

**Potential groundwater contamination from pit latrines in
Ga-Maja and Reefentse**

by

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DECLARATION

I, Ramaesela Elizabeth Molapo, declare that the thesis/dissertation, which I hereby submit for the degree MSc Water Resources Management at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature

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LIST OF ABBREVIATIONS

Ca	Calcium
CLSI	Clinical and Laboratory Standards Institute
CSIR	Council for Scientific and Industrial Research
DWAF	Department of Water Affairs and Forestry
EC	Electrical Conductivity
EIA	Environmental Impact Assessment
EUCAST	European Committee on Antimicrobial Susceptibility Testing
F	Fluoride
Fe	Iron
Mg	Magnesium
mg/L	Milligrams per Litre
mS/m	MilliSiemens per meter
NO ₃ -N	Nitrate nitrogen
Na	Sodium
PO ₄ ³⁻	Phosphate
SANS	South African National Standards
SAWQG	South African Water Quality Guidelines
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
TWQR	Target Water Quality Range
WHO	World Health Organisation
WRC	Water Research Commission

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ABSTRACT

Pit latrines are one of the most common methods of human excreta removal in low-income countries. Groundwater is a critical water resource in many peri-urban areas where there is no municipal water supply. Groundwater resources are commonly vulnerable to pollution from on-site sanitation practises, which may degrade their quality. Because groundwater is widely used for domestic purposes, maintaining groundwater quality is a vital livelihood intervention in rural areas. This study sought to assess the impacts of pit latrines on groundwater quality in Ga-Maja, Reefentse and Ga-Mothiba which was used as the reference site. For the purpose of this study, samples were collected from boreholes and a hand-dug well in the study areas. An assessment of microbial, chemical and physical qualities of groundwater in Ga-Maja, Ga-Mothiba and Reefentse was carried out to determine whether contamination of groundwater from the use of pit latrines could lead to potential human health risks. The samples were analysed for calcium (Ca), fluoride (F), iron (Fe), magnesium (Mg), nitrate nitrogen ($\text{NO}_3\text{-N}$), phosphate (PO_4^{3-}), sodium (Na), sulphate (SO_4), total organic carbon (TOC), pH, total dissolved solids (TDS) and electrical conductivity (EC). These are some important parameters for assessing the groundwater quality for drinking purposes. The South African National Standards (SANS 241) and the Department of Water Affairs and Forestry (DWAf), now known as the Department of Water and Sanitation (DWS) South African Water Quality Guidelines (SAWQG) for domestic use were used to assess groundwater's suitability for domestic and drinking purposes. This study used the Colilert™ technique to determine indicator organisms, and the agar diffusion method to determine antibiotic resistance in isolated pathogenic bacteria. Isolates were assayed against ten antibiotics using the Kirby Bauer disc diffusion technique. Identifying the sensitivity and resistance of specific pathogens to a variety of antimicrobial agents is necessary as it influences the choice of antibiotics for empiric management of infections and effective hospital infection control.

The results showed that most of the parameters were within the permissible range of DWAf guidelines for domestic water use and SANS 241 drinking water guidelines. Samples from Ga-Maja showed EC value of (76.7 mS/m), concentration of TDS (490.88 mg/L) and Ca (45 mg/L) above DWAf domestic water use guidelines. During the wet season, all samples had high total coliforms above DWAf domestic water use guidelines and SANS 241 drinking water guidelines. The presence of nitrates at high concentrations was another health concern present in Reefentse. A number of organisms that are potential pathogens were identified from the study areas. During the wet season, all isolates were resistant to amoxicillin-clavulanic acid. There was high resistance to many antibiotics particularly ampicillin (67%), ciprofloxacin (78%), erythromycin (78%), gentamicin (78%) and tetracycline (63%). During the dry season, all isolates displayed resistance to the macrolides (clarithromycin and erythromycin), amoxicillin-clavulanic acid, ciprofloxacin, and gentamicin. There



was high resistance to many antibiotics particularly ampicillin (77%), norfloxacin (85%) and tetracycline (85%). Undesirable properties of water quality caused by the presence of antibiotic-resistant bacteria can pose a negative impact on human health. The presence of total coliforms at high concentrations from all sites is a public health concern. The nitrate levels were high in Reefentse, making the groundwater unsuitable for human consumption. Water treatment and the use of alternative sources are possible solutions for improving the quality of water used for drinking and other household purposes.

Keywords: contamination, groundwater, human health, on-site sanitation, pit latrines, water quality

CHAPTER 1

INTRODUCTION

1.1. Background information

Groundwater makes up about 97 percent of all freshwater that is possibly accessible for human use and is thus essential to human life (Lawrence et al., 2001). Groundwater currently contributes between 13 and 15 percent of South Africa's total water use (Pietersen, 2005). Since it was historically easy to access and cost-effective, surface water has been the main supply of water for human consumption. However, in order to keep up with the ever-increasing demands for domestic, industrial, agricultural, and ecosystem preservation, groundwater use has increased. Rosewarne et al. (2005) reported that South Africa has limited water resources and is ranked globally amongst the twenty most water-scarce countries.

The importance of groundwater continues to grow across the country because it is easily available, cost effective and the quality of surface water resources is deteriorating due to pollution (Edokpayi et al., 2017). It is therefore very important to put in place measures to protect existing water resources from pollution (Sililo et al., 2001). Anthropogenic impacts are increasingly challenging the quality of groundwater partly due to the introduction of pathogenic microorganisms (Krauss and Griebler, 2011).

The United Nations (2010) recognises access to water and sanitation as a basic human right. Lack of access to safe, adequate, and cost-effective water and sanitation facilities has serious repercussions for the health and dignity of billions of people, as well as substantial implications for the fulfilment of other human rights. According to The United Nations (2015), every person has the right to physical and affordable sanitation that is safe, hygienic, and socially acceptable, as well as providing and ensuring privacy.

1.2. Problem statement

In most rural communities of South Africa, access to drinking water that is both clean and safe is still a major issue. To meet their daily water needs, a large number of rural communities rely on untreated surface and groundwater sources (Mpenyana-Monyatsi et al, 2012). The biggest concern with water from these sources is that they may be faecally contaminated and often lack any form of treatment. Microbiological and chemical contaminants from human activities, natural sources and on-site sanitation systems used mostly in rural areas have the potential to contaminate groundwater sources (Mpenyana-Monyatsi et al., 2012). Groundwater is considered to be a safe and dependable source of potable water, but it is vulnerable to microbial contamination by *E. coli* bacteria (Vilane and Dlamini, 2016).

Pit latrines are a type of sanitation technology that is commonly used in rural areas for onsite waste management. They comprise of a pit in the ground that can be lined or unlined, as well as a supporting material such as bricks or concrete rings to retain the human excreta. Pit toilets can last anywhere from 10 to 30 years, depending on their nature and frequency of usage, however, many are filled and must be emptied or covered after being used for less than five years (Orner et al., 2019).

According to Graham and Polizzotto (2013), approximately 21 to 40 percent of South Africans use pit latrines. Statistics South Africa (STATSSA, 2016) had reported that 16.6 percent of South African households utilise ventilated pit latrines, while 14.5 percent use non-ventilated pit latrines. A physical barrier between excreta and soil is usually absent in pit latrines, allowing bacterial pollutants to leach into the groundwater and soil (van Ryneveld and Fourie, 1997). These pollutants may pose a health risk to people as a result of contaminated well-water (Graham and Polizzotto, 2013).

In disadvantaged communities, not much attention is paid during the construction of on-site sanitation systems. Maintenance of the systems, desludging of tanks and disposal of wastewater is often not carefully considered and as a result public health issues continue. In densely populated areas where groundwater is the only source of drinking water, the risk of contamination from on-site sanitation structures reaching groundwater is often very high (Krishnan, 2011). According to Krishnan (2011), groundwater contamination could be caused by on-site sanitation systems that are not well designed and dense habitation.

Human excreta contain various chemical and pathogen species that are harmful to human health and the natural environment. Disease outbreaks such as cholera, typhoid, hepatitis A and other diarrhoeal diseases often result from water contaminated with pathogens. These diseases can also

spread by other means, but the quality of public water sources is crucial as these sources can distribute contaminated water to many people.

1.3. Research aim, objectives, and questions

1.3.1. Research aim

The aim of this project is to determine whether contamination of groundwater from the use of pit latrines could lead to potential human health risks.

1.3.2. Research objectives

- To determine whether the use of pit latrines could lead to potential contamination of groundwater in the Ga-Maja and Reefentse areas compared to a reference site in Ga-Mothiba.
- To investigate the potential health risks associated with contaminated groundwater.

1.3.3. Research questions

The research questions, specifically regarding the groundwater environment will be to answer:

- What is the extent and nature of contamination of groundwater in Ga-Maja and Reefentse compared to the reference site at Ga-Mothiba?
- Which of the selected water quality parameters are affected by groundwater contamination?
- Do the microorganisms in groundwater pose a risk to human health?

CHAPTER 2

LITERATURE REVIEW

2.1. Overview

Many rural areas in South Africa still lack access to water services and as a result, rely on untreated water sources to satisfy their daily water requirements (Mothetha et al., 2013). Groundwater contamination is a major concern, as is the possibility that on-site sanitation systems will contaminate drinking water under some circumstances (Lawrence et al., 2001). Lorentz et al. (2015) argues that the overall health and pollution impacts of on-site sanitation systems needs to be assessed. The widespread use of pit latrines has been the subject of numerous studies, but there has been no clear methodology for tracking or recording the degree of nutrient or pathogen movement. According to Graham and Polizzotto (2013), given the growing use of pit latrines and groundwater resources in developing countries, there is concern that pit latrines can pose risks to human and ecological health due to groundwater contamination by microbiological and chemical contaminants. The extent to which microorganisms can be transported from pit latrines waste and pollute groundwater is heavily influenced by the surrounding environment, especially soil and hydrogeological conditions (Graham and Polizzotto, 2013). Chemical pollutants and pathogenic bacteria discharged from on-site sanitation systems and infiltrated into nearby groundwater sources pose a greater threat in densely populated areas, where on-site sanitation facilities and drinking water supplies are close together (Shivendra and Ramaraju, 2015). According to the Institute for Resource Analysis and Policy (2010), on-site sanitation is not a major risk if groundwater is extracted from deep and confined aquifers.

A safe, cost-effective, and easily available water supply is essential for good health, yet nearly a billion people in poor nations have been without safe and sustainable water supply for decades (Hunter et al., 2010). According to the World Wide Fund for Nature (WWF-SA) (2016), groundwater is any subsurface or underground water that is stored in soils, rock pores, or aquifers. It appears in the form of springs and seeps and is extracted through boreholes or wells. Most of the time, water from these sources is used without being treated, posing the risk of faecal contamination. Because of their economic advantage, simple and inexpensive on-site sanitation techniques for disposing of faecal matter have been developed. The biggest disadvantage is their well-known ability to contaminate groundwater supplies (Lewis et al., 1980).

Because a sanitation system has the potential to pollute groundwater, the risk should be assessed and the groundwater constantly monitored, especially where water is intended for human consumption (CSIR Building and Construction Technology, 2000).

2.2. Factors affecting groundwater contamination from on-site sanitation

2.2.1. Groundwater source distance from place of sanitation

In general, large deep pits for ventilated improved pit toilets are preferable. However, if rock layers or the groundwater level are within a metre or two from the ground's surface, this may not be possible. The pit should be dug downhill and at the minimum 30 metres away from the borehole or well. It should be built on somewhat elevated terrain with solid soil (Bester and Austin, 2000).

A distance of at least 15 metres is required between a source of pollution and a water abstraction point downstream (Franceys et al., 1992). The distance may be reduced if the abstraction point is to the side or upstream, as long as the groundwater is not abstracted at a rate that causes the flow direction to be turned towards the abstraction point. Kimani-Murage and Ngindu (2007) states that, although the general rule for all soil conditions is difficult to establish, the commonly used guideline is that the well should be located in a high lying area relative to the pit latrine and should be at least two metres above the water table. However, such criteria may not be possible to meet in some areas, or the conditions of the soil such as fissured limestone, do not provide groundwater protection if these guidelines are followed (Cave and Kolsky, 1999). In South Africa, the distance between a borehole and a pit that must be maintained is generally around 30 metres, however this varies depending on the site's geotechnical characteristics (Bester and Austin, 2000).

Where groundwater pollution from ventilated improved pit (VIP) toilets could occur, it is worth considering alternative sanitation options, or the contaminated water should be treated before use. Bester and Austin (2000) suggest that the depth of pits should be kept to a minimum, thus the attenuation zone between the effluent and the water table increases. In areas where groundwater is the primary source of water supply, the toilet design must be altered to ensure that the groundwater is not contaminated or that the level of pollution is kept to a minimum (Bester and Austin, 2000). Instead of a deep single-pit VIP latrine, a shallow twin-pit VIP latrine may provide sufficient depth in the unsaturated zone (Mara, 1984). According to Wolf et al. (2015), larger separation distances usually indicate a longer period of time that pathogens travel to the well. During that travel time, more pathogens will either be inactivated or be filtered out by the soil.

2.2.2. Hydrogeological conditions

The characteristics of the subsurface geology and the water table depth have been found to influence the susceptibility of groundwater contamination. Pollutants have less of an impact on aquifer water quality if they take longer to reach the groundwater. DWAF (2003) states that water movement within the saturated zone, for instance below the water table, is usually only a few metres per day and may be as little as one metre per year. The key parameters affecting groundwater pollution are hydrogeological factors including, depth of the water table, composition of the soil matrix as well as the lateral barrier between the on-site sanitation and the source of groundwater (Lawrence et al., 2001; Lewis et al., 1980).

Because water percolates vertically through the soil, removing much of the microbial and organic contamination, the bacteriological quality of deep aquifer groundwater is usually quite good (Harikuamar and Chandran, 2013). Transportation within the aquifer is dependent on the rate of flow of groundwater and condition of the aquifer. Where karst, variable cemented limestone and sand deposits are present, typical of reef environments, solution characteristics and preferential flow paths can occur, resulting in the rapid transportation of pollutants over very lengthy distances (Dillon, 1997).

2.2.3. Nitrates and faecal coliforms

The most common reason why groundwater sources are declared unfit for drinking in South Africa is the presence of nitrate at levels above 10 mg/L (NO_3 as N) (Tredoux and Talma, 2006). Nitrates may derive from natural or anthropogenic sources. The impact of on-site sanitation on groundwater quality indicates that nitrates, chloride, and faecal coliforms are the parameters likely to be affected (Pujari et al., 2012). Agricultural practises such as excessive use of inorganic fertilizers and manures, wastewater treatment, and the oxidation of nitrogenous waste products in human and animal excreta, including septic tanks, can cause nitrates to reach both surface and groundwater (WHO, 2011a). High nitrate levels are a significant issue for bottle-fed babies, whose risk of methaemoglobinaemia increases as the nitrate levels rise and in adults, this might cause irritation of the mucous membrane (DWAF, 1996; Thompson et al., 2007). According to the WHO (2012), pharmaceuticals have also become chemicals of emerging public concern due to their potential to reach drinking water. According to Lawrence et al. (2001), each person releases approximately 4 kg of nitrogen per year, with a significant portion of that nitrogen oxidized to form nitrate under aerobic conditions. Therefore, groundwater sources are vulnerable to contamination by nitrate because of on-site sanitation systems' continuous nitrate loading.

2.3. Chemical and physical characteristics

The physical and chemical parameters play a major role in determining the quality of water. The results of the chemical and physical parameters determine the effectiveness of water treatment processes required and appropriate control measures that will render the water safe (WHO,2011). The assessment of chemical and physical parameters is important in assuring provision of safe water to communities.

2.3.1. pH

The pH of groundwater is associated with dissolved carbonates and bicarbonates, silicates, borates, fluorides and other salts in dissociated forms (Deshmukh, 2013). Organic acids may also have a significant effect on pH (Munson and Gherini, 1993). Water with a low pH can have a sour taste, whilst water with a high pH can have a bitter or soapy taste (DWAF, 1996). The pH of water as it enters the distribution system must be kept under control to prevent household water mains and pipes from corroding. The pH values of water may affect both the degree of corrosion of metals and the efficiency of disinfection (WHO, 1996).

2.3.2. Electrical conductivity

Electrical conductivity (EC) represents a measurement of water's ability to carry an electrical current (DWAF, 1996). The presence of dissolved solids such as chloride, sulphate, nitrate, carbonate, bicarbonate, sodium, potassium, calcium and magnesium in water samples creates the ability as they all carry an electrical charge (DWAF, 1996). While EC does not offer extensive information on the ionic composition of water, it may be used to determine whether the water is safe for drinking and irrigation (Elumalai et al., 2017).

2.3.3. Total dissolved solids

Total dissolved solids (TDS) represents the amount of various inorganic salts which are present as a solution in water (DWAF, 1996). TDS can also be used to assess if water is suitable for drinking and irrigation (Elumalai et al., 2017). TDS in water supplies comes from natural sources, agricultural and urban run-off, sewage and wastewater from industries (WHO, 2003). Because of excessive scaling in water pipes, heaters, and boilers, and because of the salty taste, high levels of TDS in water may be unacceptable to consumers (WHO, 2003).

2.3.4. Calcium

Calcium (Ca) is an important element for all living things, including plants and animals, and is a major component of the solutes in most natural water (Hem, 1985). One of the main contributors to water

hardness is calcium. Hard water might lead to an increase in soap use and scale formation in water distribution systems and hot water applications, resulting in insoluble metal carbonates covering surfaces and lowering heat exchanger performance (WHO, 2010).

2.3.5. Magnesium

Magnesium (Mg) is essential in plant and most living organism's nutrition (Hem, 1985). Magnesium is also a contributor to water hardness (DWAF, 1996). At high concentrations, magnesium tends to have a bitter taste and is the cause of scaling issues in appliances that use heating components and piping which transports hot water (DWAF, 1996).

2.3.6. Fluoride

The fluoride (F) concentration in groundwater has adversely affected people's lives, causing dental and skeletal fluorosis through the action of the fluoride ion on teeth and on bones (McCaffrey and Willis, 2001). Fluoride intake at high levels can harm the skeleton, causing the bones to harden, which in turn makes them brittle, this causes the bones to break easily, and crippling may occur (WRC, 1998). Low fluoride levels are associated with high levels of dental decay, although poor nutritional status is also an important contributory factor (Fawell et al., 2006). Drinking groundwater contaminated with fluoride and other harmful naturally occurring compounds is a leading cause of chronic disease, disability and death (Thompson et al., 2007).

2.3.7. Iron

All living organisms require iron (Fe) as a micronutrient (DWAF, 1996). When groundwater is extracted directly from a well, it may contain ferrous iron at certain concentrations without causing discoloration or turbidity (WHO, 2011b). When there is an excessive amount of iron in water, it produces red oxyhydroxide precipitates, which stain clothing and plumbing fixtures, making it intolerable in domestic and industrial water supplies (Hem, 1985; WHO, 2011b). If present at concentrations higher than 0.3 mg/L, iron will negatively affect the taste of water (DWAF, 1996).

2.3.8. Phosphate

Phosphate (PO_4^{3-}) may occur in groundwater due to organic matter decomposition, sewage disposal, domestic and industrial sewage effluents, detergents and use of phosphate fertilizer (Hem, 1985). It forms part of the important nutrients required for the development and nutrition of living organisms. Phosphate can contribute to a rapid growth of algae in slow-flowing or still water bodies, which may ultimately produce toxins and lead to eutrophication (Thompson et al., 2007).

2.3.9. Sodium

The most prevalent sources of increased sodium (Na) concentrations in groundwater include weathering of sodium bearing-minerals or rocks, irrigation returns, and pollution from industrial effluent and domestic sewage (Dinka et al., 2015). Human activities such as the use of salt to de-ice roads, may have a significant impact on sodium levels in surface and groundwater (Hem, 1985). Water that has been reused or recycled frequently leaves a residue with a higher concentration than the original water (Deshmukh, 2013; DWAF, 1996). Excessive levels of sodium in drinking water have been associated to hypertension and high blood pressure (WHO, 1979).

2.3.10. Sulphate

Sulphates (SO_4^{2-}) are dissolved from gypsum-containing rocks, iron sulphides and other sulphur compounds (Nolakana, 2016). Acid mine drainage and effluent return flows can pollute water, resulting in high sulphate levels (WRC, 1998). Drinking water containing sulphate can have a salty or bitter taste, and excessive concentrations may cause diarrhoea in sensitive and non-adapted individuals (WHO, 2011b; DWAF, 1996; WRC, 1998).

2.3.11. Total organic carbon

Total organic carbon (TOC) determinations can provide valuable diagnostic evidence of the extent of organic compound contamination of groundwater (Barcelona, 1984). Total organic carbon concentrations are usually lower in groundwater than in surface water (Hem, 1985). Where groundwater contains dissolved organic carbon, it is also susceptible to the formation of trihalomethanes (THMs), which are known carcinogens, during chlorination at water treatment works (Gooddy and Hinsby, 2008).

2.4. Health risks

Drinking water that is safe and clean is important to human health and can help decrease the burden of infectious diseases like diarrhoea, particularly in young children (Edokpayi et al., 2018). Lack of sanitation and poor hygiene contribute to water-borne illnesses like cholera, typhoid, diarrhoea, and several parasitic infections (United Nations-Water, 2006). It has been reported by Lukenga (2015) that more than 2.2 million people die per year from diseases that could be prevented in developing countries related to lack or no access to potable water, poor hygiene, and insufficient sanitation. Evans and Mara (2015) reported that poor hygiene, poor sanitation, and inadequate water supplies accounted for four percent of all deaths in the year 2000 and accounted for 5.7 percent of the overall global burden of disease.

The World Health Organisation (2008) has indicated that drinking water that has been contaminated with human or animal faeces poses the greatest microbial risk. Pathogenic bacteria, protozoa, viruses and helminths can all be found in faeces. Faecal-oral disease transmission and nitrate poisoning are the most common health concerns associated with water quality degradation from on-site sanitation. Consumption of water with high nitrates may lead to a fatal condition known as methaemoglobinaemia, also known as the “blue baby syndrome”. This is a disorder in which oxygen cannot be efficiently transferred or released into the bloodstream and occurs mainly in children under three months of age (Cave and Kolsky, 1999). According to Higgins (2006) newborn babies are at a high risk of methaemoglobinaemia because during foetal development and for the first three to six months of life, red blood cells contain foetal haemoglobin (Hbf) rather than adult haemoglobin (HbA).

Increased cancer risk has also been linked to high nitrate levels in drinking water. There may be an increased threat of particular cancers and birth defects when nitrate is consumed in conditions that increases the production of N-nitroso compounds (van Grinsven et al., 2006). Of all the studies that were previously conducted, thyroid disease, colorectal cancer and neural tube defects are the strongest indication for a link between nitrate in drinking water and negative health outcomes (in addition to methaemoglobinaemia) (Ward et al., 2018). The SAWQG for domestic use (DWAf, 1996) has indicated that faecal coliforms are used to indicate the presence of bacterial pathogens such as *Shigella* sp, *Campylobacter jejuni*, *Salmonella enterica*, *Vibrio cholerae*, *Yersinia enterocolitica*, *Campylobacter coli* and pathogenic *E. coli*. These organisms can be transmitted by polluted or improperly treated drinking water through faecal/oral route and have the potential to cause illnesses like, cholera, typhoid fever, dysentery, salmonellosis, and gastroenteritis.

Cholera is also one of the major illnesses caused by *Vibrio cholerae*, which can be found in contaminated food or water. Cholera outbreaks are prevalent in impoverished areas with poor sanitation facilities and limited access to safe drinking water (Okello et al., 2016). The outbreak of

cholera in Mpumalanga Province between December 2008 and March 2009 was because of contaminated and unsafe water supplies (Sigudu et al., 2015). Communities in Limpopo Province, Vhembe District Municipality, experienced an outbreak of cholera between November 2008 and April 2009, due to poor sanitation facilities and limited drinking water infrastructure (Mudau et al., 2017).

Table 1. Examples of illnesses acquired by the ingestion of faecally contaminated water (Lawrence et al., 2001; Krauss and Griebler, 2011).

<u>Pathogen</u>	<u>Disease</u>
Viruses	
Hepatitis A virus	Infectious hepatitis
Poliovirus	Poliomyelitis
Astrovirus, calcivirus, rotaviruses, noro-viruses	Diarrhoeal disease
Coxsackieviruses and echoviruses	Diarrhoeal disease
Bacteria	
<i>Campylobacter jejuni</i>	Diarrhoeal disease
Enterohaemorrhagic <i>E. coli</i> 0157	Hemorrhagic colitis
Enteroinvasive <i>E. coli</i>	Diarrhoeal disease
Enteropathogenic <i>E. coli</i>	Diarrhoeal disease
<i>Salmonella typhi</i>	Typhoid fever
<i>Shigella</i> f.	Dysentery
<i>Vibrio cholerae</i> O1	Cholera
Protozoan parasites	
<i>Cryptosporidium</i> spp.	Diarrhoeal disease
<i>Giardia lamblia</i>	Diarrhoeal disease

2.5. Guidelines/Standards for drinking-water quality

Water quality is determined by a set of physical and chemical parameters that are closely related to the intended use of the water. Solsona (2002) defined a standard as a rule or principle considered by an authority and by general consent as a basis of comparison. Furthermore, a proper standard for drinking water quality is thus the reference that ensure that the water will not be harmful for human health. The water is considered suitable for a given use if it meets the pre-defined standards for that use. If the water fails to meet these standards, it must be treated before use (Cordoba et al., 2010).

2.5.1. World Health Organization Guidelines

The primary goal of the drinking-water quality guidelines is to protect public health (WHO, 2011b). The four aspects of the WHO guidelines for drinking water quality are microbial, chemical, radiological and acceptability aspects.

The senses of touch, sight, smell and taste determine the physical properties of water (temperature, colour, taste, odour). Physical methods such as conductivity, pH, and turbidity measurement can be used to determine these properties. The chemical properties describe the nature and concentration of dissolved substances such as metals, salts and organic chemicals. Bacteria pollution is the most common cause of biological contamination of drinking water sources. Microbial contamination refers to the presence of microorganisms such as protozoa, viruses, helminths and bacteria (WHO, 2011b). The guideline values are typically the concentrations of a constituent that do not pose a significant health risk over the course of a lifetime of consumption (WHO, 2017).

2.5.2. South African Water Quality Guidelines

DWAF developed the SAWQG to serve as its primary source of information and decision-making support for determining the suitability of water for use and other water quality management purposes (DWAF, 1996). Water users can use the guidelines to learn about the physical, chemical, biological and aesthetic properties of water. The Target Water Quality Range (TWQR) is the quality criteria which specifies good or ideal water quality rather than just acceptable water quality (DWAF, 1996).

2.5.3. South African National Standards (SANS 241)

The South African National Standards for drinking water were established by the South African Bureau of Standards (SABS). The standards specify the microbiological, physical, aesthetic, and chemical characteristics of acceptable drinking water. Water that complies with the standards is deemed to present an acceptable health risk for lifetime consumption (SABS, 2015).

CHAPTER 3

MATERIALS AND METHODS

This chapter discusses the materials and methods that were used to process and analyse the data in order to achieve the aims and objectives of the study.

3.1. Study areas

Three different areas were selected for the collection of samples and the testing of various parameters. Two of the areas are highly populated (Ga-Maja and Reefentse) while the farm where livestock farming takes place (Ga-Mothiba) was used as a reference site. Ga-Maja is a rural area with agricultural activities mainly for the production of food crops. Reefentse is a peri-urban area, and according to Macagnano (2002) peri-urban areas have a rapid increase of large informal settlements with various and difficult-to-control activities such as uneconomical distribution of infrastructure. According to Baloyi and Diamond (2019), rural, agricultural and industrial settings have quite obvious sources of contaminants, while urban settings are more challenging, with numerous probable sources of contaminants.

3.1.1. Site one – Ga-Maja

The first impacted site is situated at Ga-Maja village that lies in Polokwane Local Municipality, Limpopo Province in South Africa. According to Statistics South Africa (STATSSA, 2011a), Ga-Maja has a total population of about 8 053 and approximately 2 020 households. The samples were obtained from Ga-Maja Moshate and the geographical coordinates of the sample point are - 24.184592° latitude and 29.529367° longitude (Figure 1). In Ga-Maja municipal water supply is supplied to most households by Lepelle Northern Water. However, most households have their own boreholes as the municipal source is perceived not to be reliable. The borehole depths in the village were reported to range between 60 and 100 metres below the ground level and are in most cases located near pit latrines.

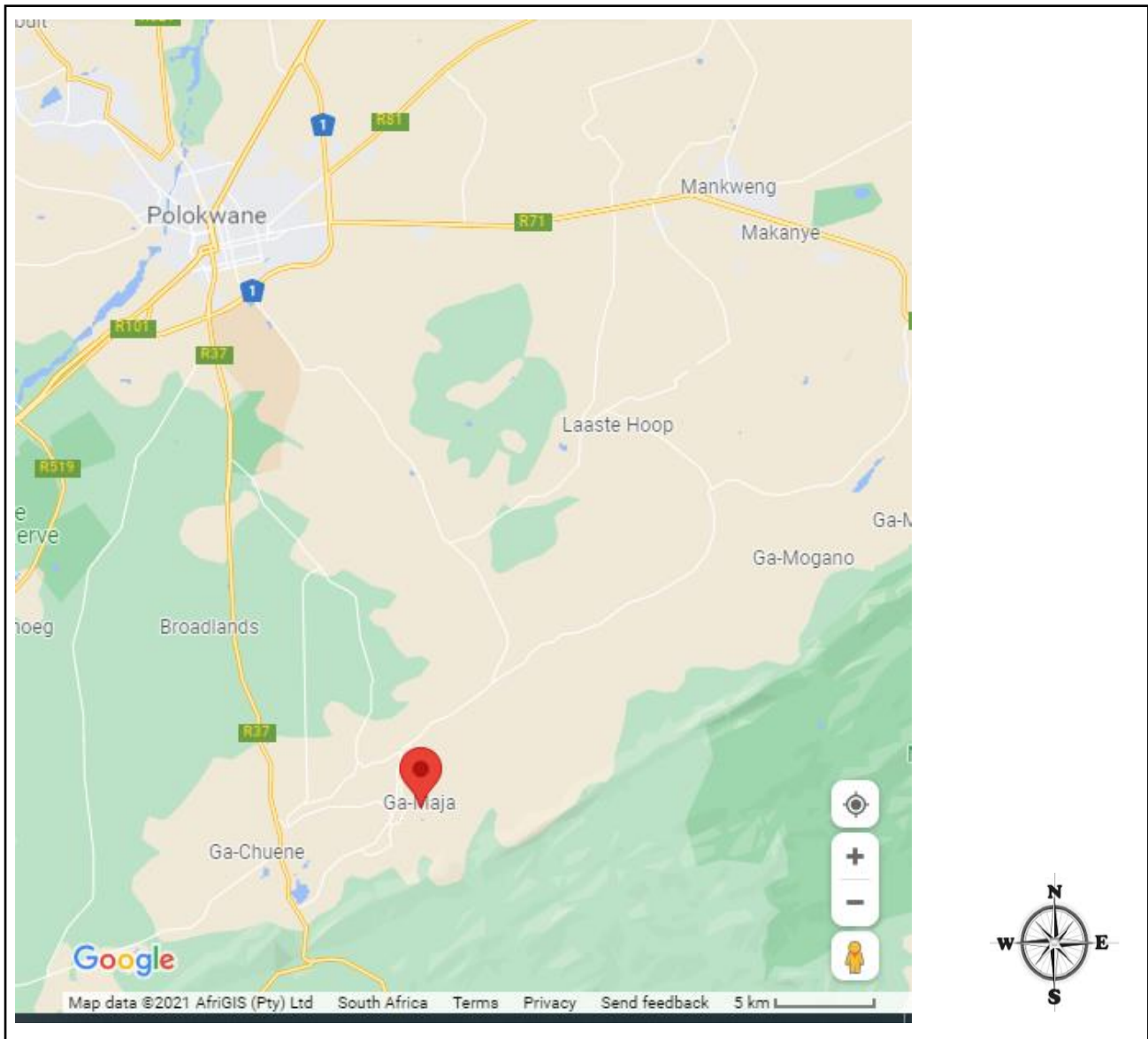


Figure 1: Map showing the geographical location of Ga-Maja, Limpopo Province, South Africa (Google Map, 2021a).

3.1.2.

3.1.3. Site two – Ga-Mothiba

The second site where samples were taken from is the reference site which lies in Ga-Mothiba Ngwanamago village and forms part of Polokwane Local Municipality, Limpopo Province in South Africa. The sampling area is located approximately 6.5 km away from the main village and has only one house used by the farmer's family. Livestock farming activities at the farm include cattle, goats,

sheep and pigs, which the owner breeds and sells to the neighbouring villages. There is low population as most of the workers spent the day in the fields looking after cattle. According to Statistics South Africa (STATSSA, 2011b), Ga-Mothiba has a total population of about 11 511 and approximately 2 825 households. The geographical coordinates of the sample point are -24.048537° latitude and 29.584139° longitude (Figure 2).

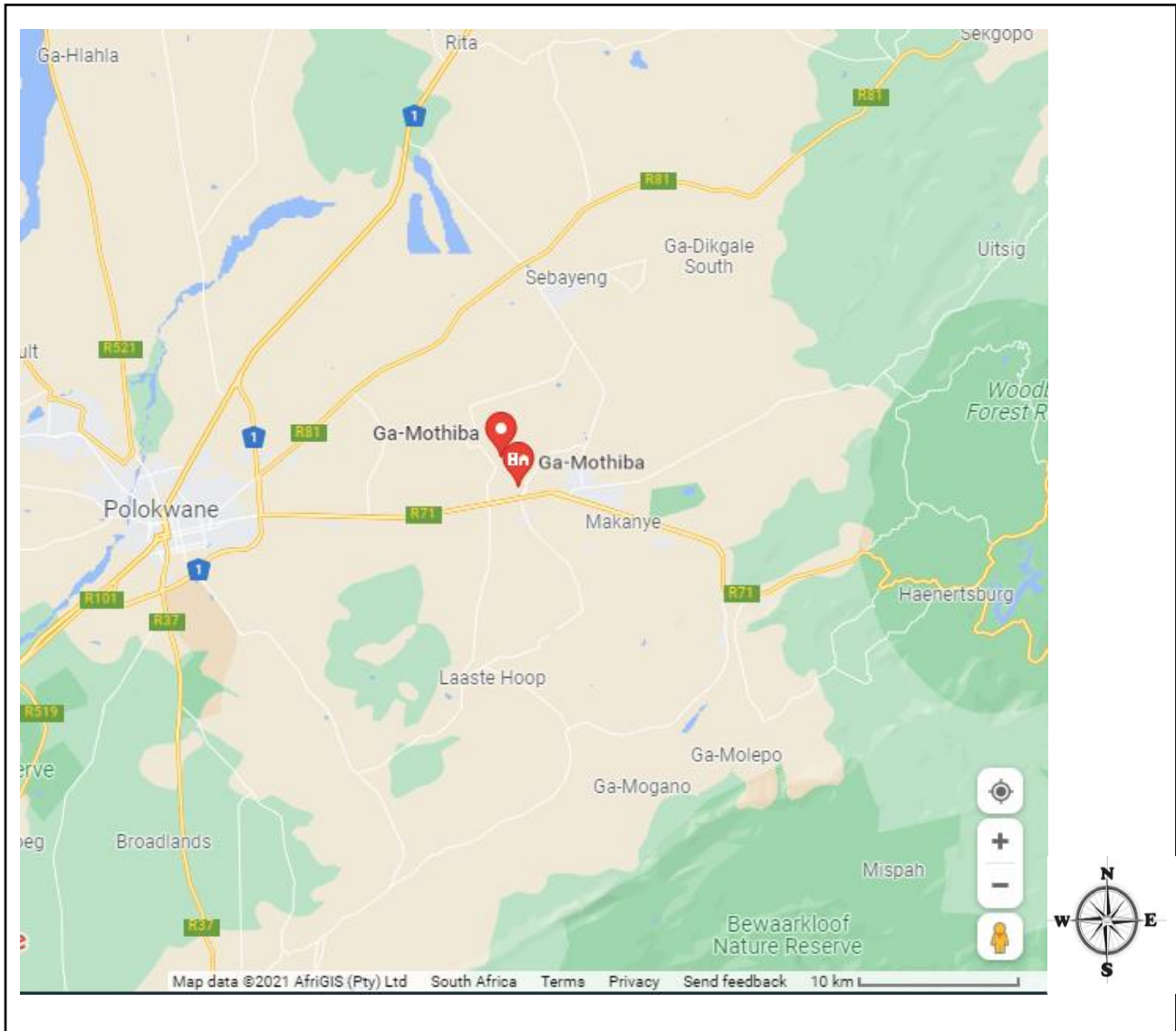


Figure 2: Map showing the geographical location of Ga-Mothiba (Klipspruit Alzu Feeds Voere), Limpopo Province, South Africa (Google Map, 2021b).

3.1.4. Site three – Reefentse

The third site which is an impacted site is Reefentse (formerly known as Stinkwater). It is about 40 km north of Pretoria and 13 km west of the N1 freeway, which runs through Hammanskraal and has access off Lucas Mangope Drive. It forms part of the City of Tshwane Metropolitan Municipality, within Gauteng Province of South Africa. The Reefentse settlement is part of the broader

Hammanskraal-Soshanguve area, a wide area of rural, low-density urban and industrial development within remnants of natural vegetation. Reefentse has municipal water supply to some households, from Magalies Water. The municipal water supply is perceived to be unreliable, and some residents are also reliant on municipal water tanker deliveries. Many residents rely on hand-dug wells or boreholes in their yards for their water because municipal water deliveries are often irregular, and the water supply is not always sufficient. A study in the Reefentse area by Abia et al. (2017) has shown that water from Reefentse's wells and boreholes is microbiologically unsafe for human consumption. According to Statistics South Africa (STATSSA, 2011c), Reefentse has a total population of 39 201 and approximately 9 213 households. The geographical coordinates of the sample point are -25.398844° latitude and 28.159426° longitude (Figure 3). The sampling site uses a hand-dug well, approximately four metres deep and is in an area that is characterized by informal housing and pit latrines are widely used in the area.

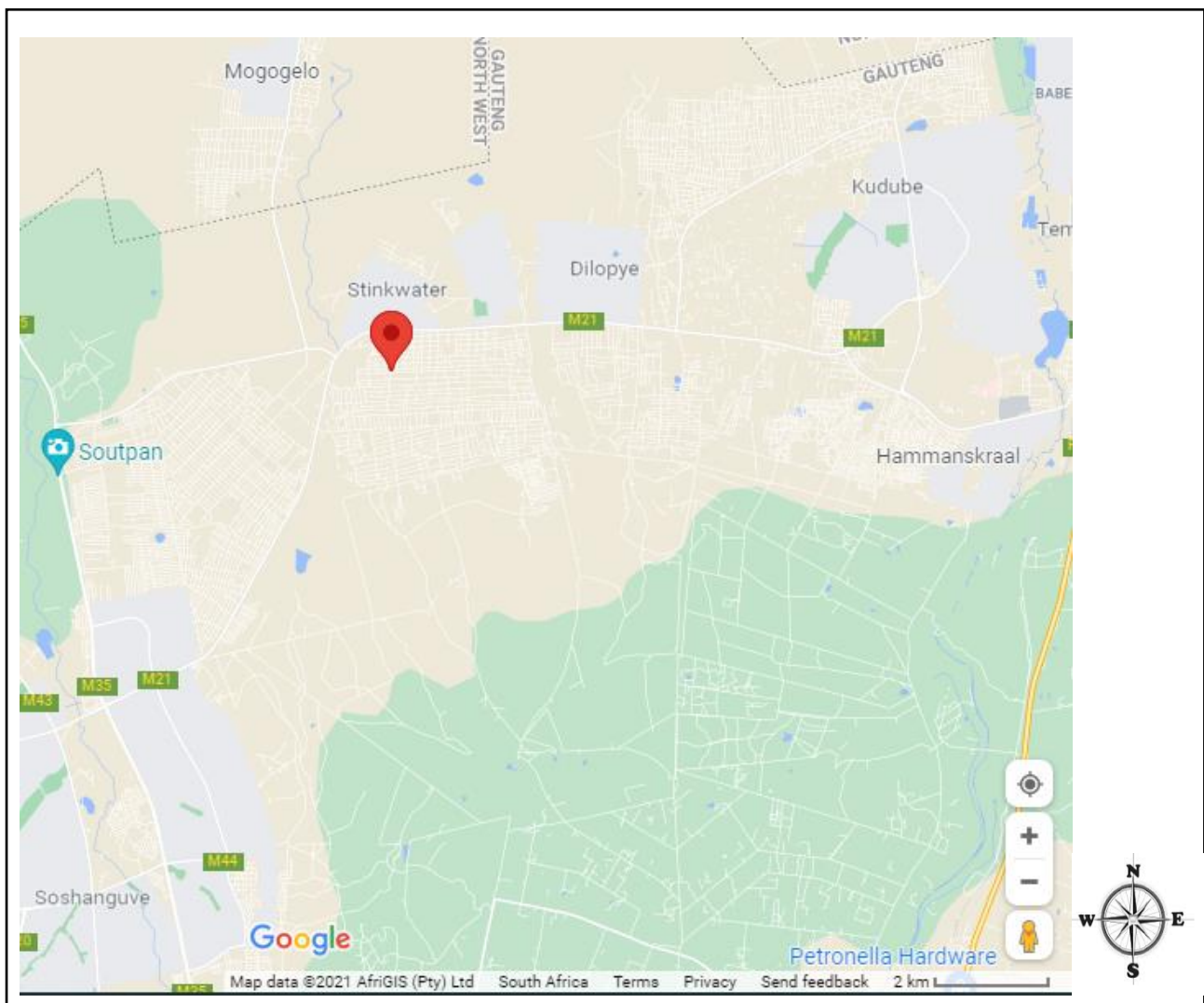


Figure 3: Map showing the geographical location of Reefentse community, Hammanskraal Gauteng Province, South Africa (Google Map, 2021c).

3.2. Sample collection

As shown in Figure 4, water was sampled from boreholes via a tap connected to the borehole water supply system. A bucket attached to a rope was used to collect samples from hand-dug wells. Samples for laboratory testing were collected on 28 April 2019 for the wet season and were collected on 20 August 2019 for dry season sampling. Water samples were placed in plastic bottles provided by Council for Scientific and Industrial Research (CSIR) and processed at the laboratory within 24 hours for all time-sensitive parameters.



Figure 4: Typical sources analysed in the target villages of the study. **(a)** borehole with a standpipe (Ga- Maja); **(b)** borehole with a standpipe (Ga-Mothiba); **(c)** hand-dug well (Reefentse).

3.3. Experimental setup

3.3.1. Total coliforms and enumeration of *E. coli*

On 29 April 2019, water samples from the three sites were cultured at CSIR premises in Pretoria. Total coliforms and *E. coli* were analysed using the Colilert™ technique (18-hour version) and made use of the Quanti-Tray 2000 system (Figure 5). For the enumeration procedure, the capsule contents were added into a 100 mL sample in a sterile vessel. The vessel was capped and then shook until the nutrients dissolved into the water. Carefully avoiding contact with the inside of the foil tab, the sample mixtures were poured directly into the Quanti-Tray 2000 and were sealed using the Quanti-Tray Sealer (Figure 5). The trays were incubated at 35 °C for 18 to 24 hours. After incubation, the yellow wells were identified as positive for total coliforms and all fluorescent blue wells (that were also yellow under normal lighting) were identified as positive for the presence of *E. coli* as shown in Table 2. Counts were recorded as the Most Probable Number (MPN) per 100 mL (MPN/100 mL) of water sample.

Table 2: Quanti-tray/2000 results interpretation table (Idexx, USA).

Appearance	Result
Less yellow than the comparator	Negative for total coliform and <i>E. coli</i>
Yellow equal to or greater than the comparator	Positive for total coliforms
Yellow and fluorescence equal to or greater than the comparator	Positive for <i>E. coli</i>

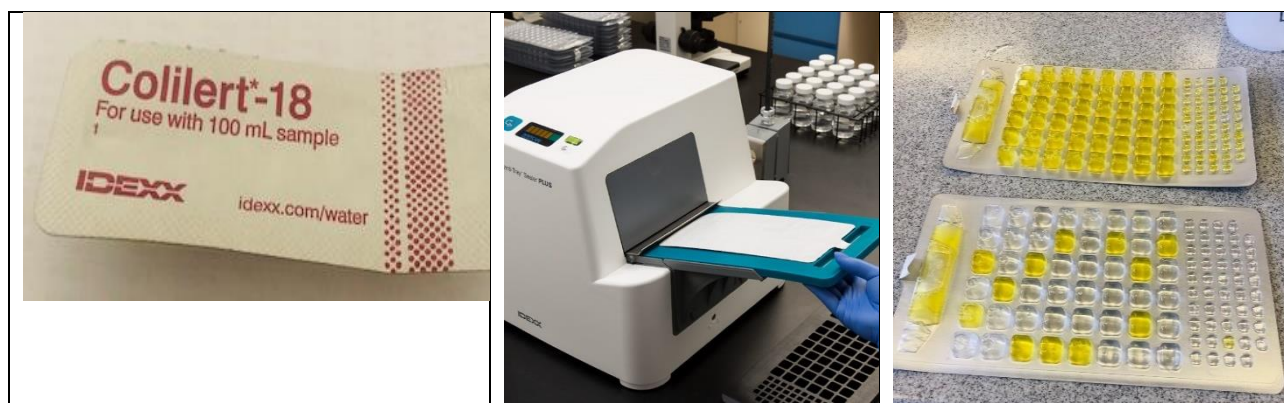


Figure 5: Experimental setup using Colilert-18, Quanti-Tray Sealer and Quanti-Tray/2000.

3.3.2. Water quality assessment

The SANS 241 (SABS, 2015), SAWQG for domestic water use (DWAF, 1996) standards and the WHO guidelines for drinking-water quality (WHO, 2011b, WHO, 2017 and WHO, 2008) were used as the basis of evaluating the suitability of groundwater for domestic purposes. The samples from the study areas were analysed for chemical and physical parameters such as calcium (Ca), fluoride (F), Iron (Fe), magnesium (Mg), nitrate nitrogen (NO₃-N), phosphate (PO₄³⁻), sodium (Na), sulphate (SO₄), total organic carbon, pH, total dissolved solids (TDS) and electrical conductivity (EC). These are some important parameters for assessing the groundwater quality for drinking purposes. The pH and EC were measured using Thermo Scientific, Orion 5-Star model (ThermoFisher Scientific, UK). The calcium, iron, magnesium, sodium, fluoride, nitrate nitrogen, ortho phosphate, and sulphate content of the water were analysed by the CSIR Environmental Laboratories (Facility T007, CSIR, Pretoria) using their SANAS accredited method. The method used to analyse total organic carbon is not SANAS accredited and is not included in the SANAS Schedule of accreditation for the CSIR Environmental Laboratories. The total dissolved solids (TDS) were calculated by $TDS (mg/L) = EC (\mu S/cm) \times 0.64$ (Lloyd and Heathcote, 1985). The exact values of the conversion factor depends on the ionic composition of the water, especially the pH and bicarbonate concentration (DWAF, 1996). Due to financial implications chemical analysis of water quality was only performed for the wet season.

3.3.3. Bacterial analysis

Hundred microliter aliquots of the sampled water was spread-plated onto nutrient agar plates, this was done in duplicate for each site. The agar plates were incubated at 35 °C for 18 to 24 hours. Results were read on 30 April 2019 for the wet season sampling and on 22 August 2019 for the dry season sampling. Phenotypically distinct colonies were selected, and sub-cultured on nutrient agar plates (Oxoid, England) to obtain pure cultures and the agar plates were incubated at 35 °C.

3.3.4. Analysis for *Vibrio cholerae*

On 21 August 2019, alkaline peptone water (APW) was prepared for testing of *Vibrio cholerae*. For a 1000 mL bottle, 5 g of sodium chloride (Merck, South Africa) and 10 g of peptone powder (Merck, South Africa) were dissolved. Five hundred millilitres of distilled water was added to make double strength of the solution. The pH of the samples was 6.73 and an adjustment was made to pH 8.6-9.00 using 1 mol/L sodium hydroxide (Merck, South Africa). *Vibrio cholerae* does not thrive well in acidic water and hence the adjustment was made. The medium was dispensed into 100 mL aliquots in screwcap bottles and sterilized by autoclaving at 110 °C-120 °C for 10-15 minutes. For the selective enrichment of *V. cholerae* 100 mL of the sampled water was added to an equal volume of

double strength APW and incubated overnight at 35 °C. Cells were harvested by extracting 1 mL of the post-incubation broth with a pipette, taking care to extract as close to the top of the broth as possible. The extracted cells were concentrated by centrifugation at 8000g for 2 minutes, followed by DNA extraction using Instagene™ matrix (Biorad, USA), following the manufacturer's protocol. *Vibrio cholerae* detection was performed using a polymerase chain reaction (PCR) assay targeting the *ompW* and *ctxAB* genes (le Roux, 2011).

3.3.5. *Salmonella* and *Shigella* analysis

Buffered peptone water (Oxoid, England) was used to enrich for *Salmonella* and *Shigella* species prior to detection by PCR. Ten grams of buffered peptone water was added to 250 mL distilled water to make a double strength solution. An equal volume of sample was added to the buffered peptone water (100 mL of sample water to 100 mL of double-strength broth) and incubated overnight at 35 °C. Cells were harvested by extracting 1 mL of the post-incubation broth with a pipette, and then concentrated by centrifugation at 8000g for 2 minutes. This was followed by DNA extraction using Instagene™ matrix (Biorad, USA). *Salmonella* sp. were detected by real-time polymerase chain reaction (PCR) targeting the *invA* gene (Malorny et al., 2003). *Shigella* (virulent types) and/or *E. coli* EIEC were detected by real-time PCR targeting the *ipaH* (invasion plasmid antigen) gene (Theron et al., 2001).

3.3.6. Antibiotic resistance profiling

Antibiotics widely used in animal and human medicine were selected for use in this study. Antibiotics used and their final concentrations in solution are shown in Table 3. The Kirby Bauer disc diffusion technique (Bauer et al., 1966) was used and zones of inhibition were measured and compared to a suitable standard (Clinical and Laboratory Standards Institute, 2017) to determine if the organisms were resistant, intermediately susceptible or susceptible to the tested antibiotics. The procedure was carried out using 1.5 mL graduated microcentrifuge tubes (Figure 6). Each tube containing a distinct bacterial culture suspension received 0.5 mL of phosphate buffered saline. The solution was mixed using a V1 plus vortex mixer (Figure 6). The mixed solution was pipetted onto 90 mm agar plates in a volume of 0.1 mL, and each isolate was tested against a battery of ten antibiotics (Figure 6) using Mastdiscs™ (Mast Diagnostics, United Kingdom). The respective bacterial isolates were subjected to the various antibiotics. The plates were incubated at 35 °C for 18 to 24 hours and plates were examined for growth. The results for the wet and dry season were read on 17 May 2019 and 29 August 2019, respectively. Following incubation, the diameter of the zone of inhibition around each antibiotic disc was measured to the closest millimetre. Zones were observed against a black, non-reflecting background illuminated with a high intensity lamp.

There is a lack of defined methods for susceptibility testing and interpretive criteria for *Bacillus* sp. (other than *Bacillus anthracis*) (CLSI, 2010). There is no disk diffusion susceptibility testing data available on *Bacillus* sp. from CLSI and EUCAST. CLSI only provides information and interpretive criteria for Minimum Inhibitory Concentration (MIC) breakpoints which was not done in this research. *Escherichia coli* data from CLSI zone diameter chart was used for determining drug sensitivity of all gram-negative bacteria and *Bacillus* data from CLSI was used to determine drug sensitivity of all gram-positive bacteria. The application of interpretation criteria developed for other distantly related species could lead to incorrect susceptibility interpretations (AgersØ et al., 2018).

Table 3: Antibiotics used in the study.

Antibiotic Name	Concentration (µg/L)	Antibiotic Class
Amoxicillin, Clavulanic Acid	20; 10	Penicillin, beta-lactamase
Ampicillin	2	Penicillin
Cefazolin	30	Cephalosporin
Ciprofloxacin	5	Fluoroquinolone
Erythromycin	15	Macrolide
Clarithromycin	15	Macrolide
Gentamicin	10	Aminoglycoside
Meropenem	10	Carbapenem
Norfloxacin	10	Fluoroquinolone
Tetracycline	30	Tetracycline



Figure 6: Experimental set-up using graduated microcentrifuge tube, V1 plus vortex mixer and antibiotics in an agar plate.

3.3.7. Identification of bacterial isolates

Identification of the bacterial isolates was done by Inqaba Biotechnical Industries (Pty) Ltd. Bacterial isolates were characterised by sequencing the 16S rDNA. Genomic DNA was extracted from the cultures using Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, USA). The 16S target region was amplified using OneTaq® Quick-Load® 2X Master Mix (New England Biolabs) with the primers presented in Table 4. The PCR products were run on a gel and gel extracted with the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, USA). The extracted fragments were sequenced in the forward and reverse direction using BrilliantDye™ Terminator Cycle Sequencing Kit V3.1 (Nimagen, Netherlands) and purified using ZR-96 DNA Sequencing Clean-up Kit™ (Zymo Research, Irvine, California). The purified fragments were analysed on the Applied Biosystems 3500xl Genetic Analyzer (ThermoFisher Scientific, UK) for each reaction for every sample, as listed in Section 1. CLC Bio Main Workbench v7.6 was used to analyse the .ab1 files generated by the ABI 3500XL Genetic Analyzer and results were obtained by a BLAST search (NCBI).

Table 4: 16S Primers sequences.

Name of Primer	Target	Sequence (5' to 3')
16S-27F	16S rDNA sequence	AGAGTTTGATCMTGGCTCAG
16S-1492R	16S rDNA sequence	CGGTTACCTTGTTACGACTT

CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents the results and discussions based on the chemical, physical and microbiological determinands that were measured in April 2019 for the wet season and August 2019 for the dry season. The results discuss the specific isolates that were identified, and antibiotic resistance patterns of bacteria isolates against antibiotics.

4.1. Chemical and physical characteristics

Physical and chemical parameters such as pH, electrical conductivity, total dissolved solids, cations like calcium, magnesium, sodium, iron and anions like sulphate, nitrate, phosphate and fluoride are some important parameters for assessing the groundwater quality for drinking purposes. The chemical and physical parameters of the groundwater samples from site one (Ga-Maja), site two (Ga-Mothiba) and site three (Reefentse) are shown in Table 5. The values in red exceed SANS 241 and/or the SAWQG for domestic use limits (SABS, 2015; DWAF, 1996).

Table 5: Wet season chemical and physical water quality results of samples analysed in Ga-Maja, Ga-Mothiba and Reefentse.

Analysis (All analysis in mg/L unless otherwise stated)	SANS 241:2015 Limits	SAWQG for domestic water use TWQR	Site 1 Ga-Maja	Site 2 Ga-Mothiba	Site 3 Reefentse
pH @ 25 °C	≥ 5 to ≤ 9.7	6.0 - 9.0	6.73	6.72	6.39
EC, mS/m @ 25 °C	≤ 170	0 - 70	76.7	36.1	58.9
TDS	≤ 1200	0 - 450	490.88	231.04	376.96
Calcium, Ca	----	0 - 32	45	20	32
Magnesium, Mg	----	0 - 30	28	12	17
Fluoride, F	≤ 1.5	0 – 1.0	0.75	0.21	< 0.2
Iron, Fe	≤ 2 / ≤ 0.3	0.1 – 0.3	0.0035	0.012	0.0084
Nitrate, as N	≤ 11	0 - 6	0.45	9.8	31
Phosphate, PO ₄ ³⁻	----	----	< 0.2	< 0.2	< 0.2
Sodium, Na	≤ 200	0 - 100	65	30	39
Sulphate, SO ₄	≤ 500 / ≤ 250	0 - 200	140	< 5	< 5
Total organic carbon, C	≤ 10	----	< 10	< 10	< 10

4.1.1. pH

The pH of water samples taken from boreholes and a hand-dug well was found to be slightly acidic, ranging from 6.39 to 6.73, with the highest value found in site one (Ga-Maja). There are no pH guidelines that are based on health, although a range of 5 to 9.7 is suggested by SANS 241 (SABS 2015). Table 5 shows that the pH values of all the water samples taken from boreholes and hand-dug well were within the suggested limit of no risk for drinking and domestic use (SABS, 2015; DWAF, 1996). Although pH does not affect consumers directly, it forms part of the significant parameters for operational water quality (WHO, 2017). At all stages of water treatment, careful pH control is required to guarantee satisfactory water clarification and disinfection. The pH for effective chlorine disinfection should preferably be less than 8 (WHO, 2011b). At low pH water may taste sour, while at high pH water tastes bitter or soapy (DWAF, 1996). The pH results obtained therefore suggest that the groundwater sources are within the range of water that does not taste sour or bitter.

4.1.2. Electrical conductivity

The electrical conductivity values of all groundwater samples ranged between 36.1 and 76.7 mS/m, as indicated in Table 5, with site one (Ga-Maja) having the highest value. This study demonstrates that the EC values of all water samples from boreholes and the hand-dug well were within the suggested limit (≤ 170 mS/m) of no risk for drinking purposes as stipulated in SANS 241 (SABS, 2015). Water samples from site one (Ga-Maja) exceeded the recommended limit (70 mS/m) of the proposed target water quality range set out by the SAWQG for domestic use (DWAF, 1996). The high level of electrical conductivity from this borehole is mainly due to the levels of sodium, sulphate, magnesium and calcium which were higher than those recorded from site two and three. A higher value of electrical conductivity does not usually pose a health risk to humans until it exceeds 450 mS/m (DWAF, 1996). At concentrations greater than 450 mS/m, water tastes extremely salty and corrosion or scaling of pipes and appliances increases (DWAF, 1996). According to the electrical conductivity values obtained from the study areas, the groundwater sources are within the range of water that does not have a salty taste and does not corrode or scale pipes and appliances.

4.1.3. Total dissolved solids

The calculated total dissolved solid concentrations of the water samples ranged from 231.04 to 490.88 mg/L as indicated in Table 5, and the highest level was observed in site one (Ga-Maja). The TDS concentrations revealed that all boreholes and the hand-dug well samples were within the suggested limit of 1 200 mg/L total dissolved solids for no risk set out by SANS 241, whereas samples collected in site one (Ga-Maja) were above the suggested limit (450 mg/L) of no risk according to the SAWQG for domestic use (SABS, 2015; DWAF, 1996). The TDS value from site one (Ga-Maja) is primarily linked to high levels of calcium, magnesium, sodium and sulphate. Although most of these parameters were within the SAWQG for domestic use (DWAF, 1996), they

were found to be closer to the recommended limits. Drinking water with a TDS level of <600 mg/L is considered to be of acceptable quality (WHO, 2011b). A higher concentration of total dissolved solids usually has a distinct salty flavour (DWAF, 1996); however, it is well tolerated with regards to the concentrations obtained from site one (Ga-Maja). According to DWAF (1996), short-term consumption at concentrations between 2 000 – 3 000 mg/L may be tolerated, but the body's salt balance is likely to be disrupted. The TDS values obtained from the study areas indicate that the salty taste is well accepted, and there are unlikely to be any negative health impacts.

4.1.4. Calcium

The calcium concentrations of all groundwater samples ranged from 20 to 45 mg/L, with the highest concentration found at site one (Ga-Maja), according to the chemical and physical parameters results as shown in Table 5. The results indicate that the values obtained from site one (45 mg/L) and site three (32 mg/L) were found to be higher than the recommended limit of 0 to 32 mg/L as indicated in the SAWQG for domestic use (DWAF, 1996). There is no health effects and no scaling evidence associated with calcium concentrations below 32 mg/L (DWAF, 1996) .

The calcium concentration recorded from site one falls outside of the TWQR of 0 to 32 mg/L recommended by the SAWQG for domestic use (DWAF,1996). Calcium does not have WHO guideline value assigned to it due to the ambiguities surrounding mineral nutrition from drinking water (WHO, 2011b). The calcium value obtained falls within the taste threshold value of 100 to 300 mg/L as per the WHO (WHO, 2011b) guidelines, however high concentrations cause the elements in hot water appliances to scale (WRC, 1998). High concentrations of calcium impair the lathering of soap, resulting in excessive use of soap used in personal hygiene (DWAF, 1996; WRC, 1998). The calcium values recorded from site two (Ga-Mothiba) and site three (Reefentse) indicate that there will be no scaling in heating water appliances. The value obtained from site one (Ga-Maja) suggests that the groundwater can cause increased scaling problems and result in excessive use of soap.

4.1.5. Magnesium

The values recorded in Table 5 for magnesium concentrations ranged from 12 to 28 mg/L. The TWQR according to the SAWQG for domestic use is 0 to 30 mg/L indicating that the water has no bitter taste, no scaling issues and no negative health effects (DWAF, 1996). The results in Table 5 showed that all of the groundwater samples were within the recommended risk limits. At high concentrations, magnesium can cause laxative effects in individuals who are not used to drinking the water (Thompson et al., 2007), particularly when present as magnesium sulphate (Deshmukh, 2013). According to the findings from the study areas, there will be no scaling issues and diarrhoea will not be experienced by new users.

4.1.6. Fluoride

The values recorded in Table 5 for fluoride concentrations were observed to range from < 0.2 to 0.75 mg/L. The fluoride concentration results showed that all the water samples were within the recommended limit of 0 to 1 mg/L as per the SAWQG for domestic use and ≤ 1.5 mg/L as per SANS 241 (DWAF, 1996; SABS, 2015). High fluoride concentrations may lead to teeth staining and fluorosis (Mpenyana-Monyatsi and Momba, 2012). The fluoride concentrations found in the study areas indicate that the users will not have stained teeth.

4.1.7. Iron

The results in Table 5 demonstrate that the iron concentrations in all of the water samples ranged from 0.0035 to 0.012 mg/L. The water samples complied with the recommended limits of ≤ 2 mg/L for chronic health risk and ≤ 0.3 mg/L for aesthetic risk as stipulated by SANS 241 guidelines (SABS, 2015). There is no health effects associated with iron at concentrations lower than 1 mg/L (DWAF, 1996).

4.1.8. Nitrate

As recorded in Table 5, all groundwater samples had nitrate concentrations ranging from 0.45 to 31 mg/L, with the highest concentration found at site three (Reefentse). The results revealed that the groundwater samples from Ga-Mothiba and Reefentse exceeded the recommended limits of (0 to 6 mg/L as N) for domestic water use (DWAF, 1996). The results from Reefentse exceeded the recommended limit of (≤ 11 mg/L as N) for drinking water as per SANS 241 guidelines and the WHO standard of 10 mg/L (SABS, 2015; WHO, 2008). The value obtained from site two (Ga-Mothiba) of 9.8 mg/L is of no greater concern as concentrations in the ranges between 6 to 10 mg/L are generally well tolerated (DWAF, 1996). Nitrate in groundwater is generally associated with agricultural activities or effluent leaks from on-site sanitation waste (Thompson et al., 2007). High nitrate levels are directly linked to methaemoglobinaemia, or “blue baby syndrome” in bottle-fed babies under three months of age and in adults, it could cause irritation of the mucous membrane (DWAF, 1996). The nitrate value obtained from site one (Ga-Maja) indicate that there will no adverse health effects for the water users. The value recorded from site two (Ga-Mothiba) may result in rare instances of methaemoglobinaemia and the nitrate concentration obtained from site three (Reefentse) may cause methaemoglobinaemia in infants.

4.1.9. Phosphate

Table 5 shows that all groundwater samples had phosphate levels of less than 0.2 mg/L. There are no phosphate limits stipulated by SANS 241 or the SAWQG for domestic use (SABS, 2015; DWAF, 1996). DWAF has approved a special effluent discharge standard of 1 mg/L orthophosphate for wastewater discharges from point sources (DWAF, 1999). Phosphate concentrations in surface and groundwater that occur naturally are not hazardous to human health, animals or the environment (Fadiran et al., 2007). High concentrations of phosphates supports the growth of bacteria and algae, leading to poor water quality and contributing to eutrophic conditions (Chapman, 1996). The phosphate concentrations recorded from the study areas are within the limits that does not support bacterial growth and algae and will not lead to poor water quality.

4.1.10. Sodium

According to the chemical and physical results shown in Table 5, the sodium levels in all groundwater samples ranged from 30 to 65 mg/L, with the highest sodium concentration found at site one (Ga-Maja). The sodium levels in the water samples were within the tolerable limit of 0 to 100 mg/L as indicated in the SAWQG for domestic use and ≤ 200 mg/L set by SANS 241 (DWAF, 1996; SABS, 2015). There are no aesthetic or health effects associated with sodium at concentrations lower than 100 mg/L (DWAF, 1996). However, high concentrations of sodium in drinking water may not be acceptable for infants who are bottle-fed due to its salty taste (DWAF, 1996; Thompson et al., 2007). High concentrations of sodium may also be a problem for adults who do not like the taste or for people with high blood pressure (DWAF, 1996; WRC, 1998). As a result of the findings, the groundwater at the study areas will not have a salty taste.

4.1.11. Sulphate

All groundwater samples had sulphate concentrations ranging from < 5 to 140 mg/L, with the highest concentration found at site one (Ga-Maja). Table 5 shows that the sulphate concentrations in all groundwater samples were within the suggested limit of no risk (0 – 200 mg/L) set out by the SAWQG for domestic use and ≤ 500 mg/L for acute health risk and ≤ 250 mg/L aesthetic risk set out by SANS 241 (DWAF, 1996; SABS, 2015). There is no health or aesthetic effects associated with sulphate concentrations analysed at the study areas. High concentrations of sulphate gives water a salty or bitter taste and causes diarrhoea in the majority of people (DWAF,1996). The sulphate concentrations recorded from the study areas indicate that the groundwater will not have a bitter or salty taste and the users are unlikely to experience diarrhoea.

4.1.12. Total organic carbon

The total organic carbon concentration results shown in Table 5 indicate that all the groundwater samples were within the recommended limit of ≤ 10 mg/L set out by SANS 241 (SABS, 2015). Total organic carbon is indicative of the suspended organic carbon found in solution (DWAF, 1996). A high TOC level increases oxidant demand, reduces sterilization efficacy, and produces toxic by-products (Miller and Fowler, 2011). The TOC values obtained from the study areas indicate that sterilization effectiveness will not be reduced, and toxic by-products will not be produced.

4.2. Microbial water quality

The microbiological determinands of the groundwater samples from site one (Ga-Maja), site two (Ga-Mothiba) and site three (Reefentse) are shown in Table 6. The values in red exceed the SANS 241 and/or the SAWQG for domestic use limits (SABS, 2015; DWAF, 1996)

Table 6: Wet and dry season microbial water quality results of samples analysed in Ga-Maja, Ga-Mothiba and Reefentse.

Analysis (All analysis in MPN/100 mL unless otherwise stated)	SANS 241:2015 Limits	SAWQG for domestic use TWQR	Wet season			Dry season		
			Site 1 Ga-Maja	Site 2 Ga-Mothiba	Site 3 Reefentse	Site 1 Ga-Maja	Site 2 Ga-Mothiba	Site 3 Reefentse
<i>E. coli</i> 100 mL	Not detected	0	0	0	110	0	0	30
Total coliform bacteria/ 100 mL	≤ 10	0-5	16	2419.6	2419.6	0	>2419.6	2419.6

4.2.1. *Escherichia coli* and total coliforms

The World Health Organisation recommends *E. coli* as the indicator organism of choice for faecal pollution (WHO, 2008). Based on this, WHO suggests that there should be no indicator organisms in water intended for human consumption (WHO, 2008). *Escherichia coli* were detected in site three (Reefentse) during both seasons. For *E. coli*, any detection is considered an exceedance, whereas the limit for total coliforms is 10 MPN/ 100 mL (SABS, 2015). Even if small amounts of water are consumed, higher levels of faecal coliforms in water will suggest a risk of contracting waterborne diseases (DWAF, 1996). The SAWQG for domestic water use (DWAF, 1996) recommend that potable water must contain zero to five total coliforms and zero *E. coli* counts per 100 mL, which translates a negligible risk of microbial infection. All the groundwater samples showed contamination by faecal matter, as the total coliform counts were very high. The presented data suggests that the groundwater sources that are used in the study areas constitute a public health concern.

Because of the barrier effects provided by the overlying soil and unsaturated zone, groundwater is perceived to be less vulnerable to the immediate influence of contamination sources. However, the results of this study areas show that some groundwater sources may have poor microbiological

quality. Excreta from pit latrines may contain pathogenic bacteria and viruses that can contaminate groundwater by passing through subsurface soils (Taonameso et al., 2019). The extent to which microbes from pit latrine wastes can be transported and contaminate groundwater is heavily influenced by the environmental context of the area, primarily the hydrological and soil conditions (Graham and Polizzotto, 2013). Borehole water is more susceptible to pit latrine contamination in regions with heavy rainfall and shallow water tables (Taonameso et al., 2019). Information on groundwater flow patterns, water table level, geological setup and soil type was not available in the study areas, this makes the association between groundwater contamination and pit latrines complex.

Total coliforms were detected from site one (Ga-Maja) during the wet season. This could be due to higher runoff or infiltration during the wet season, which transports bacteria from contaminated sources to the water body (Edokpayi et al., 2018). This finding is supported by a systematic review of water quality which showed that various types of water sources, including boreholes, suffered from greater contamination during the rainy season (Kostyla et al., 2015). The presence of total coliforms in site one (Ga-Maja) during the wet season could be associated to the close proximity of the borehole to the pit latrine. Wastewater may gain access into the groundwater through a leaching process (Potgieter et al., 2006). In most cases, an EIA is not conducted when drilling private boreholes, the quantity of boreholes drilled near pit latrines in the village attests to this. As a result, borehole water contamination is a possibility. There is a need to educate the owners of the boreholes and the rest of the community on the installation and maintenance of these boreholes. The need for appropriate technical studies before a borehole is drilled needs to be emphasized.

The high levels of total coliforms detected from site two (Ga-Mothiba) during both seasons could be attributed to poor sanitation conditions and practices at the farm. Cattle has access to waterways as there is no fencing at the farm. Leakage from manure storage piles or lagoons at animal feeding operations and land application of improperly treated wastewaters associated with animal slaughter are some of the major routes for faecal contamination to reach groundwater (Kumar et al., 2014). The site in Ga-Mothiba is used for livestock farming and other forms of agriculture. The presence of animals, including any type of animal or livestock, is positively associated with total coliform contamination (Kumpel et al., 2017).

The hand-dug well from site three (in Reefentse) was not well protected and covered. Water is extracted from the well with a rope attached to a bucket. The bucket is kept on the ground near the well under unhygienic conditions. The well is situated near a pit latrine and is used for drinking and other household purposes. Therefore, the open nature of the well, poor hygienic ways of extracting water from the well, shallow water table and the well's proximity to a pit latrine are factors that could

account for the high *E.coli* and total coliforms found at the site. Poor maintenance and unhygienic conditions have been reported as possible sources of groundwater contamination (Tamrakar et al., 2009).

Total coliform bacteria are referred to as “indicator organisms” because their presence in the water source indicates that other disease-causing microorganisms could be present. Total coliforms consist of a diverse group which include bacteria from the genera *Escherichia*, *Enterobacter*, *Citrobacter*, *Serratia*, *Klebsiella* and *Rahnella* (DWAF, 1996; WHO, 2006). Most of these bacteria belong to the family of *Enterobacteriaceae*, and they indicate the possible presence of bacterial pathogens such as *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*, *Campylobacter jejuni*, *Campylobacter coli*, *Yersinia enterocolitica* and pathogenic *Escherichia coli* (DWAF, 1996). Total coliforms may also come from soils or biofilms in the environment (Palamuleni and Akoth, 2015). The quality of groundwater may be influenced by the proximity of boreholes to solid waste dumpsites and animal waste littered nearby (Bello et al., 2013).

4.2.2. Specific isolates identified and potential health risks

Table 7 and 8 indicate various pathogenic bacteria detected by culture and 16S rDNA sequencing in the groundwater samples from site one (Ga-Maja), site two (Ga-Mothiba) and site three (Reefentse).

Table 7: Wet season pathogenic bacteria identified at each sampling site.

Name of sample	Organisms identified/ closest match(es)
Site 1 – Ga-Maja	
W1 – 1	<i>Lysinibacillus</i> sp, <i>Lysinibacillus fusiformis</i> , <i>Lysinibacillus macroides</i>
W1 – 2	<i>Bacillus</i> sp, <i>Bacillus aryabhatai</i> , <i>Bacillus megaterium</i>
W1 – 3	<i>Bacillus</i> sp, <i>Bacillus indicus</i>
W1 – 4	<i>Bacillus cereus</i>
Site 2 – Ga-Mothiba	
W2 – 1	<i>Aeromonas hydrophila</i>
W2 – 2	<i>Comamonas testosteroni</i>
Site 3 – Reefentse	
W3 – 1	<i>Aeromonas hydrophila</i>
W3 – 2	<i>Enterobacter cloacae</i>
W3 – 3	<i>Enterobacter asburiae</i> , <i>Enterobacter roggenkampii</i>

Table 8: Dry season pathogenic bacteria identified at each sampling site.

Name of sample	Organisms identified/ closest match(es)
Site 1 – Ga-Maja	
D1 – 1	<i>Bacillus flexus</i>
D1 – 2	<i>Bacillus atrophaeus</i>
D1 – 3	<i>Bacillus cibi</i>
D1 – 4	<i>Fictibacillus sp.</i>
Site 2 – Ga-Mothiba	
D2 – 1	<i>Aquitalea magnusonii</i>
D2 – 2	<i>Acinetobacter schindleri</i>
D2 – 3	<i>Bacillus cereus</i>
D2 – 4	<i>Bacillus cereus</i>
D2 – 5	<i>Enterobacter cloacae</i>
Site 3 – Reefentse	
D3 – 1	<i>Aeromonas hydrophila</i>
D3 – 2	<i>Microvirgula aerodenitrificans</i>
D3 – 3	<i>Acinetobacter junii</i>
D3 – 4	<i>Acinetobacter guillouiae</i>

The microorganisms identified with NCBI Blast were as outlined in Table 7 and 8 for the wet and dry season, respectively. *Bacillus sp.* and *Lysinibacillus sp.* were detected in site one (Ga-Maja) during the wet season sampling. *Bacillus sp.* and *Fictibacillus sp.* were detected in site one (Ga-Maja) during the dry season. *Aeromonas hydrophila* and *Comamonas testosteroni* were detected in site two (Ga-Mothiba) during the wet season sampling and *Aquitalea magnusonii*, *Acinetobacter schindleri*, *Bacillus cereus* and *Enterobacter cloacae* were detected during the dry season. *Aeromonas hydrophila* and *Enterobacter sp.* were detected in site three (Reefentse) during the wet season. *Aeromonas hydrophila*, *Microvirgula aerodenitrificans* and *Acinetobacter sp.* were detected in site three (Reefentse) during the dry season. Infectious diseases caused by pathogenic bacteria, viruses, and parasites are the most common and widespread health threat associated with drinking water (WHO, 2011b). The use of vulnerable groundwater aquifers for drinking purposes without appropriate water purification or disinfection is the primary public health concern.

Leakage from on-site sanitation systems such as septic tanks or sewers can potentially lead to faecal contamination in groundwater (Krauss and Griebler, 2011). Most of the pathogens that affect humans are derived from faeces and spread through the faecal-oral route, which includes food, water, poor personal hygiene, and flies, among other things (Lawrence et al., 2001).

The groundwater sources in the study areas are constructed in close proximity to pit latrines. Because the wastewater enters the groundwater during the leaching process, it poses a health risk due to increased contamination by human faeces. The use of pit latrines in the study areas might therefore be the main contributing factors to the contamination of the boreholes and the hand-dug well. The presence of opportunistic pathogens in all sites could also be attributed to the use of manure and compost for crops. Pathogenic organisms such as bacteria, viruses, protozoa, and helminths may be present in high concentrations in animal manure, posing a risk of zoonotic disease transmission to humans (Krauss and Griebler, 2011). During the rainy season, pollutants can seep into the groundwater through percolation and seepage (Potgieter et al., 2006). Through leaching processes, particles deposited on nearby surfaces can be washed into the water source by rain (Lester and Birkett, 1999). During dry seasons, the water table drops to low levels, and increases the rate of evaporation, lowering oxygen levels and reducing bacteria multiplication (Brady and Ray, 1996). As a result, borehole water contamination will be determined by seasonal variations and the resistance of specific bacteria to environmental conditions.

The presence of opportunistic pathogens in the groundwater samples indicates that communities in the study areas, particularly immunocompromised individuals, infants and the elderly, are at a potential risk of contracting infections and illnesses such as urinary tract infection, gastroenteritis, respiratory infections and dysentery during exposure to or consumption of groundwater from these sources (Bartram et al., 2003). Enteric pathogens such as *Bacillus cereus* and *Escherichia coli*, are known to be major causes of diarrhoea everywhere in the world (WHO, 2006). In South Africa, diarrhoeal disease is directly responsible for 20% of deaths of one- to five-year-olds (Mackintosh and Colvin, 2003). *Lysinibacillus* are pervasive bacteria that are rarely linked to human disease (Wenzler et al., 2015). Relevant clinical infections with genus *Lysinibacillus* sp. are uncommon, a rare case of *Lysinibacillus sphaericus* was demonstrated to result in two percent episodes of bacteremia in children with cancer or those receiving bone marrow transplants (Wenzler et al., 2015). Most *Bacillus* sp. are harmless, only a few are found to be pathogenic to humans and animals (WHO, 2006). *Bacillus cereus*, which was identified in site one (Ga-Maja) during the dry season and site two (Ga-Mothiba) during the wet season, causes food poisoning and is known to cause bacteraemia in immunocompromised patients resulting in symptoms such as vomiting and diarrhoea (WHO, 2006). *Aeromonas hydrophila* identified in site two (Ga-Mothiba) during the wet season and site three (Reefentse) during the dry season, can cause infections in humans such as septicaemia, wound infections and respiratory tract infections though contaminated soil and water related activities such as diving and swimming (WHO, 2006). Unlike other waterborne pathogens, no conclusive evidence of drinking water contaminated with *Aeromonas* has been linked to outbreaks of gastroenteritis (Morgan et al., 2015). *Comamonas testosteroni* is rarely recognised as a human pathogen, but after

contact with contaminated water, it appears that wound infection is a possibility (Tiwari and Nanda, 2019).

Enterobacter cloacae and most members of the family *Enterobacteriaceae* which were more prevalent during the wet season in site three (Reefentse) and identified during the dry season in site two (Ga-Mothiba), are capable of causing opportunistic infection which often arise from endogenous microflora, particularly those of the gastrointestinal tract of hospitalized or debilitated patients (Keller et al., 1998). Consumers, especially those who are immunocompromised, may be at risk of developing infectious enterocolitis due to opportunistic pathogens present in drinking water and poor sanitary conditions (De Boeck et al., 2012). *Acinetobacter* sp. which were detected during the dry season in site two (Ga-Mothiba) and site three (Reefentse) are opportunistic pathogens that can result in pneumonia, urinary tract infections, secondary meningitis, bacteraemia, and wound infections mostly in susceptible patients in hospitals (WHO, 2006). Ingestion of *Acinetobacter* in drinking-water may result in non-gastrointestinal infections in susceptible individuals, particularly in settings such as health care facilities and hospitals (WHO, 2006). *Aquitalea magnusonii* which were detected in site two (Ga-Mothiba) is a promising plant growth-promoting bacteria for duckweed (Ishizawa et al., 2017). Duckweed is a type of aquatic plant that can help with nutrient recovery from wastewater and utilized for the production of value-added products such as animal feed (Cheng and Stomp, 2009). *Microvirgula aerodenitrificans* which were detected in site three (Reefentse) had previously never been identified as a cause of clinical infection, but the first case of disease in humans attributable to the organism was reported following an infection of a vascular access device (Murphy et al., 2012).

4.2.3. Antibiotic resistance patterns of bacteria isolates against antibiotics

Table 9 and 10 indicate antibiotic susceptibility profiles of all test organisms as determined by disc diffusion assays and classified according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2010; CLSI, 2017). Antibiotics that were tested include, Amoxicillin-clavulanic acid (AMC), Ampicillin (AMP), Cefazolin (CFZ), Ciprofloxacin (CIP), Clarithromycin (CLR), Erythromycin (ERY), Gentamicin (GEN), Meropenem (MEM), Norfloxacin (NOR), Tetracycline (TET).

Table 9: Wet season: CLSI Classification.

Strain number	AMC	AMP	CFZ	CIP	CLR	ERY	GEN	MEM	NOR	TET
Ga-Maja										
W1-1 <i>Lysinibacillus sp,</i> <i>Lysinibacillus fusiformis,</i> <i>Lysinibacillus macroides</i>	R	S	S	R	I	R	S	S	R	I
W1-2 <i>Bacillus sp,</i> <i>Bacillus aryabhatai,</i> <i>Bacillus megaterium</i>	R	S	S	R	I	I	R	S	I	I
W1-3 <i>Bacillus sp,</i> <i>Bacillus indicus</i>	R	S	S	I	I	S	S	S	S	S
W1-4 <i>Bacillus cereus</i>	R	R	S	R	R	R	R	S	I	R
Ga-Mothiba										
W2-1 <i>Aeromonas hydrophila</i>	R	R	R	R	R	R	R	R	R	R
W2-2 <i>Comamonas testosteroni</i>	R	R	R	R	R	R	R	R	R	R

Table 9: Wet season: CLSI Classification.

Reefentse											
W3-1 <i>Aeromonas hydrophila</i>	R	R	R	R	R	R	R	R	R	I	R
W3-2 <i>Enterobacter cloacae</i>	R	R	R	R	R	R	R	R	R	I	R
W3 – 3 <i>Enterobacter asburiae</i> , <i>Enterobacter roggenkampii</i>	R	R	R	I	R	R	R	R	R	S	R

Resistant (R); Intermediate (I); Susceptible (S)

Table 10: Dry season: CLSI classification.

Strain number	AMC	AMP	CFZ	CIP	CLR	ERY	GEN	MEM	NOR	TET
Ga-Maja										
D1-1 <i>Bacillus flexus</i>	R	S	S	R	R	R	R	S	R	R
D1-2 <i>Bacillus atrophaeus</i>	R	S	S	R	R	R	R	S	R	R
D1-3 <i>Bacillus cibi</i>	R	S	S	R	R	R	R	S	I	R
D1-4 <i>Fictibacillus sp.</i>	R	R	S	R	R	R	R	S	I	R
Ga-Mothiba										
D2-1 <i>Aquitalea magnusonii</i>	R	R	R	R	R	R	R	R	R	I
D2-2 <i>Acinetobacter schindleri</i>	R	R	R	R	R	R	R	R	R	R

Table 10: Dry season: CLSI classification.

D2-3 <i>Bacillus cereus</i>	R	R	S	R	R	R	R	S	R	R
D2-4 <i>Bacillus cereus</i>	R	R	S	R	R	R	R	S	R	R
D2-5 <i>Enterobacter cloacae</i>	R	R	R	R	R	R	R	R	R	I
Reefentse										
D3-1 <i>Aeromonas hydrophila</i>	R	R	R	R	R	R	R	R	R	R
D3-2 <i>Microvirgula aerodenitrificans</i>	R	R	R	R	R	R	R	R	R	R
D3-3 <i>Acinetobacter junii</i>	R	R	R	R	R	R	R	R	R	R
D3-4 <i>Acinetobacter guillouiae</i>	R	R	R	R	R	R	R	R	R	R

Resistant (R); Intermediate (I); Susceptible (S)

The respective bacterial isolates were subjected to various antibiotics. Zones of inhibition were measured and compared to Clinical and Laboratory Standards Institute (CLSI) breakpoints (CLSI, 2010; CLSI, 2017). The antimicrobial susceptibility analysis from site one (Ga-Maja) as per Table 9 indicated during the wet season that, *Lysinibacillus* sp. isolates W1-1 were 40% (4/10) resistant and 40% susceptible to the tested antibiotics, while intermediate susceptibility was recorded against 20% (2/10) of the tested antibiotics. *Bacillus* sp. isolates W1-2 exhibited 40% (4/10) intermediate susceptibility to the antibiotics analysed while *Bacillus* sp. isolate W1-3 was 70% (7/10) susceptible to the analysed antibiotics. The *Bacillus cereus* isolate W1-4 exhibited high resistance 70% (7/10) towards the antibiotics analysed, while intermediate susceptibility and susceptibility was recorded against 10% (1/10) and 20% (2/10), respectively, to the tested antibiotics. The antimicrobial susceptibility analysis from site two (Ga-Mothiba) and site three (Reefentse) as per Table 9 indicated during the wet season that, *Aeromonas hydrophila* isolates W2-1 and W3-1 displayed resistance to

100% (10/10) and 90% (9/10) respectively, to the tested antibiotics. *Comamonas testosteroni* isolate W2-2 displayed resistance to all the antibiotics analysed. *Enterobacter cloacae* isolate W3-2 (with the exception of norfloxacin where an intermediate result was recorded) displayed resistance to all the antibiotics analysed. The *Enterobacter* sp. isolate W3-3 were 80% (8/10) resistant to the tested antibiotics and displayed intermediate susceptibility against ciprofloxacin and susceptibility against norfloxacin.

The antimicrobial susceptibility analysis from site one (Ga-Maja) as per Table 10 indicated during the dry season that, *Bacillus flexus* isolate D1-1 and *Bacillus atrophaeus* isolate D1-2 were 70% (7/10) resistant to the tested antibiotics and displayed susceptibility against ampicillin, cefazolin and meropenem. *Bacillus cibi* isolate D1-3 displayed 60% (6/10) resistance to the antibiotics analysed, while intermediate susceptibility and susceptibility was recorded against 10% (1/10) and 30% (3/10), respectively, to the tested antibiotics. *Fictibacillus* sp. isolate D1-4 displayed 70% (7/10) resistance to the tested antibiotics, while intermediate susceptibility and susceptibility was recorded against 10% (1/10) and 20% (2/10), respectively, to the tested antibiotics. The antimicrobial susceptibility analysis from site two (Ga-Mothiba) as per table 10 indicated during the dry season that, *Aquitalea magnusonii* isolate D2-1 were 90% (9/10) resistant to the tested antibiotics and displayed susceptibility against tetracycline. *Acinetobacter schindleri* isolate D2-2 exhibited 100% (10/10) resistance to the tested antibiotics. *Bacillus cereus* isolates D2-3 and D2-4 displayed 80% (8/10) resistance to the analysed antibiotics and were susceptible to cefazolin and meropenem. *Enterobacter cloacae* isolate D2-5 displayed 90% (9/10) resistance to the tested antibiotics and was intermediately susceptible to tetracycline. *Aeromonas hydrophila* isolate D3-1 displayed 100% (10/10) resistance to the tested antibiotics. Similarly, isolates D3-2, D3-3 and D3-4 exhibited resistance to all of the antibiotics analysed.

The level of contamination and the presence of potentially pathogenic bacteria in groundwater were investigated in this study. Findings from the antimicrobial susceptibility test revealed that all tested isolates were resistant to amoxicillin-clavulanic acid during the wet and dry season. A study by (Matanovic et al., 2010) demonstrated that excessive use of amoxicillin-clavulanic acid contributed to the high level of resistance, particularly in *Enterobacteriaceae* sp. Increased resistance to amoxicillin-clavulanic acid could be attributed to the growing consumption of the antibiotic (Oteo et al., 2008). During both seasons, the analysed isolates showed a high level of sensitivity against cephalosporin antibiotic cefazolin. Cephalosporins interfere with the bacterial cell wall synthesis by preventing the cross linking of peptide glycan units (Bagde et al., 2012).

During the dry season, the analysed isolates displayed resistance to the macrolides clarithromycin and erythromycin. Most isolates displayed intermediate susceptibility against clarithromycin during

the wet season. Macrolides are considered to be safe antibiotics that are useful for those patients who are hypersensitive to penicillins and other beta lactams (Khan et al., 2011). However, resistance by organisms against this group has also been reported (Khan et al., 2011). Physicians are advised to prescribe macrolides in the treatment of some infectious diseases when no other options are available (Khan et al., 2011). During the dry season, all isolates exhibited resistance against gentamicin. Increased resistance by organisms to aminoglycosides has been noted elsewhere (Krause et al., 2016). Improved dosing schemes may reduce aminoglycosides toxicity while maintaining efficacy (Krause et al., 2016). Extended interval dosing has shown to be more cost effective for treating neonatal patients with gentamicin, as it saves time for nursing and pharmacy staff, results in fewer doses administered per day and lowers drug supply costs (Darmstadt et al., 2008).

The study revealed that most of the analysed organisms displayed a high level of susceptibility against carbapenem and meropenem during both seasons. A study conducted by (Mohr, 2008) has shown that meropenem is more active against gram-negative pathogens and somewhat less active against gram-positive pathogens. The analysed isolates exhibited high level of resistance against tetracycline during both seasons. Tetracyclines have been used for many years to treat serious life-threatening infections, however extensive use has resulted in resistance development (Grossman, 2016). All the analysed isolates from all sites were resistant to erythromycin and tetracycline during the dry season, which was in accordance with the study conducted by Moore et al. (2010) where the majority of the isolates were also resistant to erythromycin and tetracycline. During the wet and dry seasons, approximately 80% of isolates were resistant to ampicillin. All of these findings could be attributed to the overuse of antibiotics in clinical and veterinary settings (Mulamattathil et al., 2014). Results from this study indicate that ciprofloxacin and norfloxacin (fluoroquinolones) are the drugs of choice for *E. coli* infections since none of the isolates were resistant to them. This shows the effectiveness of the fluoroquinolones and agrees with the finding which suggested that these classes of antimicrobial agents are active against a wide range of multi-resistant pathogens (Hooper, 2000). The antibiotic sensitivity patterns of the *Enterobacter* sp. (W3-3) reveals that a larger percentage (80%) of the isolate was resistant to more of the antimicrobial agents tested. Enterobacteria's resistance to ampicillin, amoxicillin-clavulanic acid and tetracycline has been reported elsewhere (Al-Kharousi et al., 2019). The presence of high levels of resistance to some of the antimicrobials used in this study suggests that the antibiotics may have been abused or overly used in the treatment of bacterial infections (Titilawo et al., 2014).

Identifying the sensitivity and resistance of specific pathogens to a variety of antimicrobial agents is necessary so that healthcare providers can institute proper and effective treatment regimens to their patients (Lagier et al., 2015). The development of antimicrobial resistance by the bacteria to these

antibiotics constitutes a serious issue in both human and animal medicine because these drugs are frequently used in animal and human medicinal procedures (Titilawo et al., 2015). The increased resistance rate observed amongst the isolates could be attributed to the pathogenic population's level of antimicrobial exposure (Havenga et al., 2019). Antibiotic resistance has become a major public health issue since bacterial pathogens that cause infections are becoming resistant to the most widely prescribed antibiotic treatments, resulting in prolonged illness and a higher risk of death (Cosgrove, 2006; Jones, 2001). Infections caused by bacteria that are resistant to antibiotics are thought to result in increased length of hospital stay, increased mortality rate and increased healthcare costs than antibiotic susceptible bacterial infections (Mauldin et al., 2010). The increased cost associated with the development of resistance during treatment was investigated (Cosgrove et al., 2002), and found that patients infected with *Enterobacter* sp., had an average hospital stay of nine days resulting in increased mortality, increased hospital stay and hospital charges. The groundwater samples from the study areas revealed high levels of antibiotic resistant during both seasons. These findings emphasize the significance of assessing antibiotic resistance when evaluating the potential risk associated with drinking water from these sources.

CHAPTER 5

5.1. Conclusions

This study aimed to determine potential groundwater contamination from pit latrines in Ga-Maja and Reefentse, with Ga-Mothiba used as a reference site and to investigate health risks associated with consumption of contaminated groundwater. To achieve this, the SAWQG for domestic use and SANS 241 drinking water guidelines were used as the basis of evaluating the groundwater for drinking and domestic use (DWAF, 1996; SABS, 2015). The water quality analysis conducted during the wet season revealed that most of the samples at all sites are within the permissible range of both the SAWQG for domestic use and SANS 241 drinking water guidelines (DWAF, 1996; SABS, 2015).

The EC, TDS and calcium values obtained from site one (Ga-Maja) were found to be above the TWQR (DWAF, 1996). However, the parameters were present at concentrations that usually poses no health threat to humans and considered acceptable for drinking water. Confirmation of the presence of total coliforms in all sites during the wet season, and from site two (Ga-Mothiba) and site three (Reefentse) during the dry season is an indication of faecal contamination and the possible presence of other enteric pathogens. Nitrate concentrations from Reefentse were found at levels that pose a potential health risk for infants and pregnant women. The tested isolates exhibited an increased presence of antibiotic resistance during both seasons, and this leads to serious health risk if the water is used for drinking. All parameters in site two (Ga-Mothiba) were found to be within the SAWQG for domestic use and SANS 241 drinking water guidelines (DWAF, 1996; SABS, 2015).

The detection of a number of opportunistic pathogens and pathogenic strains such as *Bacillus cereus*, *Bacillus indicus*, *Aeromonas hydrophila*, *Comamonas testosteroni*, *Enterobacter cloacae*, *Enterobacter asburiae*, *Enterobacter roggenkampii*, *Bacillus flexus*, *Bacillus cibi*, *Bacillus atrophaeus*, *Aquitalea magnusonii*, *Acinetobacter schindleri*, *Microvirgula aerodenitrificans*, *Acinetobacter junii* and *Acinetobacter guillouiae* indicates that communities in these study areas are at a constant risk of contracting waterborne diseases. All isolates tested in this study showed high resistance (100%) to penicillin- β -lactam class of antibiotics during both seasons. The majority of the isolates demonstrated resistance to more classes of antibiotics, including penicillin, fluoroquinolones, macrolides, aminoglycosides, and tetracycline. Resistance to antimicrobial drugs is a major public health concern. Antibiotic-resistant bacteria infections results in increased mortality, longer hospital stay, and higher costs of care (Cosgrove et al., 2002). Some of the treatment factors that may contribute to negative outcomes in patients infected with a resistant pathogen include reduced effectiveness, improper dosing of antimicrobial agents, lack of microbiologically efficient

antimicrobials and increased need for surgery and other procedures as a result of infections (Cosgrove, 2006).

Based on the outcome of the study, the specific conclusions of this research were:

- The study results highlighted the risk posed by pit latrines to groundwater quality. The shallow water table increased pollution potential from pit latrines. The pit latrines in the study areas are all situated close to the groundwater sources, increasing contamination risk.
- Pit latrines constructed near water sources can influence nitrate concentrations in groundwater. However, as the results indicate, the risk is site-specific.
- The microorganisms found in groundwater from the study areas pose a risk to human health. The presence of pathogenic bacteria indicate the potential of contracting infectious diseases such as diarrhea, pneumonia, gastroenteritis, respiratory tract infections, urinary tract infections and wound infections if water is used for human consumption.
- The high level of antibiotic resistance of isolates to most of the antibiotics used in this study increases the risk of contracting infectious diseases.

This study revealed that groundwater safety in rural and peri-urban areas cannot be guaranteed. There is a need to provide safe drinking water to communities that rely on groundwater for drinking and other domestic uses. It is recommended that in the absence of clean piped water, the communities of Ga-Maja, Ga-Mothiba and Reefentse be advised to treat the water from the boreholes and well before consumption or other domestic uses. These communities should be educated about the possible risks of using groundwater for human consumption. To avoid potentially harmful health effects, community education should include possible water treatment methods such as boiling and use of chlorine based substances where groundwater is exposed to faecal coliform contamination.

5.2. Limitations of the study

This research had some methodological limitations, which include:

- Geohydrological data was not available. Critical parameters like depth of the infiltration layer and direction of groundwater flow could be helpful during assessment of impacts of pit latrines on groundwater quality.
- Heavy metals and hazardous elements were not analysed in the groundwater samples. This could have provided more information on the extent of groundwater contamination in the study areas.
- Due to financial constraints, chemical analyses of water quality was not performed for the dry season. This was going to give a better insight on the seasonal variation of contamination.

5.3. Recommendations for future research

The following recommendations are made for future research:

- In future research, more parameters such as heavy/trace metals and organics (pharmaceuticals and pesticides) should be analysed and observed, and effects of seasonal changes or influences on the groundwater should be monitored.
- Other parameters that might be problematic with regards to on-site sanitation such as ammonium and turbidity must be monitored. Some of the parameters also tend to have an effect on the perceived water quality and health and therefore it is imperative to analyse the levels of these parameters.
- In areas where pit-latrines are widely used, hydrogeological and lithological mapping must be carried out. If the study area has a confined aquifer with sustainable yield, it could be preferable to the shallow aquifer.
- Results from this study have demonstrated that a full health risk assessment should be conducted on some of the water sources in the study areas. This is needed particularly in rural and peri-urban areas, where residents are often immunocompromised.

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