Microbial and physico-chemical quality of groundwater in the North-West Province, South Africa

By

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ABSTRACT

More than 80% of the North West Province's (NWP's) rural community solely depend on groundwater for their water needs (Kalule-Sabiti & Heath, 2008). Aquifers are exposed to pollution from anthropogenic activities, yet comprehensive data about the microbial and physico-chemical quality of this water source in the NWP is lacking. The aim of this study was to generate data indicating the microbial and physico-chemical quality of groundwater in the North West Province. Detection of faecal indicator bacteria indicates the possible presence of disease causing pathogens such as viruses (enterovirus, adenovirus and hepatitis A & B) and bacteria (*Vibrio cholerae, Shigella* spp. *Yersina* spp. and *Enterocolitica* spp.). Results may facilitate in predicting possible health risks to exposed communities. Methods included membrane filtration, culture-based methods, biochemical tests, Kirby-Bauer disk diffusion for antibiotic profiles and multiplex PCR for *E. coli* detection. Physico-chemical variables were measured with calibrated multi-meters and probes. Sampling took place during two sampling periods of 2009 and 2010. A total of 114 boreholes were tested in this study. Of the 76 boreholes tested in 2009, 49% were positive for faecal coliforms, 67% for faecal streptococci, 47% for presumptive *P. aeruginosa* and 7% for *S. aureus*. Thirty-three percent of boreholes had heterotrophic plate counts exceeding 1000cfu/ml, increasing the risk of infectious disease transmission. Detection of faecal indicators was higher in the warm, wet seasons than the cold and dry season. Members of the *Enterobacteriaceae* family were identified with the API 20E system, including *E. coli* and *K. pneumonia.* In 2010, 38 boreholes were sampled, of which 55% were positive for faecal coliforms, 63% for faecal streptococci, and 55% for *P. aeruginosa.* Based on the results obtained from MLGA medium, 34% of the 2010 sampled boreholes had *E. coli* present, whereas multiplex PCR indicated *E. coli* in 47% of boreholes. Of the 114 boreholes tested over the two sampling periods, 31% had a FC/FS ratio <0.7, indicating possible non-human source faecal contamination. Fourteen percent of these had FC/FS ratio >2, indicating human source faecal

contamination. Twenty-three percent of the total boreholes tested negative for both faecal coliforms and faecal streptococci. Several of the faecal coliform isolates tested were resistant to multiple antibiotics, especially beta-lactam antibiotics. The average MAR index for 2009 was 0.213 and 0.126 for 2010. Percentage resistance of faecal coliforms to AMOX and AMP decreased significantly in 2010 (21% and 15% respectively) compared to that of 2009 (54% and 41%). Resistance to CEP remained consistent throughout both sampling periods. Intermediate resistance to KAN increased from 4% in 2009 to 37% in 2010. The pH, EC and TDS levels were at acceptable ranges for both sampling periods. Only 28% of the total boreholes tested complied with the Department of water affairs target water quality range for nitrate (6mg/L NO3-N), and 43% of the boreholes had nitrate levels >20mg/L NO3-N. This study demonstrated that groundwater from the North West Province is vulnerable to faecal and nitrate contamination. Antibiotic resistance of indicator bacteria, and subsequently pathogens, further increase the risk to water users. Therefore, this water source should be used with care. Communities should be educated on the risks involved from using this water source for domestic purposes as well as how to minimise these risks. In conclusion, groundwater is the fibre of the socio-economics of the Province and should be managed accordingly.

Keywords: Groundwater, microbiological water quality, physico-chemical water quality, rural communities, *Pseudomonas aeruginosa, Staphylococcus aureus*, faecal contamination, mPCR, *E. coli*, *LacZ*, *mdh*, antibiotic resistance, nitrates.

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DECLARATION

I declare that this dissertation for the degree of Master of Science in Environmental Science (M.Sc Env.Sci) at the North West University: Potchefstroom Campus hereby submitted, has not been submitted by me for a degree at this or another university, that it is my own work in design and execution, and that all material contained herein has been duly acknowledged.

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Simoné Ferreira **Date**

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CHAPTER 1
INTRODUCTION

1.1 GENERAL INTRODUCTION AND PROBLEM STATEMENT

According to the World Health Organization (2003a) major obstacles to human health relates to unsafe water, poor sanitation and inappropriate hygiene. Water is sustenance and no one can survive without adequate quality and quantities thereof (Pejan *et al.*, 2007). The South-African National Water Act (No. 36 of 1998) defines equity and sustainability as the central guiding principles in the protection, use, development, conservation, management and control of water resources. The National Water Act (No. 36 of 1998) also requires that National Monitoring Programmes are established. These programmes are needed to ensure that the purpose of the National Water Act is fulfilled. One such program is the National Microbial Monitoring Program (NMMP) for groundwater (Murray *et al.* 2007). The monitoring of the quality of groundwater sources is therefore mandatory. Foster & Chilton (2003) highlighted, that unlike integrated surface water monitoring, each groundwater sample merely represents the localised state of that particular groundwater sample. Therefore, many sampling points are required to provide an adequate spatial characterization of groundwater quality. Scarcity of sufficient and reliable data reduces the ability to present a comprehensive and well substantiated statement regarding the status of groundwater quality and quantity (Foster & Chilton, 2003). According to Kalule-Sabiti & Heath (2008) there is a lack of information relating to the risk associated with using groundwater in the North West Province (NWP). The compliance of groundwater in the NWP to national standards is thus not clear (Kalule-Sabiti & Heath, 2008).

There is intense pressure on South Africa's rivers and dams to meet the demands of a fast growing population and industry. Groundwater quality is deteriorating due to anthropogenic pressures that include pollution from sewer leakage, faulty septic tank operation and landfill

leachates (Grisey *et al.*, 2010; Wakida & Lerner, 2005). Contaminated storm runoff also promotes the spread of pollutants which may ultimately enter groundwater directly at shallow water tables or infiltrate to deeper aquifers (Usher *et al.*, 2004a).

The NWP of South-Africa consists largely of semi-arid land. Mining and agriculture are the two main contributing factors impacting the quantity and quality of groundwater in the NWP (Kalule-Sabiti & Heath, 2008). The NWP has a large number of rural communities of which more than 80% solely depend on underdeveloped groundwater for their sole source of water (Kalule-Sabiti & Heath, 2008).

Knowledge of the microbiological quality of water is very important. If it is not known, then the consumption of this untreated and possibly contaminated water has little chance of being implicated in self-limiting waterborne diseases (LeChevallier & Seidler, 1980).

Nitrate is acknowledged as an important water quality parameter and also a regular chemical pollutant of groundwater worldwide (Bauchard *et al.*, 1992). When concentrations of nitrates in water exceed 20mg/l, it could cause methemoglobinemia (more widely known as 'blue baby syndrome') among infants and young children (Craun *et al.*, 1981; Sadeq *et al.*, 2008; Super *et al.*, 1981). Kwenamore (2006) analysed the groundwater quality of selected boreholes within specific areas of the NWP. The detection of faecal indicator bacteria as well as high nitrates in the groundwater from the latter study strengthened the need to determine the groundwater quality of randomly selected boreholes throughout the NWP.

Furthermore, a majority of the isolates from Kwenamore's (2006) study exhibited multiple antibiotic resistant (MAR) phenotypes. The latter ability of bacteria to adapt to a variety of antibacterial agents is also a growing health concern worldwide. Therefore, the need was also

identified to determine the degree of antibiotic resistance that faecal coliforms isolated from this study possess.

1.2 RESEARCH AIM AND OBJECTIVES

The aim of this study was to determine the microbial and physico-chemical quality of groundwater in the North West Province of South-Africa.

The objectives of this study were to:

i) measure, assess and report the status of the microbial quality of selected groundwater sources in the North West Province of South-Africa;

ii) predict the possible source of faecal contamination;

iii) determine the antibiotic resistance of faecal coliforms to selected antibiotics;

iv) measure, assess and report the status of the physio-chemical quality of selected groundwater sources in the North West Province of South-Africa; and

v) determine if groundwater is safe to use for domestic purposes by comparing the measured water quality to the target water quality range set by the Department of Water Affairs and Forestry of South Africa.

CHAPTER 2
LITERATURE REVIEW

2.1 WATER QUALITY

Water quality is a term used to describe the biological, chemical and physical characteristics of water, usually with respect to its suitability for an intended purpose (DWAF, 2005). The standards of water quality vary widely for domestic, agricultural and industrial uses (DWAF, 1996b). Furthermore, water quality differs from continent to continent, and even from region to region. This is due to differences in climate, geomorphology, geology and biotic composition (Dallas & Day, 2004). The Department of Water Affairs and Forestry (DWAF, 1996a) defines the Target Water Quality Range (TWQR) for a particular constituent and water use as the range of concentrations or levels at which the presence of the constituent would have no known adverse effects on the fitness of the water assuming long-term continuous use. Thus, good quality drinking water may be consumed in any desired amount without adverse effect on health. Such water is called "potable." It is free from harmful levels of bacteria, viruses, minerals, organic substances and is aesthetically (taste, colour, turbidity, and odours) acceptable (Haman & Bottcher, 1986; WHO 1993). Both natural and human factors can influence the quality of a water source, and it is therefore necessary to identify the factors involved that individually or jointly affect the quality of the water source. Once identified, the correct measures can be applied for the regulation and remediation of the water source (Reinert & Hroncich, 1990).

2.1.1 Microbial water quality

The Water Research Commission of South Africa defines microbial water quality as the state of the water with respect to the absence (good water quality) or presence (poor water quality) of micro-organisms (WRC, 2003). Microbial water quality is usually indicated by reporting the count of indicator organisms present in a given volume of water (WRC, 2003).

Waterborne diseases can be the result when disease causing organisms (pathogens) are transmitted via drinking water contaminated by predominately faecal material or urine (especially from mammalian source) (Ashbolt *et al.,* 2001; Grabow, 1996; Ministry of health, 2005; WRC, 2003). Acute gastroenteric illness is a common disease associated with the use of faecally polluted groundwater (Craun & Calderon, 1997). According to the World Health Organization more than 3.4 million people die as a result of water related diseases every year, making it the leading cause of disease and death around the world (WHO, 2003b). Primary waterborne transmission often goes unnoticed because a disease only manifests itself after secondary or tertiary transmission (Grabow, 1996).

Table 2.1 summarises some of the most common waterborne pathogens, diseases, symptoms of the diseases as well as the bacteria, viruses or parasites causing the disease. It is observed that diarrhoea, abdominal pain, vomiting and slight fever are the most common symptoms associated with waterborne diseases (Table 2.1). According to Duncker (2000) waterborne diseases remain a cause for concern in both developing and developed countries. Craun and Calderon (1997) as well as Reynolds *et al.* (2008) attributed a large percentage of waterborne disease outbreaks in the USA to groundwater sources. Several of the pathogens in the list in Table 2.1 are spread by faecal matter. Thus if faecal contamination of water sources occur then the chance is that these pathogens could be present causing disease symptoms listed here. However, infections from water sources are not limited to enteric diseases but may be extended to the skin, throat, ears, nose and eyes (Yoshpe-Pures & Golderman, 1987).

TABLE 2.1: Common waterborne diseases caused by the transmission of pathogens (adopted from the WRC, 2003).

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

2.1.1.1 Indicator bacteria

It is impracticable and expensive to monitor water supplies for all potential human pathogens (Barrel *et al.*, 2000; Zamxaka *et al.*, 2004). Therefore surrogates are used to indicate the possible contamination of the water with human and animal excrement, the most frequent source of health-significant microbial contamination of water supplies (Barrel *et al.*, 2000; Ministry of health, 2005). These surrogates are called indicator organisms, and they should ideally fulfil the following criteria to assess the safety of water in terms of possible pathogen

presence (DWAF, 1996b; Grabow, 1996): 1) be suitable for all types of water; 2) be present in sewage and polluted waters whenever pathogens are present and in numbers higher than the pathogens; 3) should not multiply in the aquatic environment, but must survive in the environment for at least as long as pathogens; 4) be absent from unpolluted water; 5) be detectable by practical and reliable methods; and 6) should not be pathogenic (safe to work with in the laboratory). No single indicator has been found to meet all of these requirements. Jagals *et al.* (2006) argued that testing water positive for indicators of health-threatening organisms would indeed indicate the presence of pathogens. The absence of indicator bacteria in water sources would not necessarily imply the absence of pathogens (Jagals *et al.*, 2006).

The World health Organization as well as the Department of Water Affairs and Forestry has compliance guidelines for these organisms, and states that even a single organism indicating faecal contamination in 100ml of potable water is unsatisfactory (DWAF, 1996a; Fricker & Fricker, 1996; WRC, 2003). The most commonly measured bacterial indicators are total coliforms, faecal coliforms and enterococci (Noble *et al.*, 2003). Heterotrophic plate count bacteria are also an indicator of the microbial quality of water sources (LeChevallier *et al.*, 1980; WHO, 1993).

a) Heterotrophic plate count bacteria

The heterotrophic plate counts (HPC) became one of the standard techniques for microbial water quality testing (LeChevallier *et al.*, 1980). Aerobic and facultative anaerobic bacteria found in water are enumerated on simple organic culture medium. Research by Allen *et al.* (2004) has shown that high densities of HPC bacteria in water may obstruct the accurate detection of coliform bacteria when the membrane filtration method is used for coliform detection. However, insufficient clinical and epidemiological evidence exist to conclude that

HPC bacteria in drinking water pose a health risk (Allen *et al.,* 2004; Calderon & Mood, 1991; Stelma *et al.*, 2004). Research by de Wet *et al.* (2002) and Pavlov *et al.* (2004) does not agree with the statements of the latter authors. Both these authors (de Wet *et al.*, 2002; Pavlov *et al.*, 2004) isolated HPC bacteria from South African water supplies that tested positive for pathogenesis. Resistance to multiple antibiotics amongst the HPC isolates were also found (Pavlov *et al.* 2004). De Wet *et al.* (2002) concluded that health risks may be associated with the consumption of water that has HPC bacteria present.

b) Coliform bacteria

Coliform bacteria are aerobic and facultative anaerobic, Gram negative, non-spore-forming, rod-shaped bacteria that ferment lactose and form gas (Ingraham & Ingraham, 2004). They have long been recognized as a suitable microbial indicator of drinking water quality. These bacteria are easy to detect and enumerate from water (WHO, 2003c). Although coliform bacteria may not always be directly related to the presence of faecal contamination (Stevens *et al.* 2003), its presence in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae* (da Silva *et al.*, 2008).

Microorganisms of the total coliform group are a subset of the family *Enterobactericeae* and comprises of genera such as *Escherichia coli, Citrobacter* spp., *Enterobacter* spp. and *Klebsiella* spp. (Rompré *et al.,* 2002; Stevens *et al.*, 2001). Several bacteria in the genera of *Citrobacter, Enterobacter,* and *Klebsiella* conform to the classification of coliforms, but are not of faecal origin (Edberg *et al.*, 2000). In addition to the latter, the capability of many total coliforms to grow in the environment as well as distribution systems makes it unreliable faecal indicators (Tallon *et al.*, 2005).

The faecal coliforms are a subset of organisms within the group of total coliforms. Faecal coliforms are distinguished from total coliforms by their termotrophic nature, allowing them to grow at high temperatures (44.5°C). It was recognized that faecal coliform bacteria and in particular *Escherichia coli*, is a more reliable indicator for faecal contamination than total coliforms (Edberg *et al.*, 2000; Stevenson *et al.*, 2003). However, *Klebsiella* strains are known to give rise to false positive faecal coliform tests (Duncan, 1988).

Faecal coliform counts have been widely accepted for routine monitoring of water quality, due to levels of faecal coliforms mostly being directly correlated to those of *E. coli* (WHO, 1993). However, additional biochemical tests for the confirmation of isolates as *E. coli* is recommended by the World Health Organization (1993). The most reliable way to estimate the degree of recent faecal contamination of water is to specifically test for *E. coli* (Edberg *et al.*, 2000). *E. coli* are the most predominant commensal inhabitant of the facultative anaerobic colonic micro-flora (Kaper, 2005). Similar to other faecal waterborne pathogens, *E. coli* is known to utilize environmental water as a reservoir after faecal contamination by sewage pollution (Kong *et al.*, 2002) or open defecation (Ashbolt *et al.*, 2001). The use of *E. coli* as a faecal indicator organism gives a reliable indication of the presence of faeces, since *E. coli* suits the main criteria of faecal indicator organisms (Edberg *et al.*, 2000). However, several studies have shown that *E. coli* could multiply in the environment (Hardina & Fujioka, 1991; Ishii & Sadowsky, 2008).

Although *E. coli* is commensal in the human colonic micro-flora, it can be pathogenic after infection of mucosal surfaces (Nataro & Kaper, 1998). An infection of *E. coli* occurs mostly in the debilitated or immune-suppressed host, or when gastrointestinal barriers are violated. Clinical syndromes which results from an infection with *E. coli* are sepsis, meningitis, urinary tract infection or enteric diarrheal diseases (Nataro & Kaper, 1998). The *E. coli* 0157:H7

serotype is a notorious strain known for its potential to cause disease in man (Davis, 2011). Symptoms of infection with this serotype include mild fever, nausea, vomiting, stomach cramps and bloody diarrhea (Davis, 2011). Additional complications in the immune-comprised, children and elderly include renal failure, anaemia, dehydration, organ failures and dementia in the elderly (Davis, 2011).

c) Faecal Streptococci

Faecal streptococci are a sub-group of the enterococci group of organisms. Four key points makes faecal streptococci a favourable indicator organism of faecal contamination (Ashbolt *et al.,* 2001): 1) relatively high numbers occur in the faeces of humans and other warm-blooded animals; 2) their presence in wastewaters polluted waters sources; 3) their absence from pure waters, virgin soils and environments free from human and animal contact and 4) their persistence in the environment without multiplication. The numbers of faecal streptococci in faeces is lower than that of total or faecal coliforms, however they are more resistant to environmental stressors than coliform bacteria (DWAF, 1996a). Enterococci, particularly *Enterococcus faecalis* and *Enterococcus faecium* is a leading cause of nosocomial infections (White *et al.*, 2003).

In addition to indicating faecal contamination by warm-blooded animals, faecal streptococci (FS) can also be used in combination with faecal coliform (FC) data to determine a more precise source of contamination (Csuros & Csuros, 1999). The ratios between these two are indicative of the source of pollution. Water with a FC:FS ratio >4 usually indicate faecal matter from human origin, and ratios between 2 and 4 are indicative of contamination by human waste (Geldreich & Kenner, 1969; Wyer & Kay, 1995). A ratio less than 0.7 indicate livestock and poultry wastes (Csuros & Csuros, 1999). The FC:FS ratio was used as common practise in the 1950's and 1960's (Anon, 2005a). In the 1970's however, this ratio was

removed from the "Standard Methods for Examination of Water and Wastewater" when it was determined that the different species had significantly different die off rates in water (Anon, 2005a; Feachem, 1975). This caused the FC:FS ratio to alter and significantly restricted its use (Anon, 2005a). A study by Feachem (1975) concluded that the FC:FS ratio can still be useful in that an initial high FC:FS ratio will indicate human contamination, whereas initially low ratios will indicate contamination from a non-human source.

2.1.1.2 Opportunistic pathogens

a) Staphylococcus spp.

Staphylococcus spp. is Gram positive facultative anaerobic cocci that have disease-causing potential. These organisms can grow on the skin's surface, with the highest populations in relatively moist areas such as the arm pits, around the nose, and near the anus. *S. aureus* is the most pathogenic, and is carried by 5 – 10% humans. These are normally found around the anus (Ingraham & Ingraham, 2004). Furthermore, *S. aureus* is known for its regular cause of skin and soft tissue infections of open wounds which can lead to toxic shock syndrome (Dryden, 2009). This species (*S. aureus*) can also cause more serious infectious diseases in immune-comprised humans. These infections include pneumonia (Kaye *et al.*, 1990) and *Staphylococcus aureus* bacteremia (Lowly, 1998). Both these are regular communal infections with rather high morbidity and mortality rates. This organism is expected to contaminate surface water more readily than groundwater due to the recreational human contact with surface waters.

b) *Pseudomonas aeruginosa*

The potentially pathogenic *Pseudomonas aeruginosa* is a ubiquitous environmental organism that can tolerate harsh habitats and can grow at relatively low nutrient concentrations (Grisey *et al.*, 2010; Hambsch *et al.*, 2004). It usually resides in moist soils, marshes and water. It is

not unusual to detect these organisms in groundwater as they naturally inhabit soils (Ferguson *et al.,* 2001). This species (*P. aeruginosa*) can also be associated with faecal contamination especially of human origin. The latter aspect was demonstrated by Wheater *et al.* (1980). *P. aeruginosa* is a useful indicator bacterium, indicating the potential presence of pathogenic bacteria capable to grow and multiply under nutrient-limited conditions in drinking water systems (Hambsch *et al.*, 2004). Waters containing *P. aueruginosa* should not be used by immune-c comprised individuals as drinking water and/or bath water as this species can cause infection of burn wounds, ears, urinary tract and respiratory organs (Lester & Birkett, 1999).

2.1.1.3 Bacterial antibiotic resistance

One of the fastest growing health related concerns worldwide is the remarkable ability of bacteria to adapt to lethal antimicrobial agents in their environment, including antibiotics (Barbosa & Levy, 2000). The widespread use and misuse of antibiotics by humans and veterinaries is excelling this problem (Barbosa & Levy, 2000; English & Gaur, 2010; Neely & Holder, 1999). The result will be that infections caused by resistant bacteria may become increasingly difficult to manage, leading to more frequent cases of hospitalization, illness and death (Levy & Marshall, 2004; Niederman, 2001; Sader *et al.* 2003).

Antibiotics lead to the inhibition and/or death of susceptible bacteria, while selecting for the survival and dominance of bacteria possessing the genes that code for resistance mechanisms (Levy & Marshall, 2004). These genes are thus also selected and can now spread to other bacteria in the treated individual, ultimately generating a reservoir of resistant bacteria that can be spread to the environment and other individuals (Levy & Marshall, 2004). According to a review by Kümmerer (2004) there is an undeniable problem with respect to antibiotic resistant bacteria in the environment. Therefore, resistance could be considered as an environmental pollution problem, with the target pollutants being the resistant gene vectors

(Rysz & Alvarez, 2004). Munir *et al.* (2011) observed antibiotic resistant genes and antibiotic resistant bacteria concentrations as high as 2.33 x 10^6copies/100mL and 6.10 x 10^5cfu/100ml, respectively, in treated wastewater effluent. These effluents may leach through soil pores to reach groundwater. Concentrated animal feeding operations (CAFOs) commonly use veterinary antibiotics at sub-therapeutic levels for growth promotion and at therapeutic levels for disease treatment (Falkow & Kennedy, 2001). This is another common source of antibiotic resistant bacteria in surface and groundwater down-gradient from the CAFOs (Bartelt-Hunt *et al.*, 2011; Burkholder *et al.*, 2007; Falkow & Kennedy, 2001; Gilchrist *et al.*, 2007; Sapkota *et al.,* 2007).

Multiple antibiotic resistance (MAR) indicates resistance to 2 or more antibiotics, and may be a sign of the selective pressures bacteria are exposed to in an environment (Cabrera *et al.*, 2004; Guan *et al.*, 2002). The extent of antibiotic resistance of groups of bacteria can be measured with MAR indices (Guan *et al.*, 2002). An increase in MAR Gram negative isolates is becoming more evident (O'Fallon *et al.,* 2009*)*, which is cause for great concern due to the rising inadequacy to treat bacterial infections with the antibiotics presently on hand.

Beta-lactamases is an activating enzyme that can obliterate the antimicrobial drug. The genes for beta-lactamase may be found on the bacterial chromosome or may be borne on plasmids or transposons (Davies, 1994). The latter two genetic elements are mobile and promote the sharing of this gene amongst bacteria (Davies, 1994). Research by Pitout *et al.* (1998) found a variety of extended-spectrum beta-lactamases (ESBLs) amid members of the family *Enterobacteriaceae* isolated from medical centres in South Africa. ESBLs are able to confer bacterial resistance to the penicillins as well as the first-, second-, and third-generation cephalosporins (Paterson & Bonomo, 2005). These ESBLs are commonly found in *Escherichia coli* and *Klebsiella pneumoniae* (Jacoby & Medeiros, 1991).

2.1.2 Physico-chemical water quality

2.1.2.1 Ambient water temperature

Water temperature can have an effect on the microbiological population of water. Bonton *et al.* (2010), Buckalew *et al.* (2006) and Kwenamore (2006) reported a significantly lower detection of faecal coliforms and *E. coli* counts during colder season months. Higher temperature can promote the growth as well as survival rates of microorganisms, whereas low temperatures can slow down the growth of microorganisms (Zamxaka *et al.,* 2004). Communities should always be aware of the microbiological quality of the water they use, but an increased alertness and additional safety measures should be taken in the warmer seasons.

2.1.2.2 pH

pH is determined by the concentration of hydrogen ions (H^+) in water, as well as the buffering capacity of the water body. The pH of water determines the chemical species of many elements, and thus also the potential toxicity thereof (Dallas & Day, 2004). Anthropogenic acidification (low pH; high concentration H^+-ions) of water bodies can be caused by low-pH point source effluents from industries, mine drainage and acid precipitation resulting largely from atmospheric pollution (Dallas & Day, 2004). Natural acidification of soils and water occur when acid rain falls on a catchment. The strong acids leach base cations, particularly calcium and magnesium (Cresser & Edwards, 1988). A study by Grandjean *et al.* (2005), demonstrated that water with a pH ranging from 7.7 – 8.6 has a measurable effect on the rate at which *E. coli*'s culturability decrease. The authors further demonstrated that a pH of 8.2 or higher is optimal for maintaining the culturability of *E. coli* in water. The TWQR of pH for domestic use is 6.0 - 9.0 and 6.5 - 8.4 for irrigation use (DWAF, 1996b). Deprotonated species that form at high pH's may pose a health risk to consumers, whereas foliar damage to crops may lead to a decreased yield if the TWQR for pH is not met (DWAF, 1996b).

2.1.2.3 Nitrates and nitrites

Nitrates and nitrites occur together in the environment with readily occurring inter-conversion between the two states (Anon, 2005b). Significant nitrate sources include the oxidation products of vegetable and animal debris, animal and human excrement as well as treated sewage wastes (DWAF, 1996b). Remnant nitrate fertilizers after plant and crop uptake are a common contaminant of groundwater (van der Voet *et al.*, 1996). However, a study by Conrad *et al.* (1999) showed that the nitrate found in groundwater had the isotopic character of soil nitrogen and not of fertiliser. The authors (Conrad *et al.*, 1999), therefore, concluded that the tilling of the soil caused nitrification of the soil nitrogen, causing the leaching of nitrate into the sub-surface.

Nitrate is an important water quality parameter and also a regular chemical pollutant of ground waters worldwide (Bauchard *et al.*, 1992). Nitrate is readily converted to nitrite by microflora present in the saliva and gastrointestinal tract (Lin & Lai, 1982; Lundberg *et al.*, 2004). Nitrite oxidizes haemoglobin to form methaemoglobin (Coleman & Coleman, 1996). The latter substance is incapable of carrying oxygen to body tissues, resulting in hypoxemia (Coleman & Coleman, 1996). The condition of oxygen shortage is damaging and even lethal to infants and young children due to high nitrate consumption, and is known as "blue-baby syndrome" (infantile methaemoglobinemia) (Craun *et al.*, 1981; Sadeq *et al.*, 2008; Super *et al.*, 1981). Severe cases may cause lethargy, stupor and death (Camp, 2007). Fan and Steinberg (1996) studied the results of many authors since the 1940's to make the hypothesis that methaemoglobinemia is more likely to result from water that is contaminated with both nitrates and bacteria. The proposed reason for this is that many bacteria present in the water are responsible for the conversion of nitrate to nitrite (Fan & Steinberg, 1996)..

Potentially carcinogenic nitric oxide is generated from nitrite in an acidic environment (stomach) or by bacteria that have nitrite reductase enzymes in the gastrointestinal tract (Lundberg *et al.*, 2004; Walker, 1996). The theory that nitrates causes cancer of the intestinal organs is not substantiated thus far, due to conflicting results found by various authors (Dutt *et al.*, 1987; Gulis *et al.*, 2002; Jensen, 1982; van Loon *et al.*, 1997; Yang *et al.*, 2007). Dutt *et al.* (1987) reported an average per capita intake of nitrate for people in Singapore to be 215mg. This intake was associated with contaminated vegetables, fruits and meat, and an association was drawn between this high intake and male gastric cancer incidences. The ecological data collected by Gulis *et al.* (2002) over a period of 20 years supported the hypothesis that elevated nitrate levels in drinking water could be positively associated with non-Hodgkin lymphoma and colorectal cancer. A thirty year study by Jensen (1982) concluded that several factors influenced the development of stomach cancer, and that elevated nitrate only fulfilled a possible weak role in stomach cancer incidence. Results by van Loon *et al.* (1997) indicated an inverse association between nitrate intake from foods and gastric cancer risks. Yang *et al.* (2007) also reported an inconclusive relationship between nitrate intake from drinking water and colon cancer in Taiwan.

2.1.2.4 Total dissolved solids and electrical conductivity

Total dissolved solids (TDS) concentration is a measure of the quantity of all compounds dissolved in water carrying an electrical charge (Dallas & Day, 2004; DWAF 1996b). The TDS concentration is proportional to the electrical conductivity (EC) of water. The reason for this is that electrical conductivity is a measure of the ability of water to conduct an electrical current. 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 & Day, 2004). Irrigation in hot and dry areas contributes to the transfer and deposition of inorganic compounds and salts in the unsaturated zones of soils (Papaioannou *et al.*, 2007). Evaporation then causes the concentration of salts in superficial water (Papaioannou *et al.*, 2007). The transfer of these salts to deeper layers causes the increase of salts by a factor of two or three than that of normal water (Papaioannou *et al.*, 2007).

TDS levels between 0 and 1000 mg/L are considered as aesthetically acceptable, and no known health affects will occur in this range (DWAF, 1996b). The TWQR for TDS is 0 – 450 mg/L, after which the water will become increasingly salty, hard and unpalatable. A study by Kempster *et al.* (1997) demonstrated that some long-term health problems can be expected if TDS values exceed 2450 mg/L. Corrosion of pipes and appliances will occur at levels above 1000 mg/L (DWAF, 1996b).

2.2 MONITORING PROGRAMS

The National Water Act (No. 36 of 1998) mandates that water sources should be managed by means of monitoring programs which provide data on the state of resources in terms of its quality and quantity. It is recognized by the National Water Resource Strategy (NWRS, 2004), that a variety of monitoring programs is needed to deliver a clear view on the condition of source water.

The first report of the national microbial water quality monitoring program emphasizes the importance of a monitoring program due to increasing pressures on South Africa's water (Murray *et al.*, 2004). This program ascribes this increasing pressure to the rapid expansion of dense settlements without the appropriate sanitation infrastructure. Improper maintenance and management of wastewater treatment plants, which dramatically contributes to the faecal contamination of water bodies is also implicated (DWA, 2011). The custodian of South

Africa's water resources, the Department of Water Affairs and Forestry (DWAF), used to manage South Africa's water on a national scale (Kalule-Sabiti & Heath, 2008). To do so, they required data representing the quality of water on a provincial, regional, town, and point-source scale. Like many other countries, the water resource quality management program of South Africa had the "data-rich but information-poor syndrome" (Kalule-Sabiti & Heath, 2008). This could be ascribed to unsuccessful communication routes between the data holders and sorters and the community level resource planners and managers. This stimulated new institutional arrangements which decentralised the water management portfolio from central government to provincial government (Kalule-Sabiti & Heath, 2008). The latter created a demand for relevant and significant data resources within each province (Kalule-Sabiti & Heath, 2008). Areas that are predicted to be at high risk of faecal pollution should be selected to represent a significant data resource for the microbiological quality of water (Murray *et al.*, 2002). Using basic screening methods, these areas could be predicted fairly accurately from investigating the anthropogenic practises in the catchment area (Murray *et al.*, 2004). Sampling should be done regularly in order to generate a meaningful database (Egboka *et al.*, 1989). Various parameters indicating the quality of the water should be measured. Parameters should include indicator bacteria (HPC bacteria, total- and faecal coliforms and enterococci species) and physico-chemical parameters (TDS, EC, pH, ambient water temperature, nitrates, nitrites, phosphates, etc.).

2.3 NORTH WEST PROVINCE

The North West Province (NWP) lies in the north western part of South Africa, bordering Botswana, and is known as the platinum province due to the wealth of this metal contained underground (NWDACE, 2007). It is also known for its prosperous cultivation of crops and livestock. It is the country's fourth-smallest province, constituting 8.7% of South Africa's land area and with a mid-2007 population of 3 271 million people (NWDACE, 2007).

Approximately 65% of the NWP is populated by a rural population (NWDACE, 2007) and accounts for the largest number of informal households in South Africa (StatsSA, 2007). According to Naraghi & Kebotlhale (2004), water services in rural areas of the NWP (especially in the underdeveloped western part of the Province) are for the most part underdeveloped. Therefore up to 80% of the rural population depends solely on groundwater for their water needs (Kalule-Sabiti & Heath, 2008). A community survey conducted by StatsSA (2007) established that 41.7% of households in the NWP make use of pit latrines and that 5.8% of households have no toilet facilities at all. Towns and cities such as Potchefstroom, Klerksdorp, Rustenburg, Brits and the capital Mafikeng contributes to an urban population (Kalule-Sabiti & Heath, 2008).

The landscape is largely flat with regions of scattered trees and grasslands. The NWP is a water stressed Province. Rainfall on average for the western region of the Province is less than 300mm/a, the central region receives around 550 mm/a, while the eastern and south-eastern region receives over 600 mm/a (NWPG, 2002). This is below the world average of 840 mm/a. However, the central, eastern – as well as the south-eastern region receive rainfall above the average (480 mm/a) for South Africa.

2.4 GROUNDWATER OF SOUTH AFRICA

Groundwater is a very important natural resource that offers significant economic benefits. It is often costly and unrealistic to supply dispersed rural communities with surface water. Therefore it is progressively recognised that groundwater is the only realistic option for a sustainable supply of safe water in many areas (Robins *et al.,* 2007). Despite its benefits groundwater is generally under-valued, ineffectively exploited and inadequately protected (Foster & Chilton, 2003). In terms of the overall water consumption in South Africa, groundwater contributes only some 15% of the total volume consumed (DWAF, 2002).

However, 65% of the country's population rely on groundwater as a water source (Woodford *et al.*, 2009). It is estimated that the total groundwater resource of South Africa is 19 000 Mm^3/a, of which an estimated 10 350 Mm^3/a that is considered utilizable (Woodford *et al.*, 2009). The total groundwater use of the country was estimated to be 1 770 Mm^3/a (Woodford *et al.*, 2009). From the latter it is seen that South Africa is under-utilizing this resource. In most urban areas in South-Africa, surface water is utilized more readily than groundwater. Conversely, the bulk of water used in rural communities is often groundwater. Over 300 small and/or informal towns in South Africa utilise groundwater as their only freshwater source (Woodford *et al.*, 2009). Usage of groundwater includes domestic usage, livestock watering and irrigation of cultivated land. Of the groundwater use in South Africa, 64% is for irrigation purposes (Woodford *et al.*, 2009).

2.4.1 Groundwater of the North West Province

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

Farmlands in the NWP are mainly cultivated land where crops are irrigated with groundwater, as well as livestock farms where the livestock receives water from groundwater

sources (NWDACE, 2008). The rural communities of the NWP approximately require 70 million m^3 of water per annum. Of this, 25 million m^3 per annum is used for domestic consumption and the remainder is used for livestock-watering and subsistence agriculture (NWDACE, 2008). Studies demonstrated that solid waste dumps, grey water, animal rearing activities and pit latrines are major non-point sources related to shallow groundwater quality problems in informal settlements (Bloodless *et al.*, 2006; Kulabako *et al.,* 2007).

Commercial farmers are usually better equipped financially than informal settlement dwellers. Therefore, in most instances, farmers have boreholes that are drilled much deeper into the underground aquifers. Informal dwellers usually use shallow dug wells, that are not properly sealed and which may be exposed to open defecation and/or close-by pit latrines (Grönwall *et al.* 2010). The greater vertical distance of deeper boreholes and the absence of open pit latrines, leads to the assumption that water from these boreholes may be less-contaminated than shallow wells mostly found in settlements. This is supported by studies showing that shallow boreholes (Gallegos *et al*, 1999) and boreholes in close proximity to pit latrines (Bloodless *et al.*, 2006) are vulnerable to faecal contamination.

Research by the CSIR showed that groundwater in the NWP has high concentrations of fluoride and nitrate (Ashton, 2009). The concentrations of these substances are well above the recommended maximum concentrations for human consumption in most areas (Ashton, 2009). A study monitoring the groundwater of the NWP throughout the period of 2002 to 2005 concluded that the average measured nitrate concentration of two out of the five monitored secondary drainage basins exceeded the DWAF TWQR of 6mg/L for domestic use (NWDACE, 2008). The study further established that groundwater nitrate concentration in the North West Province was higher than that of surface water. An increase in pit latrines in

rural areas together with the rapid population growth of informal settlements contributed to the latter nitrate loading of ground waters (NWDACE, 2008).

In many of the rural populations, households have to share water being pumped from the ground to communal taps and tanks. Water is mostly collected in buckets. In some of these settlements water is only pumped two to three times a week, resulting in water to stand in tanks for a period before use. A study by Momba and Kaleni (2002) concluded that if contaminated water is stored in household containers, these containers will support the growth and survival of, already present, indicator bacteria. The microbiological quality of the stored drinking groundwater will deteriorate consistently with the length of storage in the tanks (Momba & Notshe, 2003). More esteemed households may have a private shallow-well borehole that is pumped by hand. These shallow boreholes can be expected to be more contaminated than deeper boreholes due to the shorter vertical distance that microbes need to travel to reach the water table. The results of Gallegos *et al.* (1999) supports this, as the authors detected higher coliform counts in shallow (<10m) boreholes than deeper boreholes. In rural settlements, pit latrines are also frequently located in close proximity to these shallow boreholes, increasing the risk of faecal and nitrate contamination (Bloodless *et al.*, 2006; Kulabako *et al.*, 2007).

2.4.2 Groundwater quality and contamination

Contamination of groundwater and hence poor quality water, contribute to the restriction of development of all available groundwater resources. Total dissolved solids, nitrates and fluoride is considered as the most common and serious problems associated with groundwater quality (Woodford, *et al.* 2009).

In the past there was little concern with groundwater contamination. This situation was due to the perception that water is filtered through soil and thus cleaned in the process. Another aspect was the lack of methods to take appropriate samples for analysis. Soil cover serves as a natural barrier facilitating the filtration of seeping water (Egboka *et al.*, 1989). This led to the general assumption that groundwater environments are isolated from contaminants. But the reality is that if a contamination source is in close proximity to groundwater sources the successful seepage of the contaminants, including bacteria, increases greatly (Egboka *et al.*, 1989).

LeChevallier and Seidler (1980) advocated the importance of determining the microbial quality of groundwater. According to the authors (LeChevallier & Seidler, 1980), evidence is needed to estimate the incidence of waterborne disease outbreaks originating from groundwater. Pedley and Howard (1997) identified acute gastroentirits, chemical poisoning, hepatitis A and shigellosis as the illnesses most frequently transmitted through groundwater. These diseases are still under-reported, even in developed countries (US EPA, 2011).

The four main routes by which groundwater can be contaminated are, 1) infiltration; 2) direct migration; 3) inter-aquifer exchange; and 4) recharge from surface water (Barcelona *et al.*, 1988). Groundwater is recharged by rainfall and stream infiltration. Contaminants may be transported with the recharge water. Groundwater can become contaminated anywhere, but some areas are more susceptible than others due to the capacity of aquifer material to transmit water (Aichele, 2004). Coarse materials such as sand and gravel generally transmit water more rapidly than finer materials such as clay and silt (Aichele, 2004). Site-specific conditions such as soil properties, vegetation, and topography may affect contaminant transport (Aichele, 2004). Macropores present in moist soils enhances the transportation of faecal bacteria (Unc & Goss, 2003). The vertical depth of boreholes and rainfall events are

also significant factors influencing groundwater contamination (Bonton *et al.*, 2010; Cho *et al.*, 2000; Grisey *et al.*, 2010; Godfrey *et al.*, 2005; Hudak, 2000; Kundu *et al.*, 2009; Unc & Goss, 2003).

Groundwater quantity and quality may become influenced by residential areas, informal settlements, agricultural practices, industries and mining. Sources of contaminants include municipal and industrial wastes, sewer leakage, faulty septic tank operation, pit latrines, landfill leachates, storm run-off, chemical fertilizers, herbicides, pesticides, wastewater used for irrigation and livestock manure that infiltrate aquifers due to improper containment thereof (Andrade & Stigter, 2009; Babatunde *et al.*, 2009; Bloodless *et al.*, 2006; Cho *et al.*, 2000; Gallegos *et al.*, 1999; Grisey *et al.*, 2010; Howell *et al.*, 1995; Kolpin, 1997; Pitt *et al.*, 1999; Owens *et al.*, 1994; Silva *et al.*, 2006; Tissot *et al.*, 2002; Usher *et al.*, 2004a; van der Voet *et al.*, 1996). Some groundwater sources may also be subject to contamination from surface waters (Babatunde *et al.*, 2009; Fong *et al.*, 2007). Springs, infiltration galleries, shallow wells, and other collectors in subsurface aquifers may be hydraulically connected to nearby surface water sources, depending on local geology (Reinert & Hroncich, 1990).

Nitrate loading in groundwater is promoted by the pollutants mentioned above. Nitrate tends to increase in shallow groundwater sources in association with agricultural and urban runoff, especially in densely populated areas (DWAF, 1996b).

In urban areas, aquifer contamination presents a threat to the sustainability of this water resource (Usher *et al.*, 2004b). This is especially due to the high concentration of societal waste (Reinert & Hroncich, 1990), as well as poor management and services in residential areas (NWPG, 2002). The latter should not be overly generalised due to the site-specific nature of this impact. In rural regions however, the movement of animal wastes into

groundwaters is often cited as the major factor contributing to the pollution of groundwater (Doran & Linn, 1979; Gagliardi & Karns, 2000).

Restoration and replenishment of contaminated groundwater may take decades due to the slow rates of groundwater movements and relatively long subsurface residence times (Murray *et al.*, 2007; Reasoner, 1990; Tredoux *et al.*, 2004). Groundwater contamination are usually localised due to its slow flow regime (Murray *et al.,* 2004; Murray *et al.*, 2007). Contaminants may be isolated or captured in the case of surface water, but once present in an aquifer, they are very difficult to remove (Reasoner, 1990). Therefore the consequences of groundwater pollution are often more serious than for surface water.

2.5 METHODS USED FOR THE ANALYSIS OF WATER

2.5.1 Microbiological analysis of water

Due to the expense and impracticality to screen water for all potential human pathogens (Barrel *et al.*, 2000; Zamxaka *et al.*, 2004), microbiological quality of water is commonly determined by establishing the presence and numbers of faecal indicator bacteria in water (Grabow, 1996). This can be achieved by culture based or molecular based methods.

2.5.1.1 Culture based methods

The membrane filtration and multiple-tube fermentation are two commonly used culture based methods for the enumeration of indicator bacteria (Venter, 2000). The US EPA (2005) defines membrane filtration as a pressure- or vacuum-driven separation process in which particulate matter is rejected by an engineered barrier primarily through a size exclusion mechanism. The membrane filtration entails the use of a 0.45μm (pore diameter) membrane filter that concentrate and entrap bacteria. The membrane filter is then layered onto selective media in a sterile procedure, incubated at appropriate temperatures for the required periods

after which the bacterial colonies are observed. Enriched and selective media facilitate the growth of target species while inhibiting the growth of unwanted competitors (Bajeva, 2006). The results are expressed as the number of colony forming units (cfu)/100ml of water tested (Grabow, 1996). Many countries approve and employ the membrane filtration method for determining the microbiological quality of water (Rompré *et al.*, 2002).

Rompré and his colleagues (2002) named some of the advantages of the membrane filtration method: 1) the method requires limited laboratory equipment, is simple, and can be performed by a technician with basic microbiological training; 2) large volumes of water can be examined, which leads to greater sensitivity and reliability; 3) quantitative enumeration can be achieved compared to the semi-quantitative information given by the multiple-tube fermentation method. However, since this method is not sufficiently specific, confirmatory tests are needed, which prolongs the time to obtain results (Rompré *et al.*, 2002). Simpler to manage, quicker to process and easier to quantify alternative methods to the membrane filtration method are emerging. Such alternative methods include automated testing (Habash & Johns, 2009) and Colilert systems (Buckalew *et al.*, 2006; Eckner, 1998; Kämpfer *et al.* 2008). The alternative methods used by Habash and Johns (2009) and Buckalew and his colleagues (2006) performed equal to the membrane filtration method. Results from a comparative study done by Kämpfer and his colleagues (2008) as well as Eckner (1998), showed that the Colilert-system was significantly more sensitive in the detection of coliforms than the traditional membrane filtration method. Limitations of the membrane filtration method include duration of incubation, antagonistic organism interference, lack of specificity, need for confirmatory tests and poor detection of slow-growing or viable but non-culturable microorganisms (Rompré *et al.*, 2002). Burlingame *et al.* (1984) demonstrated that high numbers of HPC bacteria may decrease the recovery of coliforms on

membrane filters. Despite the mentioned alternative methods and limitations, the membrane filtration method is still used today as a reliable and preferred method.

There are a variety of selective media available for the enumeration of coliforms and faecal streptococci. A comparative study by Avila *et al.* (1989) concluded that selective mEndo agar for the enumeration of coliforms from water samples performed excellent based on specificity, selectivity, recovery efficiency and precision. The US EPA (1991) approved membrane filtration method includes the use of mEndo agar for the determination of microbiological water quality. An enriched lactose medium (m-FC) incubated at 44.5°C is used for the enumeration of faecal coliforms. Po Catalao Dionisio and Borrego (1995) evaluated the efficiency of 4 different media commonly used for the enumeration of faecal streptococci. The authors found that KF streptococcus agar were the most efficient medium for the recovery of faecal streptococci from freshwater suspensions.

2.5.1.2 Confirmation and differentiation of species

Various biochemical tests can be useful in the confirmation and differentiation of Gram-negative enteric *Enterobacteriaceae* species. The initial test for *Enterobacteriaceae* species is to establish that the isolates are Gram negative. This is achieved by staining the isolate, followed by a de-staining step and ultimately a counter stain. Gram negative bacteria have a thinner cell wall than Gram positive bacteria, and will therefore not retain the purple colour of the first stain (crystal violet) during the de-staining (acetone alcohol) step (Penny *et al.*, 2002). The counter stain (safranin) will stain the Gram negative bacteria pink (Penny *et al.*, 2002). Gram positive bacteria will remain crystal violet due to the thicker cell wall and will thus appear purple (Penny *et al.*, 2002).

The TSI test achieves differentiation amongst enteric Gram negative rods on the basis of carbohydrate (dextrose, sucrose and lactose) fermentation and the production of H_2S (Levinson, 2006). H_2S production results in the blackening of the agar. TSI agar also contains sources of carbon and energy along with the three listed carbohydrates. When these are fermented the acid production is indicated by the phenol red indicator. The colour changes to yellow for acid production and remains red or become purple for alkalinisation (Vanderzant & Splittstoesser (eds.), 1992). If an organism cannot produce acid, then an alkaline-butt as well as alkaline slant will be the result. This is already evidence enough that the organisms in not from the *Enterobacteriacea* group (Winn *et al.*, 2005). *E. coli* produces a yellow slant and yellow butt as well as evidence of gas formation (Levinson, 2006). The formation of gas can be observed as cracks in the agar, resulting in the separation of the medium.

Analytical Profile Index (API) systems can also be useful in the identification of unknown bacterial isolates. According to Shoeb (2006) the API system entails the delineation of the biochemical activities of microbial isolates. Specific groups of microorganisms can be identified since they share a common combination of metabolic and enzymatic activities prominent to these groups. Variations in biochemical activities exhibited by members of the same group of organisms can then be utilized to differentiate between these individuals and ultimately lead to identification (Shoeb, 2006).

2.5.1.3 Molecular based multiplex PCR

PCR is a commonly used molecular method used to amplify target genes. Compared to culture based methods, molecular based assays are becoming more popular in routine diagnostics owing to their specificity, sensitivity, rapid screening time and inexpensiveness (Guion *et al.*, 2008). Viable but non-culturable (VBNC) bacteria are not detected with the

culture based methods (Lleo *et al.*, 2005; Kong *et al.*, 2002; Rompré *et al.*, 2002). Therefore sample water could be tested negative for a certain bacteria when it is present in a VBNC state. When using a multiplex polymerase chain reaction (mPCR), the VBNC bacteria will be detected owing to the presence of the bacterial DNA.

Rompré and his colleagues (2002), named some of the limitations of the PCR method. Limitations of the method (PCR) when applied to natural samples include low amplification rates due to the presence of inhibitor substances as well as lack of information on the physiological activity of the cells. The PCR method requires staff with a high level of skill, dedicated laboratory space and specific reagents. Furthermore, standard PCR assays are useful for the identification as well as for absence or presence detection purposes, but not for quantifying a target bacterium (Rompré *et al.*, 2002; Williams *et al.*, 2006). The standard PCR also does not distinguish between viable and non-viable microorganisms (Williams *et al.*, 2006). However, modifications of the standard PCR assay gave rise to the real time quantitative PCR (qPCR) which can be used to determine the bacterial cell numbers of a target bacterium (William *et al.*, 2006). Reverse transcription-PCR (RT-PCR) is another modified assay allowing for a distinction to be made between viable and non-viable bacterial cells (Williams *et al.*, 2006).

Multiplex PCR simultaneously amplifies multiple selected target genes in the same reaction tube (Kong *et al.*, 2002), and are commonly used for various identification approaches for pathogenic *Escherichia coli* (Bai *et al.*, 2010; Omar *et al.*, 2010; Rodas *et al.*, 2009; Wang *et al.*, 2010). Division between the target genes is based on the base pair quantity of the PCR products (Pass *et al.*, 2000). *LacZ* and *mdh* are housekeeping genes of *E. coli* (Ram & Shanker, 2005). The presence of these genes indicates the presence of *E. coli*. The multiplex

PCR assay could be applied to detect the presence of *E. coli* directly from environmental water, thus indicating the potential hazard in terms of faecal contamination.

2.5.2 Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion technique is a method employed to determine antibiotic susceptibility of isolates (Bauer *et al.*, 1966). The aim of the test is to determine whether an organism is susceptible (S), intermediate (I), or resistant (R) to various antimicrobial agents. This is achieved by the placement of filter-paper discs impregnated with antimicrobial agents onto Petri dish inoculated with of a pure culture of bacteria. Plates are incubated at 37°C for 24 hours. By measuring (in mm) the clear zones (bacterial growth inhibited) around each disc impregnated by antimicrobial agents, the organism's susceptibility to the antimicrobial agent can be determined (Ingraham & Ingraham, 2004).

2.6 SUMMARY OF LITERATURE REVIEW

In the preceeding literature review it was demonstrated that waterborne diseases are transmitted through the use of untreated faecal contaminated water (Ashbolt *et al.* 2001; Grabow, 1996; Ministry of health, 2005; WRC, 2003). Worldwide, more than 3.4 million people die as a result of water related diseases every year (WHO, 2003b). Indicator bacteria are useful in predicting the presence of potential human pathogens in water (Barrel *et al.*, 2000; Ministry of health, 2005). However, the absence of indicator bacteria in water sources would not necessarily imply the absence of pathogens (Jagals *et al.*, 2006). Commonly screened faecal indicator bacteria include faecal coliforms, faecal streptococci, and *E. coli* (Edberg *et al.*, 2000; Stevenson *et al.*, 2003). The ability of bacteria (including pathogens) to adapt to antibiotics is also a concern for human health as these bacteria can be spread through the use of contaminated water (Barbosa & Levy, 2000).

The literature also demonstrated that monitoring programmes, such as the National Microbial Monitoring Program (Murray *et al.*, 2004) for groundwater, were developed by the Department of Water Affairs to ensure that the right of the nation is upheld. From the literature is also evident that groundwater is an important water resource in the NWP (Kalule-Sabiti & Heath, 2008). This resource is vulnerable to contamination, especially if contamination sources are in close proximity to the groundwater source (Egboka *et al.*, 1989). Amongst others, contaminants include faecal bacteria and nitrates (Cho *et al.*, 2000; Kolpin, 1997; Owens *et al.*, 1994; Silvia *et al.*, 2006; van der Voet *et al.*, 1996). Methaemoglobinemia in infants and young children may occur with the intake of high nitrates (Sadeq *et al.*, 2008). Boreholes in the North West Province have been tested to be contaminated with faecal indicator bacteria (Kwenamore, 2006) and nitrates (Ashton, 2009).

Literature was also used to demonstrate the principles of the various methods that are available to analyse the groundwater. These include culture based methods such as membrane filtration onto selective and differential agar is a commonly used to detect and quanitfy bacteria in water (Venter, 2000). PCR is also useful in the detection of target bacteria from water as it also detect the viable but non-culturable bacteria (which culture based methods can not detect) (Lleo *et al.*, 2005; Kong *et al.*, 2002; Rompré *et al.*, 2002). The Kirby-Bauer disk diffusion technique is the method employed to determine antibiotic susceptibility of isolates (Bauer *et al.*, 1966).

CHAPTER 3
METHODS

This chapter is divided into three Sections (3.1-3.3). This was due to a change in methods used in 2009 and 2010. Section 3.1 describes the methods used for groundwater sampling, strategy and a GIS-map indicating the borehole locations of both 2009 and 2010. Section 3.2 describes the methods used in 2009. Section 3.3 describes the methods used in 2010. Several of the methods used in 2009 (Section 3.2) were the same in 2010 (Section 3.3). Statistics used in this study is described in Section 3.4.

3.1 COLLECTION OF SAMPLES AND SAMPLE AREAS

3.1.1 Sample collection

Sampling was done according to the guidelines provided in the sampling guide of the Water Research Commission (WRC, 2000). In 2009, 76 groundwater samples were taken directly from boreholes in the North West Province (NWP). Sampling was conducted from February 2009 to July 2009. In 2010 selected areas were re-sampled. In this case only 38 boreholes were sampled. Sampling was from March 2010 to May 2010. Each of the boreholes was sampled once. This is the minimum allowable number of samples per year per sampling point for a borehole to still provide reliable results (WRC, 2000). Boreholes with electronic-, hand- and wind-pumps were selected randomly. The areas re-sampled in 2010 were sampled on different dates than in 2009. The latter were done to establish whether seasonal variation plays a part in groundwater quality. Not all sample areas that were sampled in the hot and wet summer the one year were sampled in the cold dry winter the next year. This was only the case for some of the sample areas (Tables 3.1 and 3.2). Boreholes in close proximity to one another were not sampled. Instead, sampling was done in a spatially separated manner in order to provide an overall status of the groundwater of the NWP and to allow for comparisons to be made between areas.

Water samples were taken with the permission of the landowners of the borehole. Boreholes were selected where water could be purged directly from the aquifer and not from a storage tank. Water from the closest possible taps or pipes were purged for 3 – 5 minutes before sampling to prevent stagnant water in the pipe lines from being sampled as well as to flush the nozzle. Caps were removed from sample bottles at the sampling sites without contaminating the inside of the cap or the neck of the sample bottles. The sterilized sample bottles were then filled without prior rinsing, and thereafter capped immediately. Sampling bottles were not filled to the top. Enough airspace was left in the bottle to allow mixing of the water before examination. Samples were transported to the laboratory on ice in sealed cooler boxes and analysed, where possible, within six hours after collection. Due to circumstances and travelling distances, samples were on occasion analysed within 8 to 12 hours.

GPS-coordinates were taken at each sampling site and utilized to assemble a GIS-map indicating the locations of the boreholes sampled in 2009 and 2010 (Figure 3.1) As the figure legend indicates, 2009's sampling sites are represented with square markers and that of 2010 with circle markers.

The major towns of the NWP can also be seen in Figure 3.1. The western part of the NWP does not have major developed towns as is the case in the rest of the province. This area mostly consists of rural settlements and agricultural land. Sample area I (Table 3.1) of 2009 represents the boreholes sampled in this area. It was not re-sampled in 2010.

3.1.2 Sample areas of 2009

In 2009 sampling took place on ten different dates. The boreholes sampled on each of these dates was grouped together to form a sampling area, thus dividing the North West Province

(NWP) into ten sampling area's (A-J). A varied number (n) of boreholes was sampled in each area depending on the accessibility to groundwater. The letters representing each sampling area are subscripted with a 09 (Ex. A_{09}) to indicate that the area 'A' was sampled in 2009. From this Table (Table 3.1) it is seen that sampling area A_{09} was the only area sampled in the wet summer season, whereas areas C_{09}-E_{09} were sampled in the drier, warm autumn season. Areas F_{09}-J_{09} was sampled in the cold and dry winter season. Different towns were sampled in each sample area with instances where two boreholes were sampled in or around the same town. This was different in the case of sample area E_{09}. Five boreholes were sampled on the same day in the same town (Hartbeespoort), resulting in sample area E_{09}. This was specifically decided due to the challenges that this town face with regards to contaminated surface and potentially groundwater.

Table 3.1: Sample areas (A-J) and the number of boreholes sampled at each area in 2009.

Sample date **Season**	**Sample Areas** **n – number of boreholes sampled in area**	**Ass. Letter (09)**
16/2/2009 Summer	Klerksdorp; Stilfontein; Orkney; Mooibank; Potchefstroom n = 8	**A**
10/3/2009 Summer	Carltonville-Ventersdorp Fochville-Welverdiend n = 5	**B**
26/3/2009 Summer	Lichtenburg; Koster; Doornkop; Makokskraal; Derby; Coligny n = 7	**C**
4/4/2009 Autumn	Hartbeespoortdam; Brits; Matrooster; Rustenburg n = 8	**D**
17/4/2009 Autumn	Hartbeespoortdam n = 5	**E**
9/5/2009 Autumn	Delareyville; Zeerust; Mafikeng; Stella; Setlagole; Ottosdal n = 8	**F**
26/5/2009 Autumn	Schweizer-Renecke; Bloemhof; Wolmaransstad; Christiana n = 5	**G**
10/6/2009 Winter	Vryburg-Ganyesa n = 4	**H**
23/6/2009	Bray; Vostershoop; Piet-Plessis; Makopeng; Molopo;Tseoge	**I**
Winter	n = 9	
26/7/2009	Taung; Salpeterspan; Sekhing; Geluk; Amalia; Reivillo	**J**
Winter	n = 9	

3.1.2 Sample areas of 2010

In 2010 fewer boreholes were sampled. Due to different circumstances and sampling opportunities, it was not possible to sample the exact same towns on a single sampling trip in 2010 as was done in 2009. However the areas sampled in 2010 still spatially correlates with that of 2009. These sampling sites were grouped into the same sample areas assigned in 2009 (A-J). Table 3.2 indicates the dates and sample sites of 2010. From Table 3.2 it is seen that area B and C of 2009 is grouped together as one ($B+C_{10}$) in the 2010 sampling regime. The reason for this is the overlap with some of the sites. Sample area I_{09} was not sampled again in 2010, for reasons provided in Chapter 4. In 2010 the Hartbeespoort area (E) was not sampled as intensive as in 2009.

Table 3.2: Sample areas (A-J) and the number of boreholes sampled at each area in 2010.

Sample date **Season**	**Sample Areas** **n – number of boreholes sampled in area**	**Ass. Letter (10)**
12/04/2010 Autumn	Klerksdorp; Stilfontein; Orkney; Potchefstroom n = 5	**A**
27/04/2010 Autumn	Ventersdorp; Coligny; Lichtenburg; Biesiesvlei; Sannieshof n = 6	**B + C**
10/05/2010 Autumn	Hartbeespoortdam; Brits; Derby; Rustenburg n = 5	**D**
18/05/2010 Autumn	Delareyville-Ottosdal; Zeerust; Mafikeng; Geysdorp; Setlagole; n = 5	**F**
23/03/2010 Autumn	Schweizer-Renecke; Bloemhof; Wolmaransstad n = 7	**G**
31/05/2010 Autumn	Vryburg; Ganyesa; Broedersput n = 4	**H**
24/05/2010	Taung; Hartswater; Sekhing; Amalia; Christiana	**J**
Autumn	n = 6	

Figure 3.1: GIS-map indicating the locations of the boreholes sampled in 2009 and 2010.

3.2 METHODS USED IN 2009 SAMPLING PERIOD

3.2.1 Enumeration of bacteria

Each borehole was sampled in triplicate. The membrane filtration technique and appropriate selective media were used to enumerate total coliforms, faecal coliforms, faecal streptococci and staphylococci. All media were Biolab products obtained from Merck (South Africa).

One hundred millilitres (100 ml) of sample water was filtered through a 0.45 micro-meter pore size Whatman filter, which were then placed on selective agar (m-Endo for total coliforms, m-Fc agar for heat tolerant faecal coliforms, mannitol salt agar for *Staphylococcus aureus* and KF-streptococcus agar for faecal streptococci). Each of these plates were incubated for the recommended incubation period at appropriate temperatures (m-Endo agar: 24h at 37°C; Mannitol Salt agar: 24h at 37°C; KF-streptococcus agar: 48h at 37°C; and m-Fc agar: 24h at 44.5°C). Following incubation, presumptive colonies (m-Endo agar plates- metallic sheen and pink-red colonies; m-Fc agar plates- blue colonies; KF-streptococcus agar plates- purple-pink colonies; Mannitol salt agar plates- small to large gold colonies surrounded with a yellow zone) observed on the surface of membranes were counted and recorded. The mean bacterial count for each borehole was then determined. The results were expressed as colony forming units per 100 ml.

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

3.2.2 Isolation and purification of faecal coliforms

From each borehole, five to six blue faecal coliform colonies was isolated from m-FC plates. This was done with a sterilized inoculation loop. An inoculation needle was used in the case of very dense bacterial growth. In order to obtain a pure culture, the selected colonies were sub-cultured three times on nutrient agar using the streak plate procedure. The streak plates were incubated for 24h at 44.5°C in order to promote the growth of the thermo-tolerant faecal coliforms while inhibiting the growth of other non-thermo-tolerant bacteria. After the second incubation period, a single colony was selected for identification.

3.2.3 Purification of presumptive Pseudomonas aeruginosa

Single colonies were selected from each plate and isolated for further purification. Presumptive *Pseudomonas aeruginosa* opportunistic pathogens were isolated from m-FC agar plates (grey colonies). Representative grey colonies from each borehole (when present) were sub-cultured onto nutrient agar plates. If the nutrient agar plates changed colour from yellow to green after a 24 hour at 37°C incubation period it was noted as potentially *P. aeruginosa*.

3.2.4 Biochemical identification of bacteria

Gram staining, TSI tests and API 20E systems were used for the biochemical identification of purified isolates.

3.2.4.1 Gram staining

Selected and purified isolates obtained from m-FC plates (blue colonies), Mannitol Salt agar (golden yellow colonies) and KF-Streptococcus agar (pink colonies) were Gram-stained using the procedure described in Harley and Prescott, (2002). As described in Section 2.5.1.2 Gram staining consist of a initial stain with crystal violet, followed by a de-

stain with acetone alcohol and a counter stain with safranin (Penny *et al.*, 2002). Faecal coliforms (Gram negative rods), *Staphylococcus* spp. (Gram positive cocci) and faecal streptococci (Gram positive cocci) were observed using a light microscope. The potential purity of the colonies was also confirmed with the Gram stain.

3.2.4.2 Triple sugar iron (TSI)

TSI agar slants were used for the differentiation and preliminary identification of Gram-negative bacilli potentially from the family *Enterobacteriaceae.* The procedure was done according to Hajna (1945). To inoculate, a sterile inoculation needle containing an isolated colony was streaked onto the slant surface in a zigzag pattern. The agar was then stabbed with the needle into the centre of the butt. The slants were incubated for 18-48 hours at 37°C and then examined for sugar fermentation, gas (splitting of agar) and H_2S production (blackening of agar). A yellow colour change in the originally red agar indicates that acid was formed (Levinson, 2006; Vanderzant & Splittstoesser (eds.), 1992). Isolates that produced a yellow butt and yellow slant together with gas production were selected for further testing.

3.2.4.3 Analytical profile index (API 20 E)

The API 20 E system (bioMerieux, Inc. Hazelwood) was the procedure followed in the identification of *Enterobacteriaceae* and other Gram-negative bacteria. Based on Gram stain and TSI results isolates were selected and inoculated in 5ml saline solution (0.89% $^{w}/_{v}$ NaCl). After the bacterial-saline suspension was mixed, a sterile pipette was used to transfer the suspension to the cupule and microtubes according to instructions of the manufacturer (bioMerieux, Inc. Hazelwood). Tap water was dispensed into the incubation tray to ensure a humid atmosphere during the 24 hours incubation period. The ADC, LDC, ODC, H_2S and URE microtubes was slightly under filled which was followed by complete filling of the

cupules' section with mineral oil to create anaerobic conditions. A lid was then placed on the incubation tray and incubated at 37°C for 24 hours. Reactions were recorded after incubation. A seven digit profile was then generated and used for identification according to the manufacturers specifications (bioMerieux, Inc. Hazelwood).

3.2.5 Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion technique was used to determine the antibiotic susceptibility of selected faecal coliform isolates from the various sites. A homogenous suspension was made by mixing distilled water with pure cultures picked from the plate. This suspension was then spread onto a Mueller Hinton agar plate, using a flame sterilized spreader. The plates were allowed to dry for 15 minutes, after which the different antibiotic disks (Mast Diagnostics, UK, supplied by Davies Diagnostics, SA) were placed on the surface of the inoculated plates with sufficient space between them. Plates were incubated for 24 hours at 37°C. Growth inhibition zones were measured in mm after incubation and compared to the NCCLS growth inhibition zone standards for *Enterobacteriacea* spp. (Table 3.3). Table 3.3 also provide a list of the antibiotics that were used in this study.

TABLE 3.3: Details of the antibiotics used in this study (NCCLS, 1999).

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

From the data obtained multiple antibiotic resistant (MAR) phenotypes and indices of each sample area were determined. Only antibiotics that had more than 20% of isolates resistant to it was included in the most prevalent MAR phenotype.

MAR indices were calculated using the following formula (Guan *et al.,* 2002):

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

3.2.6 Physico-chemical analysis of groundwater samples

Total dissolved solids (TDS), Electrical conductivity (EC), pH and temperature was determined on site with potable, calibrated field 350i multi-probe from WTW (Germany). The probe was first cleaned with distilled water before placing it in a sterile glass beaker containing the water sample. After the reading was taken, the probe was again rinsed with distilled water before re-placing the protective plastic cap. Readings was recorded in a field notebook. Nitrates were measured with a calibrated nitrate probe (pHoenix Electrode Co.) and ion meter (Oakton Instruments, USA) using ISA nitrate standards. This was done in the laboratory.

3.3 METHODS USED IN THE 2010 SAMPLING PERIOD

3.3.1 Enumeration of bacteria

The same methods were followed as described in Section 3.2.1. However a different selective agar was used to enumerate total coliforms and *E. coli.* Membrane lactose glucuronide agar (MLGA) (Oxoid, UK) was used to enumerate *E. coli* (green colonies) and total coliforms (green and yellow colonies). This agar substituted M-Endo agar for the enumeration of total coliforms, while also providing valuable *E. coli* counts. MLGA plates were incubated for 24h at 37°C. The remainder of the selective agars for bacterial enumeration was the same as those used in 2009.

3.3.2 Isolation, purification and antibiotic susceptibility testing

The same methods used in 2009 (Section 3.2.2) were performed for the isolation and purification of faecal coliforms from m-FC agar plates. The antibiotic susceptibility of the purified isolates was determined with the Kirby-Bauer disk diffusion method (Section 3.2.5).

3.3.3 Molecular methods for the detection and identification of *E. Coli* directly from water samples

Multiplex PCR (mPCR) was used to detect the presence of *E. coli* in borehole water samples. DNA was isolated directly from borehole water samples using the Nexttec™ Genomic DNA isolation kit for bacteria (Nexttec™ Biotechnology GmbH, Germany), briefly described below (Section 3.3.3.1). The mPCR simultaneously amplified fragments of the malate dehydrogenase gene (*mdh*) and of the lactose promoter (*lacZ*).

Table 3.4: Primer sets used in the multiplex PCR.

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

3.3.3.1 DNA extraction

One litre of borehole water samples were filtered through a 0.45µm Whatman filter. The filter was placed into a sterile 50ml Schott bottle. Glass beads and 5ml TE buffer (10mM Tris, 1mM EDTA; pH 8) was added, followed by incubation at 37°C while shaking for 10 min. The filter and 4.5ml of the solution containing cells were subsequently removed and placed into clean 50ml Scott bottle. To this, lysozyme (Separations, US) was added to a final concentration of 1mg/ml and incubated at 37°C for 2 hours. Proteinase K (1.8mg/ml)

was added and the mixture incubated at 56°C for 25 minutes, while shaking. Five millilitre of the solution was centrifuged at $1x10^4$ rpm. The pellet was dissolved in 15µl RNase A (20mg/ml) and incubated at 60°C for 10 minutes. DNA was bound onto the PerfectBind® Column of the NexttecTM Genomic DNA isolation kit for bacteria (NexttecTM Biotechnology GmbH, Germany). Manufacturers' instructions were followed for the washing and elution of the DNA. Positive control DNA was isolated from *E. coli* ATCC 10536 using the NexttecTM Genomic DNA isolation kit for bacteria (NexttecTM Biotechnology GmbH, Germany), following manufacturers' instructions. The quality and quantity of the isolated DNA was determined using the Nanodrop Spectophotometer ND-1000 v3.5.2 (NanoDrop Technologies, Inc., USA).

3.3.3.2 Multiplex PCR

The mPCR mix contained the *lacZ* and *mdh* primer sets (Table 3.4). The primers were synthesised by Inqaba Biotechnology (South Africa). Each reaction consisted of 1 x PCR Mastermix (2mM $MgCl_2$, 0.2mM of each dNTP, 0.025U/µl *Taq*-polymerase; Fermentas Life Sciences, US) forward and reverse primers (1µM), additional (0.5mM) $MgCl_2$ and (0.1mM) betaine. The total volume of 25µl was attained by adding RNase/DNase free PCR grade water (Fermentas Life Sciences, US). One hundred nanogram of DNA was used as template in each reaction. Cycle conditions consisted of: initial denaturation at 95°C for 300 seconds, 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 60 seconds. Final extension was at 72°C for 300 seconds. PCR reactions were processed in an ICycler (Bio-Rad, UK).

3.3.3.3 Agarose gel electrophoresis

PCR amplification products were visualized using ethidium bromide staining agarose gel electrophoresis. Electrophoresis was conducted using 1.5% (w/v) Seakem agarose gels

(WhiteSci, SA). Gels were prepared with 1 x TAE buffer (40mM Tris, 20mM Acetic acid, 1mM EDTA; pH 8.0). Twelve microliter of PCR amplification product was mixed with 5µl 6 x Orange loading dye (Fermentas Life Sciences, US) and loaded into the gel. Each gel contained a 100bp molecular marker (O'GeneRuler, Fermentas Life Sciences, US) to indicate the molecular weight of the products. Electrophoresis was performed in 1 X TAE buffer for 60 minutes at 80V. Gels were visualized with a Gene Bio Imaging System (Syngene, UK) using GeneSnap Version 6.08 (Syngene, UK).

3.3.4 Physico-chemical analysis of samples

See Section 3.2.4 for details.

3.4 STATISTICS APPLIED IN THIS STUDY

Averages and standard deviations were calculated using Microsoft Office Excel software. Canonical ordination redundancy analysis (RDA), using Canoco software version 4.5 (developed by Ter Braak, 1990), was applied to determine the correlation between the tested environmental variables (temperature, TDS, EC and nitrate-nitrogen) and the indicator bacteria tested for in each sampling period of 2009 and 2010. The results of the multivariate analysis were visualised by mean biplots. The smaller the angle between factors, the more correlated these factors are to each other. Detailed instructions is provided in the manual for Canoco Version 4.5 software (ter Braak and Smilauer, 2002).

CHAPTER 4
RESULTS

This chapter includes the measured results from both the 2009 and 2010 sampling periods. Microbiological results are given in Section 4.1. This include the bacterial counts enumerated on culture media, PCR results for the detection of *E. coli* directly from borehole water, as well as the antibiotic resistance of selected faecal coliforms. Section 4.2 will give the measured physico-chemical results. In Section 4.3 the groundwater quality of the boreholes are compared to the Department of Water Affairs and Forestry's (1996a) target water quality range (TWQR) for domestic water. Statistic analysis using CANOCO software is given in Section 4.4. A summary of the results are given in Section 4.5. Town and site names as well as the quantity of boreholes sampled in each area of 2009 are tabulated in Table 3.1 and Table 3.2 for 2010. These sampling sites can be seen on the map (Figure 3.1) of the North West Province (NWP).

From Figure 3.1 it is observed that the north-eastern part as well as the western part of the NWP was not as extensively sampled as the rest of the province. The reason for this void of sampling sites at these areas were as follow: 1) a lack of direct access to groundwater due to the sharing of a common borehole in most informal settlements; 2) in the western part of the NWP there are mostly dirt roads which made access difficult; 3) the farms in these areas are very large, and therefore the farmhouses could not be accessed without knowledge of where to locate it; 4) many farmers have an automated water level switch for turning on the pump, and therefore the pump could not be turned on manually in order to collect the sample; and 5) many of the systems were designed so that water from the boreholes are pumped directly to the storage tank, without any sample collection points before it enters the tank. The map further indicates boreholes that tested positive for faecal

coliforms (red markers) as well as the boreholes that tested negative for this group of bacteria (green markers).

4.1 MICROBIOLOGICAL RESULTS

This section includes the following: 1) minimum, maximum and average bacterial counts of each sample area sampled in 2009 and 2010 as well as FC/FS ratios (Section 4.1.1); 2) identification of faecal coliforms isolated in 2009 with API 20E system (Section 4.1.2); 3) detection of *E. coli* in 2010 directly from sample water using multiplex PCR (Section 4.1.3) and 4) the percentage of faecal coliforms from 2009 and 2010 that were resistant, intermediate resistant or susceptible to selected antibiotics (Section 4.1.4).

4.1.1 Bacterial counts and FC/FS ratio's

Membrane filtration method was used in combination with selective agars for the enumeration of the various indicator bacteria screened for in this study. Figure 3.1 indicates the faecal contaminated (red marker) boreholes and non-faecal contaminated (green marker) boreholes. At areas where the maximum count of bacteria exceeded 300, the average was calculated by using the maximum count of 300. In these instances, the average was reported as a value exceeding (>) the calculated average. Section 4.1.1.1 gives the 2009 bacterial counts, Section 4.1.1.2 the 2010 bacterial counts and Section 4.1.1.3 the FC/FS ratios of both sampling periods. The bacterial counts for each borehole sampled as well as the FC/FS ratios of each are depicted in Appendix A (see Table A.1 and A.2). These counts were the mean value of three bacterial counts for each type of bacterium at each borehole.

4.1.1.1 2009 Sampling period

During the sampling period of 2009, 76 boreholes were sampled on 10 different dates from the middle of February to the end of July. Table 4.1 summarizes the minimum, maximum and average heterotrophic plate count bacteria (HPC), total coliforms, faecal coliforms and faecal streptococci counts of each of the 10 sample areas of 2009. Samples were also screened for the presence of the opportunistic pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus.* The latter were reported as the percentage of boreholes from each sample area that had the opportunistic pathogens present.

Table 4.1: Indicator bacteria counts and % of boreholes that were positive for *S. aureus* and *P. aeruginosa* of areas A – J sampled in 2009 (HPC – Heterotrophic plate count; TC- Total coliforms; FC – Faecal coliforms; FS – Faecal streptococci; Min - minimum count; Max - maximum count; Ave - Average count; cfu - colony forming unit).

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

As expected, HPC bacteria were present in all water samples (Table 4.1). Detection varied from low counts, $<10^2$cfu/ml to counts greater than 10^2cfu/ml. The highest HPC (7 500cfu/ml) was of a borehole sampled in area F_{09}, and the lowest (10cfu/ml) of a borehole sampled in area G_{09}. Both these areas (F_{09} & G_{09}) were sampled during the winter. Area J_{09}

had the lowest average for HPC bacteria (228±122.7cfu/ml) and area F_{09} the highest (2 205±2 645.7cfu/ml).

It can also be seen in Table 4.1 that at least one of the boreholes from areas A_{09}, B_{09} and I_{09} exceeded a total coliform count of 300cfu/100ml. The average total coliform count for the 76 boreholes sampled in 2009 was >75±78.6cfu/100ml. Only one of the 76 boreholes tested negative for total coliforms. This borehole was located in Christiana (G_{09}), and also tested negative for heterotrophic plate count bacteria, faecal coliforms, faecal streptococci, *P. aeruginosa* and *S. aureus* (Table A.1 in Appendix A).

The faecal coliform bacteria were all countable as none of the boreholes exceeded the 300cfu/100ml count. Faecal coliforms were detected in at least one borehole water sample from every sample area. The average faecal coliform count for the 76 boreholes sampled in 2009 was 33±62.9cfu/100ml. Area B_{09} had the highest average count for this group of bacteria (140±97.7cfu/100ml; Table 4.1). A borehole sampled in area I_{09} had the highest faecal coliform count (280cfu/100ml; Table A.1 in Appendix A). Borehole samples taken from area J_{09} and G_{09} had the lowest average faecal coliform counts (<1±1.0cfu/100ml and 4±7.1cfu/100ml respectively).

Three sample areas (A_{09}, B_{09}, and D_{09}) had at least one borehole with faecal streptococci counts exceededing 300cfu/100ml (Table 4.1). From Table 4.1 it is further observed that the average faecal streptococci count for the 76 boreholes were >33±77.2cfu/100ml. Sample areas E_{09}, F_{09}, and H_{09} had the lowest averages for faecal streptococci (2±3.2cfu/100ml, 1±1.7cfu/100ml and 6±1.0cfu/100ml respectively).

Based on the average counts of all the indicator bacteria (Table 4.1), it is seen that area B_{09} was highly contaminated by faecal and other bacteria. This area (B_{09}) is located in the south-eastern part of the Province. On the contrary area J_{09} had the lowest overall bacterial counts. This area (J_{09}) is located in the south-western part of the Province. Area A_{09} to E_{09} was sampled in the warm and wet summer and autumn seasons whereas, F_{09} to J_{09} was sampled in the cold and dry winter season. It is seen that the average faecal coliform and faecal streptococci counts were lower in area E_{09} to J_{09} with the exception of area I_{09} which had a high average faecal coliform count (70±95.6cfu/100ml) as well as faecal streptococci (56±52.0cfu/100ml) count. This observation could statistically be linked to lower ambient and water temperatures (Figure 4.6, Section 4.4).

The opportunistic pathogen *Pseudomonas aeruginosa* was detected in 46% of the 76 boreholes sampled (Table 4.1). *P. aeruginosa* was not detected in 4 of the 10 sample areas (A_{09}, B_{09}, C_{09} & H_{09}). This species (*P. aeruginosa*) was detected in all the borehole waters sampled in area E_{09} and G_{09}. It is also seen in Table 4.1 that the opportunistic pathogen, *S. aureus*, was detected in 7% of the total 76 boreholes sampled in 2009. This species (*S. aureus*) was isolated from boreholes in areas A_{09}, C_{09}, D_{09} and G_{09}. Boreholes from the remainder of the 2009 sample areas tested negative for *S. aureus*.

4.1.1.2 2010 Sampling period

Thirty eight boreholes were sampled during the 2010 sampling period. Table 4.2 show the microbiological results of each sample area for this period. Compared to 2009's microbiological results, 2010's included *E. coli* and excluded the opportunistic pathogen *S. aureus*. Membrane Lactose Glucuronide *agar* (MLGA) was used as substitute for m-Endo agar used in 2009. MLGA selects for and differentiate between *E. coli* and total coliforms. *E. coli* is represented by green colonies and total coliforms by yellow and green colonies

collectively. In 2009 several false positives were observed with the use of Mannitol Salt *agar* (MSA) for the detection of *S. aureus*. A minority of the sites sampled in 2009 were positive for *S. aureus*. Therefore, enumeration of this opportunistic pathogen was excluded from the 2010 sampling period.

Table 4.2: Indicator organism's counts and % boreholes that were positive for *P. aueruginosa* of areas A – J sampled in 2010 (HPC – Heterotrophic plate count; TC- Total coliforms; FC – Faecal coliforms; FS – Faecal streptococci; Min - minimum count; Max - maximum count; Ave - Average count; cfu - colony forming unit).

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

During the sampling period of 2010, 38 boreholes were sampled on seven different dates (March to May). From Table 4.2 it is evident that the overall bacterial counts were lower in 2010 compared to 2009. Five of the ten sample areas in 2009 had average heterotrophic plate counts that exceeded 10^2cfu/ml, whereas only two of seven sample areas exceeded this

count in 2010. A borehole from area F_{10} had the highest count for heterotrophic plate bacteria (5500cfu/ml). A water sample from the same area, F_{09}, had the highest heterotrophic plate count (7500cfu/ml) in 2009.

The average total coliform count of all the boreholes sampled in 2010 was >46±95.6cfu/100ml. Boreholes from areas F_{10} and J_{10} also exceeded the countable number of faecal coliforms. From Table 4.8 (Section 4.3) it is observed that 80% of the boreholes from the latter two areas had faecal coliforms present, whereas area F_{09} and especially area J_{09} had less boreholes with faecal coliforms present (Table 4.7; Section 4.2). Area F_{10} is in the north central part of the Province, and area J_{10} in the south western part. Area D_{10} that is situated in the north western part of the Province also had very high total and faecal coliform counts. The average faecal coliforms for all the boreholes sampled in 2010 could not be determined as in 2009 (33cfu/100ml) due to at least one site exceeding 300cfu/100ml in areas F_{10} and J_{10}. The average faecal coliforms of boreholes from area G_{10} were less than 1cfu/100ml, as was the same for area J_{09}. The highest average *E. coli* count as well as maximum count for a borehole belonged to area D_{10} and F_{10}. Area J_{10} did not have high numbers of *E. coli* present.

In contrast to 2009 faecal streptococci were present in lower numbers for all the borehole water samples of 2010. The average faecal streptococci counts for all the boreholes sampled were 10±29.2cfu/100ml compared to the 2009 average of >33±77.2cfu/100ml. Area J_{10} had the highest average for faecal streptococci (45±66.9cfu/100ml). The remainder of the 2010 sample areas ($A_{10} - H_{10}$) had low faecal streptococci averages.

Area J_{09} was the area with the lowest bacterial counts in 2009, whereas area J_{10} was one of the areas with the highest bacterial counts in 2010. In 2009 area J was sampled in July,

whereas this area was sampled in May during the 2010 sample period. Area G_{10} was the sample area with the lowest overall bacterial counts for 2010. This area also had rather low bacterial counts in 2009. Area A + B_{10} also had low bacterial counts. The low ambient- and water temperatures during the winter sample periods may thus have had an impact on the low bacterial counts.

Despite the lower detection of indicator bacteria in samples from area G during both sampling periods, *P. aeruginosa* was detected in all of the boreholes sampled in this area. A higher percentage of boreholes had *P. aeruginosa* present in 2010 (55%) than in 2009 (46%). Only one of the sample areas of 2010 (D_{10}), tested negative for *P. aeruginosa*. In 2009 four sample areas were negative for *P. aeruginosa* (A_{09}, B_{09}, C_{09} & H_{09}).

High standard deviations were observed for each of the average bacterial counts in each of the sample areas. The standard deviation indicates the deviational counts from the mean amount of bacteria amongst boreholes sampled in each area (Koosis, 1997).

4.1.1.3 FC/FS ratios

The ratio between faecal coliforms (FC) and faecal streptococci (FS) can be used as an indication of the possible source of contamination (Csuros& Csuros, 1999; Wyer & Kay, 1995). Table 4.3 summarizes the % boreholes from each sample area of 2009 and 2010 that had a 1) FC/FS ratio <0.7, indicating possible contamination from non-human origin (Csuros & Csuros, 1999); 2) FC/FS ratios between 2 and 4, indicating possible contamination from human waste (Wyer & Kay, 1995); 3) FC/FS ratios >4, indicating possible human faecal matter contamination (Wyer & Kay, 1995) and 4) the % boreholes that tested negative for faecal coliforms as well as faecal streptococci.

Table 4.3: FC/FS ratios of 2009 and 2010

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

In Table 4.3, it is demonstrated that non-human origin (FC/FS ratio <0.7) of faecal contamination was more evident in both sampling periods than of human waste (FC/FS ratio 2-4) and human faecal matter (FC/FS ratio >4.0). In 2009, 45% and in 2010, 32% of the total boreholes tested were possibly contaminated by non-human sources. This could be ascribed to the majority of boreholes being situated on farmlands with grazing animals in the area. Sample areas A and D were the only areas from the 2010 sampling period where none of the tested boreholes were possibly contaminated from non-human sources. These two areas (A_{10} & D_{10}) as well as areas G_{10} and E_{09} had high percentages of boreholes that did not test positive for faecal coliforms or faecal streptococci.

Overall 20% of boreholes in 2009 and 8% in 2010 that were tested were possibly contaminated by human faecal waste (FC/FS ratio >4.0). This is cause for concern. Sample areas B_{09} and C_{09} had a high percentage of boreholes with possible contamination from human sources. This was not the case for area $B+C_{10}$. Areas D, F and H consistently had

20% or more boreholes that were possibly contaminated by human faecal matter (FC/FS ratio >4.0) in both sampling periods.

None of the boreholes from sample areas B_{09}, H_{09}, I_{09} and J_{10} tested negative for both faecal coliforms and faecal streprococci, indicating that boreholes from these areas were all subjected to faecal contamination. All the boreholes of the latter sample areas (B_{09}, H_{09}, I_{09} and J_{10}), except for area B_{09}, were indicated to be mainly contaminated from non-human faecal sources.

In both sampling periods of 2009 and 2010, more than two thirds of the boreholes sampled were contaminated with either faecal coliforms or faecal streptococci or both. It is also observed that 10% less boreholes sampled in 2010 were contaminated with both faecal coliforms and faecal streptococci than those sampled in 2009.

4.1.2 Identification of faecal coliforms using API 20E

After the isolation and purification of blue colonies from m-FC agar, Gram staining was used to confirm that they were Gram-negative rods. The triple sugar iron test was also performed to further confirm that the isolates were of enteric origin. API results were taken as an interpretation of the 20 biochemical reactions incorporated into test strips, which were converted to a seven-digit code. The names of bacterial species associated with each seven-digit code were obtained from a manual. Of the 110 selected faecal coliform isolates tested with the API 20E system, only 98 were accurately identified (90% or greater). Amongst these 98 representatives the following enteric bacteria from the family *Enterobacteriaceae* was confirmed with the API 20E test: *Enterobacter cloacae* (34%), *Enterobacter aerogenes* (31%), *Klebsiella pneumonia* (15%), *Escherichia coli* (10%), *Raoutella planticola* (4%), *Raoutella terrigena* (3%), and *Pantoea* spp. 2 (3%). *E. coli* identified were isolated from the

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

Species from the genus *Enterobacter* was the most recorded species. *Enterobacter* identified were isolated from Klerksdorp, Klerksdorp 2, Potch 1, Potch 2, and Stilfontein (A_{09}), Welverdiend, Carletonville, Fochville, Ventersdorp 1 and Ventersdorp 2 (B_{09}); Groenfontein, Coligny, Coligny 2, Hartbeespoort and Koster (C_{09}); Hartebeespoort, Hartebeespoort 2, Rustenburg 2 and Matrooster (D_{09}); Delareyville, Stella and Zeerust 2 (F_{09}); Schweizer Renecke (G_{09}); Ganyesa 2 (H_{09}) and Tseoge, Vostershoop, Makopeng, Morokweng2 and Molopo (I_{09}).

4.1.3 Molecular based microbiological results

Multiplex PCR (mPCR) was used to detect the presence of *E. coli* in borehole waters sampled in 2010. DNA was directly isolated from borehole water samples. The mPCR simultaneously amplified fragments of the malate dehydrogenase gene (*mdh*) and of the lactose promoter (*lacZ*). If one or both of these genes fragments were amplified it would indicate the presence of *E. coli* in the water. Agarose gel electrophoresis was used to visualize PCR amplification products.

Figures 4.1 to 4.3 are images of ethidium bromide stained 1.5% (w/v) agarose gels that illustrate the mPCR products. Thirty-two of the 38 boreholes sampled in 2010 were screened for the presence of *E. coli* using mPCR. Area H was sampled in the beginning of the year. At this early stage, DNA isolation directly from water and mPCR conditions had not been optimized. Therefore, the total DNA from the 7 boreholes of this area was not included. The remaining 32 borehole samples were clustered into three groups. This grouping was done

based on their culture based microbiological results. The mPCR was performed on each of the three groups. All the samples that had presumptive *E. coli* present (green colonies on MLGA) was grouped together (Figure 4.1). Faecal coliform positive sites (blue colonies on m-FC agar) that had no green colonies on MLGA were grouped (Figure 4.2). The third group consisted of three sites that were positive for total coliforms (yellow colonies on MLGA) but negative for faecal coliforms and *E.coli* (lane 1 – 3; Figure 4.3), and nine sites that were negative for total coliforms, faecal coliforms and *E. coli* (lane 4 – 12; Figure 4.3).

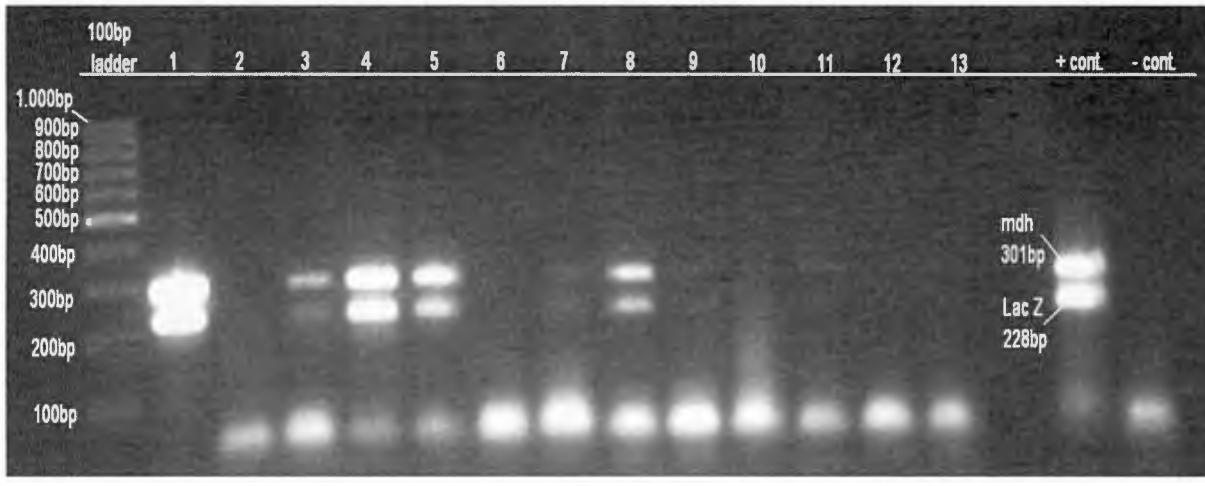

Figure 4.1: A 1.5% (w/v) Ethidium bromide stained agarose gel illustrating multiplex PCR results for boreholes that tested positive for *E. coli* on MLGA. One hundred nanograms of DNA were used as template. The left lane contains a 100bp molecular marker (O'GeneRuler™ 100bp DNA ladder, Fermentas Life Sciences, US). Lane 1 – Wolmaransstad; lane 2 - Potchefstroom2; lane 3 – Potchefstroom-Ventersdorp; lane 4 – Biesiesvlei; lane 5 – Sannieshof; lane 6 – Coligny-Biesiesvlei; lane 7 – Brits; lane 8 – Rustenburg2; lane 9 – Zeerust; lane 10 – Delarey-Ottosdal; lane 11 – Setlagole; lane 12 – Sekhing; and Lane 13 – Christiana. The last two lanes contain the positive (+cont. *E. coli* ATCC 10536) and no template controls (-cont.).

Two distinct bands of PCR product of the expected fragment size for the *mdh* and *lacZ* primer sets were visible in lanes 1, 3, 4, 5, 7, 8 and 9 of Figure 4.3.1. Faint bands were visible in lanes 6, 11, 12 and 13. Lane 2 and 10 were negative for the target *E. coli* gene fragments. The culture based method therefore gave two potentially false positives for *E.*

coli (lane 2 and 10). Potchefstroom2 (lane 2) had one green colony (presumptive *E. coli*) on MLGA in a 100ml of sample water and Delarey-Ottosdal (lane 10) had two. These results indicate that the selective and differential agar, MLGA, was 85% accurate in the positive detection *E. coli,* given that all the sites represented by lane 1- 13 tested positive for *E. coli* when MLG-agar was used. Due to the low number of *E. coli* detected in a 100ml of sample by MLGA for Potchefstroom 2 and Delarey-Ottosdal, it is possible that *E. coli* could have been detected by PCR if extraction were preceded with a 1-3 hour enrichment period.

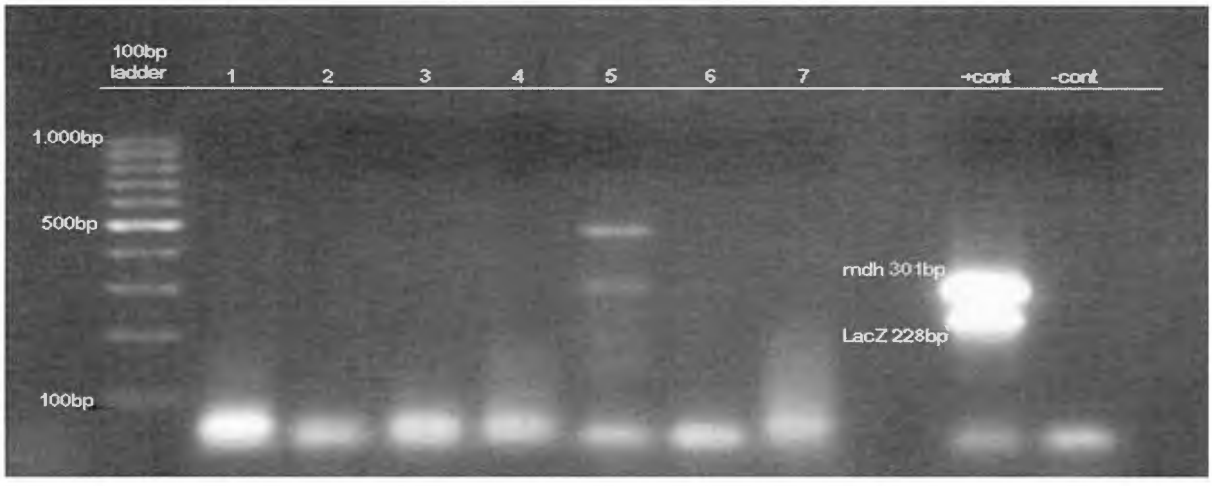

Figure 4.2: A 1.5% (w/v) ethidium bromide stained agarose gel illustrating multiplex PCR results for boreholes that tested positive for faecal coliforms on m-Fc agar but negative for *E. coli* on MLGA. One hundred nanograms of DNA were used as template. The left lane contains a 100bp molecular marker (O'GeneRuler^{TM} 100bp DNA ladder, Fermentas Life Sciences, US). Lane 1 - Klerksdorp; lane 2 – Potchefstroom1; lane 3 – Geystown; lane 4 – Taung; lane 5 – Hartswater; lane 6 – Christiana2; and lane 7 – Ganyea. The last two lanes contain the positive (+cont. *E. coli* ATCC 10536) and no template controls (-cont.).

Distinct bands indicating the PCR product for the *LacZ* and *mdh* primer sets was visible in lane 5 and 6 of Figure 4.2. Non-specific bands were also visible in lane 5. Faint bands of PCR product were observed in lane 1 and 3. The faintness of these bands could be ascribed to a low concentration of *E. coli* DNA due to a low concentration of this bacterium (*E. coli*) in these samples. Lanes 2, 4 and 7 were negative for the target *E. coli* gene fragments.

These seven sites were all positive for faecal coliforms, but negative for *E. coli* with the culture based methods. The culture based method gave a false negative result for the presence of *E. coli* for Hartswater (lane 5), Christiana2 (lane 6), Klerksdorp (lane 1) and Geystown (lane 3) samples. This could potentially be ascribed to the presence of *E. coli* bacteria in a possible viable but non-culturable state.

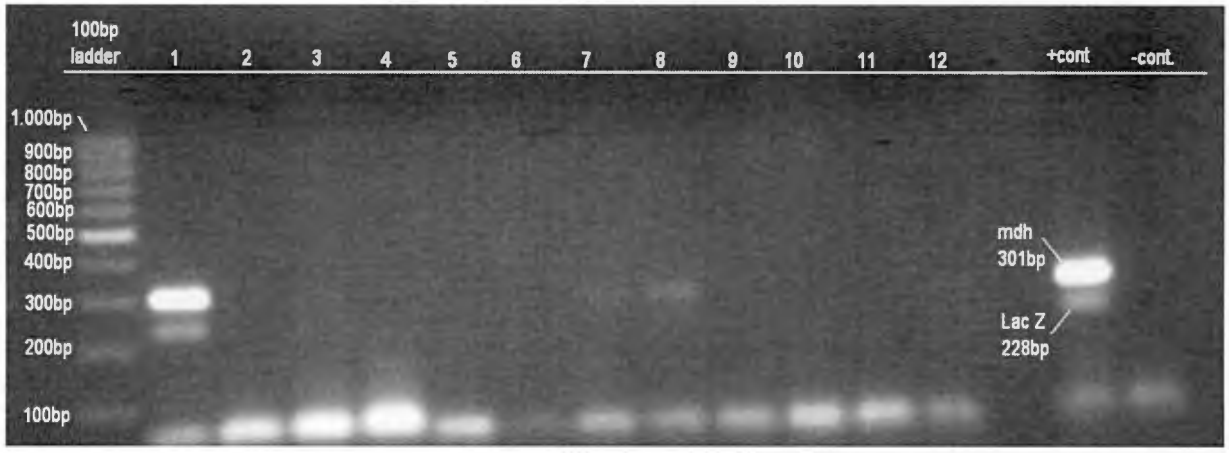

Figure 4.3: A 1.5% (w/v) ethidium bromide stained agarose gel illustrating multiplex PCR results for boreholes that tested positive for total coliforms on MLGA but negative for *E. coli* on MLGA and faecal coliforms on m-Fc (lane 1 – 3). Lane 4 – 12 represents multiplex PCR results for boreholes that tested negative for total coliforms, faecal coliforms and *E.coli*. One hundred nanograms of DNA were used as template. The left lane contains a 100bp molecular marker (O'GeneRuler^{TM} 100bp DNA ladder, Fermentas Life Sciences, US). Lane 1 - Ventersdorp-Coligny; lane 2 - Mafikeng; lane 3 – Amalia; lane 4 – Orkney; lane 5 – Stilfontein; lane 6 – Lichtenburg; lane 7 – Derby; lane 8 – Hartbeespoort; lane 9 – Rustenburg; lane 10 – Broedersput; lane 11 – Vryburg; and lane 12 – Ganyesa2. The last two lanes contain the positive (+cont. *E. coli* ATCC 10536) and no template controls (-cont.).

PCR products were visible in lane 1, 3, 4, 7, 8 and 12, indicating that *E. coli* was potentially present in these boreholes. This shows that the culture based method gave false-negative results because it did not detect *E. coli* in samples from these boreholes. PCR products were

not observed in lane 2, 5, 6, 9, 10 and 11, indicating that *E. coli* were absent in these boreholes. This result agrees with the culture-based results.

4.1.4 Antibiotic profiles of faecal coliform isolates

The susceptibility, intermediate resistance and resistance of isolated faecal coliforms to 9 common therapeutically used antibiotics were determined with the Kirby-Bauer disk diffusion method. The measured diameters (mm) of growth inhibition zone of each isolate tested from the 2009 and 2010 sampling period in response to a given antibiotic can be seen in Appendix A (Tables A.5 and A.6). For simplicity, significance and comparative reasons the isolates were divided into three spatially separated areas (1 – 3). Area 1 represents the antibiotic profiles of faecal coliforms isolated from the south-eastern part of the North West Province (sampling areas A, B & C), area 2 the north-eastern part (Sampling areas D, E & F), and area 3 the western part of the Province (Sampling areas G, J, H & I). Area 3 of 2010 was represented only by isolates from the central-south-western part of the Province (sampling areas G, H & J). The reason for this is that there were no representative faecal coliforms isolated from the north-western part (sampling area I).

Table 4.4 depicts the 2009 results of 85 faecal coliform isolates representing the percentage resistance (R), intermediate resistance (IR) and susceptibility (S) of isolates to antibiotics in an area. Table 4.5 gives these results of 60 isolates from the 2010 sample period. These tables also give the average MAR indices for each sample area (see Table A.5 and A.6 in Appendix A for the MAR indices for each isolate tested), as well as the most prevalent phenotype of antibiotics that isolates in an area were resistant to. Only antibiotics that had more than 20% of isolates resistant to it was included in the latter phenotype. Abbreviations used for the antibiotics, concentrations thereof and its inhibition zone diameter (mm) specifications are tabulated in Table 3.3.

Table 4.4: Percentage resistance (R), intermediate resistance (IR) and susceptibility (S) of selected faecal coliform isolates from the 2009 sampling period to nine antibiotics.

Sample area n FC isolates	**%SIR**	**AMOX**	**AMP**	**CEP**	**KAN**	**STREP**	**TM**	**OXY-TET**	**CIP**	**CHL**
1	% R	39	39	13	0	0	17	30	0	0
(A_{09}+B_{09}+C_{09})	% IR	39	22	31	0	0	30	61	0	0
n = 23	% S	22	39	56	100	100	53	9	100	100
MAR: 0.150	Most prevalent MAR phenotype: AMOX-AMP-OXYTET									
2	% R	57	38	38	0	0	23	43	0	10
(D_{09}+E_{09}+F_{09})	% IR	29	14	33	0	0	48	57	0	5
n = 21	% S	14	48	29	100	100	29	14	100	85
MAR: 0.233	Most prevalent MAR phenotype: AMOX-AMP-CEP-TM-OXYTET									
3 (G_{09}+J_{09}+	% R	84	49	47	0	0	5	28	0	0
H_{09}+I_{09})	% IR	8	18	20	5	0	53	49	0	8
n = 40	% S	8	33	33	95	100	42	23	100	92
MAR: 0.239	Most prevalent MAR phenotype: AMOX-AMP-CEP-OXYTET									
Total FC	% R	54	41	40	0	0	11	30	0	1
isolates	% IR	21	17	22	4	0	37	53	0	4
n = 85	% S	25	42	38	96	100	52	17	100	95
Total MAR index for 2009 sampling period: 0.213 Most Prevalent MAR phenotype: AMOX-AMP-CEP-OXYTET										

The following was observed from Table 4.4: Of the total 85 faecal coliform isolates tested, most were resistant to β-lactam antibiotics (AMOX, AMP and CEP). Only 25% of the total faecal coliform isolates tested were susceptible to AMOX. Percentage resistance to TET was also high (30%). The high intermediate resistance (53%) and low percentage susceptibility (17%) of the 85 isolates tested to OXY-TET is alarming. More than half (52%) of the isolates were susceptible to TM, with the percentage intermediate resistance to this antibiotic 37%. All isolates tested were susceptible to STREP and CIP. High

percentages of isolates were susceptible to CHL and KAN (95% and 96% respectively). No resistance to KAN and 1% resistance to CHL were observed.

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

The antibiotic profiles of the three clustered areas of the Province are also observed from Table 4.4. In all three areas, resistance to AMOX was the most prevalent. Isolates isolated from boreholes in the western part of the Province (area 3) had very high resistance to AMOX (84%). Percentage of isolates resistant to AMP was consistently high in all three areas. Isolates from area 2 and 3 had high percentage resistance to CEP (38% and 47% respectively), whereas isolates from area 1 had lower percentage resistance to this antibiotic (13%). High percentages intermediate resistance and low percentages susceptibility to OXY-TET was measured in all three areas. Area 1 had the lowest MAR index value (0.150) as well as the MAR phenotype with the least antibiotic representatives (AMOX-AMP-OXYTET) to which more than 20% of isolates were resistant to. Area 2 had a MAR index value of 0.233. More than 20% of the faecal coliforms from the latter area (area 2) were resistant to five of the nine antibiotics tested (AMOX, AMP, CEP, TM and OXY-TET). The highest MAR index value of 0.239 was observed among isolates from area 3. The most prevalent MAR phenotype for this area was AMOX-AMP-CEP-OXYTET. It is seen that faecal coliforms isolated from area 1 were resistant to a lower number of antibiotics than the isolates from area 2 and area 3.

Table 4.5: Percentage resistance (R), intermediate resistance (IR) and susceptibility (S) of selected faecal coliform isolates from the 2010 sampling period to nine antibiotics.

Sample area n FC isolates	**%SIR**	**AMOX**	**AMP**	**CEP**	**KAN**	**STREP**	**TM**	**OXY-TET**	**CIP**	**CHL**
1	% R	21	21	46	0	0	4	38	0	0
(A_{10}&B+C_{10})	% IR	54	58	41	13	0	63	45	0	0
n = 24	% S	25	21	13	87	100	33	17	100	100
MAR: 0.176	Most Prevalent MAR phenotype: AMOX-AMP-CEP-OXYTET									
2	% R	6	0	44	0	0	6	6	0	0
(D_{10} + F_{10})	% IR	44	50	12	19	0	13	69	0	13
n = 16	% S	50	50	44	81	100	81	25	100	87
MAR: 0.074	Most Prevalent MAR phenotype: CEP									
3	% R	35	25	35	0	0	15	20	0	10
(G_{10}+H_{10}+J_{10})	% IR	35	45	55	80	0	20	7 0	0	5
n = 20	% S	30	30	10	20	100	65	10	100	85
MAR: 0.155	Most Prevalent MAR phenotype: AMOX-CEP-OXYTET									
Total FC isolates	% R	21	15	42	0	0	8	22	0	3
	% IR	44	51	36	37	0	32	61	0	6
n = 60	% S	35	34	22	63	100	60	17	100	91
Total MAR index for 2010 sampling period: 0.126 Most Prevalent MAR phenotype: AMOX-CEP-TET										

The following observations were made from Table 4.5: Of the 60 faecal coliform isolates tested in 2010 more than 20% were resistant to AMOX, CEP and OXY-TET. Fourty two percent were resistant to CEP. All the isolates tested were completely susceptible to CIP and STREP. Sample area 1 had the highest MAR index value (0.176), followed by area 3 (0.155) and 2 (0.074). The average MAR index value for the isolates of the 2010 sample period were 0.126, with the most prevalent MAR phenotype being AMOX-CEP-OXYTET.

The following is a comparison between the antibiotic resistance profiles of isolates from 2009 and 2010 (Table 4.4 for 2009 results). The percentage isolates that were resistant, intermediate resistant and susceptible to STREP, CEP, TM, OXY-TET, CIP and CHL were similar in both years. Major differences include the following: 1) the percentage isolates resistant to AMOX decreased from 54% in 2009 to 21% in 2010; 2) 15% of the total 60 isolates from 2010 were resistant to AMP whereas more than 40% of the total isolates tested in 2009 were resistant to this antibiotic. This low overall percentage of isolates that were resistant to AMP in 2010 is mainly influenced by the 0% resistance to AMP in area 2, compared to the 38% of 2009. The percentage intermediate resistance to AMP increased from 17% in 2009 to 51% in 2010; 3) the percentage intermediate resistance to KAN increased from 6% in 2009 to 37% in 2010; 4) isolates from area 2 and area 3 had higher MAR index values in 2009 (0.233 and 0.239 respectively) than in 2010 (0.074 and 0.155 respectively); 5) the 2010 MAR index value of 0.126 was lower than that of 2009 (0.213).

These differences could potentially be ascribed to the sampling regime differences between the 2009 and 2010 sampling periods. Although samples were collected from the same geographical areas different boreholes were used in these two sampling periods.

4.2 PHYSICO-CHEMICAL RESULTS

Temperature, pH, total dissolved solids (TDS), electrical conductivity (EC) and nitrate was measured to characterize the water physically and chemically. Table 4.6 gives the minimum, maximum and average of each of the measured physico-chemical parameters of the water from boreholes sampled in each sampling area of the 2009 sampling period. In Table 4.7 the latter results for the 2010 sampling period are depicted.

Table 4.6: Physico-chemcial data of water from boreholes for various sampling areas measured in 2009.

Sample Area (A_{09}-J_{09})		pH	Temp (°C)	EC (µs/cm)	TDS (ppm)	Nitrate - nitrogen (mg/L NO3-N)
A_{09}	Min	6.8	20.4	347	322	1.8
	Max	7.6	24.5	1400	970	209.8
	Ave	7.1±0.3	22.9±1.4	884±410.3	609±283.9	33.1±71.4
B_{09}	Min	6.8	21.1	67	41	0.1
	Max	7.9	22.3	700	470	3.0
	Ave	7.4±0.4	21.6±0.5	447±237.2	301±160.8	0.9±1.2
C_{09}	Min	6.9	20.6	127	85	4.5
	Max	7.5	26.0	1350	906	120.9
	Ave	7.1±0.2	23.0±6.9	662±418.3	444±281.0	58.7±59.2
D_{09}	Min	7.1	20.2	489	348	0.9
	Max	8.1	24.1	1225	860	55.2
	Ave	7.3±0.4	23.0±1.8	900±264.5	611±191.3	14.1±6.9
E_{09}	Min	6.4	19.2	312	223	0.3
	Max	7.7	24.3	1146	812	8.4
	Ave	7±0.4	22.4±1.6	819.4±301.2	582.4±236.5	5.9±3.3
F_{09}	Min	6.8	21.0	214	152	0.6
	Max	7.6	22.4	1218	865	160.0
	Ave	7.2±0.3	21.6±0.9	902±327.0	635±229.8	12.4±11.5
G_{09}	Min	6.9	18.6	574	404	5.5
	Max	7.5	19.5	1758	1250	47.0
	Ave	7.1±0.4	18.7±2.1	976±489.5	691±349.1	32.2±14.9
H_{09}	Min	6.7	16.6	960	675	38.6
	Max	7	18.5	1731	1310	63.6
	Ave	6.9±0.1	17.7±0.8	1247±336.4	904±280.1	49.9±10.7
I_{09}	Min	7.1	16.2	637	624	0.5
	Max	8.2	24.0	1134	926	454.5
	Ave	7.4±04	20.6±2.6	912±163.2	701±123.1	171.6±188.2
J_{09}	Min	7	15.5	758	548	3.9
	Max	8.8	21.5	1445	1030	454.5
	Ave	7.4±0.5	19.7±2.1	1062±244.5	754±168.1	210.7±223.1
Total 76 Boreholes	Min	6.4	15.5	67	41	0.1
	Max	8.8	24.5	1758	1250	454.5
	Ave	7.2±0.4	20.9±3.2	891±357.6	636±259.1	67.0±124.3

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

The following were observed from Table 4.6: The average pH for all ten areas ranged from 6.9 to 7.4 and complied with the DWAF TWQR for domestic (pH 6.0 - 9.0) and irrigational purposes (pH 6.5 - 8.4) (DWAF, 1996b). The average groundwater temperature for the sample areas ranged from 17.7°C to 23.4°C, whereas the average temperature of all the boreholes sampled were 20.9.

Area F_{09} and H_{09} is in the northern part of the Province near the Botswana border. This could possibly explain the slight higher water temperatures due to the warmer ambient winter temperatures of this region. Of all the sampling areas only area C_{09} had average EC and TDS levels that complied with the DWAF target water quality range (TWQR) for domestic use (<700µs/cm and <450mg/l respectively). The levels of TDS and EC were within levels that could be regarded as safe for domestic consumption.

Nitrate levels, however were alarmingly high (Table 4.6). The average nitrate levels of merely two sampling areas (B_{09} and E_{09}) were within the TWQR for domestic use (< 6mg/L NO_3-N). In six of the ten sampling areas average nitrate levels exceeded 20mg/L NO_3-N. Methaemoglobinemia in infants and young children may occur if this high nitrate load is consumed over an extended period of time (Sadeq *et al.*, 2008). Furthermore, in six of the sampling areas, average nitrate concentration exceeded the maximum acceptable value of nitrate for the use of irrigation purposes (30mg/L NO_3-N; DWAF, 1996). The average nitrate level for all 76 boreholes sampled was a very high 67.0mg/L NO_3-N. The highest nitrate levels were observed in areas I_{09} (171.6±188.2) and J_{09} (210.7±223.1). These two boreholes are situated in the western part of the Province. Figure 4.4 indicates the levels of nitrates measured in 2009.

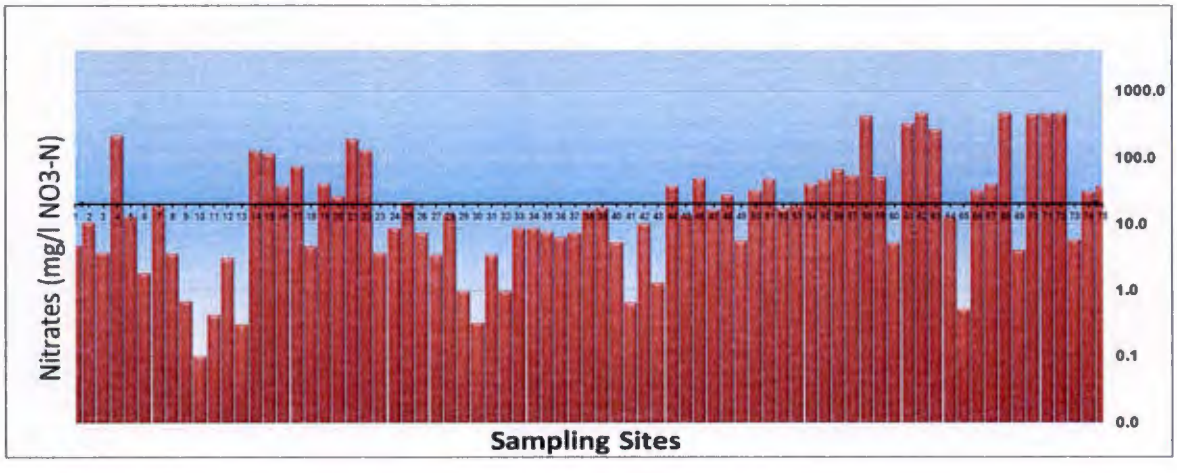

Figure 4.4: Nitrate levels of the boreholes sampled in 2009.

Each bar in Figure 4.4 represents the nitrate concentrations (logarithmic scale) of each borehole sampled individually. The black line is positioned at 20 mg/L NO_3-N. The latter is to visibly show the bars that are greater than this (20 mg/L NO_3-N) value, thereby indicating the boreholes exceeding this limit. Thirty-one (40.8%) of the total sampled boreholes had nitrate concentrations exceeding this limit that may cause methaemoglobinemia.

Table 4.7 represents the physico-chemical parameter values measured in 2010.

Table 4.7: Physico-chemical data of water from boreholes for various sampling areas measured in 2010.

Sample Area (A-K)		**pH**	**Temp** (°C)	**EC** (µs/cm)	**TDS** (ppm)	**Nitrate - nitrogen** (mg/L NO3-N)
A_{10}	Min	6.9	20.9	641	458	0.8
	Max	8.7	22	1464	1004	11.4
	Ave	7.4±0.7	21.7±0.5	1030±363.1	683±255.5	8.3±4.4
$B + C_{10}$	Min	6.8	19.5	223	157	11.1
	Max	7.3	21.1	1831	1280	74.1
	Ave	7.2±0.2	20.3±0.7	727±585.3	514±407.4	30.1±25.8
D_{10}	Min	6.7	18.8	113	80	0.7
	Max	7.6	23.5	952	675	44.3
	Ave	7.1±0.3	21.2±2.2	593±353.2	422±249.3	12.6±18.0
F_{10}	Min	7.0	18.1	624	450	8.2
	Max	7.8	20.5	2530	1790	23.9
	Ave	7.4±0.4	20.3±0.9	1342±744.2	954±522.6	15±6.5
G_{10}	Min	6.7	20.8	669	481	23.3
	Max	7.2	25.8	905	644	148.2
	Ave	7.0±0.4	23.9±2.0	771±150.6	549±107.8	44±42.0
H_{10}	Min	7.1	16.0	804	571	15.2
	Max	7.6	21.2	1259	895	28.9
	Ave	7.4±0.2	18. 6±2.4	1039±251.3	737±177.8	23.3±6.7
J_{10}	Min	6.8	19.4	861	614	1.6
	Max	7.7	24.4	2860	2003	23.2
	Ave	7.3±0.4	22.0±2.0	1490±722.7	1046±502.9	13.5±8.7
Total 38 boreholes	Min	6.7	16.0	113	80	0.7
	Max	8.7	25.8	2860	2003	74.1
	Ave	7.2±0.4	21.1±2.1	982±563.4	689±394.1	24.9±27.4

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

From Table 4.7 the following was observed: The average pH for all the sampling areas complied with the DWAF TWQR for domestic and irrigational purposes, and ranged from 6.9 to 7.4. This was also the case in 2009 (Table 4.6). Maximum pH measured in 2010 was 8.7 and the minimum pH 6.7. In 2009 pH 8.8 was the maximum and pH 6.4 the minimum (Table 4.6).

The average groundwater temperature of sample areas ranged from 18.6°C to 23.9°C, whereas it ranged from 17.7°C to 23.0°C in 2009 (Table 4.6). The lowest measured temperature of the 2010 sampling year was 0.5°C higher than the lowest temperature (15.5°C) measured in 2009. It was further observed that the highest temperature measured in 2010 (25.8°C) was 1.3°C higher than the highest measured temperature of 2009 (24.5°C). The average water temperature of all the boreholes sampled in 2010 was 21.1°C. The overall water temperature average of 2010 was 0.2°C warmer than the overall average of 2009, which was 20.9°C. This overall higher water temperature in 2010 could be ascribed to the fact that sampling was done only till the end of May 2010, whereas in 2009 sampling took place until the end of June.

In 2010, area D_{10} was the only sampling area with average EC and TDS levels that complied with the DWAF TWQR for domestic use (<700µs/cm and <450mg/l respectively). Two boreholes had EC levels exceeding 2000µs/cm (area F_{10} and J_{10}). The highest measured EC was 2860µs/cm (area J_{10}). The latter borehole from area J_{10} also had the highest TDS level (2003ppm). In 2009 (Table 4.6) area G_{09} had the highest EC level measured (1758µs/cm) as well as the highest TDS (1250ppm). However, health impacts may only occur at levels higher (EC > 4500; TDS > 3000) than the levels measured in this study (Appendix B: Table B.2; DWAF, 1996b).

In 2010 the observed nitrate levels were, in many cases, still higher than the 6mg/L NO_3-N TWQR for domestic use. However, the levels were lower than those measured in 2009. The average nitrate level for all the boreholes sampled in 2010 were 24.9±27.4 mg/L NO_3-N compared to the 67.0±124.3 mg/L NO_3-N of 2009 (Table 4.6). None of the areas sampled in 2010 had average nitrate levels below 6 mg/L NO_3-N. The highest measured nitrate level was from a borehole in area G_{10} (148.2 mg/L NO_3-N). Area I_{09} and J_{09} both had boreholes with nitrate levels at 450.0 mg/L NO_3-N or higher. The average nitrate level of sample area J, situated in the western part of the province, was much higher in 2009 than in 2010. The average for area J_{10} was 13.5±8.7mg/L and that for J_{09} 210.7±223.1mg/L NO_3-N. Area B_{09} had the lowest average nitrate (0.9±1.2mg/L NO_3-N) in 2009 but the second highest average nitrate levels (30±25.8mg/L NO_3-N) in 2010. Figure 4.5 indicates the levels of nitrates measured in 2010.

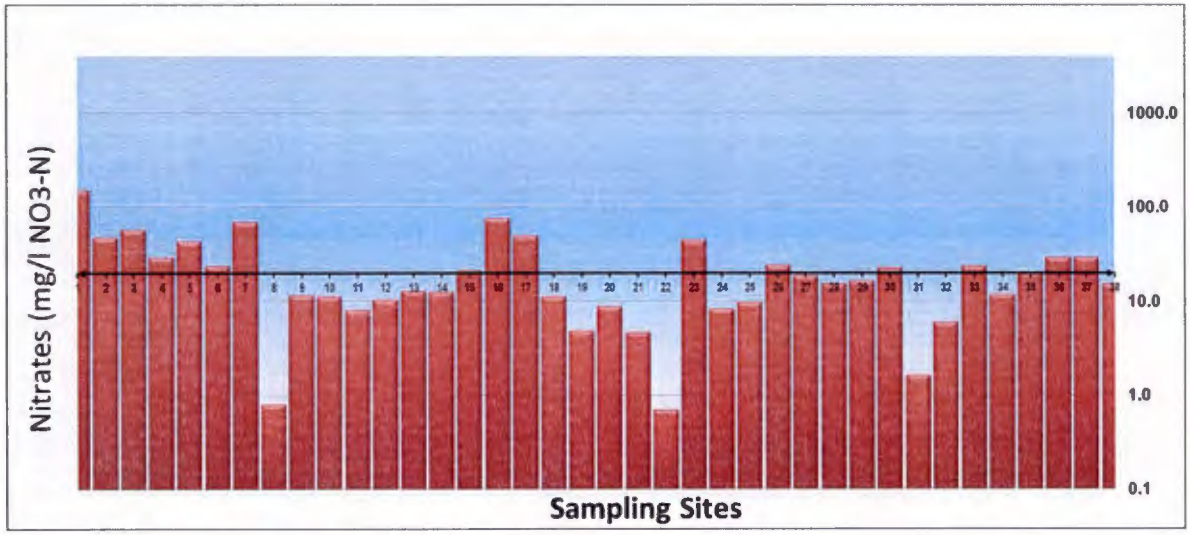

Figure 4.5: Nitrate levels of the boreholes sampled in 2010.

Each bar in Figure 4.2 represents the nitrate concentrations (logarithmic scale) of each borehole sampled individually. Sixteen (42.1%) of the boreholes sampled in 2010 exceeded the methaemoglobinemia causing limit (20mg/L NO_3-N; x-axis) whereas 40.8% of the boreholes sampled in 2009 exceeded this limit.

4.3 COMPARISON OF THE TESTED BOREHOLE WATER QUALITY TO DWAF TWQR

The Department of Water Affairs and Forestry has target water quality ranges (TWQR) for various utilizations of water (DWAF, 1996b). Table 4.8 and 4.9 gives the percentage of samples from each area of the 2009 and 2010 sampling period, respectively, which exceeded each of the TWQR for domestic use. TWQR of the measured parameters and relevant indicator bacteria are given in Table B.1 (Appendix B). The TWQR of water to be utilized for domestic purposes (such as drinking, bathing and washing), irrigation, recreation and livestock watering were included. The possible health effects that could occur if these quality ranges were exceeded are summarized in Table B.2 (appendix B). The contents of Table B.1 and B.2 were adopted from the DWAF Field Guide of 1996 (DWAF 1996b). A TWQR for faecal streptococci is only available for recreational use. Faecal streptococci are also an indicator of faecal matter, therefore, its presence in domestic water is undesirable. A water quality standard for *Staphylococcus aureus* and *Pseudomonas aeruginosa* is not provided by DWAF, but due to the pathogenic potential of these two bacterial species their presence in domestic water is not desired.

Table 4.8: Percentage boreholes of the 2009 sampling period exceeding the DWAF TWQR for domestic use.

	% boreholes exceeding DWAF TWQR						
Parameter	**pH**	**TDS**	**EC**	**Nitrate**	**HPC**	**TC**	**FC**
Sample Area							
A_{09}	0	63	63	50	88	100	63
B_{09}	0	20	20	0	60	100	100
C_{09}	0	44	44	89	89	100	63
D_{09}	0	86	71	57	100	100	57
E_{09}	0	75	63	63	75	100	14
F_{09}	0	88	88	63	88	86	50
G_{09}	0	88	75	88	75	75	38
H_{09}	0	100	100	100	100	100	25
I_{09}	0	100	89	78	100	100	78
J_{09}	0	100	100	78	78	89	11
Total 76 boreholes	**0**	**76**	**70**	**67**	**87**	**95**	**49**

From Table 4.8 it is evident that all the boreholes sampled in 2009 complied with the domestic TWQR for pH. Between 20 and 100 percent of the boreholes tested in 2009 areas exceeded the TWQR for TDS (0 - 450ppm) and EC (0 - 700µs/cm). These parameters affect the aesthetic value of the water. However, at the levels observed, these paramaters would not have any health effects (Table B.2; Appendix B). Between 50% and 100% of the boreholes in a sampling area exceeded the nitrate TWQR (0-6mg/L NO_3-N). Only in one area (B_{09}) did all the samples comply with this TWQR.

Eighty-seven percent of the 76 boreholes sampled in 2009 had heterotrophic plate count bacteria that exceeded 100cfu/ml. In areas D_{09}, H_{09} and I_{09} all samples exceeded this TWQR. From all the samples collected and analyzed in 2009, only four (5%) did not exceed the domestic TWQR for total coliforms (0 – 5cfu/ml). From all the sample areas, the percentage of boreholes that tested positive for faecal coliforms ranged from 11% to

100%. This exceeds the TWQR for faecal coliforms (0cfu/100ml). Sampling area B_{09}, which includes boreholes from Carletonville, Ventersdorp, Fochville and Makokskraal, was the only sampling area where all of the representing boreholes had faecal coliforms present but none of the boreholes exceeded the nitrate limit.

Table 4.9: Percentage boreholes of the 2010 sampling period exceeding the DWAF TWQR for domestically used water.

	% boreholes exceeding DWAF TWQR						
Parameter	**pH**	**TDS**	**EC**	**Nitrate**	**HPC**	**TC**	**FC**
Sample Area							
A_{10}	0	60	60	80	100	40	60
$B + C_{10}$	0	50	50	100	100	67	67
D_{10}	0	40	40	40	100	40	40
F_{10}	0	80	80	100	80	40	80
G_{10}	0	71	86	100	71	0	29
H_{10}	0	100	100	100	50	25	25
J_{10}	0	100	100	67	83	67	83
Total 38 boreholes	**0**	**71**	**79**	**82**	**84**	**39**	**55**

Table 4.9 demonstrates that all of the boreholes sampled in 2010 complied with the domestic TWQR for pH. This was the same for the 2009 samples (Table 4.8). Between 40 and 100 percent of boreholes from the various sampling areas exceeded the TWQR for TDS. Overall, 5% more boreholes (71%) in 2010 exceeded the TWQR for TDS than in 2009 (67%). The percentage boreholes from the various sampling areas that exceeded the EC TWQR in 2010, also ranged between 40 and 100 percent. In 2010 9% more boreholes (79%) exceeded the TWQR for EC compared to 2009 (70%). The average nitrate concentrations of water from boreholes were lower in 2009 compared to those of 2010. Despite this, 15% more boreholes exceeded the nitrate TWQR for domestic water in 2010 (82%) compared to 2009 (67%).

The 83% of boreholes exceeding the TWQR for HPC bacteria in 2010 corresponds with the 87% of boreholes that exceeded this TWQR in 2009 (Table 4.8). Non-compliance of boreholes with the TWQR for total coliforms ranged between 0 and 67 percent. The percentage boreholes that exceeded the TWQR for total coliforms in 2010 differed greatly from that of 2009. Ninety-five percent of the boreholes tested in 2009 exceeded the TWQR for total coliforms, compared to the 39% that exceeded this limit in 2010. This could be ascribed to the different selective media used to enumerate these bacteria in the two sampling years. In 2009 m-Endo agar was used, whereas MLGA was used in 2010. All of the boreholes from area G_{10} complied with the TWQR for total coliforms. The same media was used in both years for the enumeration of faecal coliforms, namely m-FC. Between 25 and 83% of boreholes from all sampling areas exceeded the TWQR for faecal coliforms. In 2010, 6% more boreholes exceeded the TWQR for faecal coliforms compared to the 2009 values. Not one sample area from 2010 totally complied with the TWQR for faecal coliforms. The later was also observed in 2009.

4.4 ORDINATION REDUNDANCY ANALYSIS

Canonical ordination redundancy analysis (RDA) was applied to determine the correlation between the tested environmental variables (temperature, TDS, EC and NO_3-N) and the indicator bacteria tested for in 2009 and 2010. Mean biplots were used to visualise the results of the multi-variate analysis. The biplots represent the joint effect of environmental variables on the selected bacteria in a single plane (Ter Braak, 1990). The angles in the biplot between the response (indicator bacteria) and environmental variables, and between the response variables themselves or environmental variables themselves, reflect their correlations. Figure 4.6 shows the RDA biplot obtained from using the environmental variable data and bacterial counts of 2009. Figure 4.7 provides the latter for the 2010 data.

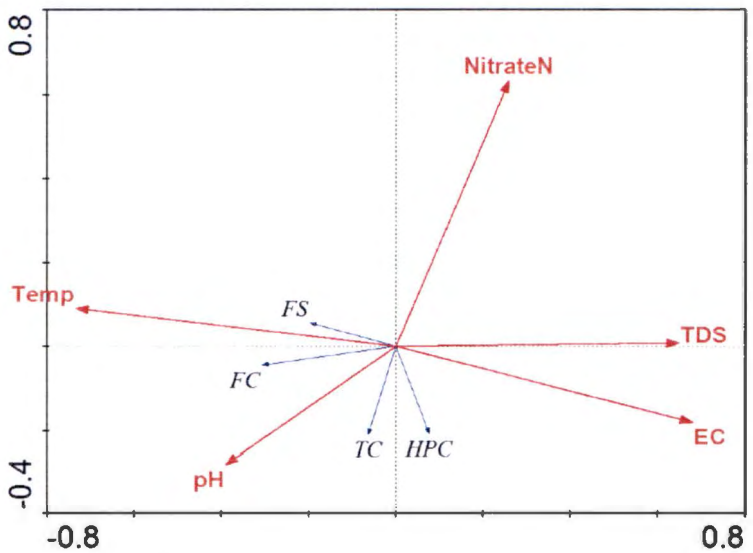

Figure 4.6: Correlation biplot indicating the influence of the environmental variables on indicator bacteria during the 2009 sample period. Environmental variables are represented by red arrows and include: water temperature (Temp), pH, total dissolved solids (TDS) and electrical conductivity (EC). Indicator bacteria are represented by blue arrows and include: faecal streptococci (FS), faecal coliforms (FC), total coliforms (TC) and heterotrophic plate count bacteria (HPC).

The biplot (Figure 4.6) shows that the two environmental variables having the closest association with faecal coliforms (FC) and faecal streptococci (FS) were temperature (Temp) and pH. This reflects that these two environmental variables exerted more effect on these bacteria (FC and FS) than the other environmental variables tested for. A strong correlation between FC and FS is also observed. Total coliforms (TC) and heterotrophic plate count (HPC) bacteria were strongly associated with each other. This was also seen for total dissolved solids (TDS) and electrical conductivity (EC). Total coliform counts were more strongly associated with pH than HPC bacteria, whereas HPC bacteria were more strongly associated with EC than TC. Nitrate-nitrogen (NO_3-N) levels did not show a correlation to any of the bacteria tested for.

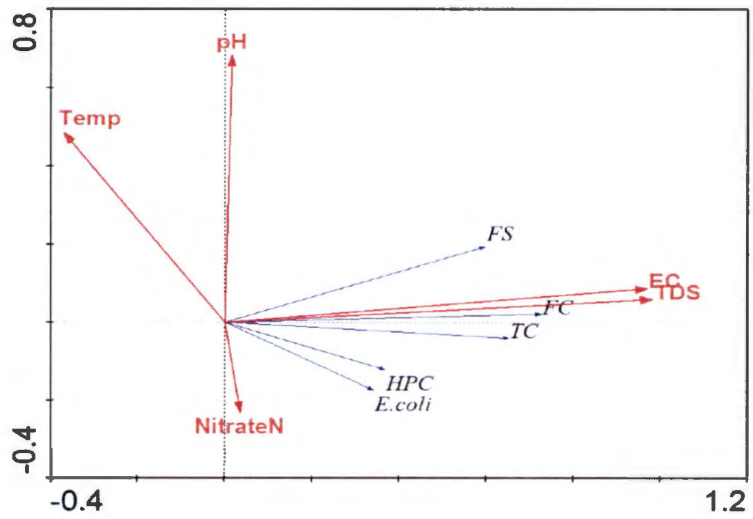

Figure 4.7: Correlation biplot indicating the influence of the environmental variables on indicator bacteria during the 2010 sample period. Environmental variables are represented by red arrows and include: water temperature (Temp), pH, total dissolved solids (TDS) and electrical conductivity (EC). Indicator bacteria are represented by blue arrows and include: *E. coli*, faecal streptococci (FS), faecal coliforms (FC), total coliforms (TC), and heterotrophic plate count bacteria (HPC).

The biplot (Figure 4.7) shows that the two environmental variables having the closest association with faecal coliforms (FC), faecal streptococci (FS) and total coliforms (TC) were electrical conductivity (EC) and total dissolved solids (TDS). This reflects that these two environmental variables exerted more effect on these bacteria (FC, FS and TC) than the other environmental variables tested for. Nitrate-nitrogen levels showed a weak correlation to *E. coli*. Furthermore, heterotrophic plate count (HPC) bacteria and *E. coli* were strongly associated with each other. This was also seen for total dissolved solids (TDS) and electrical conductivity (EC) as well as for TC en FC. None of the bacteria showed an association with temperature.

A contrast is observed between the results of the multi-variate analysis for the two sampling periods. The correlation of NO_3-N and temperature to the tested bacteria differed the most between the two sampling years. This could be ascribed to the lower overall concentrations of NO_3-N as well as temperatures measured in 2010 compared to 2009.

4.5 SUMMARY OF RESULTS

Heterotrophic plate count bacteria levels were high in most of the borehole water samples tested. In both sampling years more than 80% of the boreholes sampled had heterotrophic plate counts exceeding the DWAF TWQR of 0 – 100cfu/ml (Table B.1; Appendix B). Twenty nine percent of the 114 boreholes sampled throughout the study had heterotrophic plate counts exceeding 10^3cfu/100ml. The risk for infectious disease transmission may increase when this limit is exceeded (Table B.2; Appendix B). In 2009 and 2010, total coliform, faecal coliforms faecal streptococci and presumptive *Pseudomonas aeruginosa* were detected in many of the borehole water samples. A higher percentage of boreholes sampled in 2009 exceeded the TWQR for total coliforms (95%) than those sampled in 2010 (39%). Fifty percent of the total boreholes sampled in this study had faecal coliforms present, whereas 68% of the boreholes tested positive for the presence of faecal streptococci. Detection of faecal indicators was higher in the warm, wet seasons than the cold and dry season. Only 23% of the total boreholes sampled tested were negative for both faecal coliforms and faecal streptococci.

In 2009 *Escherichia coli* were identified from borehole water samples using the API 20E system. The latter system (API 20E) identified *E. coli* in thirteen percent of the 76 boreholes sampled in 2009. Based on the results obtained from MLGA medium, 34% of the 2010 sampled boreholes had *E. coli* present. However, multiplex PCR indicated *E. coli* in 47% of boreholes. Presumptive *P. aeruginosa* were detected in 56 (49%) of the total 114 borehole samples. *Staphylococcus aureus* was detected in 7% of the 76 boreholes sampled in 2009. FC/FS ratios were used to predict the possible source of faecal contamination (Table 4.3). The latter ratio indicated that faecal contamination from non-human sources was more frequent than contamination from human sources in both sampling years.

Several of the faecal coliform isolates tested were resistant to multiple antibiotics, especially beta-lactam antibiotics. The average MAR index for 2009 was 0.213 and 0.126 for 2010. The percentage of faecal coliforms resistant to AMOX and AMP were lower in 2010 (21% and 15 %, respectively) compared to 2009 (54% and 41%). The percentage resistance to CEP and OXY-TET was high in both sampling years, and remained consistent throughout the study. The percentage intermediate resistance to KAN increased from 4% in 2009 to 37% in 2010. None of the faecal coliform isolates tested showed resistance to STREP, KAN and CIP. Resistance to CHL was very low (1% in 2009 and 3% in 2010).

The pH, TDS and EC of the borehole water samples measured throughout this study was at acceptable levels. Water temperature ranged from 15.5°C to 25.8°C. In both sampling years high levels of nitrate-nitrogen was measured. In 2009 NO_3-N levels as high as 454.5 mg/L NO_3-N was measured and the average level was 67.0±124.3mg/L NO_3-N. The highest level of NO_3-N measured in 2010 was a much lower 74.1 mg/L NO_3-N and the average was 24.9±27.4 mg/L NO_3-N. Despite the lower nitrate levels measured in 2010 than in 2009, only 18% of the 2010 boreholes complied with the DWAF TWQR of 6 mg/L NO_3-N, whereas 33% of the 2009 boreholes complied with this standard.

CHAPTER 5
DISCUSSION

Worldwide, waterborne diseases remain a cause for concern in both developing and developed countries (Duncker, 2000). With groundwater being the only source of potable water for many low-income urban and rural households, its quality should reflect its intended use (Pedley & Howard, 1997). However, groundwater is commonly subjected to contamination (Section 2.4.2). Sources of contamination include municipal and industrial wastes, faulty septic tank operation, landfill leachates, contaminated storm run-off, animal wastes, chemical fertilizers, herbicides and pesticides (Andrade & Stigter, 2009; Babatunde *et al.*, 2009; Doran & Linn, 1979; Gagliardi & Karns, 2000; Grisey *et al.*, 2010; Silva *et al.*, 2006; Tissot *et al.*, 2002).

Throughout the study period it was observed that inhabitants of many rural communities received water from local service providers. Providers generally extract water from a borehole and distribute the water to communal taps and tanks (Mackintosh & Colvin, 2003). Other rural households made use of hand-pump operated systems in order to pump water from shallow wells into tanks or buckets. According to Grönwall *et al.* (2010) the fact that shallow wells are mostly used in rural communities should not be the core culpability of ill-health in these communities. The environmental conditions such as the lack of sanitation, drainage and solid waste disposal infrastructure along with poor hygiene awareness should be addressed to prevent diarrhoeal disease and cholera outbreaks (Grönwall *et al.*, 2010).

Mackintosh and Colvin (2003) states that South African rural communities are mostly provided with water of sub-standard quality. The authors (Mackintosh & Colvin, 2003) further established that rural water schemes dependant on groundwater resulted in a less

desirable water quality than surface water schemes. However, there is a common perception that private water systems, which mainly constitutes groundwater sources, is of better water quality than that of municipal sources (Jones *et al.*, 2005). According to Bates (2000), consumers are often ignorant of water quality. The reason for the latter is that consumers believe that water will not be supplied if it was not safe (Bates, 2000). Kolanisi (2005) used a qualitative strategy to determine the consumers' perception of rural water supply in the Madibogo district of the North West Province (NWP), South Africa. The author (Kolanisi, 2005) concluded from various interviews with rural water consumers, that the quality of the water is perceived according to its physical qualities (colour, smell and taste) and performance (lathering). The presence of bacteria and chemical constituents that may be harmful to the consumer cannot be determined from the physical appearance of water. Therefore, consumers should be educated on the potential of rural water to cause harm, as well as the safety measures that they can perform (Kolanisi, 2005). Comprehensive data on the groundwater quality is therefore needed to present relevant and insightful education to water users and water providers.

In this study the microbiological and physico-chemical parameters of selected boreholes throughout the NWP of South Africa were measured and described. The antibiotic resistance or susceptibility patterns of isolated faecal coliforms were also determined. Samples were taken during two sampling periods (February to July 2009 and February to May 2010). In this chapter the results obtained and potential implications from both sampling periods are discussed. Because there are no quality standards for private wells, specific concentrations of contaminants that indicate potability were measured according to the standards that municipalities apply. The target water quality range (TWQR) of the Department of Water Affairs and Forestry (DWAF, 1996) was used as a guide to assess the water quality of the boreholes for domestic use. The microbiological quality of the tested

boreholes as well as the resistance patterns of faecal coliforms to antibiotics are discussed in Section 5.1. Physico-chemical results are discussed in Section 5.2.

Water quality of the individual boreholes from these two sampling periods cannot be compared directly since the exact same boreholes sampled in 2009 was not re-sampled in 2010. The degree of vulnerability of each individual borehole to contaminants influences the water quality to a large extent. Furthermore, the same areas were not sampled in exactly the same time of the year. Comparisons made will thus be within the area based framework as discussed in Section 3.1.

5.1 MICROBIOLOGICAL QUALITY OF TESTED BOREHOLES

5.1.1 Heterotrophic plate count bacteria

In both sampling periods of 2009 and 2010, heterotrophic plate count bacteria were detected in all but two boreholes. The highest HPC of 2009 was 7500cfu/ml and 5500cfu/ml for 2010. There was a low percentage compliance of boreholes to the national TWQR of 10^2cfu/ml (13% in 2009 and 16% in 2010). The average detection of HPC bacteria in groundwater was fairly consistent between the two sampling years. However, considering the high standard deviations for each sample year (2009: 1068±1400.6 and 2010: 898±1293.3), it is evident that HPC bacteria counts varied considerably between boreholes. A study by Bonton *et al.* (2010) found high variability in the count of HPC bacteria in groundwater with different purge volumes. Sample counts of HPC ranged from 31cfu/ml in large purging volumes to 8400cfu/ml in small purging volumes. The results of the authors led them to the observation that HPC bacteria are able to grow in or survive in the relatively stagnant groundwater located between the top of the screen and the water table, or at the surface were the sample is taken (Bonton *et al.*, 2010). In this study the groundwater was purged for 3-5 minutes before samples were taken. Therefore, it could be concluded that the

variations observed in the counts of HPC bacteria in this study may also be as a result of different water qualities and contaminant exposures of the boreholes.

The ordination redundancy analysis (Figures 4.6 and 4.7) indicated that there was a correlation between the detection of HPC bacteria and total coliforms. A study by Cho *et al.* (2000) established a correlation coefficient of 0.37 between HPC and total coliforms in an aquifer exposed to livestock wastewater. This is a weak correlation. Many of the boreholes sampled in the present study were situated in close proximity to grazing livestock which may have contributed to the correlation observed.

According to some authors there is insufficient clinical and epidemiological evidence to conclude that HPC bacteria in drinking water pose a health risk (Allen *et al.*, 2004; Calderon & Mood, 1991; Edberg & Allen, 2004; Stelma *et al.*, 2004). However, DWAF states that numbers of these bacteria exceeding 10^3cfu/ml will increase the risk of infectious disease transmission (DWAF, 1996b). Twenty nine percent of the 114 boreholes sampled exceeded 10^3cfu/ml. Experimental results from studies done by de Wet *et al.* (2002) and Pavlov *et al.*, (2004) indicated HPC bacteria isolated from South African water supplies that tested positive for pathogenic characteristics. Pavlov *et al.* (2004) further found that several of the HPC isolates were resistant to multiple antibiotics. Due to evident potentially pathogenic properties, de Wet *et al.* (2002) suggested that HPC bacteria in drinking water (even at low levels) might pose a health risk to consumers (especially the immune-compromised). The possibility that these seemingly harmless bacteria may be pathogenic, should be reason enough to ensure their absence in potable water. Therefore, considering the results of Pavlov *et al.* (2004) and de Wet *et al.* (2002) the high detection rate of HPC in borehole water (used for domestic and drinking purposes) from this study is undesirable.

5.1.2 Faecal indicator bacteria

Faecal indicator bacteria were detected in water samples from boreholes in both the sampling periods of 2009 and 2010 (Sections 4.1.1 and 4.1.2). This was established using culture based methods and biochemical tests. Molecular based multiplex PCR amplification was utilized for the detection of *E. coli* directly from the borehole water sampled in 2010 (Section 4.1.3).

Over the two sampling periods, a total of 114 boreholes were sampled. Of these boreholes, 88% had total coliforms present, 50% thermo-tolerant faecal colifroms and 68% faecal streptococci. From these results it is observed that groundwater in the NWP was potentially contaminated by human and non-human faecal matter. The detection of faecal indicator bacteria in this study is supported by the results found by Kwenamore (2006). The author (Kwenamore, 2006) sampled 304 groundwater sources from the Ditsobotla and Molopo districts in the NWP. Samples were taken twice seasonally; winter and spring in 2003 and summer and autumn in 2004. Faecal and total coliform bacteria, *Enterococcus* spp., *Klebsiella* spp. and *Citrobacter* spp. were detected throughout the sampling period (Kwenamore, 2006).

International studies also demonstrated that faecal contamination of groundwater, as was found in the present study, is possible. Reid *et al.* (2003) analysed 243 private well samples in Aberdeen (UK). The latter study reported that 51% of the wells exceeded the TWQR for total coliforms and 31% for faecal coliforms. Howell *et al.* (1995) analysed springs and wells for faecal coliforms and faecal streptococci from two agricultural wetlands in Kentucky (USA). Sampling took place on a monthly basis for a period of three years. The authors (Howell *et al.*, 1995) concluded that the faecal indicator bacteria exceeded the standard limit between 28 and 74 percent of the time. Goss *et al.* (1998) found that 34% of the 1 292 wells tested in Ontario farmstead (USA) exceeded the maximum allowable limit

for coliform bacteria. A 12 year study period in the USA revealed that 76% of 183 documented outbreaks as well as 33% of waterborne illnesses were probably associated with the use of groundwater as a drinking water source (Reynolds *et al.*, 2008). Craun and Calderon (1997) reported 356 outbreaks associated with the use of contaminated groundwater from 1971 – 1994.

The presence of faecal indicator bacteria in groundwater is alarming, as it indicates the possible presence of disease causing pathogens (Ashbolt *et al.* 2001; Barrell *et al.*, 2000 Grabow, 1996; Jagals *et al.*, 1995). According to the South African target water quality range (TWQR) a maximum of 5cfu/100ml total coliforms and 0cfu/100ml faecal coliforms is permitted in water used for domestic purposes (DWAF, 1996b). Exposure to waters with an elevated concentration of faecal indicator bacteria (total and faecal coliforms, *E. coli* and enterococci) increases the risk of contracting gastrointestinal and respiratory illnesses (Haile *et al.*, 1999).

Indicator bacteria in the groundwater may promote additional deterioration of the water quality during its residence time in storage tanks and household containers. Studies by Momba and Kaleni (2002) and Momba and Notshe, (2003) showed that these containers will support the growth and survival of indicator bacteria and that the microbiological quality of the stored drinking groundwater will deteriorate consistently with the length of storage. In this study it was observed that groundwater was mainly stored in storage tanks. Many inhabitants of informal settlements store their water in household containers after collecting it from a central storage tank or after pumping it by hand from a shallow well. Therefore it could be expected that the water quality at point of use may be of a poorer quality than tested. In the present study water samples were taken directly from the source before it

entered storage tanks. The quality of the water in the storage tanks might have been worse. This is further cause for concern.

5.1.2.1 Trends observed in the detection of total and faecal coliforms and possible contributing factors

Total coliform counts were high for many of the boreholes sampled in 2009 with three boreholes exceeding 300cfu/100ml. Only 3% of the boreholes sampled in 2009 tested negative for this group of bacteria. However, in 2010 this scenario changed. Two boreholes from the 2010 sampling period exceeded 300cfu/100ml for total coliforms, and 34% tested negative for this group of bacteria. This could be ascribed to the different culture media used to enumerate these bacteria in the two sampling years. In 2009, m-Endo agar (Merck, SA) was used opposed to MLGA (Oxoid, UK) in 2010.

Thermo-tolerant faecal coliforms were detected in 49% of the boreholes sampled in 2009, and in 55% of the boreholes sampled in 2010. Of 11 boreholes sampled in 2009 and 10 sampled in 2010 that tested negative for faecal coliforms, high counts (>500cfu/ml) of HPC was detected. Considering the results of Allen *et al.* (2004) and Burlingame *et al.* (1984), the mentioned boreholes that tested negative for faecal coliforms may have had faecal coliforms present, but the growth of these indicators may have been suppressed by the high numbers of HPC bacteria. Differences in the trends of faecal coliform detection were observed. The trends observed in each of the sampling periods are discussed below.

During the 2009 sampling period, faecal coliforms were detected more frequently and in higher numbers from boreholes sampled during the wet summer months of February and March. These boreholes were situated in sample areas A_{09}-C_{09}. Faecal coliforms were detected in 74% of the boreholes sampled during this warmer period, with an average

faecal coliform count of 59cfu/100ml. Kwenamore (2006) reported an average faecal coliform count of 86cfu/100ml from boreholes in the Ditsobotla and 96cfu/100ml from the Molopo districts in the NWP during summer. Sample areas A_{09}-C_{09} are situated in the eastern part of the NWP, which generally has the highest mean annual precipitation in the Province (NWDACE, 2008). As for most parts of South Africa, the NWP has summer rainfall, and dry winter months. Moist soils facilitate the infiltration of bacteria (Cho *et al.*, 2000; Unc & Goss, 2003). Godfrey *et al.* (2005) reported a ten-fold increase in faecal indicator bacteria after 24 hours of heavy rainfall. This could possibly explain the high faecal bacteria counts observed in 2009 during February and April as these months fall within the rainy season. However, rainfall data for the specific areas is not available, and the claim that rainfall had an impact can therefore not be substantiated.

Fewer faecal coliform were detected during April 2009 to the end of May 2009 (autumn). Thirty nine percent of the boreholes sampled in this period had faecal coliforms present (average count of 16cfu/100ml). In support of the present study, Kwenamore (2006) reported an average of 46 and 39cfu/100ml for the Ditsobotla and Molopo districts respectively during autumn. This agrees with the findings of Bonton *et al.* (2010) and Buckalew *et al.* (2006). In both these studies the authors found that faecal coliforms and *E. coli* counts were significantly lower during colder season months. As expected, the ordination redundancy analysis (RDA) biplot of the 2009 data (Figure 4.6) indicated a correlation between water temperature and faecal coliforms. The lower water content of the soils due to decreased rainfall in the winter months could have contributed to a decreased transport of bacteria and hence a lower bacterial detection in these months.

In 2010, the first sampling took place at the end of March, and continued until the end of May. In 2009, samples were also taken during June and July. Subsequently, the water

temperature of 2010 did not decrease as much as in 2009. The average water temperature of 2010 was 21.1±2.1°C compared to 20.9±3.2°C of 2009. The lower standard deviation of 2010 also indicates less variance in the water temperature of boreholes from this sampling period. This could explain the difference observed between the ordination redundancy analysis biplot of 2010 compared to 2009. From the 2010 biplot it is seen that water temperature did not correlate with faecal coliform detection as in 2009.

The correlation biplots in Figures 4.6 and 4.7 (Section 4.4) shows a positive correlation between total and faecal coliforms. In 2010 however, this correlation was much stronger than for the 2009 data. Reid *et al.* (2003) found a significant positive relationship between the detection total and faecal coliforms. Also, Hong *et al.* (2010) showed a strong positive correlation between total and faecal coliforms in surface water. The authors (Hong *et al.*, 2010) further indicated that total coliforms were mainly associated with the physico-chemical factors of the water such as pH, temperature and total suspended solids. Faecal coliforms were more related to the external source (Hong *et al.*, 2010). In the present study, faecal coliforms were more associated with temperature and pH than total coliforms in 2009. In 2010, there was no correlation between total or faecal coliforms and temperature or pH. The latter dissimilarity in results from this study and Hong *et al.* (2010) could be that groundwater bacterial communities may be influenced by different environmental variables than surface water communities.

Selected thermo-tolerant coliforms, isolated from the 2009 water samples, were identified using TSI and API 20E tests. The species identified included *Enterobacter cloacae* (34%), *Enterobacter aerogenes* (31%), *Klebsiella pneumonia* (15%), *Escherichia coli* (10%), *Raoutella planticola* (4%), *Raoutella terrigena* (3%), and *Pantoea* spp. (3%). Kämpfer *et al.* (2008) also used the API 20E system to identify faecal coliforms isolated from

groundwater samples. The authors identified 60% of the 345 isolates tested to belong to the genus *Enterobacter*. This agrees with the results of this study, as 65% of the 98 isolates tested, also belonged to this genus (*Enterobacter*).

Enterobacter cloacae and *Enterobacter aerogenes* are the two most common species of the genus *Enterobacter* that are responsible for various nosocomial diseases as well as community acquired infections (Fraser, 2010). Community acquired infections include urinary tract, soft tissue and wound infections (Fraser, 2010). *Enterobacter* spp. possesses ß-lactamases that allow these bacteria to develop resistance to antibiotics, especially third-generation cephalosporins and penicillins (Fraser, 2010). High percentages of isolates from the present study were resistant to AMOX, AMP and CEP (Section 4.1.4). The latter could be attributed to the high percentage (65%) of the faecal coliform isolates identified belonging to the *Enterobacter* genus.

The gastro-intestinal tract serves as a reservoir for pathogenic Klebsiella, which may cause diseases such as urinary tract infections, pneumonia, septicaemias, and soft tissue infections (Podschun & Ullmann, 1998). TSI results also indicated that members of *Proteus* spp. and *Citrobacter* spp. could be amongst the isolated faecal coliforms.

5.1.2.2 *E. coli* detection

The detection of *E. coli* is a good indicator of recent faecal contamination, as it is a commensal inhabitant of the intestinal micro-flora of warm blooded animals, especially humans (Edhberg *et al.* 2000; Kaper, 2005). However, a mini-review article by Ishii and Sadowsky (2008) highlights the survival and potential replication of *E. coli* strains in water, on algae and in soils. Therefore the possibility exist that *E. coli* detected in groundwater

from this study may have been soil-naturalised *E. coli* strains that attenuated in the soil and infiltrated to the water table.

In the 2009 sampling period, 10% of the 98 faecal coliform isolates were identified with at least 90% accuracy as *E. coli*. Each borehole from the 2010 sampling period was screened for *E. coli*. This was achieved by the enumeration of *E. coli* and total coliforms on MLGA medium. This culture based method detected *E. coli* in 13 of the 38 boreholes sampled (Table B.2). In addition to this culture based method, 32 of the 38 boreholes sampled in 2010 were screened for the presence of *E. coli* using multiplex PCR. One or both of the *E. coli* specific target gene fragments (*LacZ* and *mdh*) were amplified in 22 of the 32 boreholes screened. This indicates that the MLGA had a poor sensitivity in detecting *E. coli*, resulting in false negatives. This agrees with the results of Fricker and DeSarno (2008). The authors found that 12.47% of *E.* coli strains did not produce the β-D-glucuronidase enzyme in order to form green colonies on MLGA. Instead these colonies were yellow, resulting in a false negative detection of *E. coli*. In this study, MLGA produced 23% false negative detections. If the results obtained from the culture based method (membrane filtration onto MLGA media) were performed without the confirmatory PCR technique, the microbiological risks involved in using these waters would have been underestimated. Multiplex PCR amplified fragments visualized in Figure 4.1 indicates that MLGA also produced two false positive results.

Lleo *et al.* (2005) compared culture based methods with molecular based methods for the detection of *E. coli* in groundwater. The authors demonstrated that standard culture based methods is insufficient for the total detection of the effective bacterial population in groundwater. This is potentially due to populations that may be in the viable but non-culturable state. Their study further demonstrated that molecular based PCR amplification

methods are the only method capable of detecting total bacterial population, including non-culturable populations (Lleo *et al.*, 2005). The findings of Lleo *et al.* (2005) therefore also support the higher detection of *E. coli* with PCR methods compared to culture methods in this study.

E. coli can be pathogenic after infection of mucosal surfaces (Nataro & Kaper, 1998). An infection of *E. coli* occurs mostly in the debilitated or immune-suppressed host, or when gastrointestinal barriers are violated. Clinical syndromes which result from an infection with *E. coli* are sepsis, meningitis, urinary tract infection or enteric diarrheal diseases (Nataro & Kaper, 1998). *E. coli* 0157:H7 is a pathogenic strain of *E. coli* (Table 2.1) that may cause waterborne diseases (WRC, 2003). Ateba *et al.* (2008) analysed 800 faecal samples isolated from pigs, cattle and humans in the NWP. The authors (Ateba *et al.*, 2008) positively identified 67 isolates as *E. coli* 0157:H7. Prevalence was the highest in pigs (44% – 50%), followed by cattle (5.4% - 20%) and humans (7.5%). As discussed, animal and human faecal matter contaminated groundwater sources in the present study. Therefore, there is a possibility that the *E. coli* detected in this study might be pathogenic strains such as *E. coli* 0157:H7.

5.1.1.3 Antibiotic Resistance

The resistance or susceptibility of selected faecal coliform isolates from boreholes sampled in 2009 and 2010 was tested against 9 commonly used antibiotics. Faecal coliform isolates tested, represented one of three areas the Province was divided into (Section 4.1.4). In 2009 and 2010, 85 and 60 isolates respectively were tested against the selected antibiotics. The inhibition zones that resulted from each antibiotic disc can be seen in Appendix A and Tables 4.3 and 4.4 provided the percentage resistance, intermediate resistance and susceptibility of faecal coliforms in an area to the antibiotics it was tested against.

The following trends regarding faecal coliform sensitivity to antibiotics were observed. Isolates from the 2009 sampling period were most resistant to AMOX (54%), AMP (41%), and CEP (40%). Resistance to OXY-TET (30%) and TM (11%) was also found. The 2010 isolates were most resistant to CEP (42%), OXY-TET (22%), AMOX (21%), AMP (15%) and TM (8%). The overall percentage resistance to CEP were similar for the two sampling periods, with a 14% increase in the intermediate resistance to CEP from 2009 to 2010. The percentage resistance to OXY-TET only differed with 8% between 2009 and 2010. It is observed that the major difference between resistance patterns from 2009 to 2010 is the higher intermediate resistance of isolates to AMOX and AMP and consequently a decrease in the detection of isolates resistant to these two antibiotics in 2010. Resistance to aminoglycosides (KAN and STREP), quinolones (CIP) and Chloramphenicol (CHL) was not prevalent in this study. This agrees with the findings of Hu *et al.* (2008), Servais and Passerat (2009), and Watkinson *et al.* (2007).

Kwenamore (2006) sampled boreholes, taps and windmills during a 2 year sample period in the Ditsobotla and Molopo districts of the NWP. The author (Kwenamore, 2006) also measured the antibiotic resistance of the faecal coliforms, *Klebsiella* spp. and *Citrobacter* spp. Five of the antibiotics tested by Kwenamore (2006) were equivalent to the antibiotics tested in the present study. Therefore comparisons could be made. Table A.7 (Appendix A) indicates the minimum and maximum percentages of faecal coliform isolates per sample group that were resistant to comparable antibiotics used in the present study and the study of Kwenamore (2006). From Table A.7 (Appendix A) it is observed that Kwenamore (2006) measured isolates resistant to TM, CHL, OXY-TET and STREP more frequently than in the present study. Resistance of isolates to AMOX was similar for the two studies. This variance could be credited to different geographical areas and groundwater sources. Wilcox (1998) stated that regional differences in antibiotic susceptibility might reflect

variations in antibiotic prescribing. Therefore, veterinarians and doctors may also have prescribed and administrated TM, CHL, OXY-TET and STREP more commonly in the area and/or time of Kwenamore's (2006) sample period (2003-2004). Further investigation is required to determine the exact factors that lead to a decrease in bacterial antibiotic resistance in the NWP for these antibiotics.

The presence of antibiotic resistant bacteria in groundwater sources is not unusual (McKeon *et al.*, 1995). The results of this study and Kwenamore (2006) support this. Batt *et al.,* 2006 reported the presence of sulphonamide antimicrobials in private water wells as deep as 10m in Idaho, USA. McKeon *et al.* (1995) concluded that unfavourable conditions may promote the transfer of resistance characteristic to susceptible recipients. Of the 250 coliform and non-coliform groundwater isolates the authors tested, all of the non-coliforms and 87% of the coliforms were found to be resistant to at least one of the 16 antibiotics tested. Seventy eight percent of all tested isolates were resistant to multiple antibiotics (MAR) with some being resistant to all 16 antibiotics. The authors further established that *E. coli* strains demonstrated the lowest frequency MAR as only 14% were resistant to multiple antibiotics. The origin of this resistance was not defined by the authors (McKeon *et al.*, 1995), however, possible sources included run-off from nearby farmlands and/or septic tank leakages.

Resistance to multiple antibiotics amongst Gram-negative bacteria is becoming more common (O'Fallon *et al.*, 2009). The average MAR index for the isolates of the 2009 sample period was 0.213. This index value indicates that the 85 isolates from this 2009 sampling period was resistant, on average, to more than 2 of the antibiotics tested against. The average MAR index for the 60 faecal coliforms isolated from the 2010 sampling period were on average resistant to more than 1 (MAR index: 0.126) of the antibiotics tested

against. Kwenamore (2006) reported much higher MAR indices for *Klebsiella* spp. and *Citrobacter* spp. isolated from groundwater sources in the NWP. The absence of isolates from sample area I_{10} could have contributed to this lower MAR index value of 2010, as area I_{09} of 2009 had representatives with some of the highest MAR indicies (Table A.5; Appendix A).

The beta-lactam antibiotics such as penicillin and its variants (ampicillin and amoxicillin) as well as cephalosporins are the first-line of defence for many bacterial infections in South Africa (DoH, 2006). These antibiotics cause the death of susceptible bacterial cells by interfering with cell wall formation. Due to the efficiency thereof, these antibiotics have been regularly over prescribed by doctors and in many cases misused by patients. This may have promoted the adaptation of bacteria to become resistant to these antibiotics. As found by Pitout *et al.* (1998) and Jacoby & Medeiros (1991), these resistant bacteria probably possess genes that code for a variety of extended-spectrum β-lactamases (ESBLs). ESBLs is part of the β-lactamases group, and is able to confer bacterial resistance to the penicillins as well as the first-, second-, and third-generation cephalosporins (Paterson & Bonomo, 2005).

As mentioned, isolates from area I_{09}, which represents the north-western part of the NWP had the highest MAR indices. Isolates from this area were all resistant to AMOX and most were resistant to CEP, AMP and OXY-TET. This is worrisome, because as discussed in Section 5.1, area I_{09} had high counts of faecal indicator organisms, therefore also possibly pathogens. This area has limited infrastructure and consists mainly of farmlands and rural settlements (Kalule-Sabiti & Heath, 2008). As a result, groundwater is the only water source for many households. This represents a concern to the users of these waters as they are at risk of being infected with resistant pathogens.

In many of the boreholes the FC/FS ratios (Section 4.1.1) indicated that the most probable source of contamination was of non-human origin. A study by Teuber (2001) included a review on the spread of resistant bacteria originating from animals as a result of veterinary applications. This included prevention and treatment of infections as well as to promote growth and feeding efficiency in animal farming (Singer *et al.*, 2003). Antibiotics used to treat these animals were slowly or partially metabolized especially in colder seasons, often resulting in the discharge thereof in the excreta. These un-metabolised or partially metabolised antibiotics then spread into the soil and water of the surrounding area (Dolliver & Gupta, 2008). Through molecular analysis of antibiotic resistant genes, plasmids and transposons, Teuber (2001) established that identical elements occurred in both animals and humans. This indicates the spread of resistant bacteria from animal micro-floras to human micro-floras by way of various pathways including water, further demonstrating the direct contamination of consumers. Zhang *et al.* (2009) emphasises the importance of ensuring that antibiotic resistance genes does not enter natural water resources (including groundwater) through agricultural applications of sludge and irrigation with reclaimed waste water. Therefore it is possible that the antibiotic resistant bacteria detected in this study originated from animals that were administrated antibiotics. On the other hand, Hirsch *et al.* (1999) measured the occurrence of antibiotics in aquatic environments in an agricultural region in Germany, including groundwater. Of all the numerous boreholes sampled, concentrations of antibiotics were found in only two boreholes (Hirch *et al.*, 1999). Therefore the authors concluded that the contribution of animal intake from veterinary applications to the aquatic environment were of minor importance (Hirch *et al.*, 1999).

According to a review by Kümmerer (2004) there is an undeniable problem with respect to antibiotic resistant bacteria in the environment, including soil, surface and groundwater

sources. The source of antibiotic resistant bacteria in the present study was not determined. However Kümmerer (2004) emphasise that the impact that antibiotics directly have on aquatic and soil bacteria is uncertain. Furthermore, the author stated that the most significant source of resistance in the environment is from the already resistant bacteria present in the excrement of humans and animals that used and/or was administrated antibiotics incorrectly (Kümmerer, 2004). Therefore, it is assumed that the majority of the resistant isolates from the present study originated from reservoirs (human and/or animal) that hosted resistant bacteria, instead of non-resistant bacteria that acquired the genes coding for resistance via gene transfer mechanisms.

If untreated groundwater is used for drinking purposes it could be a source for the spread and transfer of antibiotic resistant strains (Søbrum & L'Abée-Lund, 2002). Resistant strains will impair the capability of on-hand antibiotics to treat infections caused by these bacteria, giving rise to increased hospitalization, illnesses and death (Levy & Marshall, 2004; Niederman, 2001; Sader *et al.*, 2003).

5.1.2 *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Pseudomonas aeruginosa were detected in 49% of the total 114 boreholes sampled. Amongst these boreholes this bacteria was detected in conjunction with either faecal coliforms or faecal streptococci. On the other hand a total of 13 boreholes (11%) were positive for *P. aeruginosa* where no faecal coliforms were detected. Therefore it could be presumed that the pseudomonas from the latter 13 boreholes may not have originated from faecal contamination. A study by Ferguson and his colleagues (2001) included a survey on the presence of *P. aeruginosa* in natural soils. It showed that 16% of uncontaminated natural soils contained *P. aeruginosa.* Therefore, it may be presumed that infiltrating water could transport these bacteria from the soils into the groundwater.

A study by Wheater *et al.* (1980) concluded that the numbers of *P. aeruginosa* reflects the levels of domestic pollution. Therefore, the presumptive *P. aeruginosa* detected in the 33 boreholes that also had faecal indicators present, could potentially be associated with faecal contamination. These authors (Wheater *et al.,* 1980) further found significant numbers of this organism in human faecal specimens, but to a lesser extent in animal faecal specimens. Of the 56 (49%) boreholes in the present study that had *P. aeruginosa* present, 11 (20%) had FC/FS ratios >2. This ratio indicates possible contamination from human waste (Wyer & Kay, 1995). It could therefore be expected that in these cases the presence of the organism could be associated with human contamination.

According to Hardalo and Edberg (1997) it is not practical to remove this bacterium from food and water sources due to its ubiquitous nature as well as the small margin of risk it poses to healthy humans. A review by Mena and Gerba (2009) confirms that the risk of colonization from ingesting *P. aeruginosa* in drinking water is low. This risk is slightly higher if an individual is taking antibiotics that suppress the natural intestinal flora, while selecting for resistant *P. aeruginosa* (Mena & Gerba, 2009). The greatest health risk for healthy individuals in contact with water contaminated with *P. aeruginosa* is 1) skin exposure in hot tubs and 2) lung exposure from inhaling aerosols (Mena & Gerba, 2009). Immune-comprised individuals that drink water and/or bath in water contaminated with this species are at risk of infection of wounds, ears, urinary tract and respiratory organs (Lester & Birkett, 1999). Orsi *et al.* (1994) reported that *P. aeruginosa* strains isolated from the environment were more susceptible to antibiotics than clinical strains isolated from patients in health care units. The authors further established that there was little correspondence between the types of *P. aeruginosa* stains isolated from the environment and those isolated from infected patients (Orsi *et al.*, 1994). Therefore, it could be assumed that the presumptive *P. aeruginosa* isolates detected in this study may not pose a risk to water users

that are of good health. However, rural communities are often consist of a large segment of immune-comprised individuals and should therefore not be exposed to these opportunistic pathogens.

Staphylococcus aureus was detected in 7% of the 76 boreholes sampled in 2009. LeChevallier and Seidler (1980) detected *S. aureus* in 6% of the rural drinking water supplies they sampled. Lamka *et al.* (1980) detected this species in 8% of the rural households supplied with groundwater. LeChevallier and Seidler (1980) as well as Lamka *et al.* (1980) found no correlation between the simultaneous presence of coliform indicator species and staphylococci. However, the authors (LeChevallier & Seidler, 1980; Lamka *et al.*, 1980) did report high standard plate counts (also known as heterotrophic plate count) together with the detection of *S. aureus,* and they concluded that the high standard plate counts masked the presence of coliform bacteria. All of the boreholes of the present study that had *S. aureus* present also had total and faecal coliform bacteria present. Of the 5 boreholes that tested positive for *S. aureus,* 4 had FC/FS ratios that were >4. Such ratios are associated with human faecal matter (Geldreich & Kenner, 1969; Wyer & Kay, 1995). This could indicate that these staphylococci originated from human faeces, as *S. aureus* does not naturally inhabit groundwater.

S. aureus is a potential pathogen that may cause pneumonia and *Staphylococcus aureus* bacteremia in immune-comprised individuals (Kaye *et al.*, 1990; Lowly, 1998). *S. aureus* may also infect open wounds that could result in toxic shock syndrome (Dryden, 2009).

5.1.3 Possible sources of bacterial contamination and discussion of selected individual boreholes

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

The ratio between faecal coliform and faecal streptococci was used to indicate the most likely source of faecal contamination. A study by Jagals *et al.* (1995) supports the assumption that the origin of faecal contamination are more likely to come from animal waste if faecal streptococci counts are higher than that of faecal coliforms. The movement of animal wastes (frequently applied as soil fertilizers) into groundwater is often cited as a major factor contributing to the pollution of groundwater (Cho *et al.*, 2000). According to Csuros & Csuros (1999), a FC/FS ratio <0.7 correlates more strongly with non-human faecal contamination. Contamination of human origin is coupled to higher faecal coliform counts than faecal streptococci (Wyer & Kay, 1995).

As expected from most of the boreholes positions, results from Table 4.3 showed that boreholes from both sampling years were predominantly contaminated by non-human faecal matter. In 2009, 45% of the total boreholes sampled had FC/FS ratios <0.7. This was the case for 32% of the boreholes sampled in 2010. The percentage of boreholes with ratios >4 was relatively low in both years (20% in 2009 and 8% in 2010). It can therefore be concluded that the groundwater sources sampled in this study may have been subjected to contamination from non-human than human sources of faecal contamination. A review by Field and Samadpour (2007) concluded that there is insufficient information on human health risk due to non-human faecal contamination.

It is not possible to extensively discuss the possible contamination of all the boreholes sampled in this study. Therefore a few boreholes were selected for further discussion. Boreholes from area B_{09}, which was sampled in the wet and warm summer month of March, had the highest average count (140±97.7cfu/100ml; Table 4.1) for faecal coliforms. Area B_{09} was represented by 5 boreholes in the Fochville-Ventersdorp-Carltonville area in the south eastern part of the Province. Two of these boreholes were situated in close proximity to cattle feedlots, one on a small holding on the outskirts of Ventersdorp, one next to an artificial dam and the other on a farmland. The borehole from the farmland had the lowest faecal coliform count of 8cfu/100ml. This borehole was 80m deep. Deeper boreholes are expected to be less contaminated due to the longer vertical distance needed to be travelled by bacteria in order to reach the water table.

The borehole with the highest faecal coliform count from this area (260cfu/100ml) was situated on a small holding outside Ventersdorp. This borehole had not been used by the owners for an extended period due to their knowledge of its contaminated state. The FC/FS ratio of this borehole was 13.7, indicating the possible source of faecal contamination to be

of human origin. No obvious source of contamination was observed, but it was established that a septic tank was used for storage of domestic sewage. It is possible that the septic tank was leaking contaminated water. Two of the other contaminated boreholes from this sample area were situated in close proximity to cattle feedlots. As expected with animal waste contamination (Jagals *et al.*, 1995), both these boreholes had high counts of faecal streptococci, exceeding 300cfu/100ml. The FC/FS ratio of these two boreholes was 0.4 and 0.7, respectively (Table A.1).

As mentioned in Section 5.1.1.1, boreholes from sample area I_{09} sampled in the winter (June, 2009) had high faecal coliform counts. The average faecal coliform counts of boreholes sampled in this sample area were 70±95.6cfu/100ml (see Table 4.1). This was overall the second highest average for faecal coliforms during 2009 sampling period. In addition, the average faecal streptococci counts for boreholes from this area as the highest (56±52.0cfu/100ml). Possible reasons for the high numbers of faecal coliforms and faecal streptococci detected in boreholes from area I_{09} are discussed. This sample area is situated in the north western part of the Province, which mainly consists of informal settlements and small towns without extensive infrastructure and water services (Naraghi & Kebotlhale 2004). As a result, most of the representative boreholes from this area were shallow wells in close proximity to open pit latrines as well as above ground grazing and domestic animals. A study by Gallegos et al. (1999) showed that shallow wells are more commonly subjected to contamination than deeper boreholes. Higher water temperature promotes the presence of faecal bacteria (Gallegos *et al.*, 1999; Zamxaka *et al.*, 2004). The latter may also have contributed to the high bacteria counts of this area, as most of the boreholes from this sample area had relatively high water temperatures (reaching 24°C). This could be ascribed to the warmer atmospheric temperatures in this area as well as the shallow water tables of these wells.

None of the boreholes from areas A_{10} and D_{10} had FC/FS ratios indicative of non-human contamination. All but one (Derby) of the boreholes from both these sample areas were situated in towns (Klerksdorp, Potchefstroom, Orkney, Stilfontein, Rustenburg and Brits), therefore no grazing animals were found in close proximity to these boreholes. Many boreholes of these two areas were not contaminated with either faecal coliforms or faecal streptococci, which could be explained by the depth of the boreholes. Sampled boreholes situated in towns were generally deeper and better sheltered than those sampled in rural settlements. Most of the sampled boreholes situated on farms were also deep and properly sheltered. However in some cases shallow and poorly enclosed boreholes were sampled in farming areas. In these cases owners of the boreholes stated that water from these boreholes was mainly used for animal watering and irrigation.

5.2 PHYSICO-CHEMICAL PARAMATERS

Physico-chemical parameters measured in this study included pH, ambient water temperature, electrical conductivity (EC), total dissolved solids (TDS) and nitrate (NO_3-N). Measured values for these parameters for each borehole sampled is provided in Table A.3 (2009 sampling period) and A.4 (2010 sampling period) in Appendix A. Table 4.5 (2009 sampling period) and 4.6 (2010 sampling period) shows the minimum, maximum and average measurements of each parameter in a sampling group.

5.2.1 Temperature, pH, TDS and EC

The pH of all the boreholes sampled throughout the two sampling periods was within the quality range (6.0-9.0) for drinking water. This is satisfactory. The target water quality range (TWQR) for TDS is 0 – 450mg/l and 0 - 700 µs/cm for EC (DWAF, 1996a). In 2009, 76% and 70% of the boreholes exceeded the TDS and EC TWQR for domestic use (Table 4.7). In 2010 71% exceeded the TWQR for TDS and 79% for EC. Groundwater regularly

has elevated TDS and EC levels. The levels of these parameters cause the water to be aesthetically unfavourable, but still usable without any effects on health. According to Atekwana *et al.*, (2004) high TDS levels and conductivity could possibly indicate hydrocarbon contamination that is undergoing natural biodegradation by microorganisms. None of the 114 boreholes sampled throughout the study exceeded the maximum allowable level for TDS (3000 mg/l) and EC (4500 µs/cm) for domestic use (DWAF, 1996a). Values exceeding these maximum levels may cause disturbance of the salt balance in humans and animals. It may also lead to other short term health effects if exposure is continuous (Table B.2; Appendix B).

5.2.2 Nitrate concentrations

High nitrate levels were measured in both the sampling periods of 2009 and 2010. Of the 76 boreholes sampled in 2009, 67% had nitrate levels that exceeded the TWQR for domestic use of 6mg/L NO_3-N. In 2010, 82% of the 38 boreholes sampled exceeded this value. This agrees with the results of Al-Khatib & Arafat (2009). Of the 111 groundwater samples tested by the authors, 84.7% were non-compliant (Al-Khatib & Arafat, 2009). Even though more boreholes exceeded the domestic TWQR for nitrate in 2010 than in 2009, the average nitrate levels for all the boreholes sampled in 2010 were three times lower than 2009. This could be due to the large number of boreholes from the 2009 sampling period that had very high nitrate measurements. Only one of the boreholes from the 2010 sampling period had an exceptionally high nitrate measurement. Nitrate levels as high as 454.5mg/L NO_3-N were measured in 2009 and 148mg/L NO_3-N in 2010. In support of these high nitrate levels, Darwish *et al.* (2011) measured the nitrate concentration of 21 wells in a cultivated area of Lebanon-East. The authors (Darwish *et al.*, 2011) reported that 12 wells had nitrate concentrations exceeding 200mg/L.

Groundwater nitrate is expected to increase in relation to land use practices that require nitrogen application. Remnant fertilizer after plant uptake plays a major role in contributing to the high nitrates levels (van der Voet *et al.*, 1996). Trees and plants temporarily store large quantities of nutrients during the growing season, and release it again to aquifers during the following non-growing season (Walker, 1973). This could potentially explain the high nitrate levels measured in this study, since most of the sampling took place in the non-growing, dry, winter season.

The study by Limbrick (2003) found that a groundwater catchment in England, which largely remained unchanged in terms of weak arability throughout the 20th century, showed very little increase in nitrate concentrations from 1894 to 2001. Only 2% of this study area was urbanized. The author accredited this low nitrogenous content, over more than a century, to the little agriculture and human impact in this area. Research by Bonton *et al.* (2010) found that groundwater of a catchment area was greatly affected by agricultural activities. Nitrate was the main contaminant in the latter study. In this case 40% of samples exceeding the regulatory drinking water standard for nitrate (Bonton *et al.*, 2010). The authors further concluded that measured nitrate levels vary with the depth of boreholes as well as time, indicating a time dimension. In support of the latter, research done by Hudak (2000), showed that lower well dept may contribute to higher nitrate values, especially in areas with arable land. Bonton *et al.* (2010) further concluded that nitrate values measured may not only be explained by agricultural activity directly above a borehole, but also by activities located further up-gradient of a borehole. The NWP is known for its agricultural practices, which might have contributed to the high concentrations of NO_3-N measured in this study (Kalule-Sabiti & Heath, 2008),

In contradiction to the results of the previous authors (Bonton *et al.*, 2010; Hudak, 2000; Limbrick, 2003), Liu *et al.* (2005) did not find a clear correlation between land use nitrogen application rates and groundwater NO_3-N levels. The authors (Liu *et al.*, 2005) reported spatial variations in the levels of groundwater NO_3-N. Kundu *et al.* (2009) also studied the nitrate loading on groundwater sources in an intensively cultivated district of India. The authors (Kundu *et al.*, 2009) sampled 252 wells with different depths. Wells situated close to human settlements as well as wells that could not be influenced by settlements were sampled. Results showed that NO_3-N loading increased as the well depth decreased and as the nitrogenous fertilizer application increased. Nitrate-nitrogen concentrations in groundwater were also higher in wells situated close to human settlements. Overall, the nitrate load of the groundwater in this cultivated region was low. None of the 252 well sampled exceeded the WHO limit for nitrate (10mg/L as NO_3-N). The findings of Kundu *et al.* (2009) indicate that nitrogenous fertilizer used in cultivated areas does cause a NO_3-N load of ground waters. However, if the application thereof is regulated and controlled so that crops maximally utilize the nitrates for growth, it may increase but still comply with the allowable limits. Conrad *et al.*, 1999 also concluded that the nitrate detected in groundwater of a cultivated area did not originate from fertilizer, but from the soil nitrogen leaching to the subsurface as nitrate.

The North West Province is known for its extensive crop- and animal farming (Kalule-Sabiti & Heath, 2008). Sample area I_{09} and J_{09} that is situated in the western part of the Province had nitrate levels exceeding 100mg/L NO_3-N. The previously mentioned exceptionally high nitrate values measured (454.5mg/L NO_3-N in 2009 and 148mg/L NO_3-N in 2010) were from boreholes sampled in this western part of the North West Province. This region of the Province is predominantly rural and provides vast areas for agricultural activities (Kalule-Sabiti & Heath, 2008). Both areas I_{09} and J_{09} have many informal settlements and small

towns which does not all have municipal water treatment plants. Therefore most of the people in area I_{09} and J_{09} depend solely on groundwater for a source of drinking water, livestock watering and irrigation. Sample area I was not sampled in 2010. In comparison to area J_{09}, area J_{10} had much lower nitrate levels, with the average value of nitrate in area J_{10} being 13.5±8.7mg/L NO_3-N compared to 210.7±223.1mg/L NO_3-N of area J_{09}. In 2009 this area was sampled in the middle of winter (July) and in 2010 it was sampled in the end of autumn (May). Results from a study by Andrade & Stigter (2009) showed higher groundwater nitrate concentrations during the winter due to leaching of nitrate that remains in the soil after harvesting of crops. The difference in nitrate content of the same area sampled at different times agrees with the time dimension emphasized by Bonton *et al.* (2010). Spatial variations due to different sample points could also be a cause for the lower nitrate levels measured in 2010 (Liu *et al.*, 2005). Water content of the soil due to rainfall, the fertilizer application rate and the depth of the boreholes may also be implicated in the variation of nitrate content in a particular area.

In Figures 4.4 and 4.5 (Section 4.2) it is demonstrated that 40.8% of boreholes sampled in 2009 and 42.1% of the 2010 sampled boreholes exceeded the nitrate limit of 20mg/L after which methaemoglobinemia may occur Craun *et al.*, 1981; Sadeq *et al.*, 2008; Super *et al.*, 1981). The measured nitrate levels are of great concern, as infants and children exposed to these high nitrate concentrations are vulnerable to blue baby syndrome (Craun *et al.*, 1981; Sadeq *et al.*, 2008; Super *et al.*, 1981). There are also studies investigating the role of high nitrate intake to cancer of the oesophagus, stomach and nasopharynx (Dutt *et al.*, 1987; Gulis *et al.*, 2002; Jensen, 1982; Tricker & Preussmann, 1991; van Loon *et al.*, 1997; Yang *et al.*, 2007).

5.3 IS GROUNDWATER OF THE NORTH WEST PROVINCE FIT TO USE FOR DOMESTIC PURPOSES?

The custodian of the water resources of South Africa, the Department of Water Affairs, set certain target water quality ranges (TWQR's) that should be complied with in order to ensure the safety of people and animals using the water. There are different TWQR's for a variety of uses. This study focuses on the use of groundwater for domestic purposes; therefore the measured groundwater quality is compared to the TWQR for domestic use. Table B.1 (Appendix B) summarizes the TWQR relevant to this study and Table B.2 (Appendix B) summarizes the possible health risks involved if these ranges are exceeded. These tables were adopted from the DWAF (1996b) field guide and DWAF (1996a) domestic use guide. The tables also contain TWQR and risks for other uses (Tables 4.8 and 4.9 provided the percentage of boreholes from each sampling period that did not comply with the TWQR's).

Results from this study showed that many boreholes did not comply with the national TWQR for domestic use. In some cases certain variables of the borehole water complied with the TWQR whereas other tested variables did not.

The low compliance of groundwater quality to national standards is cause for concern due to the health risks that water users is exposed to. Groundwater may thus not always be fit for domestic use as assumed and at times may require treatment. People should be educated on how to minimize health risks by boiling water prior to use. Boreholes should also be contained, sealed and maintained properly. If possible, boreholes should be drilled deeper and not exposed to sources of contamination (septic tanks, open pit latrines and animal feedlots) in close proximity of boreholes. Farmers should also be diligent in fertilizer applications, and ensure that the remnant fertilizer is kept to a minimum.

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 SUMMARY

Groundwater of the North West Province is vulnerable to contamination, and restoration and replenishment of contaminated groundwater may take decades due to the slow rates of groundwater movements (Reasoner, 1990; Murray *et al.*, 2007) and relatively long subsurface residence times (Tredoux *et al.*, 2004). It is imperative to manage the groundwater resources of the North West Province in a sustainable manner. This is due to the limited surface water resources in the North West Province and subsequently the large population size depending on groundwater for their water needs. In order to implement the correct management plan the quality of groundwater as well as the factors influencing water quality should be determined.

6.2 CONCLUSION

In order to determine the microbial and physico-chemical quality of groundwater in the North West Province, 5 objectives were set. A brief discussion of the trends and conclusions for each objective is provided below.

i) **The microbial quality of selected groundwater sources in the North West Province of South Africa**

Microbiological results showed that groundwater of the North West Province is vulnerable to faecal contamination as faecal indicator bacteria were detected in a high percentage of the boreholes tested. Opportunistic pathogens associated with faecal contamination were also detected. Therefore the microbiological quality of

groundwater in the North West Province was not satisfactory. Borehole depth and water temperature seemed to influence the microbiological groundwater quality.

ii) Possible source of faecal contamination

FC/FS ratios indicated that contamination from non-human origin was more prevalent than from human-origin. This could potentially be ascribed to animals grazing close to boreholes, feedlots and storm run-off as well as septic tanks and open pit-latrines.

iii) The antibiotic resistance of faecal coliforms to selected antibiotics

Several of the faecal coliform isolates were resistant to multiple antibiotics, with the highest prevalence of resistance to beta-lactam antibiotics. Percentage resistance of faecal coliforms to AMOX and AMP were lower in 2010 (21% and 15 % respectively) compared to 2009 (54% and 41%). Resistance to CEP was high in both sampling years and remained consistent for both sampling periods. Intermediate resistance to KAN increased from 4% in 2009 to 37% in 2010. No resistance to STREP or CIP was detected in both sampling periods.

iv) The physio-chemical quality of selected groundwater sources in the North West province of South-Africa

The pH of all the sampled boreholes complied with the national TWQR. EC and TDS was mostly high, but still at acceptable levels. Nitrate contamination was found to be a major contaminant of groundwater in the North West Province, as only 28% of the total boreholes tested complied with the national nitrate limit (6mg/L NO_3N), and 43% of the boreholes had nitrate levels >20mg/L NO_3-N. Agricultural practises as well as faecal contamination could have contributed to the high levels of nitrate in the groundwater.

v) **Compared to the target water quality range set by the Department of Water Affairs and Forresty, is groundwater safe to use for domestic purposes?**

Groundwater of the North West Province is subjected to various contaminants, and should therefore be used with caution. The low compliance of the boreholes tested to national standards indicate that there are numerous health risks involved if these water is used for domestic purposes without any prior treatment. Exposed communities are mainly poor communities that have no alternatives. Therefore the need to uplift these communities by providing them with safe water, as it is a basic human right of all people to receive such water. This is established in ss. 27:1(b) of the Constitution of the Republic of South Africa (No. 108 of 1996).

6.3 RECOMMENDATIONS

In retrospection of this study the following recommendations were formulated:

i) PCR

Future studies to determine the presence of *E. coli* and other indicators in the water should be conducted using PCR methods instead of culture based methods. Quantitave analyses can also be achieved with real-time PCR. Real-time PCR may be useful in the distinction between viable and non-viable bacterial cells. PCR allows for a faster turnaround time, and may be more accurate than the traditional culture based methods.

ii) Borehole-specific studies

Boreholes should be identified that may be vulnerable to a certain contamination source. These boreholes should be sampled and analysed on a regular (weekly/two-weekly) basis. During every sample event, the surrounding area, human and environmental influences as

well as seasonal variables should be recorded. This will allow for a deeper insight into the factors promoting the contamination of groundwater.

iii) Nitrate specific study

A study that is focused on the nitrate concentrations in groundwater of the North West Province should be conducted. The dept of the boreholes, rainfall events, fertilizer application rates and the proximity of the boreholes to agricultural lands and human settlements should be considered.

iv) Education

It is imperative that communities are aware of and understand the risks involved with using untreated groundwater as a source for domestic purposes. By having knowledge of the water quality they use, communities will be encouraged to treat the water at household level (boiling of water, point-of-use chlorination, UV treatment and safe storage practices). These practises will aid in the prevention of waterborne diseases. Education on the routes and sources of contaminants will also encourage communities to take ownership of their actions.

v) Epidemiological studies

Together with data indicating the microbiological and physico-chemical quality of drinking-water boreholes, epidemiological studies should be conducted to determine how many water users served by these groundwater sources become ill each year as a result of using this source.

vi) Groundwater source protection

Policies, guidelines and programs (such as well-head protection, source-control, minimum allowable distances of contaminant sources from boreholes, etc.) should be developed and

enforced by local government and relevant stakeholders to ensure the protcetion of groundwater sources.

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APPENDIX A

Data Tables

TABLE A.1: Culture based microbiological results of boreholes sampled in 2009.

Sampling Date	Sampling Site	HPC (cfu/ml)	TC (cfu/100ml)	FC (cfu/100ml)	FS (cfu/100ml)	*FC/FS Ratio	*P. auroginosa* (D/ND)	*S. aureus* (D/ND)
16/02/09	Klerksdorp	130	35	2	4	0.5	ND	ND
16/02/09	Klerksdorp2	363	26	5	3	1.7	ND	ND
16/02/09	Mooibank	3076	76	0	0	-	ND	ND
16/02/09	Orkney	150	12	0	1	0.0	ND	ND
16/02/09	Stilfontein2	34	62	0	68	0.0	ND	ND
16/02/09	Stilfontein	1860	>300	200	1	200.0	ND	D
04/03/09	Potch1	287	43	24	>300	0.1	ND	ND
04/03/09	Potch2	487	57	44	16	2.8	ND	ND
10/03/09	Welverdiend	77	17	8	2	4.0	ND	ND
10/03/09	Carltonville	3100	153	93.3	2	46.7	ND	ND
10/03/09	Ventersdorp1	60	>300	260	19	13.7	ND	ND
10/03/09	Ventersdorp2	1253	188	133	>300	0.4	ND	ND
10/03/09	Fochville	1233	250	204	>300	0.7	ND	ND
26/03/09	Lichtenburg	3000	68	0	2	0.0	ND	D
26/03/09	Lichtenburg2	1233	45	33	0	33.0	ND	ND
26/03/09	Koster	400	54	20	0	20.0	ND	ND
26/03/09	Makokskraal	87	20	0	2	0.0	ND	ND
26/03/09	Groenfontein	4666	180	163	12	13.6	ND	D
26/03/09	Derby	1093	16	0	0	-	ND	ND
26/03/09	Doornkop	966	26	0	0	-	ND	ND
26/03/09	Coligny	1000	114	99	0	99.0	ND	ND
26/03/09	Coligny2	966	19	8	50	0.2	ND	ND
04/04/09	Hartebeespoort	230	37	15	0	15.0	D	D

Sampling Date	Sampling Site	HPC (cfu/ml)	TC (cfu/100ml)	FC (cfu/100ml)	FS (cfu/100ml)	*FC/FS Ratio	*P. auroginosa* (D/ND)	*S. aureus* (D/ND)
04/04/09	Hartebeespoort2	633	60	50	>300	0.2	D	ND
04/04/09	Rustenburg	3000	105	0	1	0.0	D	ND
04/04/09	Rustenburg2	3360	215	106	>300	0.4	D	ND
04/04/09	Matrooster	866	110	94	3	31.3	D	ND
04/04/09	Brits	753	96	0	2	0.0	ND	ND
04/04/09	Brits2	1433	23	0	0	-	ND	ND
17/04/09	HartC1	47	18	0	0	-	D	ND
17/04/09	HartC2	143	115	0	3	0.0	D	ND
17/04/09	HartA1	1366	30	0	9	0.0	D	ND
17/04/09	HartA2	143	8	0	3	0.0	D	ND
17/04/09	HartA3	67	7	0	0	-	D	ND
17/04/09	HartB1	316	22	0	0	-	D	ND
17/04/09	HartB2	190	64	0	0	-	D	ND
17/04/09	HartB3	1333	230	92	0	92.0	D	ND
09/05/09	Delareyville	3240	41	5	1	5.0	D	ND
09/05/09	Ottosdal	43	2	0	0	-	ND	ND
09/05/09	Zeerust2	276	16	4	0	4.0	D	ND
09/05/09	Zeerust1	373	28	0	0	-	D	ND
09/05/09	Mafikeng	150	61	0	1	0.0	D	ND
09/05/09	Mafikeng2	4160	23	1	2	0.5	D	ND
09/05/09	Stella	7500	220	104	5	20.8	ND	ND
09/05/09	Setlagole	1900	42	0	0	-	D	ND
26/05/09	Driefontein	400	20	2	5	0.4	D	ND
26/05/09	Christiana	950	66	20	0	20.0	ND	D
26/05/09	Christiana2	0	0	0	0	-	ND	ND
26/05/09	Schweizer-Renecke	1500	26	8	3	2.7	D	ND
26/05/09	Schweizer-Renecke2	1500	62	0	54	0.0	D	ND
26/05/09	Wolmaransstad	10	2	0	0	-	D	ND
26/05/09	Bloemhof	1200	230	0	36	0.0	D	ND

Sampling Date	Sampling Site	HPC (cfu/ml)	TC (cfu/100ml)	FC (cfu/100ml)	FS (cfu/100ml)	*FC/FS Ratio	*P. auroginosa* (D/ND)	*S. aureus* (D/ND)
26/05/09	Bloemhof2	1900	8	0	0	-	D	ND
10/06/09	Vryburg	700	30	0	6	0.0	ND	ND
10/06/09	Ganysa-Vryburg	133	12	0	5	0.0	ND	ND
10/06/09	Ganyesa	6000	6	0	7	0.0	ND	ND
10/06/09	Ganyesa2	1633	77	42	5	8.4	ND	ND
23/06/09	Piet Plessis	860	30	25	66	0.4	ND	ND
23/06/09	Vergelee	400	45	28	159	0.2	ND	ND
23/06/09	Bray	166	42	0	20	0.0	ND	ND
23/06/09	Morokweng	526	80	0	1	0.0	D	ND
23/06/09	Morokweng2	803	183	48	100	0.5	D	ND
23/06/09	Tseoge	360	211	180	5	36.0	D	ND
23/06/09	Molopo	950	>300	280	86	3.3	D	ND
23/06/09	Vostershoop	616	56	39	43	0.9	D	ND
23/06/09	Makopeng	1263	80	27	24	1.1	D	ND
26/07/09	Salpeterspan	240	70	0	94	0.0	ND	ND
26/07/09	Amalia	310	21	0	0	-	ND	ND
26/07/09	Mokgareng	50	5	0	0	-	ND	ND
26/07/09	Molatswanene	400	9	0	3	0.0	D	ND
26/07/09	Sekhing	290	50	0	2	0.0	D	ND
26/07/09	Taung	160	25	0	2	0.0	D	ND
26/07/09	Geluk	360	89	0	4	0.0	ND	ND
26/07/09	Espagsdrif	155	30	0	0	-	ND	ND
26/07/09	Reivillo	83	120	3	3	1.0	D	ND

HPC–Heterotrophic plate count; TC–Total coliforms; FC-Faecal coliforms; cfu-colony forming unit; D-Detected; ND-none detected

*If either faecal coliform or faecal streptococci were >300cfu/100ml the value was substituted with the minimum value of 300cfu/100ml in order to determine the FC/FS ratio. In these instances the actual FC/FS ratio is not represented, but it gives an indication whether the ratio is high or low.

TABLE A.2: Culture based microbiological results of boreholes sampled in 2010.

Sampling Date	Sampling Site	HPC (cfu/ml)	TC (cfu/100ml)	FC (cfu/100ml)	FS (cfu/100ml)	*FC/FS Ratio	*E. coli* (cfu/100ml)	*P. auroginosa* (D/ND)
23/03/10	Wolmarans-Schweizer	647	4	0	0	-	0	D
23/03/10	Klerks-hartbeesfontein	337	0	0	0	-	0	D
23/03/10	Schweizer-Renecke	97	2	1	6	0.2	0	D
23/03/10	Schweizer-Renecke 2	273	0	0	0	-	0	D
23/03/10	Bloemhof	320	0	0	2	0	0	D
23/03/10	Bloemhof 2	70	0	0	0	-	0	D
23/03/10	Wolmaranstad	247	5	2	8	0.3	2	D
12/04/10	Orkney	168	0	0	0	-	0	ND
12/04/10	Stilfontein	110	0	0	0	-	0	ND
12/04/10	Klerksdorp	800	21	7	2	3.5	0	D
12/04/10	Potchef-stroom 1	168	1	1	0	1	0	ND
12/04/10	Potchef-stroom 2	459	19	17	8	2.1	1	D
27/04/10	Potch-Ventersdorp	1393	65	3	10	0.3	3	D
27/04/10	Ventersdorp-Coligny	193	2	0	0	-	0	ND
27/04/10	Lichtenburg	883	0	0	2	0	0	ND
27/04/10	Biesiesvlei	363	98	15	6	2.5	12	ND
27/04/10	Sannieshof	163	20	12	4	3	7	D
27/04/10	Coligny-Biesiesvlei	287	18	5	6	0.8	2	D
10/05/10	Derby	4066	0	0	0	-	0	ND
10/05/10	Brits	443	10	3	1	3	1	ND
10/05/10	Hartbeespoort	273	0	0	0	-	0	ND
10/05/10	Rustenburg	983	0	0	0	-	0	ND
10/05/10	Rustenburg 2	3400	280	126	26	4.8	36	ND
18/05/10	Mafikeng	20	3	0	8	0	0	D
18/05/10	Zeerust	2466	5	3	3	1.0	2	D
18/05/10	Delarey-ottosdal	126	27	3	2	1.5	2	ND

Sampling Date	Sampling Site	HPC (cfu/ml)	TC (cfu/100ml)	FC (cfu/100ml)	FS (cfu/100ml)	*FC/FS Ratio	*E. coli* (cfu/100ml)	*P. auroginosa* (D/ND)
18/05/10	Setlagole	5500	>300	>300	8	37.5	34	D
18/05/10	Geystown	2533	4	2	0	-	0	ND
24/05/10	Taung	632	10	7	9	0.8	0	D
24/05/10	Hartswater	3650	>300	>300	180	1.7	0	D
24/05/10	Sekhing	485	5	3	10	0.3	2	D
24/05/10	Amalia	268	2	0	18	0	0	ND
24/05/10	Christiana	520	233	65	32	2.0	3	D
24/05/10	Christiana 2	100	26	9	18	0.5	0	D
31/05/10	Broedersput	10	0	0	0	-	0	ND
31/05/10	Vryburg	0	0	0	2	0	0	ND
31/05/10	Ganyesa	726	300	220	6	37.5	0	D
31/05/10	Ganyesa 2	966	0	0	0	0	0	ND

HPC–Heterotrophic plate count; TC–Total coliforms; FC-Faecal coliforms; cfu-colony forming unit; D-Detected; ND-none detected

*If either faecal coliform or faecal streptococci counts were >300cfu/100ml, the value was substituted with the minimum value of 300cfu/100ml in order to determine the FC/FS ratio. In these instances the actual FC/FS ratio is not represented, but it gives an indication whether the ratio is high or low.

TABLE A.3: The physico-chemical measurements of boreholes sampled in 2009.

Sample Date	Site	pH	Temp (°C)	EC (µs/cm)	TDS (mg/L)	Nitrate - nitrogen (mg/L NO_3-N)
16/02/2009	Klerksdorp	7	22	579	407	4.7
16/02/2009	Klerksdorp2	7.4	20.4	742	498	10.5
16/02/2009	Mooibank	6.8	22.7	1303	916	3.7
16/02/2009	Orkney	6.8	22.7	1400	970	209.3
16/02/2009	Stilfontein2	7	23.7	483	248	12.8
16/02/2009	Stilfontein	7.6	24.5	1300	880	1.8
04/03/2009	Potch1	6.9	24.4	347	322	18.3
04/03/2009	Potch2	7.1	22.4	920	630	3.5
10/03/2009	Welverdiend	7.2	21.1	700	470	0.7
10/03/2009	Carltonville	7.6	22.3	66.7	41	0.1
10/03/2009	Ventersdorp1	6.8	21.2	473	317	0.4
10/03/2009	Ventersdorp2	7.5	21.6	571	384	3.0
10/03/2009	Fochville	7.9	22	426	293	0.3
26/03/2009	Lichtenburg	7.5	2.5	1350	906	120.9
26/03/2009	Lichtenburg2	7.1	26	1200	804	112.0
26/03/2009	Koster	6.9	20.6	475	320	36.9
26/03/2009	Makokskraal	7.2	21	640	430	71.4
26/03/2009	Groenfontein	7	21.9	299	199	4.5
26/03/2009	Derby	7.1	22.2	127	85	39.5
26/03/2009	Doornkop	7.2	24.4	545	364	25.5
26/03/2009	Coligny	7.4	22.5	768	516	185.7
26/03/2009	Coligny2	6.8	23	1100	740	123.9
04/04/2009	Hartebeespoort3	7.4	22	690	486	55.2
04/04/2009	Hartebeespoort	7.1	24.1	1002	509	3.6
04/04/2009	Hartebeespoort2	7.3	21.9	1225	860	8.5
04/04/2009	Rustenburg	8.1	20.2	970	688	20.7
04/04/2009	Rustenburg2	7.3	23.9	1005	706	7.3
04/04/2009	Matrooster	7.6	25.6	489	348	3.3
04/04/2009	Brits	7.1	24.1	1160	823	13.6
04/04/2009	Brits2	7.2	22.7	660	468	0.9
17/04/2009	HartC1	7.7	22.9	515	370	0.3
17/04/2009	HartC2	7.5	23.4	662	911	3.4
17/04/2009	HartA1	6.8	21.9	767	547	0.9
17/04/2009	HartA2	6.6	23	966	685	8.5
17/04/2009	HartA3	6.4	21.9	312	223	8.4
17/04/2009	HartB1	7	19.2	1146	812	7.4
17/04/2009	HartB2	7	24.3	1140	808	6.2
17/04/2009	HartB3	7	23.9	984	699	7.3
09/05/2009	Delareyville	7.2	20.3	946	667	15.5
09/05/2009	Ottosdal	7	20.8	747	530	17.3
09/05/2009	Zeerust2	7.2	21.7	780	550	5.2
09/05/2009	Zeerust1	6.8	22	214	152	0.6
09/05/2009	Mafikeng	7.5	22.4	1218	865	9.8
09/05/2009	Mafikeng2	6.9	22.8	1090	760	1.3
09/05/2009	Stella	7.4	22.4	1190	841	36.4

Sample Date	Site	pH	Temp (°C)	EC (µs/cm)	TDS (mg/L)	Nitrate - nitrogen (mg/L NO_3-N)
09/05/2009	Setlagole	7.6	21	1030	713	13.3
26/05/2009	Driefontein	6.9	18.7	736	519	47.0
26/05/2009	Christiana	7.1	13.6	1830	1300	15.9
26/05/2009	Christiana2	7.1	20.1	886	630	26.8
26/05/2009	Schweizer-Renecke	8	17.1	662	469	5.5
26/05/2009	Schweizer-Renecke2	7.1	19.3	792	560	31.6
26/05/2009	Wolmaransstad	7.2	17.5	1020	720	46.6
26/05/2009	Bloemhof	7	19.5	1758	1250	16.9
26/05/2009	Bloemhof2	7.5	18.6	574	404	19.1
10/06/2009	Vryburg	6.7	17.6	1731	1310	38.6
10/06/2009	Gan-Vry	7	18	1107	780	45.2
10/06/2009	Ganyesa	7	16.6	1190	850	63.6
10/06/2009	Ganyesa2	6.9	18.5	960	675	52.3
23/06/2009	Piet Plessis	7.3	20.1	1060	750	410.5
23/06/2009	Vergelee	7.6	18.9	637	926	50.0
23/06/2009	Bray	8.1	19.7	983	702	5.0
23/06/2009	Morokweng	7.1	22.1	882	624	319.1
23/06/2009	Morokweng2	7.2	18.9	1134	810	454.5
23/06/2009	Tseoge	7.1	22.8	971	682	260.9
23/06/2009	Molopo	7.3	23.4	676	484	12.9
23/06/2009	Vostershoop	8.2	16.2	944	672	0.5
23/06/2009	Makopeng	7.3	24	922	655	31.4
26/07/2009	Salpeterspan	7.3	19.5	948	679	38.6
26/07/2009	Amalia	7	15.5	865	611	454.5
26/07/2009	Mokgareng	7.3	21.3	980	697	3.9
26/07/2009	Molatswanene	7.3	21.2	1410	961	429.5
26/07/2009	Sekhing	7.3	21.5	1445	1030	443.2
26/07/2009	Taung	7.2	20.9	1240	896	454.5
26/07/2009	Geluk	8.8	17.2	758	548	5.5
26/07/2009	Espagsdrif	7.1	19.2	1010	726	29.5
26/07/2009	Reivillo	7.4	21.1	903	634	36.8
AVERAGE		7.2	20.9	891	636	67.0
MAX		8.8	26	1830	1310	454.5
MIN		6.4	13.6	66.7	41	0.1

TABLE A.4: The physico-chemical measurements of boreholes sampled in 2010.

Sample Date	Site	pH	Temp (°C)	EC (µs/cm)	TDS (mg/L)	Nitrate nitrogen (mg/L NO_3-N)
23/03/2010	Wolmarans-Schweizer	6	21	756	536	148.0
23/03/2010	Klerksdorp-Hartbeesf	7.2	21.7	425	299	46.6
23/03/2010	Schweizer-Renecke	6.9	24.1	669	481	56.1
23/03/2010	Schweizer-Renecke 2	6.7	25.8	713	505	28.9
23/03/2010	Bloemhof	7.2	25.3	905	644	43.4
23/03/2010	Bloemhof 2	7.2	23.7	746	530	23.2
23/03/2010	Wolmaranstad	7	20.8	821	583	68.2
2010/12/04	Orkney	8.7	21.8	679	485	0.8
2010/12/04	Stilfontein	7.2	21.7	641	458	11.4
2010/12/04	Klerksdorp	6.9	22.1	1464	1004	11.1
2010/12/04	Potchefstroom 1	7.4	22	1090	559	8.0
2010/12/04	Potchefstroom 2	7	20.9	1277	913	10.2
27/04/2010	Potch-Ventersdorp	7.3	20.8	223	157	12.7
27/04/2010	Ventersdorp-Coligny	7.1	19.5	572	409	12.5
27/04/2010	Lichtenburg	7.33	19.6	709	504	21.1
27/04/2010	Biesiesvlei	6.8	20	1831	1280	74.1
27/04/2010	Sannieshof	7.3	21.1	762	543	48.9
27/04/2010	Coligny-Biesiesvlei	7.2	20.9	264	192	11.1
10/05/2010	Derby	7.1	18.8	113	80	4.8
10/05/2010	Brits	7.4	19.1	952	675	8.6
10/05/2010	Hartbeespoort	7.1	23.2	587	422	4.5
10/05/2010	Rustenburg	7.6	23.5	403	288	0.7
10/05/2010	Rustenburg 2	6.7	21.6	911	645	44.3
18/05/2010	Mafikeng	7.1	19.3	1416	997	8.2
18/05/2010	Zeerust	7.8	20.1	624	450	9.5
18/05/2010	Delarey-ottosdal	7.2	20.5	1332	948	23.9
18/05/2010	Setlagole	7	19.5	2530	1790	18.4
18/05/2010	Geystown	7.8	18.1	810	583	15.2
24/05/2010	Taung	7.7	24.4	916	650	16.4
24/05/2010	Hartswater	7.6	20.2	2860	2003	22.3
24/05/2010	Sekhing	7.3	24	1427	1007	1.6
24/05/2010	Amalia	6.8	22.3	1441	1001	5.9
24/05/2010	Christiana	7.5	19.4	1437	1002	23.2
24/05/2010	Christiana 2	6.9	22.2	861	614	11.6
31/05/2010	Broedersput	7.6	19.9	804	571	20.0
31/05/2010	Vryburg	7.2	16	1254	886	28.9
31/05/2010	Ganyesa	7.5	17.3	1259	895	28.6
31/05/2010	Ganyesa 2	7.1	21.2	840	595	15.2
	AVERAGE	7.2	21.1	982	689	24.9
	MAX	8.7	25.8	2860	2003	148.0
	MIN	6	16	113	80	0.7

TABLE A.5: Antibiotic resistance of selected faecal coliform isolates sampled in 2009.

Bore-hole	Growth inhibition zones (mm)									MAR
	STREP	AMOX	AMP	CEP	TM	KAN	OT	CIP	CHL	indices
Potch2	25	16	17	15	18	22	16	32	24	0.000
Potch2	25	18	17	15	17	21	14	32	24	0.111
Klerksd	30	10	13	24	17	25	14	33	23	0.333
Klerksd	26	0	10	24	12	24	18	30	24	0.222
Klerksd	30	17	17	18	18	20	15	36	21	0.000
Klerks2	32	15	16	17	20	20	17	36	22	0.000
Welverd	26	15	16	16	19	20	15	40	22	0.000
Welverd	25	16	12	15	18	20	14	33	23	0.222
Venter2	29	16	17	12	22	24	15	36	22	0.111
Coligny2	45	9	14	21	0	23	21	30	24	0.222
Coligny2	30	0	9	21	14	22	15	28	21	0.222
Coligny2	19	15	16	17	15	21	0	36	19	0.111
Coligny	30	0	9	22	13	22	15	32	22	0.222
Coligny	35	26	29	25	21	25	17	38	27	0.000
Coligny	35	25	26	26	20	26	17	35	25	0.000
Lichten2	38	40	28	24	20	27	19	40	26	0.000
Lichten2	26	17	18	19	10	20	15	32	20	0.111
Lichten2	24	19	19	20	16	22	15	34	22	0.000
Koster	30	16	16	17	13	20	13	33	23	0.111
Koster	26	0	10	0	11	19	14	37	23	0.444
Groenfon	25	0	0	0	10	20	14	33	21	0.444
Groenfon	25	0	0	19	0	21	15	26	20	0.333
Groenfon	25	0	0	21	11	20	15	27	22	0.222
Ave MAR for sample area 1 (A_{09}+B_{09}+C_{09}):										**0.150**
Hartbees	26	0	0	17	14	19	13	25	19	0.333
Hartbees	25	17	17	20	20	22	0	36	25	0.111
Hartbees	28	0	0	0	0	27	13	35	0	0.666
Hartbee2	24	0	0	0	0	20	11	22	0	0.666
Hartbee2	25	13	18	20	17	19	0	30	22	0.222
Hartbee2	26	15	18	16	17	20	16	30	20	0.000
Hartbee3	26	15	15	17	16	19	0	34	20	0.111
Hartbees	22	0	11	21	15	20	17	26	22	0.222
Hartbees	22	18	18	18	15	19	15	30	23	0.000
Brits	15	0	0	17	0	20	0	30	22	0.444
Brits	26	19	20	20	13	20	15	33	23	0.000
Brits	25	16	18	16	0	20	15	34	21	0.111
Rustenb2	30	20	19	19	13	19	15	33	20	0.000
Rustenb2	24	0	10	0	15	20	14	29	19	0.444
Matroost	22	0	10	0	0	20	14	30	22	0.555
Stella	27	0	11	18	17	20	17	30	25	0.222
Stella	16	12	15	0	15	19	16	27	20	0.222
Stella	20	15	17	14	20	22	19	32	24	0.111
Zeerust2	24	12	19	13	16	20	19	30	21	0.222
Zeerust2	24	15	19	15	20	20	15	23	17	0.000

Mafiken2	24	12	16	0	18	20	20	30	20	0.222
Ave MAR for sample area 2 (D_{09} + E_{09}+F_{09}):									**0.233**	
Christian	22	18	19	19	16	20	15	35	25	0.000
Christian	25	18	20	19	16	22	17	39	24	0.000
Christian	22	18	19	17	16	19	15	35	25	0.000
Christian	20	15	19	17	14	18	15	35	20	0.000
Christian	25	17	19	17	15	20	16	35	22	0.000
Schwe-R	20	0	0	25	22	17	23	33	29	0.222
Schwei-R	25	0	0	25	0	20	20	37	30	0.333
Driefonte	20	17	17	17	16	19	15	35	23	0.000
Driefonte	20	13	13	17	15	20	15	35	23	0.222
Reivillo	30	13	21	20	17	19	20	36	24	0.111
Ganyesa2	30	10	14	25	18	22	20	28	19	0.111
Ganyesa2	27	10	13	22	17	20	17	28	17	0.222
Ganyesa2	28	10	12	21	15	25	18	30	15	0.222
Ganyesa2	28	10	15	20	18	22	16	30	22	0.111
Vostersh	20	0	0	0	13	20	13	30	15	0.444
Vostersh	25	13	11	12	12	19	14	36	20	0.444
Vostersh	25	0	0	0	14	21	14	30	20	0.444
Vostersh	20	0	12	0	14	20	15	34	25	0.333
Vergelee	26	0	15	0	16	20	19	30	25	0.222
Vergelee	27	0	13	17	19	23	19	29	25	0.222
Vergelee	29	0	15	0	15	21	18	29	24	0.222
Vergelee	29	0	12	0	14	22	12	30	22	0.444
Molopo	28	10	12	25	12	20	15	26	21	0.222
Molopo	30	9	10	25	15	22	17	30	20	0.222
Molopo	28	0	11	23	14	22	17	28	20	0.222
Molopo	30	9	12	22	16	24	12	28	22	0.333
Makopen	23	10	11	0	13	17	14	35	22	0.444
Makopen	23	13	17	13	16	19	15	32	21	0.222
Piet Pless	30	0	17	0	20	20	19	32	22	0.222
Piet Pless	29	0	20	17	19	24	20	33	26	0.111
Piet Pless	25	13	15	12	15	21	15	35	25	0.222
Piet Pless	28	0	20	15	20	23	21	34	28	0.111
Morokw2	16	0	10	0	15	20	14	34	26	0.444
Morokw2	27	0	11	0	10	21	12	33	25	0.555
Morokw2	25	13	13	0	13	19	14	33	20	0.444
Morokw2	24	0	12	0	15	20	15	30	27	0.333
Tseoge	27	13	14	0	14	20	14	36	21	0.333
Tseoge	30	15	17	13	16	19	15	35	27	0.222
Tseoge	28	14	18	13	17	20	16	38	24	0.222
Tseoge	27	0	14	0	13	21	14	37	20	0.333
Ave MAR for sample area 3 (G_{09} + H_{09} + I_{09} + J_{09}):										**0.239**

TABLE A.6: Antibiotic resistance of selected faecal coliform isolates sampled in 2010.

Bore-hole	Growth inhibition zones (mm)									MAR indices
	STREP	AMOX	AMP	CEP	TM	KAN	OT	CIP	CHL	
Potchef2	24	19	18	14	18	20	13	33	24	0.222
Potchef2	19	18	18	15	15	20	13	31	25	0.111
Potchef2	27	16	16	14	17	19	13	34	24	0.222
Potchefs2	25	15	15	14	12	21	13	38	25	0.222
Potchef1	23	11	11	13	14	18	12	26	20	0.444
Klerksdor	23	0	8	18	10	18	14	24	21	0.444
Klerksdor	24	10	8	16	14	19	15	25	21	0.222
Klerksdor	26	0	8	19	10	20	14	28	22	0.444
Klerksdor	23	10	0	12	13	17	14	24	18	0.444
Pot-Vent	22	17	17	0	15	19	15	32	25	0.111
Pot-Vent	25	17	17	16	15	19	15	32	20	0.000
Pot-Vent	20	20	20	15	21	20	16	30	22	0.000
Pot-Vent	22	18	20	18	19	19	17	32	25	0.000
Sanniesh	25	17	17	16	19	18	17	35	19	0.000
Sanniesh	25	15	16	13	15	20	15	34	20	0.111
Sanniesh	25	17	17	15	14	20	14	33	20	0.111
Sanniesh	19	14	15	15	17	18	16	30	21	0.000
Biesiesvl	20	15	17	13	15	20	15	30	19	0.000
Biesiesvl	23	16	16	13	18	18	20	37	18	0.000
Biesiesvl	22	16	15	13	24	17	25	35	20	0.000
Col-Bies	27	17	15	15	15	20	15	30	25	0.000
Col-Bies	23	18	17	14	13	17	19	25	20	0.111
Col-Bies	19	16	16	15	15	18	15	30	20	0.000
Col-Bies	21	18	15	17	14	20	18	35	23	0.000
Ave MAR for sample area 1 (A_{10}+B_{10}+C_{10}):										**0.176**
Setlagod	25	15	15	0	20	14	17	30	19	0.111
Geysdor	25	0	25	14	20	20	16	31	23	0.222
Rustenb2	25	15	15	20	24	20	17	32	20	0.000
Rustenb2	25	19	18	18	18	20	17	35	22	0.000
Rustenb2	24	17	19	15	0	20	0	38	21	0.222
Rustenb2	24	16	17	14	17	19	15	30	21	0.111
Brits	25	15	14	0	18	19	17	30	20	0.111
Brits	22	18	15	10	19	18	18	24	17	0.111
Brits	28	18	18	18	21	21	17	35	24	0.000
Dela-Otto	20	18	17	20	22	15	20	30	17	0.000
Dela-Otto	25	17	19	19	15	20	17	30	24	0.000
Dela-Otto	23	14	16	0	23	20	20	30	23	0.111
Zeerust	24	18	25	17	24	20	20	30	21	0.000
Zeerust	23	21	20	19	21	15	19	30	25	0.000
Zeerust	30	21	23	14	24	23	15	36	23	0.111
Ave MAR for sample area 2 (D_{10} +F_{10}):										**0.074**
Wolmara	25	19	20	17	18	22	16	36	26	0.000
Wolmara	18	10	11	17	0	14	12	21	0	0.555
Wolmara	30	15	14	16	15	20	16	36	20	0.000

Wolmara	30	23	24	17	24	20	16	38	24	0.000
Wolmara	25	19	19	18	24	21	15	36	22	0.000
Wolmara	24	0	0	18	22	17	0	40	0	0.444
Schweiz-R	25	16	19	15	14	20	15	35	13	0.000
Ganyesa	24	14	15	15	19	20	15	28	20	0.000
Ganyesa	27	12	15	0	16	20	15	35	19	0.222
Hartswa	25	15	15	10	17	18	15	30	22	0.111
Hartswa	24	0	0	13	0	18	0	25	20	0.555
Hartswa	25	12	14	0	14	15	16	29	20	0.222
Sekhing	20	18	20	16	17	19	15	34	18	0.000
Sekhing	25	19	17	15	20	23	16	28	26	0.000
Sekhing	24	17	17	17	17	20	20	34	20	0.000
Christia1	30	10	13	14	20	21	20	37	23	0.333
Christia1	26	16	17	17	21	21	18	40	22	0.000
Christia1	28	14	17	12	14	24	17	40	25	0.000
Christia2	25	21	20	0	20	24	18	35	24	0.111
Christia2	27	0	0	0	0	14	12	25	18	0.555
Ave MAR for sample area 3 (F_{09} + G_{09} + J_{09}):										**0.155**

TABLE A.7: Comparison of percentage faecal coliforms resistant to equivalent antibiotics used in the present study and the study of Kwenamore (2006).

Antibiotic	Present Study		Kwenamore (2006)	
	Minimum and maximum % isolates in a sample group resistant			
	MIN %	MAX %	MIN %	MAX %
TM	4	23	20	80
CHL	0	10	44	100
AMOX	6	84	0	91
OXY-TET	6	43	25	100
STREP	0	0	56	100

APPENDIX B

TWQR and Possible Health Implications

Table B.1: DWAF standard TWQR's for water used for domestic, livestock watering and irrigational purposes (DWAF, 1996b).

Variable	Target water quality range (TWQR)			
	Domestic	**Recreation**	**Livestock watering**	**Irrigation**
pH	6.0 - 9.0	6.5 – 8.5	NA	6.5 – 8.4
TDS (ppm) ;	0 – 450	NA	<1000[a] <2000[b] <3000[c]	260
EC (µs/cm)	0 – 700	NA	NA	400
Nitrate (mg/L NO3-N)	0 – 6	NA	0 - 100	NA[d]
HPC (cfu/ml)	0 – 100			
Coliforms (cfu/100ml)	0 - 5 (TC) 0 (FC)	0 - 150 (FC) 0 - 130 (E.coli)	0 – 200 (FC)	<1 (FC)
Faecal Streptococci	NA	0 - 30	NA	NA
NA – Not Available; HPC – Heterotrophic Plate Counts; TC – Total Coliforms; FC – Faecal Coliforms; cfu – colony forming unit				

[a]Dairy, pigs and poultry; [b]Cattle and horses; [c]Sheep; [d]Nitrogen levels >30 mg/L will cause groundwater contamination

Table B.2: Possible health and other risks involved with parameter levels exceeding the allowable limits.

Variable	Risks Involved when limits is exceeded		
	Domestic (DWAF, 1996a)	**Livestock watering** (DWAF, 1996d)	**Irrigation** (DWAF, 1996c)
pH	**>11:** Deprotonated species danger to health	**NA**	**<6.5; > 8.4:** foliar damage decreasing crop yield
TDS (ppm) ; EC (µs/cm)	**TDS >3000 ; EC >4500:** Disturbance of bodies salt balance; short term health effects with continuous exposure	**TDS 2000 - >13000:** Reluctance to drink, therefore production decrease; adaptation may occur, depending on exposure times	**EC >5400:** Selected salt tolerant crops can still be irrigated, but sustainable irrigation decrease
Nitrate (mg/L NO3-N)	**>20:** Methaemoglobinaemia in infants, irritated mucus membranes of adults	**>400:** Frequent urination, dyspnoea, cyanosis, decreased feed and water intake	**>30:** Nitrogen application to soil may lead to groundwater contamination due to nitrate leaching
HPC (cfu/ml)	**>1000:** Increase risk of infectious disease transmission	**NA**	**NA** (High bacteria counts can lead to the clogging of irrigational drips)
Coliforms (cfu/100ml)	**TC >100 ; FC >20** Significant increased risk of infectious disease transmission	**FC 200 – 1000 for 50% of samples:** Significant risk to infection for both young and mature livestock	**FC 1 - >1000:** Likelihood of transmission of pathogens if crops are eaten raw; milk from cows grazing on contaminated pastures
NA – Not Available; HPC – Heterotrophic Plate Counts; TC – Total Coliforms; FC – Faecal Coliforms; cfu – colony forming unit			