Microbial drinking water quality of selected rural, peri-urban and urban communities and schools in the North West Province, South Africa

by

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DECLARATION

I declare that the dissertation for the Degree of Master of Environmental Science 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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ABSTRACT

Safe drinking water is a basic human right. This study mainly focused on the physico-chemical and microbiological drinking water quality of selected rural, peri-urban and urban communities and schools in the North West Province, South Africa. Parameters measured to determine the physico-chemical quality of drinking water were temperature, pH, total dissolved solids (TDS), electric conductivity, carbonate hardness, total hardness, NO_2^-, NO_3^- and chlorine. These parameters indicated hard water in the informal settlement (Sonderwater) as well as in the rural area (Ganyesa). Nitrate content were troubling for both areas, and total dissolved solids were higher than the standard in the water from Ganyesa. For microbiological quality of the water, heterotrophic plate count (HPC) bacteria, total coliforms, faecal coliforms, faecal streptococci, and staphylococci were enumerated on appropriate selective media using standard procedures. In the water from Sonderwater, faecal indicator bacteria were isolated, but none were found in the water from Ganyesa. Heterotrophic plate count bacteria and total coliforms were detected at levels above the standard in water samples from both areas. Staphylococci and faecal streptococci were present in low numbers in the water from both sites. Faecal coliforms isolated from Sonderwater showed multiple antibiotic resistances to beta-lactams. Identification of faecal coliforms from Sonderwater by API 20E strips and sequencing showed that they were *Aeromonas* spp. and *Enterobacter* spp.. Bacteria in the water from Sonderwater were tested for the potential to form biofilms. Scanning electron microscopy revealed multi-species biofilms developing in the water container after 5 days of storage. Water was sampled from four areas outside of Potchefstroom to determine a settlement gradient in water quality. Areas ranged from a formal area, through an established informal area and a newly established informal area to the newest established informal area. The water from these areas was classified as hard according to physico-chemical parameters measured, and TDS for the water from all areas were above the standard for domestic use. The established informal area had high numbers of total coliforms present in the water. Staphylococci and HPC bacteria were detected in levels higher than the standard for domestic use in all water samples. No faecal coliforms were found in the water from any of the areas. There was no visible gradient in the water quality between the areas. The water samples collected from rural, peri-urban and urban schools were also analysed in terms of physico-chemical and microbiological parameters. Water from all

schools was classified as hard water. Only one school (peri-urban) had a pH above the standard. One rural school and one peri-urban school had TDS and electrical conductivity levels above the standard for domestic use. All rural and peri-urban schools had alarmingly high levels of nitrates present in the water. These schools receive groundwater as drinking water. Total coliform bacteria were present at high levels in all water samples from the schools. Rural and peri-urban schools presented levels of staphylococci and HPC bacteria higher than the standard for domestic use. Streptococci were present in water from some of the rural and peri-urban schools and one urban school. Faecal coliform/faecal streptococci ratios for rural schools indicated faecal pollution potentially of human origin, and in other schools faecal pollution from both human and animal origin. Before the vacation, faecal coliform bacteria were detected in water from all rural schools, two peri-urban schools and one urban school. After the vacation, faecal coliforms were only detected in water from two rural schools and one peri-urban school. Faecal coliforms identified and characterized showed multiple antibiotic resistances to beta-lactams, oxy-tetracycline and trimethoprim. Identification by API 20E strips and sequencing confirmed that faecal coliforms from schools were *Escherichia coli* and *Klebsiella* spp. It was concluded that water from Sonderwater were of poor quality and water from Ganyesa were acceptable with only the nitrates a troubling factor. There was no settlement gradient observed in terms of water quality between areas. Water from rural schools were generally of unacceptable quality in terms of both physico-chemical and microbiological parameters. The water quality of these schools was also very poor when compared to urban schools. Peri-urban schools had water quality better than rural schools, but poorer than urban schools. Surveys of water quality are recommended for all areas sampled, and education on the sanitary quality of water and related health implications is advisable for residents of informal and rural areas.

Keywords: drinking water quality, North West Province, faecal indicator bacteria, schools in the North West Province

CHAPTER 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

There is an increasing awareness of the need to control the pollution of South Africa's water resources and to protect their quality (Zamxaka *et al.,* 2004; NWRS, 2004). Safe drinking-water is a basic human right and should not pose any threat to a person's health despite the amount ingested. Up to 80% of water used in the rural areas of the North West Province is from groundwater resources (NWDACE, 2008). Pollution of these resources poses a serious threat to consumers' health.

Physico-chemical parameters are important for classifying the quality of drinking water. These include parameters such as pH, temperature, total dissolved solids, electrical conductivity, total hardness, carbonate hardness, nitrate and nitrite content. Physico-chemical parameters are measured to determine the level of pollution of the water. High levels of total dissolved solids in drinking water may indicate a measure of pollution of the drinking water (Kempster *et al.*, 1997). The pH of water influences the taste of the water and also the hardness of the water. Nitrate content has a special significance when measuring the quality of drinking water. This is because infants who are constantly exposed to elevated levels of nitrates in drinking water have a high risk of developing methaemoglobinemia (Afzal, 2006).

Microbiological analyses of drinking water includes testing for the presence of indicator organisms such as faecal coliforms, total coliforms, heterotrophic plate count (HPC) bacteria and enterobacteria, using selective media. Total coliform count is one of the most useful indicators of water quality. Presence of total coliform bacteria and faecal coliform bacteria in water may be associated with faecal pollution, and the determination of the levels of these bacteria as a sanitary parameter are considered to be a reliable indicator of the quality of drinking water (DWAF, 2006; Pavlov *et al.*, 2004).

Non-detection of faecal indicator bacteria does not always indicate microbiologically safe water. Heterotrophic plate count bacteria (HPC) levels may also provide efficient

information about the level of microbial activity in water and the effectiveness of the water purification processes (Lechevallier *et al.* 1980).

Other organisms can also be used to determine the quality of drinking water and the health risk that it might pose for consumers. *Streptococcus* spp. can be used as an indicator organism for faecal contamination of drinking water. The ratio of faecal coliform bacteria/*Streptococcus* spp. indicates whether the faecal contamination of drinking water is of human or animal origin. A ratio of 4.0 or higher indicates faecal pollution from human origin, whereas a ratio of less than 0.6 indicates faecal pollution of animal origin (Gildreich and Kenner, 1969). A ration between 0.6 and 4.0 indicates faecal pollution of mixed origin. Another organism used for testing water quality is *Staphylococcus aureus*, which is a major opportunistic pathogen responsible for nosocomial and community-acquired infections (Lechevallier and Seidler, 1980).

1.2 PROBLEM STATEMENT

May (1998) stated that economics and water provision are aspects which are very closely interconnected. Peri-urban and rural areas as well as informal settlements in urban centres are also generally neglected with respect to water provision. Water distribution systems in these areas are not always up to standard. Consequently residents of rural areas are forced to rely on drinking water which is often of poor quality. Such poor quality water could cause enteric diseases and diarrhoea amongst residents (Momba *et al.,* 2006). Considering the lack of detailed information on water quality and the associated health risks of the North West Province, an investigation into drinking water quality in the North West Province is critical.

Children generally are more susceptible to diseases as they differ from adults in the ways in which they are exposed to contaminants and the ways in which they react to it (Bearer, 1995). Drinking water of poor quality thus may have a bigger impact on children than on adults. This is troubling since approximately 300 schools in the North West Province are still without access to adequate water supply and sanitation services (NWDACE, 2008). In 2007 only 62.4% of children living in the North West Province lived in houses with water on site (Children's Institute, 2009). According to these

studies many children living in the North West Province, especially in rural areas, are still subjected to water of questionable quality. An investigation into drinking water quality of rural schools in the North West Province is thus of major importance.

In 2007, 65% of the population of the North West Province lived in rural areas (NWDACE, 2007), and 80% of water used in these areas was from groundwater resources. In 2008 approximately 1 million people were still without access to basic water supply and 19% of the provincial population had no access to basic services at all or had services below basic standards (NWDACE, 2008). Studies on health risks and water quality in the North West Province remain limited and fragmented.

1.3 AIM AND OBJECTIVES

1.3.1 Aim

The aim of this study was to determine the physico-chemical and microbiological drinking water quality of selected rural, peri-urban and urban communities and schools in the North West Province, South Africa.

1.3.2 Objectives

- To determine the physico-chemical and microbiological quality of drinking water from standpipes in selected formal and informal communities in Potchefstroom.
- To determine the physico-chemical and microbiological quality of drinking water from standpipes of the rural community Ganyesa.
- To determine whether there is a settlement gradient in the water quality between formal and informal areas in the Potchefstroom municipal district.
- To determine the physico-chemical and microbiological quality of drinking water from rural schools in the Potchefstroom area and compare this to the quality of drinking water supplied to urban schools.
- To identify selected faecal coliforms and to characterize these in terms of antibiotic resistance.

- To determine the biofilm forming potential of bacteria, occurring in the drinking water from the informal settlement, Sonderwater.

CHAPTER 2

LITERATURE REVIEW

2.1 SAFE DRINKING WATER

The World Health Organization (WHO) guidelines of 1996 define safe drinking water as not representing any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages (WHO, 1996). These guidelines (WHO, 1996) also state that, should interventions be undertaken in the improvement of access to safe water, these interventions would favour the poor in particular, be they in rural or in urban areas. Such interventions could also be effective parts of poverty alleviation strategies.

Blue drop status is indicative of a municipality and water services provider`s efficacy with regards to overall management of drinking water quality. This prestigious award is only granted when 95% compliance with the Blue Drop Certification (BDC) programme criteria is met. It is important to note that if a Blue Drop status has not been achieved as yet, this should not be regarded as a reflection of the actual quality of drinking water (DWAF, 2009). In 2009, a total of 22 water supply systems around South Africa have earned "Blue Drop" status, according to a Department of Water affairs and Forestry report of the same name, released in 2009. Among these 22 water supply systems, Potchefstroom municipality was the only water supply system in the North West Province to receive this prestigious award (DWAF, 2009).

2.2 WATER QUALITY AND POVERTY: NORTH WEST PROVINCE, SOUTH AFRICA

Rural communities that are directly dependant on untreated source water for their domestic purposes are directly incapacitated by the global problem of continuous faecal pollution of source water. Elevation of the levels of indicator bacteria, such as faecal coliforms, in water may pose a public health risk (Bezuidenhout *et al*., 2002).

Economics and water provision are very closely intertwined (May, 1998). Rural areas are generally neglected with respect to water provision and have water distribution

systems which are either non-existent, very old and dilapidated or just poorly maintained. A reason for this could be the economic contribution of such communities (May, 1998), forcing the residents of rural areas to rely on drinking water which is often of very poor quality which also leads to diseases such as diarrhoea amongst residents (Momba *et al.,* 2006). Because poor people lack the technological assets to assess the quality of water they are typically deprived of the benefits of good quality drinking water (Schreiner and Van Koppen, 2002). In South Africa diarrhoea is responsible for about 20% of all deaths in the 1-5 years age group and an annual estimated 43,000 deaths (Mackintosh and Colvin, 2003). These statistics and the lack of detailed information, justifies an investigation into drinking water issues in the North West Province.

In 2007 the North West Province had a population of 3,271 million people, constituting approximately 8% of the national population. Of these, 65% lived in rural areas (NWDACE, 2007). There are 224 settlements in the North West Province and up to 80% of water used in rural communities is from groundwater resources (NWDACE, 2008). Two hundred and five (205) of the 224 settlements are categorized as either agricultural service centres or traditional rural agricultural centres. This emphasises the rural character of the North West Province.

In the North West Province, approximately 1 million people are still without access to a basic water supply and 19% of the total population has services below basic standards or has no access to basic services at all (NWDACE, 2008). Areas mostly affected are rural villages, farm settlements and informal and peri-urban settlements which form part of new developments. In 2006, a total of 49.79% of the population in the North West Province was below the RDP standards for sanitation as depicted by DWAF in 1996 and, in 2007, 23.8% of households lived in informal dwellings (NWDACE, 2008).

Only 27% of African households in the North West Province have access to safe drinking water. In 2002, the estimated unemployment rate was 38%, which was slightly higher than the national average (State of the Environment report, 2002).

However, in 2006 the official unemployment rate was 31.8% which is a definite improvement (Labour Force Survey, 2006). In 2002 almost 30% of the adult population was illiterate, the highest figure among all provinces in South Africa, whereas in 2006 the general literacy rate for this region was 57% (South African Consulate General, 2006). Rural poverty and rural-urban income differences aggravate the social problems, often resulting in violence, crime and high rates of HIV infection, estimated to be between 30-40% among some population groups (State of the Environment report, 2002). Poverty reduction through economic development and social programs is a provincial priority. However, this necessary economic development may further degrade the environment if integrated environmental management (IEM) principles are not adhered to (State of the Environment Report, 2002).

According to the StatsSA community survey (2007), there are approximately 911 120 households in the North West Province: 41.7% of which still make use of pit latrines and 5.8% of which have no toilet facilities at all. The North West Province accounts for the largest number of informal households in the country (StatsSA, 2008). This presents a challenge to the province to supply secure tenure.

Jones (2009) discussed clean South African water, reporting that South Africa's water is usually found in one of three forms: either too much, or too little, or too dirty. Another problem with South Africa's water is also the political issue of sharing water with neighbouring countries, since the majority of South Africa's rivers are in basins shared with these countries. The demand for water in South Africa is outstripping the existing technology to harvest it, a dilemma that is rapidly worsening with population growth and the imperative of providing an improved lifestyle for previously disadvantaged communities. Large areas of the country are already expressing water stress, also endangering food and energy security (Jones, 2009).

The Vaal River has a catchment area of 192 000 km^2 and has the highest concentration of urban, industrial, mining and power generation development in South Africa (Braune and Rogers, 1987). The Vaal River is also a major source of water for the North West

Province and it provides water to the Southern border towns and water supply companies such as Randwater, Midvaal and Sedibeng (Cele, 2009). In 1987, Braune and Rogers also stated that the Vaal River is the main source of water for a large part of the South African economy, especially the Pretoria-Witwatersrand-Vereeniging (PWV) complex but also totally or partially to Eastern Transvaal, the Orange Free State and the far East Rand. Cele (2009) mentioned the large industrial zones and mining activities in the river's path. Consequently, the water quality of the river deteriorates as it cascades towards the Northern Cape (Cele, 2009). Braune and Rogers (1987) also commented on this phenomenon and mentioned that in general the best quality water of the Vaal catchment is more upstream and that water quality deteriorates downstream, especially downstream of the Vaal Dam. Since water treatment is costly, it is unfair to expect people at the lower end of the river to bear all the costs. Cele (2009) also reports that a project is being undertaken by Golder into the management of the Vaal River that could benefit users of this resource.

The rapid growth in the number of mega cities and the attendant strain this puts on infrastructure, including water supply, is disturbing (Jones, 2009). Conventional methods of delivering water to households via a piped reticulation system are also impractical in rural areas. What is needed is for government to provide, or subsidise rain water collection tanks. The tanks will also engender 'pride-of-ownership' not seen with communal standpipes, which are often neglected or damaged (Jones, 2009). Lye (2009) reported that rainwater collection systems which are properly designed and maintained may provide a valuable supplement to existing water supplies. This is because it will reduce the demand on community water supplies.

In the North West Province rural users currently require about 70 million m^3 of surface water per annum, of which 25 million m^3 per annum is needed for domestic consumption (Burgess, 2009). The WHO has proposed that water safety plans (WSPs) be implemented for each country, ensuring that a sustainable water supply system is implemented and managed, thus minimizing the health risks to the consumer (Burgess, 2009). A water safety plan comprises system assessment and design, operational monitoring and management plans (Davison *et al*., 2005). According to Burgess

(2009), WSPs aim to improve water quality assurance through a multi-barrier concept, which implies that actions are required at all stages in the process of producing and distributing water in order to protect water quality. A WSP is an improved risk management tool designed to ensure the delivery of safe drinking water (Godfrey and Howard, 2004). By developing a WSP, the system managers and operators will gain a thorough understanding of their system and the risks that must be managed (Godfrey and Howard, 2004).

Naraghi and Kebotlhale (2004) studied water as a key to socio-economic development and poverty eradication in the North West Province, the findings projected across South Africa. May (1998) also state that economics and water provision are very closely intertwined. Water also plays a key role in sustaining livelihoods and ensuring social and economic development (Lake and Souare, 1997). Results from the study by Naraghi and Kebotlhale (2004) showed that the west of the North West province was underdeveloped, with a high unemployment rate, a low population density and a high resulting cost per capita for the development of infrastructure. The east of the province, however, had sufficiently high infrastructure for cross-subsidizing. Naraghi and Kebotlhale (2004) also found that historically, water services in rural areas were largely underdeveloped or ignored altogether, which confirmed the findings of May (1998) that rural areas are generally neglected with respect to water provision. The conclusion of Naraghi and Kebotlhale (2004) was that a Provincial Water and Sanitation Utility (PWSU), rather than a Regional Utility, would present an institutional structure that would be able to access commercial finance to develop the infrastructure backlog across the entire region. The PWSU would utilise the profit from the east of the province to generate income for loan repayments to develop the infrastructure in the west of the province. This concept was accepted and supported by DWAF and a new White Paper Strategy was published in 2004 to promote regional Water Utilities throughout South Africa (Naraghi and Kebotlhale, 2004).

A study by Brözel and Cloete (1991) demonstrated the effect of storage time and temperature on bacterial growth. Results obtained showed that tap water stored at 30ºC for up to 72 hours had a high number of bacteria (colony forming units (CFU)) per millilitre. These concentrations increased over time. After 216 hours, even water stored at 20ºC had so many bacteria that the levels per millilitre was too numerous to

count (Brözel and Cloete, 1991). These results are of particular importance for rural and poor urban communities, as water from communal taps is often stored unrefrigerated for long periods in the homes of residents. A study by Momba and Kaleni (2002) supported these findings. They found that regrowth of organisms occur on the surfaces of household containers after only 48 hours (Momba and Kaleni, 2002). The problem thus is that even though the tap water may be of a good quality, the storage process allows for bacterial growth (Brözel and Cloete, 1991). In previous studies of rural drinking water supplies, as many as 90% of systems studied have been found to be contaminated with coliforms (Whitsell and Hutchinson, 1973; Sandhu *et al*., 1979).

DWAF (1996) guidelines for nitrate content in drinking water allows for a maximum of 6mg/L. A study conducted in the North West Province for the period 2002 - 2005, showed groundwater nitrate concentration was higher than surface water by up to 12mg/L. Over this period of 3 years, the higher nitrate concentration in groundwater was noted as a gradually prevalent trend. This is associated with the increase in pit latrine use in rural areas and the increasing population and associated informal settlements in urban areas (NWDACE, 2007). The increase in nitrate concentration in the groundwater is troubling, since up to 80% of water used in rural areas is from groundwater resources (NWDACE, 2008). These higher nitrate levels pose a serious threat to infants living in rural areas of the North West Province.

2.3 WATER QUALITY IN SCHOOLS IN THE NORTH WEST PROVINCE

In the North West Province approximately 300 schools are still without access to adequate water supply and sanitation services (NWDACE, 2008). The distribution for children living in urban and rural areas as conducted in 2004 in the North West Province was that 33.4% lived in urban areas and 66.6% in rural areas (Children's Institute, 2009).

Table 2.1 shows that the number of children living in houses with water on site in the North West Province was 62.4% in 2007. This was higher than the 2002 average. In 2007, children in North West who were living in houses with basic sanitation were

56.4%. Although this was higher than the 2002 levels it was lower than the average for South Africa at 58.9% (Children's Institute, 2009). These figures confirm that, when referring to houses with water on site and basic sanitation, the North West Province is below the average of South Africa. Water supply and sanitation thus need attention to get them in line with the rest of South Africa.

When distances from clinics and schools were considered, Table 2.1 shows that, in 2007, 50.3% of children in the North West Province lived more than 30 minutes travel from the nearest clinic. This is much higher than the South African average of 37.8%. The number of children in the North West Province living more than 30 minutes travelling time from primary and secondary schools was 27.2% and 41.3% respectively in 2007. Again these numbers were higher than the South African average of 17% and 29.4% respectively (Children's Institute, 2009). It is disturbing to see how far below the average of South Africa the North West Province really is.

Furthermore, another aspect demonstrated in Table 2.1 is that 28.2% of children from the North West Province live in overcrowded households. This number was also higher than the average for South Africa (26.1%). In conclusion, Table 2.1 shows that, in general, more children from the North West Province are living in poverty stricken conditions than the rest of South Africa. It is thus of critical importance to improve the living conditions in the North West Province in order to be at the same living standards as the rest of South Africa. On a more positive note, however, the comparative statistics for South Africa and the North West Province in 2002 and 2007 provided in Table 2.1 reflect an improvement in the accessibility to water, the basic sanitation and poverty alleviation.

Table 2.1: **The statistics for the living conditions of children of the North West Province in 2002 and in 2007 (**Children's Institute, 2009**).**

	North West Province		South Africa	
	2002	2007	2002	2007
Children living in houses with water on site	57.0%	62.4%	60.6%	62.7%
Children living in houses with basic sanitation	43.9%	56.4%	47.4%	58.9%
Children living in income poverty*	80.1%	74.8%	76.8%	67.7%
Children living far from nearest clinic**	40.5%	50.3%	36.4%	37.8%
Children living far from nearest primary school**	20.6%	27.2%	17.1%	17.0%
Children living far from nearest secondary school**	32.5%	41.3%	28.5%	29.4%
Children living in overcrowded households	27.4%	28.2%	24.0%	26.1%

* Poverty defined as a monthly per capita income of <R350

** Far is defined as having to travel for more than 30 minutes by any means of travelling

The Save the Children organization has launched a number of School Health and Nutrition (SHN) programs. In 1998, the organization launched one of these programs in the Mangochi district of Malawi, later expanding the program to the Balaka district. In 2007 a nationwide SHN program was also implemented in Malawi (Save the Children, 2008). Another program was implemented from 2002 to 2008 in Bangladesh. With the launch of these projects, quality surveys conducted showed that, besides the dangers of drinking from unprotected shallow wells and rivers, water related problems influenced the lives of children dramatically. For example, having to travel long distances to fetch water often made them late for school, sometimes missing school altogether (Save the Children, 2009).

Although a nationwide program was started in Malawi, the provision of water and sanitation facilities remains very expensive. Unfortunately the government will probably not be able to bear the full cost to equip all schools with the adequate facilities (Save the Children, 2008). In 2006 the SHN program integrated a personal health and sanitation education program (PHASE), a hand-washing program targeting school-aged children. These two programs combined helped to improve access to and use of household water and sanitation facilities (Save the Children, 2009).

In South Africa the provision of and access to safe drinking water is a social problem, especially to poor rural communities. Remote rural schools cannot wait for Government engineers to provide them with drinking water (Roy, 2005). Rural schools often do not receive sufficient funding from government to sustain their water systems. One problem faced by rural schools is that informal settlements located near them are making use of their water facilities, resulting in escalating water accounts which schools often cannot afford to pay (Naidoo and Andrew, 2005.) For this reason, schools need an alternative in order to acquire safe water at a reasonable cost. A possible solution for rural schools to acquire more and safer drinking water is to employ the practice of rain harvesting. This is a viable alternative for schools because it is relatively inexpensive to implement and is a proven method that has been used for hundreds of years (Roy, 2005). The proven practice of collecting rainwater has already been revived to provide inexpensive drinking water for thousands of people, in many areas around the world, such as: Colombia in South America, the Atlas Mountains in North Africa, the Himalayas in India, the deserts of Rajasthan in Asia, as well as the remote Pacific island of Fiji (Roy, 2005).

In the mountainous state of Sikkim, India, rainwater harvesting is used to provide safe drinking water to isolated rural communities (Roy, 2005). With the success of the rainwater harvesting project in this area, the Chief Minister of Sikkim was so impressed that he immediately sanctioned the construction of more rooftop rainwater harvesting tanks and approved funds for rainwater harvesting in three schools (Roy, 2005).

The program of rainwater harvesting in India has been running for 20 years. In this time the projects had benefited nearly 200,000 people in 18 Indian States. In addition, through the community-managed initiatives, the Barefoot College has collected some 22 million litres of rainwater a year from the roofs of 596 rural schools and community centres in 492 villages. This has assured access to drinking water for five months of the year (Roy, 2005). This proves that even though rural schools encounter problems with water supply and quality, there are viable alternatives available for these schools.

Locally, a schools' water efficiency and awareness project was launched by the City of Cape Town, encompassing all 850 schools within the Cape Metropolitan area (Wilson, 2004). The first step of the project employed the hippo system, in which a bag was placed in the cistern of the toilet and filled with water to displace some of the water in the cistern effectively lessening the amount of water used every time the toilet is flushed. The implementation of the hippo bags alone saved 400% more than the cost of the entire project. It is thus a low-cost project with immediate and long-term benefits for all parties that could be successfully replicated across the country by any local authority (Wilson, 2004). By implementing this project in all urban schools in the North West Province, it could save such a large amount on water costs that it might enable the municipalities to allocate more money for water treatment and provision toward rural schools.

2.4 PHYSICO-CHEMICAL PARAMETERS

Physico-chemical parameters of water need to be measured in order to determine the water quality. Parameters such as pH, temperature, total dissolved solids (TDS) and the electrical conductivity (EC) need to be determined. Chemical parameters include carbonate hardness (CH), total hardness (TH), nitrite (NO_2^-), chlorine (Cl^-) and nitrate (NO_3^-) content of the water.

In the North West Province regions exist where the TDS is high. This results from natural causes as well as human impact (NWDACE, 2008). Some of the natural causes might be that the geology of the North West Province consists of yellow shifting sands, which means that nitrates and other salts will more easily leach into groundwater (State

of the Environment Report, 2002). The water in these areas is thus dolomitic due to the magnesium and calcium levels which leach through into the groundwater as a result of the geology of this area. High TDS values in drinking water may indicate a measure of pollution or poor salt removal. High levels of salts in the water also cause a high electrical conductivity and high levels of total hardness and carbonate hardness. These parameters are linked with the TDS values of the water and high levels of the one parameter will lead to high values of the other parameters. The standard for domestic use as set out in the DWAF guidelines (1996) is 450mg/ℓ. There is however a critical value for TDS above which some long-term health problems might be anticipated due to excessive concentrations of dissolved particles in the water. A study done by Kempster *et al*. (1997) showed this critical value is 2 450 mg/ℓ. The pH may influence the taste of the water and elevated temperature of water may be a more beneficial environment for growth of microorganisms. The health effects of pH is summarised in Table 1E (Appendix E).

2.5 NITRATES

Groundwater is an indispensable drinking water resource in developing countries, especially where no public water supply exists due to an inadequate infrastructure and poor economic situation. The major pollutants in groundwater worldwide are nitrate (NO_3^-) and nitrite (NO_2^-) (Umezawa *et al.*, 2008)

Effects of nitrate on human health can be classified as follows: Target water quality range is 0-6mg/ℓ, where no adverse health effects are observed. The range 6-10mg/ℓ causes rare instances of methaemoglobinemia in infants, but no effects in adults. Concentrations in this range are generally well tolerated. A 10-20mg/ℓ range is where methaemoglobinemia may occur in infants, but still no effects are observed in adults. Nitrate concentrations of more than 20mg/ℓ is more likely to once again cause methaemoglobinemia in infants. At these concentrations there might also be an occurrence of mucous membrane irritation in adults. These effects are summarised in Table 2E (Appendix E).

Due to its health implications, nitrate content of drinking water has a special significance when considering water quality. Infants who are constantly exposed to elevated levels of nitrates in drinking water have a high risk of developing methaemoglobinemia, more commonly known as "Blue Baby Syndrome" (Schoeman, 2009). Methaemoglobinemia occurs when the bacteria in the gut of an infant converts nitrates into nitrites. When this happens, the nitrites oxidize the haemoglobin of the infant, which reduces the efficiency of the haemoglobin to carry oxygen. This results in a reduced oxygen supply to the necessary organs of the infant, especially the brain, which could cause permanent damage (Wild, 1977; Afzal, 2006).

Nitrates enters the subsurface system through several pathways: (i) Directly, through the nitrogen oxides in precipitation; (ii) Leaching from nitrous fertilizers; (iii) Recharge via river water contaminated with NO_3^-; (iv) Bacterial conversion of NH_4^+ via nitrification under oxic conditions to NO_3^-, and (v) Especially in city areas, through industrial spillage and gasworks sites (Umezawa *et al.*, 2008). Nitrates are also lost from the subsurface system through uptake by plants and microbial transformation for example through autotrophic and heterotrophic denitrification (Umezawa *et al.*, 2008). Because radioactive isotopes in potential NO_3^- sources mentioned above have specific ranges of values according to the processes synthesizing NO_3^-, the use of isotopes helps to identify the source of NO_3^- contamination in water (Kendall, 1998).

In South Africa, the high nitrate levels in groundwater is the single most important reason for some groundwater sources being declared unfit for drinking, i.e. NO_3^--N (nitrate-nitrogen) exceeding 10 mg/ℓ (World Health Organisation, 1996; Marais, 1999). Although no statistics are available, it is known that infant methaemoglobinemia occurs in southern Africa (Redox *et al.* 2001). The incidence of methaemoglobinemia and the occurrence of high nitrate levels in groundwater in Namibia and South Africa have triggered epidemiological studies for investigating the effects of the sub-lethal levels of methaemoglobin on children (Tredoux *et al.* 2005). Super *et al.* (1981) conducted a study on nitrate effects on infants in Namibia. These authors discovered that regular vitamin C intake showed better effects in lowering the levels of methaemoglobin in the blood of infants than the effects of age in lowering these levels. The current technology

in South Africa to remove nitrates from groundwater is poorly developed or expensive. Methods that do remove nitrate (such as ion exchange or reverse osmosis) from either surface or waste waters produce highly saline brines that are often disposed of in potentially hazardous ways that may affect water quality (Israel *et al.*, 2009).

The ideal drinking water class ("blue", i.e. Class 0) has less than 6 mg/ℓ nitrate (plus nitrite) as N, while the maximum allowable concentration is 20 mg/ℓ as N in South Africa (DWAF 1996). This is generally in accordance with the World Health Organisation guidelines. However, in many areas of South Africa nitrate levels exceed the completely unacceptable levels of 40 mg/ℓ as N, and levels of 100 mg/ℓ or even greater than 200 mg/ℓ are found in various places, (Tredoux and Talma 2006). Water with nitrate exceeding 40 mg/ℓ belongs to the category of "dangerous" drinking water quality ("purple", i.e. Class IV) (DWAF, 1996). Such levels are an order of magnitude higher than, for example, in the European Union where water with nitrate N exceeding 5.5 mg/ℓ has to be denitrified (Tredoux and Talma 2006).

Terblanche (1990) studied the health risks of nitrate in drinking water in France, and reported that most of the high nitrate concentrations were found in groundwater, especially in wells in highly developed agricultural areas. It was also seen that in the Netherlands, 40% of urban, industrial and agricultural sources of nitrates in river water was from urban sewage effluent. The International Standing Committee on Water Quality and Treatment (1972) summarised the main sources of nitrate in groundwater. Some of these are from an excess of nitrogenous agricultural fertilisers applied to farm land, percolating from the surface into the aquifer, and broad irrigation of sewage sludge and effluent on land, with its consequent seepage into the ground (International Standing Committee on Water Quality and Treatment, 1972).

A study done by Schoeman (2009) on the removal of nitrate-nitrogen on a small scale reverse osmosis unit in a rural area clinic showed very promising results. Since this was a rural area, the water needs of the clinic were not high and the small scale, reverse osmosis technologies worked very well. This technology lowered the nitrate-nitrogen in one case from levels of 35 - 43 mg/ℓ to a concentration of between 1.4 - 5.5 mg/ℓ in

the treated water. In another case it could be reduced from 54 - 72 mg/ℓ to 12 - 17 mg/ℓ in the treated water. This method, however, was still expensive and yielded low quantities which are still problematic as stated by Israel *et al.* (2009).

2.6 COLIFORM BACTERIA IN WATER

Microbiological analyses of drinking water includes testing for the presence of indicator organisms such as faecal coliforms, total coliforms, heterotrophic plate count (HPC) bacteria and enterobacteria, using selective media. Presence of coliform bacteria in water may also be associated with faecal pollution, since some of these species are excreted in large numbers in the faeces of warm-blooded animals (Grabow and Du Preez, 1979). The total coliform group is less specific for faecal pollution because this group includes various species able to multiply in a variety of aquatic environments. However, total coliforms are still useful as a sanitary parameter for evaluating the quality of drinking water (Pavlov *et al.,* 2004). Water that presents high levels of total coliform bacteria often cause diseases such as diarrhoea and fever (Zamxaka *et al.*, 2004).

Faecal coliform bacterial levels are considered to be a reliable indicator of the sanitary quality of water (DWAF, 1996). This group of bacteria often occurs in water that is contaminated with faecal material from human or animal origin. Water contaminated with faecal bacteria may be considered high risk, since they may also contain pathogens (Harwood *et al*., 2000; Whitlock *et al.*, 2002.). *Escherichia coli* is the faecal coliform bacteria most commonly used as an indicator organism, since it is easily isolated and identified (Harwood *et al*., 2000).

Barnes *et al.* (2004) conducted a study on faecal pollution in the Plankenburg River. The study found that, in a river system with elevated levels of *E.coli,* many other pathogens may also be present. Among these other pathogens were many that could cause serious diseases in humans and animals. Many of these organisms were also found in biofilms. The range and pathogenicity of the organisms represented by the elevated *E. coli* counts were unexpected (Barnes *et al.*, 2004). The fact that almost all of the pathogens were also flourishing in the biofilms, where they were more protected

than those in planktonic form, meant that eradicating them from the river system would be a slow process. This is valuable information, since the presence of *E.coli* in the drinking water of communities or schools then serves as indication of possible presence of other pathogens. Employing *E.coli* as an indicator organism is thus a very good method in the determination of water quality. *E.coli* is also able to travel long distances underground due to its negative charge and its relatively low die-off rate and is therefore also a useful indicator of groundwater quality (Foppen and Schijven, 2006).

Streptococcus spp. can also be used as an indicator organism for faecal contamination of drinking water. Determination of the faecal coliform/*Streptococcus* spp. ratio will indicate whether the faecal contamination is of human or animal origin (Gildreich and Kenner, 1969). If the ratio is less than 0.6, the contamination may be of animal origin. If the ratio is more than 4.0, the contamination is more likely of human origin. When the ratio is between 0.6 and 4.0, the contamination is of mixed origin, in other words, both animal and human origin (Gildreich and Kenner, 1969).

Indicator organisms are identified using the Gram staining method as well as the triple sugar iron (TSI) test for *E.coli*. Gram staining is useful in identifying Gram negative bacilli, such as *E.coli*. When indicator organisms are tested for with Gram staining the expected result is a Gram negative organism. This means that the organism tests blue because of the retention of the dye, crystal violet, by the cell wall. A Gram positive organism will stain pink because of the lack of retention of the dye but the counter stain by safranine. These organisms will then be excluded from further tests as they are not used as indicator organisms for faecal pollution of water.

After Gram staining, organisms are subjected to the triple sugar iron (TSI) test. This test is used to differentiate Gram negative bacteria on their ability to ferment glucose, lactose and/or sucrose and on their ability to reduce sulphur to hydrogen sulphide.

Other than the three sugars, the TSI Agar also contains phenol red to indicate a change in pH and ferrous sulphate to demonstrate H_2S production. Fermentation is an anaerobic process. When sugar is fermented, an acid end product is produced and sometimes gas. Phenol red turns yellow under acid conditions and red under alkaline

conditions (Rollins and Joseph, 2000). Glucose is the first choice of an organism for fermentation. The acid produced will turn the agar in the tube yellow. Some organisms also produce gas from glucose fermentation. This gas may be trapped in the agar pushing the agar up in the tube or causing cracks or bubbles in the tube. Organisms that can use either sucrose or lactose (or both) will begin to ferment these sugars once the glucose has been consumed. Acid will continue to be produced as a result of the metabolism of the lactose and/or sucrose and the tube will remain yellow (Rollins and Joseph, 2000). If only glucose is fermented by an organism then the bottom of the slant will turn yellow. If all the glucose is utilized, the peptone will be used as alternative energy source and an alkaline by-product will be produced. This will turn the top part of the slant a deeper red. However, if glucose and sucrose or glucose and lactose are fermented, the entire slant will turn yellow. If gas is produced during the reaction, it can be observed as described above.

Anaerobic respiration does not require oxygen, since an inorganic salt acts as the final electron acceptor instead of oxygen. Sulphur is one of the anaerobic electron acceptors used by some facultative and obligate anaerobes. Sulphur is readily available in the media in both organic (amino acids) and inorganic (sulphates) molecules. Hydrogen sulphide is a colourless, volatile liquid. In order to detect its presence in the media, ferrous sulphate is used as an indicator. H_2S production is an anaerobic process so the black precipitate will appear only in the butt of the TSI agar (Rollins and Joseph, 2000). *E.coli* however, is an aerobic organism – thus for a positive TSI test, after incubation both the slant and the butt of the agar should be yellow and there should be gas present in the agar, but H_2S should not be detected. Interpretations of the results for TSI tests can be done as described by Rollins and Joseph (2000).

Further identification of Gram negative organisms is done with API 20E tests. API systems are standardized identification systems used to identify culturable bacteria. The API 20E strip is ideal for the identification of enteric and other related bacteria (Zamxaka *et al.*, 2004). This kit includes ID panels containing dried enzymatic and biochemical substrates. The substrates are rehydrated by means of a bacterial suspension in inoculum fluid. Tests used are based on microbial use and degradation of specific substrates detected by a variety of indicator systems. Chromogenic substrates upon hydrolysis cause visible colour changes. The resulting pattern of the reactions is

converted into a seven-digit profile number that is used as the basis for identification. An appropriate code book is then used to aid in the identification of the organisms.

2.7 HETEROTROPHIC PLATE COUNT BACTERIA

Heterotrophs are broadly defined as microorganisms that require organic carbon for growth (Bartram *et al.*, 2004). These include bacteria, yeasts and moulds. There are a variety of culture-based tests which are intended to recover a wide range of microorganisms from water. These methods are collectively referred to as "heterotrophic plate count" or "HPC test" procedures. Thus, the terms "heterotroph" and "HPC" are not synonymous. Microorganisms recovered through HPC tests generally include those that are part of the natural micro biota of water, which are typically non-hazardous, but in some instances, these counts may also include organisms derived from diverse pollutant sources (Bartram *et al.*, 2004). HPC bacteria are widely used as indicators of drinking water quality (Grabow, 1996). Analysis of HPC bacteria in water distribution systems can be helpful in assessing water quality and are useful in determining changes in water quality both during storage and distribution (Carter *et al.*, 2000)

Non-detection of faecal indicator bacteria does not always ensure microbial safe water. Heterotrophic plate count bacteria (HPC) levels may also provide efficient information about the level of microbial activity in water and the effectiveness of the water purification processes (Lechevallier *et al.* 1980). These HPC exist in all sources of water and could be present in drinking water at relatively high densities ranging from <1 to 10^4 cfu /ml (Allen *et al*., 2004). The HPC group has been associated with illnesses and infections in hospitals and could be a threat to human health (Lechevallier *et al*., 1980). It is thus important that when we investigate microbiological quality of drinking water, particularly in rural areas, that we include HPC, total coliforms and faecal coliform bacteria.

Literature documents that there is insufficient clinical and epidemiological evidence to conclude that HPC bacteria in drinking water poses a health risk to consumers (Allen *et al.*, 2004). However, research has shown that high densities of HPC bacteria in

drinking water can interfere with coliform/*E.coli* analysis by methods such as membrane filtration (Allen *et al.*, 2004). According to Geldreich *et al.* (1978), high plate counts inhibit the growth of coliform bacteria on laboratory media, thereby obscuring their presence. HPC bacteria clustering in the range of 500 – 1000 cfu/ml causes the desensitizing of coliform detection through the membrane filtration technique (Geldreich *et* al., 1978).

Other organisms can also be used to determine the quality of drinking water and the health risk that it might contain for consumers. One of these organisms is *Staphylococcus aureus*, a Gram positive coccus, which is a major opportunistic pathogen responsible for nosocomial and community-acquired infection (Lechevallier and Seidler, 1980). *Staphylococcus aureus* is one of the most common agents of food poisoning. Lamka *et al.* (1980) found that when standard plate counts were very high, *S.aureus* were very often also present in high numbers. Specific HPC genera that some consider as opportunistic pathogens enumerated by HPC methods include species of *Aeromonas*, *Klebsiella* and *Pseudomonas*. A review in 1997, "*Pseudomonas aeruginosa*: Assessment of Risk from Drinking water" (Hardalo and Edberg, 1997) stated that a small percentage of clones of *P.aeruginosa* found in drinking water, possess the required number of virulence factors to cause infection.

2.8 ANTIBIOTIC RESISTANCE OF BACTERIA

Over the past years, the antibiotic resistance dilemma has increased dramatically and the problem is getting worse by the misuse and overuse of all kinds of antimicrobial agents and environmental pollution (Pfaller *et al*., 1998). Antibiotic resistant genes can be carried on chromosomes or mobile genetic elements such as plasmids. Through transformation, conjugation and transduction, the mobile gene elements can be transferred between different species (Madigan *et al.,* 2003; Laine *et al.,* 2004).

Antibiotic resistance occurs when an organism has an enzyme or several enzymes which block the mode of action of the antibiotic. The mechanism of action of an antibiotic includes the pathway by which the inhibition of the target reactions leads to the inhibition of the reproductive capacity of the whole cell (Avalos *et al.,* 1999). This

implies that this organism acquires resistance to antibiotics which means that when a person contracts this organism, some antibiotics might not work effectively due to the organism's resistance to those antibiotics (Avalos *et al.,* 1999). Determining the antibiotic resistant phenotypes of pathogens may reveal the distribution of antibiotic resistant genes within a population and thus provide suggestions that could help in the control of antibiotic resistance (Ateba and Bezuidenhout, 2008).

An important question that arises is how pathogenic potential and antibiotic resistance patterns are linked. Levy (2000) and McDermott *et al.* (2003) mention the use of antibiotic resistance spectra of bacteria as a way to assess their potential pathogenicity. Antibiotic resistance genes are mostly located on horizontally mobile elements such as conjugative plasmids, integrons, or transposons (Heinemann, 1999; Dionisio *et al.*, 2002). Virulence genes, however, are also often found on these mobile elements (Levy, 2000; McDermott *et al.,* 2003). These genes are easily transferred from one bacterium to another by mobile elements (Heinemann, 1999; Dionisio *et al.*, 2002). It is possible that a non-pathogenic/potentially pathogenic bacterium may transfer resistance factors to more infectious bacteria, thereby making the illness caused by the specific pathogen more severe (Dzidic and Bedekovic, 2003). It is also possible that the antibiotic resistance gene and the virulence gene are present on the same mobile element. This means that if this gene is transferred to a non-pathogenic organism, this organism not only acquires antibiotic resistance, but also the virulence gene which would make it pathogenic (Levy, 2000; McDermott *et al.,* 2003).

According to Biedenbach *et al.* (2003) infections caused by resistant bacteria lead to more frequent cases of hospitalization, illness and death. As data on antibiotic resistance patterns in rural communities in the North West Province are underdetermined, any data acquired in this field would be of significant value. Ateba and Bezuidenhout (2008) did a study on the antibiotic resistance of *E.coli* in the North West Province and found that there has been an increasing report on the isolation and identification of antibiotic resistant *E.coli* strains. The authors also reported that resistant genetic elements could be transferred from these strains to other enteric

bacteria of clinical importance. This was cause for concern as these could pose health risks to humans (Ateba and Bezuidenhout, 2008).

2.9 BACTERIA IN BIOFILMS

Biofilms can be described as communities of microorganisms attached to a surface (O'Toole *et al.*, 2000). When microorganisms make the transition from planktonic, free living organisms, to cells that are part of a complex, surface attached community (biofilm), they undergo profound changes.

It is also known that biofilm bacteria are significantly less susceptible to biocides than planktonic cells (Eginton *et al.* 1998). The role of biofilm formation and development by bacteria has been suggested to be an important stage in the pathogenesis of numerous bacterial species (Costerton, 1999; Watnick and Kolter, 2000; Donlan, 2001). The establishment of biofilms by pathogenic bacteria on the tissues of susceptible hosts is believed to inhibit the effectiveness of antibiotic treatment. It also protects the organism against host defence mechanisms, and facilitates bacterial communication - leading to expression of virulence determinants (Lavender *et al.*, 2004).

Their resistance to antibiotics and biocides are accomplished by one or more mechanisms (Donlan and Costerton, 2002). Biofilms also provide a niche for the generation of resistant organisms (Donlan and Costerton, 2002; De Lancey, 2001). It was also found that biofilms can form rapidly in raw or treated potable water, even in the presence of 1 to 2 mg/litre free chlorine residual (Norton and Lechevallier, 2000).

Momba *et al.* (2006) studied the behaviour of *E.coli* in biofilms. Attachment of *E. coli* in early biofilm formation was obvious in non-disinfected water and chlorinated water distribution systems. No *E.coli* was detected on stainless steel coupons exposed to monochloramine treated water for the entire study period (96 hours). The results showed the effectiveness of monochloramine in preventing the attachment of *E. coli* during early biofilm formation, as well as the resistance of *E.coli* to chlorination and their attachment in young biofilms. This observation supported the findings of others

(Tracy *et al.,* 1966; LeChevallier *et al.*, 1988) showing the capability of coliform bacteria to survive high disinfectant doses of chlorine.

Momba and Kaleni (2002) studied the survival and regrowth of indicator organisms on the surfaces of household containers used for the storage of drinking water in rural communities in South Africa. They found that various bacteria grew on these surfaces, such as total coliforms as well as *E.coli*. These authors (Momba and Kaleni, 2002) demonstrated that regrowth of indicator organisms occurred within 48 hours on the surfaces of containers. Total coliforms were highly prevalent during the study and showed more regrowth on polyethylene containers than on galvanized steel containers. This study revealed that both types of household containers supported the growth and survival of indicator microorganisms.

The study of Momba and Kaleni (2002) was conducted on a community that uses river water and one that uses standpipes supplied with municipal water. Although the initial concentrations of total coliforms found in the water were approximately the same for both water types, the total coliform counts after 48 hours were much higher in the water from the standpipes than those in the river water. The river water did, however, show higher numbers of *E.coli* in the water initially as well as after 48 hours (Momba and Kaleni, 2002). This finding suggests that people should be aware that water can also become contaminated during storage to such an extent that it may become microbiologically as poor as untreated water. Suitable education and information programs on water safety and personal hygiene of household containers should be undertaken in rural communities in order to minimize contamination of potable water stored in containers. In this regard, it is important to keep rural communities informed about the quality of drinking water and to teach them to operate simple water treatment facilities before and during the storage thereof. This is important because identification of the source of the bacterial contamination is an essential step in seeking to control faecal pollution of water (Guan *et al.*, 2002; Momba and Kaleni, 2002).

2.10 METHODS USED FOR THE ANALYSIS OF DRINKING WATER

2.10.1 Physico-chemical analysis of drinking water

Water quality parameters [chlorine (Cl_2 – free residual chlorine), total hardness (TH), carbonate hardness (CH), nitrite (NO_2^-), and nitrate (NO_3^-)] could be measured using colorimetric multi-test strips (Merck, Germany). According to the manufacturer of the multi-test strips (Merck, Germany), the pH zone on the test strip is impregnated with phenol red and changes colour depending on the pH. Measurements of chlorine, nitrates and nitrites rest on the same basis. Calcium and magnesium ions responsible for TH react with a blue indicator and form a red-violet complex. In the CH test, hydrogen carbonate and carbonate ions react with acid and the consequent change in pH influences the colour of a mixed indicator. Measurement values are determined semi-quantitively by visually comparing the reaction zone on the test strip to a colour scale.

A handheld WTW 350i meter could be used to measure parameters such as pH, total dissolved solids (TDS) and the electrical conductivity (EC). Amounts of TDS in a solution are proportional to dissolved ionized solids such as salts and minerals. These substances contribute to the electrical conductivity of the solution. The relationship between TDS and EC is thus that the TDS meter measures the electrical conductivity of the solution which is then converted to a TDS reading (DWAF, 1996).

2.10.2 Microbiological analysis of drinking water

Zamxaka *et al.* (2004) confirms that there are various methods for the detection of the degree of water contamination. The basic technique used for microbiological assessment of water quality monitoring is the detection and enumeration of indicator organisms. The coliform group of bacteria can be defined as the principal indicators for purity of water for domestic, industrial and other uses.

Detection of indicator organisms is generally done with membrane filtration through a 0.45µm pore filter (Rompré *et al.*, 2002), which retains the bacteria but allows the water filter through. This is a cost effective method and relatively easy to analyse. Results

obtained are expressed as number of colony forming units (cfu)/100ml of water tested (Zamxaka *et al.* 2004).

The media used for detection of various organisms are as follows: m-Endo agar for total coliforms (TC), m-FC media for faecal coliforms (FC), mannitol salt agar for *Staphylococcus*, KF-Streptococcus agar for faecal *Streptococcus*, and R2A agar for heterotrophic plate count bacteria (HPC).

Detection of the various bacteria on their selective media depends on certain principles, which include the following. M-Endo agar is used for enumerating total coliforms in that they are coloured red due to the liberation of fuchsin from fuchsin-sulphite compound, which is added as a pH indicator – *E coli* colonies have a metallic sheen (Grabow and Du Preez, 1979). Colonies will appear this way after 24 hours incubation at 35˚C. Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulphite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. These are potentially *E.coli* (Grabow and Du Preez, 1979). Grabow and Du Preez (1979) established that Endo agar yielded the highest average counts for all of their samples and thus concluded that the technique of choice for routine analysis of total coliform bacteria in drinking water is membrane-filtration using m-Endo agar as a growth medium.

M-FC agar is used for the detection of faecal coliform bacteria in water. Lactose can be fermented by faecal coliforms at an elevated temperature of 44˚C, to form blue colonies on the ready medium (agar base plus rosolic acid). Other organisms show grey colonies. Grabow and Du Preez (1979) found that m-FC agar although not the most efficient media for faecal coliform isolation does have advantages, in that the medium does not require sterilization. These authors also state that the higher cost of commercial dehydrated m-FC medium is justified by the higher counts, saving in time and labour, and the convenience and advantage of using a medium of relatively homogenous composition.

Mannitol salt agar is used for the selective isolation and enumeration of *Staphylococcus* from clinical and non-clinical materials. Koch (1942) reported that only *Staphylococcus* grow on agar that contain 7.5% sodium chloride, as is the case with mannitol salt agar. This phenomenon was further studied in detail by Chapman (1945), which concluded that the addition of 7.5% sodium chloride to phenol red mannitol agar results in a medium for the isolation of plasma coagulating *Staphylococcus*. Mannitol salt agar was found by Han *et al.* (2007) to be 76.5% sensitive for *Staphylococcus aureus* at 24 hours. However, it was found that the sensitivity was increased by approximately 8%, up to 84.3% by incubating negative plates (plates with no growth) for another 24 hours. The specificity of this agar was found to be 99.6% at 24 hours and 95.8% at 48 hours. Thus it was confirmed that this agar is very suitable in the isolation of *Staphylococcus aureus*.

Niemi and Ahtiainen (1995) found that KF-Streptococcus agar yielded high recoveries of intestinal species of enterococci and *Streptococcus*, and they found that the selectivity of the agar was better at elevated incubation temperature. The target species for KF-Streptococcus agar is the *Streptococcus* species. These enterococci reduce the TTC (triphenyltetrazolium chloride) solution present in the agar and thus appear as red colonies. The TTC solution is used as a redox indicator in media used for differentiating bacteria.

2.10.3 Purification and identification of isolates

Purification of selected isolates is done prior to identification to ensure accurate identification. The streak plate method is a standard method used for purification of isolates. Primary identification of purified colonies is done with Gram staining (Prescott and Harley, 2002). This method uses a combination of stains and dyes to determine whether the organism is Gram positive or Gram negative, and also to determine the morphology of the organism, be it rods or cocci. The organisms are differentiated based on cell wall composition. A Gram positive organism tests blue in colour. This result is obtained because the cell wall of the organism retains the dye, Crystal Violet. A Gram negative organism tests pink in colour. The pink colour is caused by the fact that the cell wall does not retain the dye, but is counterstained by

Safranine and colours pink. The triple sugar iron (TSI) test is used for preliminary identification of faecal coliforms. Expected results for these two identification processes are Gram-negative rod shaped organisms which, during the TSI test, turns the agar from red to yellow and produces gas. The TSI (Triple Sugar Iron) agar provides information concerning glucose fermentation, utilization of the sugars lactose and sucrose, and the anaerobic respiratory process that uses sulphur as the final electron acceptor to produce hydrogen sulphide (Rollins and Joseph, 2000).

2.10.4 Antibiotic susceptibility testing

Antibiotic susceptibility to different antibiotics is done with the Kirby-Bauer quality-controlled disk diffusion technique that completely inhibits visible growth of the inoculums (Bauer *et al.*, 1966). The antibiotic sensitivity testing is done by filter paper disc (Mastrings-S, Mast Diagnostics, UK) and the zones of inhibition are measured, compared to NCCLS (1999) standards and then noted as resistant, intermediate or sensitive. Antibiotic sensitivity acts as a guide for choosing appropriate antimicrobial treatment. Gram-negative rods, enterococci and *Staphylococcus* have unpredictable sensitivity patterns to a variety of antibiotics, and need susceptibility testing to decide on what antimicrobial therapy to use.

2.10.5 Bacterial identification by API and 16S rDNA sequencing

The analytical Profiling Index (API) is used to classify and identify bacterial isolates based on biochemical fermentation reactions. These API systems are standardized identification systems used to identify culturable bacteria. The API 20E strip is ideal for the identification of enteric and other related bacteria (Zamxaka *et al.*, 2004). This kit includes ID panels containing dried enzymatic and biochemical substrates. The substrates are rehydrated by means of a bacterial suspension in inoculums fluid. Tests used are based on microbial use and degradation of specific substrates detected by a variety of indicator systems. Chromogenic substrates upon hydrolysis cause visible colour changes. The resulting pattern of the reactions is converted into a ten-digit profile number that is used as the basis for identification. An appropriate code book is then used to aid in the identification.

Confirmation of identities and characterization of microorganisms can be done with the analysis of 16S rDNA genes, which is aided by using PCR to amplify target sequences (Prescott and Harley, 2002; Wagner and Cloete, 2002). Analysis of 16S rDNA fragments is useful for identification of prokaryotes (Wagner and Cloete, 2002). These are amplified using specific universal primers (Hayashimoto *et al.,* 2005). The sequences of these amplicons are determined and then BLASTN searched against GenBank (http://www.ncbi.nlm.nih.gov/BLAST.cgi).

A query submitted to BLASTN search results in a list of sequences in the database which are judged as related to the specific target sequence. "Bit scores" and "E-values" are statistical values used to evaluate the relevance of the sequence matches. BLASTN presents related sequences in descending order according to bit scores. The higher the bit score, the more closely the sequence is related to the target sequence. E-values acts as an estimate of the chance occurrence of identified matches in the database. Smaller E-values indicate higher levels of confidence that similarities between two sequences are more likely caused by common descent than by chance (Ogunseitan, 2005).

2.10.6 Biofilm formation potential

Scanning electron microscopy (SEM) is used to provide detailed images of outer surfaces of microorganisms (Andermark *et al.*, 1991). The specimen is put on a stub and sputter-coated with platinum or gold under vacuum. Thereafter, the stub is put in an electron microscope containing a probe that scans the specimen.

An example in literature of investigations that used SEM in their study is that of Cortizo *et al.* (2003). They employed SEM to investigate the adhesion of motile *Pseudomonas fluorescens* on different materials, including copper. Scanning electron microscopy has also been used to observe structures of biofilms present in a local drinking water distribution system in Singapore (Feng *et al.*, 2005). Scanning electron microscopy has been used to visualize collection system biofilms that formed on inner surfaces of tubing samples during the production of maple syrup. Low temperature SEM (LTSEM) has been used to observe bacteria on spinach leaves (Babic *et al*., 1996). They concluded that frozen, hydrated leaf tissue containing bacteria could be visualized using LTSEM.

2.11 SUMMARY

Literature presented in the preceding sections indicates that physico-chemical and microbiological parameters are important for classifying the quality of drinking water. Groundwater represents an important source of drinking water and its quality is currently threatened by microbiological contamination that poses a serious public health problem. There is an increasing awareness of the need to control the pollution of South Africa's water resources and to protect their quality. The increasing population growth in the North West Province has led to a higher demand on water resources, particularly groundwater. Where people have to collect water from communal taps, sound hygiene practices should be maintained to ensure that the way people store and use water at home does not endanger their health.

Faecal coliform bacterial levels are considered to be reliable indicators of the sanitary quality of water. Heterotrophic plate count bacteria (HPC) may occur in drinking water. HPC is an important parameter to determine efficiency of water treatment. Consistent high levels of HPC in drinking water may indicate poor treatment efficiency. It may also indicate the presence of biofilm that potentially could harbour opportunistic pathogens. The study of biofilms in distribution water systems is thus important in order to understand their structure and the organisms occurring in them. The literature review also introduced various physico-chemical methods as well as microbiological methods available to study water quality. Some methods was also introduced that could be used to identify bacteria isolated from distribution water systems.

CHAPTER 3

MATERIALS AND METHODS

3.1 SAMPLING AREAS AND SITES

Drinking water samples were collected directly from taps at the sampling sites in the North West Province, South Africa. Samples were collected from three communal taps in Ikageng in the Sonderwater area (Figure 3.1). Photos of this site are in Appendix G, Figures 1G to 3G. The rural area sampled, Ganyesa, is approximately 80km from Vryburg and ± 330km from Potchefstroom (Figure 3.2). For a settlement gradient study, samples were collected from various taps in the Potchefstroom area, representing such a gradient. In this case sampling sites varied from a formal established area, through an established informal area (Figures 1G to 3G), towards a newly established informal settlement on the outskirts of Potchefstroom (Figure 3.3). Water samples were also collected from 9 schools in the Potchefstroom area (Figure 3.4). These schools included farm schools around Potchefstroom which receive groundwater as drinking water as well as urban schools that receive municipal drinking water. Sampling locations were determined using a GPS unit (Garmin), and are listed in Table 1A (Appendix A). The GPS coordinates were used to illustrate the locations on a map, constructed with Google Earth Pro.

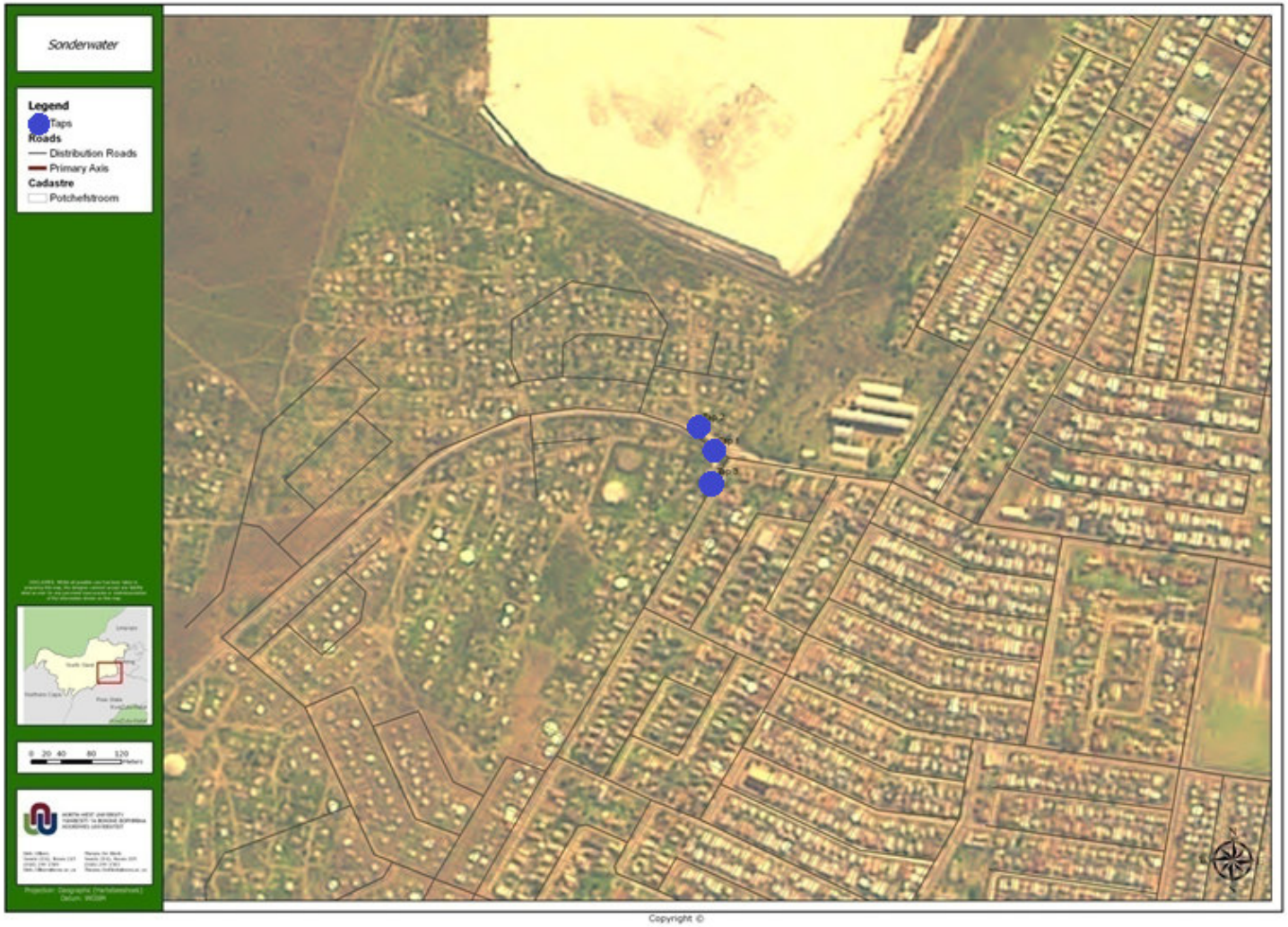

Figure 3.1: A map of the three taps sampled in Sonderwater.

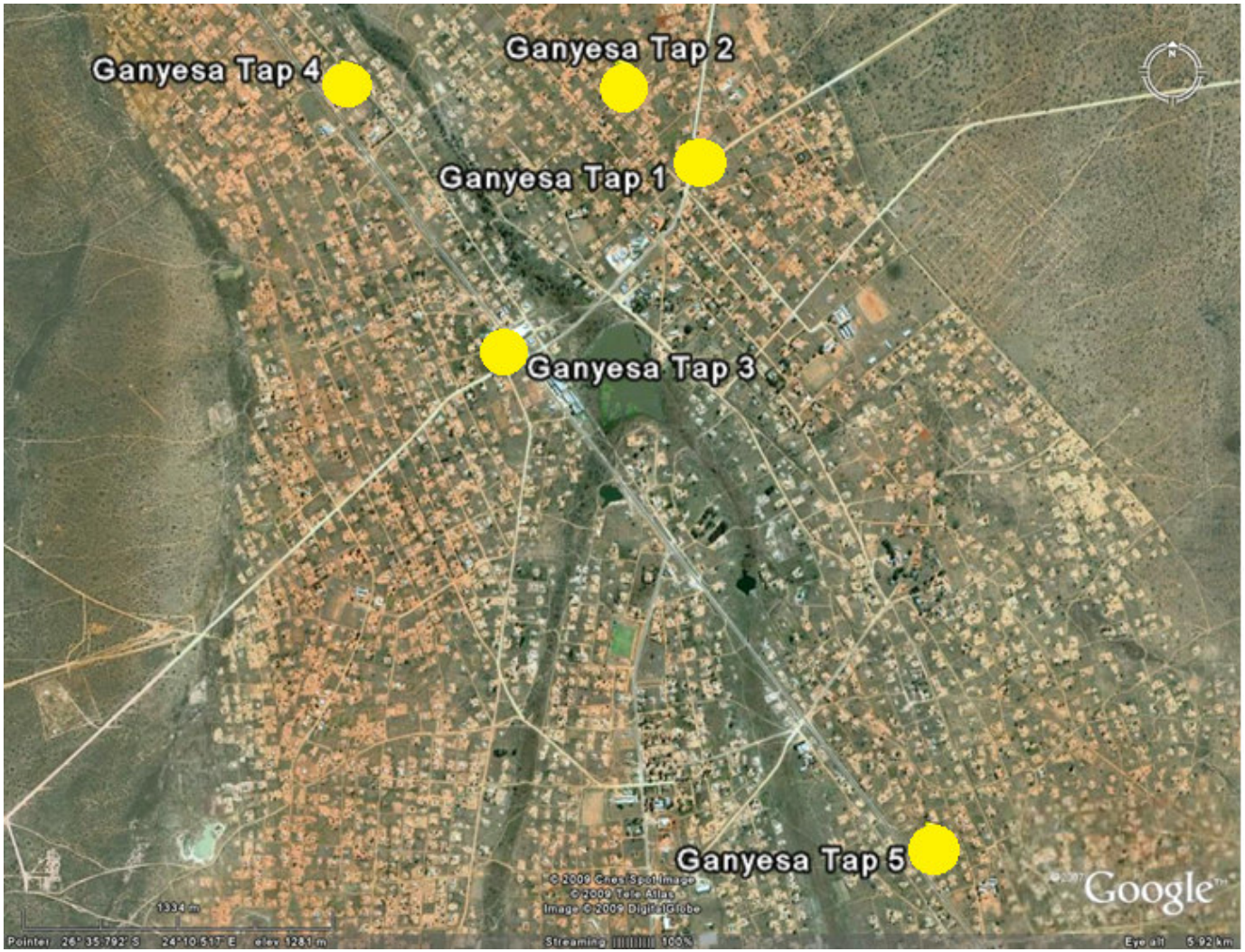

Figure 3.2: A map of the locations of the five taps sampled in Ganyesa.

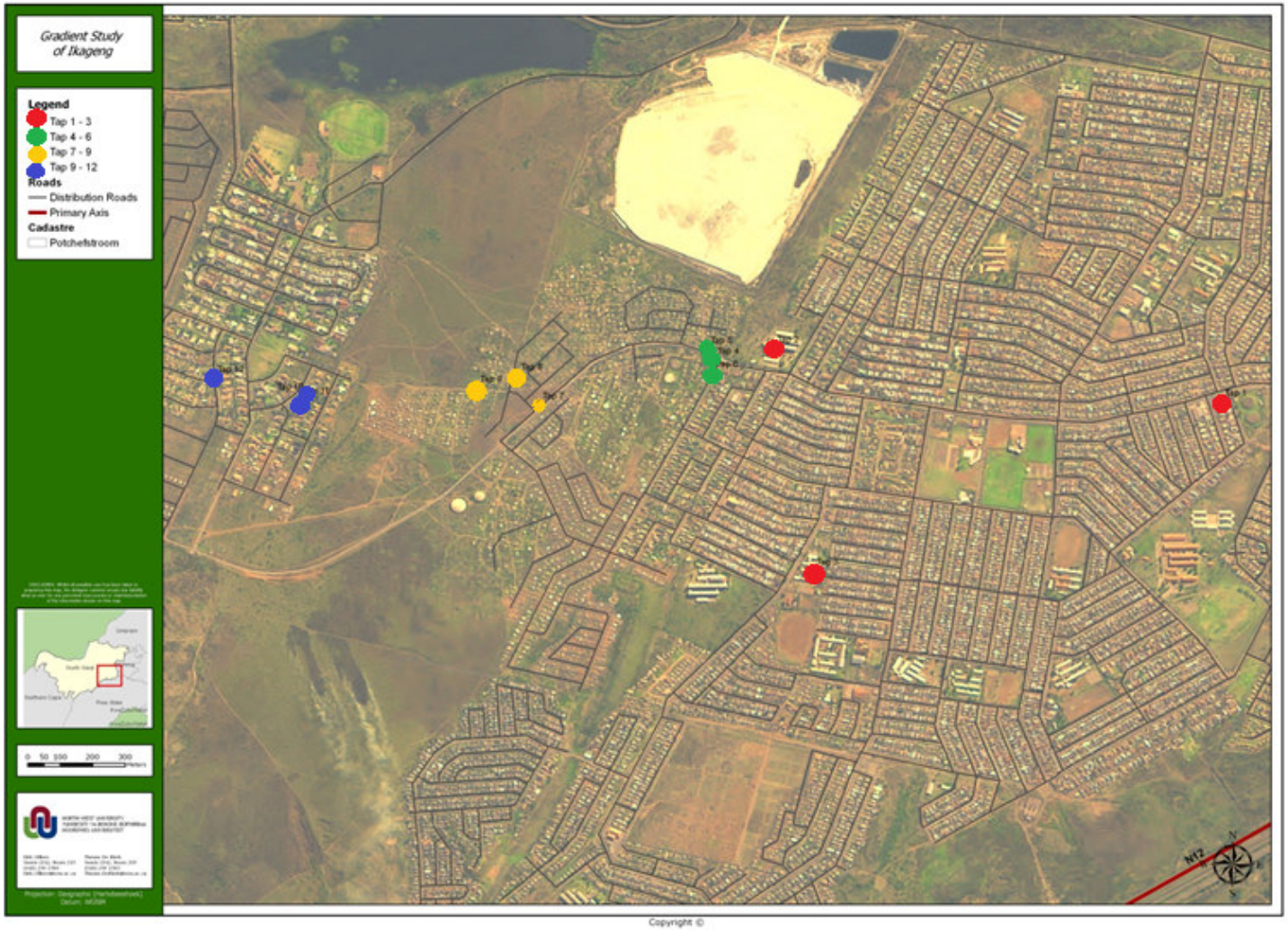

Figure 3.3: A map of the locations of the taps sampled for the settlement gradient study.

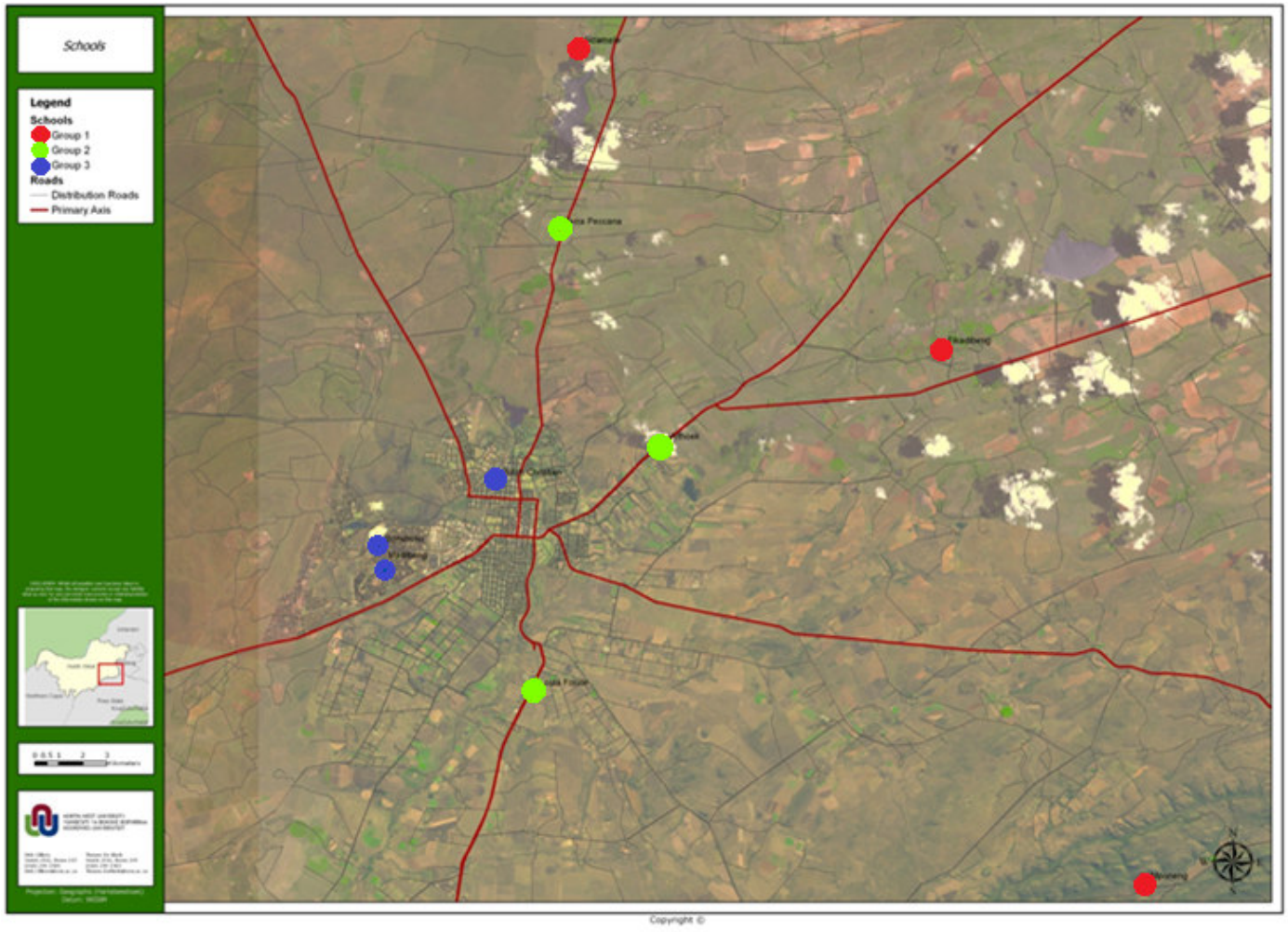

Figure 3.4: A map of the schools. Group 1 (red) in the legend indicate the rural schools. Group 2 (green) is the peri-urban schools and Group 3 (blue) is the urban schools.

3.2 SAMPLE COLLECTION

Sampling for Ikageng was done twice, once in summer (February 2009), and once in winter (May 2009). Sampling for schools were also done twice, once before school vacation (March 2009) and once after school vacation (April 2009). In the rural area, Ganyesa, samples were collected once (June 2009). Samples for the settlement gradient study were collected once, during spring (September 2009). Four areas were sampled during this settlement gradient study. Water was collected from three taps in each of the four areas.

In all cases, samples were collected in sterile 1litre glass Schott bottles, using DWAF (1996) standards. Taps were flushed for 2 minutes before collection. This flushed the interior of the nozzle and discharged stagnant water from the pipe. Taps were then turned off and heat sterilized using a gas torch (Figure 5G). Water was left to run to waste for a few seconds in order to cool the tap. The water was readjusted to a gentle stream and collected in the bottle (DWAF, 1996). Water samples were transported in a cooler box directly to the laboratory for standard microbiological analysis. Analyses were done within 6 hours of collection. The only exception was the samples from Ganyesa, which were analysed within 24 hours of sampling. This was done due to the large distance (330km) between Ganyesa and the laboratory in Potchefstroom. Samples were, however, kept on ice overnight. Another adjustment that had to be made to the sampling at Ganyesa was the numbers of bottles of water sampled from each tap. Once again, because of the large distance between Ganyesa and Potchefstroom that had to be travelled, space was limited and thus only two bottles of water could be sampled from each tap.

For the Sonderwater study, water samples collected from taps were done as follows: First, a bottle of water was collected from the tap without any prior sterilization. Then the tap was turned off and standard sterilizing procedures were followed (as described above) before collecting the second bottle. This was done in order to establish the genuine water quality which consumers experience, as well as the general quality of the water just as residents receive it from the municipality. For this reason, only one bottle of water could be collected from the non-sterilized tap, to create the exact

circumstances under which the residents collect their water, since they do not let the taps run clean before collecting water. Thus, in order to compare results for non-sterilized and sterilized taps, only one bottle was sampled from the sterilized tap. An important aspect to take note of is that when water was sampled in the winter from Ikageng, the tap at sampling site number two was in fact not a tap. Between the time of summer sampling and winter sampling, the actual tap was removed from the ground. Thus when winter sampling was conducted, there was no tap and the water was merely flowing from a pipe inside the ground (Figure 2G (a)). The water from this tap could thus not be collected from a non-sterilized and sterilized tap. Data presented for the winter samples from this tap is thus only data for a non-sterilized tap. Although this tap was removed from the ground, it was not excluded from the study because residents of this area were still collecting water from this site even though it was streaming from the ground.

For the gradient study and the schools study, water samples were collected following the guidelines as described above, and samples were collected in triplicate. Water samples from Ganyesa were also collected following the above mentioned sterilization guidelines, but samples were collected in duplicate as mentioned before.

3.3 PHYSICO-CHEMICAL ANALYSES

Physical parameters measured included temperature, pH, total dissolved solids (TDS) and electric conductivity (EC). Chemical parameters measured were carbonate hardness (CH), total hardness (TH), nitrites (NO_2^-), nitrates (NO_3^-) and chlorine (Cl^-). A WTW Multi 350i meter was used to determine the physical parameters. Colorimetric multi test strips (Merck, Germany) were used to measure the CH, TH, NO_2^- and Cl^-, and NO_3^-. Water hardness (TH and CH) was measured in German degrees (°d), where 1°d = 10mg/L CaO = 17.8mg/L $CaCO_3$. Thus 1°d = 0.178 mmol/L $CaCO_3$. The nitrate content for the water sampled from Ganyesa and Sonderwater (in the winter) was measured using a nitrate kit with a Nitrate Ion selective electrode (from Phoenix Electrode Company). For measurement of nitrate content, 25ml of the water sample is mixed with 0.5ml of the 2M $(NH_4)SO_4$ ionic strength adjuster (ISA) standard provided with the kit. The water was constantly stirred using a magnetic stirrer and nitrates were

measured with the probe. For measurement of physical parameters, 20ml of sample water was dispensed in a sterile beaker, the multimeter was suspended in the water and the measurements were taken. Chemical parameters were measured with the multi test strips and were suspended in the same water used for physical parameter measurements. Instructions of the manufacturer (Merck, Germany) were used. Briefly, the strip was dipped in the water for 5 seconds and the reaction was allowed to develop for 30 seconds outside of the water. The colour change of the test strip was compared to the colour chart on the container of the strips and the values were noted.

3.4 BACTERIAL COUNTS AND ISOLATION OF BACTERIA

The membrane filtration technique was used to enumerate faecal indicator bacteria, which included total coliforms, faecal coliforms, faecal *Streptococcus* spp. and *Staphylococcus* spp. Fifty millilitres (50 ml) of sample water was filtered through a 0.45 micro-meter pore size filter from Whatman®. Membrane filters, which retained the bacterial cells, were placed aseptically on selective agar: m-Endo agar (for total coliforms), m-FC agar (for thermo tolerant faecal coliforms), mannitol salt agar (for S*taphylococcus* spp.), and KF-streptococcus agar (for faecal *Streptococcus* spp.). All of this was done in triplicate. All media were obtained from Merck (South Africa). The plates were incubated for 24 hours at 37°C, except for the m-FC media, which was incubated for 24 hours at 44ºC (DWAF, 1996). If no growth was found on the filters, sampled water was filtered with a membrane and enriched by incubating the filter in tryptic soy broth (TSB) for 24 hours. After incubation, the TSB media was inoculated on three plates of each of the different selective agars and the growth on these plates were then counted. For isolation of heterotrophic plate count bacteria (HPC), the media used was R2A media. Spread plates for a dilution series ranging from 10^{-1} to 10^{-3} was made on R2A agar. These plates were incubated at room temperature for five days. When testing for HPC bacteria, time and temperature are very significant variables. Shorter incubation times at higher temperatures such as 35 - 37°C, for 36 – 48 hours, favours the growth of bacteria from humans and animals, whereas longer incubation at lower temperatures such as 20 - 28°C, for five to seven days, favours the growth of water-based bacteria (Allen *et al.*, 2004). For this reason, incubation at room temperature for five days was considered as ideal for the purposes of this study.

Following incubation, presumptive faecal coliform (*E.coli* specifically) colonies (m-Endo agar plates – red and metallic sheen colonies [total and faecal coliforms]; m-FC agar plates – blue colonies [faecal coliforms]; KF-streptococcus agar – brown colonies [faecal *Streptococcus* spp.]; mannitol salt agar – yellow colonies [*Staphylococcus aureus*]) observed on the surface of the membrane filters, were counted and recorded (DWAF, 1996). Results were converted and expressed as colony forming units per 100ml. Colonies formed on R2A agar were counted on day 2 and day 5 of incubation. These results were separately recorded as colony forming units per ml.

For identification and characterization of potential *E.coli*, single blue colonies from m-FC agar and metallic sheen colonies on m-Endo agar were sub-cultured on appropriate media to obtain pure cultures. For each tap sampled in the gradient study and at the schools, three bottles of water were collected. For each 1L bottle, three plates of each of the selective agars were incubated. In total nine plates for each agar type were incubated. Results presented in chapter 4 are thus an average of the nine plates for each tap. However, for the settlement gradient study, microbiological values presented are according to averages for each area, these values presented are thus averages of the 27 plates from each area (9 plates per tap, 3 taps per area = 27 plates per area). Samples collected in Ikageng during the summer and from Ganyesa were in duplicate. The values presented are thus averages of 6 plates. The samples collected from Ikageng during the winter was collected according to a different method as described in Section 3.2, and thus the results for the sterilized taps and non-sterilized taps are each the averages of the three plates for each bottle.

From each of these plates, at least 3 colonies were selected. For each tap there were thus nine plates of each agar, and 6 plates for taps in Ganyesa. From each of these, 3 colonies were selected if present, which means that for each tap, 27 colonies were purified and tested further. For each tap in Ganyesa 18 colonies from m-FC agar and 18 colonies from m-Endo agar were purified using the streak plate method.

3.5 IDENTIFICATION OF BACTERIA

Identification of the coliform bacteria was conducted using Gram staining, triple sugar iron (TSI) test and API 20E strips. Gram staining was done according to the method of Burke as described in Prescott and Harley (2002). Faecal indicator bacteria are Gram negative organisms and thus Gram staining was used as primary identification. All purified colonies that tested Gram negative were subjected to the next test for further identification.

Gram staining comprises of a bacterial smear being made of the purified colonies on sterile microscope slides. The smears are fixed in a flame after air drying. Each bacterial smear is then treated with consecutive steps of staining, destaining and counterstaining. After these steps the smear is rinsed with water and patted dry with paper towel.

Each smear is then examined with the oil immersion lense (100 x magnifications) under the microscope. If the organisms stained red, it meant that they were Gram negative, if they stained purple, they were Gram positive. Only Gram negative organisms were subjected to further identification tests. Gram staining was also used to determine the cell morphology of the organisms. Faecal indicator bacteria are small rod shaped organisms. All organisms that showed this cell morphology were subjected to further identification testing, whereas organisms of other morphology were discarded.

When testing for faecal indicator bacteria the reaction one looks for in the TSI test slant, is a complete yellow slant and gas production. This is a positive test for *Escherichia coli*. It is however also a positive test for *Serratia* spp. Interpretations of the TSI test results were done as described by Rollins and Joseph (2000). The TSI test was conducted by stab inoculation of the bacteria in the butt of the slant, but also by streaking the bacteria on the surface of the slant. The slant was incubated for 24 hours at 37°C. Results were taken and interpreted after this time.

API 20E tests were conducted according to the instructions of the manufacturer. Each selected isolate was streaked out on nutrient agar and incubated for 24 hours. After this period a loopful of the organism was taken from the agar and inoculated into sterile saline solution and shaken to create a homogenous solution. The bacterial solution was then placed in each of the mini-test tubes of the plastic API strip using a pasteur pipette. A few tubes were completely filled (CIT, VP, and GEL), whereas some other tubes were overlaid with mineral oil such that anaerobic reactions could be carried out (ADH, LDC, ODC, H2S, URE). The plastic API strip was accompanied by a cover and a tray. The tray was filled with water in order to create a humid incubation environment. The strip was then incubated in its humidity chamber at 37°C for 18-24 hours. After incubation the colour reactions were read (some with the aid of added reagents). The reactions for each of the compartments were given a (+) or a (-). Each compartment had a corresponding point. If a reaction for a certain compartment was possible, then the points were counted. Every 3 compartments (or test) had given a number. The points of every 3 set were 1, 2 and 4 (in order). All of the points of the positive (+) reactions were counted for every set of 3 test-tubes and the number it combined to, was written down. This comprised the digits of the seven-digit code. Once the reactions were converted to a seven-digit code, it was fed into the manufacturer's database. The database then returned the identification (Lindquist, 1999).

Furthermore, 16S rDNA gene sequences were then used to confirm identities. The procedure involved isolation of genomic DNA using a bacterial DNA isolation kit (PeqLab, Germany) according to the method described in the instruction manual. Briefly, the method comprised of the following: Overnight cultures were harvested by centrifugation. The bacterial cell wall was removed by lysozyme digestion, and protoplasts lysed by protease K digestion. Following lyses, binding conditions were adjusted and the sample applied to a Hibind® DNA spin-column. Two rapid wash steps removed trace salt and protein contaminants, and, finally, DNA was eluted in a TE buffer (10mM Tris, 1mM EDTA, pH 8.0). To determine the quality and estimate the quantity, purified genomic DNA samples were electrophoresed on a 1% (w/v) ethidium bromide containing agarose gel submerged in 1x TAE buffer (40mM Tris-HCl, 20mM NaOAc and 1mM EDTA), at 80 volts for 45 minutes. Gels were viewed using a Gene Genius Bio Imaging System and GeneSnap gel imaging software (version 6.00.22)

from Syngene (Synoptics, UK). This was supported by data 260nm and 280nm from a NanoDrop Spectrophotometer ND-1000 (Version 3.5.2).

DNA of samples that were clearly visible on agarose gel was selected for amplification by PCR. PCR reagents consisted of: (i) 12.5µl 2x PCR Master Mix (0.05 U/µl *Taq* polymerase, 4mm $MgCl_2$, and 0.4mm dNTPs; Fermentas, USA), (ii) 0.5µl 16S rDNA primers (GM5F: 5'-CCT ACG GGA GGC AGC AG-3' and 907R:5'-CCG TCA ATT CCT TTG AGT TT-3') synthesized by Inqaba Biotech (Pretoria), (iii) 11.0µl PCR water (Fermentas, USA) and (iv) 1.0µl genomic template DNA (~50ng) to make up a total volume of 25µl. Mixtures were briefly (3 seconds) centrifuged to ensure sufficient mixing of reagents before using the Bio-RAD C1000™ Thermal Cycler (BioRAD, UK) for amplification. The reaction mixtures were subjected to the following operational conditions: Cycle 1 (1x): 95°C for 300 seconds; cycle 2(35x cycles): 94°C for 30 seconds, 52°C for 30 seconds, 72°C for 60 seconds; cycle 3 (1x): 72°C for 600 seconds.

Amplified products were electrophoresed on a 1.5% (w/v) ethidium bromide containing agarose gel at 80 volts for 45 minutes, and viewed using a Gene Genius Bio Imaging System and GeneSnap gel imaging software (version 6.00.22) from Syngene (Synoptics, UK). Representatives of the various isolates were selected and the 16S rDNA fragments were cleaned up using a PCR cleanup kit (PeqLab, Germany) according to the method described in the instruction manual. Quantity and quality was determined using a NanoDrop Spectrophotometer ND-1000 (Version 3.5.2). This was done in order to assure the quantity and quality of the product was suitable for further use. It was also used to determine the amount of genomic template DNA that needed to be used during the presequencing PCR procedure.

Products were then subjected to a presequencing PCR procedure. PCR reagents consisted of: (i) 4µl 1:5 PCR Master Mix, (ii) 2µl Sequencing Buffer, (iii) 3.2µl Primer, (iv) 2µl genomic template DNA (as determined by the nanodrop) and (v) 8.8µl PCR water (Fermentas, USA) to make up a total volume of 20µl. Sample mixtures were then subjected to the same PCR conditions as described above. PCR products were then sequenced a using the 3130 Genetic Analyzer (Applied Biosystems, UK).

Sequenced data was used to determine the identities of the isolates. The sequences were viewed in FinchTV (version 1.4.0) software and the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST.cgi) was used to determine the identities, the percentage with which the identification was done, the accession number as well as the E-value.

3.6 ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic resistance/susceptibility patterns of faecal coliform isolates towards a selection of nine antibiotics (from seven different classes) were determined by means of the Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966; Prescott and Harley, 2002). Colonies from pure cultures were grown overnight in sterile nutrient broth. After incubation, the broth was shaken to create a homogenous suspension which was spreadplated onto Meuller-Hinton agar using a sterile swab. The plates were allowed to dry for 15 minutes. Selected antibiotics discs (Mast Diagnostics, UK, supplied by Davies Diagnostics, SA) were then placed on the surface of the inoculated plates using sterile forceps. No more than 5 different antibiotic discs were placed on a plate, to ensure adequate space for inhibition zones and to avoid overlapping of inhibition zones (Figure 6G (a)). Four discs per plate were preferable. Plates were incubated for 20-24 hours at 37°C.

Details of the antibiotics which were used are given in Table 3.1 below. Antibiotic susceptibility testing comprises of the following basis: a clear area, referred to as a zone of inhibition around the antibiotic disk (Figure 6G (b)), is an indication that the organism's growth was inhibited by the relevant antibiotic. This zone was measured to the nearest millimetre and then classified as either resistant (R), intermediate resistant (I) or susceptible (S), according to NCCLS (1999) recommendations. Antibiotic resistance data was used to determine the multiple antibiotic resistances (MAR) phenotype for each isolate. MAR indicates resistance to three or more antibiotics tested (Cabrera *et al.*, 2004). The MAR phenotype is then the combination of antibiotics to which the organism was resistant. Table 3.1 abbreviations used for the antibiotics were according to the instructions to authors for the Journal of Clinical Microbiology (http://jcm.asm.org/misc/journal-ita_abb.dtl). MAR phenotype patterns are often used

when more than one isolate has the same phenotype. When considering MAR phenotypes, it is preferable to refer to organisms which are resistant to three or more antibiotics from different classes.

Table 3.1: Details of selected antibiotics used in this study (NCCLS, 1999).

Antibiotic Classification	Antibiotics	Abbrev.	Conc.	R	I	S
Aminoglycosides	Streptomycin	STR	10 µg	≤11	12-14	≥15
	Kanamycin	KAN	30µg	≤13	14-17	≥18
Beta –lactams	Amoxicillin	AMX	10µg	≤13	14-17	≥18
	Ampicillin	AMP	10µg	≤13	14-16	≥17
Cephalosporin (with Beta-lactam base)	Cephalothin	CEP	30µg	≤14	15-17	≥18
Chloramphenicol	Chloramphenicol	CHL	30µg	≤12	13-17	≥18
Quinolones	Ciprofloxacin	CIP	30µg	≤15	16-20	≥21
Sulphonamides	Trimethoprim	TM	10µg	≤10	11-15	≥16
Tetracycline	Oxytetracycline	OXY-TET	30µg	≤14	15-18	≥19

Abbrev.–Abbreviation; Conc.–Concentration; R–Resisitance; I–Intermediate resistance; S-Sensistive

3.7 BIOFILM FORMATION POTENTIAL

To determine whether the bacteria present in the water sampled from Sonderwater had biofilm formation potential, the water samples were kept in a closed container for 1 week with a sterile microscope slide inside. After one week the water was also filtered through a 0.45µm Nucleopore filter. Both filters and microscope slides were examined by scanning electron microscopy (SEM). Before SEM examination, the filters and microscope slides were treated according to the methods from Tiedt (2009). A brief description of these procedures follows below. A full description of the method is provided in Appendix A.

Biological sample preparation for SEM comprises of the material (the filter or microscope slide) being fixed in 70% ethanol for 2-8 hours. The material was then dehydrated in an ethanol series (80%, 90%, 2x 100% for 15min each). The slide was then critically dried. This is the drying of the material in a critical drying device with

fluid carbonic acid gas. The material was then mounted on SEM buttons with double sided carbon tape. Filters were then fixed with osmium fumes and then all materials were plated with gold/palladium. After plating, material was ready for examination under the electron microscope at 2000X and 4000X magnifications.

3.8 STATISTICAL ANALYSIS

Where appropriate, averages and standard deviations were calculated with Microsoft Excel. A one tailed, two sample t-test, assuming unequal variance (95% confidence interval) was used to show correlation between physico-chemical and microbiological data sets.

CHAPTER 4

RESULTS AND INTERPRETATION

In this study municipal and groundwater samples were collected. All samples were collected from taps. Samples were collected from an informal settlement in Potchefstroom, the Sonderwater area. Three taps in Sonderwater were sampled. Samples were also collected from a rural community, Ganyesa. Five taps were selected in Ganyesa, two of which were groundwater supplied, and three taps had municipal water supply. Water samples were also collected along a settlement gradient in Potchefstroom. A total of 12 taps were selected for this study, three taps from each of four areas. All of these samples had municipal water supply. Furthermore, a set of samples were collected from urban and rural schools in the Potchefstroom area. Three of the nine schools had municipal supplied water (urban schools), whereas six of the nine schools were supplied with groundwater from boreholes (peri-urban and rural schools). All data are presented in the following order: firstly the data for Sonderwater and Ganyesa, comparing water quality of an informal settlement close to an urban area, to that of a rural area. Secondly, the data for the potential settlement gradient study, and thirdly the data for the schools are presented. The fourth section of this chapter presents the data of the identification of isolates and antibiotic tests. The fifth section presents the data for the biofilm formation studies, and the last section is a summary of all results.

4.1 THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL DATA FOR SONDERWATER AND GANYESA

This section contains the results for the studies conducted in Sonderwater and Ganyesa. Physico-chemical and microbiological data is presented for both areas in a comparative manner. In Figure 4.1 the physico-chemical data for the areas are summarised. The values given are averages for all taps sampled in the area. Standard deviations are included in these graphs. All actual values are summarised in Table 1B (Appendix B) for the summer data for Sonderwater, Table 2B (Appendix B) for the winter data for Sonderwater and Table 3B (Appendix B) for Ganyesa.

Figure 4.1(a) shows the physico-chemical data obtained for the water sampled from Sonderwater. The general trend in the data is that the physico-chemical parameters

have higher values in the winter than in the summer. Exceptions are temperature and NO_2^-, which were higher during summer (24°C; 2.5mg/L) than during winter (16.9°C; 0.5mg/L). High values for total hardness (16-20°d) and carbonate hardness (8-16°d) indicate that the water from Sonderwater can be classified as very hard water. With the exception of carbonate hardness and total hardness, all other parameters were then within the standards for domestic use. The NO_3^- levels for the water sampled during winter are a little higher than the standard for domestic use, whereas these levels were not detected in the water from sampled during the summer.

In Figure 4.1 (b) the physical-chemical data for Ganyesa are shown. Water from this area can be classified as very hard, on grounds of the high values for total hardness (20°d) and carbonate hardness (16°d) measured. Electrical conductivity measured for the water samples from Ganyesa is a bit higher (75.9mS/m) than the accepted standard for domestic use (0-70mS/m). As is the case with the water from Sonderwater, the pH, temperature and nitrites were within the standards for domestic use. The TDS for the water from Ganyesa was higher (539.8ppm) than the accepted standard (0-450ppm), which may indicate polluted water, but was lower than the critical value of 2450mg/l (ppm) which leads to long-term health effects (Kempster *et al.*, 1997). The most troubling feature of the physico-chemical data for the water from Ganyesa is that the NO_3^- levels were very high (769.7mg/L). This level of NO_3^- is dangerous to infants and may show effects on the health of adults, and it may cause methaemoglobinemia in infants and mucous membrane irritation in adults (DWAF, 1996).

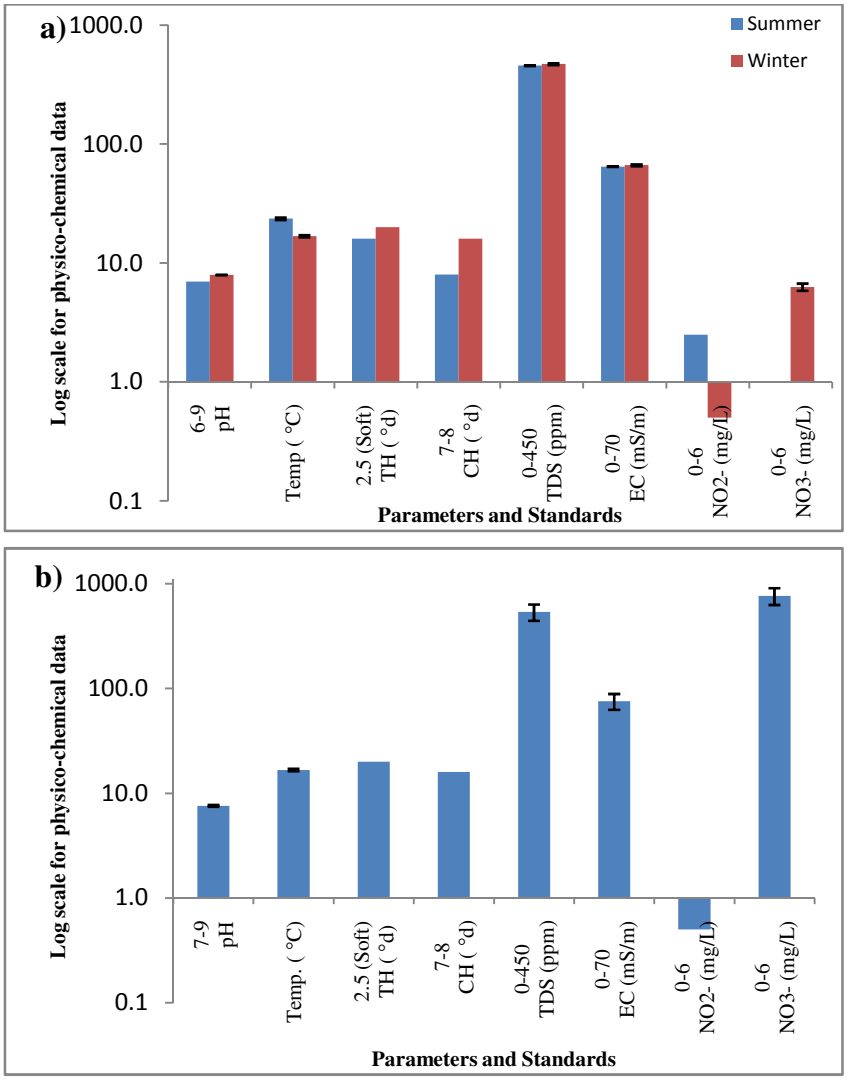

Figure 4.1: a) Physico-chemical data for water samples collected from Sonderwater during summer and winter. **b)** Physico-chemical data for water samples collected from Ganyesa in the once-off sampling in the winter.

Microbiological results are summarized in Figures 4.2 to 4.4. These graphs show the average cfu/ml for each tap sampled. Total counts for the summer data portrayed in the graphs are an average of the six plates used described in Chapter 3 (Section 3.4). Total counts for winter data portrayed in the graphs are an average of three plates as described in Chapter 3 (Section 3.4). Water samples collected from Sonderwater in the winter were membrane filtered, but did not show any growth on any of the selective media. Sampled water was then filtered with membrane filtration and enriched as described in Chapter 3 (Section 3.4). The growth on these plates was then counted for winter data for Sonderwater. During the winter sampling in Sonderwater water was

collected from non-sterilised taps as well as sterilised taps as described in Chapter 3 (Section 3.2).

Ganyesa was sampled once-off to determine the water quality of a rural community at a specific time. Quality of the water in this area thus does not portray the water quality of this region in general; it is only an indication of the water quality at that stage – which was winter. The sampling procedures were followed for the water sampled in Ganyesa as described in Chapter 3 (Section 3.2) and total counts portrayed are an average of six plates for each tap (Chapter 3, Section 3.4). Since averages were determined for each tap, it is difficult to show the standard deviation on the graphs. The standard deviation for each tap can thus be seen in Tables 1C to 4C (Appendix C) for Sonderwater and Tables 5C (Appendix C) and 6C for Ganyesa.

Figure 4.2 shows counts for faecal indicator bacteria (total coliforms and faecal coliforms) for Sonderwater and Ganyesa. In Figure 4.2 (a) the general trend observed was that total coliforms were present in large numbers during summer in all samples from Sonderwater. In winter, total coliforms were detected in samples from one tap only. Faecal coliforms were isolated from two of the three taps in the summer. In winter, no faecal coliforms were detected even after enrichment. Figure 4.2 (b) shows total coliforms present in four of five tap samples in Ganyesa. Although present, the levels were very low (3-20cfu/100ml). Faecal coliforms were only detected in samples from one of the taps, supplied with municipal water, and also at a very low level (5cfu/100ml).

Figure 4.3 depicts the results for the bacterial counts for *Staphylococcus* spp. and faecal *Streptococcus* spp. in 100ml of water for Sonderwater and for Ganyesa. Figure 4.3 (a) shows the presence of *Staphylococcus* spp. during summer and winter. During winter these species were in some cases only present in water from non-sterilised taps, whereas during summer they were isolated from sterilised taps as well. Low numbers of faecal *Streptococcus* spp. were detected in all water regardless of the season. In the winter sample from Tap 2 in Sonderwater, a large number of faecal *Streptococcus* spp. was isolated. When water samples were left to stand in a closed container for 1 week,

as described in Chapter 3 (Section 3.7), *Staphylococcus* spp. counts showed a general increase in water sampled in the summer, whereas in the winter there were none. In Figure 4.3 (b), *Staphylococcus* spp. and faecal *Streptococcus* spp. were present in three of the five samples of Ganyesa. No site had both *Staphylococcus* spp. and *Streptococcus* spp. present. *Staphylococcus* was only isolated from one tap which was supplied with groundwater. Faecal S*treptococcus* spp. was isolated from two of the five taps, which were both supplied with municipal water. Although these bacteria were present, their numbers were low (1 cfu/100ml). Determination of the faecal coliform/*Streptococcus* spp. ratio, will establish whether pollution is of animal or human origin (Gildreich and Kenner, 1969).

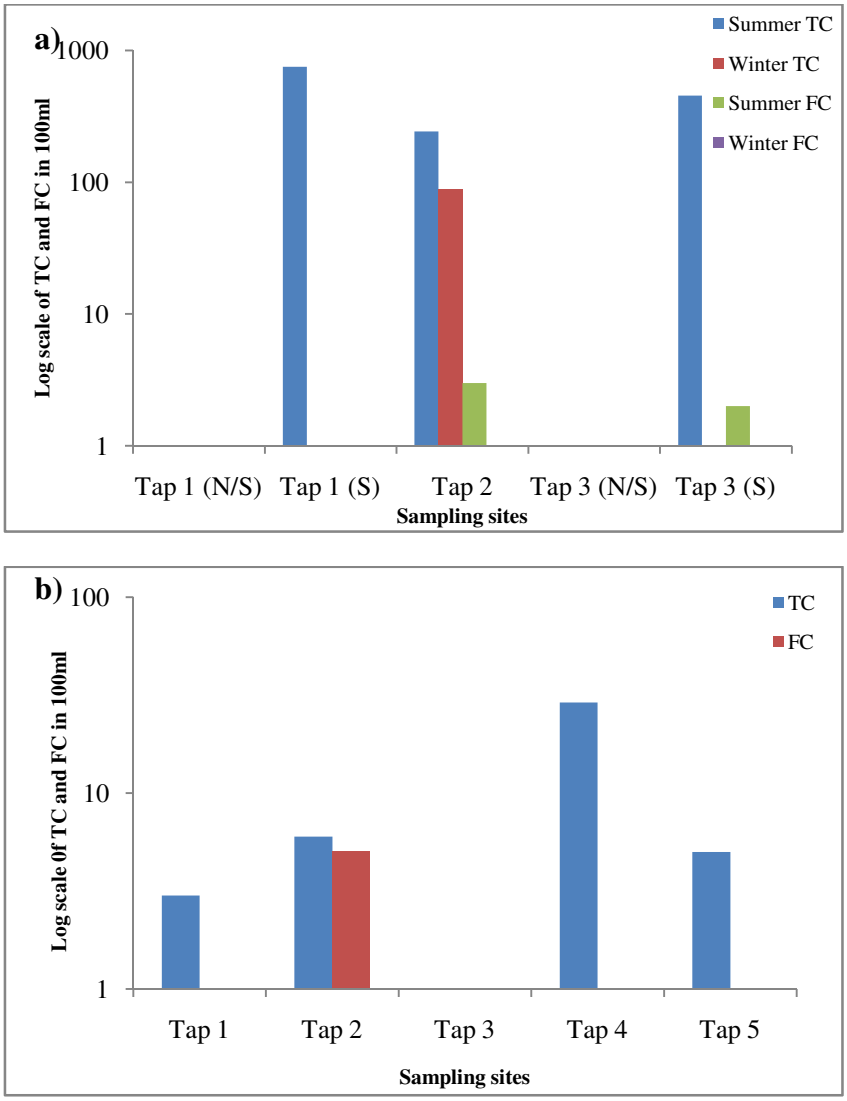

Figure 4.2: a) Total coliforms and faecal coliforms per 100ml for water samples collected from Sonderwater during the summer and winter for sterilized (S) and non-sterilised (N/S) taps. **b)** Total coliforms and faecal coliforms per 100ml for water samples collected from Ganyesa during the once-off sampling in the winter.

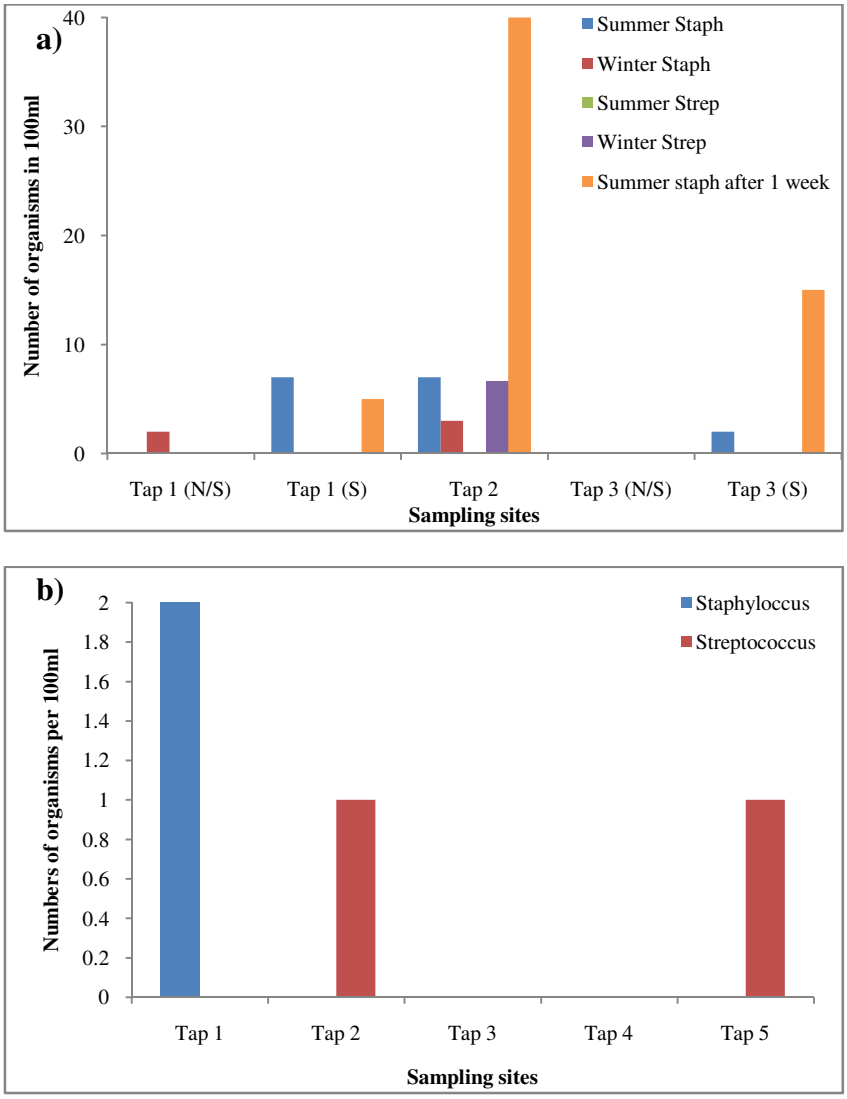

Figure 4.3: a) *Staphylococcus* spp. and faecal *Streptococcus* spp. per 100ml for water samples collected from Sonderwater during the summer and winter for sterilized (S) and non-sterilised (N/S) taps. **b)** *Staphylococcus* spp. and faecal *Streptococcus* spp. per 100ml for water samples collected from Ganyesa during the once-off sampling in the winter.

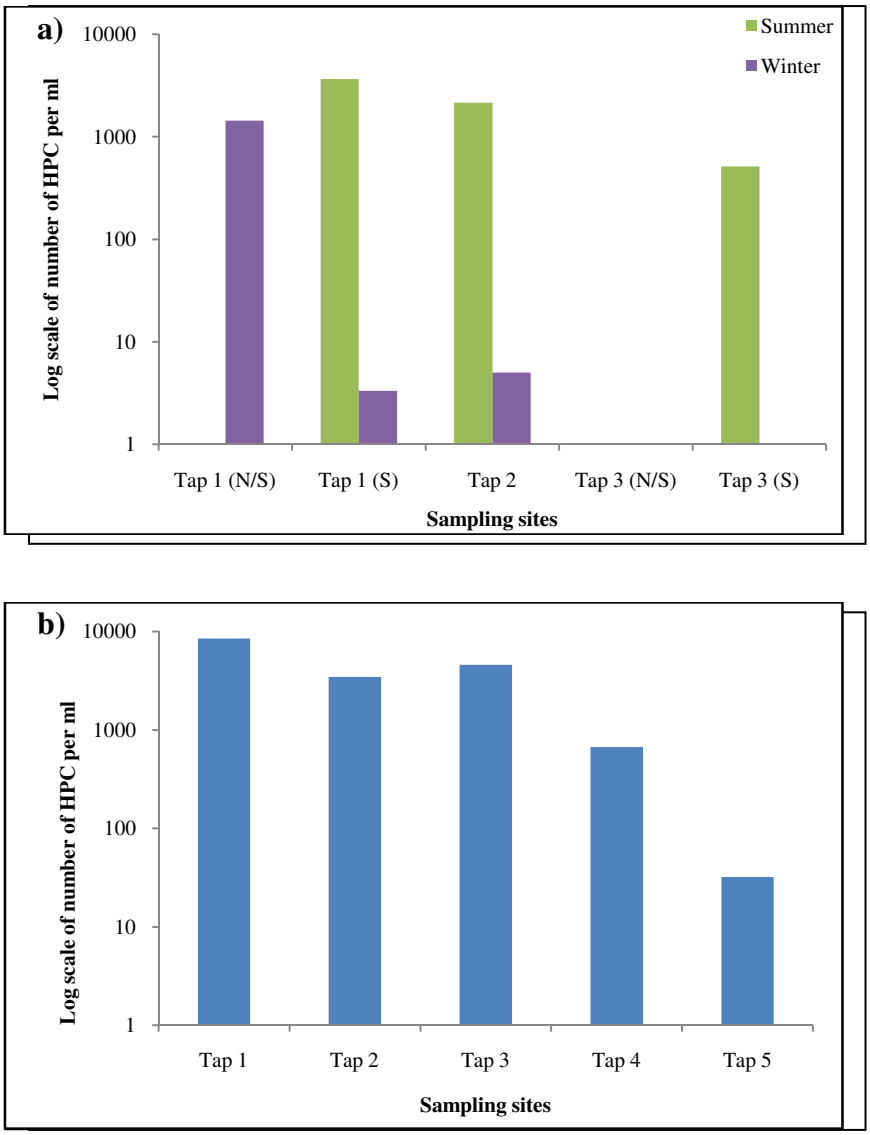

Figure 4.4: a) Heterotrophic plate count bacteria per ml for water samples collected from Sonderwater during the summer and winter, for sterilized (S) taps and non-sterilised (N/S) taps. **b)** Heterotrophic plate count bacteria per ml for water samples collected from Ganyesa in the once-off sampling during the winter.

In Figure 4.4, the counts for the heterotrophic plate count bacteria (HPC) (cfu/ml) are depicted for both Sonderwater and Ganyesa. Figure 4.4 (a) shows that the HPC count is higher during summer (3647cfu/ml) than during winter (1433cfu/ml), which indicates potential seasonal variation. During winter, the highest HPC counts were, as expected, from non-sterilised tap samples. Heterotrophic plate count bacteria for Sonderwater were much higher (3647; 4300cfu/ml) than the target quality water range (DWAF, 1996) for domestic use (0 – 100 cfu/ml). Figure 4.4 (b) represents bacterial counts for

Ganyesa. The highest levels counted were for Tap 1 (8483 cfu/ml) and Tap 3 (4588cfu/ml) which were both water from boreholes, whereas Taps 2 (3450cfu/ml), 4 (668cfu/ml) and 5 (32cfu/ml) received municipal water. Bacterial counts for Tap 5 were within the standard for domestic use.

4.2 THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL DATA FOR THE SETTLEMENT GRADIENT STUDY

In this section, the results for the settlement gradient study are presented. This study was conducted once-off in Ikageng, Potchefstroom. There were four areas sampled along a settlement gradient. The first area is a formal area, the second an established informal and the third and fourth consisted of two newly established settlements. Three taps were sampled for each of the four areas, as described in Chapter 3 (Section 3.2). Since this study was conducted once-off, the quality of the water determined was only for that specific time. Thus, it cannot be assumed that these results apply throughout the year; it is merely a portrayal of the water quality at the time of sampling. Results for another time of year might thus differ from these results.

Table 4.1 shows the physico-chemical data for this study. There is no clear gradient visible in the data, thus it is presented in a table rather than a graph. According to the values for total hardness (20°d) and carbonate hardness (16°d), the water from all areas can be classified as very hard water. The values measured for TDS and EC were all within the standard for domestic use (0-450ppm; 0-70mS/m). Nitrites and nitrates measured were also within their standards (0-6mg/L). At the time of sampling there was no gradient between the physico-chemical data for the different areas along the presumed settlement gradient.

Table 4.1: Values of the physico-chemical parameters measured for water samples collected during the once-off gradient study conducted in the spring.

Parameter	Standard for domestic use	FORMAL			ESTABLISHED INFORMAL			NEWLY ESTABLISHED INFORMAL			NEWLY ESTABLISHED INFORMAL		
		Tap 1	Tap 2	Tap 3	Tap 4	Tap 5	Tap 6	Tap 7	Tap 8	Tap 9	Tap 10	Tap 11	Tap 12
pH	6-9	7.99	8.06	7.97	8.11	8.05	8.09	8.04	8.11	7.95	8.28	8.04	8.02
Temperature (°C)	N/A	20.5	16	18	20	21	19	20	20	22.5	19	21.5	18
Total hardness (TH) (°d)	2.5 (Soft)	20	20	20	20	20	20	20	20	20	20	20	20
Carbonate hardness (CH) (°d)	7-8	16	16	16	16	16	16	16	16	16	16	16	16
Total dissolved solids (TDS) (ppm)	0-450	470	472	471	499	494	495	489	493	496	487	483	489
Electrical conductivity (EC) (mS/m)	0-70	66.1	66.2	66.3	70	69.4	69.6	68.7	69.1	70	68.5	67.7	68.8
Nitrites (NO_2^-) (mg/L)	0-6	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Nitrates (NO_3^-) (mg/L)	0-6	0	0	0	0	0	0	0	0	0	0	0	0

The microbiological results are shown in Figure 4.5. Sampling was conducted as described in Chapter 3 (Section 3.2). The microbiological data are presented as an average for each area. The actual numbers and the standard deviations are summarised in Tables 7C to 11C (Appendix C). Figure 4.5 shows the bacterial counts for the settlement gradient study conducted. There were neither faecal coliforms nor faecal *Streptococcus* spp. detected in any of the samples taken. For total coliforms, area 2 had the highest numbers (124cfu/100ml). The bacterial counts for the other areas were all within the target water quality range (0-5 cfu/100ml). *Staphylococcus* spp. was present in samples collected from all areas. Once again the samples from area 2 had the highest *Staphylococcus* spp. counts. The other areas had about equal amounts of *Staphylococcus* spp., however there was no clear gradient. For HPC bacteria, area 1 and 3 had the highest levels. In conclusion, the microbiological results obtained show no clear gradient in the water quality. This, however, is only applicable for the time sampled and does not mean that there is not a gradient present at any other time.

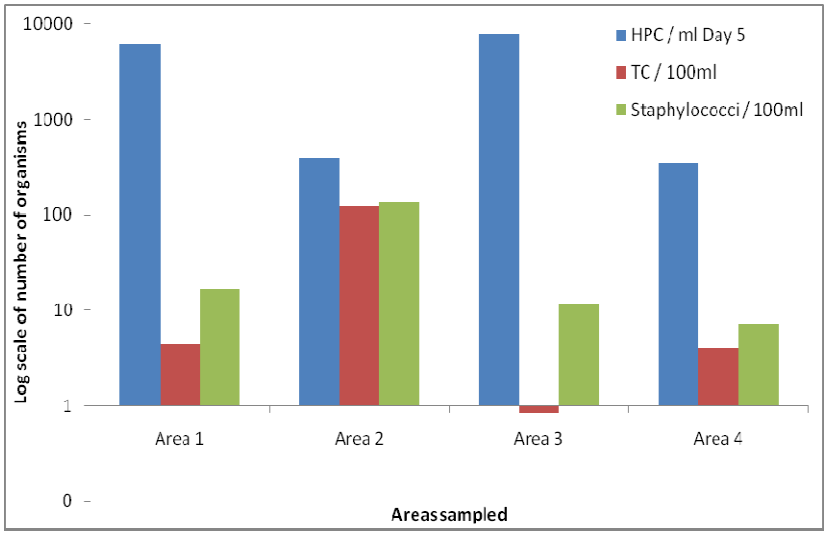

Figure 4.5: The bacterial counts for the gradient study. The values given for each area is an average of the three taps sampled in each area. There were no faecal coliforms or faecal *Streptococcus* present in the water from any of the taps.

4.3 THE PHYSICO-CHEMICAL AND MICROBIOLOGICAL DATA FOR THE SCHOOL WATER QUALITY STUDY

Nine different schools were included in this part of the study. Sampling was conducted twice, once before and once directly after the school vacation (April, 2009). This was done to determine whether the stagnant water deteriorates in quality when it is not in use for a week or two. Schools sampled were divided into three classes as described in the first part of this chapter (Chapter 4). One of the peri-urban schools, Vyfhoek, makes use of a water reservoir for their drinking water. This tank was empty at the end of the vacation – the school had a power failure at the time of sampling and thus water could not be obtained. There is resultantly only one set of data available for this school.

For each of the schools only one tap was sampled, however, samples were collected from each tap as described in Chapter 3 (Section 3.2). The physico-chemical data for the schools are summarised in Figure 4.6. This figure, however, only portrays average values for the schools, actual values are in Tables 4B to 9B (Appendix B).

Figure 4.6 depicts the physico-chemical data for the water sampled from the schools in the Potchefstroom area. Data for the rural schools are depicted in Figure 4.6 (a), in 4.6 (b) peri-urban and in 4.6 (c) urban school data. For the rural schools, there were no major differences in the physico-chemical data for the water samples taken before and after the vacation. Total hardness and carbonate hardness was very high (16°d; 14°d respectively) and high levels of NO_3^- (10mg/L) were present in the water after the vacation. In general, the electrical conductivity was acceptable (52.36-58.93mS/m), but the electrical conductivity of one rural school in particular was higher than the target quality water range both before the vacation (81.0mS/m) as well as after the vacation (101.3mS/m). The data for the peri-urban schools are depicted in Figure 4.6 (b). A generally observed trend was that the levels of the physico-chemical parameters were higher before the vacation than afterwards. Only NO_2^- level was the exception, it was higher after the vacation. The pH of one of these peri-urban schools (Terra Peccana) was high (9.2), and slightly above the standard for domestic use (7-9). Electrical conductivity for water from these schools was acceptable (31.7-46.4mS/m), except for

one school (Vyfhoek) which had an electrical conductivity of 79.9mS/m before the vacation, which is above the standard (0-70mS/m). Nitrate levels were elevated (10-15mg/L) in the water from these schools. Total hardness and carbonate hardness (14-16°d; 12-16°d) measured indicated hard water. Figure 4.6 (c) shows the data obtained for the urban schools. Values for the various parameters were similar before and after the vacation. Electrical conductivity for these schools was also acceptable (64.3-66.6mS/m). Total hardness and carbonate hardness were very high (20°d and 14-16°d respectively) indicating hard water. Most parameters were, however, essentially within their standards specified by DWAF (1996). A general trend observed in this figure is that, with some exceptions, most of the values of the parameters were slightly elevated during March 2009 (before the vacation).

Figures 4.7 to 4.11 portray the microbiological data for the schools. The numbers used to construct the graphs were averages for each school. Actual numbers, averages and standard deviations are shown in Table 12C (Appendix C) to Table 19C (Appendix C). In Figures 4.7 to 4.11, the schools are grouped on the x-axis as follows: Rural schools – Fikadibeng, Mponeng, Sizamele; Peri-urban schools – Loula Fourie, Terra Peccana, Vyfhoek; Urban schools – Boitshoko, Madibeng, Potch Christian. The schools are thus arranged from left to right, the first three being rural, the next three peri-urban and the three at the far right urban schools.

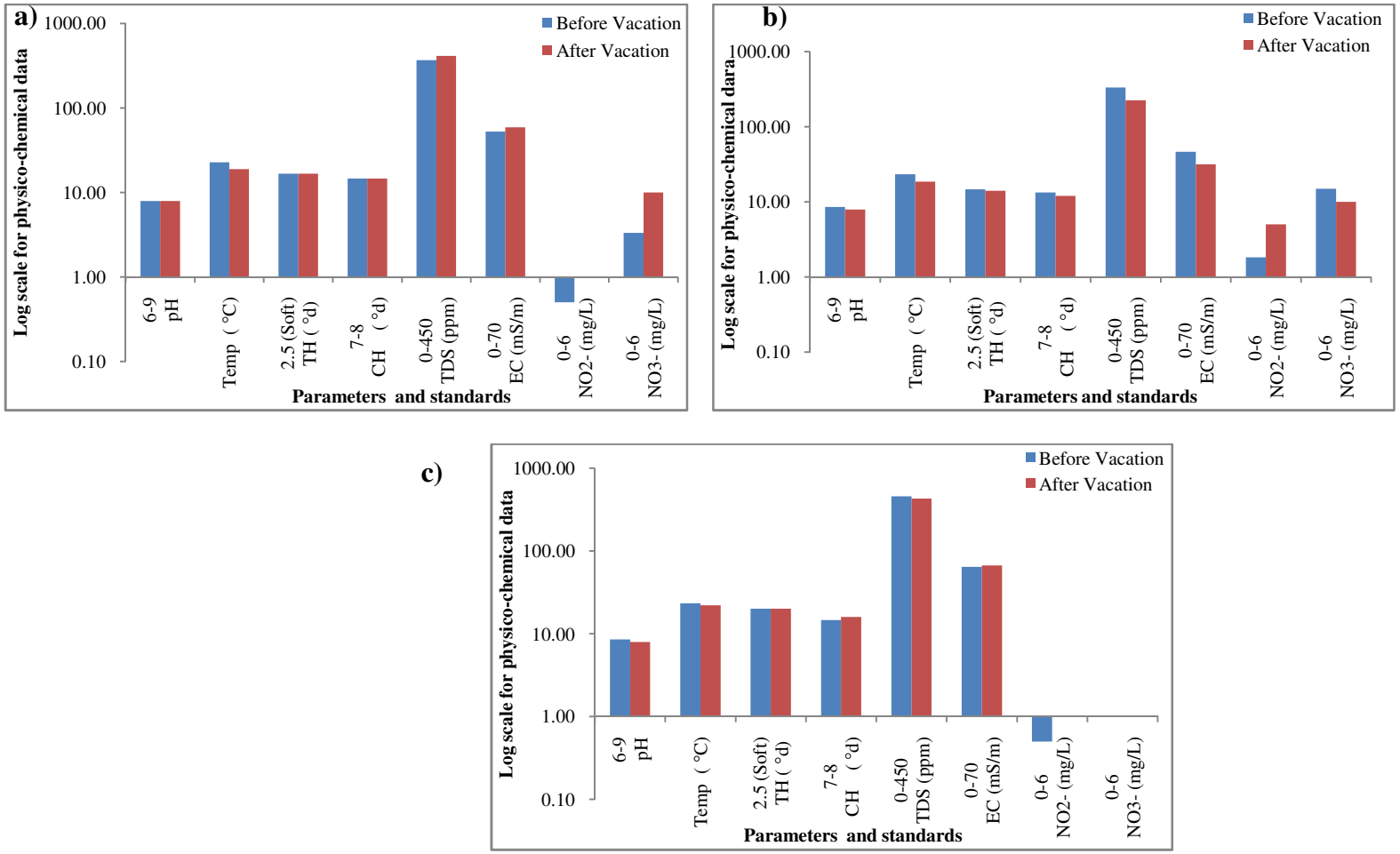

Figure 4.6: a.) Physico-chemical data measured before and after the vacation, for the rural schools. **b.)** Physico-chemical data measured before and after the vacation, for the peri-urban schools. **c.)** Physico-chemical data measured before and after the vacation, for urban schools.

Bacterial counts for total coliforms are shown in Figure 4.7. Counts were generally higher before the vacation than afterwards, but there are three exceptions (two of the rural schools [Mponeng and Sizamele] and one peri-urban school [Terra Peccana]), where the opposite scenario prevailed i.e., bacterial levels were higher after the vacation than before. The highest levels of total coliforms appear to be in the urban schools, although one peri-urban school also showed very high levels of total coliforms.

Figure 4.8 shows the levels of faecal coliforms in the water from the schools. Faecal coliform levels were higher before the vacation, than after the vacation. Two of the urban schools did not have any faecal coliforms at either of the two sampling times. Only three schools had faecal coliforms after the vacation (two rural schools [Mponeng and Sizamele] and one peri-urban school [Loula Fourie]).

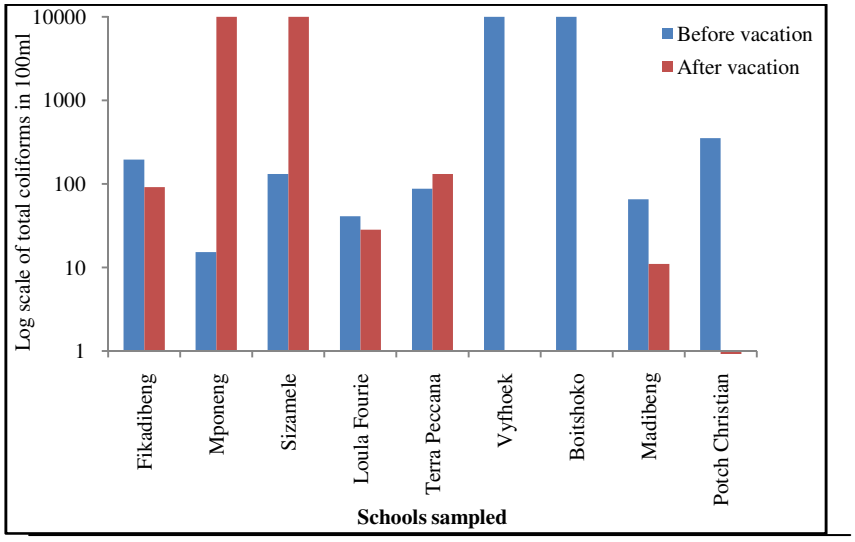

Figure 4.7: The number of total coliform bacteria from the different schools before and after the vacation.

The values before the vacation for Vyfhoek and Boitshoko, and the values after the vacation for Mponeng and Sizamele, determined were too many to count, however they were indicated in this graph as 10 000 cfu/100ml, simply to show the relationship with regards to counts obtained from other sites, and to emphasize these high numbers.

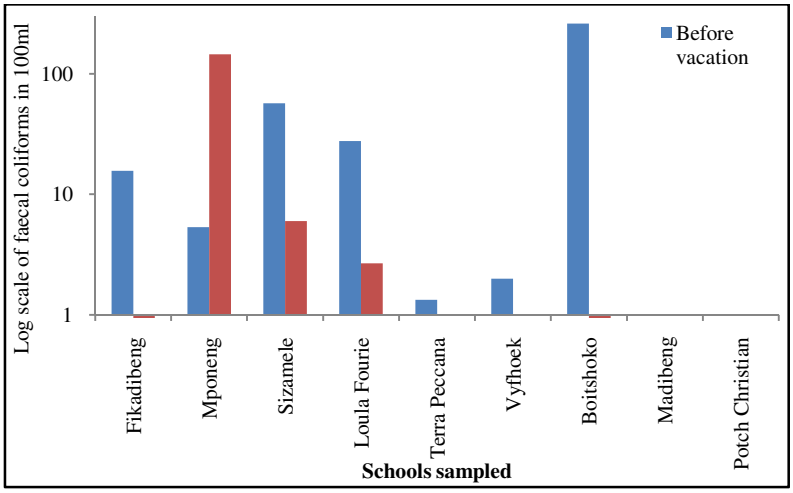

Figure 4.8: The number of faecal coliform bacteria from the different schools before and after the vacation.

Figure 4.9 shows the results for the counts of *Staphylococcus* spp. from the schools. It is demonstrated in these graphs that *Staphylococcus* spp. was present in all samples before the vacation. However, it was only present in three samples after the vacation (water from Mponeng). *Staphylococcus* spp. counts for rural and peri-urban schools were higher than the counts for the urban schools. After the vacation *Staphylococcus* spp. were present in the water of two rural schools (Mponeng and Sizamele) and one peri-urban school (Terra Peccana).

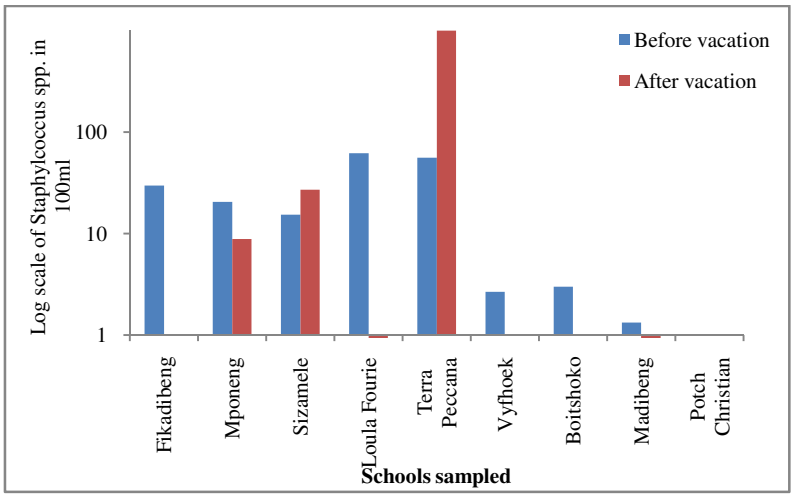

Figure 4.9: The number of *Staphylococcus* spp. from the different schools before and after the vacation.

In Figure 4.10 the counts for faecal *Streptococcus* spp. are represented. In general, faecal *Streptococcus* spp. levels were low. Before the vacation, four of the schools had

faecal *Streptococcus* spp. present in their water (two of which were rural [Mponeng and Sizamele], one peri-urban school [Loula Fourie] and one urban school [Boitshoko]). After the vacation, only two schools had faecal *Streptococcus* spp. present in the water (one rural school [Fikadibeng] and one peri-urban school [Loula Fourie]). Only one school tested positive for faecal *Streptococcus* spp. before and after the vacation (a peri-urban school – Loula Fourie). The urban school (Boitshoko) that showed an occurrence of faecal *Streptococcus* spp. makes use of a water reservoir in order to increase their water flow. Thus although they receive municipal water, the reservoir might have been contaminated, resulting in the occurrence of the *Streptococcus* spp. This is supported by the faecal coliform and *Staphylococcus* spp. data (Figures 4.8 and 4.9).

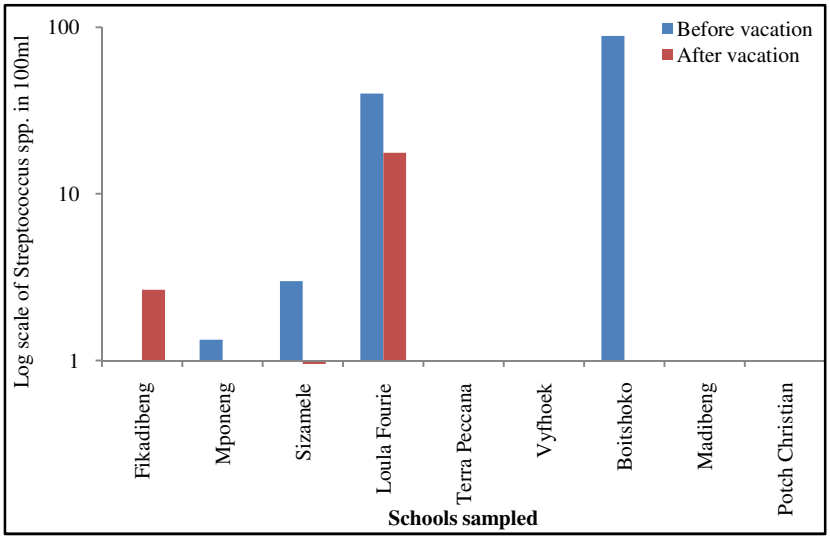

Figure 4.10: The number of faecal *Streptococcus* spp. bacteria from the different schools before and after the vacation.

In Figure 4.11 the heterotrophic plate count bacteria (HPC) levels are depicted. The general trend observed here are higher bacterial counts (one log) in the rural schools than in peri-urban (except for Terra Peccana) and urban schools (except for Madibeng). Levels after the vacation were generally also elevated when compared to the values before the vacation. There were some exceptions. Bacterial counts for urban schools were almost equal to the levels in peri-urban schools.

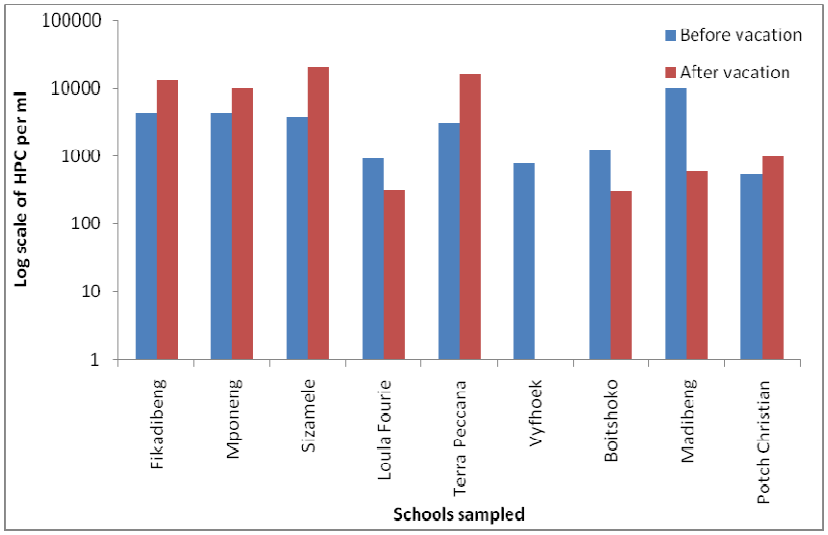

Figure 4.11: The number of heterotrophic plate count (HPC) bacteria from the different schools before and after the vacation.

4.4 IDENTIFICATION OF BACTERIA AND ANTIBIOTIC RESISTANCE PATTERNS

To isolate potential *E.coli*, only blue colonies from m-FC agar were selected for identification. At least 3 colonies per plate were selected, thus 27 colonies were selected for each tap. Primary identification was done by Gram staining and TSI testing (Chapter 3, Section 3.5). Many of the putative *E.coli* isolates were disqualified based on the results of these two tests. Antibiotic susceptibility tests were conducted on the remaining isolates (Chapter 3, Section 3.7). Water samples collected from Ganyesa and the gradient study did not have any representative isolates left after Gram staining and TSI testing was completed. The antibiotic results and API 20E results are thus only for isolates from Sonderwater and the schools. The results for the resistance patterns are shown in Tables 3D to 8D (Appendix D). The numbers of isolates tested for antibiotic resistance for each school are shown in Table 1D (Appendix D). Table 1D show that there were more isolates to be tested for antibiotic resistance before the vacation than after the vacation.

When considering the results for Sonderwater, Tap 2 did not have any representatives. There were only one representative from Tap 1 and Tap 3 each. The isolate from Tap 1 was resistant to amoxicillin, whereas the isolate from Tap 3 showed resistance to

amoxicillin, ampicillin and cephalothin. Complete results for the isolates from Sonderwater are shown in Tables 3D and 4D (Appendix D).

Table 4.2 shows the results of the antibiotic resistance testing for all the schools and complete sets of results for the isolates from the schools is shown in Tables 5D to 8D (Appendix D). This table (Table 4.2) compares the results for the isolates collected before the vacation and after the vacation. Table cells marked ND (not determined) indicate that the isolates for that school could not be classified as potential *E.coli*. Table 4.2 also shows the MAR phenotype for the organisms from each school. This table only shows results for 15 isolates. In Table 1D (Appendix D) it was shown that there were a total of 36 isolates to be tested for antibiotic susceptibility. The reason why there are only results for 15 isolates in Table 4.2 is because only 15 of the 36 in Table 1D presented multiple antibiotic resistance patterns. After the vacation, only two schools had potential *E.coli* and these were also tested for antibiotic resistance. For the samples collected after the vacation, the highest numbers of remaining isolates to be tested for antibiotic resistance were from a peri-urban school.

In Table 4.2 the most prevalent MAR phenotype is AMX-AMP-CEP. However, since amoxicillin and ampicillin are both from the class beta-lactams, this wasn't considered an example of a MAR phenotype. For a MAR phenotype there should be resistance to three antibiotics from three different active groups (Cabrera *et al.,* 2004). MAR phenotypes with resistance to antibiotics from four different active groups were observed in five of the organisms. Resistance to antibiotics from three active groups was detected in three of the organisms. Resistance to antibiotics from only two active groups was detected in seven of the organisms. Thus of the fifteen organisms, only eight portrayed true MAR phenotypes, whereas seven of the fifteen were resistant to antibiotics from only two active groups. Of the organisms with resistance to four active groups, three of the five organisms had the MAR phenotype of AMX-AMP-CEP-OXY-TET-TM. Of all isolates, 46.67% (seven of fifteen) were resistant to trimethoprim. Furthermore, six of the total number of isolates (40%) was resistant to oxytetracycline. Only one of the fifteen isolates was resistant to Kanamycin, and one to Streptomycin.

Table 4.2: The MAR phenotypes for isolates from the schools before and after the vacation. (ND=No MAR phenotypes were presented or there were no isolates to be tested for antibiotic susceptibility.)

School	Before vacation	After vacation
Fikadibeng	AMX-AMP-CEP	ND
	AMX-AMP-CEP	
	AMX-AMP-CEP-TM	
Mponeng	AMX-AMP-CEP	ND
	AMX-AMP-OXY-TET	
Sizamele	AMX-AMP-CEP-OXY-TET-TM	ND
	AMX-AMP-CEP-KAN-TM	ND
Loula Fourie	AMX-AMP-CEP-OXY-TET-TM	AMX-AMP-CEP
		AMX-AMP-CEP
		AMX-AMP-OXY-TET
	AMX-AMP-CEP-OXY-TET-TM	AMP-CEP-OXY-TET
		AMX-AMP-CEP-TM
		AMX-AMP-CEP-STR-TM

After antibiotic resistance testing representatives for each sampling site were selected and identified by API 20E (Chapter 3, Section 3.5). The results for this identification are summarised in Table 4.3.

Table 4.3: The identity of selected representative isolates for each site as determined by API 20E testing.

Site	Identity	Percentage Identification
Sonderwater Tap 1	*Aeromonas* spp.	22.7%
Sonderwater Tap 3	*Enterobacter* spp.	59.4%
Fikadibeng Before vacation	*Klebsiella* spp.	97.6%
Mponeng Before vacation	*Klebsiella* spp.	96.2%
Sizamele Before vacation	*Escherichia coli*	98.6%
Loula Fourie After vacation	*Escherichia coli*	99.9%
Boitshoko Before vacation	*Escherichia coli*	99.9%
Boitshoko After vacation	*Escherichia coli*	99.5%

Table 4.3 summarises the identification results based on API 20E. The results show that there was *E.coli* present in some of the drinking water samples tested. Identification confidence percentages were 98% and higher. Other bacteria identified were *Aeromonas* spp., *Enterobacter* spp. and *Klebsiella* spp. which are all opportunistic pathogens. The three schools that tested positive for *E.coli* were: one rural school, one peri-urban school and one urban school. Identification for the isolates from Sonderwater did not have a very high confidence percentage (22.7 to 59.4%).

4.5 IDENTIFICATION BY 16S PCR AMPLIFICATION

API 20E identities of isolates were confirmed using 16S rDNA sequence data. The results are summarised in Table 4.4. The chromatograms and raw sequences are depicted in Figures 1F to 8F (Appendix F).

Table 4.4: The identities of selected isolates as determined by 16S PCR

Site	Organism	Percentage identification	Accession number	E-value
Sonderwater Tap 1	*Aeromonas sp*. C_PIA_II1	100%	GQ983188.1	3.00E-137
Sonderwater Tap 3	*Enterobacter sp*. PR5	100%	GU086162.1	2.00E-159
Fikadibeng before vacation	*Klebsiella sp*. M-AI-2	100%	FJ828890.2	2.00E-134
Mponeng before vacation	*Escherichia sp*. Clone 7d15222	100%	GU132242.1	1.00E-121
Sizamele before vacation	*Escherichia coli* Strain A	100%	GQ983181.1	4.00E-121
Loula Fourie after vacation	*Escherichia sp*. Clone 7d14285	100%	GU132212.1	1.00E-100
Boitshoko before vacation	*Escherichia sp*. clone 7d15222	100%	GU132242.1	3.00E-173
Boitshoko after vacation	*Escherichia coli* Strain A	100%	GQ983181.1	5.00E-73

The results depicted in Table 4.4 are based on the chromatograms in Appendix (F). These chromatograms indicated that no background noise was present i.e. each of the bases formed a clear peak and could be regarded as a single base signal. The 16S rDNA sequence results confirmed all the API 20E results.

4.6 BIOFILM FORMATION

To determine whether the bacteria present in the water sampled from Sonderwater had biofilm formation potential, this water was allowed to stand in a closed container for one week as described in Chapter 3 (Section 3.8). Examination under the scanning electron microscope (SEM) yielded the micrographs shown in Figure 4.12.

Figure 4.12 shows the biofilm formed in the water from Sonderwater. Figure 4.12 (a) is a 2000x magnification and shows clusters of organisms on the microscope slide

(Chapter 3, Section 3.8). These clusters of organisms are an indication of the initial steps in biofilm formation. Figure 4.12 (b) is a 4000x magnification and shows the filter through which the water was passed. In this figure it is clear that there were a large amount of organisms present in the planktonic phase. This demonstrates that the drinking water from Sonderwater informal settlement could support the regrowth of bacteria and this could thus lead to biofilms formation in their drinking water containers.

a.) **b.)**

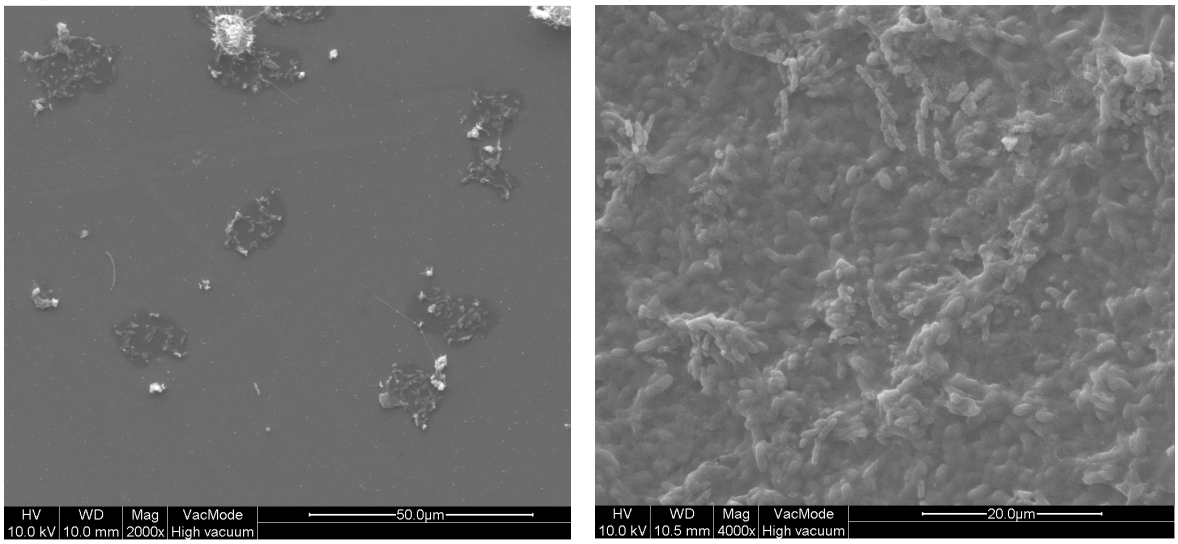

Figure 4.12: a) A scanning electron micrograph of a microscope slide left in the water for one week (2000x magnification). **b)** A scanning electron micrograph of a filter (4000x magnification).

4.7 SUMMARY OF THE RESULTS

Results presented in this chapter revealed that water sampled from Sonderwater and Ganyesa were generally hard water based on the high values measured for total and carbonate hardness. Both groundwater as well as municipal water from Ganyesa showed very high nitrate content. Indicator bacteria, (faecal coliform and total coliform bacteria) were present in some samples from both areas. These faecal coliforms were only isolated during the summer from Sonderwater tap samples. In the water of both Sonderwater and Ganyesa *Staphylococcus* spp. was present. During summer the levels were higher. After 1 week in a closed container, counts increased dramatically during this summer period. Low numbers of *Staphylococcus* spp. were detected in Ganyesa. Faecal *Streptococcus* spp. was generally detected in low numbers in both areas. HPC bacteria were prevalent in large numbers in samples from both areas. In Sonderwater, the HPC counts were higher during the summer than during winter. The highest numbers of HPC were isolated from groundwater samples of Ganyesa.

A settlement gradient study demonstrated that water obtained from all areas of Potchefstroom were of similar physico-chemical and microbiological quality, and that there was thus no gradient in water quality. Water from all sites was very hard. In all the areas sampled, the levels of HPC bacteria were high. As for *Staphylococcus* spp., they were only detected in large numbers in one of the four areas.

Schools in the Potchefstroom area generally had hard water and there were no physico-chemical variation between water sampled before and after the vacation. The schools that received groundwater had alarmingly high levels of nitrates present in their water. Total coliform bacteria were present in high numbers in the drinking water of all the schools. With three schools the exception, higher levels of total coliforms were detected before the vacation than afterwards. Indicator bacteria were present in high levels and many faecal coliforms could be isolated from the water of the schools. The general trend for the faecal coliforms was that the levels were higher before the vacation than after the vacation. *Staphylococcus* spp. was present in all samples taken before the vacation. After the vacation *Staphylococcus* spp. were detected only in the rural and peri-urban school water samples. Low levels of faecal *Streptococcus* spp.

were detected in the water from the schools. HPC bacteria were present in large numbers. Rural schools had higher levels than peri-urban and urban schools. The latter two groups had the same levels of HPC.

The main trend in the antibiotic resistance patterns was the resistance to amoxicillin, ampicillin, cephalothin, oxytetracycline and trimethoprim. Organisms with MAR phenotypes with resistance to antibiotics from four different active groups were observed in five of the organisms. Resistance to antibiotics from three active groups was detected in three of the organisms. Resistance to antibiotics from only two active groups was detected in seven of the organisms. Thus of the fifteen organisms, only eight portrayed true MAR phenotypes. Identification by API 20E strips indicated isolates were *Escherichia coli, Klebsiella* spp., *Enterobacter* spp. and *Aeromonas* spp. API 20E results were confirmed by 16S rDNA gene sequence data. Scanning electron microscopy examination of water samples from Sonderwater demonstrated the biofilm formation potential of bacteria present in the water. Such a property is also associated with pathogenicity.

CHAPTER 5

DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

The effective physico-chemical and microbiological control of water is essential for implementing efficient management of this imperative resource (Angle's d' Auriac *et al.*, 2000). Poverty and poor water quality are closely intertwined in developing areas of countries such as South Africa. Poor people lack the technological assets to assess the quality of water, or contribute to water infrastructure and are thus typically deprived of the benefits of good quality water (Schreiner and Van Koppen, 2002). Lack of efficient access to available resources may force many to drink water of inferior quality. During droughts this phenomenon is especially prevalent (Schreiner and Van Koppen, 2002). This is especially true for people in rural and informal areas. The present study compared the microbiological and physico-chemical quality of drinking water of formal and informal urban communities, rural community as well as urban, peri-urban and rural schools in the North West Province of South Africa.

5.1 SONDERWATER AND GANYESA

In the study of the water quality of Sonderwater and Ganyesa, (urban informal and rural communities respectively), it was found that water was generally classified as hard based on total hardness and carbonate hardness measured by a dip strip method (Merck, Germany). The pH, nitrite and total dissolved solids (TDS) and electrical conductivity for the water samples from these areas were all within the standards for domestic use (Figure 4.1, Table 1B and 2B). However, nitrate levels for some of the samples were slightly to extremely high. In the case of Sonderwater samples, nitrate levels detected differed considerably between summer and winter. Nitrate content measured for the summer was done using a dip-strip method (Merck, Germany), whereas a nitrate detector probe was used for the winter samples. The values of the winter samples could be regarded as more reliable, since the method using a probe is more accurate than the dip-strip method. The nitrate levels detected during winter were slightly higher (6.3-6.6mg/l) than the maximum (6.0 mg/l) for domestic use (SANS 241:2006). This water is municipal supplied and the Tlokwe City Council has Blue Drop certification for its drinking water quality and management (Blue Drop Report, 2009). The cause of this

slightly higher nitrate levels is not known but would not have any health implications for consumers.

On the other hand, some water sampled from Ganyesa had very high nitrate content (623-903 mg/l). This is cause for concern since this could be health injuring. Levels above 20 mg/l could cause methaemoglobinemia in infants and mucous membrane irritation in adults (DWAF, 1996). Furthermore, continuous consumption of water with such high nitrate levels could also lead to other medical conditions such as hypertrophy of the thyroid, gastro-intestinal cancer, Alzheimer disease, and Non-Hodgkin's lymphoma amongst others (Suthar *et al.*, 2009).

Gustafson (1983) found that sandy soils leach more nitrates to groundwater than clay soils. It might thus be the cause for the elevated nitrate content of the water samples from Ganyesa as well as the high TDS values observed in these samples. The Ganyesa community relies mainly on groundwater as their source of drinking water. The NWDACE (2007) found that for a period from 2002 to 2005 the nitrate concentrations in groundwater of certain areas of the North West Province were higher than that of surface water. Other than the soil type of the province, another possible cause for elevated nitrate levels in the water may also be the leaching from nitrous fertilizers (Umezawa *et al.*, 2008). Ganyesa is a rural community where subsistence agriculture is a large part of the community and how their income is generated. The lack of commercial farms near the sampling site of this study indicates that the geology of this region may have a greater impact on the nitrate levels in the drinking water than leaching from nitrous fertilizers. Furthermore, although not tested it is speculated that pit latrines or septic tanks may also have had an impact on the elevated nitrate levels (Wakida and Lerner, 2005). Results from a study in the Marondera district of Zimbabwe (Dzwairo *et al.*, 2006) indicated that pit latrines were microbiologically impacting on groundwater quality. The study also found that nitrate levels were elevated in some areas, but in general the levels were of no immediate threat to health. This could potentially be a similar scenario for Ganyesa and should be investigated in future studies.

A study by Choi *et al.* (2007) in rural areas of Korea showed the different amounts of nitrate contamination of groundwater in areas of mixed land-use activities. Cropping areas, cropping-livestock farming complex areas and residential areas had nitrate concentrations exceeding the national standard for drinking water. The percentage of samples that had a nitrate concentration exceeding this limit was 23, 43 and 67% respectively for the areas as mentioned above (Choi *et al.*, 2007). Thus the samples exceeding the limit for nitrate content in the water was in the residential areas. This observation is supported by Nyenje *et al.* (2010) that demonstrated the impact of a sub-Saharan mega city on leaching of nitrates and other microbial nutrients into groundwater sources. Ganyesa is a rural town and mixed land-use practices exist. The studies of Choi *et al.* (2007), Wakida and Lerner (2005) and that of Nyenje *et al.* (2010) all found high nitrate levels in their water. Their findings support the observations of high nitrate levels in the water from Ganyesa that was made in the present study.

Total dissolved solids measured for the water from Ganyesa were higher (397-599 mg/l) than the recommended standard for domestic use (450mg/l). These values were, however, lower than the critical value of 2450mg/l (Kempster *et al.,* 1997). In South Africa other regions also exist where the TDS levels are higher than the standard for domestic use. The North West Province is one of these regions where the TDS is high. This results from natural causes as well as human impact (NWDACE, 2008). The State of the Environment Report (2002) recognized that the geology of the North West Province, which consists of yellow shifting sands, could be a major cause of the high TDS values measured in the water from Ganyesa.

The microbiological quality of the water samples from Sonderwater and Ganyesa was also determined using faecal indicator bacteria (faecal coliforms and faecal streptococci), total coliforms, heterotrophic plate count bacteria and *Staphylococcus aureus* levels as parameters. Faecal coliforms were only detected in the summer water samples from Sonderwater. Some of the species detected were identified as *E.coli*. This is cause for concern. The presence of total and faecal coliforms in drinking water may indicate direct, recent faecal pollution of the water (DWAF, 1996) and suggests the possible presence of other pathogens which could transmit enteric diseases (Barnes

et al., 2004). The standard for domestic use for faecal coliforms is 0cfu/100ml. Thus detection of faecal coliforms exceeds the standard. Levels higher than 20 cfu/100ml pose a significant and increased risk of infectious disease transmission. The effects of different levels of faecal coliforms in drinking water are discussed in Table 3E (Appendix E). As faecal coliform levels increase, the amount of water ingested required to cause infection decreases (DWAF, 1996). Faecal coliforms were detected in only one of the winter water samples from Ganyesa, but none of the samples from Sonderwater. This low detection of faecal coliforms among winter samples could be ascribed to the lower environmental temperatures of the water, particularly in the case of the Ganyesa samples that were mainly groundwater samples and sampling was done in winter. Future studies should sample during both summer and winter in this region to determine whether seasonal variation in the total coliform and faecal coliform levels exist.

Faecal *Streptococcus* spp. was detected in low numbers in the samples from both areas. *Streptococcus* spp. is used as an indicator organism for faecal contamination of drinking water (Gildreich and Kenner, 1969). Determination of the faecal coliform/*Streptococcus* spp. ratio may indicate whether the faecal contamination is of human or animal origin. If the ratio is less than 0.6, the contamination may be of animal origin. If the ratio is more than 4.0, the contamination may be of human origin. When the ratio is between 0.6 and 4.0, this means that the contamination is from a mixed origin, in other words, both human and animal (Gildreich and Kenner, 1969). Only one of the tap water samples from Ganyesa tested positive for both *Streptococcus* spp. and faecal coliforms. This tap (Tap 2) was supplied by municipal water. The faecal coliform/*Streptococcus* spp. ratio determined for this tap was 5.0, which indicates faecal pollution potentially from human origin (Table 20C). However, since this result was only found in one tap and the study was only conducted once, no conclusive results were obtained. The water should be retested before any certain conclusions can be made. It can thus only be used as an indication that further studies are needed for determination of the quality of this water. The results from the present study demonstrate that some of the samples were positive for faecal indicator bacteria. A question that arose was whether this was not due to contamination of the taps. These

taps, however, were heat sterilized as indicated in one of the photos in Appendix G (Figure 5G) and it is unlikely that the contamination could have come from the taps.

Total coliform count is a useful indicator of water pollution. The presence of total coliforms in water may be used as an indicator of faecal pollution, since total coliforms are excreted from warm blooded animals (Grabow and Du Preez, 1979). It is, however, not as specific as the isolation of faecal coliforms since many species are also found in soil and aquatic environments. It is, however, considered a gold standard when used as a sanitary parameter for evaluating the quality of drinking water (Pavlov *et al.*, 2004). This is so because water that presents high levels of total coliform bacteria, often cause diseases such as diarrhoea and fever (Zamxaka *et al.*, 2004). Other health risks associated with different levels of total coliforms in drinking water are discussed in detail in Table 4E (Appendix E). This is important for the present study as total coliform levels were relatively high in the water from Sonderwater and Ganyesa. The standard for domestic use for total coliforms in drinking water is 0 - 5cfu/100ml (DWAF, 1996). If levels are higher than 100 cfu/100ml, then the water poses an increased risk of infectious disease transmission (DWAF, 1996). Levels of total coliforms in the water from Sonderwater were above this range of 5cfu/100ml and may thus, pose a threat to consumers. Water from Ganyesa had total coliform levels between 3 and 29 cfu/100ml. These values were also higher than the standard for domestic use. These findings, i.e. high total coliform levels and detection of faecal coliforms and faecal streptococci are cause for concern. Detection of faecal coliform bacteria from the Sonderwater summer samples might also be an indication of faecal pollution of the water and the non-detection of these coliforms during the winter does not necessarily mean that there were none present. Besides the low temperature of the water, Geldreich *et al.* (1978) and Allen *et al.* (2004) also demonstrated that high levels of heterotrophic bacteria, as is the case for Ganyesa, could desensitize the detection of faecal coliforms.

Martin-Dominquez *et al.* (2005) did a study on the quality of water in rural communities in Mexico. They found that only 25% of samples did not present total coliforms contamination, and that 66% of samples did not present *Escherichia coli*

contamination. The study also showed that 17% of the samples had total coliforms present in the range 2-100 MPN/100ml, and that 21% of the samples had 2-100 MPN/100ml of *E.coli* (MPN/100ml is most probable number in every 100ml of sample). Martin-Dominguez *et al.* (2005) also found that 37% of the samples showed total coliform levels in the range 101-2419 MPN/100ml and 12% of the samples had *E.coli* in the same range. This range is considered high contamination levels and coincides with the findings in Sonderwater and Ganyesa which also showed high contamination levels in their samples.

In the water samples from both Sonderwater and Ganyesa, high levels of HPC bacteria were detected. Amongst the Sonderwater samples, the HPC counts were higher during the summer than during winter. For the Ganyesa samples, the highest numbers of HPC bacteria were isolated from groundwater. Only one of the taps sampled from Ganyesa had HPC within the standard for domestic use of 0 – 100cfu/ml (DWAF, 1996). All other tap water samples from Ganyesa, as well as the water samples from Sonderwater, were above the standard for domestic use. Bacterial counts in the range of 100 – 1000 cfu/ml (DWAF, 1996) may pose a slight risk of causing a microbial infection in people who drink the water. The bacterial counts found in water from Sonderwater and Ganyesa were above this range, posing a risk of infection to consumers.

Geldreich *et al.* (1978) found that levels of HPC bacteria in the range of 500 – 1000 cfu/ml desensitize the detection of coliform bacteria and *E.coli* when the membrane filtration technique is used. These findings were confirmed by Allen *et al*. (2004) and may be of importance to the present study. Allen *et al.* (2004) stated that in the late 1980s, Edberg *et al.* (1988) provided a method for the simultaneous enumeration of coliforms and *E.coli* which was not subject to HPC interferences. This method is referred to as the Defined Substrate Technology method (Edberg *et al.*, 1988; Allen *et al.*, 2004). The Defined Substrate Technology method is based on the principle that only the target microbe, e.g. faecal coliforms, can utilize vital nutrients from the media (Rompré *et al.,* 2002). Use of this method resulted in greater confidence that negative coliform or *E.coli* results for drinking water tested reveal a more accurate microbiological quality of the water. Allen *et al.* (2004) reported that counts of

heterotrophic bacterial populations in UK water supplies are used as secondary indicators of general water quality. Typically counts in UK water supplies are low (Allen *et al.*, 2004). Allen *et al.* (2004) also stated that it is imperative that HPC analysis be performed in parallel with faecal coliform determination. It is a quality assurance approach which ensures that coliform data, especially negative results accurately reflect the true microbial quality of the tested water. Should a very low level of faecal coliforms be detected in any given sample, then high levels of HPC bacteria would still be an indication of polluted water. It is speculated that this may be so because the high density of HPC possibly repressed the detection of the faecal indicator organisms (Allen *et al*., 2004).

Heterotrophic plate count bacteria may not be as harmless as previously thought (Payment *et al.*, 1991). In a South African study, potentially pathogenic features of HPC isolates were analysed by Pavlov *et al.* (2004). Features analysed included haemolysis, potential adherence to human cells, invasiveness of human cells, as well as production of enzymes responsible for bacterial virulence. Pavlov *et al.* (2004) concluded that HPC isolates from drinking water samples were invasive to human cells and are thus potentially pathogenic to humans, especially the immune-compromised people in a community (Pavlov *et al.*, 2004).

In the water from both Sonderwater and Ganyesa, *Staphylococcus* spp. was detected. The numbers of *Staphylococcus* spp. in the water samples from Ganyesa were low. After 1 week of storage in a closed container, counts increased dramatically during the summer period for samples from Sonderwater. However, the high levels of HPC bacteria that were detected in the drinking water from Ganyesa, might also have desensitized the detection of *Staphylococcus* spp. in the water.

Previous studies detected *Staphylococcus* spp. within South African chlorinated drinking water (Muyima *et al.,* 1997; Pavlov *et al.,* 2004). The study of Muyima *et al.* (1997) was conducted in Alice, Eastern Cape and that of Pavlov *et al*. (2004) in random treated and untreated drinking water in South Africa. In both cases the municipal

supplied drinking water samples were obtained from taps in the distribution systems. Thus finding *Staphylococcus* spp. in the water samples from Ganyesa and Sonderwater was not unusual. Pavlov *et al.* (2004) found that the *Staphylococcus* spp. isolated during their study also produced disease-causing extracellular enzymes. Based on such enzymes those organisms were regarded as virulent (Pavlov *et al*., 2004).

Another aspect that was tested was the ability of the heterotrophic bacteria in the water samples from Sonderwater to form a biofilm. Using scanning electron microscopy this was demonstrated. The micrographs showed that after only one week in a closed container, there were already clusters of organisms formed on the surface of a microscope slide left in the water. This is the first step in biofilm formation (O'Toole *et al.*, 2000). When the water was filtered and the filter examined by SEM, it also showed individual organisms as well as clusters present on this filter. The fact that these bacteria thus have the potential to form biofilms, poses a potential threat to the health of consumers for numerous reasons. The role of biofilm development by bacteria has been suggested as an important stage in the pathogenesis of numerous bacterial species (Costerton, 1999; Watnick and Kolter, 2000; Donlan, 2001). Lavender *et al.* (2004) suggested that the establishment of biofilms by pathogenic bacteria on the tissues of susceptible hosts is believed to inhibit the effectiveness of antibiotic treatment, protect against host defence mechanisms and facilitate bacterial communication leading to expression of virulence determinants. This means that biofilm formation poses a serious threat to humans. Momba *et al.* (2000) stated that although detection of coliform bacteria is considered the primary concern when determining water quality, attention should also be directed towards the general bacterial population as many of these heterotrophic bacteria have been related to secondary opportunistic pathogens in humans.

In 2002 Momba and Kaleni conducted a study on the survival and regrowth of indicator organisms on the surfaces of household containers used for the storage of drinking water in rural communities in South Africa. They found that various bacteria grew on these surfaces, such as total coliforms as well as *E.coli*. They also found that regrowth of indicator organisms occurred within 48 hours on the surfaces of these containers.

Residents of Sonderwater are dependent upon communal taps for their household water and thus have to store their water in their homes for extended periods before consumption. These conditions may be ideal for biofilm formation. Results showed that on glass surfaces biofilm formation start within 48 hours. The results from the present study showed growth of total coliforms, faecal coliforms and *Staphylococci* spp. after one week in a closed container. These organisms were thus present after 48 hours in the containers, implying that observations made were supported by those reported by Momba and Kaleni (2002).

Lehloesa and Muyima (2000) reported on the groundwater supplies in a rural community in the Eastern Cape. The bacteria detected in the water in their study included heterotrophic bacteria, total coliforms, faecal coliforms and faecal streptococci. The total coliform counts in their study were high in general, and faecal coliforms detected were confirmed in many cases to be *E.coli*. Overall the microbiological water quality of this community was ruled as poor or unacceptable quality according to South African standards (Lehloesa and Muyima, 2000). There are also other South African case studies that demonstrate that water that provided to, particularly, rural communities were not of sound microbiological quality (Obi *et al.*, 2002; Zamxaka *et al.*, 2004). Obi *et al.*, (2002) did a study in rural Venda communities in South Africa, and found that the microbial quality of the water sources was poor and unacceptable for human consumption due to faecal pollution. A study by Zamxaka *et al.*, (2004) found that water from selected rural areas in the Eastern Cape was not fit for human consumption prior to sterilization. Parameters of concern were microbial contamination and turbidity. Ganyesa is a rural community supplied by groundwater although certain sections of the town receive municipal supplied treated drinking water. The water quality for this town may need a more elaborate monitoring programme.

5.2 THE SETTLEMENT GRADIENT STUDY

The once-off settlement gradient study was conducted in Potchefstroom only. It included an established urban area, one established informal settlement area (Sonderwater) and newly established formal as well as informal areas. The results showed that the water quality for physico-chemical and microbiological parameters

were similar at all sampled sites. The pH, electrical conductivity, nitrates and nitrites were all within the SANS 241 (2006) standards. Total dissolved solids measured were above the standard for domestic use, which may indicate treatment inefficiencies. The values, however, were lower than the critical value of 2450mg/l (Kempster *et al.*, 1997). Drinking water from all areas in Potchefstroom can be classified as hard water according to the measured values for total and carbonate hardness. This is not uncommon and has been reported in previous studies (Vos, 2007; Walter, 2009).

In the water collected for the settlement gradient study, no faecal coliforms were detected. Total coliforms were detected but in low numbers. In three of the four areas, the levels detected for total coliforms were within the standard. The established informal area (Area 2 - Sonderwater) had very high levels of total coliforms present in the water. A finding of high total coliform levels for water samples from Sonderwater during the settlement gradient study reflected the findings of the summer and winter sampling sessions discussed above. Large numbers of *Staphylococcus* spp. were detected. Also HPC bacteria were detected in levels higher than the standard limit of 0-100cfu/ml.

Since the water from the settlement gradient study had high levels of HPC and staphylococci present, there is a possibility that there could have been faecal coliforms and possibly *E.coli* present in this water. Potential reasons for this were discussed in the preceding section (Section 5.1). Considerations and health implications of these findings were also discussed in that section and will not be repeated here, since the same arguments hold true. A take home message from this study is that the water supplied to various communities in Potchefstroom was of similar quality when only the physico-chemical is concerned. There may be differences in the microbiological quality but faecal indicator bacteria were rarely detected in the bulk water and when detected, may be species other than *E.coli*.

5.3 RURAL, PERI-URBAN AND URBAN SHOOLS IN THE POTCHEFSTROOM AREA

Schools in the Potchefstroom area generally had hard water according to the total and carbonate hardness measured. Eight of the nine schools had a pH within the drink

water standard (pH 6-9) (SANS 241:2006). One peri-urban school (Terra Peccana), however, had a pH value that was slightly higher (pH 9.2) than the SANS 241 (2006) standard (pH 6-9) before the vacation. A pH range of 9-11 has a probability of toxic effects associated with deprotonated species increase sharply. Water also tastes bitter at a pH greater than 9 (DWAF, 1996). Four of the schools had a TDS value higher than the SANS 241 (2006) standard (450mg/l), but averages for the rural, peri-urban and urban schools were within the standard for domestic use. Electrical conductivity for the schools was within the standard, except for two schools (one rural [Fikadibeng] and one peri-urban [Vyfhoek]) which had a higher electrical conductivity. These two schools were also amongst the four schools with a higher TDS value (559 to 719 ppm), which is expected since these two parameters go hand in hand. The nitrite (NO_2^-) levels for all schools were within the acceptable standards (<6 mg/l). The two schools which presented TDS and electrical conductivity levels above the SANS 241 (2006) standard, makes use of groundwater. Since these schools are in the North West Province of South Africa, the higher TDS values are to be expected in the groundwater, potentially because of the geology of the province, as discussed in Section 5.1.

Schools which receive groundwater had alarmingly high levels of nitrates (NO_3^-) present (10-25 mg/l). This is expected since the groundwater in the North West Province tends to have high levels of nitrates present, possibly as a result of the province's geology (State of the Environment Report, 2002). The fact that the schools are all farm schools means that they are situated in an agricultural environment. As discussed in Section 5.1, this may have an effect on the nitrate levels in the groundwater. The nitrate levels in this water may thus pose the threat of methaemoglobinemia to the children as discussed in Section 5.1. Other than the nitrates, there was no variation between water sampled before and after the vacation in terms of physico-chemical parameters.

Samwel and Gabizon (2009) reported on the health of school children in the new European member states. According to their findings, little data were collected or known about the access to adequate water supply and sanitation for public institutions such as schools. Their study reported on the issues of water and sanitation in rural

areas of the new assessed European countries such as Romania and Bulgaria amongst others. They (Samwel and Gabizon, 2009) reported that in those rural areas the worst conditions of school sanitation was the pollution of groundwater through the infiltration of nitrates and microorganisms. It was also noted that in the European region, a lack of safe water and adequate sanitation has been recognized as a major cause of child mortality, especially in the eastern countries (Samwel and Gabizon, 2009). WASH Bulgaria (2004) did a survey on the status of school sanitation and hygiene and found that 21% of the schools (32% of settlements) are served on planned intermitted water supply. They found that these schools do not have water supply for 2-3 hours and sometimes even during the entire time the children spend in school.

In the present study faecal indicator bacteria were present in high levels and many faecal coliforms were isolated from the drinking water from the schools. Only one of the urban schools had faecal coliforms, and after the vacation, none of the urban schools had any faecal coliforms present. The general trend for the faecal coliforms was that the levels were much higher before the vacation than after the vacation. A lack of adequate sanitation often causes leaching of microorganisms into the groundwater which is then used as drinking water (Samwel and Gabizon, 2009). The lower levels of faecal coliforms after the school vacation may thus be an indication that in these schools the sanitary facilities may leach into the groundwater. These facilities were not used during vacations resulting in lower levels of faecal coliforms in the water. Many of the schools make use of plastic containers (5000l Jo Jo tanks) to store the water from boreholes. These containers may pose a health risk as Momba and Kaleni (2002) demonstrated that regrowth of indicator organisms such as total coliforms and *E.coli* occur on the surfaces of household containers after only 48 hours (as discussed in Section 5.1). A vacation of two weeks may thus pose the risk of biofilm formation of indicator organisms in these containers. These biofilms may then be dislodged once the tanks are in use.

Low levels of faecal *Streptococcus* spp. were detected in the water from the schools. Detection of *Streptococcus* spp. is also an indication of faecal contamination of water (Gildreich and Kenner, 1969). The faecal coliform/*Streptococcus* spp. ratio can be used

as an indication of the possible origin of faecal contamination, as mentioned in the previous section (Section 5.1). Before the vacation, the rural schools all had a ratio of more than 4.0 which indicates faecal contamination of possible human origin. After the vacation, two of the schools had a ratio above 4.0 and one had a ratio of less than 0.6. In the latter case the pollution was possibly of animal origin. The peri-urban schools had ratios between 0.6 and 4.0 and faecal pollution may thus be of both animal and human sources. One urban school that showed a presence of faecal contamination had a value of 2.9, which also indicates faecal pollution of a combination of animal and human origin (Table 20C). In these areas there may thus be both animal and human faecal pollution. Sanitary facilities of schools should thus be monitored. Many of these schools are also farm schools, and there is a real threat of animal excreta leaching into groundwater and contaminating drinking water resources.

Total coliform bacteria were present in relatively high levels in all the water samples from the schools. Levels above 100cfu/100ml were detected and could mean that there are increased risks of infectious disease transmission. The general trend observed here was that higher levels were detected before the vacation compared to after the vacation. However, there were three exceptions (two rural schools and one peri-urban school). Total coliforms are useful as a sanitary parameter for evaluating the quality of drinking water (Pavlov *et al.,* 2004); this water thus poses a threat to the wellbeing of the children of these schools (Zamxaka *et al.*, 2004).

HPC bacteria were also present in large numbers and, in general, rural schools showed higher levels than peri-urban and urban schools. The latter two groups had similar HPC levels. Another trend observed was that HPC counts were higher after the vacation for rural schools, but higher before the vacation for peri-urban and urban schools. The use of plastic containers for water storage might be the cause for the higher levels of HPC bacteria after the vacation (Momba and Kaleni, 2002). Biofilms that developed during the vacation might have been dislodged. All schools had HPC counts above the standard for domestic use, which indicates potentially polluted water. HPC counts were in the range (500-1000 cfu/ml) where the amount of HPC present desensitizes the

detection of faecal coliforms through the membrane filtration method as described in Section 5.1 (Geldreich *et al*., 1978).

Staphylococcus spp. was present in all samples taken before the vacation. In general, levels were higher in rural and peri-urban schools than for urban schools. After the vacation only some of the rural and peri-urban school samples tested positive for *Staphylococcus* spp.. The presence of these organisms poses a serious health risk to the children since certain *Staphylococcus* spp. is known human pathogens. As mentioned earlier, regrowth of total coliforms and *E. coli* occur after only 48 hours. Their presence might indicate the presence of *Staphylococcus* spp. Momba *et al.* (2000) also found that biofilm formation by heterotrophic bacteria enable opportunistic pathogens such as *Staphylococcus* spp. to more easily adhere to the biofilm and thus present a health risk to consumers. These processes might once again occur in the containers used to store the groundwater, or even in pipelines transporting the water.

Samwel and Gabizon (2009) reported that many of the rural schools of Bulgaria and Romania had such poor sanitation that the excreta of the children leached into the groundwater which is also used as drinking water. Microbial contamination of drinking water has also been recognized as a prime concern throughout the European region (WHO, 2008). It was also found that better management of water and sanitation could prevent over 30 million cases of water-related disease per year in the region (WHO, 2008). Nikolić *et al.* (2008) conducted a study on water quality in 12 schools in a suburban area in Serbia. These schools were mostly supplied with groundwater. The analysis showed that 83.8% of samples were bacteriologically contaminated and 36.8% of the samples had chemical incorrections. They also found that drinking water quality has become and ecological risk on the health of school children (Nikolić *et al.*, 2008). These findings, although in other countries, do support the findings of the present study, i.e. that water supplied to school children may be contaminated by microorganisms and chemicals that are of faecal origin.

Microbiological evidence based on heterotrophic plate count bacteria, total coliforms, faecal coliforms, streptococci, staphylococci data and biofilm potential, indicate that

water supplied to schools may not always be fit for human consumption. This is cause for concern. What is further worrying is that little data are available for water quality in South African schools. Most schools in rural areas in the country are supplied with groundwater, even though quality data for the source water is not available. Examples of studies listed (Nikolić *et al.*, 2008; Samwel and Gabizon, 2009) have demonstrated that quality of groundwater supplied to rural schools in Europe and Asia were poor, due to faecal contamination. This may thus also be the case for schools in the North West Province. An urgent detailed study on water quality in the schools supplied with groundwater should be undertaken.

There have, however, been some studies on the nutrition and associated health effects in children from informal settlements in South Africa (Kruger *et al.*, 2010). Such studies have resulted in interventions. The purpose of the proposed study on water quality issue in schools in the North West Province should also aim at resulting in appropriate interventions.

5.4 IDENTIFICATION AND CHARACTERIZATION OF ISOLATES

Primary identification tests (Gram staining and TSI tests) were used to preliminary identify faecal coliforms. Selected faecal coliforms were then identified by API 20E strips. There bacteria were identified as *Escherichia coli, Klebsiella* spp., *Enterobacter* spp. and *Aeromonas* spp. One representative belonged to the genus *Pantoea*. Identities were confirmed by 16S rDNA gene sequences analysis (Wagner and Cloete, 2002). The sequencing procedures rendered clear chromatograms without any background, and Blast analyses supported the API 20E identification. Identification of these organisms is very useful, as it may be used as an indication of the possible health risks it may pose to consumers of the water. Allen *et al.* (2004) stated that the public health significance of *Klebsiella* in water is an important concern. Serious *Klebsiella* infections were commonly seen in hospital patients whose resistance has been impaired by their primary disease condition. Furthermore, a small percentage of *Aeromonas hydrophila* isolates can cause gastroenteritis and enteritis as well as modest, self-limited infections (Allen *et al.*, 2004). *E. coli* was present among the faecal coliform isolates. Drinking water should be free from any *E. coli*. According to SANS 241 (2006), Class

I drinking water should be 0 cfu/100ml for *E. coli*. The presence of these specific bacterial species supports the conclusion that some of the drinking water sources tested was contaminated by faecal matter.

These faecal coliform organisms were then tested against several antibiotics. One main trend in the multiple antibiotic resistance patterns for the isolates from the schools was the resistance to amoxicillin, ampicillin and cephalothin, oxytetracycline and trimethoprim. The peri-urban school had isolates with resistance to 4 active antibiotic groups, as did one of the rural schools. The other rural schools had isolates with resistance to only two or three active antibiotic groups. Isolates from Sonderwater were also resistant to amoxicillin and one isolate from Sonderwater was resistant to amoxicillin, ampicillin and cephalothin (all three β-lactam antibiotics). Of all the isolates tested for antibiotic resistance, 47% showed resistance to trimethoprim and 40% were resistant to oxytetracycline. This is very important data since these antibiotics are often used in primary health care. If antibiotic resistance data is known to local clinics, appropriate antibiotics could be prescribed for successfully treating infectious diseases.

Over the past few years, the antibiotic resistance dilemma has increased dramatically and the problem is getting worse by the misuse and overuse of all kinds of antimicrobial agents and environmental pollutants (Pfaller *et al*., 1998). Ateba and Bezuidenhout (2008) did a study on the antibiotic resistance of *E.coli* isolated from pigs, cattle and humans in the North West Province. They found that the largest proportion of isolates from pigs, cattle and humans were resistant to tetracycline, erythromycin and sulphametoxazole. They (Ateba and Bezuidenhout, 2008) concluded that the level of multiple antibiotic resistant *E.coli* was high in all samples examined. The resistance patterns determined in this study are of importance since data on antibiotic resistance patterns in rural communities in the North West Province is underdetermined.

5.5 CONCLUSIONS AND RECOMMENDATIONS

5.5.1 Conclusions

The present study aimed at determining water quality of selected communities in the North West Province, South Africa. Objectives for the study are described in Section 1.3. These objectives were rephrased as headers and utilized for drawing final conclusions.

5.5.1.1 Physico-chemical and microbiological quality of drinking water from Sonderwater

Physico-chemical parameters for Sonderwater were determined and the implications of these parameters to the human consumers were demonstrated. Levels of faecal indicator and heterotrophic bacteria in the drinking water of this area were determined. Of particular concern were the high heterotrophic plate count bacteria detected and the prospects that they may have masked faecal coliform counts. The possible effects of poor microbial quality of water on consumers were discussed. Isolation and identification of indicator bacteria in drinking water from the various sites was completed and presented. Antibiotic resistance patterns of indicator organisms (faecal coliforms) were determined. Biofilm formation potential of bacterial species occurring in water from communal taps in an informal settlement (Sonderwater) was demonstrated and the implications discussed.

5.5.1.2 Physico-chemical and microbiological quality of drinking water from Ganyesa

Water from the rural community Ganyesa was sampled and analysed in terms of physico-chemical and microbiological parameters. It was found that nitrates were of a great concern in this area. Microbiological quality of the water was determined and discussed. There were large numbers of certain bacterial types detected. Implications of this observation were discussed. After initial counts on selective agar, the faecal coliform/*Streptococcus* spp. could be determined for water samples from Tap 2 in Ganyesa. However, Gram staining and TSI tests showed that none of these isolates

were *E.coli* , thus antibiotic resistance patterns for Ganyesa faecal coliforms could not be determined.

5.5.1.3 Is there a settlement gradient for water quality in Potchefstroom?

Water samples were collected along a settlement gradient in Potchefstroom and was tested for physico-chemical and microbiological parameters. At the time of sampling and analyses, all areas had similar drinking water quality for both physico-chemical and microbiological parameters. Effects of the water quality were, where applicable, discussed in terms of potential risk to consumers.

5.5.1.4 Physico-chemical and microbiological water quality of rural, peri-urban and urban schools

The schools from which water was collected and physico-chemical and microbiological quality determined were divided into the three classes as indicated above. Urban schools received municipal water and were always of high quality, particularly for physico-chemical parameters. Water from the rural and peri-urban schools was of poorer quality exceeding maximum allowable concentrations or levels for many of the parameters. Of particular concern were the potential combined effects of high concentrations of nitrate linked to high levels of heterotrophic plate count bacteria and the presence of faecal coliforms. The potential health risks of this poor water to the children were discussed.

5.5.1.5 Identification and antibiotic resistance profiles of selected faecal coliforms.

Antibiotic resistance patterns for faecal coliforms from Sonderwater and the schools were determined. It was found that in some cases they present multiple antibiotic resistance patterns, with resistance to beta-lactams the most prevalent. High resistance to trimethoprim and oxy-tetracycline was also observed. This data could prove useful in future treatment of diarrhetic illnesses for people in these areas. Results such as those presented in this study offer evidence that could be helpful in the identification of future studies and initiatives aimed at improvement of water quality for rural and poverty stricken areas as well as rural farm schools. Microbiological water quality can

be improved or other water sources can be identified for these areas, such as rainwater harvesting.

5.5.1.6 Biofilm formation potential of bacteria in water from Sonderwater

Biofilm formation potential of bacteria in the water from Sonderwater was determined by leaving water in a glass container for a period of 1 week. It was demonstrated that in this period all the water samples formed aggregates of cells on glass surfaces that represents the initial biofilm formation steps. The potential health risks associated with biofilm formation were discussed.

5.5.2 Recommendations

The following prospects of this study were identified:

5.5.2.1 Follow-up studies

Since no data could be found for microbial quality of drinking water in South African schools, this is an issue that should receive greater attention. Follow-up studies should also consider schools from a greater section of the province and country, especially schools which receive groundwater. Schools could also be involved in monitoring the quality of their drinking water. The settlement gradient study could be extended to other towns and cities in the province. It is also critically important that the issues with high nitrate levels be dealt with and where applicable, appropriate remediation measures be put in place. Follow-up studies should also be done in Ganyesa, since the nitrate levels in this area were elevated. Future studies should also sample during both summer and winter in this region to determine whether seasonal variation in the total coliform and faecal coliform levels exist.

5.5.2.2 Surveys of water quality

Surveys related to water quality and associated problems should be conducted in informal and rural settlements in the North West Province, South Africa. This should be extended to peri-urban and rural schools. Benefits of this type of research are often underestimated. Sources of contamination should also be determined in order to

circumvent these and improve water quality. Health impacts of the water used in these communities cannot be ignored. Results from the surveys should be published. Reports should be written and presented to the local and provincial government about the quality of water used by communities. Steps should be taken to improve the water quality in affected communities and schools. Children are the future of our country and, if they do not have good quality water, they cannot be expected to flourish in school. Poor water quality is thus not only obstructing the growth of children, but, in essence, also the growth of the North West Province and our country.

5.5.2.3 Education

Residents of informal settlements and rural communities such as Sonderwater and Ganyesa may need to be educated about the sanitary quality of the water in their areas and the related health implications. Lessons about sterilization of water containers and safe storage of water should also be taught. If not already in place, programs to change human behaviour to promote hygienic quality of their drinking water may have to be implemented in these communities.

5.5.2.4 Infrastructure

In the North West Province most rural areas lack proper sanitary as well as drinking water supply infrastructures (Table 2.1). This matter is of major concern. Drinking water quality may be threatened by microbiological contamination that poses serious health threats to consumers. Sanitation and activities that could contribute on maintaining the good quality of drinking water should be developed and maintained. Reports on the impacts of the lack of these basic services could highlight the plight of these communities.

Finally the physico-chemical and microbiological drinking water quality of the rural, peri-urban and urban communities in the North West Province and farm schools in the Potchefstroom area were determined and implications discussed. The aim and all objectives for this study were thus successfully achieved.

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APPENDICES

APPENDIX A

Table 1A: A list of the GPS coordinates of all the sampling locations

Site name	GPS coordinates		
	Latitude	**Longitude**	**Elevation**
Sonderwater Tap 1	26° 43.153'S	27° 2.654'E	1413m
Sonderwater Tap 2	26° 43.136'S	27° 2.643'E	1414m
Sonderwater Tap 3	26° 43.175'S	27° 2.653'E	1412m
Gradient Tap 1	26° 43.223'S	27° 3.501'E	1374m
Gradient Tap 2	26°43.134'S	27°2.754'E	1403m
Gradient Tap 3	26°43.505'S	27°2.820'E	1392m
Gradient Tap 4	26° 43.153'S	27° 2.654'E	1413m
Gradient Tap 5	26° 43.136'S	27° 2.643'E	1414m
Gradient Tap 6	26° 43.175'S	27° 2.653'E	1412m
Gradient Tap 7	26°43.228'S	27°2.363'E	1438m
Gradient Tap 8	26°43.180'S	27°2.327'E	1437m
Gradient Tap 9	26°43.200'S	27°2.259'E	1432m
Gradient Tap 10	26°43.213'S	27°1.971'E	1407m
Gradient Tap 11	26°43.220'S	27°1.965'E	1407m
Gradient Tap 12	26°43.184'S	27°1.821'E	1408m
Fikadibeng	26°38.671'S	27°15.409'E	1367m
Mponeng	26°50.720'S	27°19.983'E	1531m
Sizamele	26°31.905'S	27°7.225'E	1431m
Loula Fourie	26°46.368'S	27°6.224'E	1384m
Terra Peccana	26°35.988'S	27°6.798'E	1412m
Vyfhoek	26°40.832'S	27°9.115'E	1357m
Boitshoko	26°43.134'S	27°2.754'E	1404m
Madibeng	26°43.505'S	27°2.820'E	1394m
Potch Christian	26°41.642'S	27°5.377'E	1379m

A.1. Methods used for Scanning Electron Microscopy

The methods used for scanning electron microscopy (SEM) were obtained from Dr. L. Tiedt, at the North West University, Potchefstroom campus. The methods were obtained from the standard operating procedures (SOP) in the laboratory of Dr. L. Tiedt.

Biological sample preparation for SEM comprises of the material (the filter or microscope slide) being fixed in 70% ethanol for 2-8 hours. The material was then

dehydrated in an ethanol series (80%, 90%, 2x 100% for 15min each). During this process the sample must not have been exposed to air. The samples were then ready for CPD. Critically drying of the material in a critical drying device was done with fluid carbonic acid gas. The material was then mounted on SEM buttons with double cling carbon band.

Critical point drying was done as follows. The carbon dioxide faucet against the wall was opened. The lid of the critical-point dryer (CP-dryer) was removed and the lower valve was slowly opened until white carbon dioxide passed through. The valve was then closed. The CP-dryer was then tilted and samples were quickly transferred into the chamber of the CP-dryer. The chamber was then filled up with acetone and the metal stopper and lid was replaced. The lower valve was slowly opened until bubbling was heard, then opened entirely. The upper valve was slightly opened to allow escape of acetone. The upper valve was closed when liquid acetone had passed. Upper valve was kept closed for 2 minutes to replace acetone in the samples with carbon dioxide. Upper valve was slightly opened for 1 minute. This step was repeated 3 times until carbon dioxide released was not lumpy any more. Both valves were closed as well as the carbon dioxide faucet against the wall. Water circulation was then switched on at the wall switch and system was left for 15 minutes while the temperature reached 45°C. To reach atmospheric pressure, upper valve was slowly opened until soft blowing was heard. If valve is opened to fast, samples might be damaged. When blowing stopped, atmospheric temperature was reached. Water circulation of the system was then switched off at the wall switch. Upper valve was closed and the system was left. Valve on the CO_2 cylinder outside was then closed. Lid of the CPD was unscrewed and samples removed.

After CPD, membranes were placed in a membrane holder and 20-30ml of the suspension was extracted from the sample bottle and injected through the membrane. Push air through the membrane until dry. Remove membrane with a fine pincers and place with filtrate to above in the lid of a petri dish. A few drops of 2% osmiumtetraoxide was placed with a Pasteur pipette in the bottom of the petri dish and the lid with the membranes was replaced which were then placed in an oven at 30 -

40°C for 30min. Osmiumtetraoxide drops were removed and the lid with the membranes was placed back in the oven for 5 minutes to dry. The filters were then pasted to the SEM-button and left in the oven to dry.

For coating with gold/palladium, the start button on the EMScope was pressed to switch the system on and to open the Argon faucet against the wall. The stop button was pressed and the system was kept on to allow the Argon gas to break the vacuum in the system. Lid was removed and holder with stubs was placed on stage of metal coater. Rubber surfaces were cleaned and lid was replaced. Start button on EMScope was then pressed to restore the vacuum to <0.06 Torr. The timer was set on 3-4 minutes and Argon inlet valve was slowly opened on the lid to control the volume of the Argon, it was also ensured that the ammeter reading did not exceed 5mmA. When coating was completed, Argon valve on the lid of the coater was closed. Stop button on EMScope was pressed to allow Argon gas to break the vacuum and lid of metal coater was lifted and stubs removed. Lid was replaced and vacuum restored by pressing start button. When vacuum was restored before system was switched off. The filter was then examined under the scanning electron microscope.

APPENDIX B

PHYSICO-CHEMICAL DATA FOR WATER SAMPLES

Table 1B: The physico-chemical data for the water sampled from Sonderwater in the summer

Parameter	Standard for domestic use	Tap 1 Summer	Tap 2 Summer	Tap 3 Summer	Averages	Standard deviation
pH	6-9	7	7	7	7	0.000
Temp (℃)		23	24	24	24	0.577
TH (°d)	2.5 (Soft)	16	16	16	16	0.000
CH (°d)	7-8	8	8	8	8	0.000
TDS (ppm)	0-450	459	457	455	457	2.000
EC (mS/m)	0-70	65.1	64.6	64.5	64.7	3.215
NO_2^- (mg/L)	0-6	2.5	2.5	2.5	2.5	0.000
NO_3^- (mg/L)	0-6	0	0	0	0	0.000

Table 2B: The physico-chemical data for the water sampled from Sonderwater in the winter

Parameter	Standard for domestic use	Tap 1 Winter	Tap 2 Winter	Tap 3 Winter	Averages	Standard deviation
pH	6-9	7.97	7.98	7.93	7.96	0.026
Temp (℃)		16.9	16.5	17.2	16.9	0.351
TH (°d)	2.5 (Soft)	20	20	20	20	0.000
CH (°d)	7-8	16	16	16	16	0.000
TDS (ppm)	0-450	462	478	474	471	8.327
EC (mS/m)	0-70	65.1	67.3	67	66.5	11.930
NO_2^- (mg/L)	0-6	0.5	0.5	0.5	0.5	0.000
NO_3^- (mg/L)	0-6	6.6	6.5	5.8	6.3	0.436

Table 3B: The physico-chemical data for the water sampled from Ganyesa

Parameter	Standard for domestic use	Tap 1	Tap 2	Tap 3	Tap 4	Tap 5	Averages	Standard Deviation
pH	7-9	7.73	7.73	7.52	7.54	7.41	7.6	0.140
Temp. (°C)		17.3	16.7	16.6	16.1	16.8	16.7	0.430
TH (°d)	2.5 (Soft)	20	20	20	20	20	20.0	0.000
CH (°d)	7-8	16	16	16	16	16	16.0	0.000
TDS (ppm)	0-450		599	569	594	397	539.8	96.067
EC (mS/m)	0-70		84.6	79.1	83.1	56.6	75.9	13.042
NO_2^- (mg/L)	0-6	0.5	0.5	0.5	0.5	0.5	0.5	0.000
NO_3^- (mg/L)	0-6			783	903	623	769.7	140.475

Table 4B: Physico-chemical data for the water sampled from the rural schools before the vacation

Parameter	Standard for domestic use	Fikadibeng	Mponeng	Sizamele	Average	Standard deviation
pH	6-9	8.03	7.97	7.8	7.93	0.12
Temp. (°C)	-	23	22	23	22.67	0.58
TH (°d)	2.5 (Soft)	20	20	10	16.67	5.77
CH (°d)	7-8	16	16	12	14.67	2.31
TDS (ppm)	0-450	559	356	186	367.00	186.74
EC (mS/m)	0-70	81.0	50.0	26.1	52.36	27.52
NO_2^- (mg/L)	0-6	0.5	0.5	0.5	0.50	0.00
NO_3^- (mg/L)	0-6	0	0	10	3.33	5.77

Table 5B: Physico-chemical data for the water sampled from the rural schools after the vacation

Parameter	Standard for domestic use	Fikadibeng	Mponeng	Sizamele	Average	Standard Deviation
pH	6-9	7.99	8.02	7.77	7.93	0.14
Temp (°C)	-	18.5	20	18	18.83	1.04
TH (°d)	2.5 (Soft)	20	20	10	16.67	5.77
CH (°d)	7-8	16	16	12	14.67	2.31
TDS (ppm)	0-450	719	335	182	412.00	276.66
EC (mS/m)	0-70	101.3	49.9	25.6	58.93	38.65
NO_2^- (mg/L)	0-6	0	0	0	0.00	0.00
NO_3^- (mg/L)	0-6	10	0	10	10.00	0.00

Table 6B: Physico-chemical data for the water sampled from the peri-urban schools before the vacation

Parameter	Standard for domestic use	Loula Fourie	Terra Peccana	Vyfhoek	Average	Standard Deviation
pH	6-9	8.3	9.2	8.17	8.56	0.56
Temp. (°C)	-	22	25	23	23.33	1.53
TH (°d)	2.5 (Soft)	20	8	16	14.67	6.11
CH (°d)	7-8	12	12	16	13.33	2.31
TDS (ppm)	0-450	221	208	571	333.33	205.93
EC (mS/m)	0-70	30.9	28.4	79.9	46.4	29.04
NO_2^- (mg/L)	0-6	0.5	5	0	1.83	2.75
NO_3^- (mg/L)	0-6	10	10	25	15.00	8.66

Table 7B: Physico-chemical data for the water sampled from the peri-urban schools after the vacation

Parameter	Standard for domestic use	Loula Fourie	Terra Peccana	Average	Standard Deviation
pH	6-9	8.02	7.78	7.90	0.17
Temp (°C)	-	17	20	18.50	2.12
TH (°d)	2.5 (Soft)	20	8	14.00	8.49
CH (°d)	7-8	12	12	12.00	0.00
TDS (ppm)	0-450	213	237	225.00	16.97
EC (mS/m)	0-70	30.1	33.4	31.75	2.33
NO_2^- (mg/L)	0-6	0	5	5.00	Not calculable
NO_3^- (mg/L)	0-6	10	10	10.00	0.00

Table 8B: Physico-chemical data for the water sampled from the urban schools before the vacation

Parameter	Standard for domestic use	Boitshoko	Madibeng	Potch Christian	Average	Standard Deviation
pH	6-9	8.59	8.51	8.54	8.55	0.04
Temp. (°C)	-	23	25	22	23.33	1.53
TH (°d)	2.5 (Soft)	20	20	20	20.00	0.00
CH (°d)	7-8	16	16	12	14.67	2.31
TDS (ppm)	0-450	449	459	462	456.67	6.81
EC (mS/m)	0-70	62.9	64.6	65.4	64.30	1.27
NO_2^- (mg/L)	0-6	0.5	0.5	0.5	0.50	0.00
NO_3^- (mg/L)	0-6	0	0	0	0.00	0.00

Table 9B: Physico-chemical data for the water sampled from the urban schools after the vacation

Parameter	Standard for domestic use	Boitshoko	Madibeng	Potch Christian	Average	Standard Deviation
pH	6-9	8.06	7.97	7.91	7.98	0.08
Temp (°C)	-	20	22	24	22.00	2.00
TH (°d)	2.5 (Soft)	20	20	20	20.00	0.00
CH (°d)	7-8	16	16	16	16.00	0.00
TDS (ppm)	0-450	331	478	474	427.67	83.74
EC (mS/m)	0-70	66.0	67.3	66.6	66.63	0.65
NO_2^- (mg/L)	0-6	0	0	0	0.00	0.00
NO_3^- (mg/L)	0-6	0	0	0	0.00	0.00

APPENDIX C

MICROBIOLOGICAL DATA FOR WATER SAMPLES

Table 1C: The microbiological data for the water sampled from Sonderwater in the summer

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 1	40	1180	860	0	0	0
	500	5700	560	0	14	0
	No growth	8000	610	0	6	0
	40	500	874	0	4	0
	No growth	2500	824	0	2	0
	3000	4000	786	0	0	0
Tap 2	210	1030	260	0	2	0
	400	2500	228	2	2	0
	No growth	4000	286	0	4	0
	50	220	290	4	12	0
	No growth	100	184	0	14	0
	1000	5000	210	0	8	0
Tap 3	210	620	518	0	0	0
	No growth	600	492	0	0	0
	No growth	No growth	454	0	0	0
	250	340	316	2	4	0
	No growth	500	526	4	2	0
	No growth	T.M.T.C	416	0	0	0

Table 2C: Averages, standard deviation and t-test values for the data from Sonderwater sampled in the summer

Site	T-Test Day 2 and Day 5 HPC	Avg. Day 2 HPC	Avg. Day 5 HPC	Standard deviation	Avg. Total coliforms	Avg. Faecal coliforms	Avg. Staphy-lococci	Staphylococci / 100ml after 1 week
Tap 1	0.17	895.00	3646.67	2716.73	752.33	0.00	4.33	5.00
Tap 2	0.13	415.00	2141.67	1946.97	243.00	1.00	7.00	40.00
Tap 3	0.36	230.00	515.00	118.44	453.67	1.00	1.00	15.00

Table 3C: The microbiological data for the water sampled from Sonderwater in the winter

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 1 Sterilised	0	0	0	0	0	0
	0	0	0	0	0	0
	0	10	0	0	0	0
Tap 1 Non-Sterilised	4300	4300	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	6	0
Tap 2 Non-Sterilised	0	0	480	0	0	36
	0	0	48	0	0	2
	0	0	0	0	0	0
	10	10	0	0	0	0
	0	10	0	0	0	2
	0	10	0	0	18	0
Tap 3 Sterilised	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
Tap 3 Non-Sterilised	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0

Table 4C: Averages, standard deviation and t-test values for the data from Sonderwater in the winter

Site	T-Test Day 2 and Day 5 HPC	Avg. Day 2 HPC	Avg. Day 5 HPC	Standard deviation	Avg. Total coli-forms	Avg. Faecal coli-forms	Avg. Staphy-lococci	Staphy-lococci / 100ml after 1 week
Tap 1 Sterilised	0.42	0.00	3.33	5.16	0	0	0	0
Tap 1 Non-Sterilised	Not calcula-ble	1433.33	1433.33	2220.51	0	0	2	0
Tap 2 Non-Sterilised	0.17	1.67	5	5.22	88	0	3	0
Tap 3 Sterilised	Not calcula-ble	0.00	0.00	0.00	0	0	0	0
Tap 3 Non-Sterilised	Not calcula-ble	0.00	0.00	0.00	0	0	0	0

Table 5C: The microbiological data for the water sampled from Ganyesa

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 1 A	925	9300	4	0	0	0
	1020	10100	4	0	2	0
	3000	7000	6	2	0	0
Tap 1 B	2300	6800	0	0	4	0
	3000	8000	0	0	0	0
	5040	9700	6	2	4	0
Tap 2 A	350	1740	0	10	0	6
	220	1100	0	6	0	0
	180	2190	0	10	2	2
Tap 2 B	1270	13500	0	2	0	0
	140	1070	22	2	0	0
	90	1100	16	0	0	0
Tap 3 A	40	230	0	0	0	0
	0	0	0	0	0	2
	12	280	18	0	0	0
Tap 3 B	150	26600	16	0	0	0
	36	220	48	0	0	0
	10	200	42	0	0	0
Tap 4 A	26	200	38	0	0	0
	47	600	20	0	0	0
	65	740	18	0	0	0
Tap 4 B	89	1400	28	0	0	0
	97	TMTC	26	0	0	0
	37	400	44	0	0	0
Tap 5 A	2	30	4	0	0	4
	11	100	6	0	0	0
	0	0	2	0	0	0
Tap 5 B	23	TMTC	6	0	0	0
	3	30	8	0	0	0
	0	0	6	0	0	0

Table 6C: Averages, standard deviation and t-test values for the data from Ganyesa

Site	Average HPC	Average Total Coliforms	Average Faecal Coliforms	Average Staphylococci	Average Streptococci	Standard Deviation HPC	t-test Day 2 and Day 5 HPC
Tap 1	8483.33	3.33	0.67	1.67	0	1416.22	0
Tap 2	3450.00	6.33	5.00	0.33	1.33	4944.16	0.16
Tap 3	4588.33	20.67	0	0	0.33	10783.90	0.35
Tap 4	668.00	29	0	0	0	457.30	0.03
Tap 5	32.00	5.33	0	0	0.67	40.87	0.15

Table 7C: The microbiological data for the water sampled in the settlement gradient study – Area 1

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 1	0	10	18	0	28	0
	0	0	0	0	5	0
	0	0	0	0	10	0
	0	0	23	0	13	0
	0	0	8	0	10	0
	0	0	5	0	28	0
Tap 2	0	TMTC	0	0	13	0
	0	0	0	0	5	0
	1000	2000	0	0	8	0
	0	0	0	0	15	0
	0	0	0	0	5	0
	0	0	0	0	15	0
Tap 3	TMTC	TMTC	TMTC	0	33	3
	10000	10000	TMTC	0	38	0
	12000	29000	TMTC	0	18	0
	TMTC	TMTC	TMTC	0	20	3
	11200	11200	TMTC	0	28	3
	22000	22000	TMTC	0	13	0

Table 8C: The microbiological data for the water sampled in the settlement gradient study – Area 2

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 4	600	600	240	0	283	0
	200	200	118	0	TMTC	0
	TMTC	TMTC	333	0	TMTC	0
	100	120	215	0	508	0
	320	320	333	0	TMTC	0
	2000	2000	308	0	343	0
Tap 5	20	50	178	0	13	0
	100	200	8	0	50	0
	1000	1000	218	0	75	0
	0	0	70	0	8	0
	100	100	58	0	10	0
	0	0	3	0	23	0
Tap 6	110	130	8	0	8	0
	0	100	3	0	8	0
	0	1000	40	0	8	0
	120	130	13	0	8	0
	400	600	43	0	3	0
	0	0	45	0	3	0

Table 9C: The microbiological data for the water sampled in the settlement gradient study – Area 3

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 7	100	TMTC	0	0	10	0
	100	2150	3	0	8	0
	0	40000	0	0	8	0
	10	TMTC	3	0	5	0
	200	23300	0	0	3	0
	0	29000	3	0	10	0
Tap 8	10	30	0	0	5	0
	0	0	0	0	8	0
	0	0	0	0	33	0
	0	10	0	0	8	0
	0	0	0	0	5	0
	1000	1000	0	0	8	0
Tap 9	10	210	3	0	10	0
	0	0	0	0	30	0
	0	0	0	0	0	0
	0	0	3	0	8	0
	TMTC	TMTC	0	0	5	0
	0	0	3	0	48	0

Table 10C: The microbiological data for the water sampled in the settlement gradient study – Area 4

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Tap 10	0	30	10	0	18	0
	0	0	5	0	5	0
	0	0	25	0	13	0
	0	TMTC	10	0	13	3
	0	0	10	0	5	0
	0	0	0	0	5	0
Tap 11	0	TMTC	5	0	3	0
	0	300	0	0	8	0
	0	0	0	0	0	0
	0	TMTC	0	0	13	0
	0	800	3	0	5	0
	0	1000	5	0	15	0
Tap 12	30	TMTC	0	0	5	0
	0	0	0	0	5	0
	0	0	0	0	3	0
	10	TMTC	0	0	8	0
	0	0	0	0	5	0
	0	2000	0	0	5	0

Table 11C: Averages, standard deviation and t-test values for the data from the settlement gradient study

Site	T-Test Day 2 and Day 5 HPC	Average Day 2 HPC	Average Day 5 HPC	Standard deviation for HPC day 5	Average Total coliforms	Average Faecal coliforms	Average Staphy-lococci	Staphy-lococci / 100ml after 1 week
Tap 1	0.36	0	1.67	3.89	8.75	0	15.42	0
Tap 2	0.37	166.67	400	843.27	0	0	10	0
Tap 3	0.39	13800	18050	8404.59	TMTC	0	24.58	0
Tap 4	0.37	644	648	732.92	257.50	0	377.50	0
Tap 5	0.24	203.33	225	368.97	88.75	0	29.58	0
Tap 6	0.22	105	326.67	372.59	25	0	5.83	0
Tap 7	0.06	68.33	23612.50	14719.39	1.25	0	7.08	0
Tap 8	0.20	168.33	173.33	386.30	0	0	10.83	0
Tap 9	0.37	2	42	88.54	1.25	0	16.67	0
Tap 10	0.37	0	6	12.65	10	0	9.58	0
Tap 11	0.11	0	525	423.42	2.08	0	7.08	0
Tap 12	0.39	6.67	500	925.82	0	0	5	0

Table 12C: The microbiological data for the water sampled from the rural schools before the vacation

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylo-cocci / 100ml	Faecal Streptococci / 100ml
Fikadibeng	3800	4600	105	0	24	0
			185	7	23	0
			435	4	19	0
	3300	4400	4	6	33	0
			18	9	36	0
			25	10	45	0
	2800	4000	25	8	34	0
			36	11	24	0
			46	9	29	0
Mponeng	1060	4935	7	5	10	0
			5	1	22	1
			7	3	30	0
	890	5330	6	0	10	0
			0	1	0	0
			4	1	0	0
	265	2365	9	4	19	1
			0	0	16	0
			14	0	37	0
Sizamele	3300	4000	140	73	27	1
			85	56	10	1
			111	27	8	2
	2100	3000	37	35	19	1
			5	9	10	1
			33	31	14	1
	2700	4100	78	3	17	1
			68	16	5	0
			32	6	28	3

Table 13C: The microbiological data for the water sampled from the rural schools after the vacation

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylococci / 100ml	Faecal Streptococci / 100ml
Fikadibeng	13200	13833	123	0	0	4
			44	1	0	0
			60	0	0	2
	32800	TMTC	9	0	0	0
			23	0	0	1
			14	0	0	0
	11800	12400	9	0	0	1
			TMTC	0	0	0
			TMTC	0	0	1
Mponeng	TMTC	TMTC	TMTC	66	0	0
			TMTC	67	0	0
			TMTC	75	0	0
	TMTC	TMTC	TMTC	42	5	0
			TMTC	82	7	0
			TMTC	60	8	0
	TMTC	TMTC	TMTC	80	34	0
			TMTC	90	22	0
			TMTC	88	3	0
Sizamele	18900	20833	TMTC	3	0	0
			TMTC	2	0	0
			TMTC	4	29	0
	TMTC	TMTC	TMTC	3	35	0
			TMTC	0	37	0
			TMTC	0	37	0
	TMTC	TMTC	TMTC	0	20	1
			TMTC	1	50	0
			TMTC	5	34	0

Table 14C: The microbiological data for the water sampled from the peri-urban schools before the vacation

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylo-cocci / 100ml	Faecal Streptococci / 100ml
Loula Fourie	370	745	31	11	18	16
			3	32	36	0
			38	17	58	8
	80	1055	21	5	34	16
			19	12	63	9
			27	19	67	19
	170	400	17	13	86	36
			18	6	87	30
			10	0	107	34
Terra Peccana	2100	2900	35	0	57	0
			31	0	56	0
			29	2	14	0
	3500	4100	29	0	69	0
			42	0	88	0
			77	0	0	0
	1700	2200	31	0	41	0
			49	0	76	0
			72	0	104	0
Vyfhoek	340	510	TMTC	8	6	0
			TMTC	3	0	0
			TMTC	0	1	0
	440	545	TMTC	3	0	3
			TMTC	0	3	0
			TMTC	0	5	0
	1050	1290	TMTC	0	1	0
			TMTC	2	2	0
			TMTC	0	6	0

Table 15C: The microbiological data for the water sampled from the peri-urban schools after the vacation

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylo-cocci / 100ml	Faecal Streptococci / 100ml
Loula Fourie	50	160	20	4	0	0
			25	0	0	0
			17	1	0	0
	202	468	25	0	1	0
			25	1	0	0
			16	1	0	0
	120	273	37	2	0	16
			19	2	0	18
			23	0	0	19
Terra Peccana	16600	20533	52	0	TMTC	0
			154	0	TMTC	0
			124	0	TMTC	0
	13500	17333	19	0	TMTC	0
			24	0	TMTC	0
			25	0	TMTC	0
	9800	11700	91	0	TMTC	0
			60	0	TMTC	0
			43	0	TMTC	0

Table 16C: The microbiological data for the water sampled from the urban schools before the vacation

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylo-cocci / 100ml	Faecal Streptococci / 100ml
Boitshoko	1240	1570	TMTC	137	5	38
			TMTC	111	1	47
			TMTC	134	5	37
	790	865	TMTC	130	4	42
			TMTC	124	1	32
			TMTC	133	6	34
	350	600	TMTC	118	1	51
			TMTC	106	3	71
			TMTC	112	1	48
Madibeng	20	100	1	0	5	0
			0	0	2	0
			0	0	2	0
	215	600	77	0	1	0
			118	0	2	0
			44	0	0	0
	30	200	7	0	0	0
			12	0	0	0
			35	0	0	0
Potch Christian	165	725	180	0	0	0
			189	0	0	0
			192	0	0	0
	105	385	152	0	0	0
			168	0	0	0
			163	0	0	0
	60	465	181	0	0	0
			179	0	0	0
			184	0	0	0

Table 17C: The microbiological data for the water sampled from the urban schools after the vacation

Site	HPC / ml Day 2	HPC / ml Day 5	Total Coliforms / 100ml	Faecal Coliforms / 100ml	Staphylo-cocci / 100ml	Faecal Streptococci / 100ml
Boitshoko	133	180	1	0	0	0
			0	0	0	0
			0	0	0	0
	380	427	2	0	0	0
			1	0	0	0
			0	1	0	0
	542	570	0	0	0	0
			0	0	0	0
			0	0	0	0
Madibeng	590	1692	20	0	0	0
			14	0	0	0
			15	0	0	0
	17	17	0	0	0	0
			0	0	0	0
			0	0	0	0
	82	102	0	0	0	0
			0	0	0	0
			0	0	0	0
Potch Christian	144	580	0	0	0	0
			2	0	0	0
			0	0	0	0
	1092	1635	0	0	0	0
			0	0	0	0
			0	0	0	0
	225	695	0	0	0	0
			0	0	1	0
			0	0	0	0

Table 18C: The averages, standard deviations and t-test values for the data from the schools before the vacation

Site	T-Test Day 2 and Day 5 HPC	Average Day 2 HPC	Average Day 5 HPC	Standard deviation	Average Total coliforms	Average Faecal coliforms	Average Staphy-lococci	Staphylococci / 100ml after 1 week
Fikadibeng	0.01	3300	4333.33	273.25	97.67	7.11	29.67	0
Mponeng	0.04	738.33	4210	1440.01	5.78	1.67	20.57	0
Sizamele	0.04	2700	3700	544.06	65.44	28.44	15.33	0
Loula Fourie	0.15	206.67	733.33	293.06	20.44	12.78	61.78	15
Terra Peccana	0.02	2433.33	3066.67	859.46	43.89	0.22	56.11	0
Vyfhoek	0.05	610	781.67	394.06	TMTC	1.78	2.67	0
Boitshoko	0.10	793.33	1011.67	448.43	TMTC	122.78	3	0
Madibeng	0.14	88.33	300	236.64	32.67	0	1.33	10
Potch Christian	0.04	110	525	159.00	176.44	0	0	0

Table 19C: The averages, standard deviations and t-test values for the data from the schools after the vacation

Site	T-Test Day 2 and Day 5 HPC	Average Day 2 HPC	Average Day 5 HPC	Standard deviation	Average Total coliforms	Average Faecal coliforms	Average Staphylococci
Fikadibeng	0.02	19266.67	13116.5	827.34	40.29	0.11	0
Mponeng	Not calculable	TMTC	TMTC	Not calculable	TMTC	72.22	8.78
Sizamele	Not calculable	18900	20833	0	TMTC	2	26.89
Loula Fourie	0.06	124	300.33	139.36	23	2.7	0.11
Terra Peccana	0.04	13300	16522	3999.88	65.78	0	TMTC
Boitshoko	0.02	351.67	392.33	176.47	TMTC	0.11	0
Madibeng	0.41	229.67	603.67	843.88	5.44	0	0
Potch Christian	0.00	487	970	517.67	0.22	0	0.11

Table 20C: The faecal coliform/faecal *Streptococci* ratio for the water from Ganyesa and the schools, before the vacation (BV) and after the vacation (AV) where determinable

Site		**Average Faecal Coliforms**	**Average Faecal *Streptococci***	**Faecal coliform / Faecal *Streptococci* ratio**	**Origin of pollution**
Ganyesa	Tap 2	5	1	5	Human
Boitshoko	BV	123	44	2.8	Human and animal
Loula Fourie	BV	13	19	0.7	Human and animal
Mponeng	BV	2	0	8	Human
Sizamele	BV	28	1	23	Human
Vyfhoek	BV	2	0	5	Human
Loula Fourie	AV	2.7	17.7	0.2	Animal
Sizamele	AV	2	0	18	Human

APPENDIX D

ANTIBIOTIC SUSCEPTIBILITY RESULTS

Table 1D: The number of isolates from schools before and after the vacation tested for antibiotic resistance

Site	Number of isolates tested	
	Before vacation	**After vacation**
Fikadibeng	6	0
Mponeng	3	0
Sizamele	6	0
Loula Fourie	3	7
Boitshoko	10	1

Table 2D: The names and abbreviations of all antibiotics used, as well as the interpretation values of the inhibition zones

Antibiotic name		Amoxicillin	Ampicillin	Cephalo-thin	Chloram-phenicol	Cipro-floxacin	Kanamycin	Oxytetra-cycline	Strepto-mycin	Trime-thoprim
Abbreviation		AMX	AMP	CEP	CHL	CIP	KAN	OXY-TET	STR	TM
Concentration		10 µg	10 µg	30 µg	30 µg	5 µg	30 µg	30 µg	300 µg	2.5 µg
Inhibition zones	Resistant (R)	≤ 13	≤ 13	≤ 14	≤ 10	≤ 12	≤ 16	≤ 14	≤ 13	≤ 14
	Intermediate resistant (I)	14-17	14-16	15-17	11-16	13-19	Not Applicable	15-18	14-16	Not Applicable
	Susceptible (S)	> 17	> 16	> 17	> 16	> 19	≥ 17	> 18	> 16	≥ 15

Table 3D: Initial inhibition zones of antibiotics tested for the isolates from Sonderwater (summer)

Site	AMX 10µg		AMP 10µg		CEP 30µg		CHL 30µg		CIP 5µg		KAN 30µg		OXY-TET 30µg		STR 300µg		TM 2.5µg	
	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S	Zone in mm	I/R/S
Tap 1	9	R	14	I	15	I	25	S	30	S	20	S	21	S	27	S	20	S
Tap 2	15	I	15	I	15	I	28	S	28	S	19	S	21	S	22	S	20	S
Tap 3	8	R	12	R	12	R	21	S	31	S	19	S	18	I	21	S	17	S

Table 4D: Antibiotic susceptibility data and MAR phenotypes for the isolates from Sonderwater (summer)

The antibiotic resistance/susceptibility data for faecal coliform isolates from Sonderwater (1 indicates resistance and 0 indicates susceptibility)										
SITE	**AMX**	**AMP**	**CEP**	**CHL**	**CIP**	**KAN**	**OXY-TET**	**STR**	**TM**	**MAR PHENOTYPE**
Tap 1	**1**	0	0	0	0	0	0	0	0	AMX
Tap 2	0	0	0	0	0	0	0	0	0	
Tap 3	**1**	**1**	**1**	0	0	0	0	0	0	AMX-AMP-CEP
Total	2	1	1	0	0	0	0	0	0	
% Resistant (R/N)*	66.67%	33.33%	33.33%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	*(R/N = Total resistant / Total number of sites)

Table 5D: Initial inhibition zones of antibiotics tested for the isolates from the water samples collected from the schools before the vacation.

School	Sample name	AMX 10µg		AMP 10µg		CEP 30µg		CHL 30µg		CIP 5µg		KAN 30µg		OXY-TET 30µg		STR 300µg		TM 2.5µg	
		Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S
Fikadibeng	FK 2.1	11	R	13	R	12	R	26	S	33	S	19	S	16	I	30	S	15	I
	FK 2.2	15	I	16	I	14	R	25	S	35	S	18	S	16	I	27	S	10	R
	FK 3.2	7	R	7	R	7	R	11	I	25	S	19	S	17	I	22	S	0	R
	FK 2.3	10	R	11	R	14	R	22	S	27	S	16	I	19	S	22	S	17	S
	FK 3.1	16	I	15	I	14	R	22	S	36	S	16	I	17	I	23	S	17	S
Mponeng	VFS 1.2	8	R	10	R	17	I	23	S	28	S	22	S	11	R	30	S	15	I
	VFS 1.1	16	I	17	S	17	I	22	S	30	S	18	S	15	I	35	S	14	I
	VFS 2.3	11	R	10	R	8	R	28	S	30	S	20	S	18	I	28	S	13	I
Sizamele	RB 1.3	10	R	14	I	15	I	24	S	32	S	20	S	16	I	25	S	15	I
	RB 2.1	0	R	0	R	8	R	22	S	25	S	0	R	16	I	26	S	0	R
	RB 2.2	16	I	13	R	17	I	26	S	36	S	21	S	15	I	28	S	15	I
	RB 3.1	11	R	9	R	10	R	21	S	20	S	15	I	13	R	25	S	0	R
	RB 3.3	18	S	18	S	18	S	23	S	35	S	20	S	15	I	25	S	12	R
	RB 3.1	16	I	16	I	16	I	20	S	35	S	20	S	16	I	23	S	15	I
Loula Fourie	CCLF 1.1	0	R	7	R	0	R	22	S	29	S	16	I	14	R	30	S	0	R
	CCLF 1.2	8	R	0	R	20	S	22	S	28	S	21	S	16	I	29	S	17	S
	CCLF 3.1	8	R	10	R	0	R	16	I	21	S	13	I	12	R	28	S	0	R

Table 5D (continues): Initial inhibition zones of antibiotics tested for the isolates from the water samples collected from the schools before the vacation.

School	Sample name	AMX 10µg		AMP 10µg		CEP 30µg		CHL 30µg		CIP 5µg		KAN 30µg		OXY-TET 30µg		STR 300µg		TM 2.5µg	
		Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S
Boitshoko	**B 3.2.2**	17	I	16	I	17	I	23	S	35	S	20	S	7	R	25	S	7	R
	B 3.3	15	I	15	I	15	I	21	S	30	S	15	I	12	R	25	S	13	I
	B 1.1.2	17	I	16	I	17	I	21	S	36	S	20	S	14	R	28	S	16	I
	B 1.2.1	16	I	18	S	17	I	22	S	32	S	19	S	16	I	26	S	16	I
	B 1.2.2	16	I	18	S	12	R	23	S	34	S	20	S	15	I	27	S	15	I
	B 1.3.1	17	I	14	I	7	R	21	S	35	S	20	S	16	I	25	S	18	S
	B 1.3.2	16	I	17	S	15	I	21	S	28	S	20	S	15	I	22	S	14	I
	B 2.1.2	14	I	16	I	10	R	22	S	29	S	18	S	15	I	27	S	19	S
	B 2.3.1	17	I	19	S	15	I	21	S	33	S	20	S	16	I	26	S	11	R
	B 3.3.1	17	I	16	I	16	I	22	S	33	S	18	S	16	I	27	S	15	I
Madibeng	**MAD 1.1**	8	R	9	R	10	R	22	S	23	S	14	I	18	I	29	S	0	R

Table 6D: Antibiotic susceptibility data and MAR phenotypes for the isolates from water samples collected from schools before the vacation

School	The antibiotic resistance/susceptibility data for faecal coliform isolates from schools (1 indicates resistance and 0 indicates susceptibility)										
	SITE	**AMOX**	**AMP**	**CEP**	**CHL**	**CIP**	**KAN**	**OXY-TET**	**STR**	**TM**	**MAR PHENOTYPES**
Fikadibeng	**FK 2.1**	**1**	**1**	**1**	0	0	0	0	0	0	AMX-AMP-CEP
	FK 2.2	0	0	**1**	0	0	0	0	0	**1**	CEP-TM
	FK 3.2	**1**	**1**	**1**	0	0	0	0	0	**1**	AMX-AMP-CEP-TM
	FK 2.3	**1**	**1**	**1**	0	0	0	0	0	0	AMX-AMP-CEP
	FK 3.1	0	0	**1**	0	0	0	0	0	0	CEP
Mponeng	**VFS 1.2**	**1**	**1**	0	0	0	0	**1**	0	0	AMX-AMP-OXY-TET
	VFS 1.1	0	0	0	0	0	0	0	0	0	
	VFS 2.3	**1**	**1**	**1**	0	0	0	0	0	0	AMX-AMP-CEP
Sizamele	**RB 1.3**	**1**	0	0	0	0	0	0	0	0	AMX
	RB 2.1	**1**	**1**	**1**	0	0	**1**	0	0	**1**	AMX-AMP-CEP-KAN-TM
	RB 2.2	0	**1**	0	0	0	0	0	0	0	AMP
	RB 3.1	**1**	**1**	**1**	0	0	0	**1**	0	**1**	AMX-AMP-CEP-OXY-TET--TM
	RB 3.3	0	0	0	0	0	0	0	0	**1**	TM
	RB 3.1	0	0	0	0	0	0	0	0	0	
Loula Fourie	**CCLF 1.1**	**1**	**1**	**1**	0	0	0	**1**	0	**1**	AMX-AMP-CEP-OXY-TET-TM
	CCLF 1.2	**1**	**1**	0	0	0	0	0	0	0	AMX-AMP
	CCLF 3.1	**1**	**1**	**1**	0	0	0	**1**	0	**1**	AMX-AMP-CEP-OXY-TET-TM

Table 6D (continues): Antibiotic susceptibility data and MAR phenotypes for the isolates from water samples collected from schools before the vacation

School	The antibiotic resistance/susceptibility data for faecal coliform isolates from schools (1 indicates resistance and 0 indicates susceptibility)										
	SITE	**AMOX**	**AMP**	**CEP**	**CHL**	**CIP**	**KAN**	**OXY**	**STR**	**TM**	**MAR PHENOTYPES**
Boitshoko	**B 3.2.2**	0	0	0	0	0	0	**1**	0	**1**	OXY-TET-TM
	B 3.3	0	0	0	0	0	0	**1**	0	0	OXY-TET
	B 1.1.2	0	0	0	0	0	0	**1**	0	0	OXY-TET
	B 1.2.1	0	0	0	0	0	0	0	0	0	
	B 1.2.2	0	0	**1**	0	0	0	0	0	0	CEP
	B 1.3.1	0	0	**1**	0	0	0	0	0	0	CEP
	B 1.3.2	0	0	0	0	0	0	0	0	0	
	B 2.1.2	0	0	**1**	0	0	0	0	0	0	CEP
	B 2.3.1	0	0	0	0	0	0	0	0	**1**	TM
	B 3.3.1	0	0	0	0	0	0	0	0	0	
Madibeng	**MAD 1.1**	**1**	**1**	**1**	0	0	0	0	0	**1**	AMOX-AMP-CEP-TM
	Total	12	12	14	0	0	1	7	0	10	
	% Resistant (R/N)*	42.86%	42.86%	50.00%	0.00%	0.00%	3.57%	25.00%	0.00%	35.71%	*(R/N = Total resistant / Total number of sites)

Table 7D: Initial inhibition zones of antibiotics tested for the isolates from the water samples collected from the schools after the vacation.

School	Sample name	AMX 10µg		AMP 10µg		CEP 30µg		CHL 30µg		CIP 5µg		KAN 30µg		OXY-TET 30µg		STR 300µg		TM 2.5µg	
		Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S	Zone in mm	I/ R/ S
Boitshoko	**BOI 2.3**	17	I	20	S	17	I	22	S	28	S	15	I	18	I	26	S	21	S
Loula Fourie	**CCLF 1.1**	13	R	13	R	15	I	18	S	25	S	14	I	13	R	22	S	18	S
	CCLF 1.2	0	R	0	R	10	R	21	S	22	S	14	I	17	I	20	S	0	R
	CCLF 2.2	12	R	10	R	12	R	21	S	20	S	16	I	31	S	13	R	0	R
	CCLF 2.2	11	R	0	R	11	R	23	S	21	S	15	I	15	I	26	S	20	S
	CCLF 2.3	0	R	0	R	9	R	21	S	30	S	20	S	16	I	20	S	16	I
	CCLF 3.1	12	R	14	I	15	I	20	S	32	S	22	S	13	R	26	S	13	I
	CCLF 3.1	14	I	12	R	14	R	18	S	32	S	17	S	14	R	24	S	19	S
Mponeng	**MPO 2.3**	0	R	7	R	18	S	19	S	17	I	20	S	16	I	25	S	15	I

Table 8D: Antibiotic susceptibility data and MAR phenotypes for the isolates from water samples collected from schools after the vacation

School	The antibiotic resistance/susceptibility data for faecal coliform isolates from schools (1 indicates resistance and 0 indicates susceptibility)										
	SITE	**AMOX**	**AMP**	**CEP**	**CHL**	**CIP**	**KAN**	**OXY**	**STR**	**TM**	**MAR PHENOTYPES**
Mponeng	**MPO 2.3**	**1**	**1**	0	0	0	0	0	0	0	AMOX-AMP
Loula Fourie	**CCLF 1.1**	**1**	**1**	0	0	0	0	**1**	0	0	AMOX-AMP-OXY
	CCLF 1.2	**1**	**1**	**1**	0	0	0	0	0	**1**	AMOX-AMP-CEP-TM
	CCLF 2.2	**1**	**1**	**1**	0	0	0	0	**1**	**1**	AMOX-AMP-CEP-STR-TM
	CCLF 2.2	**1**	**1**	**1**	0	0	0	0	0	0	AMOX-AMP-CEP
	CCLF 2.3	**1**	**1**	**1**	0	0	0	0	0	0	AMOX-AMP-CEP
	CCLF 3.1	**1**	0	0	0	0	0	**1**	0	0	AMOX-OXY
	CCLF 3.1	0	**1**	**1**	0	0	0	**1**	0	0	AMP-CEP-OXY
Boitshoko	**BOI 2.3**	0	0	0	0	0	0	0	0	0	
	Total	7	7	5	0	0	0	3	1	2	
	% Resistant (R/N)*	77.78%	77.78%	55.56%	0.00%	0.00%	0.00%	33.33%	11.11%	22.22%	*(R/N = Total resistant / Total number of sites)

APPENDIX E

PHYSICO-CHEMICAL AND MICROBIOLOGICAL GUIDELINES FOR DRINKING WATER

Table 1E: The effects of pH on human health (DWAF, 1996)

pH Range (pH units)	**Effects**
< 4.0	Severe danger of health effects due to dissolved toxic metal ions. Water taste sour.
4.0 - 6.0	Toxic effects associated with dissolved metals, including lead, are likely to occur at a pH of less than 6. Water taste slightly sour.
Target Water Quality Range (6.0 - 9.0)	No significant effects on health due to toxicity of dissolved metal ions and protonated species, or on taste are expected. Metal ions (except manganese) are likely to dissolve readily unless complexing ions or agents are present. Slightly metal solubility may occur at the extremes of this range. Very slight effects on taste may be noticed on occasion.
9.0 - 11.0	Probability of toxic effects associated with deprotonated species increase sharply. Water tastes bitter at a pH greater than 9
>11.0	Severe danger of health effects due to deprotonated species. Water tastes soapy at pH of greater than 11.

Table 2E: The effects of nitrate on human health (DWAF, 1996)

Nitrate Range (mg/l)	**Effects**
Target Water Quality Range (0- 6)	No adverse health effects
6 - 10	Rare instances of methaemoglobinemia in infants, no effects in adults. Concentrations in this range generally well tolerated
10 - 20	Methaemoglobinemia may occur in infants. No effects in adults
>20	Methaemoglobinemia may occur in infants. Occurrence of mucous membrane irritation in adults

Table 3E: The effects of faecal coliforms on human health (DWAF, 1996)

Faecal Coliform Range (counts/100 mg/l)	**Effects**
Target Water Quality Range 0	Negligible risk of microbial infection
0 - 10	Slight risk of microbial infection with continuous exposure, negligible effects with occasional or short-term exposure
10 - 20	Risk of infectious disease transmission with continuous exposure, slight risk with occasional exposure
> 20	Significant and increasing risk of infectious disease transmission. As faecal coliform levels increase, the amount of water ingested required to cause infection decreases

Table 4E: The effects of total coliforms on human health (DWAF, 1996)

Total Coliform Range (counts/100 mg/l)	**Effects**
Target Water Quality Range 0	Negligible risk of microbial infection
5 - 500	Indicative of an adequate treatment, post-treatment contamination or growth in the distribution system. Risk of infectious disease transmission with continuous exposure and a slight risk with occasional exposure
>100	Indicative of poor treatment, contamination or definite growth in the water distribution system. Significant and increasing risk of infectious disease transmission

APPENDIX F

PCR RESULTS

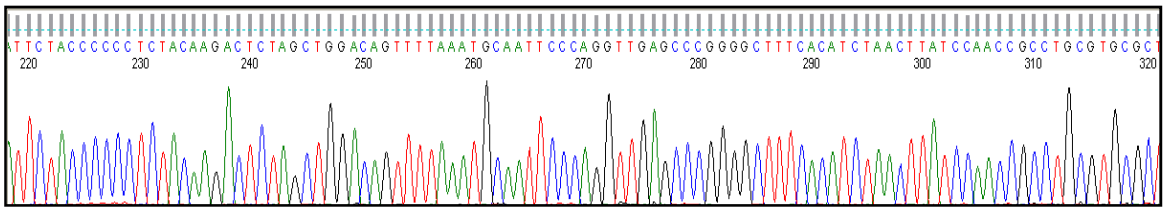


Figure 1F: The chromatograph of the sequence of the isolate from Sonderwater Tap 1 – *Aeromonas sp.* (Accession number: GQ983188)

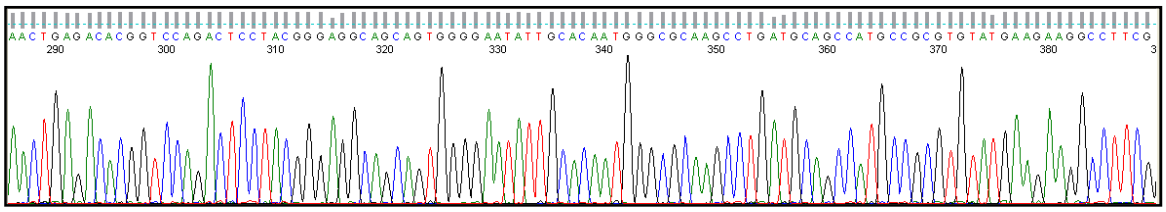


Figure 2F: The chromatograph of the sequence of the isolate from Sonderwater Tap 3 – *Enterobacter sp.* (Accession number: GU086162)

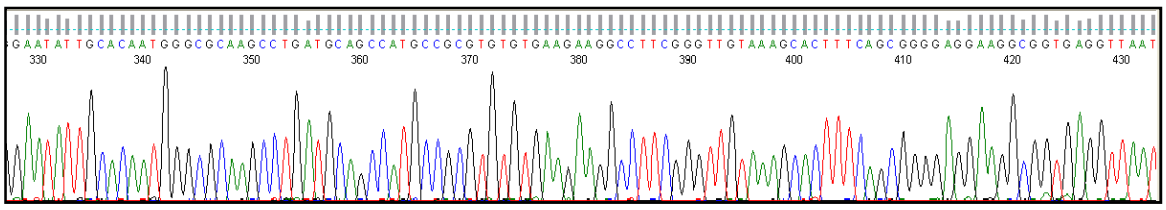


Figure 3F: The chromatograph of the sequence of the isolate from Fikadibeng before the vacation – *Klebsiella sp.* (Accession number: FJ828890)

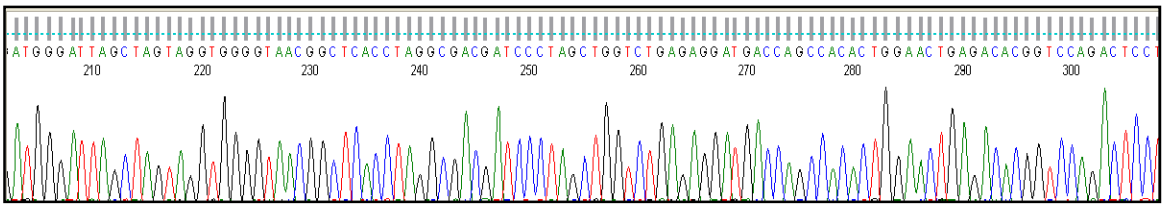


Figure 4F: The chromatograph of the sequence of the isolate from Mponeng before the vacation – *Escherichia sp.* (Accession number: GU132242)

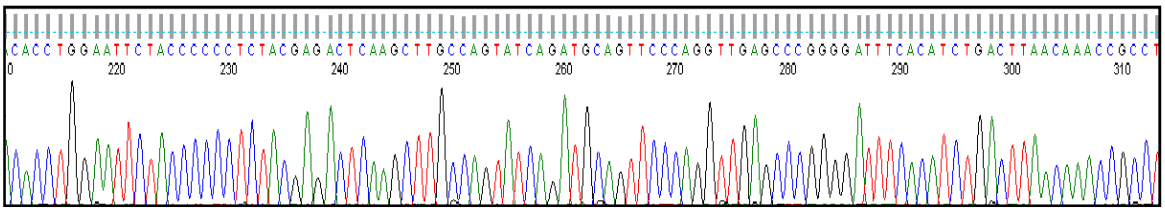


Figure 5F: The chromatograph of the sequence of the isolate from Sizamele before the vacation – *Escherichia coli* (Accession number: GQ983181)

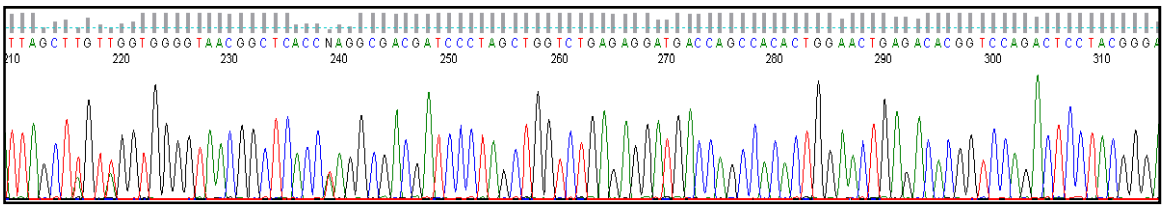


Figure 6F: The chromatograph of the sequence of the isolate from Loula Fourie after the vacation – *Escherichia sp.* (Accession number: GU132212)

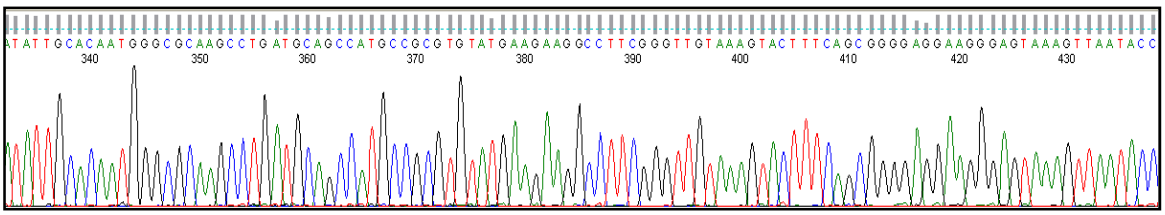


Figure 7F: The chromatograph of the sequence of the isolate from Boitshoko before the vacation – *Escherichia sp.* (Accession number: GU132242)

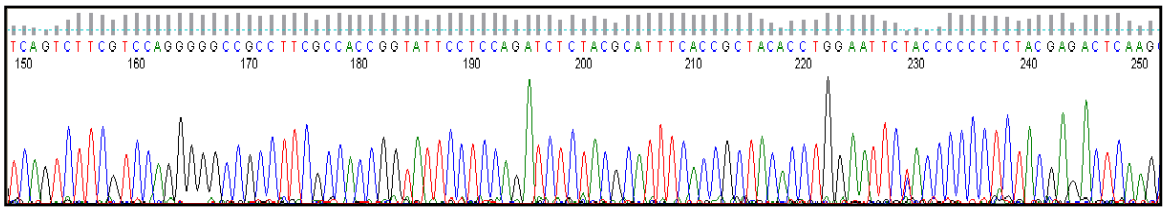


Figure 8F: The chromatograph of the sequence of the isolate from Boitshoko after the vacation – *Escherichia coli* (Accession number: GQ983181)

APPENDIX G

PHOTOS

a) **b)**

Figure 1G: **a)** A photo of the location of Tap 1 in Sonderwater, and **b)** a local resident collecting water at Tap 1 in Sonderwater.

a) **b)**

Figure 2G: a) A photo of the location of Tap 2 in Sonderwater, and **b)** a local resident drinking from Tap 2 in Sonderwater

a)

b)

Figure 3G: a) A photo of Tap 2 in Sonderwater during the second sampling session, when the actual tap was removed and the water was streaming from under a rock, and **b)** the location of Tap 3 in Sonderwater

Figure 4G: A photo of how a tap was sterilized before collection of water

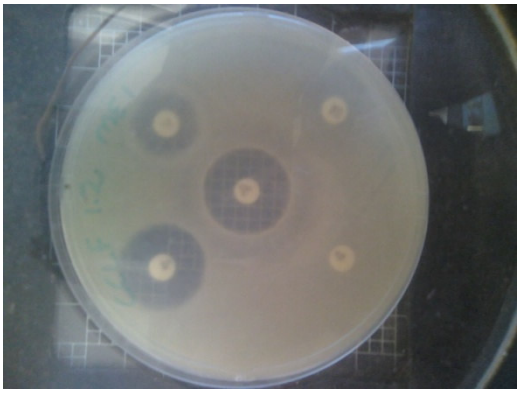
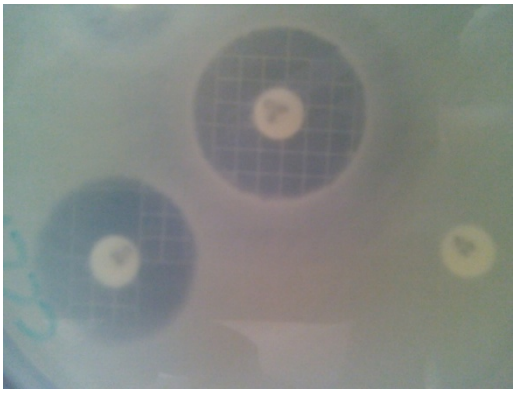
a)  **b)** 

Figure 5G: a) A photo of the placement of antibiotic discs on a plate so that there is no overlapping of inhibition zones, and **b)** a closer view of the inhibition zone of an antibiotic disc.