Surface water quality of the North West Province based on physico-chemical properties and faecal streptococci levels

By

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20318634

Submitted in fulfilment of the requirement for the degree of

MAGISTER OF SCIENCE IN ENVIRONMENTAL SCIENCE

(M.Sc Env. Sci)

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May 2012

ABSTRACT

Water resources in the North West Province are characterised by an overall scarcity due to non-perennial rivers being the dominating water sources. However, increases in water demand from all social, environmental and economic entities have resulted in the inevitable deterioration of surface waters resulting in increasing threats of faecal and chemical pollution. The quality of 5 surface water systems in the North West Province was assessed by monitoring the physico-chemical properties of the water as well as the levels and diversity of faecal streptococci. The Mooi, Harts, Schoonspruit and Vaal Rivers were sampled during one warm-rainy season in 2010 and one cold-dry season in 2011. Dissimilarly, the opposite was employed for Barberspan as it was sampled during one cold-dry season in 2010 and one warm-rainy season in 2011. The average physico-chemical and microbial levels measured at some sites during both seasons were elevated and exceeded the acceptable South African Target Water Quality Range (TWQR) for full and intermediate recreational contact, livestock watering and irrigation. Seasonal variation patterns were observed in both physico-chemical and microbial levels. All surface water systems had relatively lower pH and electrical conductivity levels during the warm-rainy season as compared to the cold-dry season. In addition, water samples collected during the warm-rainy season from the Harts River, Barberspan and Vaal River had higher bacterial levels (total coliforms, faecal coliforms, *E. coli* and faecal streptococci) compared to samples collected during the cold-dry season. The Schoonspruit River had higher bacterial levels during the cold-dry season compared to the warm-rainy season. A total of 80 and 59 presumptive faecal streptococci isolates were obtained in 2010 and 2011, respectively. Biochemical tests and 16S rRNA gene sequencing were used to identify all faecal streptococci isolates. A total of 6 faecal streptococci species were identified and these included: *Enterococcus faecium, E. faecalis, E. mundtii, E. casseliflavus, E. gallinarum and E hirae.* Haemolysis and antibiotic resistant

patterns were used for the characterisation of all faecal streptococci isolates. Of the 80 and 59 faecal streptococci isolates obtained, 32.5% and 6.8% displayed β-haemolysis. Furthermore, dominant multiple antibiotic resistance patterns were observed for faecal streptococci isolates at most sites in both years. Faecal streptococci isolates obtained in 2010 were resistant to Penicillin G (10 µg), Neomycin (30 µg) and Vancomycin (30 µg) and susceptible to Amoxicillin (10 µg) and Streptomycin (300 µg). Isolates obtained in 2011 were also resistant to Penicillin G (10 µg) and Neomycin (30 µg) but also to Amoxicillin (10 µg) and Ciprofloxacin (5 µg). Furthermore, the 2011 isolates were susceptible to Chloramphenicol (30 µg) and Trimethoprim (2.5 µg). The physico-chemical and microbial levels measured at several sites exceeded acceptable limits and proved unsuitable for applications such as full and intermediate recreational activities, livestock watering as well as irrigation. In addition, it appears that the physico-chemical and microbial levels were influenced by the season of collection. The multiple antibiotic resistance (MAR) phenotype data of faecal streptococci isolates obtained for the different sites suggests that their exposure history to several antibiotics was similar. This is most probably due to a uniform pollution pattern along the system. The presence and isolation of faecal streptococci, particularly those capable of causing β-haemolysis, in surface water systems used for livestock watering and cultural activities is an important health care concern. The significance is outlined when the serious nature of the diseases and infections that could be caused by some of the pathogens (*E. faecalis* and *E. faecium*) isolated in this study as well as their emerging antimicrobial resistances is considered.

Keywords: faecal streptococci, *Enterococcus* spp. β-haemolysis, antibiotic resistance.

This work is dedicated to my parents, Sarah and Lekopane Molale, the reason for my existence. Thank you for a lifetime of love and allowing me to realize my own potential. The love, devotion, support and guidance you have provided me over the years is by far the greatest gift anyone has ever given me. I am humbled to be an imprint of your seed.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation and gratitude to the following people and institutions for their contribution and support towards the completion of this study:

My maker and heavely father for his never ending mercies and favour. It is by grace alone that I have made it this far. Thank you Lord for being a light unto my feet and a lamp unto my path.

Prof. C.C. Bezuidenhout for his patience, guidance, encouragement, time, support and valuable input into making this possible.

The Water Research Commission (WRC) of South Africa (K8/853 & K5/1966), the National Research Foundation-Deutscher Akademischer Austausch Dienst (NRF-DAAD) and the North West University post graduate (PUK-bursary) for financing this study.

Mr. Mathews Makinta Managing Director of MKM Data Services cc. for his assistance with the map.

My parents Mama Sarah and Papa-Dad for their prayers, love, emotional and financial support as well as believing in me always.

My sister, Magai for her support and words of encouragement when all seemed impossible.

My younger siblings, Nobuhle and Mamkeli, for always being there.

My friends Maggy, Deidré, Abraham, Moses and Nkosinathi for their motivation and assistance with this dissertation.

DECLARATION

I, Lesego Gertrude Molale, declare that this dissertation is my own work in design and execution. It is being submitted for the degree Master of Science in Environmental Science at the North West University, Potchefstroom Campus. It has not been submitted before for any degree or examination at this or any other university. All material contained herein has been duly acknowledged.

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Lesego Molale **Date**

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CHAPTER 1

INTRODUCTION

1.1 General introduction and problem statement

South Africa is a water scarce country with a pronounced spatial and temporal variability (Stats SA, 2010a). Due to its extreme climate and rainfall fluctuations, South Africa is ranked the 35th driest country in the world (DWAF, 2010). Furthermore, the country faces a number of inter-related water crises that are described by Herold (200 9) as “potential show stoppers”. The most threatening of all problems is not only that the country has almost exhausted its available surface water, but also limited space is available for construction of new dams (CSIR, 2010a). As a result, an imbalance is found between water availability and demand, and consequently demand undoubtedly exceeds supply (DWAF, 2004a). According to Oberholster and Ashton (2008), when considering the current and probable future population growth rates as well as the expected socio-economic development trends, it is highly unlikely that South Africa’s water resources will be able to sustain current patterns of water use and waste discharge.

Water is vital for the sustenance of all living matter and its risk against over-exploitation and pollution requires careful management (Stats SA, 2010a). Section 24 of the South African Constitution (108/1996) declares that every inhabitant has the right to a clean and healthy environment, which is protected for the benefit of present and future generations. However, an escalation in demography and water demand has resulted in the deterioration of surface waters (Oberholster and Ashton, 2008). Therefore, the protection and management of South Africa’s water resources from degradation of pollution cannot be overemphasized (Fatoki *et al*., 2001).

The importance of integrating water resource management with regular monitoring of water resource quality is outlined in chapter 14 of South Africa's National Water Act (Act 36/1998). This chapter is, according to DWAF (2004b), based upon a principle that quotes "If you can't measure it, you can't manage it". Fatoki and co-workers (2001) suggested that the construction of records aimed at explaining water pollution sources and trends is an important aspect of water quality monitoring. The latter avoids the danger of not knowing a rivers water quality status which often leads to problems that arise unexpectedly consequently limiting the implementation of remedial action.

Water pollution is defined by DWAF (2002a) as an alteration in the properties of a water resource so as to make it, among others, harmful or potentially harmful to the welfare, health or safety of human beings. Since water is an excellent solvent and transport medium for particulates it can easily be contaminated by physical soil as well as clay particles, chemical constituents and microorganisms (Hohls *et al*., 2002). Subsequently the term 'water quality' has been defined as the chemical, physical and biological characteristics of water with respect to its suitability for a specific purpose (DWAF, 2004a). Furthermore, surface water systems are predominately affected by untreated wastewaters, industrial, agricultural and domestic wastes decanting into them (Haller *et al*., 2009). As a result, the exposure of humans to polluted water systems often results in infections and waterborne diseases (Darakas *et al*., 2008; Cho *et al*., 2010). The World Health Organization (2008) stated that numerous water related health problems are a result of microbial and chemical contaminants in water systems.

The researchers from the Council for Scientific and Industrial Research (CSIR, 2010a) stated that South Africa's water resources will progressively worsen if major improvements are not made regarding the manner in which water quality management is approached. It has however been suggested that the quality of water for various use in South Africa should be managed according to scientific principles (WRC, 1998). The National Water Resource Strategy (DWAF, 2004a) stipulates that a wide range of variables should be analysed since different

ecosystems and different user groups have widely variable water quality requirements.

Several approaches have been made available for water quality monitoring in South Africa and they include the Target Water Quality Range (TWQR) values for physico-chemical properties and microbial levels (1996a-e). Chapter 14 of the National Water Act (Act 36 of 1998) calls for the establishment of co-ordinated national monitoring systems in order to deal with water pollution in South African water systems. For this reason, several national water resource quality monitoring programmes such as the National Chemical Monitoring Programme and the National Microbial Monitoring Programme have been developed (Van Niekerk, 2004).

Temperature, pH, total dissolved solids (TDS) and electrical conductivity (EC) are the commonly assessed physical parameters during water quality studies throughout the world and South Africa (Igbinosa and Okoh, 2009; Krishnan *et al*., 2007; Venkatesharaju *et al*., 2010). Furthermore, eutrophication, that is influenced by various nutrient levels, is not only a problem in many South African rivers but also sub-Saharan Africa as a whole (Oberholster and Ashton, 2008; Nyenje *et al*., 2010). Thus, nitrogen and phosphorous are important nutrients recommended for chemical water quality monitoring (Hill and Olckers, 2001; Van Niekerk, 2004).

According to George and co-workers (2002), determining the microbiological safety of surface waters by analyzing them for the presence of specific pathogens is vital. Microbiological methods suggest the detection of a single microorganism which is hosted specifically in a pollution source (human or animal). This host specificity comes on top of other prerequisites for a good indicator of faecal contamination such as persistence in environmental waters or the absence of growth outside hosts (Wéry *et al*. 2009). Total and faecal coliforms are widely used as indices to measure the quality of surface and groundwater (Murray *et al*., 1999; WHO, 2008). However, the applicability of solely using coliform bacteria as indicators of faecal pollution has been extensively argued. It has been suggested

that faecal streptococci be included as they have proven to be more reliable (WHO, 2008).

Nevertheless, measuring only the presence of indicator bacteria in water sources is not sufficient to determine the potential sources of pollution (Whitlock, *et al*., 2002). Thus, bacterial source tracking methods can be used to determine the source of pollution (Field and Samadpour, 2007). Antibiotic resistance patterns can be used to determine potential sources of pollution (Harwood *et al*., 2000). The latter can make use of cluster analysis which aids in determining the frequency of pollution along the different sites by grouping the antibiotic resistant patterns of selected bacteria into classes such that bacteria with similar resistant patterns are grouped together (Ateba and Bezuidenhout, 2008; Wose Kinge *et al*., 2010).

Studies assessing the microbial quality of surface water systems are important in South Africa particularly in the North West Province. This is because a central source of information for assessing the potential health risks associated with natural waters contaminated with faecal pollution in the country is sparingly available (DWAF, 2002a; Kalule-Sabiti and Heath, 2008). This is even worrisome when considering the significant impact of water-borne diseases in South Africa (Mackintosh and Colvin, 2003; DWAF, 2010). Waterborne diseases are contracted via the faecal-oral route and are predominantly caused by pathogens associated with faecal contamination in water systems. The management thereof may require antibiotic therapy, however; the overuse of antibiotics has contributed to multiple antibiotic resistances in normal enteric and pathogenic bacteria (Al-Bahry, 2009). As a result clinical methods applied to cure infections caused by these bacteria may prove to be challenging (WHO, 2000).

1.2 Research Aim and Objectives

The aim of the study was to assess the surface water quality of the North West Province (South Africa) based on physico-chemical properties, the levels of indicator bacteria and characteristics of faecal streptococci.

The specific objectives of the study were to:

I. determine the physico-chemical and microbial quality of selected surface water systems in the North West Province;

II. determine the prevalence and seasonal variation of total coliforms, faecal coliforms, *E. coli* and faecal streptococci, in surface water systems of interest;

III. confirm *E. coli* identity using biochemical techniques;

IV. identify faecal streptococci using biochemical and DNA sequencing data;

V. characterise faecal streptococci by determining their haemolysis and antibiotic resistance patterns.

CHAPTER 2

LITERATURE REVIEW

2.1 Current water situation in South Africa

South Africa is a semi-arid land predominated by high evaporation rates and an average rainfall of 450 mm per annum (mm/a) (DWAF, 2004a). Comparative to the world average rainfall of 860 mm/a, South Africa's average rainfall is far below. In addition, South Africa shares water from four of its main rivers (Inkomati, Pongola, Orange and Limpopo) with neighbouring countries. Furthermore, Statistics South Africa (2010a) reported that only 1 100 cubic meters (m^3) of water is available for one person per annum.

The country's water resources are in the form of rivers, dams, lakes, wetlands and subsurface aquifers (DWAF, 2004a). However, the major needs of South Africa's water requirements are provided by surface water supplies from rivers and dams (Stats SA, 2010a). Surface water resources contribute 77% of the total water needs across the country. Groundwater contributes 9% while 14% is water which has been made available for re-use from return flows (DNT, 2011). However, a considerable amount of water in the form of return flows should be returned to streams after use if the quality of the water satisfies the relevant user requirements (DWAF, 2004a).

Of the available water resources, agriculture and irrigation consume 60% of the nation's total water demand. Municipal and domestic demands further utilize 27% of the available water resources which is divided into 24% for urban areas and 3% for rural areas. Furthermore, afforestation; industrial; livestock watering and nature conservation each respectively account for 3% of the total water demand. While, mining and power generation each use 2% of the total water demand (DWAF,

2010). Indisputably, water is 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's Human Reserve is required to satisfy basic human needs by securing a basic water supply, for people who are now or who will, in the near future, be: (i) relying upon; (ii) taking water from; or (iii) being supplied with water from, relevant sources (DWAF, 2010). However, the country's water resources are a social, environmental and economic entity. Hence, once the basic human needs and requirements for maintaining a healthy ecosystem have been met, there inevitably is always competition for access to the remaining available water (DWAF, 2004b).

In a briefing during parliament, Moloko Matlala stated that the health status of South Africa's rivers was deteriorating (Water and Sanitation Africa, 2011). Matlala affirmed that faecal pollution, eutrophication high salinity and toxicity were among the major challenges. This proves worrisome particularly when considerations are made regarding the existent link between the state of the environment as well as the well-being of humans (CSIR, 2010b). As a result, measures regarding the protection and quality of South Africa's water resources are imperative when considering the current state thereof.

2.2 Water situation in the North West Province

Many challenges faced in South Africa, regarding water and its availability, are also experienced in the North West Province (NWP). Surface water systems in the NWP consist of rivers, dams, pans and wetlands; however, many of the rivers in the province are non-perennial (NWDACE-SoER, 2008). Consequently, the total amount of available water in the province is regarded a key limiting factor for development (NWP-SoER, 2002).

A large portion of the available surface water contributes to the mining, agricultural, industrial and property sectors (Kalule-Sabiti and Heath, 2008). Furthermore, water in the NWP and South Africa as a whole, is a key resource that plays a central role in many cultural ceremonies as well as religious rights and beliefs (Zenani and Mistri, 2005). In terms of Section 31 of the South African Constitution (108/1996), all persons belonging to a cultural, religious or linguistic community may not be denied the right, with other members of the community, to enjoy their culture, practice their religion and use their language. Thus, water from rivers, streams, dams and springs may be utilised for cultural and religious purposes such as baptismal and initiation ceremonies (Zenani and Mistri, 2005).

Surface water quality in the NWP is impacted by various point source factors such as acid mine drainage, domestic and industrial sewage effluents, as well as non-point source pollutants such as agricultural and storm water runoff (NWDAC-SoER, 2008). Additionally, when municipal raw water is discharged from specific point-sources and channelled into rivers, a wide range of potentially infectious agents are introduced to the rivers (Awofolu *et al*., 2007). Kalule-Sabiti and Heath (2008) stated that variables such as faecal coliforms are not being monitored on a routine basis even though they are required to be represented in the North West Department of Agriculture, Conservations and Environment-State of the Environment Report (NWDACE-SoER). Therefore, much attention needs to be given to surface water resources in the NWP because not only are they responsible for socio-economic growth and poverty reduction. They also play a central role of providing water for basic human need, recreational, cultural and religious activities.

2.3 Selected rivers in the North West Province

For management reasons, South Africa's water resources have been decentralized into 19 water management areas (WMA's) (DWAF, 2010). This study focuses on 4 rivers and an Inland Lake located in the Upper, Middle and

Lower Vaal WMA's. According to Kalule-Sabiti and Heath (2008), several regions in the North West Province have been labelled as "Hot Spots" recommended for surface and Groundwater monitoring. Among these are the Middle Vaal, Schoonspruit, Harts and Mooi Rivers. Moreover, Kalule-Sabiti and Heath (2008) added that there is currently no wetland monitoring programme in the NWP which is why many wetlands have been seriously degraded and lost.

2.3.1 Mooi River

The Mooi River catchment falls under the Upper Vaal WMA and runs from the North West Province of South Africa into the eastern part. It is divided into three sub-catchments: the Mooi River, Wonderfontein Spruit and the Loop Spruit (Wade *et al*., 2002; van der Walt *et al*., 2002). This catchment area is regulated by the Klerkskraal, Klipdrift, Boskop and Potchefstroom Dams (Wade *et al*., 2000; van der Walt *et al.*, 2002). The Mooi River catchment has a total surface area of 1800 km^2 within which only 44.2% yields run-off. Majority of its precipitation, however, ends up as groundwater recharge which feeds the Mooi River along with its tributaries via dolomitic eyes (van der Walt *et al*., 2002).

The River supports farming activities located around it such as livestock watering and domestic supplies (DWAF, Anon). Furthermore, water abstracted from Klerkskraal Dam in the Mooi River is used for irrigation purposes (le Roux, 2005). Recreational activities such as swimming, fishing and angling are supported by and occur along the Mooi River (le Roux, 2005; Pantshwa, 2006).

Water management challenges in the Mooi River catchment are becoming increasingly complex due to an increase in the demand of water usage together with the current and historic negligent pollution of water (le Roux, 2005). Currie (2001) explains that environmental problems experienced within the Mooi River are a result of the impact of extensive gold mining, population growth and increased water utilization. All major Gold mining activities carried out in this catchment have the potential of polluting surface and groundwater sources

(DWAF, 1999). le Roux (2005) elaborates on the latter by adding that water quality in the Mooi River catchment is impacted on by water from the Wonderfontein Spruit tributary, mainly due to the regular pollution of gold mining industries, associated abandoned infrastructures and deposits. le Roux (2005) further added that pollution of both the surface water and the underlying dolomitic water resources is due to the discharge of polluted underground mine water within the Wonderfontein Spruit catchment, together with underground flooding of some abandoned mines. According to van der Walt and co-workers (2002), development of the mines in this catchment area during the 1930's resulted in the rapid development of informal and formal settlements. These developments gave rise to the possibility of inhabitants consuming untreated surface and groundwater while also having a negative influence on the quality of the water resources surrounding the settlements (DWAF, 1999).

It is thus clear that mining practices in the Mooi River catchment area have adversely affected the quality of water in this region and its beneficial use. This however, 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 from the Boskop Dam for industrial and agricultural use (DWAF, 1999; le Roux, 2001).

2.3.2 Harts River

The Harts River falls under the Lower Vaal WMA and is situated in the north-western part of South Africa (DWAF, 2002b). This river flows in a south westerly direction past Barberspan (DWAF, 2009). Furthermore, this catchment area is regulated by the Taung and Spitskop dams as well as the Little Harts River flowing nearby Coligny and the Great Harts River flowing from Lichtenberg (DWAF, 2009c). The Great Harts River is one of the most significant tributaries of the Vaal River (DWAF, 2009). The Dry Harts River located near Taung is a seasonal river and comprises headwaters in the Vryburg area.

The Harts River is located in a semi-arid to arid region and has rainfall levels ranging from 100 mm to 500 mm per year with evaporation rates reaching 2 800 mm per year along the western parts of the Lower Vaal WMA (DWAF, 2002b). One of the world's largest irrigation schemes, the Vaalharts irrigation scheme, is located close to where the Vaal and Harts rivers confluence and is managed by Vaalharts Water (Ferreira, 2008). This irrigation scheme was developed in 1933 with a main purpose of eradicating poverty amongst whites, which emanated from unemployment and the great depression (WRC, 2010).

Intensive irrigation practices occur in this region and they include, among others, planting and harvesting of cotton; maize; wheat and groundnuts (Ferreira, 2008). Furthermore, alluvial diamond mining occurs on ancient river beds along the Harts River and cement is produced in Lichtenburg (NWP-SoER, 2002). Other land use activities include extensive livestock farming of beef, dairy, cattle, goats, sheep, pigs and ostriches (DWAF, 2004a).

The quality of water in the Harts River is impacted upon by irrigation return flows as well as water usage in the Upper and Middle Vaal WMA's. According to Ellington (2003), the Vaalharts irrigation scheme contributes 50 000 t/a of fertilizer while 130 000 t/a of salts move towards the Harts River, from the Upper and Middle Vaal WMA's. Water downstream of the irrigation scheme has been reported to be high in salinity due to large saline leachates (DWAF, 2004b).

The Harts River is connected with an off channel-pan, Baberspan, via a channel (DWAF, 2004a). Lying 9m below the Harts river, the pan is situated ±17 km north-east of Delareyville and is the largest wetland in province (NWP-SoeR, 2002). Compared to other pans in the region Barberspan is a perennial water body with a water depth of 10m (Swart and Cowan, 1994). As a result, 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, and is currently used for bird watching and angling (Swart and Cowan, 1994). Land use activities surrounding the pan include cattle and maize farming (Swart and Cowan, 1994).

2.3.3 Schoonspruit River

The Schoonspruit River is a tributary of the Vaal River and has a catchment area of 325 $km^{2.}$ It is located in a sub humid, warm, wet region that has cool dry winters. This river is located in the Middle Vaal WMA and is one of the 5 major rivers in this WMA. The Schoonspruit River has been divided into distinct reaches according to the riparian zone vegetation as well as the type of ecosystem dominating the catchment. The upper Schoonspruit is characterised by a wetland habitat. Below Ventersdorp, a narrow channel is found and the riparian zone is dominated by willows and white poplars. The lower Schoonspruit is an extensive wetland system (DWA, 2007).

The Schoonspruit River is fed by dolomite springs in the upper regions of this catchment (DWAF, 2004c). It supplies a huge part of Ventersdorp with its urban and irrigation water requirements. Pollution in the Schoonspruit River has largely been contributed by diamond digging operations located on the bank of the River (DWAF, 2004c). As a result, of these mining operations, the Schoonspruit River impacts water quality in the main stem of the Vaal River immensely (WRC, 2008). Thus, boreholes in the Schoonspruit dolomite compartments, located at mines and municipalities, are monitored regularly for the purpose of compliance monitoring (DWAF, 2004c). This is justified by beliefs that the interaction of groundwater and surface water occurring within the dolomites in the Schoonspruit area, contribute close to 26.7 million m^3/annum of water associated with salt loadings to the Vaal River. Little data is available on the Schoonspruit River, however, a catchment management strategy is being developed (DWAF, 2004c).

2.3.4 Vaal River

The Vaal River is South Africa's largest catchment and is divided into three WMA's: Upper, Middle and Lower Vaal (DWAF, 2009). It originates from the Drakensburg escarpment and drains majority of South Africa's central Highveld. The Vaal River catchment area flows through five provinces namely: parts of

Gauteng, Free State, Mpumalanga, North West and the Northern Cape. The Vaal River is the largest tributary of the Orange River in South Africa and is used to meet the industrial needs of the Johannesburg area and a large part of the Free State Province. Water flow in the Vaal River is regulated by the Grootdraai, Vaal and Bloemhof Dams as well as a number of weirs. The large weirs include the Vaal Barrage, Vaalharts, and Douglas Barrage (Hendriks and Rossouw, 2009). Water drawn from the Vaal River supports 12 million consumers in Gauteng as well as smaller towns in the NWP including Vryburg. Pollution in the Vaal River, as a result of ongoing sewerage spills, is a serious problem and places huge stress on water supply in the country.

2.3.4.1 Upper Vaal Water Management Area

The Upper Vaal WMA covers part of Gauteng, Free State Province, Mpumalanga Province and North West Province. Major rivers in this WMA include Wilge, Klip, Liebenbersvlei, Waterval, Suikerbosrand and the Mooi River. The Upper Vaal WMA is regulated by the Grootdraai, Vaal and Sterkfontein Dams (Hendriks and Rossouw, 2009).

Climate in the Upper Vaal WMA is characterised by seasonal (summer) rainfall patterns which peak during December and January. The mean annual temperature ranges between 16°C in the west to 12°C in the east (Grobler and Ntsaba, 2004; Hendriks and Rossouw, 2009).

Land use activities occurring between Grootdraai and the Vaal Dam include agriculture (maize, wheat) as well as urban centres in Bethlehem and Harrismith (Hendriks and Rossouw, 2009). The predominant minerals found in the Upper Vaal WMA include gold, uranium, base metals, semi-precious stones and industrial minerals. Gold, uranium and coal mining are, however, of particular economic importance (DWAF, 2004). Water quality in this WMA is impacted by seepage from waste facilities, industries, waste water treatment plant discharges

and the mining of the above mentioned minerals (DWAF, 2004a; 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 WMA's in the country (DWAF, 2004a).

2.3.4.2 Middle Vaal Water Management Area

The Middle Vaal WMA flows from upstream Bloemhof right to the confluence of the Vaal and Rietspruit Rivers, and is located in a semi-arid region (DWAF, 2002b). It runs through the North West and Free State Provinces and is integrally connected to the Upper and Lower Vaal WMA's. Major rivers in this WMA include Schoonspruit, Rhenoster, Vals, Vet and Vaal Rivers. 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-Orkney as well as the Welkom-Virginia areas (DWAF, 2009c).

Land use activities in the Middle Vaal WMA contribute 4% of South Africa's GDP. Furthermore, the land use activities in this WMA include extensive dry land agriculture (wheat, maize, sorghum, sunflower), and gold mining (DWAF, 2002). Water quality is impacted by return flows in the form of treated effluent from urban areas and mines. In addition, high nutrient wash outs from the agricultural lands result in periodic algal blooms which have aesthetic effects on the water in the WMA. Water is also subjected to high TDS levels (DWAF, 2009).

2.3.4.3 Lower Vaal Water Management Area

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

Vaal Rivers (Grobler and Ntsaba, 2004). Water flowing into this WMA is dependent on water releases from the Middle Vaal WMA. Therefore, water quality in this region is strongly influenced by the high salty water leached from the Vaalharts irrigation scheme and water flowing from the Upper and Middle Vaal WMA. Land use activities occurring in the Lower Vaal WMA comprise livestock agriculture (beef, cattle and goats), stock farming, dry land cropping (maize, ground nuts and vegetables) and irrigation. In addition, there is mining of diamonds, iron ore, manganese and minerals such as dolomite and lime (Grobler and Ntsaba, 2004).

2.4 Water borne diseases

The South African National Environmental Management Act (Act 107/1998) stipulates that everyone has the right to an environment that is not harmful to their health and wellbeing. As a result, an all inclusive understanding of the inter-relationship found between water and human health is essential for the sustainable management of water quality, so as to attain optimum human health gains (CSIR, 2010b). In South Africa, water-borne diseases are a major concern (DNT, 2011). The National Environmental Health Policy (DoH, 2011), states that South Africa's location is encompassed by developing countries that have environmental health challenges such as water-borne diseases. Further, it adds that the population migration across the borders is an influencing factor in the spread of diseases. Disease causing microbes are easily spread in the environment while some communities are still reliant on untreated water from easily accessible surface water systems. This places the inhabitants at a vulnerable position especially with the case of water-borne diseases (DWAF, 2002a).

The National Environmental Health Policy (DoH, 2011), states that water-borne diseases are transmitted mainly through poor water quality, human contact, eating using contaminated utensils, food and soil. It has been 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 five years while 60% are related to people receiving lower than the acceptable basic level of service (DWAF, 2010). The National Environmental Health Policy (DoH, 2011) explains that children of 5 years and younger are the most susceptible to diarrhoea caused by amongst others lack of potable water. According to the mortality and causes report issued by Statistics South Africa (2010b), intestinal infectious diseases are among the leading underlying natural causes of death through all age groups. Additionally, this report confirmed that the leading cause of infant mortality (22.4%) in 2008 was attributed to intestinal infectious diseases. While deaths for children aged 1-4 years (27.3%) were due to intestinal infectious diseases.

Although not reported in South Africa, incidents have been found in many parts of the world where epidemics relating to contact water sports have been found (WHO, 2005). Several studies have illustrated that human and animal waste discarded in water used for full body contact activities can have an effect on humans using the water mostly resulting in gastroenteritis, acute respiratory disease, eye, ear and skin infections (Saliba and Helmer, 1990; Dwight *et al*., 2004).

2.5 Water quality monitoring

In water resource management, a lot of emphasis revolves around ensuring that users have sufficient quantities of water. However, surfacing threats of water scarcity, as well as increases in the amount of water being used and re-used daily, it is the quality thereof that begins to take a dominant role (DWAF, 2004a). Water quality is defined on the basis of its physical, chemical, biological and aesthetic characteristics and determines the health and integrity of an aquatic ecosystem (DWAF, 1996a). According to Lemarchand and co-workers (2004), water quality is taken for granted even though health risks from polluted water remain a major public concern. The lack of access to good quality drinking water and sanitation are the cause of huge health impacts such as diarrhoeal diseases (Rijisberman, 2006).

In 2003, the World Health Organisation (WHO) reported that 1.2 billion people in the world lacked access to safe and affordable water for domestic purposes. Furthermore, it has recently been estimated that by the year 2025 the demand of water in South Africa will exceed its supply (DWAF, 2010). This validates the statement made by DWAF that water quality has still not yet taken its rightful place in integrated water resource management (DWAF, 2004a).

The main objective for water quality monitoring is to control and minimize the incidence of pollutant-oriented problems, so as to provide good quality water for drinking, irrigation and other purposes (Boyacioglu, 2006). Baltaci and co-workers (2006) stated that consistent and comparable long-term water quality monitoring is essential in order to:

- describe the status and trends of a water resource;
- identify existing and emerging water quality issues as well as
- determine compliance with regulations

Water quality monitoring is imperative for the protection of surface water resources. It provides water resource managers and politicians with information they need to make all necessary decisions regarding the equitable access, sustainable, efficient and effective use of water. Much of the information needed will be generated by water quality monitoring programmes (Van Niekerk, 2004).

According to Plummer and Long (2007), monitoring programs often include a large number of water quality parameters in order to fully capture contaminant issues in a watershed. These authors list the physical and chemical parameters generally monitored as pH, temperature, dissolved oxygen (DO), organic matter, particulate matter and electrical conductance. While the most commonly used measures of microbiological water quality assessments are indicator organisms such as total and faecal coliforms (Plummer and Long, 2007).

As the custodian of South Africa's water resources, the Department of Water Affairs and Forestry (DWAF), is responsible for ensuring that the country's water

systems are fit for various use while remaining sustainable (Murray, 1999). In addition, surface water systems in South Africa, are usually assessed for their suitability of domestic and irrigation water use (Hohls *et al*., 2002). A number of physical, chemical and biological constituents are usually assessed as they may possibly have an effect on the suitability of water for a specific use (DWAF, 2004a). As a result, a number of water quality monitoring programmes have been set up and are functioning.

2.5.1 National Chemical Monitoring Programme

This programme was started with the aim of monitoring water quality in the country with initially only pH, EC and inorganic ions being the measured variables. However, an increase in the amount of nutrients flowing into the rivers from all non-point and point source pollutants resulted in many rivers becoming eutrophic (Van Niekerk, 2004). Thus, there arose a need to expand this programme adding total and dissolved phosphate, ammonium and total nitrogen. (www.dwa.gov.za/iwqs/water_quality/NCMP/default.htm).

2.5.1.1 Physical variables monitored during water quality monitoring

2.5.1.1.1 Temperature

According to Ahipathy (2006), temperature levels of rivers are influenced by factors such as the geographic location of the river, season of sample collection as well as the temperature of effluents entering the river. Makhlough (2008) further listed latitude, altitude, time of day, air circulation, water flow (laminar, stagnant etc) and the depth of a water body as factors influencing temperature levels. However, changes in water temperature can lead to changes in an aquatic community's abundance, diversity and species composition (Dallas and Day, 2004). Thus, temperature levels of a water body are important and should always be measured since many other water quality variables such as pH, electrical

conductivity, nitrate and phosphate are influenced by it (DWAF, 1996; Jensen and Anderson, 1992). According to the WHO (2008), elevated water temperatures enhance the growth of microorganisms which may have an effect on the aesthetic properties of water.

2.5.1.1.2 pH

The pH of a water resource is an indication and measurement of the waters hydrogen ion activity (DWAF, 1996b). pH levels in various parts of the country are generally influenced by surrounding anthropogenic and biological activities (Dallas and Day, 2004). During water quality monitoring, pH measurements of a water resources may prove to be useful and important for the following reasons:

- pH controls the solubility of many metallic elements whose concentrations in natural waters are pH-dependent (Frengstad *et al*. 2001; Banks *et al,.* 2004).
- pH determines the suitability of water for various purposes such as toxicity to animals and plants (Venkatesharaju *et al.*, 2010).

High pH values with a median of 8.3 are observed in rivers, streams and surface waters in some semi-arid parts of the world (Banks *et al*., 2004). Whereas, the sources of low pH values are attributed to by chemical pulp, paper, leather industries as well as acid mine drainage from coal and gold mine dumps located near the surface waters (Dallas and Day, 2004). Neutral pH values of 7.0 in water sources result from complex acid-base equilibria found in various dissolved compounds such as carbon dioxide bicarbonate-carbonate equilibrium system at a temperature of 24°C (DWAF, 1996c). However, the change of a soils pH into more acidic forms will affect metals such as Fe and Mn oxides in sediment resulting in their mobilisation (Levy *et al*., 1992). Furthermore, the pH and redox potential of these sediments may be altered due to dredging and can thus influence mobilization of sediment bound metals (Cappuyns and Swennen, 2005).

The South African pH Target Water Quality Range is 6.0-9.0 and consumption of water within this range will have no significant impacts on health. Levels of pH 11.0 and greater will pose severe danger to health as a result of the effects of consumption of deprotonated species (DWAF, 1996c).

2.5.1.1.3 Total Dissolved Solids and Electrical Conductivity

Total dissolved solids (TDS) and electrical conductivity (EC) are all inorganic salts and small traces of organic matter present in a solution of water (DWAF 1996a; WHO, 2008). The TDS value of most water bodies originates from natural sources such as sewage, industrial wastewater, urban and agricultural run-off (WHO 2003a). Dallas and Day (2004) listed all soluble matter constituents as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium, all of which carry an electrical charge. In addition Dallas and Day (2004) stated that the amount of TDS arising from natural sources is usually determined by characteristics of geological formations found, either in the water or where the water is in contact with the formation/s. Thus, the amount of TDS in different water bodies is variable due to the solubility of the minerals found in rocks, soils and plant material within those areas.

The National Water Resource Quality Status Report (Hohls, 2002) stated that the main water quality problem for domestic use, related to the widespread and elevated salt levels (high TDS) throughout the country. The current South African TWQR for TDS levels in water used for livestock watering of cattle and horses in purposes is 0-2000 mg/L while it is 0-3000 mg/L for sheep.

While TDS itself may be only an aesthetic and technical factor, high concentrations of TDS are an indication that harmful contaminants such as iron, manganese, sulphate, bromide and arsenic can also be present in the water (Dallas and Day, 2004). Particularly when the excessive dissolved solids are added to the water as human pollution, through runoff and wastewater discharges (Dallas and Day, 2004).

Electrical conductivity (EC) is directly proportional to TDS and is a measure of the ability of water to conduct an electrical current (Dallas and Day, 2004). However, EC is much easier to measure than TDS and is thus used as an estimate of the TDS concentration. The higher the conductivity, the greater the number of ions, and thus also the dissolved concentration of salts, such as carbonate, bicarbonate, chloride, sulphate, nitrate, sodium, potassium, calcium and magnesium, all of which carry an electrical charge. A measure of conductivity does not include un-ionized solutes, such as dissolved organic carbon (Dallas and Day, 2004). The current South African TWQR of EC in water for use in irrigation is 0-40 mS/m (DWAF, 1996c).

2.5.1.2 Chemical variables monitored during water quality monitoring

Nutrients are chemical compounds or elements that can be utilised by plants for growth (Dallas and Day, 2004). Industrial, agricultural and medical sectors have introduced many chemicals into the environment (UNDP, 2008). While human activities require the use of products such as pesticides, household detergents and pharmaceuticals (CSIR, 2010a). These products however contain nutrients such as nitrogen and phosphorus which are released into the environment and easily converted into their available forms (Dallas and Day, 2004).

According to Hill and Olckers (2001) South African rivers and dams usually receive nitrates and phosphates from anthropogenic activities. However, when found in high concentrations within a water system these nutrients may result in eutrophication altering the structure and functioning of biotic communities (Davies *et al*., 1998). Walmsley (2000) defines eutrophication as a process where a water body is enriched with plant nutrients. Eutrophication is a widespread global problem and a serious problem in numerous catchments in South Africa (Nyengye and co-workers, 2010; van Ginkel, 2011).

A surface water system is said to be eutrophic when metabolic products increase such that the occurrence of cyanobacterial bloom forming species are enhanced

(Oberholster and Ashton, 2008). The problem however, is that cyanobacteria are capable of producing toxic secondary metabolites known as cyanotoxins which present a potential risk to public health (Paerl *et al.* 2001). According to Codd and co-workers (1999), cyanobacterial toxins are a human health threat when ingested accidentally during recreational activities. However, cases of skin irritation and allergic reactions subsequent to contact with cyanobacteria during water contact sports have been reported (Bell and Codd, 1994; Pilotto *et al*., 1997). Codd and co-workers (1999) further listed several major reported symptoms and these included mouth blisters, dermatitis, ear irritations and eye inflammation.

2.5.1.2.1 Nitrites and Nitrites

Nitrogen is an important nutrient in the eutrophication process (Walmsley, 2000). Furthermore, this nutrient is found in water sources due to natural and anthropogenic sources including agricultural run-off (Yang *et al.,* 2007). The most common ionic reactive forms of dissolved inorganic nitrogen in aquatic ecosystems include Ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) (Wetzel, 2001). Under aerobic conditions, ammonium is oxidized to nitrate in a two step process known as nitrification within which nitrite is the intermediate product (Dallas and Day, 2004).

Besides their central role in eutrophication, these nutrients have several human health effects when found in drinking water sources. According to Honikel (2008), nitrites are more toxic than nitrates when absorbed into the bloodstream of humans because they can convert hemoglobin to methemoglobin. Nitrite is also a precursor of toxic and carcinogenic *N*-nitrosamines (Bassir and Maduagwu, 1978; Walker, 1990). These *N*-nitroso compounds are capable of inducing tumours at multiple organ sites including the colon (Ward *et al*., 2005).

However, Gatseva and co-workers (2008) have argued that nitrate is the most common chemical contaminant that is harmful to human health because its toxicity is manifested by increased levels of methemoglobin and formation of carcinogenic

compounds (Gatseva *et al*., 2008). While Sadeq and co-workers further validated that problems associated with nitrate in drinking water are particularly alarming when considering its effects on infants 6 months and younger. Infants are sensitive to nitrate because it induces methemoglobinemia in their bodies due to factors such the high foetal haemoglobin content in their bodies (Sadeq *et al*. 2008). Lastly, it has been proven that increased nitrate intake can affect the thyroid function as nitrate in drinking water has been recognized as a factor for enhanced goitre (Gatseva *et al*. 1998; 2008).

2.5.1.2.2 Phosphates

Phosphorous as phosphate is one of the limiting nutrients which can over fertilize aquatic plants resulting in eutrophication (Oberholster and Ashton, 2008). High concentrations of phosphate occur in waters that receive sewage, crop residues, leaching human and animal wastes as well as runoff from cultivated lands (Dallas and Day, 2004).

Measuring phosphate during water quality studies is important because phosphate levels can be used as a tool to understand the health of a system and help identify possible sources for phosphate introduction to surface waters. Compared to nitrate, phosphate is regarded more of a limiting factor in eutrophication because some bacteria and algae are able to fix atmospheric nitrogen and convert it to more oxidisable states of nitrates and nitrites (Wentzel, 1990).

2.5.2 National Microbial Monitoring Programme

The National Microbial Monitoring Programme was initiated in 1994 with its main monitoring variables being *E. coli* and faecal coliforms, which are monitored bi-weekly. The aim of this programme was to aid in the assessment of management effectiveness of faecal pollution.

In 1994, it was proposed that the trends of faecal pollution as well as its status should be assessed (Kühn *et al*., 2000). In 1996 a conceptual design for monitoring the microbial quality of national surface waters was compiled (Kühn *et al*., 2000). The national microbial water quality monitoring program was designed upon the recognition that information on the microbial water quality status of South Africa's water resources was limited (DWAF, 2002c).

The program was implemented on a pilot scale in the beginning stages. However, its goal at large is to provide the necessary information required, on a national level, to assess and manage faecally polluted surface water systems while considering the potential health risks of water users when exposed to these waters (Murray, 1999).

The NMMP is centralised around two objectives which aim to:

- provide information on the microbial quality of surface water resources in priority areas by reporting on the status and trends as well as extent of faecal pollution in priority areas;
- provide information which will elucidate the potential health risk of humans associated with the possible use of faecally polluted water resources (Murray, 1999).

In addition, the program was designed in such a way that it focuses on priority areas only. This was decided upon considerations such as the unevenly distributed nature of microbial pollution. The priority areas are identified by two general criteria:

- land-uses that are typically associated with faecal pollution of water resources;
- the number of people likely to be impacted upon by exposure to water of poor microbial quality as a consequence of the way they use the water. (DWAF, 2002c).

The NMMP is still operational and bi-monthly reports are made available by DWA online. Priority areas chosen on all 19 WMA's are assessed and variables such as *E. coli*, pH, temperature and turbidity are measured. Thereafter, information on the suitability of water for drinking prior to treatment, full or partial contact, irrigation and drinking after treatment is provided at the Department of Water Affairs and Forestry website: (http://www.dwaf.gov.za/iwqs/microbio/nmmp.aspx). Based upon the success of the current implementation, the full scope will eventually include groundwater resources used for domestic, recreational, and irrigation of edible crops consumed raw. It is also planned that estuaries predominantly used for recreational purposes will also be included (DWAF, 2002c).

Water derived from open sources such as rivers, is frequently exposed to faecal pollution (Fremaux *et al*., 2008). Concerns over water quality have greatly risen over the years due to the increasing bacterial, viral and protozoan pathogens found in open water sources. This has inevitably increased the transmission of pathogens and water borne diseases occurring via the faecal oral route (Savichtcheva *et al*., 2006). Jamieson and co-workers (2006) suggested that the primary source of microbial pollution is faecal matter generated from livestock production. Kirschner and co-workers (2009) further suggested that microbiological loading into water resources occurs from point sources discharging both treated and untreated sewage from human and/or livestock while the non-point sources are classified as urban and agricultural runoff. As a result untreated surface water may contain faecal matter which constitutes human pathogens such as: pathogenic *E. coli* and faecal streptococci strains. Improvement of water resources would include controlling microbial levels at the source in order to provide microbiologically and chemically safe drinking water (Hong *et al.,* 2010).

2.5.2.1 Concept of indicator organisms

The concept of indicator organisms in water microbiology is well established and has provided a large framework for assessing the microbial quality of drinking

water. Microbiological methods imply the detection of a single micro-organism which is hosted specifically by one of the pollution sources. Faecal indicator bacteria (FIB) residing in the gastrointestinal tracts of humans and animals, are commonly used to assess the microbiological safety of drinking and recreational waters (Haller *et al.,* 2009). Various indicator organism levels are used to distinguish between faecal pollution of humans and animal origin during the wet and dry season (Sankarmakrishnan and Guo, 2005). Jagals and co-workers (2006) emphasized that testing water for the presence of indicators and health-threatening contaminants may be an indication of the presence of pathogens and that negative testing for the presence of indicators does not necessarily imply the absence of pathogens.

Compulsory laws generally monitor and enforce standards for drinking water quality, using bacterial indicators of faecal pollution and pathogens (Wilkes *et al*., 2009). Host specificity comes on top of other prerequisites for a good indicator of faecal contamination such as persistence in environmental waters or the absence of growth outside hosts (Wéry *et al.,* 2009). The Department of Water Affairs and Forestry (1996a) defined requirements that indicator organisms should fulfil and each organism should:

- be suitable for all types of water;
- be present in sewage and polluted waters whenever pathogens are present;
- be present in numbers that correlate with the degree of pollution;
- be present in numbers higher than those of pathogens;
- not multiply in the aquatic environment;
- survive in the environment for at least as long as pathogens;
- be absent from unpolluted water;
- be detectable by practical and reliable methods and
- not be pathogenic making them safe to work with in the laboratory.

However, indicator organisms used for water quality monitoring programs do not satisfy all the requirements. Instead a wide variety of indicators commonly used

have their own advantages and disadvantages. Therefore, routine water monitoring programs include appropriate combinations of indicators that test for the presence of pathogens of major importance (DWAF, 2004). Indicator organisms generally recommended for assessment of the microbiological quality of domestic, surface, and Groundwater for contamination include: Heterotrophic plate count bacteria, total coliform bacteria, faecal coliform bacteria (*E. coli*), enterococci (faecal streptococci) and Bacteriophages (Kim *et al.,* 2005; DWAF, 2004).

2.5.2.1.1 Total coliforms

Total coliforms are gram negative, non-spore forming bacilli, aerobic or facultative rod shaped anaerobic bacteria that ferment lactose with gas formation within 48h at 35°C. A well defined characteristic of total coliforms is their ability to produce the enzyme β-galactosidase during lactose fermentation (WHO, 2008). Total coliforms are normally inhabitants of the intestine of humans and warm blooded animals, thus they are usually sources of faecal pollution (Rompré *et al*. 2001; Wutor *et al*. 2009).

Studies have indicated that coliform bacterial levels in surface water often increase after a rain event (Kistemann *et al*., 2002; Mehaffey *et al.,* 2005). Furthermore studies in the past have indicated that the rate at which coliform bacteria decay, is strongly associated and increases with elevated water temperatures, pH and nutrient scarcity (Flint, 1987; Curtis *et al.,* 1992; Davies *et al.,* 1995). Hong and co-workers (2010) found that the physico-chemical properties of water correlated highly with the survival, decay and growth rate of coliform bacteria. In addition, total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms.

The coliform group is used widely as an indicator of water quality (Rompré *et al.,* 2001). However, the suitability of coliform bacteria as an indicator of faecal contamination in aquatic systems has been a subject of debate recently. Arguments regarding the appropriateness of total coliforms as indicators of faecal

pollution have come up because some studies have revealed they can be of an environmental origin (Evans *et al*., 1968; WHO 2008). Rompré and co-workers (2001) stated that total coliforms are usually found in diversified natural environments. According to the WHO (2008) total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters and that even though total coliforms are excreted in the faeces of humans and animals, some are heterotrophic and able to multiply in water and soil environments. Thus the presence of total coliforms during water quality monitoring should not be used as an indicator of faecal contamination but rather an indicator of to what extent water should be treated.

The South African water quality guidelines for domestic use (DWAF, 1996a) reported that total coliform bacteria are primarily used as practical indicators of the microbial quality of water which are mainly used in routine monitoring of drinking water supplies. Epidemiological studies have documented an increased risk of contracting gastrointestinal and respiratory illnesses after contact with waters with elevated concentrations of coliform bacteria (Dewailly *et al*., 1986; Haile *et al*., 1999).

2.5.2.1.2 Faecal coliforms

Faecal coliforms are a subgroup of total coliforms, they are derived from the intestine of warm blooded animals and include thermo tolerant coliforms which have the ability to grow at a temperature of 44.5°C (WHO, 2001). A study by Hong and co-workers (2010) illustrated that higher levels of faecal coliforms were observed during the wet than the dry season which could be explained by a suggestion that storm water runoff served as an important carrier of coliforms flushed into reservoirs during the wet season (Hong *et al*., 2010).

The use of faecal coliforms as indicators of faecal pollution has been questioned and limitations have been found in their use and applicability (US EPA, 2000;

Boehm *et al*., 2009). Tyagi and co-workers (2006) stated that faecal coliforms are unreliable indicators of faecal pollution and this statement has been justified by numerous studies. Faecal coliforms have been reported to possess the ability to multiply in water systems (Evans *et al*., 1968; Desmarais *et al.,* 2002; Solo-Gabriele *et al*., 2000). Furthermore, several studies indicated that faecal coliforms have a short survival in water systems making their detection erroneous (Savichtcheva and Okabi, 2006). It has also been suggested that not all faecal coliforms are of faecal origin (Scott *et al*., 2002; Simpson *et. al*., 2002).

Escherichia coli are a species group belonging to the faecal coliform bacteria and they are commonly used as indicators of faecal pollution in contaminated surface waters (Garizo-Hadzik *et al.,* 2010). According to Pachepsky and Shelton (2011), *E. coli* are frequently also used during microbial source tracking studies because the various pathogenic strains such as enteropathogenic, enterotoxigenic, enterohemorragic *E. coli* make them appropriate for such studies.

2.5.2.1.3 Faecal streptococci

Faecal streptococci are Gram-positive; catalase negative cocci that are not inhibited by bile salts (Lemarchand *et al*., 2004). The WHO (2008) described this group as relatively tolerant of sodium chloride and alkaline pH levels. Faecal streptococci are members of the human and animal gastrointestinal flora and are therefore present in high numbers in faeces and can be found in food and water (Rincé *et al*., 2003).

The faecal streptococci group has undergone several taxonomic changes in recent years due to a progressive increase in the number of novel species (Naser *et al*., 2005). Schleifer and Kilpper-Bälz (1984) stated that streptococci belonging to the serological group D can be divided into two physiologically different groups the: *Streptococcus faecalis* and *Streptococcus faecium* which are placed in the Enterococcus division of the Streptococci. Enterococci and their subset faecal streptococci have the ability to grow in a medium containing 6,5% NaCl, in an agar

medium with a pH of 9.6 as well as a wide temperature range 10°- 45°C (Harwood *et al*. 2000).

According to Bitton (2005), the faecal streptococci group consists of *Streptococcus bovis,* S. *equinus,* S. *alactolyticus,* S. *intestinalis,* S. *hyointestinalis* and S. *acidominumus* however, Holt and co-workers (1993) further list *Enterococci faecium, E. gallinarum, E. durans, E. faecalis, E. cecorum,* and *E. hirae* as subsets within the faecal streptococci species group. In addition it has been proposed that some of the species (*Enterococcus casseliflavus, E. gallinarum, E. mundtii, E. sulphureus*) classified within this group are of environmental origin and their placement there in is highly questionable.

The current doubts regarding applicability of using faecal coliforms as indicators of faecal pollution have resulted in recommendations that faecal streptococci be used as a supplementary indicator (WHO, 1996). Faecal streptococci are used as an index of faecal contamination in water because they are easily expelled into the environment via human and animal excretes (Rincé *et al*., 2003). A study by Pinto and co-workers (1999) showed that more than 80% of faecal streptococci isolated from a variety of polluted water sources were true faecal species (Pinto *et al.*, 1999).

The survival of this group has been reported to be longer than that of faecal coliforms while they have shown persistence patterns similar to those of waterborne pathogenic bacteria (Dionisio and Borrego, 1995). Furthermore it has been proven that faecal streptococci are more resistant to drying and chlorination while with most of the species not able to multiply in the environment (WHO, 2008).

Faecal streptococci are responsible for serious human and animal diseases such as endocarditis, meningitis, intra-abdominal, urinary tract, wound and other infections (Rincé *et al*., 2003). Beta (β) hemolytic streptococci have been associated with weakening disease processes such as pneumonia, septicaemia and opportunistic infections.

2.6 Methods used for the identification of faecal streptococci

The conventional methods used for the routine identification of species are based on physiological and molecular characteristics (Gledreich and Kenner, 1969; Manero and Blanch, 2002). Furthermore, the ability of faecal streptococci to grow under particular conditions is widely used in their selective isolation (Knudston and Hartman, 1992). Several proposed biochemical tests used during the identification of faecal streptococci are available in tables or biochemical keys (Devriese *et al*., 1993; Knudtson, and Hartman, 1992).

2.6.1 Biochemical identification of faecal streptococci

2.6.1.1 Gram staining

Gram staining is a differential stain used to classify bacteria into 2 groups: Gram-positives and Gram-negatives and serves as an important primary taxonomic tool (Beveridge, 2001). It is based on the ability of bacteria to retain the colour of the stains used such that their morphology and arrangement may be observed under a light microscope (Barthomew and Mittwer, 1952; Facklam and Elliot, 1995).

Structural differences are found between the cell wall anatomy of Gram positive and Gram negative bacteria (Salton, 1961). Gram positive walls are thick, highly impermeable as it is composed of approximately 25 layers of peptidoglycan with secondary polymers (Beveridge, 2001). The Gram negative wall is however more complex because it consists of a thinner peptidoglycan which does not have secondary polymers but an outer membrane made up of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol phospholipids as well as outer membrane proteins (Beveridge, 2001). According to Burke and Barnes (1929), the Gram reaction of bacteria is determined by permeability of Gram positive and Gram negative isolates which distinctly differ in cell wall composition. Due to the nature of their cell wall composition, Gram negative bacteria cannot retain crystal violet, are easily disrupted by organic

solvents such as ethanol and easily damaged during decolourization (Prescott *et al*., 2005).

2.6.1.2 Catalase activity in *Streptococcus*

Some bacteria contain flavoproteins which have the ability to reduce O_2 while producing hydrogen peroxide (H_2O_2) or superoxide (O_2^-). These products of aerobic metabolisms are toxic and powerful oxidizing agents (Harley and Prescott, 1999). Studies in the past have illustrated that hydrogen peroxide and superoxide have the ability to inactivate enzymes, cause DNA damage and lipid peroxidase injury (Flint *et al*., 1993; Huycke, 2002; Almagor *et al.* 1983).

Thus many bacteria contain enzymes that protect them against these toxic O_2 products. Catalase is an enzyme frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide (Serra, 2008). Therefore catalase activity by bacterial cells can be used for differentiating between bacteria that are morphologically similar such as *Enterococcus* (catalase negative) and *Staphylococcus* (catalase positive) (Fidanoski and Fidanoski, 2007).

2.6.1.3 Bile salts response found in faecal streptococci

The ability of streptococci to grow in a medium containing bile is one of the crucial biochemical tests during identification of faecal streptococci. The presence of bile salts in a medium inhibits the growth of Gram positive organisms other than group D streptococci being the faecal streptococci (Facklam *et al*., 1982). Rincé and co-workers (2003) stated that *Enterococcus faecalis* can be considered an interesting model for bacterial stress response studies because it is capable of growing under hostile conditions as well as survive many harsh environments that are usually detrimental for the development of mesophillic microorganisms. The main reason being that faecal streptococci are capable of developing a general resistance during their stationary growth phase as a result of glucose exhaustion or

oligotrophic conditions towards diverse stress factors such as heat, acid, H_2O_2 hypersmolarity, NaCl and UV radiation (Giard *et al*., 1997; Hartke *et al*., 1998).

2.6.2 Molecular identification of faecal streptococci

The identification of faecal streptococci using biochemical tests requires a large number of tests, and can prove difficult and time consuming for routine application (Moore *et al*., 2006). According to Manero and Blanch (1999) some faecal streptococci species lack phenotypic characteristics that typically define the species group (Manero and Blanch, 1999). Moore and co-workers (2006) explained that faecal strepococci species may vary by one phenotypic trait which can lead to possible errors when large numbers of isolates are biochemically tested. As a result, although traditional methods have been used for microbial identification, full and partial 16S rRNA gene sequencing methods have emerged as useful tools for identifying microorganisms (Petti *et al*., 2005). It has also been argued that the genotypic identification of microorganisms by 16S rRNA gene sequencing is more objective, accurate and reliable (Clarridge, 2004). Major advantages of using the 16S rRNA gene include the ability to identify species not normally seen or expected as well as the high possibility of identifying significant difference between the various faecal streptococci species (Kim *et al.,* 2010).

2.7 Characterisation of faecal streptococci

2.7.1 Haemolysis

Bacteria such as pneumococci, streptococci and staphylococci produce leukocidins known as membrane disrupting toxins that kill phagocycotic leukocytes. While other toxins such as haemolysins are secreted by pathogenic bacteria (Prescott *et al.*, 2005). According to Fidanoski and Fidanoski (2007), determination of the type of haemolysis a streptococcal species produces on blood agar is one of the first steps involved during accurate biochemical identification.

This test has been used as a diagnostic description characteristic of various *Streptococcus* species (Imai *et al*., 2009; Rahkila *et al*., 2011).

Three types of haemolysis patterns have been reported: alpha (α) haemolysis, β-haemolysis and Gamma (γ) haemolysis (Prescott *et al.,* 2005). β-haemolysis is indicated by a zone of complete lysis where bacterial growth had occurred on a blood agar plate. Furthermore, α-haemolysis is indicated by partial clearing of the blood and a green colour clearing on the bacterial colony growing on the blood agar. Organisms illustrating γ-haemolysis do not destroy blood cells and are illustrated by a white/greyish colony on the blood agar plates (Whiley *et al.,* 1990).

The detection of β-haemolytic organisms is an integral part of microbial water monitoring. Bhadaki and co-workers (1994) stated that most medically significant pathogens can produce pore forming proteins and induce haemolysis. Biedenbach and co-workers (2002) further added that diseases caused by β-haemolytic streptococci remain an important global health concern. β-haemolytic streptococci are responsible for acute and chronic disease including respiratory tract infections, endocarditis and meningitis while being associated with pneumonia and septicaemia (Ruoff, 2002; Johnson *et al.,* 2006; Kuiken *et al*., 2006).

2.7.2 Antibiotics

In recent years methods aimed at determining sources of pollution in a water system have been suggested, and the use of antibiotic resistance patterns has shown the most potential for success (Burnes, 2003). Several studies support the use of antibiotic resistance patterns in faecal streptococci to determine sources of faecal pollution in water over faecal coliforms (Wiggings, 1996; Hagedorn *et al*., 1999). The presence of antibiotic resistant bacteria in the natural environment was already marked in the 1990's (Davison, 1999). According to Barbosa and co-workers (2000), the wide spread use and application of antibiotics in agriculture, aquaculture, veterinary medicine and food technology was the main culprit.

According to Harwood and co-workers (2000), antibiotic resistant patterns in the flora of humans and animals are reflective of the extent to which exposure to antibiotics has occurred. Jiang and co-workers (2007) suggested that the use of multiple antibiotic resistance analysis is simple and inexpensive. These authors further explain that methods used for identification, of both point and non-point pollution, are grouped into three categories. These include the database dependent genotypic, the phenotypic typing and the library independent biomarkers (Jiang *et al*., 2007).

2.8 Summary

The literature has shown that South African water resources including those in the North West Province have been regarded as limiting. Furthermore, water resources in the country are regarded a social, environmental and economic entity. A large amount of the available water in the North West Province supplies agricultural, mining and industrial sectors with their water demands. However, increases in water demand, have increased the amount of point and non-point source pollution resulting in the deterioration of surface water systems.

It was also demonstrated that surface water resource's physical, chemical and biological aspects are used to determine the health and quality of a system. Therefore, a complete water quality assessment should include all three aspects in order to determine the integrity of a system, while also determining the possible sources of pollution.

The concept of indicator bacteria has been made available for the determination of the biological aspects. Most microbial water quality studies have used coliform bacteria as indicators of faecal pollution. However, several limitations have been found in their use and brought up arguments regarding their reliability as single indicators of water quality assessments. As a result, faecal streptococci have been recommended as they have proven to be more reliable indicators of faecal

pollution than faecal coliforms. Therefore, a section of the importance of faecal streptococci in water quality studies has also been included.

The determination of faecal indicator bacteria has also proved to be insufficient in determining all potential pollution sources. As a result, bacterial source tracking methods have been made available. These include determining the phenotypic as well as physiological characteristics. An overview of methods which could be used during physico-chemical and microbial water quality assessments have been discussed in the literature review.

CHAPTER 3

MATERIALS AND METHODS

The objective of this chapter is to outline all specialized materials and general procedures used in order to achieve the specific aim and objectives of the study.

3.1 Study area

The areas of interest were 4 rivers (Mooi, Harts, Schoonspruit and Vaal Rivers) and an Inland Lake (Barberspan) in the North West Province (Figure 3.1). Surface water samples were collected during one warm-rainy and one cold-dry season during 2010 and 2011. The geographical co-ordinates of all sampling sites were obtained using a Garmin nüvi 1310 (Garmin, US) Global Positioning System (GPS). More detail regarding the study areas is provided in Section 2.3. The co-ordinates of each sampling site are listed in Appendix A.

3.2 Sample collection

Surface water samples were collected between March and July 2010 as well as April and August 2011. This was aimed at ensuring that seasonal trends regarding the physico-chemical and microbial surface water quality were obtained for the sites. Two water collection techniques were employed and the use thereof was determined by the physical setting of each sampling site (US EPA, 1994). The direct sampling technique was employed at sites where water samples could be collected close to the river bank. However, where direct access to the surface water systems was limited, the dip sampling technique was employed. All surface water samples were collected aseptically in a sterile glass bottle and filled to the brim. Sterilised bottles were labelled before sampling and gloves were worn when handling the bottles. All samples were kept on ice in a sealed cooler box and analyzed within 6 (at most 12) hours of collection.

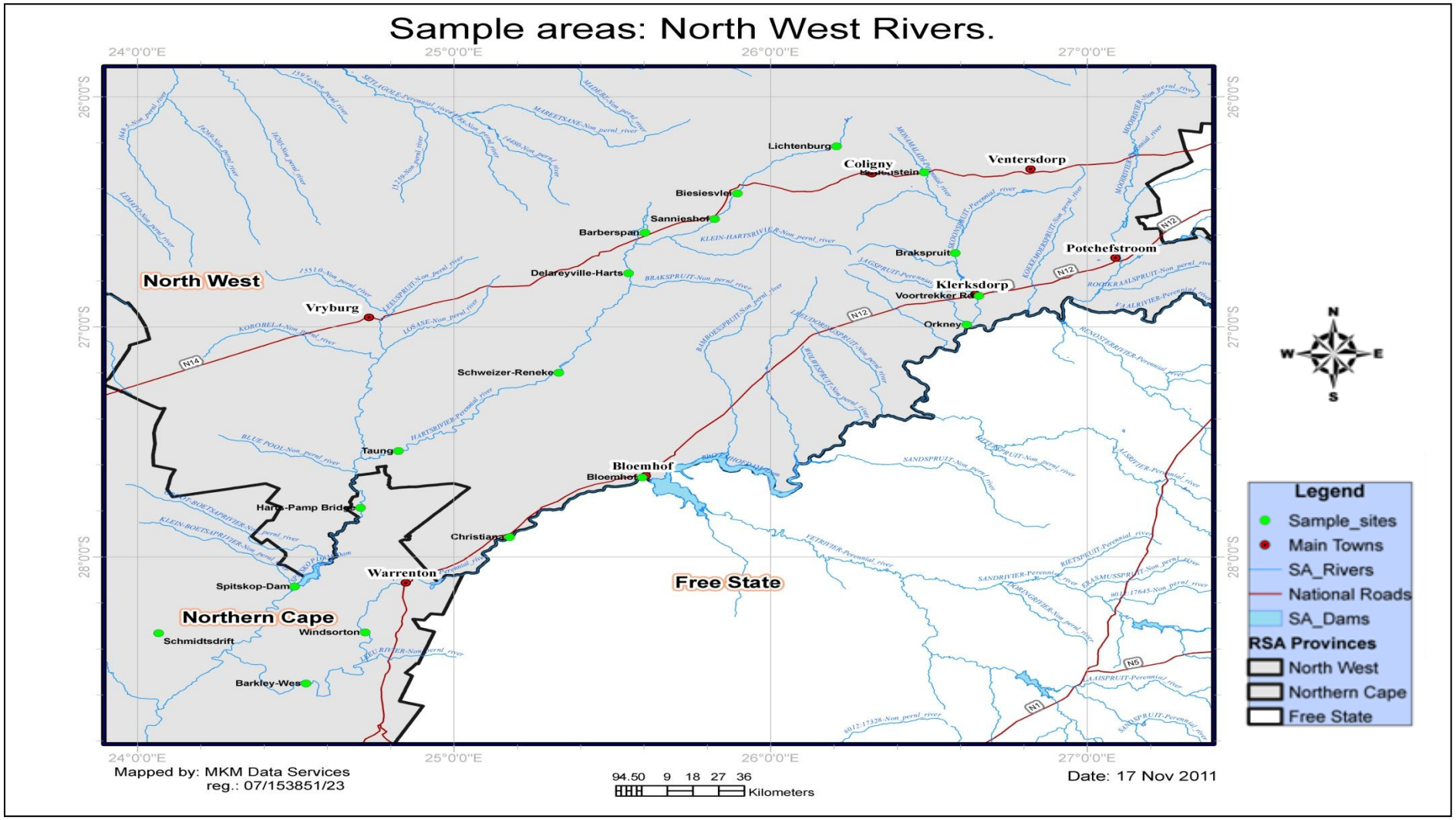

Figure 3.1: Map illustrating the spatial distribution and geographical location of each sampling site within the surface water systems of interest.

3.3 Physical and chemical analysis of surface water samples

The physical properties (pH, temperature, total dissolved solids and electrical conductivity) of the surface water systems were determined on site using a calibrated Oakton PCStestr^{TM} 35 waterproof field multi-parameter probe (Thermo Fisher scientific, US). The probe was rinsed with distilled water prior and after use. The chemical properties of the surface water systems [nitrites (NO_2^-), (CAT No: 21071-69); nitrates (NO_3^-), (CAT No: 21061-69) and phosphates (PO_4^{2-}), (CAT No: 2236-32)] were measured in the laboratory as milligrams per litre (mg/l) using a HACH DR 2800^{TM} (HACH, US). All physico-chemical values obtained were compared to the South African Target Water Quality Range (TWQR) for full and intermediate recreational contact, livestock watering and irrigation (DWAF, 1996d). All the applicable TWQR as set by the Department of Water Affairs and Forestry are summarised in Appendix B.

3.4 Microbiological analysis of surface water samples

Membrane filtration was used to isolate and enumerate all faecal streptococci, total coliform, faecal coliform and *E. coli* from the sampled surface water systems according to APHA (1998). Triplicates of 100ml water samples were filtered through 0.45 μm diameter, 47 mm grid PALL Corporation sterilised filter membranes [(PALL Life Sciences, Mexico) (CAT No: GN-6 Metricel Membrane 66191)] and placed on selective media: m-FC agar (Merck, Germany), MLG agar (Oxoid, UK) and KF-Streptococcus agar containing 1ml of 2,3,5-Triphenyltetrazolium chloride (TTC) per 100ml (Sigma Aldrich, South Africa). The filter funnel apparatus was treated with 70% Ethanol (ETOH) and washed thoroughly with sterile distilled water when analysing 2 different samples.

All m-FC agar plates were incubated at 44.5°C for 24 hours while all MLG agar plates were incubated at 37°C for 24 hours. The KF-Streptoccus agar plates were

incubated at 37°C for 48 hours. Colonies of interest on the surface of the filter membranes (blue and yellow colonies on m-FC agar as total coliforms, blue colonies on m-FC agar as faecal coliforms, green colonies on MLG agar as *E. coli* and pink colonies on KF-Streptococcus agar as presumptive faecal streptococci) were observed, counted and recorded as colony forming units (cfu) per 100ml.

3.5 Isolation and purification of presumptive *E. coli* and faecal streptococci

For isolation and purification purposes, selected pink colonies on KF-Streptococcus and green colonies on MLG agars were aseptically sub-cultured at least 3 times on nutrient agar using the streak plate technique and incubated for 24 hours at 37ºC.

3.6 Identification and confirmation of presumptive *E. coli*

Gram staining was used as the first step for confirming all presumptive *E. coli* isolates (see Section 3.7.1). The triple sugar iron (TSI) agar (Merck, Germany) test used for the further confirmation of presumptive *E. coli* isolates was performed according to (Fankhauser, 2001). Briefly, purified presumptive *E. coli* isolates were inoculated into a TSI agar slant using a zig-zag streak pattern and stabbed using an inoculation needle. These slants were incubated at 37°C for 18 hours. A positive TSI agar reaction test was indicated by a change in the colour of the agar from red to yellow as well the development of an air bubble in the test tube and the absence of a black precipitate indicative of prodcution of Hydrogen sulfide (H_2S).

3.7 Preliminary identification of presumptive faecal streptococci

3.7.1 Gram reaction

In this study, 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. Briefly, a bacterial smear was heat fixed and stained with a drop of crystal violet for 1 minute and rinsed with distilled water. A drop of Grams iodine was placed for 1 minute and rinsed with distilled water. Thereafter the smear was destained with ethanol for 10 seconds and washed with distilled water again. Lastly, the smear was counterstained with safranin for 1 minute and rinsed with distilled water once more. Schleifer and Kilpper-Bälz (1984) described the observation of a faecal streptococci isolate under a microscope as Gram positive, elongated, ovoid shaped cells in pairs or short chains. This staining procedure was done according to the method prescribed in Pandolfi and Pons (2004). Expected results for all presumptive faecal streptococci were indicated by a bacterial mat composed of deep purple stained cocci arranged in chains.

3.7.2 Catalase activity

Catalase and peroxidase are enzymes produced by obligate aerobes and facultative anaerobes (Rolfe *et al*., 1978). These enzymes hydrolyse hydrogen peroxide (H_2O_2) and yield water and free oxygen as end products. The catalase activity of all presumptive faecal streptococci isolates was determined using the agar slant method described by the Health Protection Agency (2010a). An isolate that liberated gas bubbles when exposed to 3% H_2O_2 was regarded catalase positive. Faecal streptococci are known to be catalase negative.

3.7.3 Bile solubility

Some *Streptococcus* species have autolytic enzymes capable of causing cell wall lysis during cellular division (Health Protection Agency, 2010b). The addition of bile

salts to these bacteria result in cell lysis. Faecal streptococci, however, do not disintegrate when exposed to bile salts. The bile solubility colony procedure was performed as prescribed by the Health Protection Agency (2010). Briefly, overnight bacterial cultures were spot inoculated onto 5% (v/v) sheep blood agar (National Health Laboratories, SA) and incubated at 37°C for 24 hours. One drop of a 2% (w/v) sodium deoxycholate (Sigma-Aldreich, US) solution in water was placed directly on all α-haemolytic colonies and incubated at 37°C for 30 minutes. All plates were incubated in an un-inverted position.

3.7.4 Temperature and salt tolerance test

The ability of the presumptive faecal streptococci isolates to grow at their diagnostic temperatures was determined. Isolates were streaked onto nutrient agar and incubated at 10°C and 45°C for 24 hours (Slantez and Bartley, 1964; Geldreich and Kenner, 1969). A positive result was illustrated by growth on the nutrient agar plate.

The tolerance of all presumptive faecal streptococci isolates to high salt concentrations was determined by their ability to grow in Brain Heart Infusion Broth (BHI) containing 6.5% (w/v) sodium chloride (NaCl). The BHI was prepared according to the manufacturers specifications and supplemented with (6.5%) NaCl. The BHI was obtained from Merck (Germany) while the NaCl was obtained from Sigma Aldrich (US).

3.8 Confirmation of faecal streptococci

Faecal streptococci isolates identified by biochemical tests were selected and 16S rRNA gene fragments were sequenced. The sequence data was used to confirm their identity.

3.8.1 Total genomic DNA isolation

Specific information on the protocol used during total DNA extraction, according to the manufacturer's extraction kit instructions is listed in Appendix D. Briefly, pure faecal streptococci isolates were cultured overnight at 37°C in BHI broth and then harvested by centrifugation. A genomic DNA isolation kit was used to extract total DNA [(Macherey-Nagel, Germany) (NucleoSpin® Tissue CAT No: 740 952.250)]. The concentration and quality (A_{260nm}:A_{280nm}) of the total genomic DNA isolated was determined using a NanoDrop TM 1000 Spectrophotometer (Thermo Fischer Scientific, US).

3.8.2 DNA amplification of presumptive faecal streptococci isolates

All PCR reactions were prepared in a laminar flow (Bioflow I) cabinet and DNA was amplified using an ICycler (Bio-Rad, UK) thermal cycler. A PCR reaction mixture with a final volume of 25µl was used and it contained RNase/DNase free water H_2O (Fermentas Life Sciences, US), 12.5 µl 2x PCR Master Mix (0.05U/µl *Taq* DNA Polymerase in reaction buffer, 0.4mM of each dNTP, 4mM MgCl2) (Fermentas Life Sciences, US), 0.5 µl primer (50µM/50pmol) and 1µl bacterial DNA template (50-100 ng/µl). The primer set used in this study had an expected amplicon size of 550bp. In addition, the primer sequences (5'-3') used were 341F:5'-CCTACGGGAGGCAGCAG-3' and 907R:5'CCGTCAATTCCTTTGAGTTT-3' (Muyzer *et al*., 1993). The PCR reaction was set such that initial denaturation occurred at 95°C for 300 seconds. Thereafter, the PCR mixture was subjected to 35 cycles of 30 seconds at 95°C, 30 seconds at 52°C and 60 seconds at 72°C, followed by a final extension step of 180 seconds at 72°C.

3.8.3 Agarose gel electrophoresis

All PCR amplifications were confirmed using a 1.5% (w/v) agarose gel (peqLab, Germany) in 1 x TAE buffer [40 mM Tris (Sigma Aldrich, US), 20 mM Acetic acid

(Merck, Germany), 1 mM EDTA, at pH 8.0 (Merck, Germany)]. A total of 1µg/ml Ethidium Bromide (Bio-Rad, UK) was added to the gel for visualisation of the PCR amplified fragments under UV light. Five microliters of the PCR product and 5 µl 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 amlification products. Electrophoresis was performed for 45 min at 80V using 1 x TAE buffer. A GeneGenius Bio Imaging System (Syngene, Synoptics, UK) was used to capture images using GeneSnap (version 6.00.22) software as prescribed by (Bezuidenhout *et al.,* 2006).

3.8.4 Sequencing and phylogenetic analysis

Amplified faecal streptococci fragments were sequenced by Inqaba Biotech (South Africa, Pretoria). 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).

3.9 Characterisation of faecal streptococci

3.9.1 Haemolysis patterns

The haemolytic activity of all faecal streptococci identified was determined in order to establish if they were potentially pathogenic. Haemolysis test 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. Haemolysis patterns were examined from subsurface colonies and all green colonies surrounded by a clear

margin were identified as α-haemolytic. Isolates showing β-haemolysis caused complete cell lysis and were identified by a clear zone where inoculation occurred. While, where no change occurred on the blood agar the isolate was classified γ-haemolytic (Health Protection Agency, 2008).

3.9.2 Antibiotic resistance patterns

Antibiotic susceptibility tests were performed on faecal streptococci isolates using the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). Overnight cultures of faecal streptococci were grown in Brain Heart Infusion Broth (BHI) (Merck, Germany) at 37°C and spread onto Mueller Hinton agar (Merck, Germany). The 10 antibiotics used included: Ampicillin (10 µg), Amoxicillin (10 µg), Penicillin G (10 µg), Neomycin (30 µg), Streptomycin (300 µg), Vancomycin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Oxy-tetracycline (30 µg), and Trimethoprim (2.5 µg). 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). All faecal streptococci isolates displaying resistance and susceptibility were recorded. A table regarding the zone diameter interpretive standards of *Streptococcus* can be found in Appendix C.

3.10 Statistical analysis

The statistical analysis of all physico-chemcial 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. In addition, the multiple antibiotic resistant cluster analysis of faecal streptococci isolates was determined using Ward's method and Euclidean distances in Statistica version 10 (StatSoft, US).

Canoco (Canoco for Windows Version 4.0, GLW-CPRO ©) was used to create Redundancy Analysis (RDA) multivariate ordination diagrams (Ter Braak, 1990). The RDA's were used to determine the relationship between dominant physico-chemical parameters and bacterial species isolated from the all 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).

CHAPTER 4

RESULTS

The combination of faecal indicator bacteria and physico-chemical levels used to assess water quality of selected surface water systems, over the study period enabled us to draw a clear picture of the faecal pollution patterns along their respective sampling sites. This chapter illustrates the results which are presented in three sections. The first subset reports the results of the 2010 physico-chemical and microbial water quality of 4 rivers (Mooi, Lower Harts, Schoonspruit and Vaal Rivers) sampled once during the warm-rainy season, and Barberspan sampled once in winter.

The second section of this chapter reports the 2011 physico-chemical and microbial water quality results of 3 rivers (Lower Harts, Schoonspruit and Vaal River) sampled during one cold-dry season in, as well as 1 river (Upper Harts River) and Barberspan which were sampled once during summer. In the last section, results are combined and compared.

4.1 Water samples collected in 2010

This section addresses the physico-chemical and microbiological quality of 5 surface waters sampled in 2010. The Mooi River was used as a preliminary study in 2010 and water samples were collected once a month for three consecutive months. However, only results from the third sampling trip have been presented below. Water samples from the Mooi River were used to optimise all methods outlined in Chapter 3. As a result, water quality results of the Mooi River have not been omitted but only presented with the 2010 physico-chemical and microbial quality results.

4.1.1 Physico-chemical analysis

Table 4.1 below briefly summarizes the physico-chemical parameters, temperature; pH; total dissolved solids (TDS); electrical conductivity (EC); nitrite (NO_2^-); nitrate (NO_3^-) and phosphate (PO_4^{2-}), of the 5 surface water systems sampled in 2010 based on values from each sampling site. Temperature values of the sampled surface water systems ranged from 12.00 to 26.00°C. These temperature ranges reflect the sampling period. Barberspan had the lowest overall average water temperature (13.90±1.41°C) as it was sampled in winter. The Mooi, Lower Harts, Schoonspruit and Vaal Rivers sampled during the warm-rainy season all had high temperature levels. However, the highest temperatures were observed at the Mooi River (24.23±1.08°C) and Lower Harts River (24.05±0.07°C).

It is evident from Table 4.1 that pH values of the 5 surface water systems ranged from 7.61 to 9.23. As depicted in Table 4.1, Schoonspruit River had the lowest overall average pH level (7.83±0.19) while Barberspan had the highest (8.76±0.31). The overall average pH levels of the Mooi, Lower Harts, Schoonspruit and Vaal Rivers supported the use of river water for full contact recreational activities as well as irrigation (Appendix B, DWAF, 1996d). However, the overall average pH level of Barberspan was unsuitable for the use of water during full contact recreational activities and irrigation.

Similarly in Table 4.1, TDS levels of the 5 surface water systems were measured and they ranged from 22.50 to 961.00 mg/l. The Vaal River had on average the lowest TDS levels (264.20±11.67 mg/l) and Barberspan the highest (899.75±70.00 mg/l). These differences were also reflected in the EC values. These values were suitable for livestock watering while only the values from the Vaal River indicated that the water from this river was suitable for irrigation (Appendix B, DWAF, 1996d). Variation in the pH, TDS and EC levels measured during the warm-rainy and cold-dry periods could be ascribed to the differences in water temperature.

Table 4.1: The physico-chemical and microbiological water quality analysis of each sampling site within the 5 respective surface water systems sampled in 2010.

River system	Sampling Site	Temperature (°C)	pH	TDS (mg/l)	EC (mS/m)	NO_2^- (mg/l)	NO_3^- (mg/l)	PO_4^{2-} (mg/l)	Total coliforms (cfu/100ml)	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100ml)	Faecal streptococci (cfu/100ml)
Mooi River	Klerkskraal Dam	23.60	8.36	265.00	39.00	2.00	0.50	1.42	154.30	52.00	6.00	17.30
	Muiskraal	23.10	8.01	306.00	47.00	0.00	0.20	0.86	65.30	51.30	10.00	62.00
	Around the World Bridge	23.00	7.76	466.00	70.00	4.00	0.60	0.68	69.30	60.00	17.00	25.00
	Thabo Mbeki Drive	24.30	8.22	427.00	64.00	3.00	0.10	0.81	78.00	59.30	6.00	19.30
	Trimpark North Bridge	24.60	8.25	443.00	65.00	3.00	0.40	0.02	118.00	55.00	1.30	42.00
	Pedestrian Bridge	25.00	8.25	443.00	66.00	9.00	0.30	0.12	116.30	59.00	12.00	147.00
	Viljoen Road Bridge	26.00	8.11	406.00	61.00	11.00	0.10	1.80	154.30	52.00	12.00	17.30
Mean		**24.23±1.08**	**8.14±0.20**	**391.43±75.55**	**58.86±11.39**	**4.57±3.95**	**0.31±0.19**	**0.82±0.64**	**107.93±38.03**	**55.51±3.86**	**9.19±5.16**	**47.13±47.02**
Lower Harts River	Dam 8	24.00	8.23	437.00	62.00	0.00	0.00	0.00	70.00	20.60	22.00	287.30
	Harts-Pamp Bridge	24.10	7.90	960.00	135.00	2.00	3.50	1.70	>315.00	242.00	76.00	44.30
Mean		**24.05±0.07**	**8.07±0.23**	**698.50±369.82**	**98.50±51.62**	**1.00±1.41**	**1.75±2.47**	**0.85±1.20**	**192.50±173.24**	**131.30±156.55**	**49.00±38.18**	**165.80±171.83**
Barberspan	Town-Harts	13.70	8.62	862.00	121.00	0.70	3.00	1.68	270.30	130.00	33.60	127.30
	Inflow	15.20	8.58	820.00	115.00	0.00	4.00	1.02	27.00	14.67	4.60	36.00
	Hotel	14.70	9.23	961.00	132.00	0.00	0.00	0.11	19.30	1.00	0.30	50.00
	Outflow	12.00	8.61	956.00	147.00	1.20	4.00	0.19	4.00	1.00	0.00	61.30
Mean		**13.90±1.41**	**8.76±0.31**	**899.75±70.00**	**128.75±14.06**	**0.48±0.59**	**2.75±1.89**	**0.75±0.74**	**80.15±127.13**	**36.67±62.55**	**9.63±16.12**	**68.65±40.45**
Schoonspruit River	Bodenstein	20.40	7.91	432.00	60.00	4.00	0.00	0.63	252.60	72.00	54.00	75.30
	Brakspruit	21.10	8.05	381.00	52.00	3.00	0.30	0.55	196.60	80.00	45.00	82.00
	Voortrekker	22.50	7.74	22.50	39.00	2.00	0.00	0.41	>315.00	134.30	44.00	56.00
	Orkney	21.70	7.61	367.00	52.00	3.00	1.70	2.44	>315.00	110.00	34.00	44.60
Mean		**21.43±0.89**	**7.83±0.19**	**300.63±187.51**	**50.75±8.69**	**3.00±0.82**	**0.50±0.81**	**1.00±0.96**	**269.80±56.98**	**99.08±28.62**	**44.25±8.18**	**64.48±17.24**
Vaal River	Bloemhof	18.00	8.05	265.00	37.00	3.00	0.00	0.00	132.00	86.60	20.30	66.60
	Christiana	19.00	7.86	**256.00**	36.00	4.00	0.40	0.00	120.00	78.60	34.00	165.30
	Windsorton	21.10	8.23	**256.00**	36.00	1.00	0.00	0.00	74.60	52.30	33.30	64.60
	Barkley-Wes	19.30	8.47	**260.00**	40.00	0.00	0.00	0.00	114.60	61.30	17.30	70.60
	Schmidtsdrift	18.70	8.80	**284.00**	40.00	8.00	0.20	0.00	77.30	42.60	36.00	81.30
Mean		**19.22±1.16**	**8.28±0.37**	**264.20±11.67**	**37.80±2.05**	**2.80±3.70**	**0.12±0.18**	**0.00±0.00**	**103.70±26.12**	**64.28±18.20**	**28.18±8.68**	**89.68±42.76**

Values of levels at all sites, as well as average values of levels of all sampling sites per River are presented and ± standard deviation values. Measured values of >315cfu/100ml were noted where colonies on the selective medium exceeded 315 cfu/100ml. TDS – Total Dissolved Solids; EC - Electrical Conductivity, NO_2^- - Nitrite; NO_3^- - Nitrate, PO_4^{2-} - Phosphate.

Measured NO_2^- levels of the 5 surface water systems ranged from 0.00 to 11.00 mg/l. Barberspan had the lowest overall average NO_2^- level (0.48±0.59 mg/l) and the Mooi River the highest level (4.75±3.95 mg/l). In addition, the measured NO_3^- levels of all sites in the respective rivers ranged from 0.00 to 4.00 mg/l. The lowest NO_3^- levels were measured at the Vaal River (0.12±0.18 mg/l) while the highest were measured at Barberspan (2.75±1.89 mg/l). The overall average NO_2^- and NO_3^- levels measured at all 5 surface water systems were within the livestock watering TWQR. However, even though the NO_2^- levels measured in the water systems were not high, the concentration of this nutrient measured at the rivers (excluding Barberspan) did not support use of water for irrigation (Appendix B, DWAF, 1996d).

All measured PO_4^{2-} levels of the 5 surface water systems ranged from 0.00 to 2.44 mg/l. the Vaal River had the lowest overall average PO_4^{2-} levels (0.00±0.00 mg/l) whereas the Schoonspruit River had the highest (1.00±0.96 mg/l).

Overall, the Vaal River had better water quality as compared to the other surface water systems when pH, TDS, NO_2^-, NO_3^- and PO_4^{2-} are considered. Comparatively, Barberspan had the lowest quality of water when pH, TDS, EC and NO_3^- are considered.

4.1.2 Microbiological analysis

Table 4.1 above also presents the average bacterial levels found per site and surface water system sampled in 2010. It is evident from Table 4.1 that the average total coliforms levels recorded at all 5 surface water systems ranged from 4.00 to >315.00 cfu/100ml. Barberspan had on average the lowest total coliform levels (80.15±127.13 cfu/100ml) while the Schoonspruit River had the highest (269.80±56.98 cfu/100ml).

The average faecal coliform levels were measured and ranged from 1.00 to 242.00 cfu/100 ml. Furthermore, the lowest faecal coliform levels were measured at

Barberspan (36.67±62.55 cfu/100ml) and the higest at the Lower Harts River (131.30±156.55 cfu/100ml). The overall average faecal coliform bacterial levels measured at all surface water systems were suitable for full and intermediate contact recreational activities as well as livestock watering. However, these levels were unsuitable for irrigation (Appendix B, DWAF, 1996d).

As depicted in Table 4.1, the average *E. coli* bacterial levels ranged from 0.00 to 76.00 cfu/100ml. The lowest overall average *E. coli* levels were measured at the Mooi River (9.19±5.16 cfu/100ml) and the highest at the Lower Harts River (49.00±38.18 cfu/100ml). These *E. coli* levels were suitable for full contact recreational water activities (Appendix B, DWAF, 1996d).

The average faecal streptococci levels within all surface water systems ranged from 17.30 to 287.30 cfu/100ml. The lowest overall average faecal streptococci levels (47.13±47.02 cfu/100ml) were measured at the Mooi River and the highest were measured at the Lower Harts River (165.80±171.83 cfu/100ml). The overall average faecal streptococci levels measured in all surface water systems were suitable for intermediate contact recreational activities. These levels were however, unsuitable for full contact recreational activities (Appendix B, DWAF, 1996d).

Total coliform, faecal coliform, *E. coli* and faecal streptococci were present at all surface water systems. In terms of the overall microbial quality of water, Barberspan and the Mooi River had the better water quality. While, the Lower Harts River had the poorest water quality due to high levels of faecal coliform, *E. coli* and faecal streptococci.

4.1.3 Correlation of physico-chemical parameters and bacterial levels

A redundancy analysis (RDA) ordination plot, presented below, was used to directly relate dominant physico-chemical parameters to faecal indicator bacteria in the 5 surface water systems sampled in 2010.

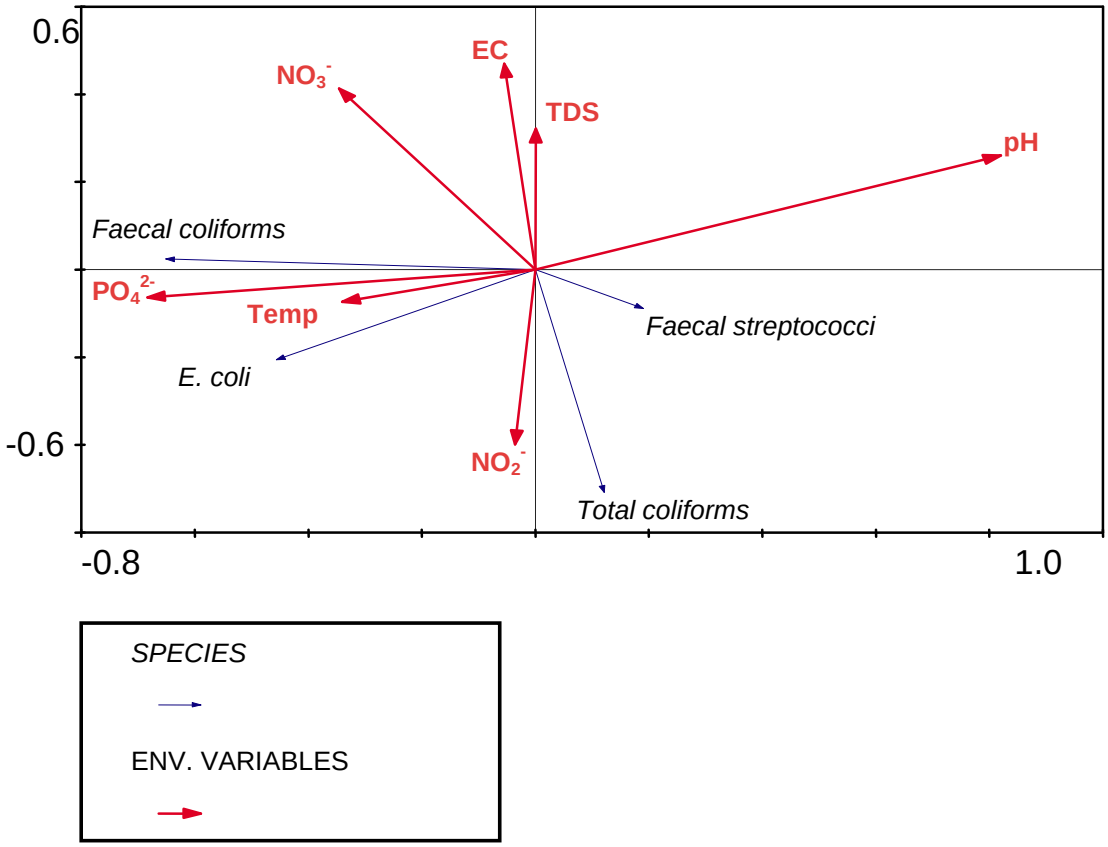

Figure 4.1: Correlation biplot illustrating the relationship between dominant physico-chemical parameters (pH, Temperature, TDS, EC, NO_2^-, NO_3^- and PO_4^{2-}) and microbial levels (total coliforms, faecal coliforms, *E. coli* and faecal streptococci), measured and isolated in 2010 from 5 surface water sources, in the ordination space of first and second axis. The physico-chemical parameters are represented by red arrows, while the species are represented by blue arrows.

According to Rahman and co-workers (2008), a strong positive correlation exists between any two variables provided a small angle is observed between them. Furthermore, an angle larger than 90° between two variables indicates a negative correlation. In Figure 4.1 a statistically significant positive correlation is observed between temperature and total coliforms, faecal coliforms as well as *E. coli*. While a negative correlation is observed between temperature and faecal streptococci. Statistically significant strong negative correlations are observed between pH and faecal coliforms and *E. coli* while to a lesser extent a negative correlation is observed between pH and total coliforms. Furthermore, pH positively correlates to

faecal streptococci in Figure 4.1. Negative correlations are observed between electrical conductivity and total coliforms, *E. coli* and faecal streptococci where as a positive correlation is observed for *E. coli* and faecal coliforms. In Figure 4.1, nitrite has a significant positive correlation with total coliforms, faecal coliforms as well as *E. coli*. While a positive correlation is observed between nitrite and faecal streptococci. A negative correlation exists between nitrate and total coliforms as well as faecal streptococci. Lastly, a positive correlation exists between phosphate and total coliforms, faecal coliforms as well as *E. coli*. A negative correlation is observed between phosphate and faecal streptococci.

4.2 Water samples collected in 2011

This section of the chapter reports the physico-chemical and microbiological quality results of 5 surface water systems sampled in 2011. During 2011, more sampling sites were added to the Lower Harts River and Barberspan. The Upper Harts River which flows into Barberspan was sampled concurrently with Barberspan. These sample sites belong to the Lower Harts River however, in Table 4.2 below they have been grouped with the Barberspan. This is because sampling of sites in the Lower Harts River took place during a different period to the Upper Harts River. Consequently, to prevent confusion of varying temperature levels, these sites have been grouped under Barberspan but will be referred to as the Upper Harts River sampling sites. Furthermore, sampling sites of the Lower Harts River were also extended in order to connect the Upper Harts River, Barberspan and the sites located in the Northern Cape.

4.2.1 Physico-chemical analysis

A summary of the physico-chemical parameters (Temperature, TDS, EC, NO_2^-, NO_3^-, PO_4^{2-}) measured at 5 surface water systems in 2011, with respect to each sampling site, is presented below in Table 4.2.

Table 4.2: The physico-chemical and microbiological water quality analysis of each sampling site within the 5 respective surface water systems sampled in 2011.

River system	Sampling Site	Temperature (°C)	pH	TDS (mg/l)	EC (mS/m)	NO_2^- (mg/l)	NO_3^- (mg/l)	PO_4^{2-} (mg/l)	Total coliforms (cfu/100ml)	Faecal coliforms (cfu/100ml)	*E. coli* (cfu/100ml)	Faecal streptococci (cfu/100ml)
Lower Harts River	Delareyville-Harts	13.10	8.75	781.00	110.00	4.00	0.40	0.70	26.00	36.60	24.30	1.30
	Schweizer-Reneke	13.70	8.33	562.00	79.00	4.00	0.10	0.34	95.30	45.30	37.30	47.00
	Taung	11.80	7.95	339.00	48.00	3.00	0.30	0.43	66.60	34.60	26.60	29.30
	Harts-Pamp Bridge	15.40	8.28	955.00	133.00	4.00	0.10	0.33	>315.00	139.60	128.60	252.00
	Spitskop Dam	16.40	8.25	601.00	84.00	2.00	0.00	0.44	102.00	54.60	2.00	24.60
Mean		**14.08±1.83**	**8.31±0.29**	**647.60±232.93**	**90.80±32.28**	**3.40±0.89**	**0.36±0.39**	**0.45±0.15**	**120.98±112.52**	**62.14±44.02**	**43.76±49.13**	**70.84±102.58**
Upper Harts River	Lichtenburg	23.30	8.02	927.00	130.00	4.00	0.40	2.97	302.00	191.30	178.00	202.00
	Biesiesvlei	22.70	7.91	432.00	61.00	2.00	0.00	2.65	274.30	216.00	140.00	109.30
	Sannieshof	23.60	7.58	324.00	45.00	3.00	0.10	2.19	241.30	75.60	38.00	86.60
Barberspan	Town-Harts	22.40	7.92	336.00	47.00	5.00	0.80	1.74	>315.00	287.00	143.30	228.60
	Inflow	22.00	7.51	269.00	38.00	2.00	0.20	1.60	156.00	44.00	14.00	109.30
	Hotel	22.60	8.75	511.00	100.00	1.00	0.20	0	293.00	132.60	90.00	195.30
	Outflow	23.50	8.60	728.00	102.00	2.00	2.00	0.52	254.60	76.00	32.00	180.00
Mean		**22.87±0.60**	**8.04±0.47**	**503.86±241.59**	**74.70±35.65**	**2.71±1.38**	**0.53±0.69**	**1.67±1.08**	**262.31±53.60**	**146.07±88.76**	**90.76±64.45**	**158.73±55.3**
Schoonspruit River	Bodenstein	17.00	7.92	456.00	64.00	3.00	0.80	1.03	202.30	174.00	91.30	76.00
	Brakspruit	18.00	8.01	386.00	54.00	4.00	0.30	0.58	>315.00	>315.00	>315.00	295.33
	Voortrekker	18.90	7.85	307.00	43.00	2.00	0.10	0.86	>315.00	>315.00	>315.00	>315.00
	Orkney	18.90	7.88	250.00	51.00	3.00	0.20	0.33	>315.00	>315.00	>315.00	>315.00
Mean		**18.20±0.91**	**7.92±0.07**	**349.75±90.15**	**53.00±8.68**	**3.00±0.82**	**0.35±0.31**	**0.70±0.31**	**286.83±56.35**	**279.75±70.50**	**259.08±111.5**	**250.33±116.59**
Vaal River	Bloemhof	11.20	8.58	255.00	36.00	4.00	0.30	0.25	115.30	18.00	18.60	18.60
	Christiana	11.20	9.08	192.00	40.00	0.00	0.00	0.00	64.30	44.60	22.60	50.60
	Windsorton	16.10	7.73	274.00	39.00	0.00	0.00	0.10	146.00	10.60	4.00	4.00
	Barkley-Wes	17.00	8.47	286.00	40.00	3.00	0.00	0.45	83.30	22.60	2.00	3.30
	Schmidtsdrift	15.50	9.06	425.00	60.00	2.00	0.00	0.16	73.30	27.60	18.67	3.30
Mean		**14.20±2.79**	**8.58±0.55**	**285.80±85.54**	**43.00±9.64**	**1.80±1.79**	**0.06±0.13**	**0.19±0.17**	**96.44±33.74**	**24.68±12.77**	**13.17±9.45**	**15.96±20.44**

Values of levels at all sites, as well as average values of levels of all sampling sites per River are presented and ± standard deviation values. Measured values of >315 cfu/100ml were noted where colonies on the selective medium exceeded 315 cfu/100ml. TDS – Total Dissolved Solids; EC - Electrical Conductivity, NO_2^- - Nitrite, NO_3^- - Nitrate, PO_4^{2-} - Phosphate.

Illustrated above in Table 4.2, the water temperature measured at all respective sites in the 5 surface water systems ranged from 11.20 to 23.60°C. The lowest overall average temperature levels were measured at the Lower Harts River (14.08±1.83°C) and the highest at Barberspan including the Upper Harts River (22.87±0.60°C). The Lower Harts, Schoonspruit and Vaal Rivers were sampled during the cold-dry season and had lower temperatures. Furthermore, the Upper Harts River and Barberspan had the highest temperature levels because they were sampled during the warm-rainy season.

As depicted in Table 4.2, pH levels of the 5 surface water systems ranged from 7.51 to 9.08. Schoonspruit River had the lowest overall average pH levels (7.92±0.07) while the Vaal River had the highest (8.58±0.55). The overall average pH levels measured at the surface water systems with the exception of the Vaal River permitted the use of water for full contact recreational activities and irrigation (Appendix B, DWAF, 1996d).

The TDS levels measured at the 5 surface water systems ranged from 192.00 to 955.00 mg/l. The lowest TDS values were measured at the Vaal River (285.80±85.54 mg/l), and the highest at the Lower Harts River (647.60±232.93 mg/l). These differences were also reflected in the EC values. These values were suitable for livestock watering and unsuitable for irrigation (Appendix B, DWAF, 1996d).

The NO_2^- levels of the 5 surface water systems varied from 0.00 to 5.00 mg/l. The Lower Harts River had the lowest overall NO_2^- levels (1.80±1.79 mS/m) and Vaal River the highest (3.40±0.89 mS/m). Nitrate (NO_3^-) levels measured in the 5 surface water systems ranged from 0.00 to 2.00 mg/l. The lowest overall NO_3^- levels were measured at the Vaal River (0.06±0.13 mg/l), and the highest at Barberspan (0.53±0.69 mg/l). The NO_2^- and NO_3^- levels were suitable for livestock watering. Furthermore, the NO_2^- levels measured at all surface water systems indicated that the water was not suitable for use during irrigation (Appendix B, DWAF, 1996d).

Measured PO_4^{2-} levels of all sampling sites in the 5 respective surface water systems ranged from 0.00 to 2.97 mg/l. The lowest overall average PO_4^{2-} levels were measured at the Vaal River (0.19 ±0.17 mg/l) while Barberspan had the highest (1.66±1.08 mg/l).

The Vaal River had the better water quality in 2011 when TDS, EC, NO_2^-, NO_3^- and PO_4^2 are considered. Barberspan including the Upper Harts River and the Lower Harts River had the lowest quality of water when TDS, EC, NO_2^-, NO_3^- and $PO_4^{2-}$are considered.

4.2.2 Microbiological analysis

Above in Table 4.2 a summary of average bacterial levels measured at 5 surface water systems in 2011, based on values from each sampling site is presented. According to Table 4.2, the average total coliform bacterial levels ranged from 26.00 to >315.00 cfu/100ml. The Vaal River had the lowest overall total coliform bacterial levels (96.44±33.74 cfu/100ml) whereas the highest were observed at the Schoonspruit River (286.83±56.35 cfu/100ml).

As illustrated in Table 4.2, the average faecal coliform levels measured at all sampled sites in 2011 ranged from 10.60 to >315.00 cfu/100ml. The lowest overall average faecal coliform levels were measured at the Vaal River (24.68±12.77 cfu/100ml) and highest were measured at the Schoonspruit River (279.75 ± 70.50 cfu/100ml). The overall average faecal coliform levels measured at all surface water systems were suitable for intermediate recreational activities. However, these levels illustrated that the water was unsuitable for irrigation. Furthermore, faecal coliform levels measured at the Schoonspruit River showed that the water at this river was unsuitable for full contact recreational activities and livestock watering (Appendix B, DWAF, 1996d).

All measured average *E. coli* levels ranged from 2.00 to >315.00 cfu/100ml. The lowest overall average *E. coli* levels were measured at the Vaal River (13.17±9.45

cfu/100ml) and the highest at the Schoonspruit River (259.08±111.85 cfu/100ml). The overall average *E. coli* levels measured at the sampled surface water systems with the exception of Schoonspruit River, had water suitable for full contact recreational activities (Appendix B, DWAF, 1996d).

Also depicted Table 4.1, the measured average faecal streptococci levels ranged from 1.30 to >315.00 cfu/100ml. The Vaal River had the lowest overall average faecal streptococci level (15.96±20.44 cfu/100ml) while the Schoonspruit River had the highest (250.33±116.59 cfu/100ml). The Vaal River was the only surface water system with an average faecal streptococci level that permitted the use of water during full contact recreational activities. However, Schoonspruit River was the only surface water system with an overall average faecal streptococci level unsuitable for the use of water during intermediate contact recreational activities (Appendix B, DWAF, 1996d).

Total coliform, faecal coliform, *E. coli* and faecal streptococci were present at all surface water systems. Overall, the Schoonspruit River had the poorest water quality in terms of the high total coliform, faecal coliform *E. coli* and faecal streptococci levels prevalent. However, the Vaal River had the best microbial water quality in terms of Total coliform, faecal coliform, *E. coli* and faecal streptococci measured.

4.2.3 Correlation of physico-chemical parameters and bacterial levels

Figure 4.2 below is a redundancy analysis (RDA) ordination plot which was used to directly relate dominant physico-chemical parameters to faecal indicator bacteria in the 5 surface water systems sampled in 2011. Significant positive correlations were observed between temperature and total coliforms, faecal coliforms, *E. coli* and faecal streptococci. A negative correlation is observed between pH and total coliforms, faecal coliforms, *E. coli* as well as faecal streptococci.

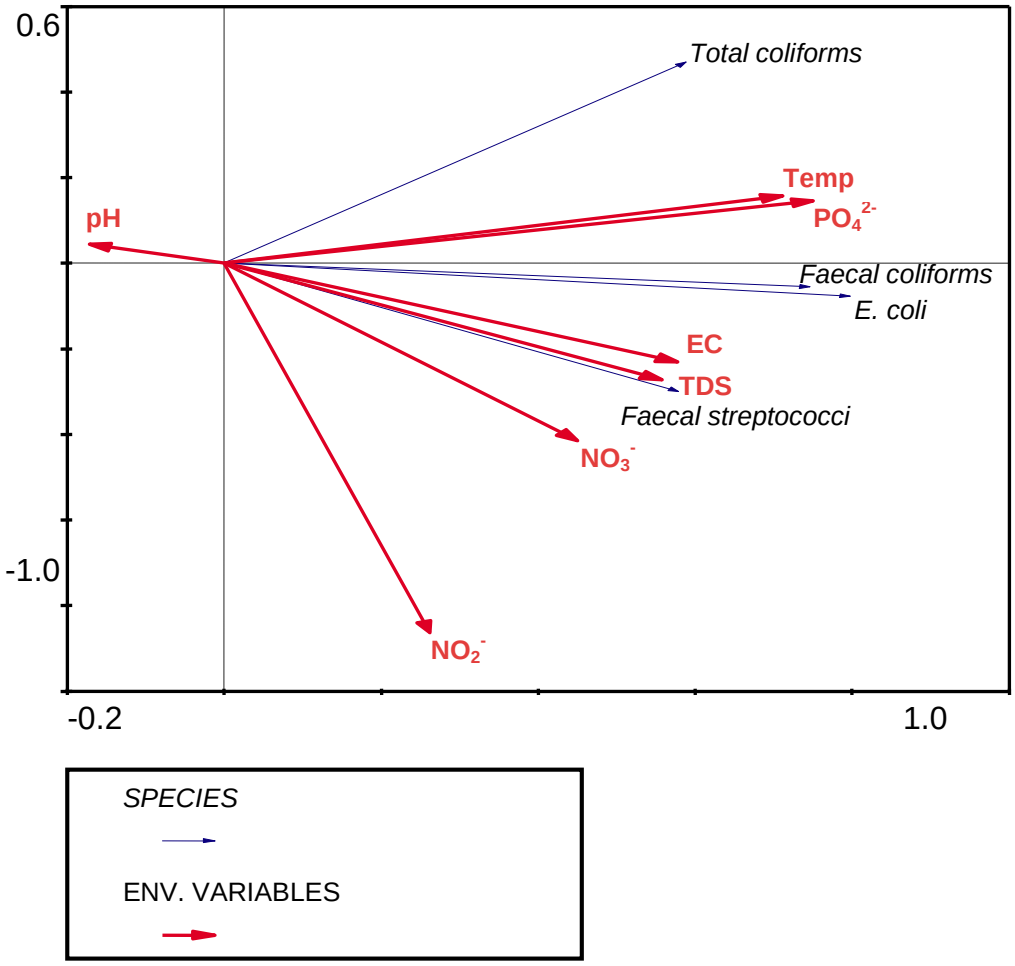

Figure 4.2: Correlation biplot illustrating the relationship between dominant physico-chemical parameters (pH, Temperature, TDS, EC, NO_2^-, NO_3^- and PO_4^{2-}) and microbial levels (total coliforms, faecal coliforms, *E. coli* and faecal streptococci), measured and isolated in 2011 from 5 surface water sources, in the ordination space of first and second axis. The physico-chemical parameters are represented by red arrows, while the species are represented by blue arrows.

Electrical conductivity and total dissolved solids correlated positively with total coliforms, faecal coliforms, *E. coli* and faecal streptococci. A negative correlation exists between nitrites and total coliforms. Significant positive correlations are observed between nitrites and faecal coliforms, *E. coli* as well as faecal streptococci. A strong positive correlation is observed between nitrates and faecal

streptococci. To a lesser extent a positive correlation exists between total coliforms, faecal coliforms and *E. coli*.

4.3 Preliminary identification and confirmation of *E. coli*

Isolate specific information such as sample site, isolate identification, Gram staining and Triple Sugar Iron (TSI) agar test reactions for 2010 and 2011 are listed in Appendix E. A total of 54 presumptive *E. coli* obtained from the 5 surface water systems sampled in 2010 were purified and confirmed using TSI agar reactions. All 54 (100%) presumptive *E. coli* were Gram negative rods while only 42 (78%) were preliminary confirmed as *E. coli*. The remaining 12 (22%) isolates produced H_2S and were regarded species other than *E. coli*. No further identification tests were conducted on these (Appendix E).

Similarly, in 2011, a total of 63 presumptive *E. coli* were obtained from the 5 respective surface water systems analysed were purified and confirmed using TSI agar reactions. All 63 were Gram negative rods. The TSI agar reaction test confirmed that of the 63 presumptive *E. coli*, 59 (93.7%) were potentially once again *E. coli*. The remaining 3 (4.8%) isolates also produced H_2S gas and were also regarded as species other than *E. coli*. One of the isolates did not give any reaction for the TSI test and was also discarded. Similarly, no further identification tests were conducted on these isolates (Appendix E).

4.4 Identification and confirmation of faecal streptococci isolates

4.4.1 Preliminary identification of faecal streptococci

Table 4.3 below summarizes the results of all biochemical tests performed during the preliminary identification of faecal streptococci collected in 2010 and 2011. As depicted below in Table 4.3, a total of 82 presumptive faecal streptococci isolates were obtained from the 5 surface water systems sampled in 2010. All 82 (100%)

isolates were Gram positive cocci while 80 (98%) were catalase negative. Two (2%) isolates from the Vaal River were catalase positive.

Table 4.3 Summary of all biochemical tests performed on the 2010 and 2011 faecal streptococci isolates.

River system	Year	N	Gram reaction	Catalase activity	Bile solubility	Growth at		
			+	-	-	6,5% NaCl	10°C	45°C
Mooi River	**2010**	22	22	22	17	15	15	14
	2011	-	-	-	-	-	-	-
Lower Harts River	**2010**	8	8	8	4	5	5	5
	2011	14	14	14	14	13	14	14
Barberspan	**2010**	16	16	16	16	16	16	16
	2011	21	21	21	16	21	19	21
Schoonspruit River	**2010**	16	16	16	5	7	6	7
	2011	12	12	11	3	11	11	9
Vaal River	**2010**	20	20	18	11	17	14	17
	2011	15	15	13	13	13	13	13

No analysis was conducted in the Mooi River in 2011.

The solubility of 80 presumptive streptococci to bile salts was determined and 53 (66%) were insoluble when exposed to 2% (w/v) sodium deoxycholate. This indicates that unlike other seriological groups of streptococci, faecal streptococci do not possess active autolytic enzymes which cause cell lysis when exposed to bile salts.

Similarly, above in Table 4.3, a total of 62 presumptive faecal streptococci were isolated from the surface water systems sampled in 2011and were identified using biochemical tests. Fifty nine (95%) were Gram positive and catalase negative. One (1.6%) isolate from the Schoonspruit River as well as 2 (32%) isolates from the Vaal River were catalase positive. The solubility of 59 presumptive faecal streptococci to bile salts was determined and 53 (89%) were insoluble when exposed to 2% (w/v) sodium deoxycholate. In addition, the ability of all presumptive faecal streptococci to grow in 6.5% NaCl BHI (w/v) and at 10°C and

45°C was determined. Isolates growing under the latter conditions were classified as faecal streptococci (Slantez & Bartley, 1964; Geldreich & Kenner, 1969) and their identity confirmed using 16S rRNA gene sequences. The 2010 and 2011 isolate specific information for faecal streptococci (site, isolate number and specific biochemical test results) is listed in Appendix F.

4.4.2 16S rRNA sequencing confirmation of faecal streptococci

4.4.2.1 DNA extraction

The quality of DNA extracted was considered good since the A_{260}:A_{280} readings were between 1.71 and 2.06. The DNA concentrations ranged from 5.41 ng/µl to 202.84 ng/µl. In addition, the PCR amplification process was consistent when the quantity of DNA was kept between 50 – 100 ng/µl.

4.4.2.2 DNA amplification

16S rDNA from the genomic DNA of pure cultures was amplified using the PCR process described in Section 3.8.2. Figure 4.3 below illustrates that the amplified fragment length was of the expected 550bp size. Figure 4.3 is an example of the amplified 16S rDNA products. The product yield of all amplified products was similar for all the isolates. No primer dimers or non-specific products were observed in any of the products when the amplification conditions described above in Section 3.8.2 were used.

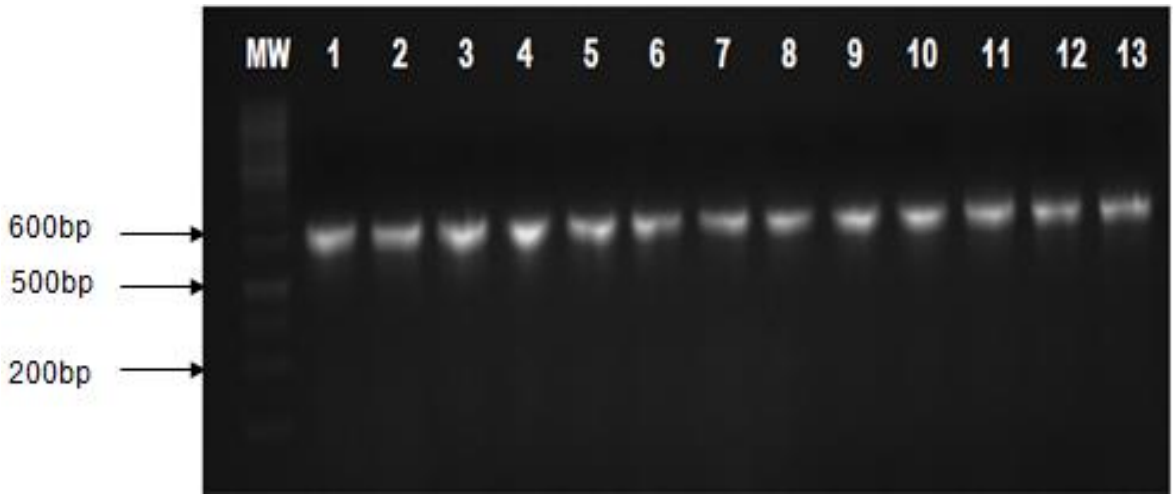

Figure 4.3: A 1.5% ethidium bromide stained agarose gel indicating the amplified 16S rDNA products of pure cultures isolated from the Vaal River sampled in 2011. The MW represents a 100bp molecular weight marker (O'GeneRuler^{TM} 100bp DNA ladder, Fermentas Life Sciences, US).

4.4.2.3 Sequencing and phylogenetic analysis

Sequencing was outsourced to Inqaba Biotech (South Africa, Pretoria). Selected isolates classified as faecal streptococci in Section 4.4.1 were sequenced and sequence data was used to confirm their identity. The amplified sequences were analysed by Genbank BLASTN searches (http://www.ncbi.nlm.nih.gov/BLAST). A summary of sequencing identification of isolates from the various sampling sites within the respective surface water systems is presented in Table 4.4. Specific information such as band ID, Genbank accession number, closest Genbank match, type strain and percentage (%) identity for all 2010 and 2011 isolates is presented in Appendix G. The sequences obtained in this study have not yet been submitted to Genbank, however the closest Genbank match has been used for all analyses.

Table 4.4 below, presents a total of 6 species that were identified during this study. 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%), respectively.

Enterococcus gallinarum (17 %) species were also isolated in 2010 however at lower prevalence levels.

Table 4.4: Distribution of *Enterococcus* species amongst the sites sampled in 2010 and 2011.

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

All Barberspan values included species isolated from the Upper Harts River due to sampling collection.

Depicted above in Table 4.4, the most frequently isolated species in 2011 were *E. mundtii* (37%)*, E. faecalis* (33%) and *E. faecium* (21%). *E. casseliflavus, E. gallinarum and E hirae* were less frequently isolated in 2011 with prevalence levels of 7% and below. *Enterococcus faecium* was isolated from all the water systems, for each sampling period in 2010 and 2011. Whereas, *E. faecalis* were only isolated in 2011.

Figure 4.4, is a neighbor-joining phylogenetic tree of faecal streptococci species isolated from 5 surface water systems in 2010. 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 consists of *E. mundtii* and *E. faecium* species where as sub-cluster A2 is composed of *E. faecium* species*.* Furthermore, cluster B is composed of *E. casseliflavus* and *E. gallinarum* species grouped into one cluster. Furthermore, the *E. faecium* group in cluster A is further grouped into 2 sub-clusters differentiated

by distinct branches. It appears that clustering was according to species and strains but also according to geographical origin. This could be attributed to the type of point source and non-point source pollutants occuring at the different sites.

In Figure 4.4, a high bootstrap support and sequence similarity of 99% is observed for the *E. mundtii* strain type Aq5 group. On the basis of differentiation from the other species strains, the *E. mundtii* group were predominately isolated from Barberspan (Appendix G). Furthermore, the *E. mundtii* group is closely associated with the *E. faecium* sub-cluster composed of strains SJL302 and 044, supported by a 98% bootstrap confidence. In addition, a *E. hirae* species type strain M10_2A was clustered within the *E. faecium* species group cluster and showed a distance relatedness to *E. faecium* SJL302, the bootstrap support for this relatedness was 78%. All *E. faecium* species in this cluster were predominantly isolated from the Mooi River. The second sub-cluster (A2) is predominated by *E. faecium* isolates type strain CE3.A (Fig 4.4) and is supported by a 99% bootstrap confidence value. The *E. faecium* strain CE3.A was predominately isolated from the upstream sampling sites of the Mooi River (Appendix G).

Cluster B is made up of *E. casseliflavus* and *E. gallinarum* species which are supported by a bootstrap confidence of 100%. The *E. casseliflavus* cluster species group is composed of two dominant strains: LHICA_41_6 and RZC110. The *E. gallinarum* species group cluster was strongly associated with *E. casseliflavus* type strain LHICA_41_6. *E. casseliflavus* and *E. gallinarum* were most frequently isolated at Schoonspruit River and Vaal River (Appendix G).

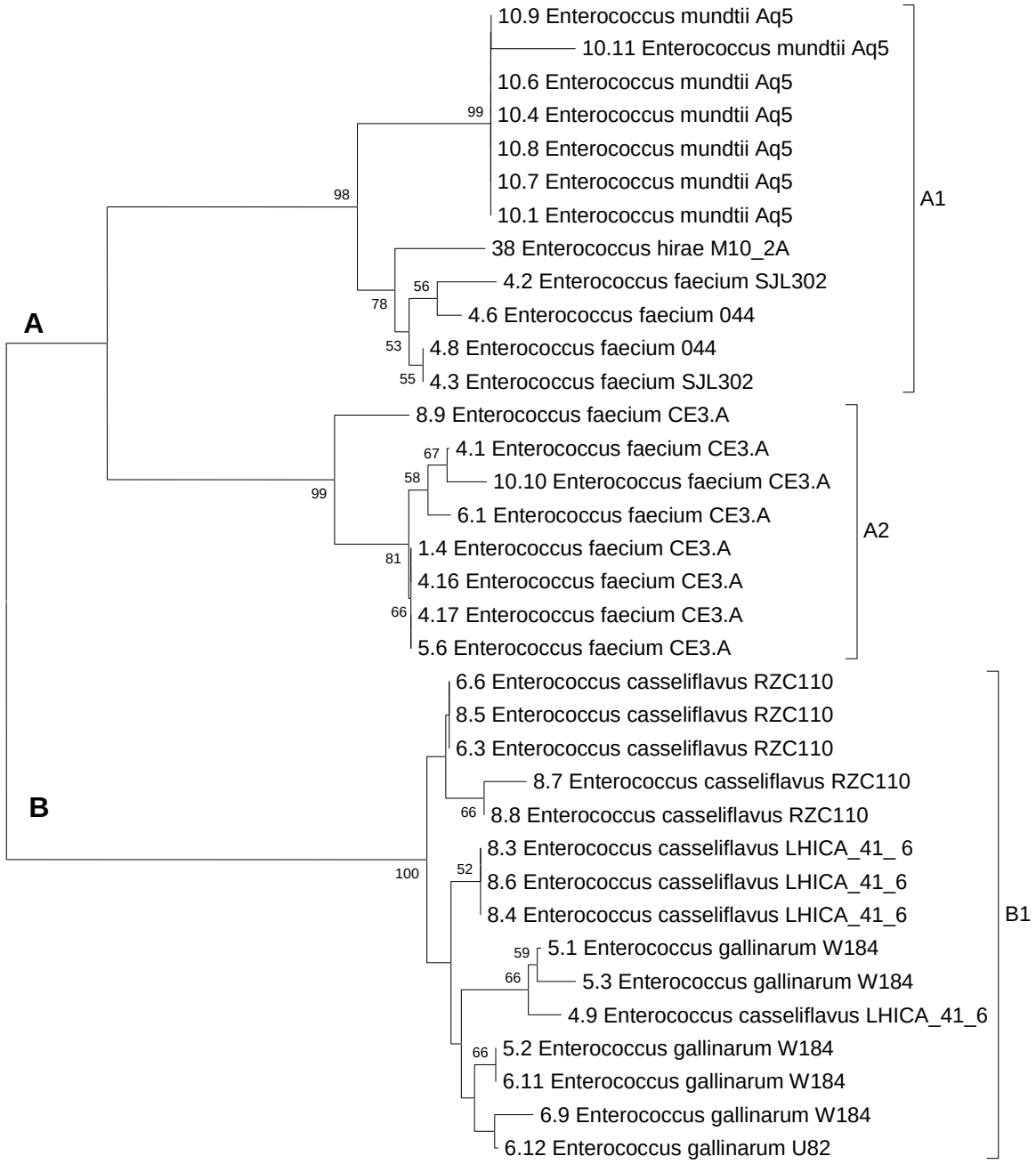

Figure 4.4: Neighbour-joining tree showing the phylogenetic relationships of 35 faecal streptococci species isolated from 5 surface water systems in 2010. The Jukes Cantor model and 1000 bootstraps were used to generate this tree in MEGA 5.0. Percentages are indicated at the branching points of the dendrogram.

Figure 4.5 is a neighbor-joining phylogenetic tree of faecal streptococci species isolated from 5 surface water systems during 2011. Similar to the 2010 tree, 2 main clusters, A and B, are found in the 2011 tree (Figure 4.5). Cluster A is composed of 3 sub-clusters A1, A2 and A3. The latter sub-clusters are composed of *Enterococcus mundtii*, *E. faecium* and *E. casseliflavus* groups with a bootstrap support of 99%. Cluster B is composed of the *E. faecalis* group. In Figure 4.5, the *E. mundtii* group, in cluster A1, is composed of isolates with a high sequence similarity as illustrated by their bootstrap confidence of 95%. The *E. mundtii* species group is predominated by type strain Aq5 with 1 isolate being of a different strain HAMB13965. The *E. mundtii* species were isolated more frequently at the Lower Harts River and Barberspan (Appendix G).

Similar to the 2010 species tree, it is visible from Figure 4.5 that at an interspecies level, *E. mundtii* group was associated with the *E. faecium* cluster. In Figure 4.5 the *E. faecium* cluster group is supported by a bootstrap confidence value of 74%. This cluster was predominated by 2 type strains, SJL302 and 044. *E. faecium* species were isolated predominantly from the Vaal River (Appendix G). Species belonging to the *E. casseliflavus* species group were less frequently isolated in 2011 as compared to isolates obtained in 2010. Nevertheless, *E. casseliflavus* and *E. gallinarum* species were closely related in their sub-cluster and supported by a bootstrap confidence of 99%.

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 a 88% bootstrap confidence. One strain E12 was identified in this species group.

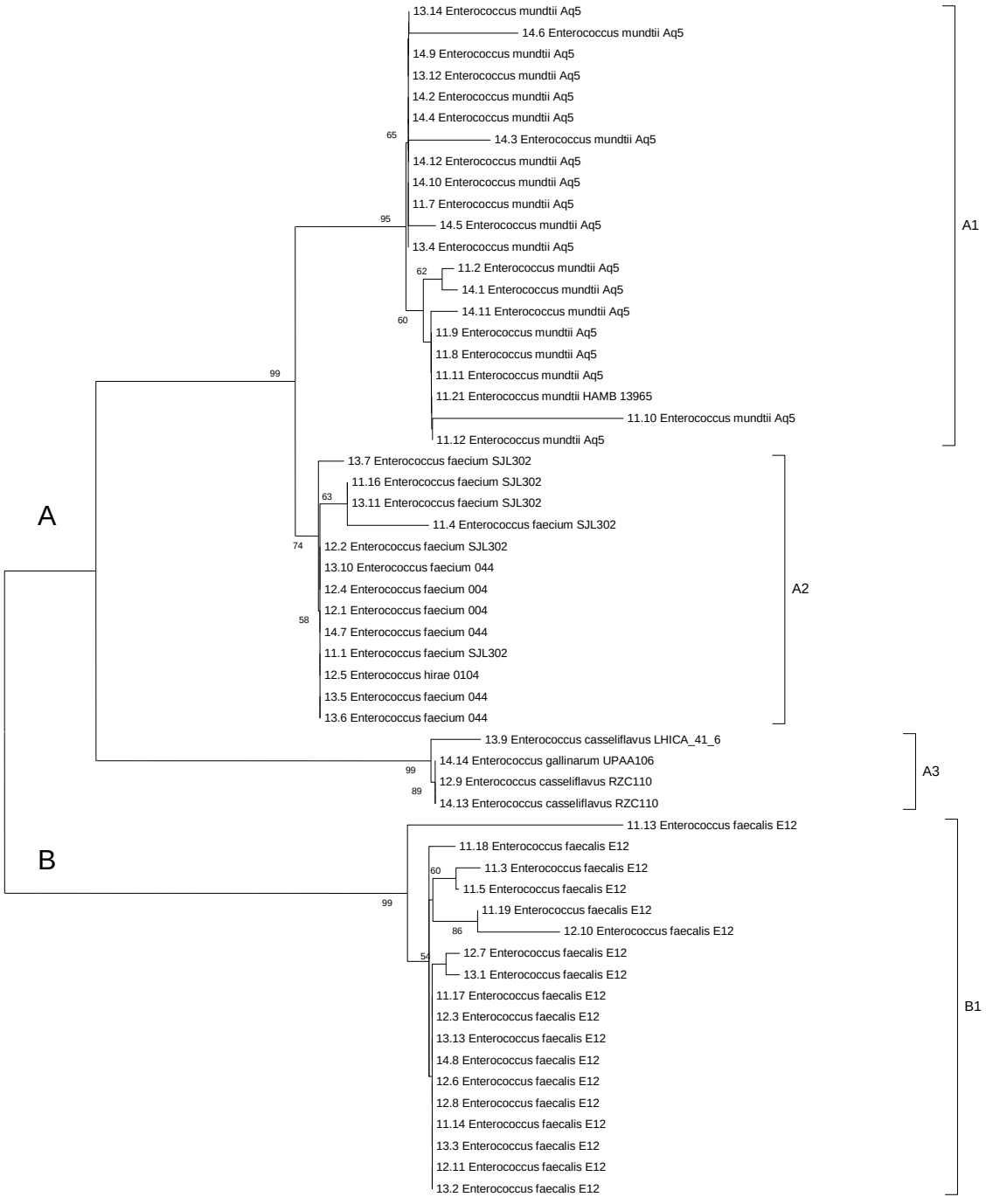

Figure 4.5: Neighbour-joining tree showing the phylogenetic relationships of 56 faecal streptococci species isolated from 5 surface water systems in 2011. The Jukes Cantor model and 1000 bootstraps were used to generate this tree in MEGA 5.0. Percentages are indicated at the branching points of the dendrogram.

4.5 Haemolysis and antibiotic susceptibility characterisation of faecal streptococci

4.5.1 2010 isolates

The summary of haemolysis patterns and multiple antibiotic resistant phenotypes of 80 faecal streptococci isolates obtained in 2010 are presented in Table 4.5.

Table 4.5 Haemolysis patterns and major multiple antibiotic resistant phenotypes for 80 faecal streptococci 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.

Isolate specific information on sample site, isolate number and haemolysis production pattern for all 2010 isolates is presented in Appendix F.

4.5.1.1 Haemolysis patterns of 2010 isolates

In Table 4.5 above, a total of 38 (47.5%) faecal streptococci isolates caused α-haemolysis, 26 (32.5%) caused β-haemolysis while 16 (20.0%) were γ haemolytic. The Mooi River had the highest number of faecal streptococci isolates causing α-haemolysis (prevalence of 18.7%). In addition, the highest number of faecal streptococci causing β- haemolysis (12.5%) were isolated from the Schoonspruit River. The 16 (20%) non-haemolytic faecal streptococci were obtained from Barberspan.

4.5.1.2 Antibiotic resistant patterns of 2010 isolates

In Table 4.5 above, a total of 80 faecal streptococci isolates obtained in 2010 were screened for their resistance to 10 antibiotics. Among the 80 faecal streptococci isolates 55 (68.8%) were resistant to Penicillin G (10 µg), 52 (65%) to Neomycin (30 µg) and 40 (50%) to Vancomycin (30 µg). In addition, 62 (78%) of these isolates were susceptible to Amoxicillin (10 µg) while 79 (98.8%) were susceptible to Streptomycin (100 µg). Not all the isolates that were resistant to Penicillin G (10 µg) were resistant to Amoxicillin (10 µg) or Ampicillin (10 µg) and vice versa. There were thus varying β-lactam resistance phenotypes observed among the isolates.

Dominant multiple antibiotic resistance (MAR) phenotypes were determined using triplicate faecal streptococci isolates obtained at each site within the respective surface water systems. MAR phenotypes of three or more antibiotics are presented in Table 4.5. Faecal streptococci isolates obtained from Klerkskraal Dam, Muiskraal and Around the World Bridge in the Mooi River had relatively similar MAR phenotypes. These sites are relatively close to each other and this could explain the similar antibiotic resistant phenotypes. Faecal streptococci isolates from all three sites were resistant to Ampicillin (10 µg), while isolates from two sites were resistant to Penicillin G (10 µg) and Neomycin (30 µg), respectively. In addition, isolates from Pedestrian Bridge had a similar MAR phenotype (PG-NE-

TM) to isolates obtained at Around the World Bridge. These two sites are physically quite far from each other. There are also two dams (Boskop and Potchefstroom dams) located between the two sites. Similarity, the antibiotic resistance patterns between these two sites can thus not be explained by spatial separation.

The resistance of faecal streptococci isolates to three or more antibiotics was only observed at Dam 8 in the Lower Harts River. The dominant MAR phenotype of isolates obtained was Amp-PG-NE-TM.

Faecal streptococci isolates obtained at all Barberspan sites were predominantly resistant to Amoxicillin (10 µg). Isolates from Town-Harts had a similar MAR phenotype to isolates from site Inflow (AMOX-AMP-VA), with isolates from Inflow being additionally resistant to Neomycin (30 µg). Similarly, isolates from Hotel had a similar MAR phenotype to isolates obtained at Inflow (AMOX-NE-VA). These sites are in close proximity and this could explain the similar antibiotic resistance pattern. Site Inflow is half-way between the Town-Harts sample site and the hotel site. In addition to this is that, isolates obtained from site Hotel were also resistant to Penicillin G (10 µg). However, of contrast to the 3 sampling sites in Barberspan, faecal streptococci isolates from Outflow were resistant to a broader spectrum of antibiotics. It could be that due to the flow regime in Baberspan (from Town-Harts to Outflow site) the streptococci potentially accumulated at the Outflow site.

As depicted in Table 4.5, faecal streptococci isolates from Bodenstein, and Brakspruit in the Schoonspruit River had the same MAR phenotype (PG- NE -CIP -VAN). Isolates from Voortrekker also had a similar MAR phenotype to the MAR phenotype of isolates obtained from the 2 upstream sites. However, these isolates were resistant to Trimethroprim (2.5 µg) and not Vancomycin (30 µg). These three sites are in closer proximity than the Orkney site. The MAR phenotypes observed at site Orkney were different to the three upstream sites. Two dominant phenotypes were observed for faecal streptococci obtained at this site (Table 4.5). This indicates that potentially different pollution events may be responsible for streptococci pollution at the Orkney site.

Of the 5 sites sampled in the Vaal River, only 3 had faecal streptococci that were resistant to 3 or more antibiotics. Isolates obtained from Bloemhof had a different MAR phenotype compared to the 2 sites downstream (Table 4.5). Isolates from Christiana and Windsorton had a similar MAR phenotype (AMP-PG-NE-VAN-TM). However, isolates from Windsorton and Bloemhof were resistant to Ciprofloxacin (5 µg).

4.5.1.3 Cluster analysis of antibiotic resistant patterns observed in faecal streptococci obtained in 2010

A total of 80 faecal streptococci isolated from 5 surface water systems sampled in 2010 were subjected to cluster analysis. The dendrogram was assembled using the antibiotic inhibition zone diameter data in order to determine the similarities of antibiotic resistant patterns observed amongst the 80 isolates.

The tree is composed of mainly 2 clusters, A and B which are further divided into 3 sub-clusters for cluster A and 2 sub-clusters for cluster B. Sub-cluster A1 is composed of minor sub-clusters, A1a and A1b, which are both composed of isolates from all 5 surface water systems sampled. Sub-cluster A2 is predominantly composed of isolates from the Mooi River and one Vaal River isolate. Similarly, sub-cluster A3 is composed of isolates from the Mooi and Vaal River.

Sub-cluster BI is composed of 2 minor sub-clusters, B1a and B1b. Minor sub-cluster B1a is composed of isolates from Barberspan, Schoonspruit and Vaal Rives. Similarly, minor sub-cluster B1b is composed of isolates from Barberspan, Schoonspruit and Vaal Rivers as well as an isolate from the Lower Harts River. Sub-cluster B2 is composed of 2 minor sub-clusters, B2a and B2b. The first minor sub-cluster, B2a, is composed of isolates from the Lower-Harts River, Barberspan and Schoonspruit River. The second minor sub-cluster, B2b, is predominantly composed of isolates from the Mooi River as well as 1 isolate from the Vaal and Schoonspruit River.

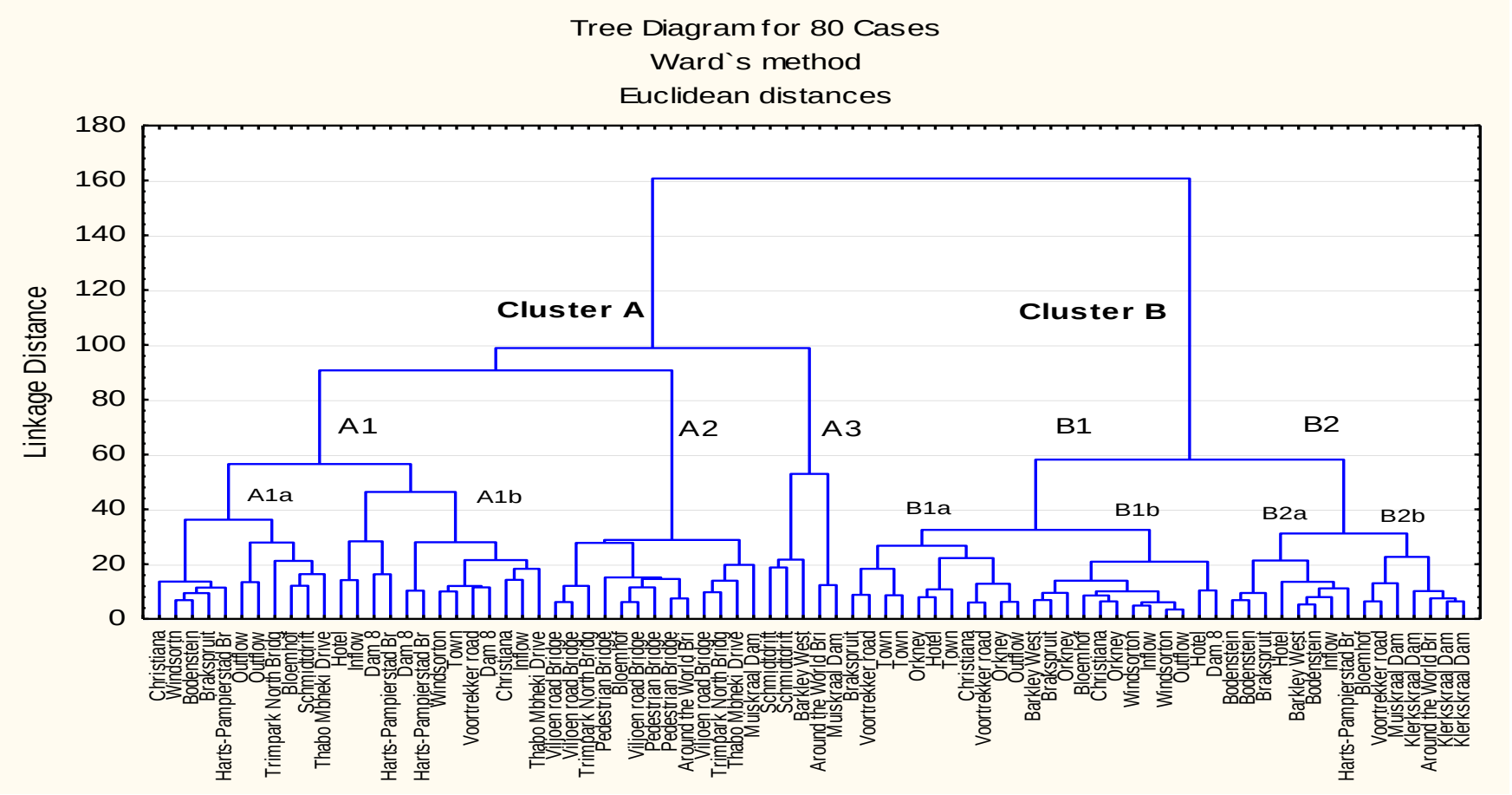

Figure 4.6: Dendrogram showing the relationship of 80 faecal streptococci isolates obtained from 5 surface water systems in 2010. Antibiotic inhibition zone diameter data was used to compile the cluster formation using Ward's method and Euclidean distances in Statistica, version 10 (Statsoft, US). The description for each line indicates the sampling site.

The use of cluster analysis to determine the similarities of antibiotic resistant patterns for 80 faecal streptococci isolates, yielded different results to the MAR phenotype results (Section 4.5.1.2). The grouping of faecal streptococci using the inhibition zone diameter data did not fully illustrate spatial separation. Nevertheless, partial grouping of isolates obtained at each surface water system appeared.

4.5.2 2011 isolates

4.5.2.1 Haemolysis patterns of 2011 isolates

Table 4.6 below is a summary of the haemolysis and antibiotic resistant patterns of faecal streptococci isolates obtained in 2011.

Table 4.6: Haemolysis patterns and major multiple antibiotic resistant phenotypes for 59 faecal streptococci isolated from the 5 surface water systems sampled 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.

As illustrated in Table 4.6, 55 (93.2%) faecal streptococci isolates illustrated α-haemolysis production while only 4 (6.8%) displayed β-haemolysis production. Barberspan had the highest number of α-haemolysis producing isolates (prevalence of 38%). The Lower Harts and Schoonspruit Rivers had β-haemolysis producing isolates (prevalence of 3.3% each). No γ non-haemolysis producing isolates were recorded in the 59 faecal streptococci isolates obtained in the 5 surface water systems sampled during 2011. Isolate specific information on sample site, isolate number and haemolysis production pattern for all 2011 isolates is presented in Appendix F.

4.5.2.2 Antibiotic resistant patterns of 2011 isolates

Presented above in Table 4.6, a total of 59 faecal streptococci isolates were screened for resistance to 10 antibiotics. Amongst the 59 faecal streptococci obtained 24 (40.7%) 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). However, of the 59 faecal streptococci isolates tested, 16 (27.1%) were susceptible to Chloramphenicol (30 µg) while 26 (44.1%) were susceptible to Trimethroprim (2.5 µg). All 4 β-haemolysis producing isolates, which are characterised by a complete zone of clearing around the bacterial colony, displayed resistance to 3 and more antibiotics. Of the 4 β-haemolysin producing isolates, 3 (75%) were resistant to Ampicillin (10 µg); Amoxicillin (10 µg); Vancomycin (30 µg); and Chloramphenicol (30 µg). Resistance to Penicillin G (10 µg), Streptomycin (300 µg), and Ciprofloxacin (5 µg) was observed in 2 (50%) of the β-haemolysin producing isolates while resistance to Neomycin (30 µg) and Oxy-tetracycline (30 µg) was observed in only 1 (25%) of the 4 isolates.

The dominant MAR phenotypes determined using triplicate faecal streptococci isolated from each sampling site within the respective surface water systems are listed in Table 4.6. In 2011, each site at the Lower Harts River had isolates illustrating different MAR phenotypes (Table 4.6). This could be as a result of the wide spatial distance between the sampling sites. However, marked resistances

for Ciprofloxacin (5 µg) and Vancomycin (30 µg) were found in 4 and 3 sites respectively. Furthermore, faecal streptococci isolated from Delarey-Harts and Spitskop dam had the greatest resistance patterns, being resistant to 6 of the 10 assessed antibiotics (Table 4.6). It is evident that the dam at site Spitskop Dam, in the Lower Harts River, could be a contributing factor to the increase in MAR phenotypes. Faecal streptococci isolates found after the dam have a wide MAR phenotype. In addition, a large MAR phenotype is observed at site Delareyville-Harts which is the first point to which water flows directly from Barberspan into the Lower Harts River.

Faecal streptococci from Lichtenburg and Biesiesvlei in the Upper Harts River both had the same MAR phenotype (AMOX-NE-CIP). However, isolates from Sannieshof had a different MAR phenotype to the 2 upstream sites while showing only similar resistance to Neomycin (30 µg). This indicates that potentially different pollution inputs may be occuring at site Sannieshof. Isolates from 3 sites in Barberspan all had different MAR phenotypes which could be a result of the flow regime at the inland lake.

Faecal streptococci isolates obtained at Brakspruit in the Schoonspruit River had a MAR phenotype of 6 antibiotics (AMOX-PG-NE-STREP-CHLOR-CIP) belonging to 4 antimicrobial classes. Isolates from sites Voortrekker and Orkney, (downstream Brakspruit) had MAR phenotypes composed of antibiotics found in the Brakspruit isolates MAR phenotype (Table 4.6). This could potentially mean a pollution source occurs between sites Bodenstein and Brakspruit. Furthermore, sites Voortrekker and Orkney are located fairly far from Brakspruit which could potentially explain the decrease in MAR phenotypes at these sites. Similar to the Lower Harts River, faecal streptococci isolates obtained from 4 sites in the Vaal River all had different MAR phenotypes. Isolates from Schmidtsdrift had a similar MAR phenotype observed in the Barkley-Wes isolates (PG-NE-CIP).

4.5.2.3 Cluster analysis of antibiotic resistant patterns observed in faecal streptococci obtained in 2011

A total of 59 faecal streptococci isolated from 5 surface water systems in 2011 were subjected to cluster analysis. The antibiotic inhibition zone diameter was used so as to indicate similar antibiotic resistant patterns observed amongst the 59 isolates.

The tree is composed of 2 main clusters, cluster A and B. These clusters are further divided into two sub-clusters A1 and A2 as well as B1 and B2. Sub-cluster A1 is composed of 3 minor sub-clusters: A1a, A1b and A1c. Minor sub-cluster A1a is composed of isolates obtained from the Upper Harts River, Barberspan and Vaal River. Clustering of faecal streptococci isolates in minor sub-cluster A1a is composed of isolates obtained from subsequent sites (Biesiesvlei, Sannieshof and Town-Harts) located in close proximity to one another. Minor sub-cluster A1b is composed predominantly of isolates from the Lower and Upper Harts River as well as Barberspan. One isolate within this cluster was from the Vaal River. Lastly, minor sub-cluster A1c is composed of isolates from the Upper and Lower Harts, Vaal and Schoonspruit Rivers. Sub-cluster A2 is composed of isolates from Barberspan, Vaal and Schoonspruit Rivers.

Sub-cluster B1, of cluster B, is composed of isolates from the Lower Harts River, Barberspan and Schoonspruit River. In addition, sub-cluster B2 is composed of 2 minor sub-clusters, B2a and B2b. Minor sub-cluster B2a is composed of isolates from the Lower and Upper Harts River, Schoonspruit and Vaal Rivers. Minor sub-cluster B2b is composed of isolates from the Lower Harts River, Barberspan, Schoonspruit and Vaal Rivers.

Similar to the 2010 cluster analysis, spatial separation of 59 faecal streptococci based on inhibition zone diameter data yielded different results to the MAR phenotype results (Section 4.5.2.2). Similarities in the antibiotic resistant patterns of isolates obtained in 2011 also showed minimal spatial differentiation.

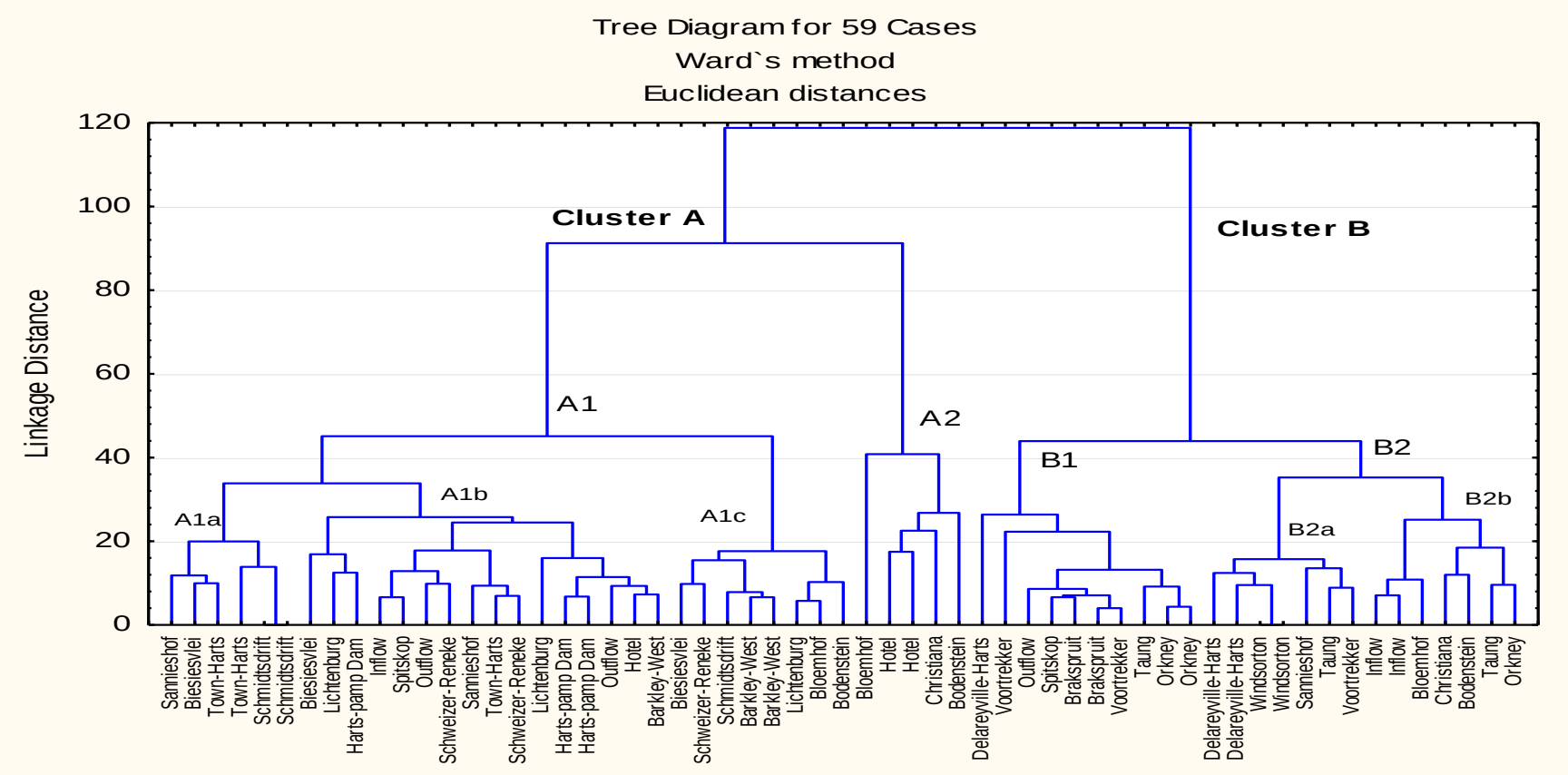

Figure 4.7: Dendrogram showing the relationship of 59 faecal streptococci isolates obtained from 5 surface water systems in 2011. Inhibition zone diameter data was used to compile the cluster formation using Ward's method and Euclidean distances on Statistica, version 10 (Statsoft, US). The description for each line indicates the sampling site.

4.6 Summary

The quality of 5 surface water systems was successfully determined by the use of physico-chemical and microbial parameters. In 2010, water samples were collected during one warm-rainy season from the Mooi, Lower Harts, Schoonspruit and Vaal Rivers while Barberspan was sampled during winter. In 2011, water samples were collected during one cold-dry season at the Lower Harts, Schoonspruit and Vaal Rivers, while the Upper Harts River and Barberspan were sampled during summer. The variation in water quality between the two seasons was observed.

The Vaal River had better physico-chemical water quality as compared to the other surface water systems in 2010. These trends were also observed in 2011. Barberspan had the lowest physico-chemical water quality in 2010. Similarly, Barberspan, the Upper and Lower Harts River had poor physico-chemical water quality in 2011. In 2010 and 2011, total coliforms, faecal coliforms, *E. coli* and faecal streptococci were detected in all 5 assessed surface water systems. The Lower Harts and Schoonspruit Rivers had the highest levels of bacteria measured in 2010 while Barberspan and the Schoonspruit River had the highest in 2011. In 2010, Barberspan and the Mooi River had the best overall microbial water quality. The Vaal River had the best microbial water quality in 2011. Significant differences were observed in both physio-chemical levels and microbial levels between 2010 and 2011. These were confirmed by the positive and negative correlations existent between temperature and the physico-chemical as well as microbial parameters.

The confirmation of *E. coli* species was achieved by biochemical methods. A total of 80 and 59 presumptive faecal streptococci were identified, by a series of biochemical tests, in 2010 and 2011 respectively. The latter isolates were confirmed using 16S rRNA sequencing and a total of 6 species belonging to the faecal streptococci species group were identified as *Enterococcus mundtii, E. faecalis, E. faecium E. casseliflavus, E. gallinarum* and *E hirae.* The phylogenetic relationship of all isolated species was determined using neighbour joining trees. In both years, the *Enterococcus mundtii* species group was strongly associated

with *E. faecium* species. Similarly, the *E casseliflavus* species group was strongly associated with the *E. gallinarum* species group.

The identifed faecal streptococci were tested for their ability to lyse blood cells in order to determine their pathogenic potential. Results indicated that 26 of 80 faecal streptococci isolated in 2010 and 4 of 59 obtained in 2011 caused β-haemolysis. Furthermore, the resistance of the identified faecal streptococci isolates to 10 antibiotics was determined using the Kirby-Bauer disk diffusion method. The antibiotic resistant patterns of isolates obtained in 2010 showed predominant resistance to Penicillin G (10 µg), Neomycin (30 µg) and Vancomycin (30 µg). Whereas faecal streptococci isolates obtained in 2011, showed high resistance to Amoxicillin (10 µg), Penicillin G (10 µg), Neomycin (30 µg) and Ciprofloxacin (5 µg).

Multiple antibiotic resistant phenotypes were documented for isolates obtained at each site with resistance to 3 or more antibiotics. Significant pollution patterns along the surface water systems were observed upon analysis of the MAR phenotypes. Furthermore, cluster analysis was performed on 80 and 59 faecal streptococci isolates identified by biochemical tests. This analysis was used in order to determine the similarity of faecal streptococci isolates obtained per surface water system. Clustering was however inadequate for this and only showed partial grouping of isolates.

CHAPTER 5

DISCUSSION

In this chapter, the results obtained will be used to determine whether the quality of water from the assessed surface water systems is safe for use of various functions and activities. Where possible, the physico-chemical and microbiological quality of the assessed surface water systems will be brought into relation with various land use activities surrounding sample sites. In addition, seasonal variation trends visible in the physico-chemical and microbial levels will be discussed. The sanitary risk of humans and animals using and exposed to these surface water systems will also be discussed by taking into account the presence of potential pathogens and identified species with possible virulent traits.

5.1 Water quality analysis for 2010 and 2011

5.1.1 Physico-chemical surface water quality analysis

5.1.1.1 pH

The pH of a surface water system can be a useful indicator of water quality because it determines the suitability of water for various purposes (Nola *et al*., 2002). Anthropogenic activities which can have an influence on a surface water system's pH include: runoff from agricultural fields and mines as well as infiltration of untreated wastewater (Dallas and Day, 2004; Monwq, 2007). Furthermore, influences of human activities on an aquatic ecosystem's water quality are usually evident by pH values that exceed the permissible range (Dallas and Day, 2004).

The overall average pH levels measured at all 5 surface water systems sampled in 2010 were slightly alkaline. Based on values from each sampling site, the permissible pH Target Water Quality Range (TWQR) for full contact recreational

activities (6.5-8.5) and irrigation (6.5-8.4) was exceeded in all Barberspan sites and site Schmidtsdrift (Vaal River). In addition, water samples collected at Barkley-Wes (Vaal River) also exceeded the acceptable pH TWQR for irrigation.

The measured overall average pH levels of 5 surface water systems assessed in 2011 based on the overall average values were also slightly alkaline. pH levels measured at sites Delarey-Harts (Harts River), Hotel and Outflow (Barberspan), as well as Bloemhof, Christiana and Schmidtsdrift (Vaal River) exceeded the TWQR for full contact recreational water use (6.5-8.5) as well as irrigation (6.5-8.4). Once again Barkely-Wes (Vaal River) had a pH value that exceeded the TWQR for irrigation.

Humans directly exposed to water at sites exceeding the permissible limit of full contact recreational activities may suffer from mild effects. Their eyes, skin, ears, nose, mouth and throat mucous membranes may be slightly irritable upon contact with water (DWAF, 1996c; WHO, 2003b). Furthermore, water found at sites with elevated pH levels exceeding the TWQR for irrigation will have an effect on crop yield while the encrustation of irrigation pipes is also likely to occur (DWAF, 1996b). Also, good quality irrigation water is crucial, particularly in semi-arid regions like the North West Province, where competition for good quality surface water prevails amongst the agricultural, urban, mining and industrial entities (DWAF, 2004a; NWDACE-SoER, 2008; Valdez-Aguilar *et al*., 2009).

5.1.1.2 Temperature

According to Ahipathy (2006), temperature levels in a river are dependent and influenced by factors such as its geographic location, the season, as well as the temperature of effluents entering the river. The trends and influence of temperature on the measured water quality variables is discussed in section 5.2.

5.1.1.3 Total Dissolved Solids and Electrical Conductivity

According to DWAF (1996c), the TDS concentration of a water body is directly proportional to its electrical conductivity provided the water temperature is at 25°C. The known permissible TDS TWQR for livestock watering of cattle and horses as well as sheep is 0-2000 mg/l and 0-3000 mg/l, respectively. In 2010 and 2011, the measured TDS and EC levels of all sites within the 5 surface water systems assessed, were within the permissible TWQR for livestock watering of cattle and horses as well as sheep.

The permissible EC TWQR for irrigation is ≤ 40 mS/m (DWAF, 1996d). Of the surface water systems sampled in 2010, sites in the Mooi River (excluding site Klerkskraal Dam), Harts River, Barberspan and Schoonspruit River exceeded this range. Furthermore, in 2011, water samples collected at all sites in the Lower Harts River, Upper Harts River, Barberspan (excluding site Inflow), Schoonspruit River and Schmidtsdrift (Vaal River) exceeded the permissible TWQR for irrigation. A number of farms using irrigation for watering of crops are found along these surface water systems. Thus, the elevated EC levels which indicate high ion concentrations may have a profound impact on crop production occurring on these farms (Grattan, 2002).

5.1.1.4 Nitrites and Nitrates

The water quality irrigation guideline by DWAF (1996d) refers to nitrogen as all inorganic forms of nitrogen present in water. These include ammonia, ammonium, nitrite and nitrate. The irrigation TWQR of inorganic nitrogen is ≤ 0.05 mg/l. In 2010, most sites in the surface water systems exceeded this range except for Dam 8 (Harts River), Hotel (Barberspan), and Barkley-Wes (Vaal River). Similarly, in 2011, most sites in the surface water systems also exceeded this range except for Christiana and Windsorton (Vaal River). Furthermore, the permissible Nitrite (NO_2^-) TWQR for livestock watering is 0-10 mg/l and water collected at site Viljoen Road Bridge (Mooi River) in 2010 exceeded this range. In 2011, NO_2^- levels measured

from all sites in the 5 surface water systems were however within the NO_2^- livestock watering TWQR.

5.1.1.5 Phosphates

Due to the unavailability of phosphate (PO_4^{2-}) Target Water Quality Ranges in South Africa, the suitability of surface water systems for use in various purposes cannot be reported. Nonetheless the highest measured PO_4^{2-} levels were observed at all Schoonspruit River sites while none were detected at the Vaal River in 2010. In 2011, the Upper Harts River and Barberspan had the highest PO_4^{2-} levels while the lowest were observed at the Vaal River.

It was observed that the highest phosphate levels were measured during the warm-rainy season at sites located in regions of intensive agricultural activities. These included sites Orkney (Schoonspruit River), site Town-Harts (Barberspan) and all 3 Upper Harts River sites. This could be an indication that possible chemical pollution is occurring as a result of the agricultural activities taking place along the latter listed surface water systems. This pollution is likely because run-off is an important carrier of non-point source agricultural pollutants to surface water sources (Carpenter *et al*., 1998). Several fertilizers used in modern agricultural practices contain high levels of plant nutrients, particularly nitrogen and phosphorus, which enhance a high crop yield (Walmsley, 2000). However, when found in high concentrations within a water system these nutrients may result in eutrophication, altering the structure and functioning of biotic communities (Diaz and Rosenberg, 2008). According to Oberholster and Ashton (2008), eutrophication is characteristic of enhanced cyanobacterial blooms causing discolouration, poor light penetration and decreased oxygen levels. According to van Ginkel (2011), extensive eutrophication problems are experienced in South Africa particularly in dams sourced for water supplies. Thus, if pollution of phosphate from the agricultural activities into the surface water systems persists,

and the levels elevate such that its concentration supports the growth of cyanobacteria, the surface water systems may end up eutrophic.

5.1.2 Microbial water quality analysis

5.1.2.1 Total and faecal coliforms

The present study assessed surface waters that are predominately used for recreational activities, livestock watering and irrigation. Based on all faecal coliform levels measured per site in 2010, site Harts-Pamp Bridge (Lower Harts River) exceeded the TWQR for full contact recreational activities (≤150 cfu/100ml) and livestock watering (≤200 cfu/100ml). Furthermore, the overall average faecal coliform levels measured at all sites in all surface water systems during 2010, exceeded the TWQR for irrigation (<1 cfu/100ml).

All faecal coliform levels measured at all sites in 2011 exceeded the TWQR for Irrigation (<1 cfu/100ml). The faecal coliform levels of water samples collected at Lichtenburg and Biesiesvlei sites (Upper Harts River) as well as all Schoonspruit River sites exceeded the TWQR for full contact recreational activities (0-150 cfu/100ml). Furthermore, apart from the Bodenstein sample (Schoonspruit River), all other water samples from the various sample sites in all the surface water systems exceeded the TWQR for livestock watering (0-200 cfu/100ml).

Areas frequented by cattle had the greatest degree of coliform contamination. These included sites Biesiesvlei (Upper Harts River) and Orkney (Schoonspruit River) (Appendix H1 a-c). This suggests that cattle around these sites were significant contributors of bacteria measured in the water. These findings are supported by Bachoon and co-workers (2010) who stated that the contamination of water systems by animal farming is increasing worldwide at an alarming rate. In addition, Jenkins and co-workers (2009) suggested that in such areas significant correlations may be present between excreted faeces from animals and water runoff. Thus, allowing microorganisms from the excreta to easily be transferred into the water systems. Thus, the poor microbial water quality of these surface

water systems at specific sites may possibly be attributed to agricultural activities, even though cattle are an important livestock asset for the socio-economic spectrum in many African countries (Jenkins *et al*., 2009).

This trend was however not observed at site Taung where cows and goats were found close to the river and found drinking water from the Harts River. Nevertheless, visual observations upon sampling at Taung illustrated that the river had a large amount of red and green algae that was in the process of breaking down (Appendix H1 d). According to Kloot (2007) non-point pollutants such as faeces from cattle also pose serious eutrophication threats to both surface water sources. In addition, low levels of bacteria were observed at Lower Harts River sites Taung, Delarey-Harts and Schweizer-Reneke (Appendix, H1 d-f) although algae was observed at these sites. Schindler and co-workers (2008) stated that floating algal blooms are one of the most visible characteristics of eutrophication. Eutrophication decreases a water systems dissolved oxygen levels resulting in anoxic and hypoxic conditions (Diaz and Rosenburg, 2008). Since total and faecal coliforms are aerobic and facultative anaerobes (Prescott *et al*., 2005), it seems algae in water could have had an influence on the low total and faecal coliform levels.

The effects of full and intermediate recreational contact to faecal indicator bacteria in surface waters will be discussed in sections 5.1.2.2 and 5.1.2.3. According to DWAF (1996e) the potability of water for livestock is defined according to the palatability of the water which would affect intake and production. The TWQR of livestock watering for faecal coliforms primarily focuses on the toxicological effects associated with ingestion of waterborne pathogens present in water (DWAF, 1996e). Water collected from site Harts-Pamp Bridge in the (Harts River), in 2010, was unsuitable for livestock watering. The adverse effects of elevated faecal coliform levels in water used for livestock watering are usually observed in young stock. No livestock was observed near the Harts-Pamp Bridge (Appendix H1 a), however, traces of animal faeces were observed not far from the sampling site.

According to Gemmell and Schmidt (2011) wastewater in South Africa is widely used for irrigation purposes. However, a wide range of problems may arise when the quality of water used for irrigation does not meet acceptable or permissible standards (Gemmell and Schmidt, 2010). According to the WHO (2001) food-borne illness are frequently caused by marine and fresh water that is faecally contaminated. According to DWAF (1996b), the level to which humans risk being infected by contaminants from irrigation water is determined by two factors. DWAF (1996b) defines the latter as the degree to which irrigation water is contaminated and it's inevitable influence on fresh vegetables consumed by humans. The problem with contaminated irrigation water is that it acts as a vehicle and facilitates the diffusion of viral and bacterial pathogens to fresh produce and minimal processed produce (Heaton and Jones, 2007; Steele and Odumeru, 2004). According to DWAF (1996b) the biggest challenge faced with contaminated irrigation water is that fresh produce is likely to be consumed without any micobial lethal processing. Results of the present study indicate that the faecal coliform bacterial levels measured in water collected at all sampling sites throughout 2010 and 2011 were unsatisfactory for the use in irrigation. The faecal coliform bacterial group includes species such as *Shigella* and *E. coli. Shigella* sp. and some *E. coli* strains are bacterial pathogens and have been responsible for numerous bacterial water borne diseases (WHO, 2001).

5.1.2.2 *E. coli*

According to DWAF (1996c), the permissible *E. coli* TWQR for full contact recreational water use is 0-130 cfu/100ml. In 2010, all average *E. coli* levels measured per site were within this range. However, based on values from all sites *E. coli* levels measured in water from Lichtenburg (Upper Harts River), as well as Orkney, Voortrekker and Brakspruit (Schoonspruit River) exceeded the TWQR for full contact recreational water activities (0-130 cfu/100ml).

Water resources used for recreational activities may contain a vast combination of pathogenic and non-pathogenic microorganisms sourced from various anthropogenic activities (WHO, 2003b). Of major concern upon exposure to full human body contact are the pathogenic organisms present in these waters which can cause infections in the intestine upon ingestion, upper respiratory tract, ears and eyes (WHO, 2003b). Recreational exposure to water also offers opportunities to micro-organisms which are members of the normal human microbial flora to cause secondary infections in wounds, exposed tissue or individuals with reduced resistance (DWAF, 1996c).

However, of major concern is the possible presence of Verocytotoxin-producing *E. coli* known as Enterohemorrhagic *E. coli* (EHEC) in surface waters used for recreational or full body human contact (WHO, 2005). The EHEC group is particularly specific to *E. coli* O157. According to Garizo-Hadzik and co-workers (2010), considerable numbers of *E. coli* may reach surface waters from runoff in agricultural watersheds, sediments, fecal material grazing lands and wildlife excreta. A study by Ateba and co-workers (2008) isolated *E. coli* O157 from cattle, pigs and humans in the North West Province. This latter study illustrated that cattle, rather than pigs, have been identified as the main reservoir of *E. coli* O157 strains. The study of Ateba and co-workers (2008) is supported by previous reports (Schouten *et al*., 2004; WHO 2005). This suggests that these pathogens could be present in the 5 surface water systems, particularly at sites frequented by cattle or where there was evidence (cattle faeces and tracts) of cattle using the water sources. *E. coli* O157:H7 is capable of causing haemorrhagic enteritis and haemolytic uraemic syndrome in humans (Hunter, 2003). Accidental ingestion of water that is contaminated by cattle faecal matter could thus potentially cause severe gastroenteritis.

5.1.2.3 Faecal streptococci

In 2010, faecal streptococci levels measured at sites Muiskraal, Trimpark North Bridge, and Pedestrian Bridge (Mooi River) as well as all sampling sites in the Harts River, Barberspan, Schoonspruit River and Vaal River exceeded the TWQR for full contact recreational use (0-30 cfu/100ml). Water collected from Dam 8 (Harts River) was the only site amongst all sites that exceeded the permissible faecal streptococci TWQR for intermediate contact (≤ 230 cfu/100ml) (DWAF, 1996c).

Faecal streptococci levels measured in 2011 which did not comply with the TWQR for full contact recreational water use (0-30 CFU/100ml) were found at all Upper Harts River, Barberspan and Schoonspruit River sites. In addition sites Schweizer-Reneke and Harts-Pamp Bridge (Harts River) and site Christiana (Vaal River) also exceeded this range. Furthermore, the measured faecal streptococci at Harts-Pamp Bridge (Harts River) and sites Orkney, Voortrekker and Brakspruit (Schoonspruit River) exceeded the faecal streptococci recreational intermediate contact TWQR (0-230 CFU/100ml).

The presence of faecal streptococci bacteria that exceeded the TWQR for full contact and intermediate contact recreational water use in the Schoonspruit and Vaal Rivers is worrisome. This is because fishing activities are taking place at one Schoonspruit site and several Vaal River sampling sites (Appendix H2 a-c). The incidence of human pathogenic bacteria such as faecal streptococci in the surface water systems is indicative of their possible presence in the fish obtained therein. This is in accordance to studies that isolated human pathogenic bacteria from aquatic foods found in polluted waters (Olayemi *et al*., 1990; Ndip *et a*l., 2002). Furthermore, what seemed like grey water coming from an unknown source was flowing into one of these sites where fishing was taking place in the Vaal River (Appendix H2 d- e). According to Given and co-workers (2006), the contamination of recreational waters by sewage or runoff can lead to increased swimmer illness from exposure of water-borne pathogens.

In addition to fishing activities, cultural activities such as baptisms were taking place at some sampling sites such as Inflow at Barberspan and Harts-Pamp Bridge in the Harts River. For privacy and respect, photos for illustrative purposes were not obtained. It is well documented that exposure to waters with high concentrations of *E. coli* and faecal streptococci is associated with an increased risk of contracting gastrointestinal and respiratory illnesses (Kay *et al*., 1994; Haile *et al*., 1999). Clark and co-workers (2003) stated that although the risks associated with swimming in microbiologically polluted rivers are usually not life threatening, they may have significant consequences on children and immuno-compromised individuals.

5.2 Seasonal variation

In 2010, samples from the Harts, Schoonspruit and Vaal Rivers were collected during one warm-rainy season while samples from Barberspan were collected during one cold-dry season. The inverse was done in 2011. As a result, seasonal variation with regard to physico-chemcial parameters and microbial levels is evident. Analysis of correlations between the physico-chemical and microbiological data as well as the season which sampling took place is described in sections 4.1.3 and 4.2.3.

5.2.1 Influence of season on physico-chemical water quality

In 2010, water samples collected during the warm-rainy season at all the rivers (Mooi, Harts, Schoonspruit, and Vaal Rivers) had relatively lower pH levels than samples collected during the cold-dry season in 2011. Similarly, the pH levels of water samples collected at Barberspan during the warm-rainy season in 2011 were relatively lower than the measured pH levels of water samples collected during the cold-dry season. Similar results have been observed previously in several studies where elevated pH levels were measured during the colder dry

seasons as compared to the warmer rainy seasons (Skoroszeweski, 1999; Matowanyika, 2010; Ideriah *et al*., 2010). Skoroszeweski (1999) explains this phenomenon of decreased pH levels during the colder dry seasons as a result or influence of rainfall levels affecting a rivers flow rates.

Seasonal variation in measured EC was evident in the 2010 and 2011 samples. Thus, the observed seasonal variation for electrical conductivity levels in the surface water systems is an indication of the role season has on the ionic distribution in the sampled surface water systems of the NWP.

The EC levels of samples collected at Schoonspruit and Vaal Rivers during the cold-dry season in 2011 were relatively higher than EC levels measured at these rivers during the warm-rainy season in 2010. Similarly, EC levels measured at Barberspan during the cold-dry season in 2010 were higher than the EC levels measured during the warm-rainy season in 2011.

Studies done by Ideriah and co-workers (2010) as well as Kacar (2011) found that conductivity decreased during the rainy season and increased during the dry season. As suggested by Ideriah and co-workers (2010) the high electrical conductivity levels measured during the dry season could be attributed to low precipitation rates, and increased atmospheric temperatures which, when combined, result in elevated evaporation and transpiration rates as well as higher ionic concentrations. Furthermore it could be suggested that the salts and ions in the sampled surface water systems were diluted with rain while they may have increased during dry periods. However, this seasonal variation trend was not observed in the measured EC levels of the Harts River samples. Water samples that were collected during the warm-rainy season in 2010 had higher EC levels than samples collected during the cold-dry season in 2011. Thus, the season of water collection did have an influence on the physico-chemical water quality.

5.2.2 Influence of season on microbial water quality

During the analysis, similar trends were found each year with respect to the prevalence of total coliforms, faecal coliforms, *E. coli* and faecal streptococci measured in all surface water systems. However, marked differences were observed in the levels of bacteria measured during 2010 and 2011. This was expected as a result of the sampling regime.

In 2010, water samples collected from the Harts and Vaal Rivers had higher levels of total coliforms, faecal coliforms, *E. coli* and faecal streptococci during the warm-rainy season as compared to the cold-dry season in 2011. Similarly, samples collected at Barberspan during the warm-rainy season in 2011 had higher levels of total coliforms, faecal coliforms, *E. coli* and faecal streptococci as compared to samples collected in 2010 during the cold-dry season. These surface water systems are surrounded by agricultural activities and most sites are located in rural settings.

According to Hong and co-workers (2010) the observation of higher coliform levels during the rainy season may be attributed to storm water runoff flushing coliforms into receiving waters. Thus, rainfall could have been a significant contributor of faecal contamination via storm water runoff. Additionally, Tiefenthaler and co-workers (2009) illustrated that faecal indicator bacteria in water systems can be found at slightly elevated levels during the warm summer months. These authors proposed that, when high bacterial levels are observed during the warm seasons, certain factors which promote bacterial growth and regrowth exist in the water systems. Ibekwe and Lyon (2008) suggested that, the concentration of indicator bacteria in surface water may be higher during summer months due to higher temperatures that may possibly enhance and favour the growth of indicator bacteria. Even so, these authors pointed out that an increased availability of nutrients during warm summer months may also be an influencing factor.

Schoonspruit River, however, showed different trends to the other surface water systems because samples collected during the warm-rainy season in 2010 had

lower total coliforms, faecal coliforms, *E. coli* and faecal streptococci levels than samples collected during the cold-dry season in 2011. Chua and co-workers (2010) explained that this phenomenon is comprehensible since dry weather flow can contribute as much as or even more contaminants into a receiving water body than storm flows. An and co-workers (2002) suggested that their observed lower *E. coli* levels during the warm season could have resulted from a decreased loading of faecal material during summer, more vigorous grazing by protozoa, as well as the decreased viability of bacteria in warm water. In addition, several studies have demonstrated that dry weather can be a significant contributor to the total load occurring in a water body (Peterson *et al*., 2005; Stein and Tiefenthaler, 2005).

Several factors could have influenced the increased levels of bacteria in the Schoonspruit River during the cold-dry season. To mention a few: the catchment is located in a region characterized by a high population density, intense industrial and agriculture activities. It is well understood that contamination of surface water systems can originate from a wide variety of point source and non-point source pollutants. Mallin and co-workers (2000) illustrated that non-point source pollutants of surface waters are influenced by both the demography and land use activities of the area within which the surface water system is located. The Schoonspruit River sampling sites are located around urban, industrial and agricultural activities (Appendix H3 a-c). Visual observations at Voortrekker sampling site indicated drainage designs on the road which facilitate untreated runoff from the road and pavement, surface directly down into the river (Appendix H3 a). Mallin and co-workers (2000) explained that the deposition of faecal bacteria on or near impervious surfaces, exposes downstream water bodies to contamination by bacteria and other pollutants which are rapidly transported and easily facilitated by these impervious surfaces to downstream water bodies. As a result, receiving waters are impacted upon, end up with a degraded water quality and increased potential health concerns (Coulliette and Noble, 2008). Thus human development and anthropogenic activities along the Schoonspruit River could have had a

possible influence on the declining water quality observed in the river over a temporal scale of April 2010 to May 2011.

5.3 Identification and confirmation of faecal streptococci isolates

Several studies support the identification of faecal strepotococci by using biochemical tests (Facklam and Collins 1989; Knudtson and Hartman, 1992; Devriese *et al*. 1993; Manero and Blanch, 1999). However, Devriese and co-workers (1993) stated that the characterisation of environmental samples by simple physiological tests is limited. As a result the 16S rRNA gene has proven to be a very useful tool for discriminating between the main groups of enterococci (Naser *et al*., 2005; Petti *et al*., 2005).

In the present study a total of 6 species were isolated and identified by both biochemical techniques and confirmed using 16S rRNA sequencing. However, several shortcomings were found with the use of biochemical tests. Upon examination, several presumptive faecal streptococci isolates yielded a catalase positive reaction. However, the 16S rRNA sequencing confirmed these as *Enterococci casseliflavus* species. In addition, some isolates could not grow at 10° or 45°C but were confirmed as faecal streptococci using 16S rRNA sequencing data. These results are supported by similar findings in previous studies (Manero and Blanch, 1999; Müller *et al*., 2001).

The faecal streptococci species isolated from the 5 surface water systems of interest included *Enterococcus mundtii, E. faecalis, E. faecium, E. casseliflavus, E. gallinarum* and *E. hirae*. Due to the nature of the study, it is important to discuss where each faecal streptococci species was predominantly isolated as well as the implications thereof when water is used for various purposes.

Enterococcus mundtii species identified in this study were predominately isolated from Barberspan and at the Harts River in 2010 and 2011. Although *Enterococcus mundtii* have previously been isolated from humans, these species are mainly isolated from plants, soil and cattle manure (Collins *et al*., 1986; Soupir *et al*.,

2006). Thus the surface runoff and cattle manure could have been the possible source of contamination at Barberspan, the upper Harts and the Harts River. *Enterococci mundtii* has been reported to cause endophthalmitis in a healthy individual (Higashide *et al*., 2005). The presence of this pathogen in surface water sources is thus cause for concern as some of these sites are used for recreational purposes.

The *Enterococcus faecium* species group was predominately isolated in the Mooi River during 2010 and the Vaal River in 2011. In addition, *Enterococcus faecalis* species were not isolated in 2010 but frequently isolated from the Upper Harts, Schoonspruit and Vaal Rivers in 2011. Isolates belonging to these two species have previously been isolated from soil, recreational waters human faeces and sewage (Pinto *et al*., 1999; Müller *et al*., 2001; Ferguson *et al*., 2005). In addition, *E. faecium* species account for 5–15% while *E. faecalis* account for 80–90% of clinical isolates. *E. faecalis* and *E. faecium* are among the leading causes of nosocomial infections and may cause endocarditis, urinary tract infections and bacteraemia (Ratanasuwan *et al*., 1999; Saxena *et al*., 2003; Fernandez-Guerrero *et al*., 2002). Therefore, humans using water containing *E. faecalis* and *E. faecium* for full contact recreational or cultural activities are at risk of contracting infections.

Species belonging to *Enterococcus gallinarum* and *E. casseliflavus* were mostly isolated from the Schoonspruit and Vaal Rivers in both 2010 and 2011, as well as the Mooi River in 2010 and the Upper Harts River in 2011. Their isolation in 2011 was less frequent compared to the 2010 isolates. According to the phylogenetic relationship (neighbor joining trees in Section 4.4.2.3) *Enterococcus gallinarum* and *E. casseliflavus* species showed a strong grouping which was supported by 100% and 99% bootstrap values. This relationship was is characteristic of the two species because *Enterococcus gallinarum* and *E. casseliflavus* are phylogenetically and phenotypically closely related and belong to the *E. gallinarum* species group (Devriese *et al*., 1993). *E. gallinarum* and *E. casseliflavus* have been previously isolated from a variety of sources such as clinical specimens, a

subsurface flow constructed wetland, wastewater and chicken faeces (Reid *et al*., 2001; Graves and Weaver, 2009; Harwood *et al*., 2001).

Studies which isolated these two species have indicated that *E. gallinarum* and *E. casseliflavus* normally colonize the gastrointestinal tracts of both hospitalized patients and non-hospitalized healthy individuals (Toye *et al*., 1997; Iaria *et al*., 2005). *Enterococcus gallinarum* and *E. casseliflavus* are capable of causing serious persistent diseases and have been implicated in a wide variety of infections particularly in immuno-compromised individuals (Reid *et al*., 2001). Infections caused by *E. gallinarum* and *E. casseliflavus*, such as meningitis, have been associated with high mortality rates (Iaria *et al*., 2005).

Similar to Ibekwe and Lyon (2008), the species composition of some faecal streptococci isolated in this study strongly suggested that intestinal microflora from humans and animals may have been sources of these bacteria in the surface water systems. In addition, plants and soil runoff may have also been the possible source of faecal streptococci isolated in this study. What is of concern is the potential of the various species to cause severe diseases in healthy and immuno-compromised individuals using these water sources for recreational and other purposes.

5.4 Characterisation of faecal streptococci

5.4.1 Haemolysis patterns

In the 1900's haemolysin production was reported a common trait among Gram positive and negative bacteria. However, its role in virulence was unclear (Ike *et al*., 1987). Since then several studies have attempted to determine the association between haemolysin production and virulence in bacteria such as *Streptococci* and *E .coli.* Several β-haemolysis producing faecal streptococci isolates were found amongst both the 2010 and 2011 samples.

Poeta and co-workers (2008) detected and proved that a wide variety of genes of the *cly* operon in enterococci isolates are mostly found in *Enterococcus faecalis, E.*

faecium, and *E. hirae* species. Cytolysin is a toxin responsible for the rupture of a variety of target membranes resulting in the lysis of erythrocytes causing a β-hemolytic reaction usually identified as a zone of clearing on some types of blood agar (Huycke *et al*., 1998). This characteristic found in faecal streptococci isolated in the 5 surface water systems together with their ability to cause infections shows the added risks of using these surface water systems for full body contact activities.

5.4.2 Antibiotic resistance patterns

The determination of non-point sources of pollution by using only faecal indicator bacteria is challenging (Harwood *et al*., 2005). However, because antibiotic resistant patterns in bacteria are geographically specific due to their differing application regimes, non-point source pollutants can be detected using antibiotic resistant patterns (Burnes, 2003).

Faecal streptococci isolates obtained from Pedestrian Bridge were resistant to three or more antibiotics while faecal streptococci isolated from the two sites upstream (Thabo Mbeki Drive and Trimpark North Bridge) showed minimal resistance to antibiotics. This suggests that water flowing from Trimpark through the Mooi River Mall is impacted upon by humans. Trimpark is an area that is frequented by vagrants that live in the nearby bushes.

Faecal streptococci isolates obtained from site Outflow were resistant to a larger number of antibiotics compared to isolates obtained from the sites upstream. In addition, the antibiotics composing the MAR phenotypes of faecal streptococci isolates obtained in the upstream sites were also visible in the MAR phenotypes of faecal streptococci isolates obtained from site Outflow. Thus, the similarity of antibiotics resistance is a possible indicator that pollution occurring at Barberspan is relatively uniform. However, the increase in the number of antibiotics composing the MAR phenotype, as well as, the close proximity of site Outflow and Hotel is a possible indicator that human activities occurring at site Hotel, added to the

already existing pollutants, have an impact on water quality at Outflow. Correlations between the presence of antibiotic resistant bacteria in surface waters with human activities occurring close to a system have previously been demonstrated (Boon and Cattanach, 1999).

The MAR phenotypes of faecal streptococci isolated at Lichtenburg and Biesiesvlei in the Upper Harts River were the same, suggesting that pollution occurring along these sites was similar. However, the MAR phenotype of faecal streptotocci isolates obtained at Sannieshof in the Upper Harts River was different to those of the 2 upstream sites. This is an indication that certain activities, located downstream of Biesiesvlei, were impacting water quality. This is observed in the sudden change of antibiotics making-up the MAR phenotype of isolates obtained at site Sannieshof. Similarly, the MAR phenotype of faecal streptococci isolates obtained from Town-Harts of Barberspan located downstream was different to that of isolates obtained at its upstream Sannieshof sampling site in the Upper Harts River. Town-Harts site is located just after the confluence of the Upper Harts River and the Klein Harts River. This suggests that water flowing from the latter, could have had an influence and impacted water quality at site Town-Harts and caused the different MAR phenotype in isolates obtained at this site. Furthermore, faecal streptococci isolates obtained from the remaining Inflow and Outflow sites of Baberspan both showed different MAR phenotypes. This suggests that pollution occurring upstream each site was different. However, isolates from Hotel were not resistant to three or more isolates but isolates at Outflow located downstream from the Hotel were resistant to three antibiotics. This could mean that the Outflow was impacted upon by another source of pollution which channels in after the Hotel site or that the Outflow site was a reservoir of several antibiotic resistant genes.

The MAR phenotypes of faecal streptococci obtained from all Schoonspruit River sites differed considerably. In 2010, the MAR phenotype of faecal streptococci isolates obtained at Brakspruit and Bodenstein were the same. This suggests that the type of pollution taking place at these two sites was similar. In addition, the MAR phenotype of faecal streptococci isolates obtained from Voortrekker road site

was similar to that of the two upstream sites. This is probably due to the close proximity of the sites upstream to Voortrekker road site. However, the MAR phenotypes of faecal streptococci isolates obtained from site Orkney were different to those of the 3 upstream sites. Activities contributing to pollution could include livestock farming, agricultural and industrial activities occurring around Orkney. These findings were similar to those of Lin and co-workers (2004), who observed higher MAR patterns for enteric bacteria isolated in downstream sites that were characterized by urban and industrial activities compared to the upper rural areas. In 2010 faecal streptococci isolated from Bodenstein were not resistant to three or more antibiotics. However, the MAR phenotype of faecal streptococci isolated from this Brakspruit was composed of 6 antibiotics. This suggests that a source of pollution arises downstream from Bodenstein and influences water quality at Brakspruit. The intensity of pollution seems to be reduced and uniform downstream Bodenstein because faecal streptococci isolates from sites Voortrekker and Orkney showed MAR phenotypes of 3 antibiotics each that were similar.

The dominant MAR phenotype observed at Bloemhof was different to that observed at Christiana and Windsorton. This suggests that pollution occurring at Bloemhof in 2010 was different to the pollution occurring at the two downstream sites. The difference in MAR phenotypes observed at Bloemhof and at the two downstream sites could be attributed to Bloemhof Dam which is located upstream of the Bloemhof sample site. Isolates obtained from Christiana and Windsorton had a similar MAR phenotype which suggests that these isolates had similar chemical exposure histories. In 2011, faecal streptococci isolates obtained from Bloemhof were not resistant to 3 or more antibiotics, while those from Christiana were. It seemed that pollution impactors along the Vaal River increased after Christiana. Sites Windsorton and Barkley-Wes had MAR phenotypes composed of 5 and 6 antibiotics respectively. Dominant resistance to antibiotics varied between these 3 sites. However, a decrease in the number of antibiotic resistant isolates was observed at Schmidtsdrift.

The significance of measuring antibiotic resistant bacteria in surface waters is also outlined when the type of species identified in the water systems is considered. Antibiotic test results of environmental bacterial strains may possibly assist in epidemiological studies as well as determine the role these strains have during the distribution of antibiotic resistant genes (Junco *et al*., 2001). Faecal streptococci possess numerous mechanisms which aid with resistance of most antimicrobial drugs currently approved for therapeutic use in humans (Shepard and Gilmore, 2002). This bacterial group is intrinsically resistant to some multiple antibiotics and this therapeutic challenge has brought the focus of their role as important nosocomial pathogens into an even greater concern where treatment of the infections is concerned (Huyke *et al*., 1998; Jankosa, 2008; Upadhygaya *et al*., 2011). It has been suggested that even though the emergence of enterococci in nosocomial infections is probably caused by a variety of factors, antibiotic resistance appears to be a primary cause (Klare and Witte 1997; Wade, 1997).

5.4.3 Parallel action of haemolysis and multiple antibiotic resistances

The large number of antibiotic resistant bacteria found in untreated water supplies is a major public health concern (Lawrence *et al*., 2010). The implications of virulent traits such as haemolysis are an even greater cause for concern when the isolates have acquired resistance to multiple antibiotics belonging to one or more antimicrobial classes (DePaola *et al*., 1995; Ogan and Nwiika, 1993). Franz and co-workers (2003) noted that among the concerns of the dual role played by these bacteria is the risk and ability of potentially virulent strains and antibiotic resistance traits to spread outside hospital environments. The isolation of faecal streptococci that caused α -and β- haemolysis and showed multiple antibiotic resistance from surface water systems in the North West Province is a cause for concern. Particularly in areas where full human body contact to water for recreational, religious and cultural activities occurs. The virulence characteristics expressed by the isolated enterococcal species may have health implications for immuno-compromised individuals (Souto and Colombo, 2008; Baldassarri *et a*l., 2001).

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The aim of the study was to assess the surface water quality of 5 surface water systems in the North West Province. This was achieved by assessing the physico-chemical and microbial levels in the surface water systems. In addition, a total of 5 objectives were formulated to achieve the aim of this study. Below, the findings of each objective are briefly discussed.

I. Determining the physico-chemical and microbial quality of selected surface water systems in the North West Province

The quality of water in 4 rivers and an inland lake (Baberspan) situated in the North West Province was successfully determined by the use of physico-chemical and microbial parameters. Physico-chemical and microbial levels measured during the analysis were compared to target water quality ranges of various applications as set by DWAF (1996d). Their comparison to these standards yielded results of whether or not water was suitable for full and intermediate contact recreational activities, livestock watering and irrigation.

Some water sources had physico-chemical values exceeding TWQRs for full contact recreational activities and irrigation. Furthermore, seasonal variations in pH and EC levels were evident. The pH levels of water samples collected at all surface water systems in the cold-dry season were higher than the warm-rainy season. A similar pattern was observed for EC levels measured at the Harts River and Barberspan. EC levels measured at the Schoonspruit and Vaal River showed

different trends. Water samples collected during the warm rainy season in these rivers had higher levels of EC than the cold-dry season. These opposite trends could not be explained and needs further investigation.

II. Determining the prevalence and seasonal variation of total coliforms, faecal coliforms, *E. coli* and faecal streptococci, in surface water systems of interest

The prevalence of total coliforms, faecal coliforms, *E. coli* and faecal streptococci in the 5 surface water systems of interest, was successfully determined using the membrane filtration technique together with selective agars (m-FC, MLGA and KF-Streptococcus agars). The results indicated significant differences in coliform levels during the warm-rainy and cold-dry season. Water samples collected from the Lower Harts River, Barberspan and Vaal River during the warm-rainy season had higher levels of total coliforms, faecal coliforms, *E. coli* and faecal streptococci. However Schoonspruit River showed different trends of higher bacterial levels in the cold-dry season.

III. Confirmation of *E. coli* using biochemical techniques

MLG agar was used for the isolation of *E. coli* from the various surface water systems. Thereafter the TSI test was used for preliminary confirmation of *E. coli* identities. A total of 54 *E. coli* were isolated from MLG agar in 2010 and 42 were confirmed by TSI agar. Similarly, a total of 63 *E. coli* were isolated from MLG agar in 2011 and 59 were confirmed by the TSI agar. Although these were preliminary identification, they confirmed that the isolates were most likely *E. coli* and not another species. Since the focus of the study was not on *E. coli* these isolates were not further identified using more elaborate biochemical test and PCR based methods.

IV. Identification of faecal streptococci using biochemical and DNA sequencing data

The successful identification of faecal streptococci was through a series of biochemical tests and 16S rRNA gene sequencing. Diagnostic characteristics of faecal streptococci were used during their preliminary identification. These include their description as Gram positive, catalase negative bacteria that are insoluble when exposed to bile salts but have the ability to grow at 10 and 45 °C as well as 6.5% NaCl.

All isolates that were positive for all the diagnostic features were confirmed by sequencing analysis as faecal streptococci (enterococci species). The 6 enterococci species identified were *Enterococcus mundtii, E. faecalis, E. faecium, E. casseliflavus, E. gallinarum and E hirae*. Predominantly isolated species in 2010 were *E. faecium, E. casseliflavus and E. mundtii.* Similarly, the most frequently isolated species in 2011 were *E. mundtii, E. faecalis and E. faecium.*

In the 2010 phylogenetic analysis, *E. mundtii* was predominantly isolated from Barberspan, *E. faecium* from the Mooi River, *E. gallinarum* from the Schoonspruit River while *E. casseliflavus species* were isolated from the Schoonspruit and Vaal Rivers. In 2011, *Enterococcus mundtii* was isolated from the Lower Harts River, Barberspan and the Upper Harts River while *Enterococci faecium* was mostly isolated from Barberspan and the Vaal River. Lastly, *Enterococci faecalis* species were isolated from the Upper Harts, Schoonspruit and Vaal Rivers respectively.

Faecal streptococci isolates identified in this study are known to cause nosocomial infections, endocarditis, urinary tract infections, bacteraemia, meningitis and a wide variety of infections in immuno-compromised individuals. Although faecal streptococci are inhabitants of the normal human flora their infections can be exogenously acquired (Kayser, 2003). Therefore, the implications of faecal streptococci should be considered when the microbial quality of surface waters is monitored. Furthermore, the presence of these species in surface water systems

used for recreational, cultural and agricultural activities is a cause for concern as they could have serious human and animal health implications.

V. Characterisation of faecal streptococci by determining their haemolysis and antibiotic resistance patterns

The ability of isolated faecal streptococci species to cause haemolysis was successfully determined. From the 80 isolates obtained in 2010, 38 caused α-haemolysis, 26 caused β-haemolysis while 16 were non-haemolytic. Similarly, of the 59 faecal streptococci obtained in 2011, 55 caused α-haemolysis while 4 caused β-haemolysis. In addition, the resistance and susceptibility patterns of isolated faecal streptococci to 10 antibiotics were successfully determined. A large number of the 2010 isolates were resistant to Penicillin G (10 µg), Neomycin (30 µg) and Vancomycin (30 µg), while a large proportion was susceptible to Amoxicillin (10 µg) and Streptomycin (300 µg). On the other hand, a large number of the 2011 Isolates were resistant to Amoxicillin (10 µg), Penicillin G (10 µg), Neomycin (30 µg) and Ciprofloxacin (5 µg). In 2011, most of the isolates were susceptible to Chloramphenicol (30 µg) and Trimethoprim (2.5 µg).

The presence of β-haemolytic positive faecal streptococci showing resistance to multiple antibiotics in surface waters used for recreational, cultural and religious activities in the North West Province is another cause for concern. This expression of potential virulence characteristics may have health implications particularly for immuno-compromised individuals. Furthermore, the management of bacterial infections acquired through the faecal oral route may require antibiotic therapy. The latter may prove challenging when organisms causing infections are resistant to antimicrobial therapeutic agents.

6.2 Recommendations

With reference to this study, the following recommendations are suggested:

1 Parameters such as Chemical Oxygen Demand (COD) and Biological Oxygen Demand should be monitored. COD and BOD are indicators of organic pollution measured during water quality studies because they provide information that indicates the toxic conditions of a water system while reporting on the potential presence of biologically resistant organic substances (Venkatesharaju *et al*., 2010).

2 The physico-chemical and microbial levels should be correlated to rainfall patterns as well as the river flow conditions. This correlation will help explain seasonal variations and trends observed in the surface water systems.

3 *E. coli* species confirmed by biochemical techniques (such as API, Entropleuri tests) and the identification should be further confirmed by molecular methods. A polymerase chain reaction (PCR) amplification procedure that uses *E. coli* housekeeping genes such as *lacZ* and *mdh* could be used for this purpose (Bej *et al*., 1991).

4 Biochemical techniques used in this study for the identification of faecal streptococci proved to be limiting. Sequencing data was more confirmatory and should always be used for the identification of DNA. Molecular profiling techniques such as BOX-PCR and ERIC-PCR could be used to investigate the population structure of the various faecal streptococci species that were isolated in this study. Such data will provide insights into the spatial and temporal variation of such species in the surface water systems in the North West Province.

5 The use of antibiotic MAR phentoypes can be improved by increasing the range of antibiotic concentrations. This will allow for discriminant analysis which

categorises the antibiotic resistant patterns with a known database thus providing results of a high certainty (Hagedorn, *et al*., 1999; Burnes, 2003).

Finally, this study presented some baseline data on the relationship between physico-chemical water quality and microbiological parameters of 5 surface water systems in the North West Province. In addition, attention was drawn to the faecal pollution trends in the surface water systems as well as their potential risk when water is used for full and intermediate contact recreational activities, livestock watering and irrigation. Faecal streptococci have been reported to be a major cause of various infections. The presence of multiple antibiotic resistant β-haemolytic species in surface water in the North West Province is a major cause for concern and should be addressed.

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APPENDICES

Appendix A: GPS co-ordinates of all sampling sites.

River system	Sample site	GPS Co-ordinates	
		Longitude (S)	**Latitude (E)**
Mooi River	Klerkskraal Dam	S 26° 15’ 15.9”	E 027° 06’ 43.2”
	Muiskraal Dam	S 26° 26’ 70.4”	E 027° 07’10.0”
	Thabo Mbheki Drive	S 26° 41’ 05.2”	E 027° 06’ 00.8”
	Trimpark North Bridge	S 26° 42’ 29.3”	E 027 06’ 20.6”
	Pedestrian Bridge	S 26° 43’ 29.2”	E 027 06’ 21.6”
	Viljoen Road Bridge	S 26° 49’ 09.2”	E 027 06’ 00.9”
Lower Harts River	Delareyville-Harts	S 26° 46’ 01.5”	E 025° 33’ 05.9”
	Schweizer-Reneke	S 27° 11’ 57.1”	E 025° 19’ 51.5”
	Taung	S 27° 32’ 26.8”	E 024° 49’ 29.4”
	Harts-Pamp Bridge	S 27° 47’ 12.2”	E 024°42’ 19.1”
	Spitskop-Dam	S 28° 07’ 43.7”	E 024° 29’ 48.8”
	Dam 8	S 27° 50’ 56.8”	E 024° 45’ 49.7”
Lower Harts River	Delareyville-Harts	S 26° 46’ 01.5”	E 025° 33’ 05.9”
	Schweizer-Reneke	S 27° 11’ 57.1”	E 025° 19’ 51.5”
	Taung	S 27° 32’ 26.8”	E 024° 49’ 29.4”

River system	Sample site	GPS Co-ordinates	
		Longitude (S)	**Latitude (E)**
Barberspan	Town-Harts	S 26° 38’ 37.2”	E 25° 36’ 58.8”
	Inflow	S 26° 37’ 06.6”	E 25° 34’ 40.0”
	Hotel	S 26° 35’ 26.1”	E 025° 36’ 10.9”
	Outflow	S 26° 33’ 00.8”	E 025° 35’ 48.1”
Schoonspruit River	Orkney	S 26° 59’ 144”	E 26° 37’ 906”
	Voortrekker	S 26° 51’ 899”	E 26° 39’ 541”
	Brakspruit	S 26° 40’ 759”	E 26° 34’ 957”
	Bodenstein	S 26° 19’ 717”	E 26° 29’ 181”
Vaal River	Bloemhof	S 27° 39’ 14.7”	E 025° 35’ 41.1”
	Christiana	S 27° 54’ 50.3”	E 025° 10’ 32.9”
	Windsorton	S 28° 19’ 41.3”	E 024° 43’ 10.4”
	Barkley-Wes	S 28° 32’ 59.9”	E 024° 31’ 58.8”
	Schmidtsdrift	S 28° 19’ 41.3”	E 024° 04’ 29.9’

Appendix B: Target Water Quality Variables.

Variable		**Target Water Quality Range (TWQR)**				
		Domestic	**Recreation**		**Livestock watering**	**Irrigation**
			Full contact	**Intermediate contact**		
pH (pH units)		6-9	6.5-8.5	NA	NA	6.5-8.4
Electrical Conductivity(mS/m)		0-70	NA	NA	NA	0-40
Nitrite (mg/l)		0-6	NA	NA	0-10	0-0.5
Faecal streptococci (cfu/100 ml)		NA	0-30	0-230	NA	NA
Coliforms (cfu/100 ml)	Total coliforms	0-5	NA	NA	NA	NA
	faecal coliforms	0	0-150	0-1000	0-200	<1
	E. coli	NA	0-130	NA	NA	NA

Appendix C: Antibiotic zone diameter interpretative standards *Streptococcus* spp.

Antibiotic class	Antibiotic	Abbreviation	Concentration [µg]	R	I	S
β-lactams	Ampicillin	AMP	10	≤16	-	≥17
	Amoxicillin	AMOX	10	≤16	-	≥17
	Penicillin G	PG	10	≤16	-	≥17
Aminoglycosides	Neomycin	NE	30	≤ 13	14-16	≥ 17
	Streptomycin	STREP	300	≤ 6	7-9	≥ 10
Glycopeptides	Vancomycin	VA	30	≤ 14	15-16	≥ 17
Chloramphenicol	Chloramphenicol	CHL	30	≤ 12	13-17	≥ 18
Quilone	Ciprofloxacin	CIP	5	≤ 15	16-20	≥ 21
Tetracycline	Oxy-Tetracycline	OT	30	≤ 14	15-18	≥ 19
Trimethroprim	Trimethoprim	TM	2.5	≤15	16-18	≥19

Appendix D: Genomic DNA isolation protocol.

Overnight cultures of faecal streptococci were grown in 10ml Brain Heart Infusion Broth (BHI) at 37°C shaking at 100rpm per minute. Bacterial cells were harvested by centrifuging 100 µl of overnight culture at 11, 000 x *g* for 5 minutes at 8, 000 x *g* and the remainder supernatant was removed. The samples were pre-lysed by resuspending the harvested cells in 180µl Buffer T1 as well as 25µl Proteinase K and incubated for 1 hour at 56°C. The samples were then vortexed; 200 µl of Buffer B3 was added and incubated at 70°C for 10 minutes. DNA binding conditions were adjusted by adding 210µl ethanol (96-100%) and vortexing the samples. Each sample was placed in a NucleoSpin® Tissue column supported by a collection tube. DNA was bound by centrifuging the sample for 1 minute at 13, 000 x *g.* The flow thru was discarded and the silica membrane was washed. Buffer BW (500 µl) was added and centrifuged at 11, 000 x *g* for 1 minute and 600µl of Buffer B5 was added to the column and centrifuged for 1 minute. The flow thru was discarded, thereafter the silica membrane was dried and residual ethanol removed by centrifuging the column at 11, 000 x *g* for 1 minute. The NucleoSpin® Tissue was placed in a 1.5ml microcentrifuge tube, 100µl pre-warmed Buffer BE (70°C) was incubated at room temperature and centrifuged 11, 000 x *g* for 1 minute respectively. The DNA samples were stored at 4°C until required.

Appendix E: *E. coli* biochemical tests results for 2010 and 2011.

River system	Isolate	Sample site	Gram reaction	TSI		
				Colour	Gas	H_2S
Mooi River 2010	2.1	Klerkskraal Dam	-	Yellow	+	-
	2.2	Klerkskraal Dam	-	Yellow	+	-
	2.3	Klerkskraal Dam	-	Yellow	+	-
	2.4	Muiskraal Dam	-	Yellow	+	-
	2.5	Muiskraal Dam	-	Red, Black precipitate	-	+
	2.6	Muiskraal Dam	-	Yellow	+	-
	2.7	Around the World Bridge	-	Yellow	+	-
	2.8	Around the World Bridge	-	Yellow	+	-
	2.9	Around the World Bridge	-	Yellow	+	-
	2.10	Thabo Mbheki Drive	-	Red, Black precipitate	-	+
	2.11	Thabo Mbheki Drive	-	Yellow	+	-
	2.12	Thabo Mbheki Drive	-	Red, Black precipitate	-	+
	2.13	Trimpark North Bridge	-	Red, Black precipitate	-	+
	2.14	Trimpark North Bridge	-	Red, Black precipitate	-	+
	2.15	Trimpark North Bridge	-	Yellow	+	-
	2.16	Pedestrian Bridge	-	Yellow	+	-
	2.17	Pedestrian Bridge	-	Red, Black precipitate	-	+
	2.18	Viljoen road Bridge	-	Red, Black precipitate	-	+
	2.19	Viljoen road Bridge	-	Yellow	+	-

River system	Isolate	Sample site	Gram reaction	TSI		
				Colour	Gas	H_2S
Harts River 2010	5.8	Harts-pamp Dam	-	Yellow	+	-
	5.9	Harts-pamp Dam	-	Yellow	+	-
	5.10	Harts-pamp Dam	-	Yellow	+	-
	5.11	Dam 8	-	Red, Black precipitate	-	+
	5.12	Dam 8	-	Yellow	+	-
Barberspan 2010	10.11	Inflow	-	Yellow	+	-
	10.12	Inflow	-	Red, Black precipitate	-	+
	10.13	Inflow	-	Yellow	+	-
	10.14	Town	-	Yellow	+	-
	10.15	Town	-	Yellow	+	-
	10.16	Town	-	Yellow	+	-
	10.17	Hotel	-	Yellow	+	-
Schoonspruit River 2010	7.1	Orkney	-	Yellow	+	-
	7.2	Orkney	-	Red, Black precipitate	-	+
	7.3	Orkney	-	Yellow	+	-
	7.4	Voortrekker	-	Yellow	+	-
	7.5	Voortrekker	-	Yellow	+	-
	7.6	Voortrekkker	-	Yellow	+	-
	7.7	Brakspruit	-	Yellow	+	-
	7.8	Bodenstein	-	Yellow	+	-
	7.9	Bodenstein	-	Yellow	+	-

River system	Isolate	Sample site	Gram reaction	TSI		
				Colour	Gas	H_2S
Vaal River 2010	9.1	Windsorton	-	Yellow	+	-
	9.2	Windsorton	-	Yellow	+	-
	9.3	Windsorton	-	Yellow	+	-
	9.4	Schmidtsdrift	-	Red, Black precipitate	-	+
	9.5	Schmidtsdrift	-	Yellow	+	-
	9.6	Schmidtsdrift	-	Yellow	+	-
	9.7	Bloemhof	-	Yellow	+	-
	9.8	Bloemhof	-	Yellow	+	-
	9.9	Bloemhof	-	Yellow, Black precipitate	-	+
	9.10	Christiana	-	Yellow	+	-
	9.11	Christiana	-	Yellow	+	-
	9.12	Barkley-Wes	-	Yellow	+	-
	9.13	Barkley-Wes	-	Yellow	+	-
	9.14	Barkley-Wes	-	Yellow	+	-

River system	Isolate	Sample site	Gram reaction	TSI		
				Colour	Gas	H_2S
Lower Harts River 2011	14.16	Delareyville-Harts	-	Yellow	+	-
	14.17	Delareyville-Harts	-	Yellow	+	-
	14.18	Delareyville-Harts	-	Yellow	+	-
	14.19	Schweizer-Reneke	-	Yellow	+	-
	14.20	Schweizer-Reneke	-	Yellow	+	-
	14.21	Schweizer-Reneke	-	Yellow	+	-
	14.22	Taung	-	Yellow	+	-
	14.23	Taung	-	Yellow	+	-
	14.24	Taung	-	Yellow	+	-
	14.25	Harts-pamp Dam	-	Yellow	+	-
	14.26	Harts-pamp Dam	-	Yellow	+	-
	14.27	Harts-pamp Dam	-	Yellow	+	-
	14.28	Spitskop Dam	-	Yellow	+	-
	14.29	Spitskop Dam	-	Yellow	+	-
	14.30	Spitskop Dam	-	Yellow	+	-
Barberspan 2011	11.15	Town-Harts	-	Yellow	+	-
	11.16	Town-Harts	-	Yellow	+	-
	11.17	Town-Harts	-	Yellow	+	-
	11.18	Inflow	-	Yellow	+	-
	11.19	Inflow	-	Yellow	+	-
	11.20	Inflow	-	Yellow	+	-

River system	Isolate	Sample site	Gram reaction	TSI		
				Colour	Gas	H_2S
Barberspan 2011	11.21	Hotel	-	Red, Black precipitate	-	+
	11.22	Hotel	-	Yellow	+	-
	11.23	Hotel	-	Red, Black precipitate	-	+
	11.24	Outflow	-	Yellow	+	-
	11.25	Outflow	-	Yellow	+	-
	11.26	Outflow	-	Yellow	+	-
Upper Harts River 2011	11.27	Biesiesvlei	-	Yellow	+	-
	11.28	Biesiesvlei	-	Yellow	+	-
	11.29	Biesiesvlei	-	Yellow	+	-
	11.30	Sannieshof	-	Yellow	+	-
	11.31	Sannieshof	-	Yellow	+	-
	11.32	Sannieshof	-	Yellow	+	-
	11.33	Lichtenburg	-	Yellow	+	-
	11.34	Lichtenburg	-	Yellow	+	-
	11.35	Lichtenburg	-	Yellow	+	-
Schoonspruit River 2011	12.13	Orkney	-	Yellow	+	-
	12.14	Orkney	-	Yellow	+	-
	12.15	Orkney	-	Yellow	+	-
	12.16	Klerksdorp	-	Yellow	+	-
	12.17	Klerksdorp	-	Yellow	+	-
	12.18	Klerksdorp	-	Yellow	+	-

River system	Isolate	Sample site	Gram reaction	TSI		
				Colour	Gas	H_2S
Schoonspruit 2011	12.19	Brakspruit	-	Yellow	+	-
	12.20	Brakspruit	-	Yellow	+	-
	12.21	Brakspruit	-	Yellow	+	-
	12.22	Bodenstein	-	Yellow	+	-
	12.23	Bodenstein	-	Yellow	+	-
	12.24	Bodenstein	-	Yellow	+	-
Vaal River 2011	13.16	Bloemhof	-	Yellow	+	-
	13.17	Bloemhof	-	Yellow	+	-
	13.18	Bloemhof	-	Yellow, Black precipitate	-	+
	13.19	Christiana	-	Yellow	+	-
	13.20	Christiana	-	Yellow	+	-
	13.21	Christiana	-	Yellow	+	-
	13.22	Windsorton	-	Yellow	+	-
	13.23	Windsorton	-	Yellow	+	-
	13.24	Windsorton	-	Yellow	+	-
	13.25	Barkley-Wes	-	Yellow	+	-
	13.26	Barkley-Wes	-	Yellow	+	-
	13.27	Barkley-Wes	-	Yellow, Black precipitate	-	+
	13.28	Schmidtsdrift	-	Yellow	+	-
	13.29	Schmidtsdrift	-	Yellow	+	-
	13.30	Schmidtsdrift	-	Yellow	+	-

Appendix F: Faecal streptococci biochemical tests results for 2010 and 2011.

River system	Isolate	Sample site	Gram reaction	Catalase activity	Bile solubility	Growth 6,5% NaCl	Growth 10°C	Growth 45°C	Haemolysis α,β,γ
Mooi River 2010	4.1	Klerkskraal Dam	+	-	-	+	+	+	α
	4.2	Klerkskraal Dam	+	-	-	+	+	+	α
	4.3	Klerkskraal Dam	+	-	-	-	-	-	α
	4.6	Muiskraal Dam	+	-	-	+	-	-	α
	4.7	Muiskraal Dam	+	-	-	-	+	+	α
	4.8	Muiskraal Dam	+	-	-	-	-	-	α
	4.16	Around the World Bridge	+	-	-	+	+	+	α
	4.17	Around the World Bridge	+	-	-	+	+	+	α
	4.18	Around the World Bridge	+	-	+	+	+	+	β
	4.9	Thabo Mbheki Drive	+	-	-	+	+	-	α
	4.10	Thabo Mbheki Drive	+	-	-	+	+	+	α
	4.11	Thabo Mbheki Drive	+	-	+	-	+	+	β
	4.13	Trimpark North Bridge	+	-	-	+	+	+	α
	4.14	Trimpark North Bridge	+	-	+	+	+	+	β
	4.15	Trimpark North Bridge	+	-	+	+	+	+	β
	1.4	Pedestrian Bridge	+	-	-	-	-	-	α
	1.7	Pedestrian Bridge	+	-	+	+	+	+	β
	1.13	Pedestrian Bridge	+	-	-	-	-	-	α
	36	Viljoen road Bridge	+	-	-	+	+	+	α
	37	Viljoen road Bridge	+	-	-	-	-	-	α
	38	Viljoen road Bridge	+	-	-	+	+	+	α
	39	Viljoen road Bridge	+	-	-	+	-	-	α

River system	Isolate	Sample site	Gram reaction	Catalase activity	Bile solubility	Growth 6,5% NaCl	Growth 10°C	Growth 45°C	Haemolysis α,β,γ
Upper Harts River 2010	5.1	Harts-Pampierstad Bridge	+	-	+	+	+	+	β
	5.2	Harts-Pampierstad Bridge	+	-	-	-	+	-	α
	5.3	Harts-Pampierstad Bridge	+	-	+	+	-	+	β
	HP-R	Harts-Pampierstad Bridge	+	-	-	+	+	-	α
	5.5	Dam 8	+	-	+	-	-	+	α
	5.6	Dam 8	+	-	-	-	+	-	α
	5.7	Dam 8	+	-	-	+	-	+	α
	D8-R	Dam 8	+	-	+	+	+	+	β
Barberspan 2010	10.1	Outflow	+	-	-	+	+	+	γ
	10.2	Outflow	+	-	-	+	+	+	γ
	10.3	Outflow	+	-	-	+	+	+	γ
	Out-R	Outflow	+	-	-	+	+	+	γ
	10.4	Inflow	+	-	-	+	+	+	γ
	10.5	Inflow	+	-	-	+	+	+	γ
	10.6	Inflow	+	-	-	+	+	+	γ
	Inf-R	Inflow	+	-	-	+	+	+	γ
	10.7	Town	+	-	-	+	+	+	γ
	10.8	Town	+	-	-	+	+	+	γ
	10.9	Town	+	-	-	+	+	+	γ
	Tow-R	Town	+	-	-	+	+	+	γ
	10.10	Hotel	+	-	-	+	+	+	γ
	10.11	Hotel	+	-	-	+	+	+	γ
	10.12	Hotel	+	-	-	+	+	+	γ
	Hot-R	Hotel	+	-	-	+	+	+	γ
Schoonspruit River 2010	6.1	Orkney	+	-	+	+	+	+	β
	6.2	Orkney	+	-	+	+	-	-	β
	6.3	Orkney	+	-	+	+	-	+	β
	Ork-R	Orkney	+	-	-	+	+	+	α

River system	Isolate	Sample site	Gram reaction	Catalase activity	Bile solubility	Growth 6,5% NaCl	Growth 10°C	Growth 45°C	Haemolysis α,β,γ
Schoonspruit River 2010	6.4	Voortrekker road	+	-	-	+	+	+	α
	6.5	Voortrekker road	+	-	-	-	-	-	α
	6.6	Voortrekker road	+	-	+	-	-	-	β
	VT-R	Voortrekker road	+	-	+	-	+	+	α
	6.7	Brakspruit	+	-	+	-	-	-	β
	6.8	Brakspruit	+	-	+	-	-	-	β
	6.9	Brakspruit	+	-	+	-	-	-	β
	Brak-R	Brakspruit	+	-	-	-	-	-	α
	6.10	Bodenstein	+	-	+	+	+	+	β
	6.11	Bodenstein	+	-	+	-	-	-	β
	6.12	Bodenstein	+	-	+	+	+	+	β
	Bod-R	Bodenstein	+	-	-	-	+	-	α
Vaal River 2010	8.1	Barkley-Wes	+	+	+				
	8.2	Barkley-Wes	+	-	-	+	+	+	α
	8.3	Barkley-Wes	+	-	-	+	+	+	α
	Bar-R	Barkley-Wes	+	-	+	+	+	+	β
	8.4	Windsorton	+	-	-	+	+	+	α
	8.5	Windsortn	+	-	+	+	+	+	β
	8.6	Windsorton	+	-	+	+	+	+	β
	Wind-R	Windsorton	+	-	+	-	+	+	β
	8.7	Christiana	+	-	-	+	+	+	α
	8.8	Christiana	+	-	-	+	+	+	α
	8.9	Christiana	+	-	-	+	-	+	α
	Chris-R	Christiana	+	-	-	+	+	+	α
	8.10	Schmidtdrift	+	-	+	-	-	-	β
	8.11	Schmidtdrift	+	-	+	-	-	-	β
	8.12	Schmidtdrift	+	-	-	-	-	-	α
	Schmi-R	Schmidtdrift	+	+	+	-	-	-	
	8.13	Bloemhof	+	-	-	+	+	+	α
	8.14	Bloemhof	+	-	-	+	-	+	α
	8.15	Bloemhof	+	-	-	+	+	+	α
	Bloem-R	Bloemhof	+	-	+	+	-	+	β

River system	Isolate	Sample site	Gram reaction	Catalase activity	Bile solubility	Growth 6,5% NaCl	Growth 10°C	Growth 45°C	Haemolysis α,β,γ
Lower Harts River 2011	14.1	Delareyville-Harts	+	-	-	+	+	+	α
	14.2	Delareyville-Harts	+	-	-	+	+	+	α
	14.3	Delareyville-Harts	+	-	-	+	+	+	α
	14.4	Schweizer-Reneke	+	-	-	+	+	+	α
	14.5	Schweizer-Reneke	+	-	-	+	+	+	α
	14.6	Schweizer-Reneke	+	-	-	+	+	+	α
	14.7	Taung	+	-	-	+	+	+	α
	14.8	Taung	+	-	-	+	+	+	α
	14.9	Taung	+	-	-	-	+	+	α
	14.10	Harts-pamp Dam	+	-	-	+	+	+	α
	14.11	Harts-pamp Dam	+	-	-	+	+	+	α
	14.12	Harts-pamp Dam	+	-	-	+	+	+	α
	14.13	Spitskop Dam	+	-	-	+	+	+	β
	14.14	Spitskop Dam	+	-	-	+	+	+	β
Barberspan 2011	11.1	Town-Harts	+	-	-	+	+	+	α
	11.2	Town-Harts	+	-	-	+	+	+	α
	11.3	Town-Harts	+	-	-	+	+	+	α
	11.4	Inflow	+	-	-	+	+	+	α
	11.5	Inflow	+	-	-	+	+	+	α
	11.6	Inflow	+	-	-	+	+	+	α
	11.7	Hotel	+	-	-	+	+	+	α
	11.8	Hotel	+	-	-	+	+	+	α
	11.9	Hotel	+	-	-	+	+	+	α
	11.10	Outflow	+	-	-	+	+	+	α
	11.11	Outflow	+	-	-	+	+	+	α
	11.12	Outflow	+	-	+	+	+	+	α
Upper Harts River 2011	11.13	Biesiesvlei	+	-	+	+	+	+	α
	11.14	Biesiesvlei	+	-	-	+	+	+	α
	11.15	Biesiesvlei	+	-	+	+	+	+	α
	11.16	Sannieshof	+	-	-	+	+	+	α
	11.17	Sannieshof	+	-	-	+	+	+	α
	11.18	Sannieshof	+	-	+	+	-	+	α

River system	Isolate	Sample site	Gram reaction	Catalase activity	Bile solubility	Growth 6,5% NaCl	Growth 10°C	Growth 45°C	Haemolysis α,β,γ
Upper Harts River 2011	11.19	Lichtenburg	+	-	+	+	+	+	α
	11.20	Lichtenburg	+	-	-	+	+	+	α
	11.21	Lichtenburg	+	-	-	+	-	+	α
Schoonspruit River 2011	12.1	Orkney	+	-	-	+	+	+	α
	12.2	Orkney	+	-	+	+	+	+	α
	12.3	Orkney	+	-	+	+	+	+	β
	12.4	Voortrekker	+	-	+	+	+	+	α
	12.5	Voortrekker	+	-	+	+	+	+	α
	12.6	Voortrekker	+	-	+	+	+	+	α
	12.7	Brakspruit	+	-	+	+	+	-	α
	12.8	Brakspruit	+	-	-	+	+	-	α
	12.9	Brakspruit	+	+	-	-	-	-	α
	12.10	Bodenstein	+	-	+	+	+	+	β
	12.11	Bodenstein	+	-	+	+	+	+	α
	12.12	Bodenstein	+	-	-	+	+	+	α
Vaal River 2011	13.1	Bloemhof	+	-	-	+	+	+	α
	13.2	Bloemhof	+	-	-	+	+	+	α
	13.3	Bloenhof	+	-	-	+	+	+	α
	13.4	Christiana	+	-	-	+	+	+	α
	13.5	Christiana	+	-	-	+	+	+	α
	13.6	Christiana	+	-	-	+	+	+	α
	13.7	Windsorton	+	-	-	+	+	+	α
	13.8	Windsorton	+	-	-	-	-	-	α
	13.9	Windsorton	+	+	-	+	+	+	α
	13.10	Barkley-Wes	+	-	-	+	+	+	α
	13.11	Barkley-Wes	+	-	-	+	+	+	α
	13.12	Barkley-Wes	+	-	-	+	+	+	α
	13.13	Schmidtsdrift	+	-	-	+	+	+	α
	13.14	Schmidtsdrift	+	-	-	+	+	+	α
	13.15	Schmidtsdrift	+	+	-	-	-	-	α

Appendix G: GenBank identification of the amplified faecal streptococci isolates from the various surface water systems.

River system	Band ID	Accession number of Genbank match	Closest Genbank match	Strain	% identity (no of bp)	Source
Mooi River 2010	4.1	JN653465.1	Enterococcus faecium	CE3.A	100 (1364bp)	Epiphyte from plant surface
	4.2	AB671573.1	Enterococcus faecium	SJL302	100 (858bp)	Fermented zoned cerith
	4.6	JN560911.1	Enterococcus faecium	044	99 (1452)	Unknown
	4.16	JN653465.1	Enterococcus faecium	CE3.A	99 (1364)	Epiphyte from plant surface
	4.17	JN653465.1	Enterococcus faecium	CE3.A	99 (1364)	Epiphyte from plant surface
	4.8	JN560911.1	Enterococcus faecium	044	100 (1452)	Unknown
	4.9	FJ656729.1	Enterococcus casseliflavus	LHICA_41_6	100 (778bp)	Unknown
	4.13	JN560911.1	Enterococcus faecium	044	100 (1452)	Unknown
	1.4	JN653465.1	Enterococcus faecium	CE3.A	99 (1364)	Epiphyte from plant surface
	38	JN644509.1	Enterococcus hirae	M10_2A	100 (1525)	Unknown
Lower Harts River 2010	5.1	JN020631.1	Enterococcus gallinarum	W184	100 (1552)	West African soft cheese
	5.2	JN020631.1	Enterococcus gallinarum	W184	99 (1552)	West African soft cheese
	5.3	JN020631.1	Enterococcus gallinarum	W184	100 (1552)	West African soft cheese
	5.6	JN653465.1	Enterococcus faecium	CE3.A	99 (1364)	Epiphyte from plant surface
Barberspan 2010	10.1	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	10.4	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	10.6	JN257085.1	Enterococcus mundtii	Aq5	99 (1436)	Unknown
	10.7	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	10.8	JN257085.1	Enterococcus mundtii	Aq5	98 (1436)	Unknown
	10.9	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	10.10	JN653465.1	Enterococcus faecium	CE3.A	100 (1364)	Epiphyte from plant surface
	10.11	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown

River system	Band ID	Accession number of Genbank match	Closest Genbank match	Strain	% identity (no of bp)	Source
Schoonspruit River 2010	6.1	JN653465.1	Enterococcus faecium	CE3.A	99 (1364)	Epiphyte from plant surface
	6.3	AB671565.1	Enterococcus casseliflavus	RZC110	100 (853)	Raw zoned cerith
	6.6	AB671565.1	Enterococcus casseliflavus	RZC110	99 (853)	Raw zoned cerith
	6.9	JN020631.1	Enterococcus gallinarum	W184	99 (1552)	West African soft cheese
	6.11	JN020631.1	Enterococcus gallinarum	W184	99 (1552)	West African soft cheese
	6.12	JF774412.1	Enterococcus gallinarum	U82	99 (1560)	Ugba (Nigeria)
Vaal River 2010	8.3	FJ656729.1	Enterococcus casseliflavus	LHICA_41_6	100 (778)	Packaged meat
	8.4	FJ656729.1	Enterococcus casseliflavus	LHICA_41_6	100 (778)	Packaged meat
	8.5	AB671565.1	Enterococcus casseliflavus	RZC110	100 (853)	Raw zoned cerith
	8.6	FJ656729.1	Enterococcus casseliflavus	LHICA_41_6	99(778)	Packaged meat
	8.7	AB671565.1	Enterococcus casseliflavus	RZC110	99 (853)	Raw zoned cerith
	8.8	AB671565.1	Enterococcus casseliflavus	RZC110	99 (853)	Raw zoned cerith
	8.9	JN653465.1	Enterococcus faecium	CE3.A	92 (1364)	Epiphyte from plant surface

River system	Band ID	Accession number of Genbank match	Closest Genbank match	Strain	% identity (no of bp)	Source
Lower Harts River 2011	14.1	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.2	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.3	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.4	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.5	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.6	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.7	JN560911.1	Enterococcus faecium	044	100 (1452)	Unknown
	14.8	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	14.9	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.10	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.11	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.12	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	14.13	AB671565.1	Enterococcus casseliflavus	RZC110	100 (853)	Raw zoned cerith
	14.14	HQ450720.1	Enterococcus gallinarum	UPAA106	99 (786)	Digestive tract

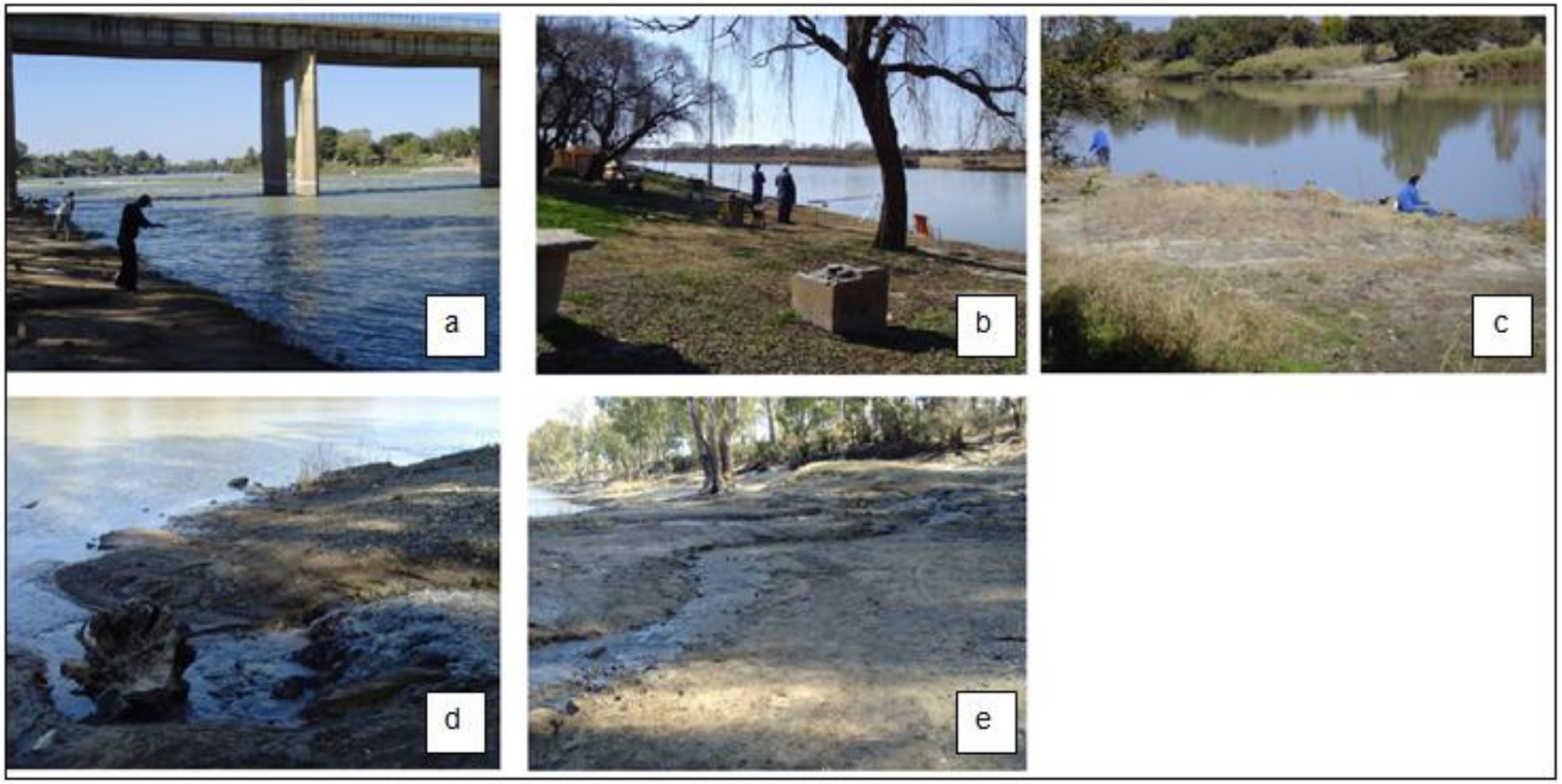
River system	Band ID	Accession number of Genbank match	Closest Genbank match	Strain	% identity (no of bp)	Source
Barberspan 2011	11.1	AB671573.1	Enterococcus faecium	SJL302	100 (858)	Fermented zoned cerith
	11.2	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	11.3	JN587279.1	Enterococcus faecalis	E12	100 (854)	Unknown
	11.4	AB671573.1	Enterococcus faecium	SJL302	100 (858)	Unknown
	11.5	JN587279.1	Enterococcus faecalis	E12	100 (854)	Unknown
	11.7	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	11.8	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	11.9	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	11.10	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	11.11	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	11.12	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
Upper Harts River 2011	11.13	JN587279.1	Enterococcus faecalis	E12	93 (854)	Unknown
	11.14	JN587279.1	Enterococcus faecalis	E12	100 (854)	Unknown
	11.16	AB671573.1	Enterococcus faecium	SJL302	100 (858)	Unknown
	11.17	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	11.18	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	11.19	JN587279.1	Enterococcus faecalis	E12	99 (854)	Vaginal tract
	11.21	FN822767.1	Enterococcus mundtii	HAMB13965	100 (1479)	Unknown

River system	Band ID	Accession number of Genbank matc	Closest Genbank match	Strain	% identity (no of bp)	Source
Schoonspruit River 2011	12.1	JN560911.1	Enterococcus faecium	044	100 (1452)	Unknown
	12.2	AB671573.1	Enterococcus faecium	SJL302	99 (858)	Fermented zoned cerith
	12.3	JN587279.1	Enterococcus faecalis	E12	99 (854)	Vaginal tract
	12.4	JN506911.1	Enterococcus faecium	044	100 (1452)	Unknown
	12.5	JN560885.1	Enterococcus hirae	0104	99 (1443)	Unknown
	12.6	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	12.7	JN587279.1	Enterococcus faecalis	E12	99 (854)	Vaginal tract
	12.8	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	12.9	AB671565.1	Enterococcus casseliflavus	RZC110	99 (853)	Raw zoned cerith
	12.10	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	12.11	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract

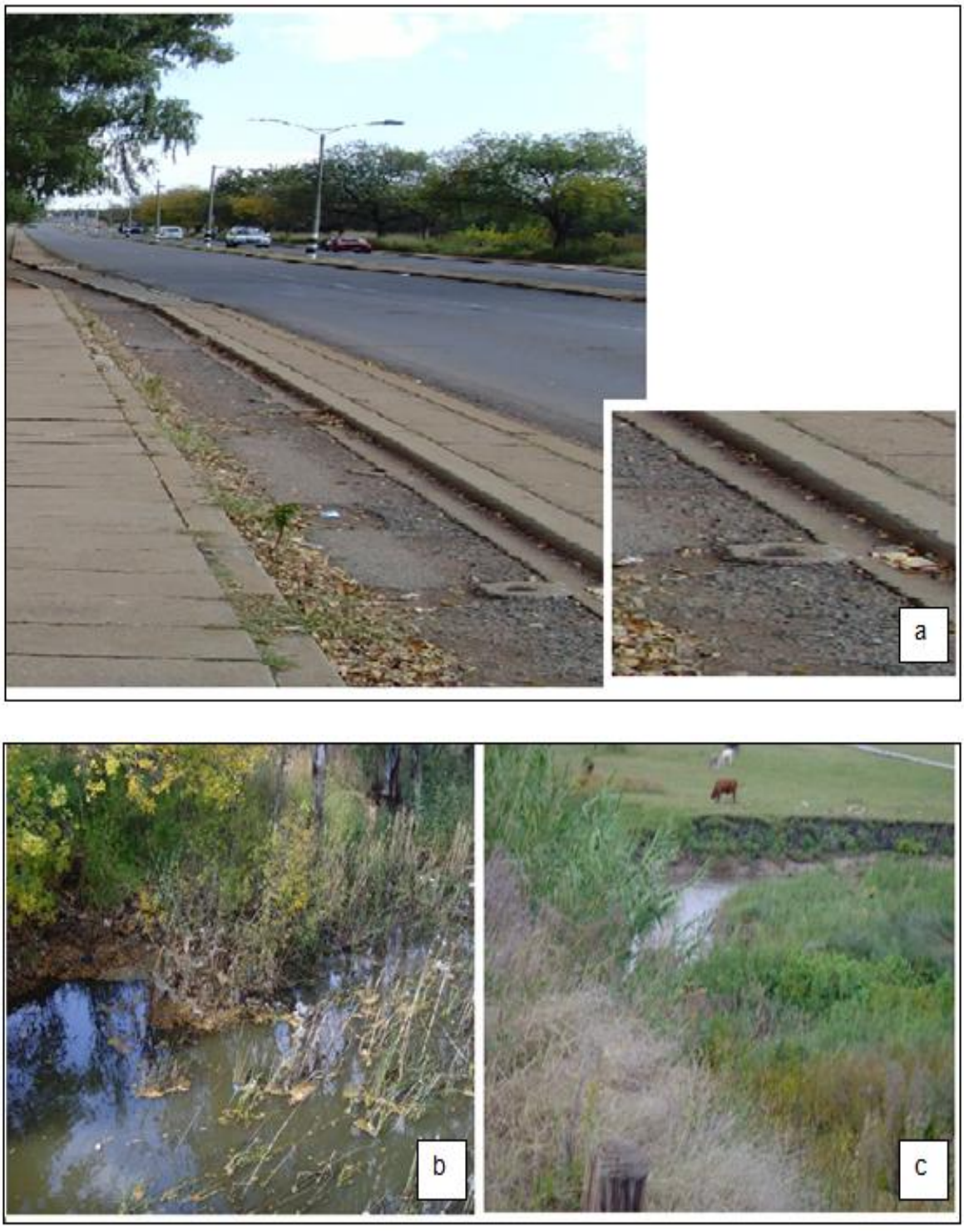
River system	Band ID	Accession number of Genbank matc	Closest Genbank match	Strain	% identity (no of bp)	Source
Vaal River 2011	13.1	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	13.2	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	13.3	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	13.4	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	13.5	JN560911.1	Enterococcus faecium	044	100 (1452)	Unknown
	13.6	JN560911.1	Enterococcus faecium	044	99 (1452)	Unknown
	13.7	AB671573.1	Enterococcus faecium	SJL302	100 (858)	Fermented zoned cerith
	13.9	FJ656729.1	Enterococcus casseliflavus	LHICA_41_6	100 (778)	Packaged meat
	13.10	JJN560911.1	Enterococcus faecium	044	99 (1452)	Unknown
	13.11	AB671573.1	Enterococcus faecium	SJL302	100 (858)	Fermented zoned cerith
	13.12	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown
	13.13	JN587279.1	Enterococcus faecalis	E12	100 (854)	Vaginal tract
	13.14	JN257085.1	Enterococcus mundtii	Aq5	100 (1436)	Unknown

Appendix H: Relevant photos of sampling sites discussed in chapter 5.

H1 a-f Visual observations of sample sites a. Harts-Pamp Bridge, b. Biesiesvlei, c. Orkney, d. Taung, e. Delarey-Harts and f. Schweizer-Reneke.



H2 a-e: Visual observations of sample sites a. Bloemhof, b. Christiana, c. Windsorton, d & e. Bloemhof.



H3 a-c: Visual observations of sample sites a & b Voortrekker, c. Orkney.