E_{09}	Max	7.7	24.3	**114.6**	8.4
	Ave±SD	7±0.4	22.4±1.6	**81.9**±30.1	5.9±3.3
	Min	6.8	21.0	21.4	0.6
F_{09}	Max	7.6	22.4	**121.8**	**160.0**
	Ave±SD	7.2±0.3	21.6±0.9	**90.2**±32.7	12.4±11.5
	Min	6.9	18.6	57.4	5.5
G_{09}	Max	7.5	19.5	**175.8**	**47.0**
	Ave±SD	7.1±0.4	18.7±2.1	**97.6**±48.9	**32.2**±14.9
	Min	6.7	16.6	96.0	**38.6**
H_{09}	Max	7.0	18.5	**173.1**	**63.6**
	Ave±SD	6.9±0.1	17.7±0.8	**124.7**±33.6	**49.9**±10.7
	Min	7.1	16.2	63.7	0.5
I_{09}	Max	8.2	24.0	**113.4**	**454.5**
	Ave±SD	7.4±04	20.6±2.6	**91.2**±16.3	**171.6**±188.2
	Min	7.0	15.5	75.8	3.9
J_{09}	Max	8.8	21.5	**144.5**	**454.5**
	Ave±SD	7.4±0.5	19.7±2.1	**106.2**±24.5	**210.7±223.1**

	Min	6.4	15.5	6.7	0.1
Total 76	Max	8.8	24.5	**175.8**	**454.5**
Boreholes	Ave±SD	7.2±0.4	20.9±3.2	**89.1**±35.8.	**67.0±124.3**

Min – minimum; Max – maximum; SD – standard deviation Ave – average; Temp – temperature; EC – electrical conductivity; TDS – total dissolved solids

Table 24: Physico-chemical data of water from boreholes for various sampling areas (A_{10} – J_{10}) measured in 2010. The details of the area location are found in Table 18. Highlighted values exceeded TWQR for domestic use.

Sample Area		**pH**	**Temp** (°C)	**EC** (mS/m)	**Nitrate – nitrogen** (mg/ℓ)
TWQR		**6-9**		**0-70** mS/m	**0-6** mg/ℓ
A_{10}	Min	6.9	20.9	64.1	0.8
	Max	8.7	22	**146.4**	**11.4**
	Ave	7.4±0.7	21.7±0.5	**103.0**±36.3	**8.3±4.4**
B + C_{10}	Min	6.8	19.5	22.3	**11.1**
	Max	7.3	21.1	**183.1**	**74.1**
	Ave	7.2±0.2	20.3±0.7	**72.7**±58.5	**30.1±25.8**
D_{10}	Min	6.7	18.8	11.3	0.7
	Max	7.6	23.5	**95.2**	**44.3**
	Ave	7.1±0.3	21.2±2.2	59.3±35.3	**12.6±18.0**
F_{10}	Min	7.0	18.1	62.4	**8.2**
	Max	7.8	20.5	**253.0**	**23.9**
	Ave	7.4±0.4	20.3±0.9	**134.2**±74.4	**15±6.5**
G_{10}	Min	6.7	20.8	66.9	**23.3**
	Max	7.2	25.8	**90.5**	**148.2**
	Ave	7.0±0.4	23.9±2.0	**77.1**±15.1	**44±42.0**
H_{10}	Min	7.1	16.0	**80.4**	**15.2**
	Max	7.6	21.2	**125.9**	**28.9**
	Ave	7.4±0.2	18. 6±2.4	**103.9**±25.1	**23.3±6.7**
J_{10}	Min	6.8	19.4	**86.1**	1.6
	Max	7.7	24.4	**286.0**	**23.2**
	Ave	7.3±0.4	22.0±2.0	**149.0±72.3**	**13.5±8.7**
	Min	6.7	16.0	11.3	0.7
Total 38	Max	8.7	25.8	**286.0**	**74.1**
Boreholes	Ave	7.2±0.4	21.1±2.1	**98.2**±56.3	**24.9±27.4**

Min – minimum; Max – maximum; SD – standard deviation Ave – average; Temp – temperature; EC – electrical conductivity; TDS – total dissolved solids.

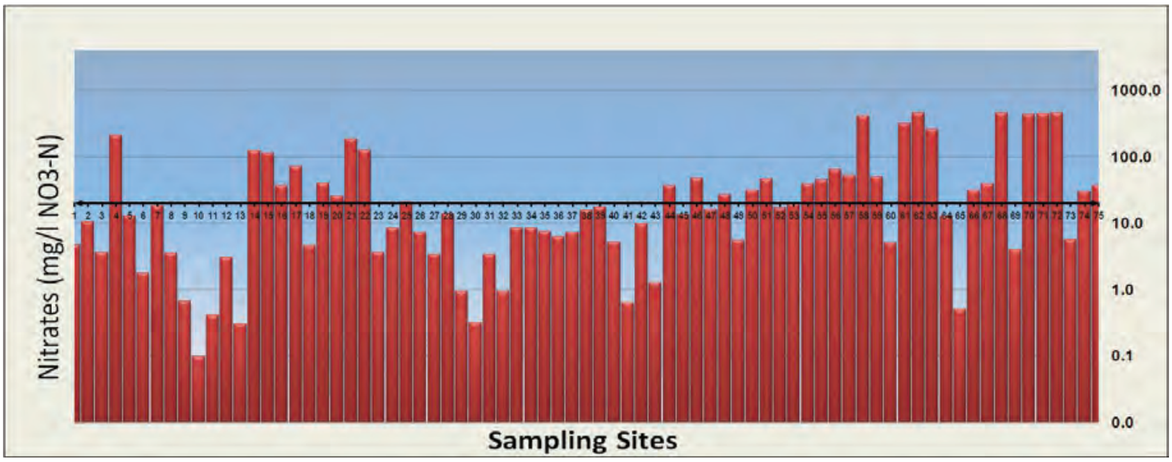


Figure 20: A log graph of the nitrate levels of the boreholes sampled in 2009. The x-axis represents the individual boreholes (1 to 76) and the horizontal line represents that 20 mg/ℓ level above which methaemoglobinemia is most likely to occur if young children and infants were to consume the water.

Each bar in Figures 19 and 20 represents the nitrate concentrations (logarithmic scale) of each borehole sampled individually. Figure 19 indicates the levels of nitrates measured in 2009. In some cases nitrate levels were alarmingly high. The average nitrate levels of only two sampling areas (B_{09} and E_{09}) were within the TWQR for domestic use (<6 mg/ℓ NO_3-N). In six of the ten sampling areas average nitrate levels exceeded 20 mg/ℓ NO_3-N. A total of thirty-one (40.8%) of the boreholes sampled in 2009 had nitrate concentrations exceeding this limit. Methaemoglobinemia in infants and young children may occur if this high nitrate load is consumed over an extended period of time (Sadeq et al*.*, 2008). Furthermore, in six of the sampling areas, average nitrate concentration exceeded the maximum acceptable value of nitrate for the use of irrigation purposes (30 mg/ℓ NO_3-N; DWAF, 1996). The average nitrate level for all 76 boreholes sampled was a very high (67.0 mg/ℓ NO_3-N). In areas I_{09} and J_{09} the highest nitrate levels (171.6±188.2 and 210.7±223.1, respectively) were measured.

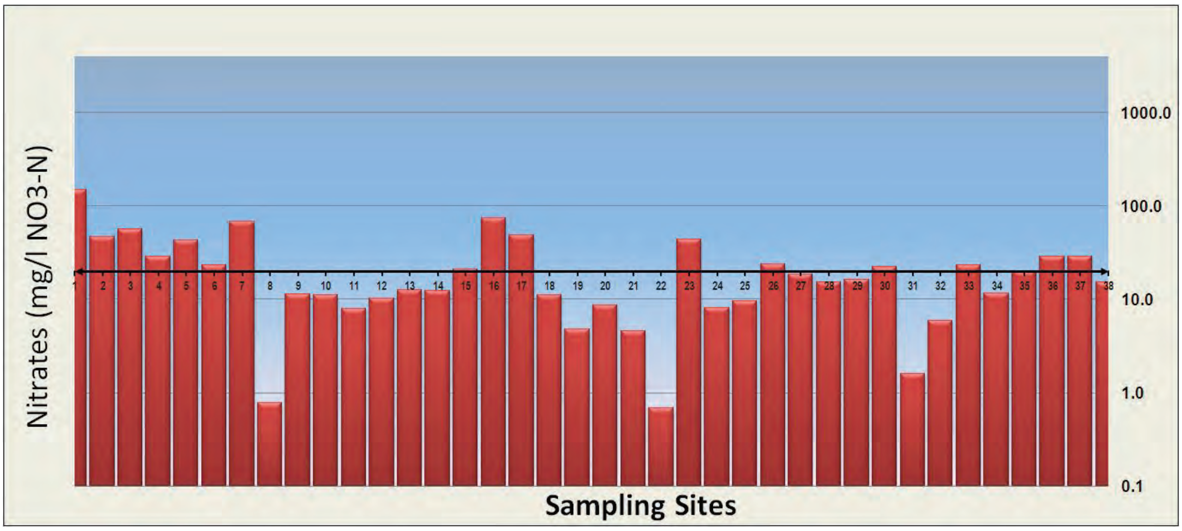


Figure 21: A log graph of the nitrate levels of the boreholes sampled in 2010. The x-axis represents the individual boreholes (1 to 38) and the horizontal line represents that 20 mg/ℓ level above which methaemogloninemia is most likely to occur if young children and infants were to consume the water.

In 2010 the observed nitrate levels were, in many cases, higher than the 6 mg/ℓ NO_3-N TWQR for domestic use (Figure 21). However, the levels were lower than those measured in 2009. The average nitrate level for all the boreholes sampled in 2010 were 24.9±27.4 mg/ℓ NO_3-N compared to the 67.0±124.3 mg/ℓ NO_3-N of 2009 (Table 22). None of the areas sampled in 2010 had average nitrate levels below 6 mg/ℓ NO_3-N. The highest measured nitrate level was from a borehole in area G_{10} (148.2 mg/ℓ NO_3-N). Areas I_{09} and J_{09} both had boreholes with nitrate levels at 450.0 mg/ℓ NO_3-N or higher. The average nitrate level of sample area J, situated in the western part of the province, was much higher in 2009 than in 2010. The average for area J_{10} was 13.5±8.7 mg/ℓ and that for J_{09} 210.7±223.1 mg/ℓ NO_3-N. Although the boreholes sampled during these two periods were in the same sampling areas, they were not exactly the same ones. This finding, however, indicates that some boreholes within sample areas had water of different qualities that could potentially be ascribed different aquifers. Boreholes from B_{09} had the lowest average nitrate levels (0.9±1.2 mg/ℓ NO_3-N) in 2009 but the second highest average nitrate levels (30±25.8 mg/ℓ NO_3-N) in 2010. Sixteen (42.1%) of the boreholes sampled in 2010 exceeded the critical methaemoglobinemia causing limit (20 mg/ℓ NO_3-N; x-axis) whereas 40.8% of the boreholes sampled in 2009 exceeded this limit (Table 25).

Table 25: Summary of percentage boreholes that exceeded TWQR values as well as those exceeding levels that are most likely to cause methaemoglobinemia.

	2009	2010
% Boreholes exceeding domestic TWQR (6 mg/ℓ)	67.0	82.0
% Boreholes exceeding definite methaemoglobinemia causing levels (20 mg/ℓ)	40.8	42.1

3.3.2 Bacteriological quality of borehole water

Table 24 summarises the minimum, maximum and average heterotrophic plate count bacteria (HPC), total coliforms, faecal coliforms and enterococci counts of each of the 10 sample areas of 2009. Samples were also screened for the presence of the opportunistic pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus.* Results for the latter two were reported as the percentage of boreholes from each sample area that tested positive for the opportunistic pathogens.

Faecal coliforms were detected in at least one borehole water sample from every sample area. Three sample areas (A_{09}, B_{09}, and D_{09}) had at least one borehole with enterococci counts exceeding 300 cfu/100 mℓ (Table 24). Based on the average counts of all the indicator bacteria (Table 24), it is demonstrated that area B_{09} was highly contaminated by faecal and other bacteria. This area (B_{09}) is located in the south-eastern part of the North West Province and includes Ventersdorp, Carletonville and Fochville. These are urban areas but recently there had been sanitation and water quality issues in some of the towns (http://www.pmg.org.za/print/29951). Area J_{09} had the lowest overall bacterial counts. This area (J_{09}) is located in the south-western part of the Province and the boreholes are generally deep. Area A_{09} to E_{09} was sampled in the warm and wet season and F_{09} to J_{09} was sampled in the cold and dry winter season. Average faecal coliform and enterococci counts were lower in area E_{09} to J_{09} with the exception of area I_{09} which had a high average faecal coliform (70±95.6 cfu/100 mℓ) as well as enterococci (56±52.0 cfu/100 mℓ) count. This observation could statistically be linked to lower ambient and water temperature (Figure 25).

Pseudomonas aeruginosa was detected in 46% of the 76 boreholes sampled and *Staphylococcus aureus* in 7% (Table 26). *Staph. aureus* were detected in only four of the 10 sample areas. A future study on occurrence and distribution of *Staph. aureus* in groundwater in the province could focus on the areas that were positive and determine the potential source of the pollution.

Table 26: Indicator bacteria counts and % of boreholes that were positive for *S. aureus* and *P. aeruginosa* of areas A-J sampled in 2009.

Sampling Area		**HPC**	**TC**	**FC**	**FS**	***P. aeruginosa***	***S. aureus***
		cfu/mℓ	**cfu/100 mℓ**	**cfu/100 mℓ**	**cfu/100 mℓ**	**%Present**	**%Present**
TWQR - Domestic		**0-100**	**0-5**	**0**	**N/A**		
A_{09}	Min	34	**12**	0	0	0%	**13%**
	Max	**3076**	**>300**	**200**	**>300**		
	Ave±SD	**798±1089.2**	**>76±92.7**	**34±68.7**	**>49±104.0**		
B_{09}	Min	60	**17**	8	2	0%	0%
	Max	**3100**	**>300**	**260**	**>300**		
	Ave±SD	**1145±1240.9**	**>182±108.0**	**140±97.7**	**>125±160.3**		
C_{09}	Min	87	**16**	0	0	0%	**22%**
	Max	**4666**	**180**	**163**	**50**		
	Ave±SD	**1490±1437.7**	**60±54.8**	**36±57.3**	**7±16.5**		
D_{09}	Min	180	**23**	0	0	**75%**	**14%**
	Max	**3360**	**215**	**106**	**>300**		
	Ave±SD	**1468±1226.9**	**92±63.8**	**38±46.1**	**>87±145.8**		
E_{09}	Min	47	**7**	0	0	**100%**	0%
	Max	**1333**	**230**	**92**	**9**		
	Ave±SD	**451±560.9**	**62±77.0**	**12±32.5**	**2±3.2**		
F_{09}	Min	43	2	0	0	**75%**	0%
	Max	**7500**	**220**	**104**	**5**		
	Ave±SD	**2205±2645.7**	**54±69.4**	**14±36.3**	**1±1.7**		
G_{09}	Min	10	0	0	0	**100%**	**13%**
	Max	**1900**	**230**	**20**	**54**		
	Ave±SD	**933±722.7**	**52±76.4**	**4±7.1**	**12±21.9**		
H_{09}	Min	133	**6**	0	5	0%	0%
	Max	**3000**	**77**	**42**	**7**		
	Ave±SD	**2117±2661.8**	**31±32.2**	**11±21.0**	**6±1.0**		
I_{09}	Min	166	**30**	0	1	**75%**	0%
	Max	**1263**	**>300**	**280**	**159**		
	Ave±SD	**660±340.9**	**>114±94.5**	**70±95.6**	**56±52.0**		
J_{09}	Min	50	**5**	0	0	**44%**	0%
	Max	**400**	**120**	**3**	**94**		
	Ave±SD	**228±122.7**	**47±39.2**	**<1±1.0**	**12±30.8**		
Overall	Min	10	0	0	0	**46%**	**7%**
	Max	**7500**	**>300**	**280**	**>300**		
	Ave±SD	**1068±1400.6**	**>75±78.6**	**33±62.9**	**>33±77.2**		

HPC – Heterotrophic plate count; TC – Total coliforms; FC – Faecal coliforms; FS – Enterococci; Min – minimum count; Max – maximum count; Ave – Average count; SD –Standard deviation; cfu – colony forming unit.

Thirty eight boreholes were sampled during the 2010 sampling period. Table 25 shows the microbiological results of each sample area for this period. Compared to 2009 microbiological results the 2010 results included *E. coli* and excluded the opportunistic pathogen *S. aureus*. Membrane Lactose Glucuronide *agar* (MLGA) was used as substitute for m-Endo agar that was used in 2009. MLGA selects for and differentiate between *E. coli* and total coliforms. In 2009 several false positives were observed with the use of Mannitol Salt agar (MSA) for the detection of *S. aureus*. Also, a minority of the sites sampled in 2009 were positive for *S. aureus*. Therefore, enumeration of this opportunistic pathogen was excluded from the 2010 sampling period.

Table 27: Indicator organism's counts and % boreholes that were positive for *P. aueruginosa* of areas A-J sampled in 2010.

Area		**HPC**	**TC**	**FC**	***E. coil***	**FS**	***P. aeruginosa***
		cfu/mℓ	cfu/100 mℓ	cfu/100 mℓ	cfu/100 mℓ	cfu/100	%Present
TWQR - Domestic		0-100	0-5	0	0	N/A	
A_{10}	Min	110	0	0	0	2	**40%**
	Max	**800**	**21**	**17**	1	**8**	
	Ave±SD	**341±290.6**	**8.2±10.8**	**5±7.3**	<1±0.4	**2±3.5**	
$B+C_{10}$	Min	163	0	0	0	0	**50%**
	Max	**1 393**	**98**	**15**	**12**	**10**	
	Ave±SD	**547±490.5**	**34±39.2**	**6±6.3**	**4±4.0**	**5±3.5**	
D_{10}	Min	273	0	0	0	0	0%
	Max	**4 066**	**280**	**126**	**36**	**26**	
	Ave±SD	**1833±1769.9**	**58±124.1**	**26±56.0**	**7±16.0**	**5±11.5**	
F_{10}	Min	20	3	0	0	0	**60%**
	Max	**5 500**	**>300**	**>300**	**34**	**8**	
	Ave±SD	**2129±2241.7**	**>62±133.3**	**>68±130.2**	**8±14.8**	**4±3.8**	
G_{10}	Min	70	0	0	0	0	**100%**
	Max	**647**	**5**	**2**	**2**	8	
	Ave±SD	**208±190.6**	1±2.1	**<1±0.8**	<1**±0.8**	**3±3.4**	
H_{10}	Min	0	0	0	0	0	**25%**
	Max	**966**	**300**	**220**	0	**6**	
	Ave±SD	**425±495.4**	**75±150**	**55±110**	0	**2±2.8**	
J_{10}	Min	100	2	0	0	**10**	**83%**
	Max	**3 650**	**>300**	**>300**	**3**	**180**	
	Ave±SD	**943±1340.1**	**>96±134.0**	**>64±118.1**	**<1±1.4**	**45±66.9**	
Overall	Min	0	0	0	0	0	**55%**
	Max	**5 500**	**>300**	**>300**	**36**	**180**	
	Ave±SD	**898±1293.3**	**>46±95.6**	**>29±76.6**	**3±8.0**	**10±29.2**	

HPC – Heterotrophic plate count; TC – Total coliforms; FC – Faecal coliforms; FS – Enterococci; Min – minimum count; Max – maximum count; Ave – Average count; SD – Standard deviation; cfu – colony forming unit.

A large proportion of the boreholes sampled in 2010 had faecal coliforms present. Of particular concern was that some of the boreholes from areas F_{10} and J_{10} had faecal coliforms levels exceeding 300 cfu/100 mℓ (Table 27). The boreholes from J_{09} (Table 26) had the least number of faecal coliforms present during the 2009 sampling period. This discrepancy was due to the inclusion of the Hartswater samples in the 2010 sampling period. Water samples from boreholes from this area had very high levels of faecal coliforms and other bacteria.

Enterococci levels in borehole water samples were lower 2010 compared to the 2009 samples. The average 2010 enterococci counts for all the boreholes sampled were 10±29.2

cfu/100 mℓ compared to the 2009 average of >33±77.2 cfu/100 mℓ. Area J_{10} had the highest average for enterococci (45±66.9 cfu/100 mℓ). This could once again be ascribed to the Hartswater samples. The remainder of the 2010 sample areas (A_{10} – H_{10}) had low enterococci averages.

A higher percentage of boreholes had *P. aeruginosa* present in 2010 (55%) compared to 2009 (46%). Only one of the sample areas of 2010 (D_{10}), tested negative for *P. aeruginosa* and four (A_{09}, B_{09}, C_{09} & H_{09}) in 2009. *P. aeruginosa* was detected in all of the boreholes sampled in area G_{10}.

Over the two sampling periods, a total of 114 boreholes were sampled. Of these boreholes, 88% tested positive for total coliforms, 50% for thermo-tolerant faecal coliforms and 68% for enterococci. These results indicate that several groundwater sources in the NWP are contaminated by faecal matter that could be of human or non-human origin. The results of this study are reflective of the results of Kwenamore (2006). That study sampled 304 groundwater sources from the Ditsobotla and Molopo districts in the NWP. Samples were taken twice seasonally; winter and spring in 2003 and summer and autumn in 2004. Faecal and total coliform bacteria, *Enterococcus* spp., *Klebsiella* spp. and *Citrobacter* spp. were detected throughout the sampling period (Kwenamore, 2006).

Indicator bacteria in the groundwater may promote additional deterioration of the water quality during its residence time in storage tanks and household containers. Studies by Momba and Kaleni (2002) and Momba and Notshe, (2003) showed that these containers will support the growth and survival of indicator bacteria and that the microbiological quality of the stored drinking groundwater will deteriorate consistently with the length of storage. Groundwater as a source for domestic use is almost always stored in large storage tanks. Inhabitants of informal settlements in rural areas usually store their water in smaller household containers (e.g. 20ℓ). The drinking water containers of communities in the North West Province may thus harbour various indicator and other bacterial species. This is an aspect that could be further explored in future studies.

With the groundwater study the focus was on determining the identity of faecal coliforms using API 20 E. Of the 110 selected faecal coliform isolates tested with the API 20 E system, 98 were identified at 90% or greater accuracy. Amongst these 98 representatives the following enteric bacteria from the family *Enterobacteriaceae* was confirmed. *Enterobacter cloacae* (34%), *Enterobacter aerogenes* (31%), *Klebsiella pneumonia* (15%), *Escherichia coli* (10%), *Raoutella planticola* (4%), *Raoutella terrigena* (3%), and *Pantoea* spp. 2 (3%).

E. coli identified were isolated from the following boreholes and sample areas: Stilfontein (A_{09}); Fochville, Ventersdorp 1 and Ventersdorp 2 (B_{09}); Groenfontein (C_{09}); Matrooster (D_{09}); Stella (F_{09}); Christiana 1 (G_{09}); and Tseoge and Molopo (I_{09}).

Thirty-two of the 38 boreholes sampled in 2010 were screened for the presence of *E. coli* using mPCR. Area H was sampled in the beginning 2010 when the DNA isolation directly from water and mPCR conditions had not been optimised. Therefore, this area was excluded. The remaining 32 borehole samples were clustered into three groups. This grouping was done based on their culture based microbiological results. The mPCR was performed on each of the three groups. All the samples that had presumptive *E. coli* present (green colonies on MLGA) were grouped (Figure 22). Faecal coliform positive sites (blue colonies on m-FC agar) that had no green colonies on MLGA were grouped (Figure 23). The third group consisted of three sites that were positive for total coliforms (yellow colonies on MLGA) but negative for faecal coliforms and *E. coli* (lane 1-3; Figure 24), and nine sites that were negative for total coliforms, faecal coliforms and *E. coli* (lane 4-12; Figure 24).

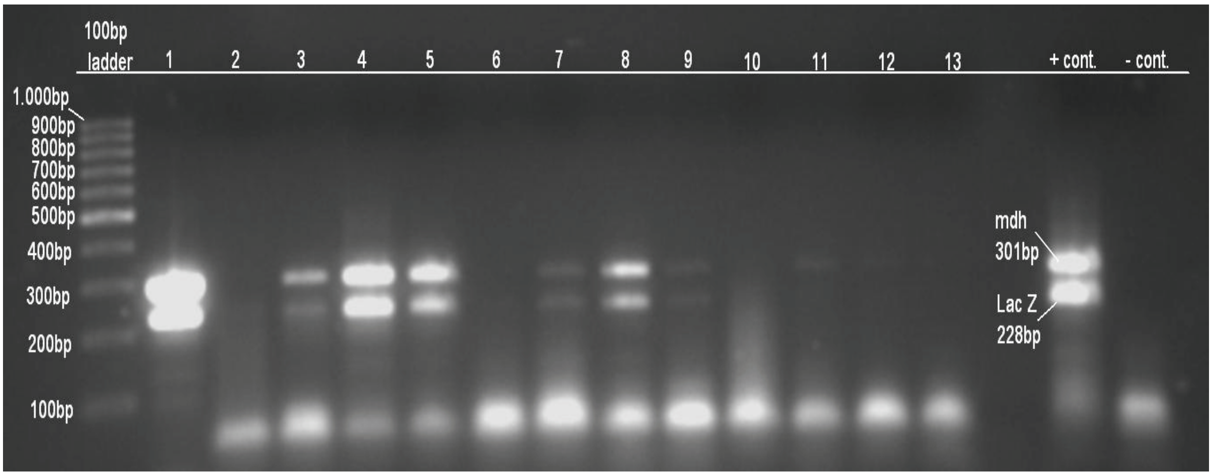


Figure 22: A 1.5% (w/v) Ethidium bromide stained agarose gel illustrating multiplex PCR results for boreholes that tested positive for *E. coli* on MLGA. One hundred nanograms of DNA were used as template. The left lane contains a 100bp molecular marker (O'GeneRuler^{TM} 100bp DNA ladder, Fermentas Life Sciences, US). Lane 1 – Wolmaransstad; lane 2 **–** Potchefstroom2; lane 3 – Potchefstroom-Ventersdorp; lane 4 – Biesiesvlei; lane 5 – Sannieshof; lane 6 – Coligny-Biesiesvlei; lane 7 – Brits; lane 8 – Rustenburg2; lane 9 – Zeerust; lane 10 – Delarey-Ottosdal; lane 11 – Setlagole; lane 12 – Sekhing; and Lane 13 – Christiana. The last two lanes contain the positive (+cont. *E. coli* ATCC 10536) and no template controls (-cont.).

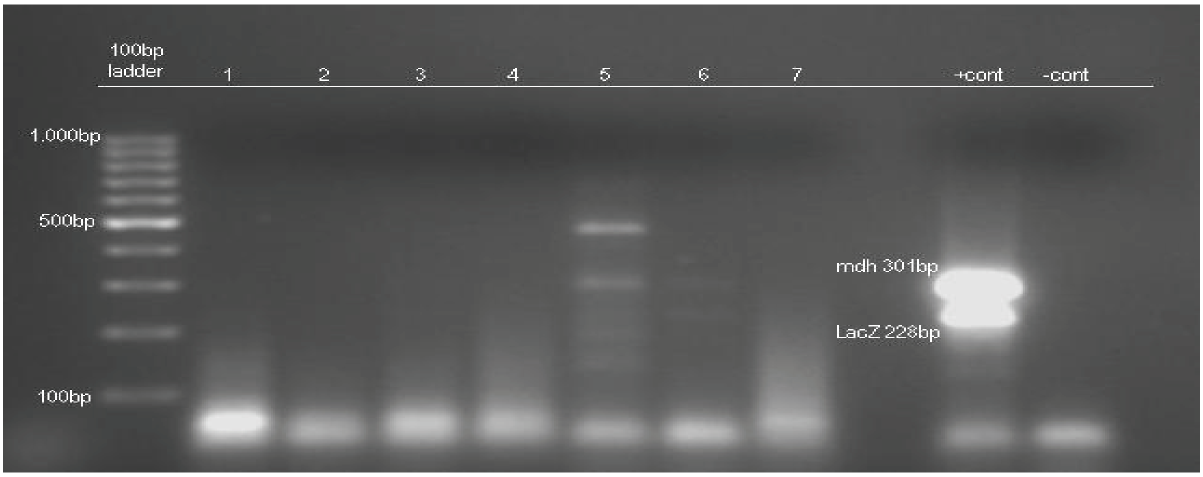


Figure 23: A 1.5% (w/v) ethidium bromide stained agarose gel illustrating multiplex PCR results for boreholes that tested positive for faecal coliforms on m-Fc agar but negative for *E. coli* on MLGA. One hundred nanograms of DNA were used as template. The left lane contains a 100bp molecular marker (O'GeneRuler^{TM} 100bp DNA ladder, Fermentas Life Sciences, US). Lane 1 **–** Klerksdorp; lane 2 – Potchefstroom1; lane 3 – Geystown; lane 4 – Taung; lane 5 – Hartswater; lane 6 – Christiana2; and lane 7 – Ganyea. The last two lanes contain the positive (+cont. *E. coli* ATCC 10536) and no template controls (-cont.).

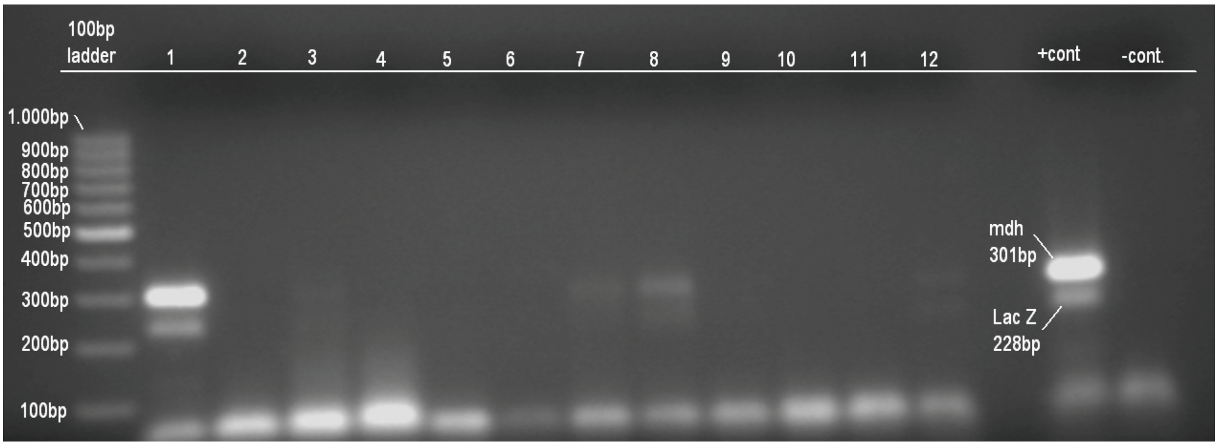


Figure 24: A 1.5% (w/v) ethidium bromide stained agarose gel illustrating multiplex PCR results for boreholes that tested positive for total coliforms on MLGA but negative for *E. coli* on MLGA and faecal coliforms on m-Fc (lane 1-3). Lane 4-12 represents multiplex PCR results for boreholes that tested negative for total coliforms, faecal coliforms and *E. Coli*. One hundred nanograms of DNA were used as template. The left lane contains a 100bp molecular marker (O'GeneRuler^{TM} 100bp DNA ladder, Fermentas Life Sciences, US). Lane 1 – Ventersdorp-Coligny; lane 2 – Mafikeng; lane 3 – Amalia; lane 4 – Orkney; lane 5 – Stilfontein; lane 6 – Lichtenburg; lane 7 – Derby; lane 8 – Hartbeespoort; lane 9 – Rustenburg; lane 10 – Broedersput; lane 11 – Vryburg; and lane 12 – Ganyesa2. The last two lanes contain the positive (+cont. *E. coli* ATCC 10536) and no template controls (-cont.).

Two distinct bands of PCR product of the expected fragment size for the *mdh* and *lacZ* primer sets were visible in lanes 1, 3, 4, 5, 7, 8 and 9 of Figure 22. Faint bands were visible in lanes 6, 11, 12 and 13. Lane 2 and 10 were negative for the target *E. coli* gene fragments. The culture based method therefore gave two potentially false positives for *E. coli* (lane 2 and 10). Potchefstroom2 (lane 2) had one green colony (presumptive *E. coli*) on MLGA in a 100 ml of sample water and Delarey-Ottosdal (lane 10) had two. Overall results indicate that the selective and differential agar, MLGA, was 85% accurate in the positive detection *E. coli,* given that all the sites represented by lane 1-13 tested positive for *E. coli* when MLG-agar was used. Due to the low number of *E. coli* detected in a 100 ml of sample by MLGA for Potchefstroom 2 and Delarey-Ottosdal, it is possible that *E. coli* could have been detected by PCR if extraction were preceded with a 1-3 hour enrichment period.

Boreholes sampled in both the sampling periods (2009 and 2010) were situated on farms as well as rural settlements. Almost all the boreholes from rural settlements were shallow wells pumped by hand. The remainder of the boreholes sampled were situated on small-holdings and towns. It was expected that the main source of faecal contamination would be from animal origin. Farm animals (cows, chickens, pigs and horses) were found grazing in close proximity to most of the boreholes. In the case of the shallow wells found in the rural settlements, faecal contamination from human and animal origin was expected. Most of the shallow wells sampled in rural settlements were situated in close proximity to open pit latrines. Water abstracted from a shallow well within a lateral distance of about 25 m from a pit latrine may be contaminated by faecal bacteria (Dzwairo et al., 2006). In most cases these shallow wells are not maintained or sealed properly at the ground surface, increasing the potential for contamination (Gallegos et al., 1999).

The ratio between faecal coliform and enterococci was used to indicate the most likely source of faecal contamination. A study by Jagals et al. (1995) supports the assumption that the origin of faecal contamination is more likely to be animal waste if enterococci counts are higher than that of faecal coliforms. The movement of animal wastes (frequently applied as soil fertilisers) into groundwater is often cited as a major factor contributing to the pollution of groundwater (Cho et al*.,* 2000). Even though there were mixed indications of the origin of faecal pollution, it was established that water sources were polluted and that the species isolated could have health implications for humans.

The faecal coliforms (FC) and faecal streptococci (FS) ratio can be used as an indication of the possible source of contamination (Csuros& Csuros, 1999; Wyer & Kay, 1995). Table 28 summarises the % boreholes from each sample area that had a 1) FC/FS ratio <0.7, indicating possible contamination from non-human origin; 2) FC/FS ratios between 2 and 4, indicating possible contamination from human waste; 3) FC/FS ratios >4, indicating possible human faecal matter contamination (Csuros& Csuros, 1999; Wyer & Kay, 1995) and 4) the % boreholes that were negative for faecal coliforms as well as faecal streptococci.

Table 28: FC/FS ratios of 2009 and 2010**.**

2009 Sampling period	**% FC/FS ratio**			**% boreholes no FC or FS detected**	**2010 Sampling period**	**% FC/FS ratio**			**% boreholes no FC or FS detected**
	<0.7	**2-4**	**>4**			**<0.7**	**2-4**	**>4**	
A_{09}	50	13	13	13	A_{10}	0	40	0	40
B_{09}	20	20	40	0	$B+C_{10}$	33	33	0	17
C_{09}	33	0	44	22	-	-	-	-	-
D_{09}	57	0	29	14	D_{10}	0	20	20	60
E_{09}	38	0	13	50	-	-	-	-	-
F_{09}	25	13	25	38	F_{10}	20	0	20	20
G_{09}	38	13	13	38	G_{10}	43	0	0	57
H_{09}	75	0	25	0	H_{10}	50	0	25	25
I_{09}	55	11	11	0	-	-	-	-	-
J_{09}	55	0	0	33	J_{10}	50	17	0	0
Total 76 boreholes	45	7	20	22	Total 38 boreholes	32	16	8	32

In Table 28 it is demonstrated that non-human origin (FC/FS ratio <0.7) of faecal contamination was more common in both sampling compared to contamination by human waste (FC/FS ratio 2-4) or human faecal matter (FC/FS ratio >4). This may be so because the majority of boreholes sampled were situated on farms and rural areas. Areas B_{09}, C_{09} had a high percentage of boreholes with possible contamination from human sources. This was not the case for area $B+C_{10}$. None of the boreholes from sample areas B_{09}, H_{09}, I_{09} and J_{10} tested negative for both faecal coliforms and faecal streprococci, indicating that boreholes from these areas were all subjected to faecal contamination. All the boreholes of the latter sample areas (B_{09}, H_{09}, I_{09} and J_{10}), except for area B_{09}, were indicated to be mainly contaminated from non-human faecal sources. It was also observed that the samples from area C_{09} were predominantly contaminated by human faecal waste. The overall 20% of boreholes in 2009 and 8% in 2010 that had FC/FS ratio >4 is cause for concern and needs to be further investigated.

Canonical ordination redundancy analysis (RDA) was applied to determine the correlation between the environmental variables (temperature, TDS, EC and NO_3-N) and the indicator bacteria levels detected in 2009 and 2010. Mean biplots were used to visualise the results of the multi-variate analysis. The biplots represent the joint effect of environmental variables on the selected bacteria in a single plane (Ter Braak, 1990). The angles in the biplot between the response (indicator bacteria) and environmental variables, and between the response variables themselves or environmental variables themselves, reflect their correlations. Figure 25 shows the RDA biplot obtained from using the environmental variable data and bacterial counts of 2009 and Figure 26 provides the latter for the 2010 data.

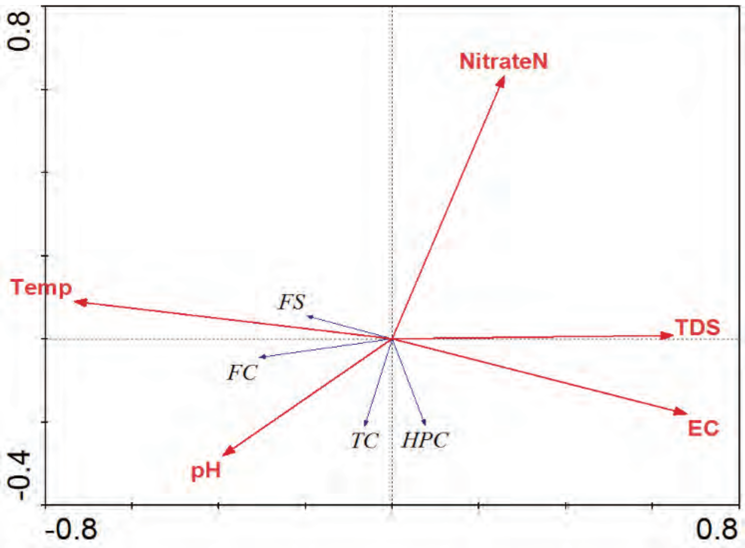


Figure 25: Correlation biplot of the relationship between the environmental variables and indicator bacteria levels measured during the 2009 sample period. Environmental variables are represented by red arrows and include: water temperature (Temp), pH, total dissolved solids (TDS) and electrical conductivity (EC). Indicator bacteria are represented by blue arrows and include: faecal streptococci (FS), faecal coliforms (FC), total coliforms (TC) and heterotrophic plate count bacteria (HPC).

The biplot (Figure 25) shows that the two environmental variables having the closest correlation with faecal coliforms (FC) and faecal streptococci (FS) were temperature (Temp) and pH. Furthermore, a strong correlation also existed between FC and FS. Total coliforms (TC) and heterotrophic plate count (HPC) bacteria were strongly correlated. Total coliform counts were more strongly correlated with pH than HPC bacteria. On the other hand HPC bacterial levels were strongly correlated with EC than TC. Nitrate-nitrogen (NO_3-N) levels did not show a correlation to levels of any of the bacterial tested for. Biplots of the 2010 results are shown in Figure 26.

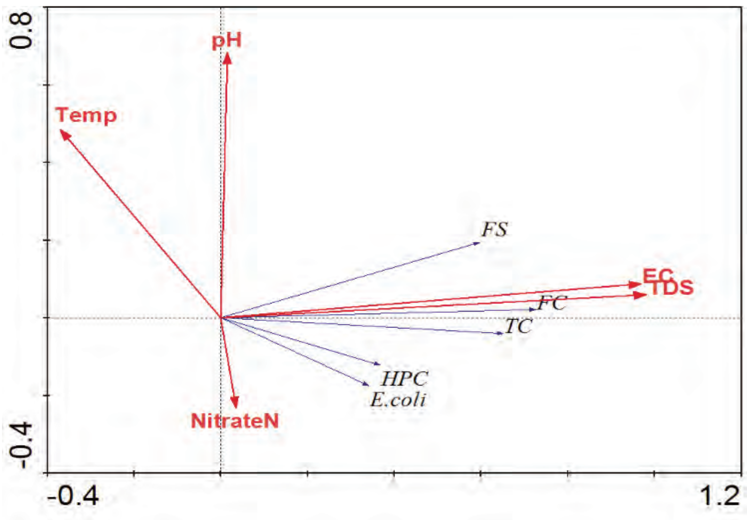


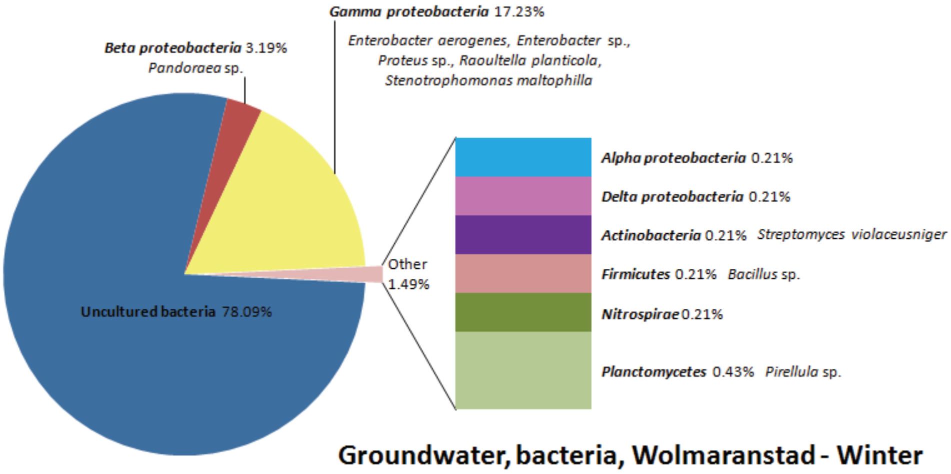
Figure 26: Correlation biplot of the relationship between the environmental variables and indicator bacteria levels measured during the 2010 sample period. Environmental variables are represented by red arrows and include: water temperature (Temp), pH, total dissolved solids (TDS) and electrical conductivity (EC). Indicator bacteria are represented by blue arrows and include: *E. coli*, faecal streptococci (FS), faecal coliforms (FC), total coliforms (TC), and heterotrophic plate count bacteria (HPC).

The biplot (Figure 26) shows that the environmental variable having the closest correlation with faecal coliforms (FC), faecal streptococci (FS) and total coliforms (TC) were electrical conductivity (EC). Nitrate-nitrogen levels showed a weak correlation with *E. coli*. Furthermore, a strong correlation between heterotrophic plate count (HPC) bacteria and *E. coli* is also observed. This was also seen for TC and FC. In the 2010 analysis none of the bacteria showed a correlation with temperature.

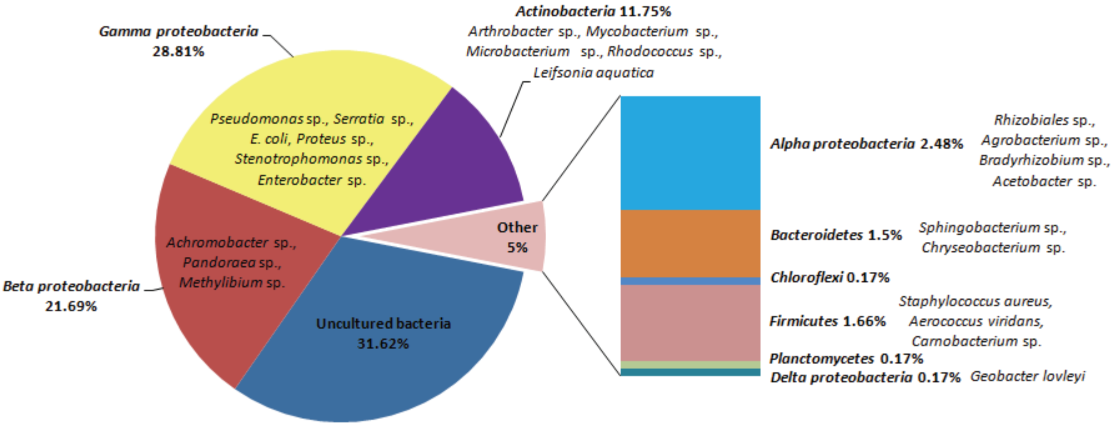
3.3.3 Bacterial diversity by High Through-put sequencing

The relative abundance of various bacterial phylogenetic groups in the water of the boreholes are depicted in Figures 27 and 28. In both cases the uncultured bacterial component was greater (70.55-78.09%) in the winter when compared to the summer samples (31.65 to 34.79%). In the summer samples of both the boreholes the Gamma and Beta proteobacteria as well as Actinobacteria made up greater proportions of the bacterial community structure. Among the Gamma proteobacteria, *Enterobacter* sp., *Proteus* sp., *Stenotrophomonas maltophilia* are species that were always detected. There were also several other species that were consistent with the culture dependent results. Sequences from *E. coli* as well as *Pseudomonas* sp. and *Staph aureus* were also detected in the summer samples.

The most important message from these results is that HTS is a powerful tool to provide data on the microbial diversity in borehole water. Results are generated much quicker and presence of indicator bacteria were also demonstrated.



Groundwater, bacteria, Wolmaranstad - Winter



Groundwater, Bacteria, Wolmaranstad - Summer

Figure 27: The relative abundance and composition of the dominant bacterial phylogenetic groups in the groundwater obtained through high-throughput sequencing for the Wolmaransstad borehole.

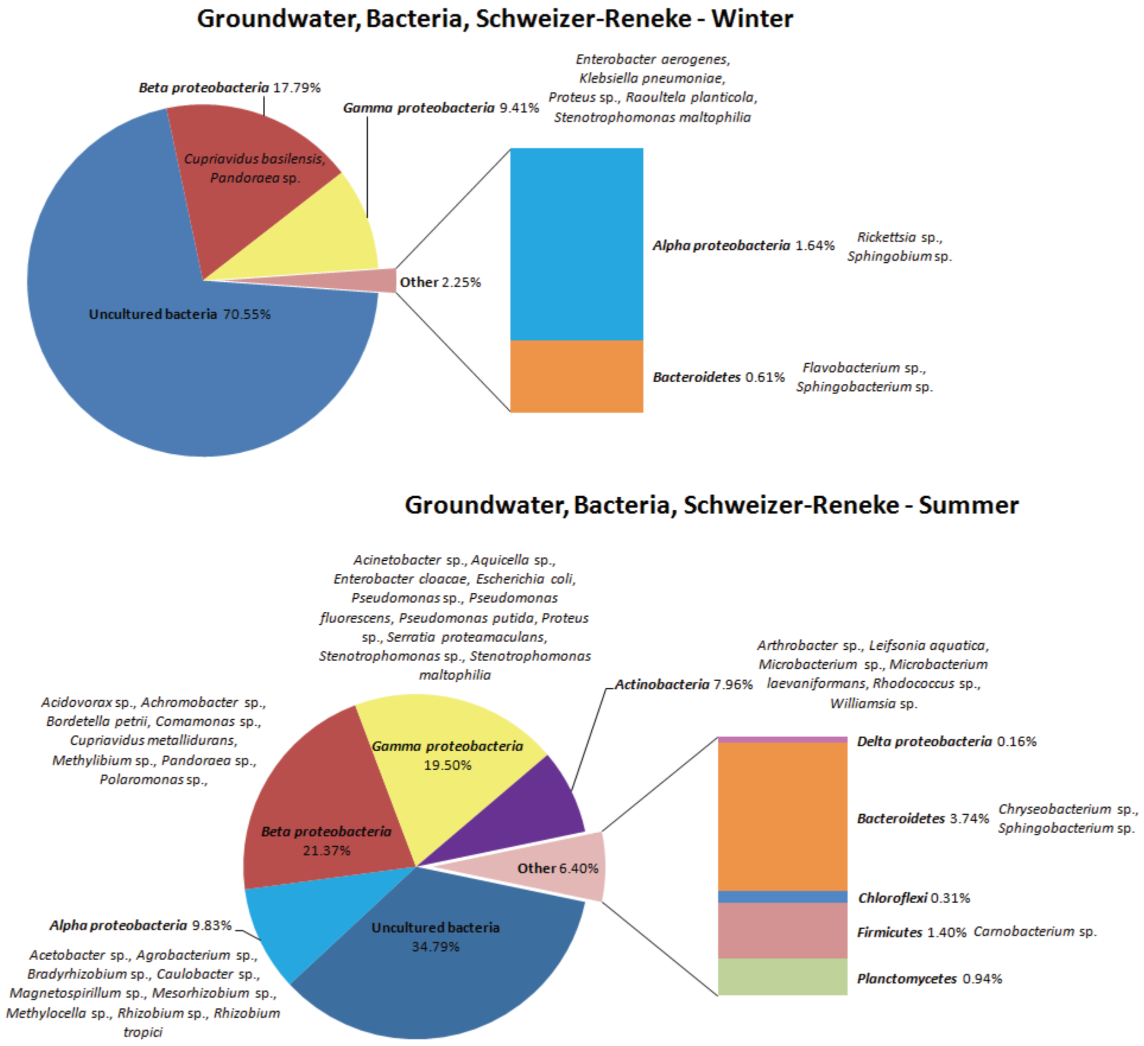


Figure 28: The relative abundance and composition of the dominant bacterial phylogenetic groups in the groundwater obtained through high-throughput sequencing for the Schweizer-Reneke borehole.

3.4 Conclusion

- All water samples complied with the domestic use TWQR for pH (DWAF, 1996). Although EC readings were mostly high, the values were also at acceptable levels for domestic use. Nitrate contamination was the major contaminant. Twenty eight percent of all the boreholes tested complied with the national nitrate limit (6 mg/L NO_3N) for drinking water. Forty three percent of the boreholes had nitrate levels greater than 20 mg/L NO_3-N. Some of the levels were greater than 400 mg/L NO_3-N.
- Microbiological results showed that groundwater of the North West Province may be prone to contamination from faecal sources. The study also showed that opportunistic pathogens (*P. auruginosa* and *Staph aureus*) associated with faecal pollution are present in the water from some boreholes.

- Next generation sequencing (or high through-put sequencing) results provided data on the microbial diversity in borehole water. This method is fast in comparison to culture based methods. It was demonstrated that this method holds promise to be used in monitoring indicator bacterial species in groundwater.

CHAPTER 4

QUANTIFICATION OF FAECAL STEROLS IN ENVIRONMENTAL WATERS

4.1 Introduction

Sewage tracers can be used to examine a) the presence of current, past, sewage inputs and b) the distribution and transport of sewage in the environment (Pratt, 2005). In this respect, the biomarkers are organic compounds that, once released into the environment, retain their structural integrity for their source to be recognised (Leeming et al., 1996). The distribution of sterols found in faeces and hence their source-specificity, depends on the animal's ability to synthesise its own sterols as well as the intestinal micro-biota (Leeming, 2006). Combinations of these factors determine "the sterol fingerprint". Field studies (Leeming, 1996) and a major validation study (Leeming et al., 1998) highlighted the possibility of using sterols in combination with bacterial indicator concentrations as faecal pollution indicators in any water column.

The principal human faecal sterol, coprostanol (COP; 5β(H)-cholestan-3β-ol) constitutes about 60% of the total sterols found in human faeces. Coprostanol itself is not a contaminant of concern, but it provides the opportunity to assess other particulate bound contaminants such as viruses, PCB's, bacteria, hydrocarbons and endocrine disrupters (Writer et al., 1995). Coprostanol is much more stable and resistant to environmental changes compared to faecal coliform bacteria (Nishimura and Koyama, 1977). The C_{29} homologue of coprostanol is 24-ethylcoprostanol (24-ethyl-5β(H)-cholestan-3β-ol) which is the main faecal sterol excreted by herbivores. Both coprostanol and 24-ethylcoprostanol are present in different ratios in both human and animal faeces. It is possible to determine the relative contributions by calculating the ratio of coprostanol to 24-ethylcoprostanol in human and herbivore (e.g. sheep, cow, and possum) faeces (Leeming et al., 1996). Cholesterol (CHL; 5-cholesten-3β-ol) is the main sterol synthesised in the body of animals, and is absent from prokaryotes (bacteria) and plants. It is not completely absorbed by the body and is also excreted as a component of bile (Anon^2, 2007).

Stigmasterol (SROL; 3β-hydroxy-24-ethyl-5,22-cholestadiene) is an un-saturated plant sterol occurring in plant fats and oils (Baxter et al., 1999), whereas stigmastanol (SNOL; β-sitostanol) is an algae sterol and is found in reducing environments. Stigmastanol's presence in water indicates algae presence and does not signify human or animal waste (Pratt et al., 2007). β-sitosterol (β-SIT; 24β-ethylcholesterol) is a phytosterol. It originates from a wheat germ, peanuts, soybeans, pumpkin seeds and corn oil. The presence β-sitosterol in water thus indicates sterols originating from plants and not humans or animals (Lam, 2009).

The naturally occurring stanol in unspoiled environments is 5α-cholestanol (DCHL; dehydrocholesterol). It is thermodynamically the most stable isomer. By comparing ratios of coprostanol to 5α-cholestanol (Leeming and Nichols, 1995), one can determine if coprostanol found in organic–rich, or anaerobic sediments are of faecal origin. Dehydrocholesterol is found in trace amounts (relative to coprostanol) in human faeces, but increases in relative proportions in digested sewage sludges (Leeming, 2006). Increased amounts of dehydrocholesterol relative to coprostanol indicate older human faecal contamination (such as faecal sludges from sewer pipes). Measurement of these sterols from a non-faecal origin in the water column indicates that organic matter had been re-suspended from highly anaerobic sediments (Leeming, 2006).

Sterols are extracted and isolated from surface water samples and wastewater samples by liquid-liquid extraction using dichloromethane. Coprostanol, however, has an a-polar character, which slightly reduces its solubility in dichloromethane, making it more difficult to recover. A surrogate solution is added (perylene) in order to test the recovery of the sterols in the water samples. An internal standard (perylene-d12) is added to quantitate the sterols that elute (Szűcs et al., 2006). Because significant differences occur in individual composition of human and animal faeces due to alterations in dietary intake, sterol patterns in water can provide information to distinguish, and determine the origin of faecal pollution. High ratios of coprostanol could be associated with anthropogenic faecal contamination in water, while abundances in cholesterol and β-sitosterol are typical sterols from dogs or birds (Szűcs et al., 2006). In accordance, herbivore profiles have terrestrial sterols and phytosterol abundances.

Faecal sterols in biological and environmental samples are usually quantitated by using gas chromatographic (GC) techniques such as, GC with flame ionisation detection (FID) (Batta et al., 2002) and GC-electron capture detection (Jayasinghe *et al.*, 1998). Identification is confirmed by GC-MS (mass spectrometry) (Batta et al., 2002). Because cost and effectiveness is of concern when testing environmental water samples for faecal sterol content, the identification and quantitation of sterols in a single chromatographic run is of importance. The objectives of this study was to use the validated Szűcs method (Szűcs *et al.,* 2006) in determining faecal sterol fingerprints for various animal species and humans, as well as the quantitation of these sterol profiles and the differences between each of the profiles. This method was also evaluated for the presence of faecal sterols in selected surface and groundwater sources.

4.2 Experimental procedures

The method used was from Szűcs et al. (2006), with some modifications. This (Szűcs et al., 2006) was used to validate simultaneous determination of faecal sterols in water by gas chromatography-mass spectrometry. Only six target sterols (COP, CHL, DCHL, SROL, SNOL, β-SITO) were analysed in this method.

Dichloromethane (HPLC grade), acetonitrile (HPLC grade), sodium hydroxide, and standards of faecal sterols (COP, CHL, DHCL, SROL, SNOL, β-SIT) as well as the surrogate standard solution (Perylene), and the internal standard (Perylene-d12) were all provided by Sigma-Aldrich (Stenheim, Germany). N,O-bis-trimethylsilyl-trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was from Supelco (Germany).

4.2.1 Preparation of standard solutions

For optimisation of GC-MS conditions, a standard mixture of COP, CHL, DCHL, SROL, β-SIT, SNOL, and perylene (surrogate standard) was prepared in dichloromethane at a concentration of 1.0 mg/mℓ for each component. For quantitative determination, perylene-d12 was used as an internal standard (IS) and dissolved in dichloromethane to obtain a stock solution of 1.0 mg/mℓ. In order to determine the extraction efficiency (recovery) of the faecal sterols in unknown water samples, a 0.25 mg/mℓ perylene surrogate standard solution was prepared in acetonitrile.

4.2.2 Derivatisation procedure

The GC-MS analysis of target sterols was carried out after derivatisation with BSTFA-TMCS. A 25 μℓ aliquot of the standard mixture as well as 20 μℓ of IS solution was pipetted into 1.5 mℓ auto sampler vial (Supelco, Germany) and dried completely under nitrogen flow. The residue was reconstituted in 125 μℓ of dichloromethane and mixed with 125 μℓ of BTSFA-TMCS. The vial was closed with a Teflon-lined screw cap, and the sample was silylated at 70˚C for 30 min. Following derivatisation, 1 μℓ of the mixture was injected into the GC-MS.

4.2.3 Instrumentation and GC-MS conditions

The samples were analysed on a Hewlett-Packard (Palo Alto, CA) GC-MS system consisting of an HP 6890 GC with an HP 5973 mass selective detector (MSD) and an Agilent 7683 automatic liquid sampler (Agilent Technologies, Palo Alto, CA). Separations were accomplished using an Supelco Equity-5 fused silica capillary column (Hewlett-Packard) coated with phenylmethylsiloxane (30 m x 0.25 mm x 0.25 μm film thickness) using the Environmental Protection Agency (EPA) Method 8270 with minor modifications.

Briefly, the GC-MS parameters were as follow: Carrier gas: helium 1.4 mℓ/min, constant flow; injection: splitless, 1 μℓ sample; inlet temperature: 280˚C; oven ramps, 150˚C for 0.5 min, 150˚-300˚C at 20˚C/min, and 300˚C-310˚C at 25˚C/min; GC-MS interface temperature: 280˚C; MSD ion source temperature: 230˚C; MSD quadrupole temperature: 150˚C; ionisation energy: 70 eV; and solvent delay: 7.0 min. System control, data acquisition, and analysis were performed with the HP G1701AA MSD Productivity ChemStation software, Rev. A.03.01 (Agilent) on a HP (Hewlett-Packard) computer. Data was acquired in the full scan mode between ions of *m/z* 50 and 550.

4.2.4 Preparation of calibrators and calibration curves

Target sterols and surrogate standard were dissolved in dichloromethane to prepare the standards as 1.0 mg/mℓ stock solution. Calibrators were prepared by measuring 20 μℓ of IS and 5-, 10-, 15-, 20-, 25-, 30-, 35-, and 40 μℓ aliquots of the stock solution into auto sampler vials and dried under nitrogen flow. Residues were constituted in 125 μℓ of dichloromethane, mixed with 125 μℓ of BSTFA-TMCS, and derivatised as described previously. Final mass concentrations of calibration standards were 20, 40, 60, 80, 100, 120, 140, and 160 ng/ℓ for each of the components and that of IS was 80 ng in 1μℓ injected volume. Calibration curves were obtained by plotting the target compound: IS response ratios of characteristic ions selected from the mass spectra as a function of the respective concentrations of the individual components. Target (T) and qualifier ions of m/z (Q1 and Q2) applied for the GC-MS quantitation are shown in Table 29.

Table 29: Target (T) and qualifier (Q1 and Q2) ions of m/z applied for GC-MS quantitation of faecal sterols.

Target Compound	T (m/z)	Q1 (m/z)	Q2 (m/z)
Perylene	252	253	250
Perylene-d12	264	260	132
Coprostanol	370	215	355
Cholesterol	329	368	353
Dehydrocholesterol	215	445	355
Stigmasterol	255	394	484
ß-sitosterol	357	396	381
Stigmastanol	215	473	383

Identity of the target sterols was examined by comparing the obtained mass spectra with those of the corresponding reference substances using the HP 5973 MSD reference collection, NIST MS search program, and spectrald database collection, Version 1.5 (1996) (Hewlett-Packard).

4.2.5 Preparation of water samples for GC-MS analysis

A raw domestic wastewater sample (influent) was obtained from an urban sewage treatment plant (Potchefstroom Wastewater Treatment Plant) as well as a treated wastewater sample (effluent). Surface water samples were also spiked with faeces from animals (cattle, chickens, horses, pigs and sheep). Samples were processed for extraction within 24-36 h. Water samples (300 mℓ) were mixed with 200 µℓ of 0.125 mg/mℓ surrogate standard solution and 10 g NaOH. Samples were then saponified in a shaking incubator at 60˚C for 1 h. Following saponification, the spiked water samples and the effluent were extracted (see section on recovery), derivatised (see section on derivatisation procedure), and analysed by GC-MS (see section on instrumentation and GC-MS conditions). The untreated wastewater sample (influent) was prepared for GC-MS in the same manner, but it was mixed with 50 mℓ of dichloromethane and ultra-sonicated with 10µm amplitude for 10 min before extraction. Concentrations of target sterols and surrogate were calculated on the basis of calibration curves by the data analysis software (HP ChemStation)

Groundwater samples (300 ml) were collected from boreholes and surface water samples from several sites in the North West Province and analysed by using the Szucs et al. (2006) method.

4.3 Results and Discussion

Standard curves (Figure 29) were constructed by dividing the area of the peak for perylene-d12 (IS), by the area of the peak for each of the target sterols (COP, CHL, DCHL, SROL, SNOL, β-SIT). This was done for each concentration of the standard solution. The ratios were determined and plotted on a concentration/response graph.

The calibration curves for COP, CHL, DCHL, SROL, β-SITO and SNOL were obtained from three different series. Target compound-IS response ratios were linear up to 160ng for each target sterol molecule. Lines of best fit obtained by linear regression were described by:
COP: $y = 0.0311x – 0.1999$, where $r^2 = 0.9094$; CHL: $y = 0.0311x – 0.2285$, where $r^2 = 0.9044$; DCHL: $y = 0.0499x – 0.2802$, where $r^2 = 0.9339$; SROL: $y = 0.0353x – 0.2711$, where $r^2 = 0.8995$; B-SITO: $y = 0.0268x – 0.2131$, where $r^2 = 0.8981$;
SNOL: $y = 0.0504x – 0.3302$, where $r^2 = 0.9250$.

These equations were used in the determination of concentrations of the various sterols.

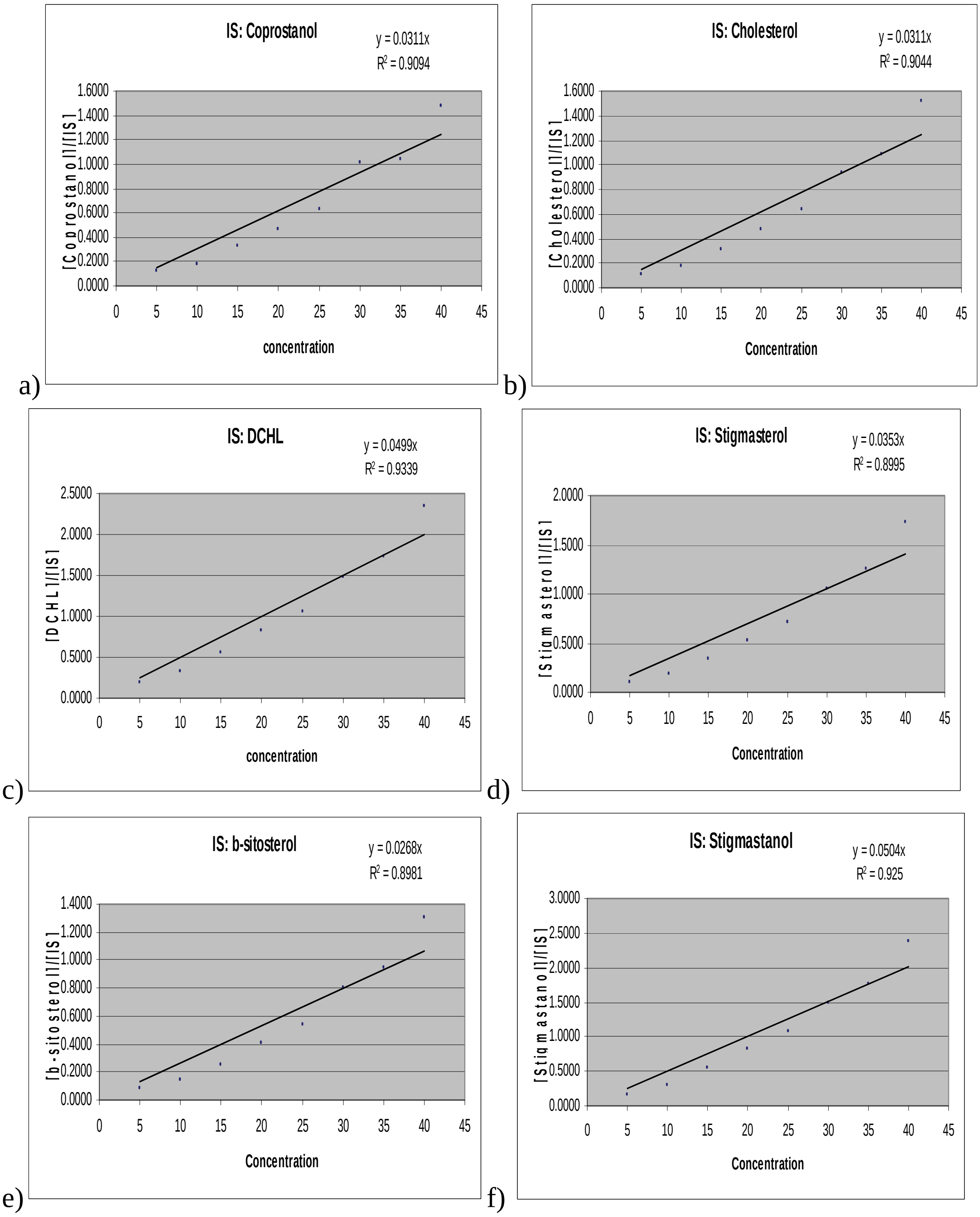


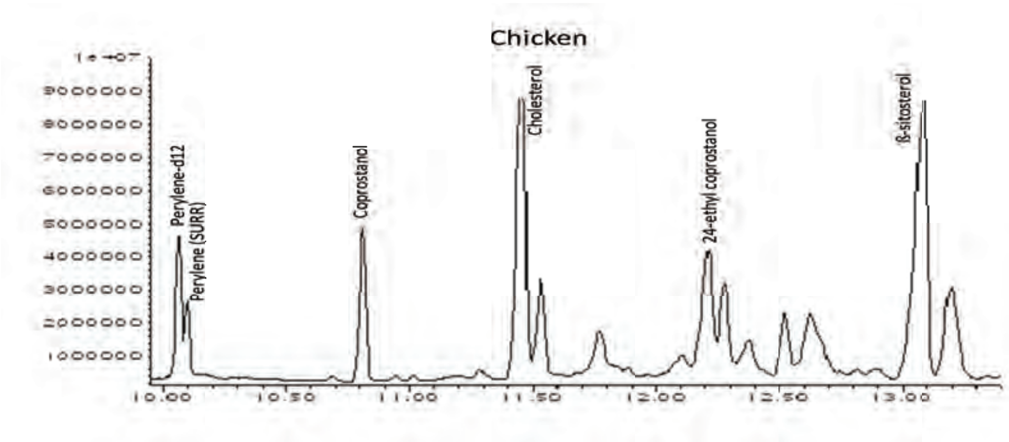
Figure 29: Standard curves of all sterols that where tested for with the Szűcs method.

4.3.1 Chromatograms

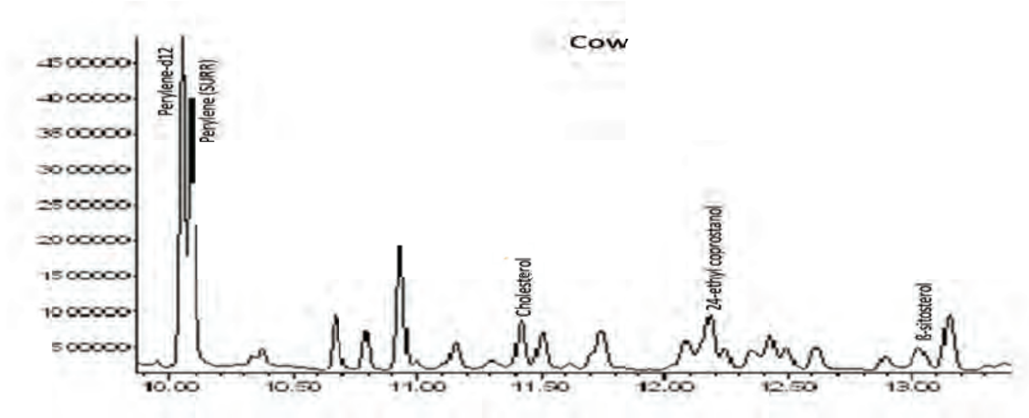
The following chromatograms (Figures 30 and 31) indicate preliminary results in determining the sterol fingerprint for the six species examined. Elution of perylene (surrogate) indicates that the recovery of the sterols was successful.

Although sterol profiles may look similar and peaks elute at the same retention times, it may not be the same sterol. Identification of the target sterols are done by the obtained mass spectra for each eluted compound and not by retention times. This is necessary because some compound may overlap during elution. Target and qualifier ions (Table 28), obtained by the MS detector, were used to identify target sterols of all species.

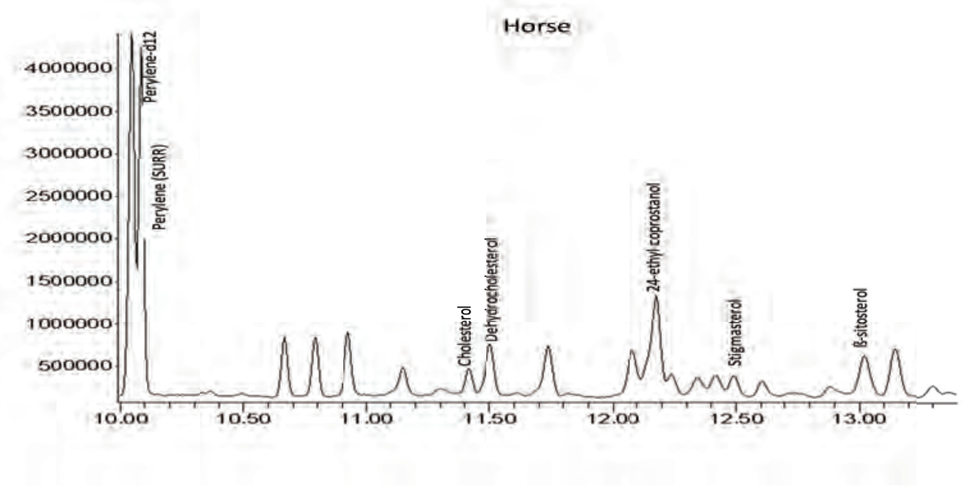
Establishing sterol fingerprints is necessary because it forms the baseline for the faecal sterol analysis and helps with understanding the origin of faecal pollution in a water body (Leeming et al., 1996). GC-MS conditions were optimised with a standard mixture of six sterols and one surrogate. Peaks of all the target sterols could be seen (Figures 30 and 31).



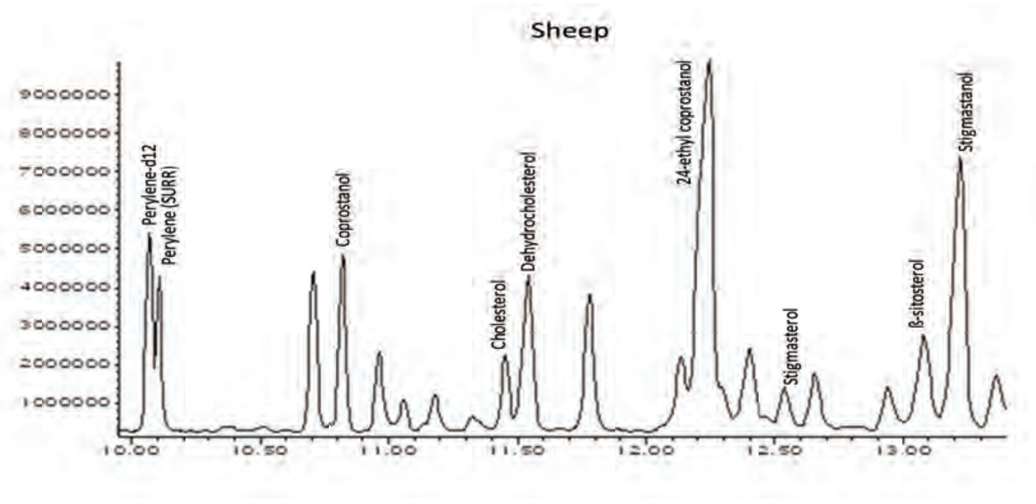
a)



b)



c)



d)

Figure 30: The sterol profiles of the herbivore species; a.) chicken, b.) cattle, c.) horse and d.) sheep.

Figure 30a shows higher abundance of cholesterol and β-sitosterol was observed compared to levels of coprostanol. This is in agreement with Szűcs et al., (2006), which suggested such results indicate faecal pollution from birds. This phenomenon is also demonstrated in Table 2.

Elution of cholesterol and the phytosterol, β-sitosterol, occurs in all sterol profiles, at low abundances. Those of cattle (Figure 30b) and horse (Figure 30c) profile were of such low levels that their quantities could not be determined. Horse sterol profile (Figure 30c) also eluted dehydrocholesterol and stigmasterol that was not found in the sterol profile of the cow faeces. These sterols were detected in sheep sterol profile (Figure 30d). The sterols profiles of pigs and humans are provided in Figure 31.

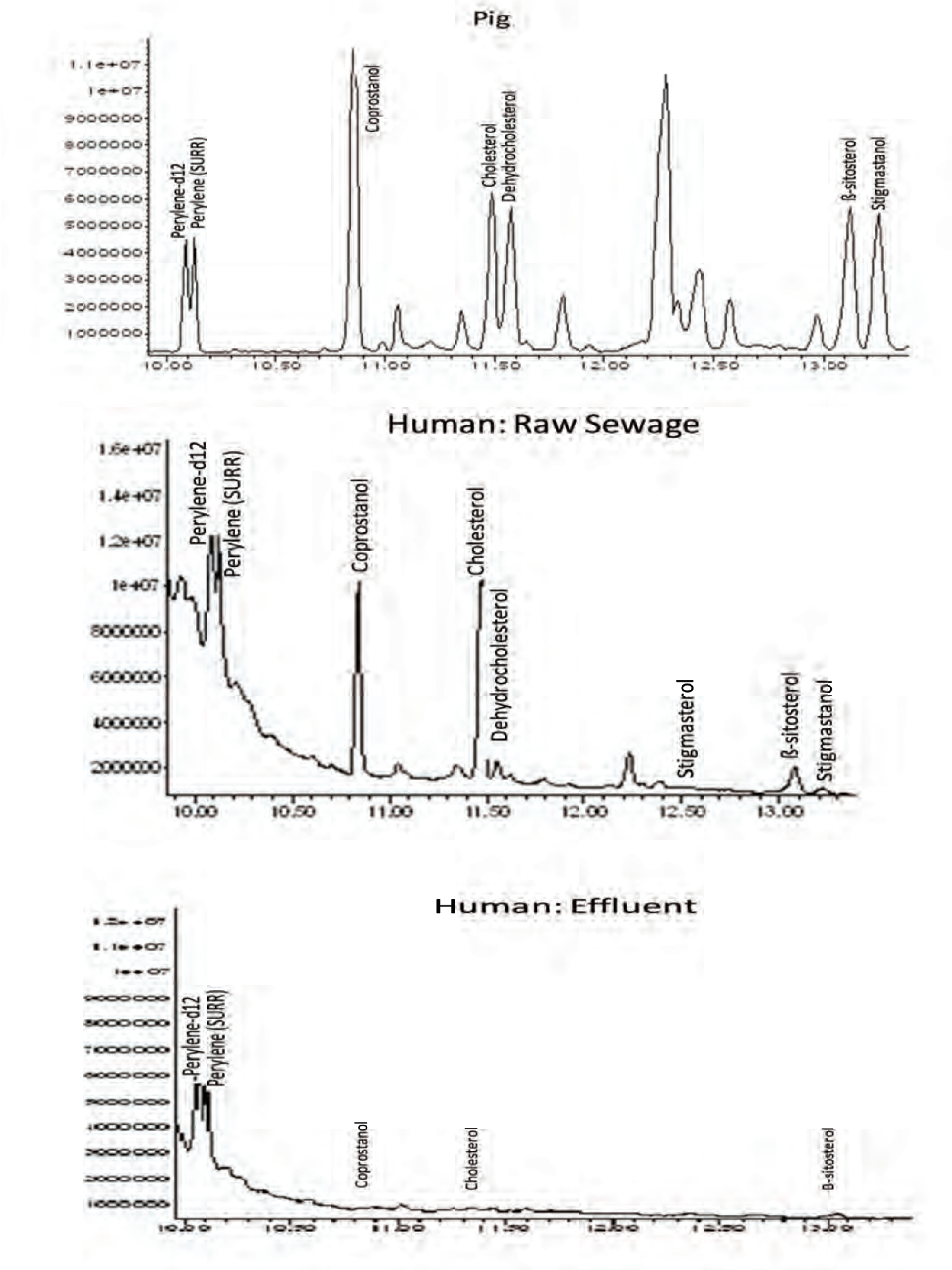


Figure 31: Sterol profiles of a.) pig, b.) untreated influent, and c.) treated effluent.

The sterol profile of the pig faeces (Figure 31a) shows very high peaks of coprostanol, cholesterol, β-sitosterol and stigmastanol. In WWTP influent (Figure 31b), cholesterol and coprostanol was found. Coprostanol is the main human faecal sterol biomarker, indicating fresh human faecal contamination (Leeming, 1996; Szűcs et al., 2006). All other five sterols were found in varying concentrations. Coprostanol, cholesterol and β-sitosterol was found in very low levels in WWTP effluent (Figure 31c).

Comparing the sterol profiles of pigs (Figure 31a) and sewage (Figure 31b), a distinct difference between the two profiles can be seen. All target sterols eluted in the sewage sterol profile (Figure 31b). Levels of β-sitosterol and the terrestrial sterols (stigmastanol and stigmasterol) were all low. Coprostanol and cholesterol were present at higher concentration. The pig sterol profile (Figure 31a) had high coprostanol but low cholesterol levels. The concentration of dehydrocholesterol and stigmastanol were much higher in the pig profile than in the WWTP influent profile (Figure 31b).

Comparing the WWTP influent sterol profile (Figure 31b) to the treated effluent (Figure 31c), it can be deducted that the target sterols tested for in this study has been radically decreased or completely removed. There were also fewer peaks in the treated water demonstrating that the wastewater treatment works was removing sterols from wastewater. This result implies that one could evaluate the efficiency of wastewater treatment plants using GC-MS analysis of faecal sterols.

In all the samples that contained mainly human faecal matter, the concentration of coprostanol and cholesterol concentrations were higher than the other sterols. In the case of cow, horse, sheep and chicken faecal samples, the concentration of coprostanol was lower than other sterols. This observation forms the basis for differentiating human from non-human sources of faecal contamination in environmental water samples.

Concentrations of the various sterols that eluted are important because from the concentrations, ratios can be established (Leeming, 2006). The ratios of the concentrations are used to determine the origin of faecal pollution. Concentrations of the target sterols were calculated by integrating the peaks that eluted for each species and determining the area of each peak. Using the standard curve's y-values and r^2-values, the concentrations of each sterol that eluted for each of the various species could be calculated by the straight line equation: $y = mx + c$. The values are depicted in Table 30**.**

Table 30: Concentrations of all marker sterols that eluted for the seven samples analysed.

	COP (ppm)	**CHL (ppm)**	**DCHL (ppm)**	**SROL (ppm)**	**ß-SITO (ppm)**	**SNOL (ppm)**
Chicken	29.998	81.126	0	0	119.828	0
Cow	0	5.859	0	0	4.875	0
Horse	0	3.785	4.740	3.078	8.024	0
Sheep	26.995	12.698	21.227	8.442	28.126	74.828
Pig	111.008	49.280	34.957	0	14.070	68.336
Sewage Influent	14.297	15.012	1.685	0.636	3.513	1.012
Sewage Effluent	1.368	2.004	0	0	2.887	0

Table 30 indicates that the treated effluent had very low concentrations of sterol to be of any consequence. All species had cholesterol and β-sitosterol in their profiles. Sheep, horse, pig and raw sewage samples had dehydrocholesterol and are consistent with observations by Leeming, (2006). The WWTP influent, horse and sheep faecal samples were the only profiles that had stigmasterols present. Large amounts of β-sitosterol were found among horse and sheep profiles, but less in cow faeces. This is contradictory since these species are all herbivores. All the herbivore species had large amounts of stigmastanol and stigmasterol compared to the other species. This is because of their diet, as stigmasterol and stigmastanol are both terrestrial sterol biomarkers (Leeming, 2006).

4.3.2 Surface and groundwater

Water samples collected from boreholes were subjected to analysis for faecal sterols. The same groundwater samples were also analysed for physico-chemical properties and bacteriological quality (Ferreira, 2011). Tables 32 and 33 are summaries of the results. The electrical conductivity and nitrate levels were high, mostly exceeding the drinking water TWQR (DWAF, 1996). These are discussed in Chapter 3 and implications further explored in Chapter 5.

Table 31: Physico-chemical properties of the groundwater samples taken at the various sites.

Site	pH	Temp (°C)	EC (mS/m)	NO_3-N (mg/ℓ)
Mafikeng	7.1	19.3	141.6	8.2
Zeerust	7.8	20.1	62.4	9.5
Delarey-Ottosdal	7.2	20.5	133.2	23.9
Setlagole	7.0	19.5	253.0	18.4
Geystown	7.8	18.1	810.0	15.2
Taung	7.7	24.4	916.0	16.4
Hartswater	7.6	20.2	286.0	22.3
Sekhing	7.3	24.0	142.7	1.6
Christiana	7.5	19.4	143.7	23.2
Christiana 2	6.9	22.2	86.1	11.6
Vryburg	7.2	16.0	125.4	28.9
Ganyessa	7.5	17.3	125.9	28.6
Ganyessa 2	7.1	21.2	84.0	15.2

mS/m = micro Siemens per metre; mg/ℓ= milligram per litre; NO_3-N=Nitrate-nitrogen

The values of the bacteriological counts are provided in Table 29. Very high total coliform and faecal coliforms were detected in four of the boreholes samples. In the case of the Hartswater sample the faecal streptococci levels were also high. These samples were also analysed for the presence of faecal sterols. Most of the samples tested negative. Chromatograms for the samples that were positive are shown in Figures 31 to 33**.**

Table 32: Bacterial counts for groundwater samples.

Site	TC (cfu/100 ml)	FC (cfu/100 ml)	*E. coli* (cfu/100 ml)	FS (cfu/100 ml)
Mafikeng	3	0	0	8
Zeerust	5	3	2	3
Delarey-Ottosdal	27	3	2	2
Setlagole	>300	>300	34	8
Geystown	4	2	0	0
Taung	10	7	0	9
Hartswater	>300	>300	0	180
Sekhing	5	3	2	10
Christiana	233	65	3	32
Christiana 2	26	9	0	18

Site	TC (cfu/100 ml)	FC (cfu/100 ml)	*E. coli* (cfu/100 ml)	FS (cfu/100 ml)
Vryburg	0	0	0	2
Ganyessa	>300	220	0	6
Ganyessa 2	0	0	0	0

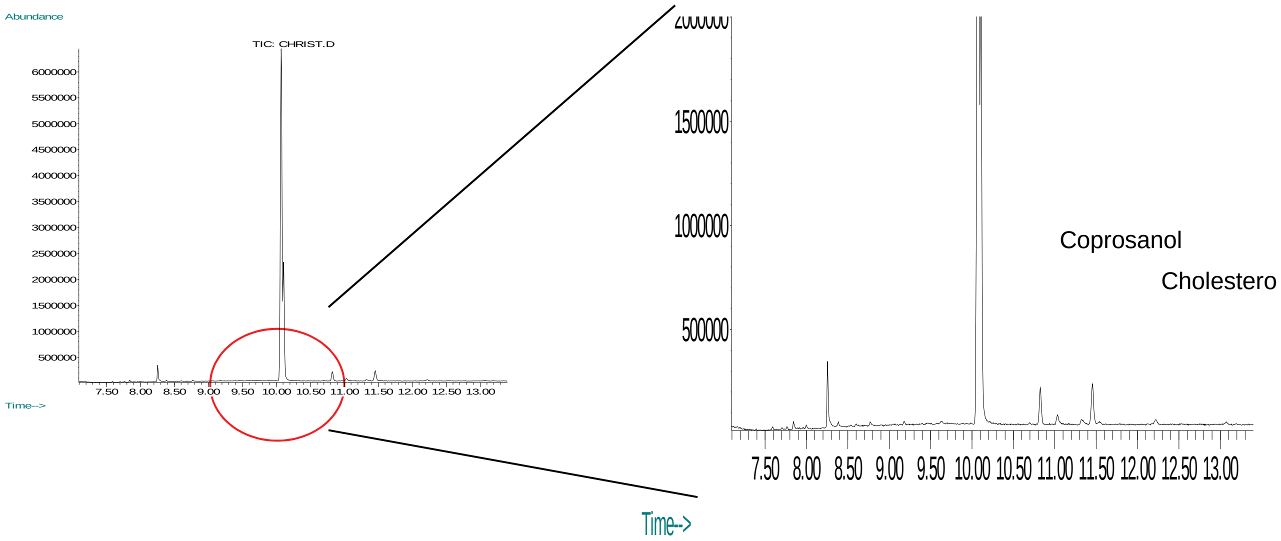


Figure 32: The GC-MS Chromatogram of the Christiana groundwater sample that was analysed for faecal sterols.

The chromatogram (Figure 32) showed peaks for coprosanol and cholesterol in the groundwater sample from Christiana. Coprostanol may indicate human and cholesterol human or animal faecal pollution. These faecal sterols were also present in the GC-MS chromatograms of Geystown and Taung samples (Figure 33). Both these samples had very low levels of total coliforms (Table 31). They also did not have any *E. coli* present. Since sterols interact with and become particle bound it was not expected to find any of these organic substances in the groundwater. These positive results should be further explored in future studies.

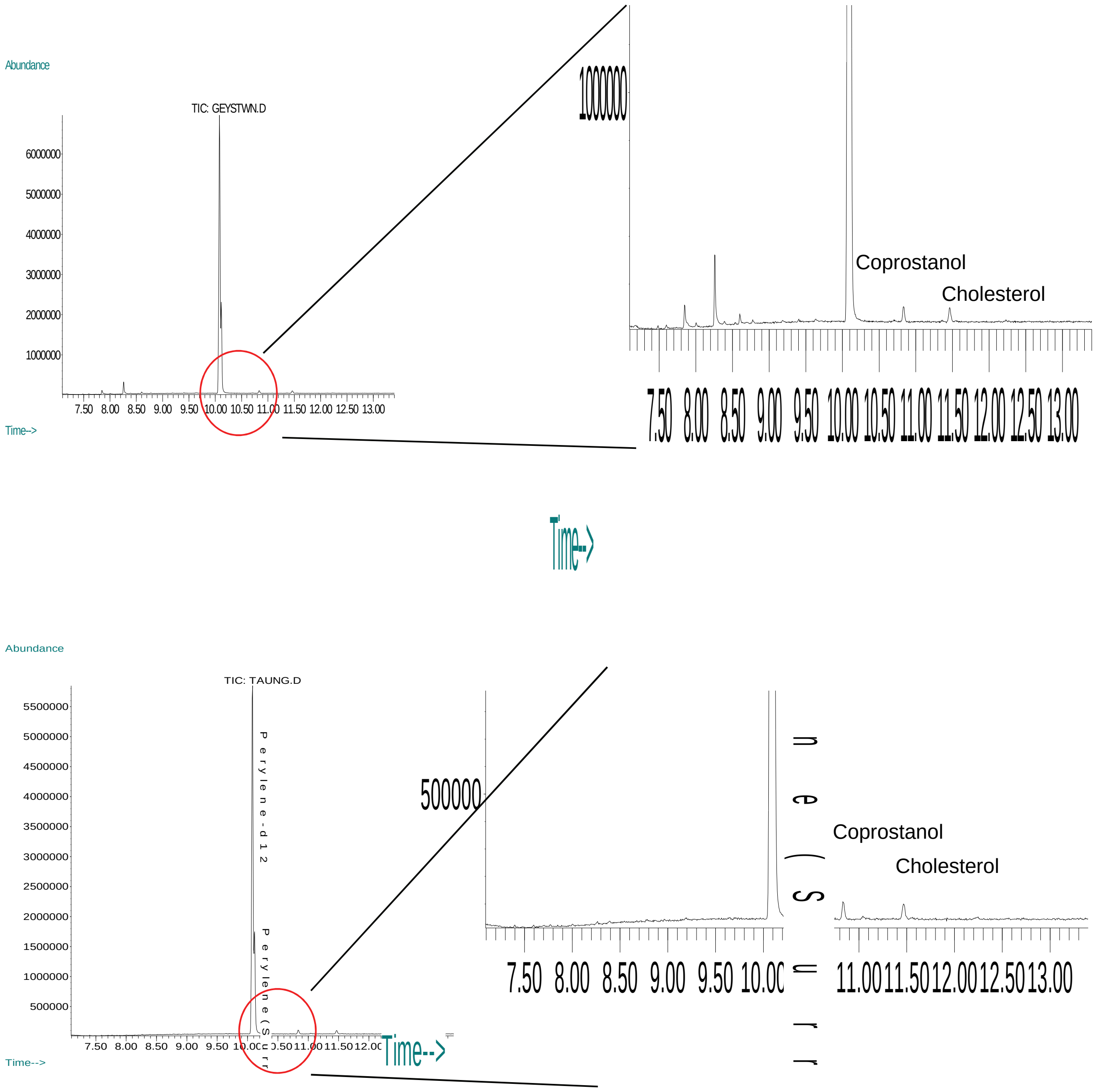


Figure 33: The GC-MS chromatograms of the (a) Geystown and (b) Taung groundwater samples that were analysed for faecal sterols**.**

Surface water samples were also collected and analysed for the presence of faecal sterols. These surface water samples were collected from the Baberspan Bird Sanctuary, previously described. They were also analysed for their physico-chemical properties and levels of bacteria (Molale, 2012). From Table 33 it is evident that the electrical conductivity for these sites was high and that the nitrate levels were very low.

Table 33: Physico-chemical properties of the Baberspan samples.

Baberspan sampling	pH	Temp (°C)	EC (mS/m)	NO_3-N (mg/ℓ)
Harts river	8.6	12.0	147.1	1.2
Inflow	9.2	14.7	135.1	0.5
Hotel	8.6	15.2	115.2	0
Outflow	8.6	13.7	120.9	0.7

Table 34: Bacterial counts for the Baberspan water samples.

Baberspan sampling	TC (cfu/100ml)	FC (cfu/100ml)	*E. coli* (cfu/100ml)	FS (cfu/100ml)	Cholesterol (ppm)
Harts river	265	130	3	127	0
Inflow	27	7	3	18	6.212
Hotel	19	1	1	50	6.008
Outflow	4	0	0	61	7.258

Bacterial counts for the Baberspan water samples were low, except for the Harts River site (Table 34). All sites were positive for faecal streptococci. Low levels of faecal coliforms and *E. coli* were detected at the inflow and the hotel sites. Water samples collected at the Baberspan Bird Sanctuary were also analysed for faecal sterols using the Szucs method (Szucs *et. al*., 2006). The only faecal sterol that was detected was cholesterol at the inflow, hotel and outflow sites. No faecal sterols were detected at the Harts river site.

4.4 Conclusions

- Using water samples that were spiked with agricultural animal faeces as well as two samples (influent and effluent) from a water treatment plant. faecal sterol profiles that were animal species specific were generated. This demonstrated the potential of using such an approach in source tracking faecal pollution in river systems in the NWP.
- The potential of using this method for surface and groundwater studies to track pollution was also demonstrated.
- Another potential application of the faecal sterol analysis is to use it to evaluate the efficiency of wastewater treatment plants since it is a relatively quick method and small amounts of water is needed to be analysed. This could prove to be very beneficial since South Africa is a country of coherent wastewater treatment plant failures (DWAF, 2009).

CHAPTER 5

WATER-RELATED HEALTH RISK POTENTIAL

5.1 Introduction

There is a close relationship between poverty and poor water quality available for domestic use (Schreiner *et al.,* 2002). In many cases poor communities are deprived of the benefits of good quality water because they lack the finances as well as technological assets to determine the quality of the water available of drinking purposes and, if necessary, initiate remediation processes. They are thus forced to drink water, particularly surface water, of low quality (Schreiner *et al.,* 2002). In South Africa, in the recent past, several service delivery social upheavals occurred due to the inability of local authorities to provide sufficient quantity and acceptable quality of drinking water to these communities (e.g. Gaoganediwe, 2006; GCIS, 2005; Landman, 2009; Tempelhoff, 2009). Such urban South African communities in these modern times are still deprived of sufficient water that is safe for human consumption. This includes several communities in the North West Province and these community members may be reliant on untreated water sources.

Health risks associated with untreated drinking water cause diseases such as shigellosis, cholera, salmonellosis, diarrhoea and a variety of fungal, parasitic, bacterial and viral infections (Zamxaka et al., 2004). A list of enteric diseases is presented in Table 1. The majority infections from consuming untreated polluted water are associated with secondary infections that are due to weakened defence mechanisms. People who are particularly susceptible include very young children with underdeveloped immune systems, individuals with compromised immune systems and the elderly with weakened immune systems (WHO & UN-WATER, 2010). Patients under medical treatment after an organ transplant or immune-compromising infections, such as AIDS (Acquired Immune Deficiency Syndrome), are also at risk of infection (Pavlov et al., 2004). It is recognised that microbial flora of humans play an important role in immunity to microorganisms (Candela et al., 2010). However, individuals that are immune-compromised may not have such microbial flora. Such immune-compromised individuals may even be infected by microorganisms that generally have very low virulence and that would not typically infect healthy individuals (Glasmacher et al., 2003). Studies on water quality and the impacts thereof should thus also determine the general diversity of organisms present in the water samples. For this culture dependent as well as culture independent methods are available. Both of these methods have advantages as well as disadvantages when used in a study where the aim is to investigate potential health impacts.

E. coli is used when the microbiological quality of water is determined (DWAF, 1996; Kong et al., 2002; Lleo et al., 2005; Oliver et al., 2005). Although it is a commensal bacterium of various animals inhabiting the colon, certain strains are pathogenic and infect mucosal surfaces of the intestines (Kaper and Nataro 1998). Five groups of pathogenic *E. coli* are known may use environmental water as a reservoir and transport agent after faecal contamination (Kong et al. 2001; Ashbolt, 2004). These groups of *E. coli* are: (i) Entero-Pathogenic *E. coli* (EPEC), (ii) Entero-Toxigenic *E. coli* (ETEC), (iii) Entero-Hemorrhagic *E. coli* (EHEC), (iv) Entero-Invasive *E. coli* (EIEC) and (v) Entero-Aggregative *E. coli* (EAEC) (Kaper, 2005; Nataro and Kaper, 1998). Under certain conditions chlorine treatment induces *E. coli* into a viable but non-cultureable (VBNC) state (Oliver et al., 2005). This also holds true for certain environmental conditions. The VBNC *E. coli* will not be detected by culture based methods but will be able to cause infection (Lleo et al., 2005, Kong et al., 2002). Polymerase chain reaction methods are available for the detection of *E. coli*. Multiplex PCR methods had been developed for the identification of pathogenic *E. coli* from environmental water sources (Omar et al., 2010; Bai et al., 2010; Wang et al., 2010; Rodas et al., 2009). This is based on the direct PCR detection of several DNA markers by a single PCR (Omar et al., 2010).

Besides bacteria, environmental water can also contain viruses and bacteriophages. Municipal wastewater treatment systems, even in developed countries are capable of reducing the load of these but in many cases cannot completely remove them from the effluent (Carducci et al., 2008). This is particularly of concern if the poor state of waste water treatment plants in South Africa is considered, particularly those decanting into the Vaal River and its tributaries (DWA, 2009). In many cases these systems are exceeding their design flow capacity, are not maintained, etc. (DWAF, 2012). These problems lead to poorly treated (or untreated) sewage reaching the receiving waters. Within this matrix there will be viruses that could have human health implications.

Yeasts of various types have been reported to have been isolated from lakes and ponds (Sláviková & Vadkertiová, 1997; Medeiros et al., 2008), estuaries, coasts and mangrove areas (van Uden & Fell, 1968) as well as oceans and the deep sea (Nagahama et al., 2003). Only a few studies have reported on the presence of yeasts in river water (Sláviková & Vadkertiová, 1997; Medeiros et al., 2008; van Wyk et al., 2012). Furthermore, more than 100 yeast species that are known to occur in environmental water sources have been identified as human pathogens (Fromtling et al., 2003). A large percentage of these pathogens are classified in the genus *Candida*. Hurley et al. (1987) list following pathogenic yeasts that may be causal agents for candidosis: *C. albicans, C. tropicalis, C. stellatoidea, C. glabrata, C. krusei, C. parapsilosis, C. guilliermondii, C. viswanathii, Clavispora lusitaniae (Candida lusitaniae)* and *Rhodotorula mucilaginosa (Rh.rubra)*.

There are major health risks associated with untreated drinking water because it may cause diseases such as shigellosis, cholera, salmonellosis, diarrhoea and a variety of fungal, parasitic, bacterial and viral infections (Zamxaka et al., 2004). Infectious particles and pathogenic (potential pathogenic) microorganisms may cause various impacts on mucosal cells of the intestine. Some of the impacts may be cytotoxic. In order for consumed microbes to cause gastrointestinal diseases they must meet a number of virulence characteristics: secrete extracellular enzymes, be cytotoxic to cells, adhere to cells, and survive passing through the gastric fluids (Yuk, & Marshall, 2004). Various methodologies are available to investigate cytotoxic effects. Each of these has their own advantages and disadvantages (Lyle and Dufour, 1991).

This chapter is a focus on potential health implication of water samples collected during this study and is based on potential use (general domestic and recreational). The objectives were to:

1) use the microbial and physico-chemical quality data to determine if there are any health risks associated if this water is used for domestic and recreational purposes;

2) determine antibiotic resistance and haemolytic potential of selected isolates and comment on the potential human health risks;

3) investigate if pathogenic strains of *E. coli* are present in selected environmental water sources;

4) determine the general microbial diversity in surface and groundwater sources by using culture independent methods and whether amongst these there may be potential pathogens.

5) comment on the detection of enteroviruses in treated sewage and sewage-contaminated water sources in selected water sources in the North West Province, South Africa;

6) develop and test a MTT method to determine the cytotoxicity effects on intestinal cells.

5.2 Experimental procedures

5.2.1 Physico-chemical parameters

Details of investigation are provided in Chapters 2, 3 and 4

5.2.2 Culturable bacteriological parameters

Details of investigation are provided in Chapters 2 and 3

5.2.3 Antibiotic resistance and haemolytic patterns

Antibiotic susceptibility tests were performed on faecal coliforms and enterococci isolates using the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). Overnight cultures of faecal coliforms and enterococci were grown in Brain Heart Infusion Broth (BHI) (Merck, Germany) at 37°C and spread onto Mueller Hinton agar (Merck, Germany). In addition to the antibiotics listed in Table 35, Neomycin (30 µg) Penicillin G (10 µg) and Vancomycin (30 µg) were used for enterococci. For this group (enterococci) Cephalothin and Kanamycin was not included. All antibiotics were obtained from Mast Diagnostics (UK). After incubation inhibition zones were measured in millimetres and compared to the NCCLS growth inhibition zone standards for Streptococci (NCCLS. 1999).

Table 35: Details of the antibiotics used in this study. The susceptibility specifications are only for *E. coli* (NCCLS, 1999).

Antibiotic Classification	Antibiotics	Abb	Conc (µg)	Resistant (mm)	Intermediate Resistant (mm)	Susceptible (mm)
Penicillin B-lactam& derivatives	Ampicillin	AMP	10	≤ 13	14-16	≥ 17
	Cephalothin	CEP	30	≤ 14	15-17	≥ 18
	Amoxicillin	AMOX	20	≤ 13	14-17	≥ 18
Amino-glycosides	Kanamysin	KAN	30	≤ 13	14-17	≥ 18
	Streptomycin	STR	10	≤ 11	12-14	≥ 15
Quinolones	Ciprofloxacin	CIP	5	≤ 15	16-20	≥ 21
Trimethoprim	Trimethoprim	TM	1.25	≤ 10	11-15	≥ 16
Chloram-phenicol	Chloram-phenicol	CHL	30	≤ 12	13-17	≥ 18
Tetracyclines	Oxy-Tetracycline	OXY-TET	30	≤ 14	15-18	≥ 19

From the data obtained multiple antibiotic resistant (MAR) phenotypes and indices of sample area in the groundwater study were determined. Only antibiotics to which more than

20% of isolates were resistant to was included in determining the most prevalent MAR phenotype. MAR indices were calculated only for faecal coliforms using the following formula (Guan *et al.,* 2002):

$$\text{MAR indices} = \frac{\text{Number of isolates in a specific population resistant}}{(\text{number of antibiotics tested})\text{x}\ (\text{total number of organisms in sample})}$$

Haemolysis was performed as described by Frobisher and co-workers (1928). Briefly, purified overnight cultures were spot inoculated on 5% (v/v) sheep blood agar plates (National Health Laboratories, SA) and incubated at 37°C for 24 hours. All green colonies surrounded by a clear margin were identified as α-haemolytic. The β-haemolysis isolates caused complete cell lysis and were identified by in a clear zone around the colony. Where no change occurred on the blood agar the isolate was classified γ-haemolytic (Health Protection Agency, 2008)

5.2.4 Pathogenic *E. coli*

E. coli isolates were obtained from surface and groundwater samples. When *E. coli* isolates were characterised for pathogenicity, genomic and plasmid DNA was isolated. The *E. coli* strains were incubated at 37°C for 18 hours in Luria broth. Genomic DNA and plasmid DNA were isolated using the NucleoSpin® Tissue Kit (Separations, US) and NucleoSpin® Plasmid DNA Purification Kit (Separations, US), respectively.

Multiplex PCR using the Qiagen® multiplex PCR Kit (Southern Cross Biotechnology, SA) was conducted according to the instructions of the manufacturer. Optimised reactions contained 1 X multiplex PCR master mix, 1 X Q-solution, 2.5 mM $MgCl_2$, 0.5 µM of *mdh* and *lacZ* and 1.5µM of *Eagg*, *LT* and *ST* (See Table 36 for sequences) and 100-150 ng template DNA. Cycle conditions consisted of 95°C for 15 minutes, 40 cycles of 94°C for 30 seconds, 56°C for 90 seconds and 68°C for 90 seconds. This was followed by a final extension at 72°C for 10 minutes. A C1000^{TM} Thermal Cycler (Bio-Rad, UK) was used and PCR fragments were resolved on 2.5% ($^{w}/_{v}$) Seakem Agarose (WhiteSci, SA) gels and 1 X TBE (89.0 mM Tris-base, 89.0 mM boric acid, 2.0 mM EDTA, pH 8.0) as electrophoresis buffer. The gels contained 1µg/ml Ethidium Bromide (Bio-Rad, UK) for visualisation of the PCR amplified fragments under UV light after electrophoresis. Five microliters of the PCR product and 5 µl of 6x Orange Loading Dye (Fermentas Life Sciences, US) were mixed and loaded into wells in the gel. A 2.5µl 100bp molecular weight marker (O'GeneRuler, Fermentas Life Sciences, US) was used to confirm the size of the amplification products. Electrophoresis was performed for 45 min at 80V. A GeneGenius Bio Imaging System (Syngene, Synoptics, UK) was used to capture gel images using GeneSnap (version 6.00.22) software (Bezuidenhout *et al.,* 2006).

Table 36: Primers used in the multiplex PCR reaction for detection of pathogenic *E. coli.*

Name	Specificity	Sequence (5’→3’)	Size	Reference
Mdh	*E. coli*	**FW** GGTATGGATCGTTCCGACCT **RV** GGCAGAATGGTAACACCAGAGT	301bp	Tarr et al., 2002
lacZ	*E. coli*	**FW** CTGGCGTAATAGCGAAGAGG **RV** GGATTGACCGTAATGGGATATG	228bp	Ram & Shanker, 2005
ST	ETEC	**FW** TTTCCCCTCTTTTAGTCAGTCAACTG **RV** GGCAGGATTACAACAAAGTTCACA	159bp	Pass et al., 2000
LT	ETEC	**FW** GGCGACAGATTATACCGTGC **RV** CGGTCTCTATATTCCCTGTT	440bp	Lopez-Saucedo et al., 2003
Eagg	EAEC	**FW** AGACTCTGGCGAAAGACTGTATC **RV** ATGGCTGTCTGTAATAGATGAGAAC	193bp	Pass et al., 2000

5.2.5 Culture independent microbial diversity

Details of investigation are provided in Chapters 2.

5.2.6 Enterovirus and bacteriophages

Details of investigation are provided in Chapters 2.

5.2.7 Cytotoxicity of culturable bacteria

HuTu-80 cells were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) (Sigma D2902) with a pH of 7.1 and supplemented with 10% $(^{V}/_{V})$ foetal bovine serum (FBS) (Thermo Scientific Hyclone) and 0.08 N $NaHCO_3$. The cells were grown in tissue culture dishes (90 mm x 20 mm) (Tooltech Pty) in a humidified atmosphere at 37°C and 5% CO_2 with media changes every 2-3 days (Reidling et al., 2006). Cells were passaged when confluent.

One litre of water was collected from the various sources (taps, dam, river, sewage wastewater effluent, boreholes). Water samples were filtered through 0.22 µm filters. Unfiltered and filtered water were used to make-up the media for the cells. De-ionised water was used as a control.

The cells were exposed to the media that contained the various filtered and unfiltered water. After 24 hour of exposure, the cell debris was removed and the media containing the exudates were used to expose freshly prepared cells. After exposure these cells that were exposed to the excudates were then analysed by the MTT test according to Vistejnova et al. (2009). Analysis was conducted after 6 and again after 12 hours.

An internal control (total carbon) for each of the water types was determined. The TC content was determined by the Eco-Analytica Laboratory (Potchefstroom, South Africa) on a LECO TruSpec CN analyser with an elemental infra-red detector.

Appropriate statistics using SPSS software (SPSS, 2011) were used to interpret the results. Sample size dictated that non-parametric tests should be performed. *p*-values less than 0.05 were considered statistically significant. In some cases where *p*-values were greater than 0.05, effect sizes were determined. Effect sizes indicate the practical significance and were determined according to Ellis and Steyn (2003).

5.3 Results and Discussion

5.3.1 Physico-chemical parameters

Physico-chemical quality of surface water was generally within TWQR for recreation as well as agricultural applications (Chapter 2). In some cases the electrical conductivity was very high. This however, does not pose human health risks. The scenario with groundwater was similar except for very high nitrate levels in some cases for the groundwater that were used for drinking purposes.

Table 37: Summary of percentage boreholes that exceeded TWQR values as well as those exceeding levels that are most likely to cause methaemoglobinemia.

	2009	2010
% Boreholes exceeding domestic TWQR (6 mg/ℓ)	67.0	82.0
% Boreholes exceeding definite methaemoglobinemia causing levels (20 mg/ℓ)	40.8	42.1

High nitrate levels were measured in borehole water during both the 2009 and 2010 sampling periods. Table 37 indicates that 67% of the 2009 samples had nitrate levels that exceeded the TWQR for domestic use (6 mg/ℓ NO_3-N). In 2010, 82% of exceeded this value. This observation is similar to the results of Al-Khatib & Arafat (2009). These authors tested 111 groundwater samples and found that 84.7% were non-compliant with WHO standards for nitrates (Al-Khatib & Arafat, 2009).

In Table 37 it is shown that 40.8% of boreholes sampled in the present study in 2009 and 42.1% of the 2010 boreholes exceeded the nitrate limit of 20 mg/ℓ above which methaemoglobinemia may occur (Craun et al., 1981; Sadeq et al., 2008; Super et al., 1981). Only one of the boreholes from the 2010 sampling period had an exceptionally high nitrate measurement. Nitrate levels as high as 454.5 mg/ℓ NO_3-N were measured in 2009 and 148 mg/ℓ NO_3-N in 2010. Darwish et al. (2011) measured the nitrate concentration of 21 wells in a cultivated area of Lebanon-East. The authors reported that 12 (more than 50%) wells had nitrate concentrations exceeding 200 mg/ℓ. Extremely high nitrate levels in water sources of rural communities had thus been reported elsewhere.

Groundwater nitrate levels may be impacted on by land use practices that require nitrogen application. Remnant fertiliser plays a major role in contributing to the high nitrates levels (van der Voet et al., 1996). The NWP is known for its Agricultural practices that use large quantities of fertilisers (Kalule-Sabiti & Heath, 2008). This might have contributed to the high concentrations of NO_3-N measured in this study. Other potential contributors to the high nitrates levels may be faecal pollution or the geology of the region (Kalule-Sabiti & Heath, 2008). Only by careful designed experiments can one determine which factor is the major contributor in a particular area. Another impact that also requires a careful experimental approach is epidemiological studies of the consequences of the high nitrate levels.

5.3.2 Culturable bacteriological parameters

Species from the genus *Enterobacter* was the most frequently detected amongst the 98 faecal coliforms isolated and purified in this study. *Enterobacter cloacae* and *Enterobacter aerogenes* were the two most common species identified. These species are responsible for various nosocomial diseases as well as community acquired infections (Fraser, 2010). Such infections include urinary tract, soft tissue and wound infections *Enterobacter* spp. possesses ß-lactamases that allow these bacteria to develop resistance to third-generation cephalosporins and penicillins (Fraser, 2010).

Fifteen percent of the faecal coliforms were identified as *Klebsiella pneumonia*. The gastro-intestinal tract of animals serves as a reservoir for pathogenic Klebsiella. The latter may cause urinary tract infections, pneumonia, septicaemias and soft tissue infections (Podschun & Ullmann, 1998). Finding this bacterium in the groundwater that is used for drinking and other domestic purposes is cause for concern.

The detection of *E. coli* in many of the surface and groundwater sources may be an indication of recent faecal contamination in those sources (Edhberg et al. 2000; Kaper, 2005). The survival and potential replication of *E. coli* strains in water, on algae and in soils is known (Ishii and Sadowsky, 2008). Therefore the possibility exists that *E. coli* detected in surface and groundwater may be soil-naturalised *E. coli* strains. These may have attenuated in soil and infiltrated to the water table. Detection of *E. coli* in surface and groundwater may thus not necessarily indicate recent faecal pollution.

E. coli may become pathogenic after infection (co-infection) of mucosal surfaces (Nataro & Kaper, 1998). *E. coli* infections occur mostly in the debilitated or immune-suppressed host. It may also occur when gastrointestinal barriers are violated. Clinical syndromes which result from an infection with *E. coli* are sepsis, meningitis, urinary tract infection or enteric diarrheal diseases (Nataro & Kaper, 1998). These are not as a result of known pathogenic *E. coli* strains. Thus whenever studies are conducted and the samples are positive for *E. coli* then tests should be conducted to determine if these are not one of the pathogenic strains.

In 49% of the 114 boreholes sampled, *Pseudomonas aeruginosa* were detected in conjunction with either faecal coliforms or enterococci (Chapter 3). A total of 13 boreholes (11%) were positive for *P. aeruginosa* where no faecal coliforms were detected. Therefore it could be presumed that the pseudomonas from the latter 13 boreholes may not have originated from faecal contamination. In the present study *P. auruginosa* detected could have been due to water infiltrating the water table. The presumptive *P. aeruginosa* detected in the 33 boreholes that also had faecal indicators present, could potentially be associated with faecal contamination. Wheater et al. (1980) found significant numbers of *P. auruginosa* in human faecal specimens, but to a lesser extent in animal faeces. According to Hardalo and Edberg (1997) it is not practical to remove this bacterium from food and water sources. This is due to its ubiquitous nature as well as the small margin of risk it poses to healthy human subjects. A review by Mena and Gerba (2009) confirmed that the risk from ingesting *P. aeruginosa* in drinking water is low. This risk is slightly elevated if an individual is on an antibiotics treatment course that suppresses the natural intestinal flora (Mena & Gerba, 2009). The greatest health risk for healthy individuals in contact with water contaminated with *P. aeruginosa* is skin exposure in hot tubs and lung exposure from inhaling aerosols (Mena & Gerba, 2009). Immune-comprised individuals that drink water and/or bath in water contaminated with this species are at risk of infection of wounds, ears, urinary tract and respiratory organs (Lester & Birkett, 1999).

It can be assumed that the presumptive *P. aeruginosa* isolates detected in the present study may not pose a risk to water users that are healthy. Poor immune-comprised individuals often inhabit rural communities. These individuals are at risk and should therefore not be exposed to these opportunistic pathogens.

Staphylococcus aureus was detected in 7% of the 76 boreholes sampled in 2009 (Chapter 3). LeChevallier and Seidler (1980) and Lamka et al. (1980) detected *S. aureus* in small percentage of samples from rural drinking water supplies they sampled (<10%). Both these authors found no correlation between the simultaneous presence of coliform indicator species and staphylococci. However, the authors (LeChevallier & Seidler, 1980; Lamka et al., 1980) reported that high heterotrophic plate counts could be linked to the detection of *S. aureus*. All of the boreholes in the present study that had *S. aureus* present also had total and faecal coliform bacteria present.

S. aureus is a potential pathogen that may cause pneumonia and *Staphylococcus aureus* bacteremia in immune-comprised individuals (Kaye et al., 1990; Lowly, 1998). *S. aureus* may also infect open wounds and could result in toxic shock syndrome (Dryden, 2009). The presence of this bacterium in water sources is thus cause for concern and it is particularly immune compromised individuals that are at greater risk.

5.3.3 Antibiotic resistance and haemolytic patterns

a) Antibiotic resistance of isolated faecal coliforms in groundwater

Another aspect that is of concern is antibiotic resistance amongst bacteria occurring in water sources. Infections caused by these organisms may be difficult to treat using general antibiotics of therapeutic importance. Tables 39 and 40 present the antibiotic resistance data for faecal coliforms.

Table 38: Percentage resistance (R), intermediate resistance (IR) and susceptibility (S) of selected faecal coliform isolated from groundwater during the 2009 sampling period.

Sample area **n FC isolates**		**AMOX**	**AMP**	**CEP**	**KAN**	**STREP**	**TM**	**OXY-TET**	**CIP**	**CHL**
1	% R	39	39	13	0	0	17	30	0	0
(A_{09}+B_{09}+C_{09})	% IR	39	22	31	0	0	30	61	0	0
n = 23	% S	22	39	56	100	100	53	9	100	100
MAR: 0.150	Most prevalent MAR phenotype: AMOX-AMP-OXYTET									
2	% R	57	38	38	0	0	23	43	0	10
(D_{09}+E_{09}+F_{09})	% IR	29	14	33	0	0	48	57	0	5
n = 21	% S	14	48	29	100	100	29	14	100	85
MAR: 0.233	Most prevalent MAR phenotype: AMOX-AMP-CEP-TM-OXYTET									
3 (G_{09}+ H_{09}+I_{09}	% R	84	49	47	0	0	5	28	0	0
+J_{09})	% IR	8	18	20	5	0	53	49	0	8
n = 40	% S	8	33	33	95	100	42	23	100	92

MAR: 0.239	Most prevalent MAR phenotype: AMOX-AMP-CEP-OXYTET									
Total FC isolates n = 85	% R	54	41	40	0	0	11	30	0	1
	% IR	21	17	22	4	0	37	53	0	4
	% S	25	42	38	96	100	52	17	100	95
Total MAR index for 2009 sampling period: 0.213 Most Prevalent MAR phenotype: AMOX-AMP-CEP-OXYTET										

A total 85 faecal coliform isolates were tested against 9 antibiotics (Table 38). A large percentage of these (40% to 54%) were resistant to β-lactam antibiotics (AMOX, AMP and CEP). Percentage of isolates that were resistant to OXY-TET was also high (30%). The high percentage of the isolates that had intermediate resistance (53%) to OXY-TET is alarming. More than half (52%) of the isolates were susceptible to TM, however, 37% of the isolates had intermediate resistance to this antibiotic. All isolates tested were susceptible to STREP and CIP.

The MAR index for all 85 isolates tested was 0.213. A value of 1 would indicate that all isolates were resistant to the 9 antibiotics used, whereas 0 would indicate that none of the isolates were resistant to any of the antibiotics used. Thus, on average, each isolate was resistant to just more than two of the antibiotics tested.

Table 39: Percentage resistance (R), intermediate resistance (IR) and susceptibility (S) of selected faecal coliform isolated from groundwater during the 2010 sampling period.

Sample area **n FC isolates**		**AMOX**	**AMP**	**CEP**	**KAN**	**STREP**	**TM**	**OXY-TET**	**CIP**	**CHL**
1 (A_{10}&B+C_{10}) n = 24	% R	21	21	46	0	0	4	38	0	0
	% IR	54	58	41	13	0	63	45	0	0
	% S	25	21	13	87	100	33	17	100	100
MAR: 0.176	Most Prevalent MAR phenotype: AMOX-AMP-CEP-OXYTET									
2 (D_{10} + F_{10}) n = 16	% R	6	0	44	0	0	6	6	0	0
	% IR	44	50	12	19	0	13	69	0	13
	% S	50	50	44	81	100	81	25	100	87
MAR: 0.074	Most Prevalent MAR phenotype: CEP									
3 (G_{10}+H_{10}+J_{10}) n = 20	% R	35	25	35	0	0	15	20	0	10
	% IR	35	45	55	80	0	20	7 0	0	5
	% S	30	30	10	20	100	65	10	100	85

MAR: 0.155	Most Prevalent MAR phenotype: AMOX-CEP-OXYTET									
Total FC	% R	21	15	42	0	0	8	22	0	3
isolates	% IR	44	51	36	37	0	32	61	0	6
n = 60	% S	35	34	22	63	100	60	17	100	91
Total MAR index for 2010 sampling period: 0.126										
Most Prevalent MAR phenotype: AMOX-CEP-TET										

The following observations can be made from Table 39: Among the 60 faecal coliform isolates tested in 2010 more between 15 and 42% were resistant to β-lactam antibiotics (AMOX, CEP and OXY-TET). This is less than the 2009 levels. Of concern was the fact that 42% of the isolates were resistant to CEP. All the isolates tested were completely susceptible to CIP and STREP. This observation was similar for the 2009 sampling period (Table 39). In 2010, sample area 1 (A_{10}&B+C_{10}) had the highest MAR index value (0.176), followed by area 3 (G_{10}+H_{10}+J_{10}; 0.155) and 2 (D_{10} + F_{10}; 0.074). The 2009 results were different and the MAR indices were higher. In this case area 3 (G_{09}+H_{09}+I_{09}+J_{09}; 0.239) followed area 2 (D_{09}+E_{09}+F_{09}; 0.233) had the highest MAR index values and sample area 1 (A_{09}+B_{09}+C_{09}; 0.15) the lowest (Table 39).

The average MAR index value for the isolates of the 2010 sample period were 0.126, with the most prevalent MAR phenotype being AMOX-CEP-OXY-TET. The most prevalent MAR in 2009 was AMOX-AMP-CEP-OXY-TET. Resistance to β-lactam and tetracycline antibiotics was thus generally observed among faecal coliforms isolated from groundwater sources. An aspect that is of concern is that a high percentage of the isolates had intermediate resistance to several antibiotics. This could indicate that the resistance to these antibiotics is emerging and could become a problem in the future.

b) Antibiotic resistance and haemolysis of isolated enterocci

A further concern is the number of multiple antibiotic resistant enterococci that were isolated from the water sources. Resistance to β-lactam antibiotics were generally observed at most of the sites (Tables 41 and 42). In addition to this, several of the enterococci that were isolated from surface water sources during 2010 and 2011 were also resistant to trimetroprim, neomycin and vancomycin. Many of these isolates were also haemolytic. This implies that these bacteria produce enzymes that are could render virulence capabilities. Detailed virulence profiles were not determined for the isolates from the present study.

Table 40: Haemolysis patterns and major multiple antibiotic resistant phenotypes for 80 enterococci isolated from the 5 surface water systems in 2010.

River system	N	Sample site	Haemolysis: α,β,γ	MAR phenotype
Mooi river	3	Klerkskraal Dam	α,α,α	AMP-PG-CIP
	3	Muiskraal	α,α,α	AMP-NE-TM
	2	Around the World Bridge	α,α	AMP-PG-NE-TM
	3	Thabo Mbeki Drive	α, α, β	-
	3	Trimpark North Bridge	α, β, β	-
	3	Pedestrian Bridge	α, β, β	PG-NE-TM-OT
	3	Viljoen road Bridge	α, α, α	-
Lower Harts river	4	Harts-Pampierstad Bridge	β, α , β, α	-
	4	Dam 8	α, α, α, β	AMP-PG-NE-TM
Barberspan	4	Town-Harts	γ, γ, γ, γ	AMOX-AMP-VAN
	4	Inflow	γ, γ, γ, γ	AMOX-AMP-NE-VAN
	4	Hotel	γ, γ, γ, γ	AMOX-PG-NE-VAN
	4	Outflow	γ, γ, γ,γ	AMOX-AMP-PG-CIP-NE-VAN
Schoonspruit river	4	Bodenstein	β, β, β, α	PG-NE-CIP-VAN
	4	Brakspruit	β, β, β, α	PG-NE-CIP-VAN
	4	Voortrekker	α, α, β, α	PG-CIP-NE-TM
	4	Orkney	β, β, β, α	CIP-NE-VAN AMOX-PG-TM
Vaal river	4	Bloemhof	α, α, α, β	CIP-NE-VAN-TM
	4	Christiana	α, α, α, α	AMP-PG-NE-VAN-TM
	4	Windsorton	α, β, β, β	AMP-PG-NE-VAN-CIP-TM
	4	Barkley-Wes	β, α, α, β	-
	4	Schmidtsdrift	β, β, α, α	-

α – alpha, β-beta, γ –gamma haemolysis. Multiple antibiotic resistant (MAR) phenotypes were determined for isolates showing resistance to three or more antibiotics per site. AMP-Ampicillin, AMOX-Amoxicillin, PG-Penicillin G, NE-Neomycin, STREP-Streptomycin, VAN-Vancomycin, CHLOR-Chloramphenicol, CIP-Ciprofloxacin, OT-Oxy-tetracycline, TM-Trimethoprim.

Enterococci isolates obtained in 2010 were tested for susceptibility to 10 antibiotics. Among the isolates 55 (68.8%) were resistant to Penicillin G (10 µg), 52 (65%) to Neomycin (30 µg) and 40 (50%) to Vancomycin (30 µg). Seventy eight percent of these isolates were susceptible to Amoxicillin (10 µg) while 79 (98.8%) were susceptible to Streptomycin (100 µg). There were thus varying β-lactam resistance phenotypes observed among the isolates. Not all the isolates that were resistant to Penicillin G were resistant to Amoxicillin or Ampicillin and vice versa. Forty seven percent of the enterococci isolates (Table 38) caused α-haemolysis, 26 (32.5%) β-haemolysis while 16 (20.0%) were γ haemolytic. The highest number of enterococci causing β-haemolysis (12.5%) was isolated from the Schoonspruit River. All the γ non-haemolysis producing enterococci were obtained from Barberspan. The antibiotic susceptibility and haemolysis data of 2011 are presented in Table 41.

Table 41: Haemolysis patterns and major multiple antibiotic resistant phenotypes for enterococci isolated from the 5 surface water systems in 2011.

River system	N	Sample site	Haemolysis: α,β,γ	MAR phenotype
Lower Harts river	3	Delareyville-Harts	α, α, α	STREP-VAN-CHLOR-CIP-OT-TM
	3	Schweizer-Reneke	α, α, α	AMOX-VAN-CIP
	3	Taung	α, α, α	NE-STREP-CIP
	3	Harts-pamp Dam	α, α, α	PG-NE-OT
	2	Spitskop Dam	β, β	AMOX-AMP-PG-VAN-CHLOR-CIP
Upper Harts river	3	Lichtenburg	α, α, α	AMOX-NE-CIP
	3	Biesiesvlei	α, α, α	AMOX-NE-CIP
	3	Sannieshof	α, α, α	NE-VAN-TM
Barberspan	3	Town-Harts	α, α, α	PG-VAN-CIP
	3	Inflow	α, α, α	AMOX -AMP-NE
	3	Hotel	α, α, α	-
	3	Outflow	α, α, α	AMP-PG-OT
Schoonspruit river	3	Bodenstein	β, α, α	-
	2	Brakspruit	α, α	AMOX-PG-NE-STREP-CHLOR-CIP
	3	Voortrekker	α, α, α	NE-STREP-CIP
	3	Orkney	α, α, β	NE-STREP-CHLOR
Vaal river	3	Bloemhof	α, α, α	-
	3	Christiana	α, α, α	AMOX-AMP-TM
	2	Windsorton	α, α	PG-NE-STREP-VAN-TM
	3	Barkley-Wes	α, α, α	AMOX -AMP-PG-NE-CIP-OT
	2	Schmidtsdrift	α, α	PG-NE-CIP

α- alpha, β-beta, γ-gamma haemolysis. Multiple antibiotic resistant (MAR) pattern/phenotypes were determined for isolates showing resistance to three or more antibiotics per site. AMP-Ampicillin, AMOX-Amoxicillin, PG-Penicillin G, NE-Neomycin, STREP-Streptomycin, VAN-Vancomycin, CHLOR-Chloramphenicol, CIP-Ciprofloxacin, OT-Oxy-tetracycline, TM-Trimethoprim

Twenty four (40.7%) enterococci isolated in 2011 were resistant to Amoxicillin (10 µg), 32 (54.2%) to Penicillin G (10 µg), 40 (67.8%) to Neomycin (30 µg) and 33 (55.9%) to Ciprofloxacin (5 µg). Sixteen (27.1%) were susceptible to Chloramphenicol (30 µg) while 26 (44.1%) to Trimethroprim (2.5 µg). All 4 β-haemolysis positive isolates were resistant to 3 or more antibiotics including amongst others, Vancomycin (30 µg) and Penicillin G (10 µg). Among the 2011 enterococci isolates, α-haemolysis was the major haemolysis phenotype observed. Only 4 (6.8%) displayed β-haemolysis. This is less than the proportion among the 2010 isolates. Unlike the 2010 isolates where all the isolates displayed γ-hemolysis, 21 of the Barberspan 2011 isolates displayed α-haemolysis. There was no γ-hemolysis amongst the isolates.

Haemolysis activity is a virulence factor and is based on the secretion of haemolysin by the bacterium which is involved in the lysis of erythrocytes. Positive (β- and α-haemolysis) results indicate potential pathogenicity. This is a first tier screening for pathogenicity. To confirm this feature more extracellular enzyme production of bacteria needs to be conducted. Antibiotic resistance of bacterial isolates may not have direct human health impacts. However, antibiotic resistance become a problem during therapeutic interventions of bacterial infections. The results demonstrated that Gram negative as well as Gram positive bacteria from the North West Province may be resistant to β-lactam antibiotics. Among the Gram negative, bacteria Tetracycline resistance may also be common. The Gram positive bacteria that are resistant to trimetroprim, neomycin and vancomycin resistance may also be a challenge particularly if one considers that some of the β-haemolysis positive enterococci isolated in this study were resistant to these (3 and more) antibiotics.

5.3.4 Pathogenic *E. coli*

The purified *E. coli* strains that were isolated from environmental sources were incubated in Luria broth and incubated at 37°C for 18 hours. Genomic and plasmid DNA were isolated using NucleoSpin® Tissue Kit (Separations, US) and NucleoSpin® Plasmid DNA Purification Kit (Separations, US), respectively. Examples of results after multiplex PCR is provided in Figure 34.

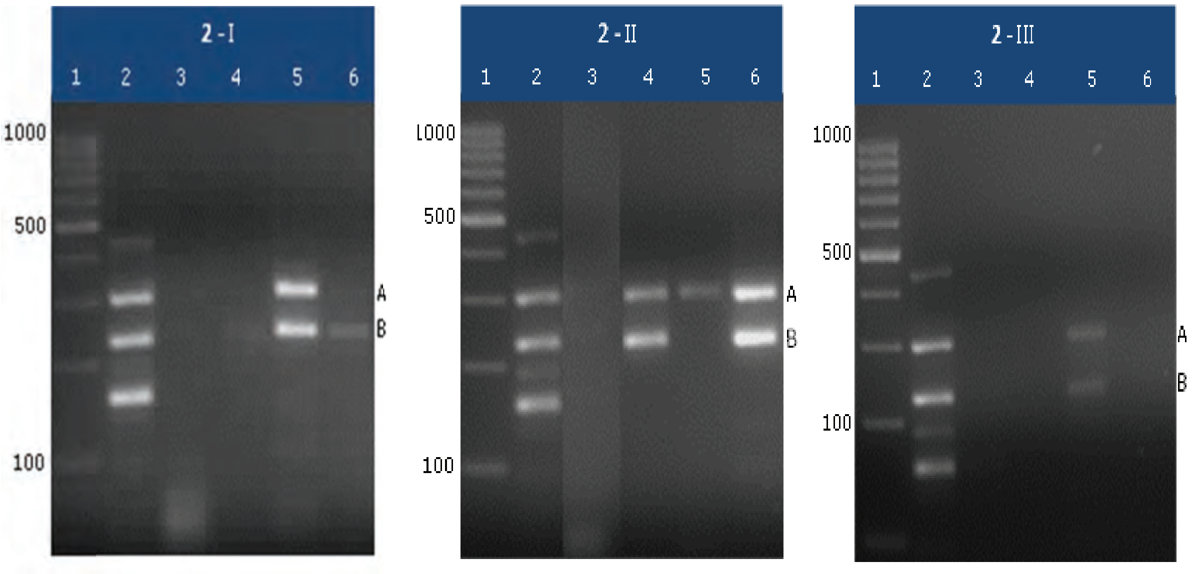


Figure 34: Three 2.5% $(^{W}/_{V})$ agarose gels showing environmental *E. coli* PCR products of; (I) genomic and plasmid, (II) genomic, and (III) plasmid templates. Lane 1 contains 100bp molecular weight marker (O'GeneRuler, Fermentas Life Sciences, US), lane 2 and 3 the positive and negative controls, respectively. Lane 4 to 6 contains PCR products of genomic and/or plasmid DNA of environmental isolated *E. coli*.

A summary of the PCR results are provided in Table 42. The results demonstrated that all genomic DNA samples tested positive for the *mdh* gene. This was expected for one of the housekeeping genes of *E. coli*. However, 90.5% of the same samples tested positive for the *lac*Z genes, another housekeeping genes.

Table 42: Detection percentage of genes in environmental *E. coli.*

	Template	mdh(%)	lacZ(%)	Eagg(%)	ST(%)	LT(%)
I.	Genomic + plasmids DNA	42.8	47.6	0	0	0
II.	Genomic DNA	100	90.5	0	4.8	0
III.	Plasmid DNA	38.1	38.1	0	0	0

A large proportion of the plasmid DNA also tested positive for both *mdh* and *lac*Z genes. When the same genomic and plasmids DNA samples were combined, less than 50% of the samples were positive for the *mdh* and *lac*Z genes. The plasmid DNA thus appeared to have had an inhibitory effect on the PCR of *mdh* and *lac*Z genes. Among the environmental water samples 4.8% tested positive for the shigatoxin gene. This result demonstrated that potential pathogenic *E. coli* species are present in water sources of the North West Province. The low frequency is consistent with Ateba and Mbewe (2011) who also detected low levels of pathogenic *E. coli* in water samples of the same province.

5.3.5 Culturable independent microbial diversity

HTS analysis showed that DNA of opportunistic pathogens were present in low densities (few sequences detected) at Vaal Barrage, Parys and Scandinawieë Drift included *Roseomonas* sp., *Ralstonia* sp., *Serratia* sp. and *Stenotrophomonas* sp. Human infections and diseases are often associated with these opportunistic pathogens (Berg et al., 2005; Mahlen, 2011) and have caused mortalities in immunocompromised individuals (Fergie et al., 1994; Paez and Costa, 2008). The relative abundance of these pathogens was low. In healthy individuals many of these species will not have a direct health impact. However, their impact on immune-compromised is undetermined (WHO, 2008).

5.3.6 Enterovirus and bacteriophages

Viral genomes were frequently detected in treated sewage effluent of three WWTPs that discharged into rivers in the North West Province. Two of these plants could not effectively remove the virus particles and levels of up to 2.54×10^5. Infectivity of the detected viruses could not be confirmed. However, previous studies have established a link between qPCR results and data from cell culture methods. The frequent detection of viral genome in sewage effluents suggests that treated sewage may represent a source of environmental contamination with potentially infectious enteroviruses.

5.3.7 Cytotoxicity of culturable bacteria

Water from different sources was subjected to a MTT based assay to establish the ability of this assay to differentiate between water of varying microbial quality. For this assay to work effectively cells had to be exposed to the test water first. Microbial exudates from this first step are harvested and added to sterile media. A new set of cells is then exposed to this combined media-exudate-complex. The difference in cell viability is then measured by an MTT colorimetric assay. Viability assay results in this preliminary study were statistically significant for all types of water except for borehole water (Table 43). Differences in viabilities indicated that treated sewage, dam, river and both types of tap water decreased cell viability over a 6 and 12 hour period. The effect was particularly severe for the treated sewage, dam and river water where the entire population of exposed cells was destroyed after a 6 h exposure period.

In this study a cytotoxicity index was developed as a tool for use when prioritising water sources for treatment. Essentially, the cytotoxicity index is an indication of how many times a specific water samples is less toxic than the negative control (no cells found). The cytotoxic index (C*i*) varies between 0 and 1 where 1 is the maximum toxicity. For example, treated sewage was as toxic as the negative control (Table 40) but the filtered tap water after 12 h was only 0.2 times as toxic. The C*i* was calculated after adjusted % viability was inverted to % cytotoxicity by subtracting from 100.

$$\mathrm{C}i = \frac{\%\ \text{cytotoxicity of water sample}}{\%\ \text{cytotoxicity of negative control}}$$

Table 43: The cytotoxicity index (C*i*) for each water sample at two exposure periods.

Water type	Time (h)	Adj. % viability	% cytotoxicity	C*i*
Positive control	6	100	0	0
	12	100	0	0
Borehole	6	82	18	0.2
	12	79	28	0.2
Treated sewage	6	0	100	1
	12	0	100	1
Dam	6	0	100	1
	12	0	100	1
river	6	0	100	1
	12	0	100	1
Unfiltered tap	6	71	29	0.3
	12	62	39	0.4
Filtered tap	6	95	5	0.1
	12	78	3	0.2
Negative control	6	0	100	1
	12	0	100	1

The C*i* for treated sewage, dam and river water indicates that those water samples were as toxic to the intestinal human cells as the negative control. The filtered tap water had the lowest C*i* after 6 h exposure, but toxicity was higher after 12 h exposure. The unfiltered tap water had a higher C*i* than the filtered tap water for both exposure periods. This was even higher than the borehole water.

This test is being further developed. The development includes the use of an xCELLigence RTCA system (Roche, Germany) to determine cell viability after exposure to the different water types. This system measures electrical impedance across microelectrodes integrated to the bottom of these E-plates where the cells attach and can provide information on the cell numbers, morpholgy and viability in real-time.

5.4 Conclusion

The major questions of this chapter are whether groundwater of the North West Province is safe to use for domestic purposes and surface water for recreation and full contact activities.

- From the results it is evident that the groundwater in several areas is subjected to various contaminants, particularly nitrates and various bacterial species. *E. coli* and enterococci were isolated from surface and groundwater sources, indicating faecal pollution of these water sources. Surface water is not recommended for direct use. It should be treated before being provided as potable water.
- Several bacterial species were also isolated from several of the groundwater sources using selective culture media. Culture independent methods (PCR-DGGE and HTS) supported groundwater results for the presence of faecal indicator bacteria. Additional pathogenic and potential pathogenic species were also identified by these methods. These species pose a health risk to individuals that are exposed to such water sources.
- Several yeast species that are human pathogens/opportunistic pathogens were isolated from surface water sources. This demonstrates that the water poses a potential health risk during exposure when participating in full contact recreational activities.
- Bacteriophage analysis supported the bacteriological observation, i.e. that surface and groundwater sources are in many cases contaminated with faecal matter. Enterovirus (qPCR) analysis at WWTPs demonstrated that the possibility exists that enteric viruses may be present in the surface water sources. This may be due to poorly or untreated sewage being discharged into such water sources.

- Environmental *Escherichia coli* was successfully identified by multiplex PCR when using the *mdh* and *lacZ* and genomic DNA as template. Almost 5% of the *E. coli* strains tested were enterotoxigenic and the remainder was neither ETEC nor EAEC. This part of the study demonstrated that pathogenic *E. coli* (although at low frequency) were present among environmental *E. coli* isolated from surface water of the North West Province. This is a real cause for concern since some of the pathogenic strains could cause disease at very low dosage (10-100 cells/intake).

- From the results of the study it is evident that faecal pollution of the water sources is a major problem that could be addressed by improving the quality of sewage and preventing sewage spills. This impact that faecal pollution has on surface water is mainly with respect to direct contact such as recreation and religious practices.

- The perception that groundwater is free from contamination is refuted by results presented here and elsewhere. It is thus important that groundwater in this province be tested and appropriately treated before use. The low compliance of the boreholes tested to national standards indicates that there are health risks if this water is used for domestic purposes without any prior treatment.

- A cytotoxicity test developed in this study demonstrated that it could act as a first tier test in evaluating the potential of water for negative health impacts. Such a method needs to be further refined and could be a useful system for a widespread application to water in the North West Province and elsewhere. All samples showing cytotoxicity could then be further investigated for the actual causes.

CHAPTER 6

SOCIAL CONTEXT OF WATER

6.1 Introduction

Water has been and is still used by all humans to process and prepare food, for washing, for its heat exchange properties, for transportation and even for recreational purposes, to name just a few. Humans also have a special connection and affinity for water, something that often manifests in their cultural, social and political orientations (Biswas, 2004). However, humans not only use it and need it, but they have a dramatic effect on water and are often responsible for wasting or polluting it (Gleick, 2003). The latter may have a negative effect on the health and survival of other life forms. Scientists have, therefore, focused their research attention on aspects such as water quality, pollution and the associated risks to human health.

Seeing that water is so important to all humans, the need to understand how they interact with water has become apparent. The purpose of this study was, therefore, to explore how and for what reasons people in the North West Province (NWP) of South Africa interact with water, especially on a community level.

6.1.1 Research questions

Three research questions guided this study:

Research question 1: How and for what reasons do communities in the North West Province interact with water? (Qualitative question)

Research question 2: Is there a difference between the interactions of communities with water in the Dr. Kenneth Kaunda District (high rainfall where water is perceived to be more abundant) compared to the Bophirima District (with a lower rainfall where water is perceived to be less abundant)? (Quantitative question)

Research question 3: To what extent do the quantitative results confirm the qualitative results? (Mixed-methods question)

The aim of the second question is to quantify and verify the results in order to identify the most important aspects for future research and interventions in the province. Recommendations for future research and interventions will, therefore, be based on the quantitative results of the study.

6.2 Experimental procedures

6.2.1 Sampling area

The NWP has four district municipalities, namely Dr. Kenneth Kaunda (Southern District), Bophirima, Dr. Ngaka Modiri Molema (Central District) and Bojanala (e.g. 21 local municipalities). Figure 34 illustrates the municipal areas and locations of the six communities that formed part of this study.

Figure 35: Map of study area.

Six communities, four from the Dr. Kenneth Kaunda District (Southern District) and two from the Bophirima District, were included in the study (Figure 34). Magopa is a small rural village, situated to the north of Ventersdorp. Tigane is the former township area of the town Hartebeesfontein, situated just to the north of it on the R503, the road that leads to Ottosdal. Kgakala is the former township area of the town Leeudoringstad, situated north-east of the town between the R502 and the R504. Lebaleng is the former township area of the town Maquassi, situated just to the south of the town on the R502. Setshing and Tlbabologang are both large rural villages situated near Taung. Residents in all six communities speak a combination of Setswana, Afrikaans and English (Website: www.researchlogistics.co.za).

A sequential exploratory mixed-methods design was used. This kind of design enabled the researchers to use the qualitative findings to guide the development and implementation of a quantitative instrument (Creswell and Piano Clark, 2007). A mixed-methods approach includes the use of both qualitative and quantitative research methods to collect, analyse and interpret the research data (Figure 2).

Figure 36: Diagrammatic presentation of research design.

The benefits of using a mixed-method approach are that it provides a more detailed understanding of any research topic compared to a single approach. In short, it allows ‘multiple angles’ that provide different ‘pictures’, and it is more naturalistic in the sense that ‘it is more intuitive’ by basically mirroring ‘real life’ (Creswell and Piano Clark, 2007).

6.2.2 Participants (samples)

During the qualitative phase of the study, 25 participants were selected by means of purposeful sampling. Purposeful sampling refers to the selection of specific individuals who could potentially give an informed opinion about a specific research topic and who are familiar with the unique historical, socio-cultural and political context in which the research is embedded. The final group of participants included a selection of community activists, traditional leaders (tribal authorities), political leaders (municipal ward councillors and committee members), religious leaders (pastors), educational leaders (school principals and vice-principals), youth leaders and health practitioners (i.e. social workers and nurses at primary healthcare facilities).

During the quantitative phase, a sample of 1000 participants was proportionately divided between the six different communities (according to their size) and systematically drawn to further explore their interactions with water. Their basic socio-demographic profile is as follows:

a) Age

In the total group, 29% of the participants were between 41 and 50 while the second largest group of 25%, was between the ages of 31 and 40. This indicates good age distribution since the opinions of older people, who are usually more familiar with the cultural, social and political orientation in their particular community, made up a large percentage of the total group. Of similar importance was the fact that a fair portion of the group consisted of younger people (26-30 = 15%; 18-25 = 8%). The rest of the group consisted of individuals between the ages of 51-60 years (14%) and those above 61 years of age (9%).

b) Gender

In the total group, there were 49% male and 51% female participants.

c) Marital status

The largest proportion of the participants appear to be unmarried (35%) or are living together (24%). Eleven percent were widowed and another 11% were married and living with their spouse. Ten percent were, divorced and 9% married but not living with their spouse.

d) Nationality

The majority of the participants were South African nationals (88.3%).

e) Level of education

The level of education varied, with the most participants (44%) having completed their secondary education, followed by having some secondary education (29%). Those with only primary education constituted 15%) and those with no education at all consisted of 11%. A further 11% of the total group had some kind of tertiary education.

f) Occupation

Most of the participants were self-employed (30%) or had a part-time job (26%). Twenty six percent had full-time employment and only 16.7% were unemployed.

g) Income

Income levels were calculated for people who live in the same household and share at least three meals per week with the rest of the household. For the total group, most participants (32%) had a monthly household income ranging between R2001.00 to R5000.00. A large proportion (29%) had an income of R1001.00 to R2000.00. Smaller proportions of the participants had a monthly income of R5001.00 to R10000.00 (16%) or more than

R10000.00 (6%). Less than 9% of the participants had income of less than R1000.00 per month.

h) Number of dependants

Most of the participants (46%) had one or two dependants. Twenty two percent had, three dependants, 18% had no dependants and a further 18% had four or more.

6.2.3 Procedure

A list with the contact details for ward councillors in each municipal area was obtained during the qualitative phase from their respective local municipal offices. First contact with the participants was made by means of a general telephonic conversation. The aim of this conversation was to determine each participant's willingness to take part in the research, to inform him/her about the broad objectives of the study and to discuss the nature and duration of his/her participation, should the participant agree to take part in the research.

Meetings were then scheduled for face-to-face interviews and focus groups with participants (at a time and place most convenient to each of them) during which the details of the study were discussed before participants were asked to tell the researchers about their community and, more specifically, their interactions with water in their particular community.

During the quantitative phase, local community members were recruited as fieldworkers and trained in the questionnaire and on how to conduct a community survey. In proclaimed areas where street names and numbers are present, the sample was pre-planned (systematically conducted and proportionately allocated according to the number of households in each community). In unproclaimed areas, the number of households was first counted, the sample proportionately allocated (according to its size) and then systematically conducted.

6.2.4 Data gathering methods

Three data gathering methods (de Vos et al., 2007) were used during the qualitative phase, namely in-depth interviews with key informants, focus group discussions and observations. All verbal data (interviews) were tape-recorded and transcribed to ensure accuracy. To ensure validity in the qualitative findings, Guba's model of trustworthiness (Kreftin, 1991), consisting of four criteria (credibility, transferability, dependability and confirmability), was applied using strategies proposed by Shenton (2004). A research questionnaire (Appendix 2) was then developed and used in a survey of the communities.

In-depth interviews: Face-to-face interviews were conducted with each participant, because of their usefulness for gathering data in a person's own, verbalised words. A benefit of using interviews as a data gathering method is that it permits immediate follow-up questions that can be used for clarifications. They can therefore be regarded as a flexible mode of data collection that not only contributes to the trustworthiness of the data but the general credibility of the study. Open-ended questions were used to ask participants to share information about their community (interactions with water).

Focus group discussions: Small groups of between two and five participants were asked to express their opinions on a specific set of open-ended questions regarding the community in general, as well as their community's interactions with water. Focus groups are more cost- and time effective than individual interviews.

Observations: General non-participant observations/field notes were made in each community to supplement or support the data generated by interviews and to ensure that the results of the study were context specific.

During the quantitative phase (community survey), a consent form (Appendix 1), a page consisting of eight demographic questions and a semi-structured questionnaire were developed for use in the second phase of the research (Appendix 2). The demographic questionnaire was used to collect basic demographic/background information (age, gender, marital status, nationality, level of education, occupation, average household income per month, number of dependants in household).

6.2.5 Data analysis

Thematic content analysis was used to analyse the verbal data during the qualitative phase. Each transcribed text was first read and studied in detail to gain a broad overview of the data. Units of meaning, including sentences or paragraphs relating to the topic, were then identified in each of the texts and coded accordingly. This was followed by grouping related units of meaning under a descriptive theme. Each theme was then again carefully studied in detail to check that the original data truly supported the theme identified as well as to identify the units of meaning and links with other themes.

The statistical consultation services of the North-West University were used for the statistical analysis in this study. Descriptive statistics, including frequency tables, were calculated for all themes and subthemes in each community and also for the weighted population of the total group. Crosstabs and Cramer's V test was used to determine if there were any statistical or practical differences between the two districts. Statistical significant differences are indicated by $p < 0.05$, with 0.10 indicating a small difference, 0.30 a medium difference and 0.50 a large difference (that is practical significant). Cramer's V becomes significant at 0.25.

6.3 Qualitative results

Five different themes emerged in relation to the interactions of communities with water. Table 44 indicates these themes and the subthemes that emerged and gives verbatim examples to support each theme or subtheme.

Table 44: Overview of qualitative results.

Theme	Subtheme	Verbatim example
Perceptions about water	*Quality*	*"Here in our village water is scarce, but very good. We drink it. Our livestock drink it. No problem."*
	Availability	"*Water is a scarce resource and it must be conserved*"
	Management	*"Sometimes we have problems, but in general there is no problems"*
Beliefs/attitudes towards water	*Water should be free*	"*We all need water. Us humans and our livestock. It is government's responsibility"*
	Spiritual and cultural connections	*"Water is very important to our people (culture)"*
Sources of water		(most came up with a list)
Uses of water	*Physical (physiological) needs*	*"In our community we can fish for food and we drink the water every day"* *"We use it for everything, from washing to cooking and building"*
	Everyday household use *Recreational purposes*	*"The small children swim here (pointing to a small pond) after school"*
	Religious/spiritual and cultural purposes	(same as under beliefs)

Water management	*“Now we are lucky. In the past we used to walk far. Now we have a tap in the yard”*

6.3.1 Perceptions about water

Quality: The quality of water according to most participants refers to aspects such as the colour (cleanliness and brightness), smell and composition (if there are debris of plants or animal material) of their water. Perceptions of the quality of water in each community varied considerably from poor to very good or excellent. One of the participants captured the general opinion of the group very well when he said: “*Here in our village water is scarce, but very good. We drink it. Our livestock drink it. No problem”,* while a small group of participants in another community said: “*We don’t like the water here. It is not good*.”

Availability: In some of the communities in the Dr. Kenneth Kaunda District, participants often commented on the availability of water, including that it is freely available compared to communities in the Bophirima District where it was often said that “*water is a scarce resource and it must be conserved*”.

Management: Most participants thought that their water resources were managed effectively and optimally by local and provincial authorities. Most participants said that they do “*encounter problems every now and then”* but, in general, they can’t complain.

6.3.2 Beliefs about and attitudes towards water

Water should be free. Participants often referred to water as a “shared resource” that should be available free of charge. In other words, that no person should be expected to pay for water and that it is government’s prerogative to provide people with water and for other water-related uses such as sanitary purposes. One participant made this clear when he said: “*We all need water. Us humans and our livestock. It is government’s responsibility.”*

Spiritual and cultural connections: Many participants indicated that they or “the people of their community” have a strong spiritual connection with water. They therefore use water in rituals or ceremonial acts to cleanse themselves/others, for example after a funeral, or to get in contact with their ancestors. One participant said it best when he noted that: *“Water is very important to our people (culture). We use it to make contact with our ancestors. We also use it to wash people before church or after a funeral.”*

6.3.3 Sources of water

A number of sources of water were indicated in each community, including by pumping water from a borehole/well (e.g. draw-well, pit, etc.) with a windmill or an electrical pump (motor), by collecting rain water (rain harvesting) or by obtaining water from a cave or underground source (Tigane), dam, fountain, pan (seasonal or permanent) or river or by making use of municipal water ("tap water").

6.3.4 Uses of water

Physical (physiological) needs: Participants use water for a variety of reasons related to physical needs, for example to drink on a daily basis, to fish in, or to harvest plants/other edible creatures (for food). One participant described it best when he said: *"In our community we can fish for food and we drink the water every day."*

Everyday household use: According to the participants, water for everyday household use includes using it to build, by using a mixture of soil and water (mud) for building or construction purposes (e.g. houses), for cooking food, to flush their toilets, to give to their livestock (to drink), to grow/water crops (larger scale farming), wash their clothes in, to wash goods (cleaning of physical objects other that themselves), to wash themselves (bath) or their hands before or after eating, to water their gardens (domestic plants) and for example to water their vegetables (on smaller scale/for subsistence). One participant summarised it when she said: *"We use it for everything, from washing to cooking and building."*

Recreational purposes: Participants, especially the youth in their communities, use water for swimming and/fishing (recreational purposes). This appears to be more common in the rural areas, especially in Bophirima. A teacher from one of the schools confirmed this when he said that: *"The small children swim here (pointing to a small pond) after school."*

Religious/spiritual and cultural purposes: This includes the use of water to wash their/other's feet before church or to wash themselves/others after a funeral service, the use of water to drive out evil spirits/to remove evil spirits (cleansing themselves/members of their family or house), to help them when they are fasting, to initiate a traditional healer/s in their community or to make traditional medicine (to steam, drink or mix with other herbs, etc.), and for example to make traditional beer (*umqhombothi*).

6.3.5 Water management

The theme water management includes how water is obtained and managed after it has been obtained. For example, one of the participants said: *"Now we are lucky. In the past we* *used to walk far. No we have a tap in the yard."* It the past, people often had to travel large distances to get water. Nowadays, in most cases, it appears as if they get most of their water directly from a tap in the house/homestead, or from a tap in their yard, or by using a communal tap for which they have to walk a distance of between less than 50 m from homestead to larger distances of more than 50-100 m. Some communities experience water shortages (from time to time) or lack infrastructure and therefore have to depend on water that is delivered to them by a water tank/truck. Once the water is obtained, it is generally either stored in a container in the house where it may be cooled down (e.g. by keeping it in a fridge) or it is simply kept in a container outside the home.

6.4 Quantitative results

Results for the total group are reported, except where there was a significant statistical or practical difference between the two districts. (All y-axis are percentages).

a) Perceptions about water

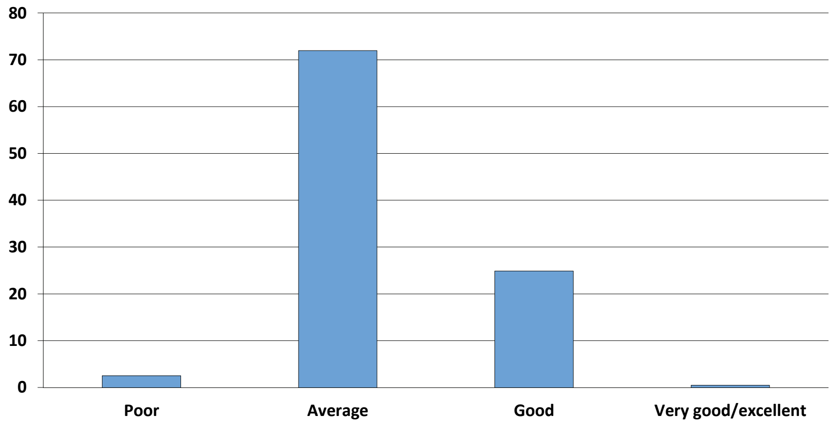


Figure 37: Perceptions about water quality.

There were no meaningful differences between the two districts regarding their perceptions about the quality of their water or its availability. The majority of the participants perceived the quality of their water to be average (72%), while 25% thought that their water is of good quality. A small percentage (0.5%) thought that their water is of very good or excellent quality and only 2.5% that it is of poor quality (Figure 37).

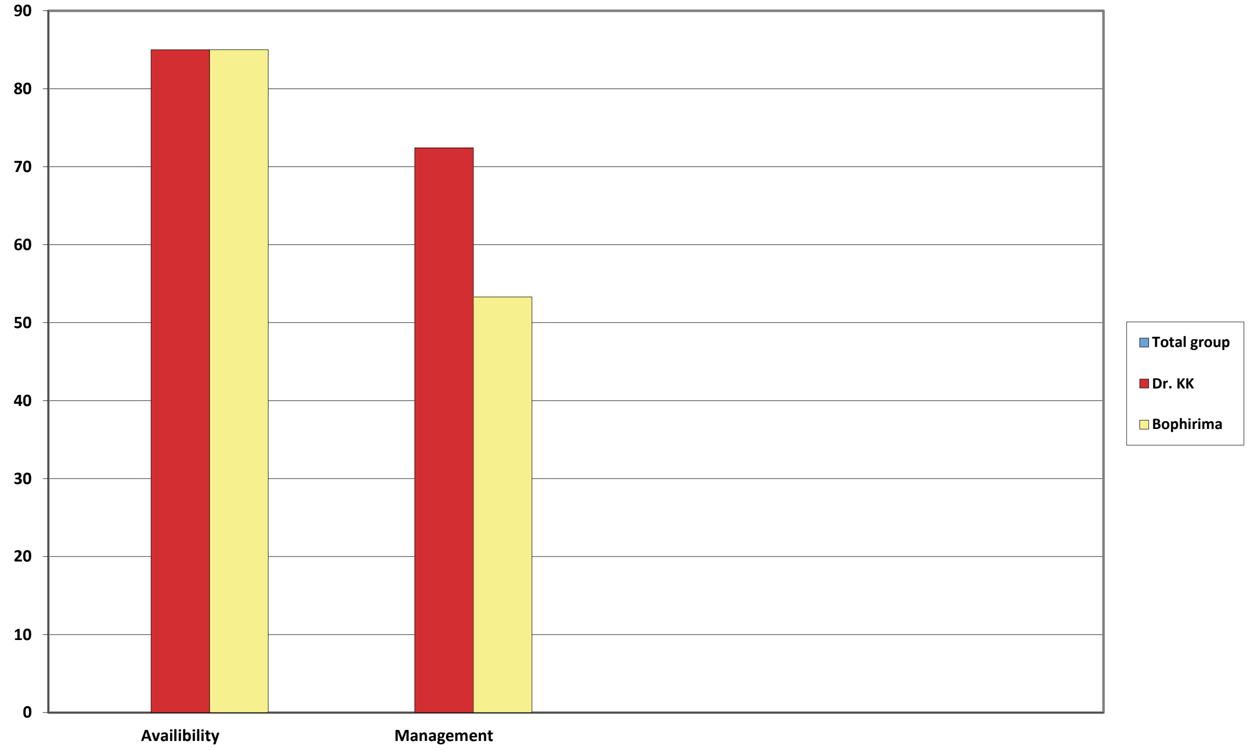


Figure 38: Perceptions related to the availability (scarce resource) and management of water.

The majority of the participants in each of the two districts perceives their water to be a scarce resource (Dr Kenneth Kaunda district = 84.6%; and Bophirima district = 85.2%) but differed in their opinion regarding how their water is managed (Figure 38). In Bophirima, almost half of the participants (47%) said that they think their water is not managed correctly, while the majority of participants in Kenneth Kaunda (72%) thought their water is managed correctly ($p < 0.05$; Cramer's V = 0.20).

b) Beliefs about and attitudes towards water

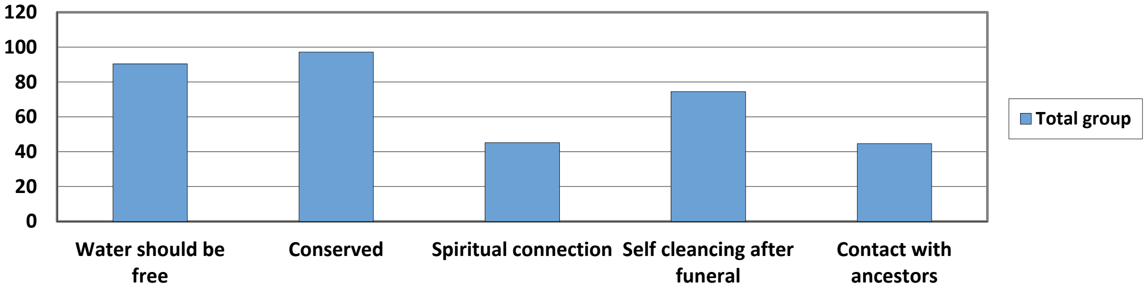


Figure 39: Beliefs and attitudes towards water.

Ninety percent of participants felt that they should not have to pay for water. A larger percentage (97%) felt that water must be used sparsely or should be conserved. Forty percent of the participants (said that they have a spiritual connection with water. A large percentage (75%) indicated that they use water for example to cleanse themselves or others after a funeral or burial ceremony. Forty five percent use water to make contact with their ancestors (Figure 39).

c) Sources of water

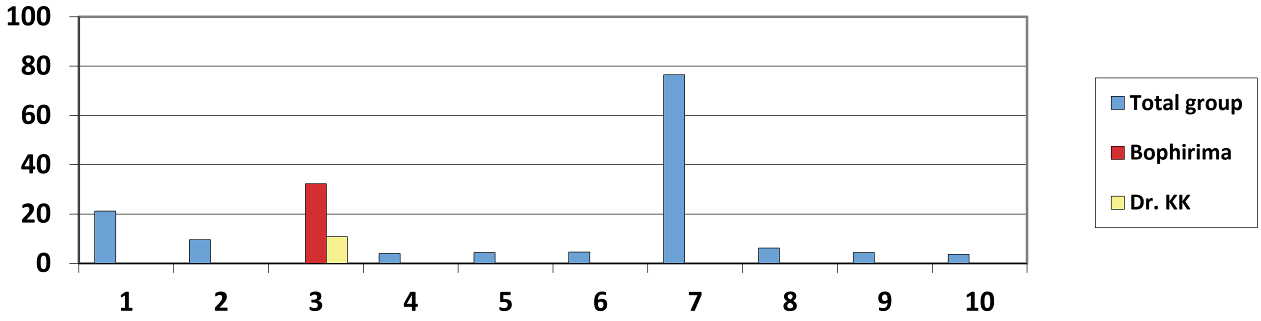


Figure 40: Sources of water (1 = Borehole with a windmill; 2 = Borehole with an electrical pump (motor); 3 = By collecting rain water; 4 = Cave or underground source; 5 = Dam; 6 = Fountain; 7 = Municipal water (tap); 8 = Pan (seasonal or permanent); 9 = River; 10 = Well (e.g. draw-well, pit, etc.)).

The majority of households (76.5%) make use of municipal water. Sources of water did not differ greatly between the two districts, except in regard to the harvesting of rain water. In the Bophirima district, more of the participants make more use of rain water harvesting (32.3%), compared to the Dr Kenneth Kaunda district (32% vs. 11%). Other sources of water include boreholes with windmills (21%), or borehole with electrical pumps (10%), seasonal pans (6%), fountains (5%), dams and rivers (4%) as well as wells (4%) (Figure 40).

d) Uses of water

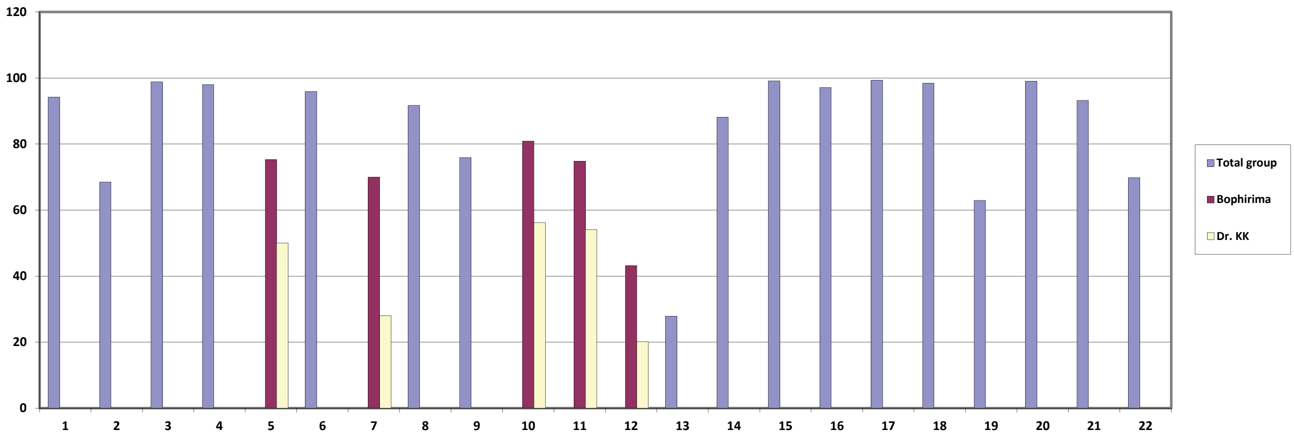


Figure 41: Uses of water (1 = Build our house/other physical structure (for example in combination with mud); 2= Cleanse myself/ourselves from the inside (enema); 3 = Cook my/our food; 4 = Drink (when I am/we are thirsty); 5= Drive out evil spirits/to remove evil spirits (cleansing myself/members of my family or house); 6 = Flush my/our toilet; 7 = Give to my/our livestock (to drink); 8 = Help me when I am fasting; 9 = Initiate the traditional healer/s in our community; 10 = Make traditional medicine (to steam, drink, or mix with other herbs, etc.); 11 = Swim or fish in (recreational purposes); 12 = Fish in, to harvest plants from or any other edible creatures (for food); 13 = Grow/water crops (larger scale farming); 14 = Make traditional beer (*umqhombothi*);15 = Wash my clothes in;16 = Wash my/other's feet before church;17 = Wash my/our goods (cleaning of physical objects other that myself); 18 = Wash myself (bath); 19 = Wash myself/others after a funeral service; 20 = Wash my/our hands; 21 = Water my garden (domestic plants); 22 = Water my vegetables (on smaller scale/for subsistence)).

The number of uses of water differed statistically and practically between the two districts (Figure 41). When comparing the Bophirima and Dr Kenneth Kaunda districts the following pattern emerges:

- traditional uses such as using water to drive out evil spirits or to remove evil spirits (cleansing themselves or members of their family or house) (75% vs. 50%; $p < 0.05$; Cramer's V = 0.25)
- making traditional medicine (to steam, drink, or mix with other herbs, etc.) (81% vs. 56%; $p < 0.05$; Cramer's V = 0.26),
- swim or fish in (recreational purposes) (75% vs. 54%; $p < 0.05$; Cramer's V = 0.21)

- to fish in (to harvest plants from or any other edible creatures (for food) (43% vs. 20%; $p < 0.05$; Cramer's V = 0.25) and
- livestock watering (70% vs. 28%; $p < 0.05$; Cramer's V = 0.41).

Other popular uses of water that 88% more of the participants mentioned include
the use of water for house building or other physical structures (for example in combination with soil) (94%),

- to cook their food (99%), to drink (98%),
- to flush their toilet (96%),
- to help them when they are fasting (92%),
- to wash their clothes (99%),
- to wash their brother's feet before church (97%),
- to wash their goods (cleaning of physical objects other that themselves) (99%),
- to wash themselves (bath) (98%),
- wash their hands (99%) and
- to water their garden (domestic plants) (93) and to make traditional beer (*umqhombothi*) (88%).

Other uses of water include:

- self-cleansing (enema) (68%),
- to initiate the traditional healer/s in their community (75.9%),
- larger scale farming (grow/water crops) (27.8%),
- to wash themselves or others after a funeral service (62.9%) and
- to use water for smaller scale/subsistence farming (to grow vegetables (69.8) (Figure 41).

e) Water management

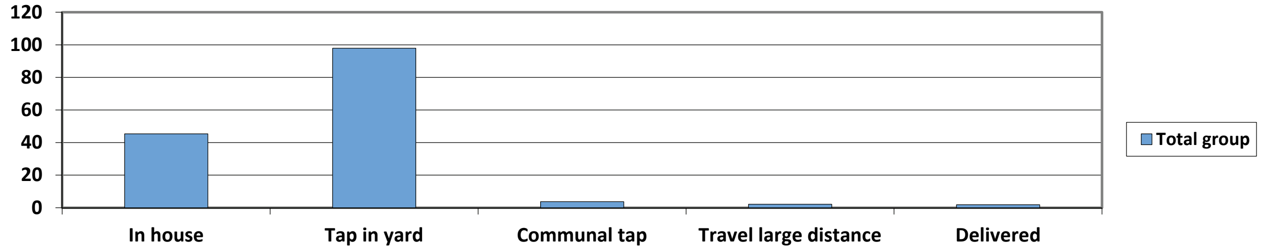


Figure 42: Water availability and management.

The majority of households (97%) appear to make use of a tap in their yard even though 45% have water in their houses. Very small percentage of participants use a communal tap (4%) for which they have to travel less than 50 meters. Two percent have to fetch water more than 50 meters from their dwelling and another 2% gets water that is delivered to them (Figure 42).

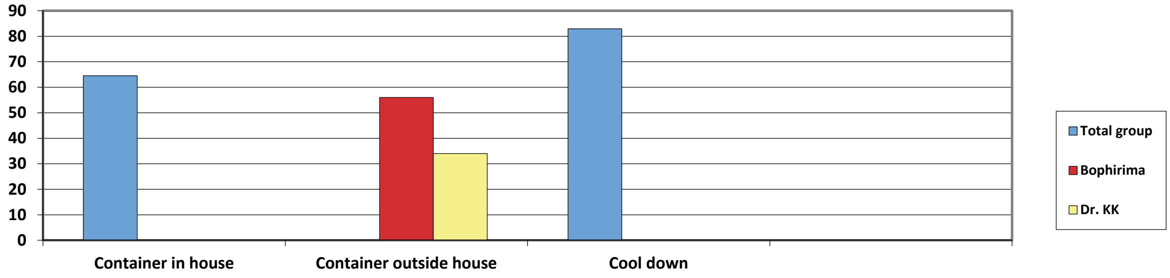


Figure 43: Storage and management of water.

Most households appear to store water inside their homes (64.5%) where it is cooled down in most cases (82.9%). Communities in the Bophirima district tend to store water more regularly in containers outside their house compared to the Dr Kenneth Kaunda district participants (56% vs. 34%; $p < 0.05$; Cramer's V = 0.30; Figure 43).

6.5 Discussion and conclusion

From the findings it is clear that people's interactions with water elicit certain perceptions, beliefs and attitudes and behaviours (e.g. uses of water, water management, etc.). There were, however, no meaningful differences between people's (i.e. the different communities') perceptions regarding the quality of their water. This is something that is subjective and it will be interesting to compare this to actual water quality data in the different communities. The management and availability of water are more objective, because it is something that is experienced by the communities on a daily basis. The good news is that people in these communities appear to realise that water is a scarce resource and that it should be conserved and used sparsely. In terms of future interventions, there is, therefore, no need to start education or awareness campaigns in these communities. Education programmes could build on the existing knowledge and understanding of community members.

There were, however, differences regarding people's perceptions about how their water is managed. This is something that needs to be taken up with local authorities, where they can come up with better strategies to involve the local communities in their planning. A good public participation process will make the people from these communities feel more involved or will at least sort out misperceptions if they exist.

One aspect that people felt very strongly about is that they should not be expected to pay for water. Water is often regarded as a shared resource that should be respected, but something that belongs to everyone. It is no surprise in a democratic country such as South Africa that people are aware and willing to stand up for their basic rights, like in this case free access to water. The South African government currently provides a certain amount of water free to all the people of South Africa (ANC freedom charter), but the amount of free water is often considered insufficient for everyday household use. From the results it is evident that the average number of dependants per household is small. It could thus be argued that due to this average households should have sufficient free water. Government has also come a long way to provide access to water, to households. However, this study has shown that most households make use of a tap in their yard. Similar results were found by Coetzee and Du Toit (2011) in the rest of the province.

An interesting result is that there are differences between communities regarding the harvesting of rainwater. More people in communities in Bophirima district tend to harvest water compared to people living in communities in Dr. Kenneth Kaunda district. Water is a scarce resource and education and awareness programmes related to water harvesting in the Dr. Kenneth Kaunda district will definitely help to provide and use water in the district in a more sustainable manner.

Other interesting results are the fact that a significant number of people in the communities that were surveyed still make use of borehole water that is powered by a windmill. This is presumably as a secondary source of water but it needs to be confirmed whether or not community members consider this as the provision of water from local government. It also opens up the possibility to introduce more sustainable sources of water provision and even ways to generate energy in communities in the NWP, seeing that there is no shortage of sunshine and wind. If added up, it also appears as if a significant number of people in the province, especially in rural areas, still use natural sources of water such as pans, dams and rivers to get water. This makes them vulnerable to water pollution and the associated risks that go with it (Beekman, 1998)

In general, the uses of water appear to be similar in most communities and compare well with more common uses documented in the literature (Coetsee, 2011). Some uses such using water for transportation was not found, because the North West Province has very few permanent water bodies like rivers.

In order to answer the rest of the second research question, it was found that some uses of water differed significantly between the communities, for example uses of a more cultural nature, e.g. to drive out evil spirits/to remove evil spirits (cleansing themselves/members of their family or house) and to make traditional medicine. The use of traditional medicine is not always common knowledge especially in more urban areas where traditional knowledge is often lost due to acculturation (people becoming more westernised) and enculturation (knowledge not being transferred) (Reeder, 2011). This result can perhaps be explained by the fact that the two communities in the Bophirima district are more rural compared to the semi-urban communities in the Dr. Kenneth Kaunda district and the aforementioned reasons. Furthermore, rural communities also often have different values than people in more urban areas (with a different set of social and political dynamics) which could explain why children tend to swim or fish more for recreational purposes or to harvest plants or other edible creatures from it. People in rural areas also tend to keep more livestock due to the availability of communal grazing areas.

Finally, the quantitative results confirmed that most people in the communities that were surveyed have a strong spiritual and cultural connection with water. Some of the more spiritual and cultural interactions that people have with water, in terms of uses, include the use of water to wash themselves before and after funeral services. This is presumably as a sign of respect for the deceased. It is something that is prominent in many African cultures (Reeder, 2011). This is an aspect that needs further exploration if culture sensitive interventions need to be developed in future.

In conclusion, this study has shown that people interact with water in various ways and that certain perceptions, beliefs and behaviours are associated with these interactions. It has also shown that water has important social and cultural meanings to people and also the biggest difference when communities from Dr. Kenneth Kaunda are compared to Bophirima. Finally, although the study has limitations, in general, it provides an excellent platform for future research and interventions in the province.

6.6 Limitations

- The biggest limitation is that, due to funding and other restraints, not more communities could be included in the research. Six out of a potential 50 communities (own estimate) could be included. The communities that were included do, however, appear to be fairly representative in terms of general size and structure and dynamics when compared to other communities in the province.

- Other limitations include the fact that this is an *ad hoc* study and it is a pity that more social-orientated studies are not done in the province.
- A final limitation is the fact that the current project does not allow a report back to all the communities that were surveyed to share with them what has been learned.

6.7 Recommendations

- A study to compare the perceptions of the six communities that were included in this study of the quality of their water to the actual water quality should be conducted. In the present study the actual water quality of the communities were not determined. Actual microbiological and physico-chemical quality data would provide insights into the perception of the community members. This study on water quality perceptions and actual water quality should then be repeated in new communities that were not previously included.
- A study to confirm the amount of water needed by communities on a household level per day should also be conducted. This should be done over a larger number of communities particularly those on rural and peri-urban areas. In the present study most of the communities were in reasonable close proximity of potable water sources. This could be accepted as the norm for the North West Province.
- More in-depth research on the spiritual and cultural connection that people have with water in this province is needed. Some interesting uses of water with regards to religious, traditional and cultural practices were mentioned by some of the participants. There could be other uses as well such as full-body baptisms of adults that were not mentioned. A study that focuses on these aspects should provide a more in-depth knowledge of the association the people of the North West Province and water.
- A study to determine the perceptions of the urban populations of the North West Province on water quality, quantity interactions and management of water should also be done. This was not initially part of the current study but this part of the provincial population should be included in future studies.
- Study to investigate the feasibility of providing alternative water sources and sources of energy (e.g. to pump or manage water) to communities should be conducted. For example a programme in the harvesting of rain water should be considered. The province is in a summer rainfall region and large quantities of water could be

harvested during this period. This water could then be used for various purposes during the dry winter period.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

The overall purpose was to conduct a large-scale study of microbial and physico-chemical quality of groundwater and surface water in the North West Province, South Africa and to provide a report of the results. The following conclusions were drawn from the results:

- Target water quality range comparisons (DWAF, 1996) of physico-chemical parameters of selected surface water sources indicated that this water was generally suitable for several uses including recreational and domestic uses. In some cases electrical conductivity was elevated but not to an extent where negative health implications would be associated with the consumption of the water. This elevated EC could be indicative of increasing salinisation.

- *Enterococcus* spp. as well as *Escherichia coli,* were isolated demonstrating that the specific surface water sources are contaminated by various faecal indicator bacteria. Several of the enterococci species identified are potentially pathogenic to humans and animals. A very small percentage of *E. coli* isolates were entero-pathogenic strains and could have human and animal health implications.

- *Candida*, *Clavispora, Cryptococcus, Cystofilobasidium Hanseniaspora*, *Meyerozyma*, *Pichia*, *Rhodotorula, Saccharomyces*, *Sporidiobolis*, and *Wickerhamomyces* are various yeast genera that were isolated. Some these species of these genera may cause invasive infections in sensitive individuals.

- Baseline culture independent molecular profiling data of the dominant bacterial communities in the Vaal River were also provided by DGGE and HTS profiling. These methods allowed for the identification of the bacterial community composition and dynamics in the planktonic component in this river system. Next generation sequencing or (HTS, high through-put sequencing) analysis presented a better resolution of the bacterial diversity and dynamics in the Vaal River compared to DGGE.

- The bacteriophage data supported the general findings of the bacterial analysis i.e. that many of the water sources in the North West Province have some form of faecal pollution. Phages were also isolated from potable water sources and this is a cause for concern.

- Boreholes that were sampled generally complied with the drinking water TWQR (DWAF, 1996) accept for nitrates. Less than 30% all boreholes tested complied with <6 mg/ℓ NO_3N for drinking water (DWAF, 1996). Some of the levels were greater than 400 mg/ℓ NO_3-N. Such high levels of nitrates could have human health implications.

- Faecal contamination of the groundwater was demonstrated by data from indicator bacteria, bacteriophage and faecal sterol data. In addition to bacterial faecal indicators, *P. auruginosa* and *Staph aureus* were also found in many borehole water samples. Next generation sequencing (or high through-put sequencing) results provided supported the culture dependent data.

- Animal species-specific faecal sterol profiles were generated using the Szuc method. This method demonstrated the potential of using such an approach in source tracking faecal pollution in the North West Province for local application.

- Another potential application of the faecal sterol analysis is to use it to evaluate the efficiency of wastewater treatment plants. However, more accurate sterol recovery methods and larger sample volumes needs to be considered (Leeming, 2006) and the method optimised and validated.

- Sterol analysis may also aid in the water quality determination of both ground- and surface water. It can is used to determine the source of faecal pollution by utilising the faecal sterol ratios.

- Culture independent methods (PCR-DGGE and HTS) supported groundwater bacteriological plating results for faecal indicator bacteria. Additional pathogenic and potential pathogenic species were also identified by these methods.

- The results indicated that several human pathogens as well as opportunistic yeast pathogens were present in the water sources. Enterovirus (qPCR) analysis demonstrated that WWTPs may be decanting large amounts of this (and potentially other viruses) into receiving water bodies if poorly or untreated sewage is allowed into such water sources.

- Results of a cytotoxicity test developed during this study demonstrated that such a test could act as a first tier evaluation of the potential of water for negative health impacts. Once refined it could be a useful system for a widespread application to water in the North West Province and elsewhere. All flagged samples could then be further investigated for the actual causes of cytotoxicity.

- The social and water management part of the study has shown that people interact with water in various ways. It also demonstrated that certain perceptions, beliefs and behaviours are associated with these interactions. It was demonstrated that water has important social and cultural meaning. Responses from people in the communities surveyed indicated that they realise that water is a scarce resource and that it should be conserved and used sparingly.

7.2 RECOMMENDATIONS

- Microbiological and physico-chemical quality of drinking-water boreholes should be combined with epidemiological studies to determine how many water users served by these groundwater sources become ill each year as a result of using this source. The present study, as well as previous ones, has demonstrated that several of the surface waters and groundwater sources are polluted by faecal matter. These results have indicated that nitrate concentrations are high in some areas. However, no epidemiology data is available to demonstrate conclusively that the pollution is linked to disease burden. Disease states should include diarrhoea, physiological conditions as well as skin and invasive infections. A study that is focused on the nitrate concentrations in groundwater of the North West Province should also be conducted. The geology of the area, depth of the boreholes, rainfall events, fertiliser application rates and the proximity of the boreholes to agricultural lands and human settlements should be considered.

- Another aspect that needs to be addressed is the elevated electrical conductivity that was observed at several of the sites. Sources of these high EC values should be investigated as this could have serious implications on soil quality in the province and by implication also food security. The North West Province is one of the main grain producers in the country producing a large percentage of maize and sorghum, amongst others. In many cases these crops are under irrigation. Salinisation of soils will reduce the carrying capacity thereof and eventually render it unproductive. In regions of the Vaalharts irrigation scheme, salinisation of soils has resulted in large sections becoming waterlogged which are now completely unproductive. This could also happen in the North West Province if early interventions are not put in place. Elevated electrical conductivity will also impact negatively on livestock production. If environmental water salt levels become such that additional treatment is required then this will add cost to such operations. This will also have impacts on food affordability and security. Continuously increasing EC will eventually also impact on drinking water production in the process will add costs to drinking water. Although elevated electrical conductivity does not appear to be a threat at the moment, it should be carefully monitored, sources of the increases identified and appropriately managed.

- Boreholes should be identified that may be vulnerable to certain types of contamination. Site-specific conditions such as soil properties, vegetation, and topography may affect contaminant transport at boreholes. Various sources could further contribute to nitrate contamination of boreholes. These include the geology, agricultural practices such as high density feed lots, use of un-composted faecal material as well as fertiliser remnants in soil. Some of the factors and sources mentioned could contribute to high levels of nitrates and faecal matter in groundwater in the North West Province (NWP-SOER, 2002). Causes of contamination of groundwater should not be generalised and specific ones affecting specific boreholes investigated. Large sections of the rural communities in the North West Province are dependent on groundwater for all their domestic needs. Thus when groundwater is supplied to communities and schools in this province, a detailed analysis of the water quality should be conducted. Regular tests should also be done by the supply water authorities to ensure that the water is of suitable quality for the intended use. In cases where the nitrate and electrical conductivity levels are high purification steps should be considered. These boreholes should be sampled and analysed on a regular basis. During every sample event, the surrounding area, human and environmental influences as well as seasonal variables should be recorded. This will allow for insights into the factors promoting the contamination of the groundwater and preventative or remedial processes could be put in place.

- Future studies to determine the presence of *E. coli* and other indicators in the water should be conducted also using PCR methods instead of only culture based methods. The latter methods are tedious and confirmation of identities takes several days. False negative results are a cause for concern particularly if pathogenic *E. coli* may be present in the samples. PCR methods will only give qualitative results i.e. present or absent but results will be available in a much shorter time frame than what is feasible with culture dependent methods. Also, quantitative analyses may be achieved using real-time PCR technologies. Reverse Transcription-qPCR may be useful to distinguish between viable and non-viable bacteria. PCR based methods allow for a faster turnaround time of results and may provide more accurate results than the traditional culture based methods.

- The traditional culture based methods provided microbiologists with a number of organisms that could be used to indicate pollution events. Such methods mainly focus on the microbial quality of water for specific uses. The methods also do not have sufficient resolution to provide insights into the important ecological processes occurring within aquatic ecosystems. This is because cultivation procedures allow for a small number of microbes to be cultivated. Estimating the total microbial diversity and identifying microbial assemblages as well as proposing their role at community level is thus not possible. Understanding the microbial community dynamics in aquatic systems may provide microbiologists with better approaches to determine and predict the effects of pollution. Since organisms normally act within in communities their abundance will then be dependent on the selection forces prevailing within the community and environment. In the present study two culture independent approaches demonstrated that the traditional indicator bacterial species were not detected in large quantities. Both these approaches found various other bacterial phylogenetic groups present of which some uncultured species were previously detected in aquatic ecosystems. Future work in this regard is thus necessary. It should concentrate on in-depth HTS analysis of the microbial diversity and water quality in the surface and groundwater with the focal point on the identification of specific species within the major key groups. Identification may phylogenetically affiliate uncultured bacteria with known bacterial genera or species. It may thus be possible to cultivate some of these previously uncultured bacteria using specific nutrient, pH and temperature conditions. Morphological descriptions may then be possible. Such species may then be further exploited as indicators species of specific pollution event

- Further research into the levels and diversity of yeasts in the North West Province should be conducted. Their potential sources should also be investigated. The association of yeast and organic carbon pollution should also be investigated. What also needs to be determined is an equivalent level of risk of illness. There is presently no regulation or risk model for yeasts in aquatic ecosystems. The various yeast genera and species identified in the present study are mostly associated with opportunistic infections in immune-compromised. *Cryptococcus neoformans* is one species of particular concern. However, in the present study a species that causes the same symptoms as *C. neoformans* was isolated. The levels and distribution of this species is unknown. There may also be other species that are of clinical importance that could be present in the water source of the North West Province. This is against the back drop of numerous waste water treatment plants in the province not operating optimally and in many of these cases, untreated sewage is allowed to enter waterways. In the present study the number of sampling sites and isolates identified were limited. A large-scale study should be designed particularly around the areas that are most frequently used by the community of the North West Province for full contact activities. This will provide clearer insights into the actual infection risks of exposure to the various yeast species.

- A need exists to gain more knowledge about the social context and management of water at a community level in the North West Province. In the present study rural and peri-urban communities were targeted. Future studies should include urban communities a larger cross section to include all the economic groups. Such a study should also include a section of the water needs and efforts that community members make to conserve water. More in-depth research on the spiritual and cultural connection that people have with water should also be considered. A study to investigate the feasibility of providing alternative sources energy to pump (or manage) water to communities. Education and awareness programmes to promote the harvesting and use of rain water should also be considered, particularly for the western part of the province that borders on and is part of the Kalahari Desert. It is imperative that communities are made aware of, and understand the risks involved with using untreated groundwater as a source for domestic purposes. By having knowledge of the water quality they use, communities should be encouraged to treat the water at household level (boiling of water, point-of-use chlorination, UV treatment and safe storage practices). These practises will aid in the prevention of waterborne diseases. Education on the routes and sources of contaminants should also encourage communities to take ownership of their health.

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ANNEXURE 1

INFORMED CONSENT[1]

An exploration of people's interactions with water in the NWP

I have been informed that the purpose of the research is to explore people's interactions with water in the North West Province. My participation will involve answering questions related to my/our household's (myself and the members of my family's) interactions with water and that the interview will take approximately 15-30 minutes.

I understand that there are no foreseeable risks or discomforts if I agree to participate in the study.

I understand that the results of the study may be published but that my name or identity will not be revealed. I also understand that the results of the study may be used for secondary studies connected to this project, but that my name or identity will not be revealed. The North-West University will maintain confidentiality of all records and materials.

I have been informed that I will not be compensated for my participation. I have been informed that any questions I have concerning this research study or my participation in it before or after my consent will be answered by the investigators of this study. I understand that I may withdraw my consent and discontinue participation at any time without penalty or loss of benefit to myself. In signing this consent form, I am not waiving any legal claims, rights or remedies.

I, the undersigned, _________________________________ (full names), have read and understand the above information and by signing this form indicate that I will participate in the research voluntarily.

___________________ ____________________

Participant's signature Date

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[1] All prescribed ethical guidelines were adhered to before, during and after the research. Informed consent was obtained from all participants and information communicated included the voluntary nature of their participation, possible risk factors, factors which may cause discomfort, the expected benefits of taking part in the research, their right to withdraw at any stage from the research without having to give reasons for doing so and that all information shared with the researchers will be considered confidential and anonymous.

ANNEXURE 2

A. What is your age?

18-25	1
26-30	2
31-40	3
41-50	4
51-60	5
61+	6

B. Gender (do not ask!)

Male	1
Female	2

C. Marital status

Married, living with spouse	1
Married, not living with spouse	2
Cohabiting	3
Unmarried	4
Divorced	5
Widowed	6

D. Nationality

RSA	Other

E. Level of education

None ☐ Primary ☐ Some secondary ☐ Completed secondary ☐ Tertiary ☐

F. Occupation

Unemployed ☐ Self-employed ☐ Part-time job ☐ Full-time job ☐

G. Average household income per month

R0-100 ☐ R101-200 ☐ R201-300 ☐ R301-500 ☐ R501-800 ☐ R801-1000 ☐

R1001-2000 ☐ 2001-5000 ☐ 5001-10000 ☐ More than R10,000.00 ☐

H. Number of dependants in household (living in the same household and sharing at least 3 meals per week with the rest of the household)

None ☐ One ☐ Two ☐ Three ☐ Four or more ☐

<u>Please answer the following questions:</u>

1. **The quality of the water in my community is…**

1 Poor	2 Average	3 Good	4 Very good/excellent

2. I have the following beliefs and/attitudes about water:

a) That water is freely available in my community	1 Yes	2 No
b) That the water source/s in my community is/are managed correctly	1 Yes	2 No
c) That water should be free (we should not pay for it)	1 Yes	2 No
d) That I/we must use water sparsely/conserve water	1 Yes	2 No
e) That I have a spiritual connection with water	1 Yes	2 No
f) That I must cleanse myself/others with water after a funeral	1 Yes	2 No
g) That I can get in contact with my ancestors through water	1 Yes	2 No

3. I/we use water to:

a) Build our house/other physical structures (for example in combination with mud) If other that house, what?..	1 Yes	2 No
b) Cleanse myself/ourselves from the inside (enema)	1 Yes	2 No
c) Cook my/our food	1 Yes	2 No
d) Drink (when I/we am/are thirsty)	1 Yes	2 No
e) Drive out evil spirits/to remove evil spirits (cleansing myself/members of my family or house)	1 Yes	2 No
f) Flush my/our toilet	1 Yes	2 No
g) Give to my/our livestock (to drink)	1 Yes	2 No
h) Help me when I am fasting	1 Yes	2 No
i) Initiate the traditional healer/s in our community	1 Yes	2 No
j) Make traditional medicine (to steam, drink or mix with other herbs, etc)	1 Yes	2 No
k) Swim or fish in (recreational purposes)	1 Yes	2 No
l) Fish in, to harvest plants from or any other edible creatures (for food)	1 Yes	2 No
m) Grow/water crops (larger scale farming)	1 Yes	2 No
n) Make traditional beer (*umqhombothi*)	1 Yes	2 No
o) Wash my clothes in	1 Yes	2 No

p) Wash my/other’s feet before church	1 Yes	2 No
q) Wash my/our goods (cleaning of physical objects other that myself)	1 Yes	2 No
r) Wash myself (bath)	1 Yes	2 No
s) Wash myself/others after a funeral service	1 Yes	2 No
t) Wash my/our hands	1 Yes	2 No
u) Water my garden (domestic plants)	1 Yes	2 No
v) Water my vegetables (on smaller scale/for subsistence)	1 Yes	2 No

4. I/we get my/our water from the following sources:

a) Borehole with a windmill	1 Yes	2 No
b) Borehole with an electrical pump (motor)	1 Yes	2 No
c) By collecting rain water	1 Yes	2 No
d) Cave or underground source	1 Yes	2 No
e) Dam	1 Yes	2 No
f) Fountain	1 Yes	2 No
g) Municipal water (tap)	1 Yes	2 No
h) Pan (seasonal or permanent)	1 Yes	2 No
i) River	1 Yes	2 No
j) Well (e.g. draw-well, pit, etc.)	1 Yes	2 No

5. I/we have water in my/our house	1 Yes	2 No
6. I have water (a tap) in my yard	1 Yes	2 No
7. I use a communal tap less than 50 m from house	1 Yes	2 No
8. I/we have to travel large distances to get water (further than 50 meters)	1 Yes	2 No
9. Someone delivers water to our community (water tank/truck)	1 Yes	2 No

10. When I/we get my/our water we:

Store it in a container in the house	1 Yes	2 No
Store it in a container outside the house	1 Yes	2 No
We cool it down/keep it in a fridge	1 Yes	2 No
Other:		