SILVER NANOPARTICLE-RESIN FILTER SYSTEM FOR DRINKING WATER DISINFECTION AND INHIBITION OF BIOFILM FORMATION

by

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DECLARATION BY CANDIDATE

"I hereby declare that the thesis submitted for the degree D Tech: Water Care, at Tshwane University of Technology, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited, or quoted, are indicated and acknowledged by means of a comprehensive list of references."

L. Mpenyana-Monyatsi

DEDICATION

I dedicate this work to my Heavenly Father, God Almighty for providing me with strength and wisdom throughout my studies Without His grace; all this would not have been possible.

To my loving husband, Stephen Monyatsi, for his support and understanding during my late nights, and his unconditional love. To my son, Katlego, and my daughter, Kgalalelo, for being patient during the execution of this investigation.

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PRESENTATIONS AND PUBLICATIONS

The work contained in this thesis has previously been presented in conferences and has also been submitted for publication in peer reviewed journals as indicated below.

CONFERENCE PRESENTATIONS

1. **MPENYANA-MONYATSI**, L., NAMENI, G., ONYANGO, M. & MOMBA, M. 2009. Ag modified oligodynamic nanoparticle impregnated systems and utility in water disinfection. *Poster Presentation, Nanotech Europe 2009 Conference and Exhibition. 28-30 September, 2009. Berlin, Germany.*

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2. **MPENYANA-MONYATSI, L.**, MOMBA, M.N.B., ONYANGO, M., NAMENI, G. & MADOROBA, E. 2009. Groundwater quality supply in rural community of North West Province: A possible threat to public health. *Oral Presentation, Groundwater Biennial Conference. 16-18 November, 2009. Somerset West. Western Cape Province, South Africa.*

3. NAMENI, G., **MPENYANA-MONYATSI**, L., ONYANGO, M. & MOMBA, M. 2010. Cost effectiveness of pathogenic microorganisms using oligodynamic nanoparticle impregnated natural zeolites and sand. *Oral Presentation, WISA 2010 Biennial Conference. 18-22 April, 2010. Durban, South Africa.*

4. MTHOMBENI, N.H., **MPENYANA-MONYATSI, L**., MOMBA, M.N.B. & ONYANGO, M.S. 2010. Deactivation of microbes in drinking water using silver nanoparticles-impregnated media. *Oral presentation, Moi University 6th Annual International Conference. 7-11 September, 2010. Margaret Thatcher Library, Kenya.*

5. **MPENYANA-MONYATSI**, **L.,** ONYANGO, M.S. & MOMBA, M.N.B. 2010. Groundwater quality supply in rural community of Mpumalanga Province: A possible threat for public health. *Poster Presentation, 11th WaterNet/WARFSA/GWP-SA Symposium. 27-29 October, 2010. Victoria Falls, Zimbabwe.*

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PUBLICATIONS

1. **MPENYANA-MONYATSI, L**., MTHOMBENI, N.H., ONYANGO, M.S. & MOMBA, M.N.B. 2012. Cost-effective filter materials coated with silver nanoparticles for the removal of pathogenic bacteria in groundwater. *International Journal of Environmental Research and Public Health*, *9*:244-271.

2. **MPENYANA-MONYATSI, L**. & MOMBA, M.N.B. 2012. *Assessment of groundwater quality in the rural areas of the North West Province, South Africa. Scientific Research and Essays, 7(8):903-914.*

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silver nanoparticles coated resin beads in fixed-bed column. *Journal of Hazardous Materials, 217:133–140.*

5. **MPENYANA-MONYATSI, L.,** MTHOMBENI, N.H., ONYANGO, M.S. & MOMBA, M.N.B. The effects of material loading and flow rate on the disinfection of pathogenic microorganisms using silver nanoparticle-cation resin filter system. *Under review in Journal of Nanotechnology.*

6. **MPENYANA-MONYATSI, L.** & MOMBA, M.N.B. Silver nanoparticles-resin filters system for the production of drinking water free from viruses and protozoan parasites. *Under review in Journal of Antimicrobial Agents and chemotheraphy.*

7. **MPENYANA-MONYATSI, L.,** ONYANGO, M.S. & MOMBA, M.N.B. Performance of silver nanoparticles-cation resin filter in inhibiting bacterial regrowth and biofilm formation in drinking water supply systems. *Under review in Plos One Journal.*

SUMMARY

Groundwater is the main source of drinking in most rural areas of South Africa and is supplied to the communities without prior treatment. However, the contamination of groundwater sources by pathogenic bacteria poses a public health concern to these communities. This study was aimed at developing and evaluating the effectiveness of filter materials coated with silver nanoparticles for the removal of pathogenic microorganisms from groundwater as well as the inhibition of biofilm formation in drinking water systems.

The study was divided into three phases. In the first phase, a survey of 200 boreholes was conducted so as to assess the quality of groundwater in various places in two provinces of South Africa: North West Province (100 boreholes) and Mpumalanga Province (100 boreholes). Culture-based methods and molecular techniques were applied in order to assess the quality of these water sources. The outcomes of this part of the study revealed high concentrations of fluoride, nitrate, magnesium, calcium, totally dissolved solids (TDS) and turbidity, which by far exceeded the limits recommended by the South African guidelines. The results also indicated that 23 % and 86 % of the boreholes in North West and 78 % and 81 % of the boreholes in Mpumalanga did not comply with the limits set by the national guidelines (SANS 241 and DWAF) in terms of faecal and total coliforms, respectively. Various opportunistic pathogens and pathogenic strains such as *Serratia marcescens*, *Citrobacter freundii*, *Salmonella enteric and Shigella dysenteriae*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Shigella flexineri*,

Pseudomonas maltophilia, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Morganella morganii, Aeromonas veronii* and *Cronobacter sakazakii* were found in some of the boreholes were revealed by molecular study. Consequently, treatment of these water sources prior to consumption is of great importance for the protection of public health.

The second phase focused on the development and evaluation of the effectiveness of filter materials coated with silver nanoparticles for the removal of pathogenic microorganisms found in groundwater. Five substrates such as sand, zeolite, fibreglass, anion resin, and cation resin with various concentrations (0.01 mM, 0.03 mM, 0.05 mM, and 0.1 mM) of $AgNO_3$ were successfully synthesised by employing hydrothermal reduction and chemical reduction methods. The characterisation of the substrates was carried out by X-ray diffraction (XRD) analyses, scanning electron microscopy (SEM) supported by energy dispersive X-ray spectroscopy (EDS), and transmission electron microscope (TEM) analysis in order to confirm the presence of silver nanoparticles on these substrates. Laboratory-scale filter systems packed with silver nanoparticle substrates demonstrated that 0.1 mM of silver nanoparticles in all the substrates was effective in decreasing the concentration of *E.coli* in synthetic groundwater. Moreover, the effectiveness of the five substrates coated with 0.1 mM of silver nanoparticles were tested against four different bacterial species found in groundwater sources, including *E.coli*, *S. typhimurium, S. dysenteriae* and *V. cholerae*. The results revealed that the silver nanoparticle- cation resin filter completely (99.9 %) removed all the tested bacterial species. The performance of this filter system

depended on the amount of bed mass used as well as the flow rate. It was found that the breakthrough curves, obtained at different bed mass levels, resulted in an increase of breakthrough time with an increase in bed mass. The higher flow rate decreased the performance of the filter, while the lower flow rate increased the contact time between the bacteria and bed mass material. The highest performance of the silver nanoparticles resin filter system was also noted in the complete (99.9 %) removal of somatic coliphages during the first five runs filtering 600 mL of contaminated water. With the protozoan parasites, the highest performance of the removal (99.9 %) of 2 log oocysts (*Cryptosporidium* spp.) and 2 log cysts (*Giardia* spp.*)* was achieved during the first three cycles of filter runs, filtering 15 L. The filter system also demonstrated that it was capable of producing drinking water exhibiting turbidity values of less than 1 NTU throughout the filter runs.

In the last phase, a groundwater source was used to evaluate the impact of a silver nanoparticle-cation resin filter by inhibiting bacterial regrowth and biofilm formation in potable water distribution systems using a laboratory-scale unit. Plastic-based pipe materials (polyvinyl chloride-PVC) and metallic-based pipe materials (galvanised steel-GS) used for drinking water distribution systems were used in this part of the study. The effectiveness of silver nanoparticle-cation resin filter was compared to that of chlorine. Scanning electron microscopy was used to view the bacterial regrowth and formation of biofilm on the PVC and GS piping materials. Results within the first day indicated the adhesion and regrowth of heterotrophic plate count bacteria on both piping materials exposed to untreated

and chlorinated water systems, with an average count of 1 log cfu/cm^2 of heterotrophic bacteria attached to piping materials. The use of a silver nanoparticles-resin system resulted in the inhibition of heterotrophic bacteria from attaching to pipe materials. However, the biofilm formation occurred on the surface of pipe materials between day 28 and day 30, due to the depletion of silver ion residuals. Bacterial counts were significantly higher on GS pipes when compared to PVC pipes.

The filter system using silver nanoparticles-cation resin substrates has demonstrated its capability to produce safe drinking water and to preserve the integrity of potable water distribution systems by inhibiting the phenomenon of bacterial regrowth and bacterial adhesion on plastic- and metallic-based pipe materials. Results of this study suggest the possibility of the silver nanoparticle filter system being considered a potential alternative cost-effective filter for the disinfection of groundwater.

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
Ag	Analysis Of Variance
BET	Brunauer-Emmett-Teller
cm	Centimetre
CAWST	Center for Affordable Water and Sanitation Technology
CDC	Centers for Disease Control and Prevention
CFU	Colony Forming Unit
d	Days
DALY	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
DWAF	Department of Water Affairs and Forestry
E.coli	Escherichia Coli
EDS	Energy Dispersive Spectroscopy
EPA	Environmental Protection Agency (United States)
Eq.	Equation
FC	Fecal Coliform
G	Gram
H	Hours
HIV-AIDS	Human Immunodeficiency Virus-Acquired Immune Deficiency Syndrome
HWTS	Household Water Treatment Systems
L	Litre

M	Molar
µm	Micrometer
MDG	Millennium Development Goal
min	Minutes
MF	Microfiltration
MPN	Most Probable Number
m-FC	Medium for detection of Fecal Coliform
mg	Milligram
mL	Millilitre
NF	Nanofiltration
NOAEL	No-observed-adverse-effect-level
NTU	nephelometric turbidity units
POU	Point-Of-Use
PFP	Potters for Peace
PVC	polyvinyl chloride
RNA	Ribonucleic Acid
RO	Reverse Osmosis
rpm	Revolution Per Minute
SADC	Southern African Developing Community
SANS	South African National Standard
SEM	Scanning Electron Microscopy
SIPP	Silver Impregnated Porous Pot
SODIS	Solar Water Disinfection
SWS	Safe Water System

TC	Total Coliform
TDS	Total Dissolved Solids
TOC	Total Organic Carbon
THM	Trihalomethanes
UN	United Nations
UNICEF	United Nations Children’s Fund
UF	Ultrafiltration
USAID	United States Agency for International Development
USEPA	United States Environmental Protection Agency
UV	Ultra-Violet
WHO	World Health Organization
XRD	X-ray Diffraction

CHAPTER 1

GENERAL INTRODUCTION

Water quality has long been regarded as the primary indicator of health and well-being and it is crucial for the economic development of a country. Polluted water, on the contrary, not only has the potential to cause human suffering, but also to reduce individual productivity. Families experience diminished disposable income due to payments for medical treatment, and valuable time and energy are spent on efforts to secure their water supply. The most vulnerable circmstances are the deaths of thousands of people, especially children, due to a lack of clean and safe water (WHO, 2002). Data by the WHO (2004) on the burden of disease indicate that approximately 1.8 million deaths and 61.9 million disability adjusted-life years (DALYs) worldwide are attributed to unsafe water, sanitation and poor hygiene. An estimated 99 % of such deaths occurs in developing countries with children ranking (90 %) as the first victims.

The vital role that safe drinking water plays in human health and well-being has been globally recognised for decades. As a result, in 2002, the United Nations Millennium Development Goals (MDG) firmly established the issue of water and sanitation on the global agenda. The vision of the MDG is to halve the proportion of people without access to safe drinking water and sanitation by 2015 (WHO and UNICEF, 2010). Tremendous progress has been made to date, with 87 % of the world's population using improved sources of drinking water; however, the WHO

and the United Nations Children's Fund (UNICEF) have indicated that 884 million people across the world still lack access to drinking water from improved sources. In most of the cases, non-potable drinking water supplies are currently found in rural communities that are widely dispersed as well as in informal peri-urban communities that are continuously expanding. Consequently, these communities living in rural areas depend on groundwater sources as their main source of water.

Groundwater, which is considered to be the main water source supply in many disadvantaged communities, is consumed without any prior treatment. These communities receive their drinking water directly from uncovered or covered boreholes and wells, whereas for some, water is drawn from boreholes (using pumps (Momba & Notshe, 2003). It has been reported by the WHO (2007) that groundwater sources are often contaminated with pathogenic organisms. Pathogenic organisms are bacteria that are able to cause diseases. They contain virulence factors that distinguish them from nonpathogens (Peterson, 1996). The virulence factors are typically consists of proteins and other molecules that are synthesised by enzymes. These proteins are coded for by genes in chromosomal DNA, bacteriophage DNA or plasmids (Wikipedia, 2008). These pathogens are bacteria, viruses, and protozoa which originate from faecal pollution and are disease-causing organisms transmitted through drinking water (Ashbolt *et al*., 2001; Hunter *et al*., 2002). Studies conducted by Momba and co-workers from 1998 to May 2006 have indicated that such water sources are not fit for human consumption as they contain high counts of faecal coliforms, especially *Escherichia coli*, and other pathogenic microorganisms such as *Vibrio cholerae,*

Aeromonas hydrophila, Shigella dysenteria, Salmonella tyhimurium, Pseudomonas spp, Klebsiella spp (Momba & Mnqumevu, 2000; Momba & Notshe, 2003; Momba *et al*., 2006). Protozoan parasites which contaminate groundwater systems include *Cryptosporidium parvum* and *Giardia spp* and they cause health problems as well (Schijven, 2001).

To render drinking water safe and prevent or reduce the risk of waterborne diseases, many water utilities use various treatment processes, such as coagulation or flocculation, sedimentation, filtration and disinfection (Parsons & Jefferson, 2006). Disinfection processes, which include chlorination, ozonation and ultraviolet radiation (UV) are the most important steps in the production of safe drinking water. Currently chlorine is the most widely used drinking water disinfectant because of its relatively low running costs. This disinfectant has made an immense contribution to the safety of drinking water supplies, yet, the limitations of chlorine and some disadvantages linked to its use have been widely publicised (WHO, 2003a). Chlorine produces disinfection by-products (DBPs) such as trihalomethanes (THMs), which are known to be carcinogenic (WHO, 2003a; Van der Walt *et al*., 2009). Although chlorine is effective against most vegetative bacteria and viruses when used at the normal concentration for treatment, it is unable to inactivate protozoan parasites like *Cryptosporidium* and *Giardia* (Hambidge, 2001; Li *et al*., 2002; Arnold & Colford, 2007). Furthermore, chlorine has a very limited effect upon pathogens growing in biofilms. It was reported that the bacterial survival after disinfection is a problem for potable water utilities (Momba *et al*., 2000). Therefore, bacteria will continue to contaminate piped water

supplies. Although ozonation and UV are excellent disinfection processes in the inactivation of pathogens, they have been reported to be costly. Moreover, previous investigators have pointed out that ozone does not provide a stable residual, while UV does not have any residuals (Solsona & Méndez, 2003).

Nanotechnology is an emerging technology that covers a wide range of disciplines, including design, synthesis, characterisation, manipulation and exploitation of materials at nanoscale with at least 1 nm-100 nm (Schutte & Focke, 2007). It has shown a huge potential in various areas such as drug development, information and communication technologies, production of stronger and lighter materials, human health care, and water treatment (Koch, 2002; Cross *et al*., 2009). Nanotechnology can be useful in resolving current problems in water treatment (Bottero *et al*., 2006; Savage & Diallo, 2005). Various forms of nanotechnology such as nanoadsorbents, nanocatalyst, nanostructured membrane and nanobiocides are used in water treatment (Schutte & Focke, 2007). They can be applied for chemical decontamination, desalination, and water filtration. Nanobiocides used for water disinfection include: metals or metal oxides such as silver, gold, zinc, tin, iron, nickel, and copper.

Silver ion (Ag+) has long been known to be a potential antimicrobial agent used in wound dressings for the prevention of infections in burn patients, to prevent blindness in newborns, in severe chronic osteomyelitis and urinary infection, to control *Legionella* bacteria in hospitals, and to enhance the performance of drinking water filters (Klasen, 2000; Bosetti *et al*., 2002; Richard *et al*., 2002;

Niven, 2005). Silver in the form of nanoparticles which release silver ions more effectively has a better bactericidal activity due to its high surface area-to-volume ratio (Kumar *et al*., 2008). Recent studies have indicated that distinctively prepared silver nanoparticles display good antibacterial activity (Matsumura *et al*., 2003; Sondi & Salopek-Sondi, 2004). As a result, researchers have considered silver nanoparticles for drinking water treatment due to its strong and wide spectrum of antimicrobial activities (Stoimenov *et al*., 2002; Cho *et al*., 2005; Jain & Pradeep, 2005). With the advancement of materials development, silver nanoparticles can be coated onto or impregnated into materials or substrates for the deactivation of microorganisms.

Several studies have shown the removal of microorganisms in water filtration by using silver nanoparticles coated onto or impregnated into various materials or substrates such as fibreglass (Nangmenyi *et al*., 2009; Li, 2010), polysulfone ultrafiltration membranes (Zodrow *et al*., 2009), polyurethane foams (Jain & Pradeep, 2005), ceramic filters (Oyanedel-Craver & Smith, 2008), sand (Mahmood et al., 1993) and zeolite (Matsumura *et al*., 2003). Although nanoparticle filter systems have been employed in bacterial removal, there is a need to develop new cost-effective filter systems for the removal of various pathogenic microorganisms from groundwater and for the protection of water distribution systems against bacterial regrowth and biofilm formation. The new system can also be implemented as a point-of-use (POU) water treatment filtration system.

1.1 PROBLEM STATEMENT AND JUSTIFICATION

The availability of safe and clean water is generally not a problem in South African Metropolitan areas and in the areas provided with water from the major water boards, where consumers generally receive a constant supply of high quality water (Momba *et al*., 2006). However, in many rural communities and small towns, the situation is very different. In those areas where water supply or treatment systems are non-existent or inadequate, the communities depend on groundwater sources. These communities are exposed to many water-related diseases due to the poor water quality they consume daily. Pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella typhimurium, Shigella dysenteriae and Vibrio cholera* are transmitted by water, infect the gastrointestinal tract and are excreted in the faeces of infected humans and other animals (Momba & Kaleni, 2000; Edberg *et al*., 2000; Enriquez *et al*., 2001; Momba & Notshe, 2003; Momba *et al*., 2006).

The use of chlorine as a disinfection method has some disadvantages associated with it such as the formation of disinfection by-products, which are toxic to humans. There is a need to identify an alternative material for water disinfection which is non toxic to humans, cost-effective and can be used at POU water treatment systems for communities living in rural areas. Even though a number of studies have been conducted on the removal of microrganisms with Ag-zeolite, Ag-sand, Ag-fibreglass, and Ag-resin nanoparticle substrates, there is no information on comparative studies related to the use of these technologies for the removal of pathogenic organisms from drinking water sources and especially for

the inhibition of bacterial regrowth and biofilm formation in distribution systems. This study therefore focused on the development of the substrates that were modified with silver nanoparticles and compared their effectiveness in removing not only pathogenic bacteria (*Escherichia coli*, *Vibrio cholerae*, *Shigella dysenterae, and Salmonella typhimurium*), but also viral indicators (somatic coliphages) and protozoan parasites (*Cryptosporidium* and *Giardia* spp.) from polluted groundwater sources. Our main intention was to find an alternative cost-effective technology with the best concentration of silver nanoparticles loaded onto the substrates, which could completely remove pathogenic organisms from water samples and result in the production of safe drinking water for rural communities as well as protect the distribution system.

1.2 GENERAL AIM AND OBJECTIVES

The aim of the present study was to develop filter materials coated with silver nanoparticles and investigate the effectiveness of this technology for the removal of pathogenic microorganisms from groundwater and the inhibition of biofilm formation in drinking water systems. The following specific objectives were pursued:

- To assess the general quality of groundwater in Mpumalanga and North West Provinces.
- To synthesise and characterise cation resins and other materials such as zeolite, sand, and fibreglass with silver nanoparticles and compare their

effectiveness in removing pathogenic bacteria (*Escherichia coli*, *Vibrio cholerae*, *Shigella dysenterae,* and *Salmonella typhimurium*) from polluted groundwater sources.

- To ascertain the lifespan of the resin-silver nanoparticle filter system in producing adequate and safe drinking water at a POU water treatment system for rural communities.
- To conduct a comparative study and assess whether filter materials coated with silver nanoparticles are effective in removing viral indicators (coliphages) and protozoan parasites (*Giardia* spp. and *Cryptosporidium* spp).
- To investigate the performance of filter materials packed with cation resin-coated with silver nanoparticles in inhibiting bacterial regrowth and biofilm formation on piping materials used in water supply systems.

CHAPTER 2

LITERATURE REVIEW

2.1 SCARCITY OF FRESH WATER WORLDWIDE

Historically, water has been regarded as an infinite resource. It is a key element for safe and healthy life as well as for the maintenance of sustainable socio-economic development. Currently, the world faces formidable challenges in meeting society's rising demand for clean water. The available sources of fresh water are decreasing because of extended and worsening droughts, continued population growth, more stringent health-based regulations, and competing demands for water from a variety of users (Savage & Diallo, 2005). This water crisis has been aggravated by water pollution, which may stem from various causes, for which some are natural while others are a result of human activities (Kongolo, 2011).

Pollution of water sources has created many challenges for the purification of safe drinking water. These challenges have become accute especially in developing countries without access to proper infrastructures. It has been pointed out that at least one-sixth of the world's population lacks access to safe drinking water (Li *et al.,* 2008), and two-fifths suffer the consequences of unacceptable sanitary conditions (UNESCO, 2003). Ecological and human health crises are related to inadequate access to, or inappropriate management of clean fresh water (Gleick, 1998). As the human population increases, economic development accelerates,

and regional conflicts over water, ecological degradation, and human sickness and death are becoming more frequent and serious (Gleick, 1998; Kataoka, 2002). Consequently, these factors have caused an imbalance between water availability and water demand, which has led to a water crisis in many regions of the world.

2.1.1 Distribution of world water sources

Water covers nearly 70 % of the surface of the planet (Kataoka, 2002). Figure 1.1 illustrates that 97 % of the water on the Earth is salt water, with only 3 % being fresh water. Over two-thirds (69 %) of this fresh water is locked up in glaciers and polar ice caps and the remaining fresh water is found mainly in groundwater (30 %). Consequently, of all the fresh water on Earth, only about 0.3 % is contained in rivers and lakes, which exist as fresh surface water (Gleick, 1996). Humans are dependent on only 1 % of the Earth's fresh water which is considered to be a natural resource that is available for use (Kataoka, 2002).

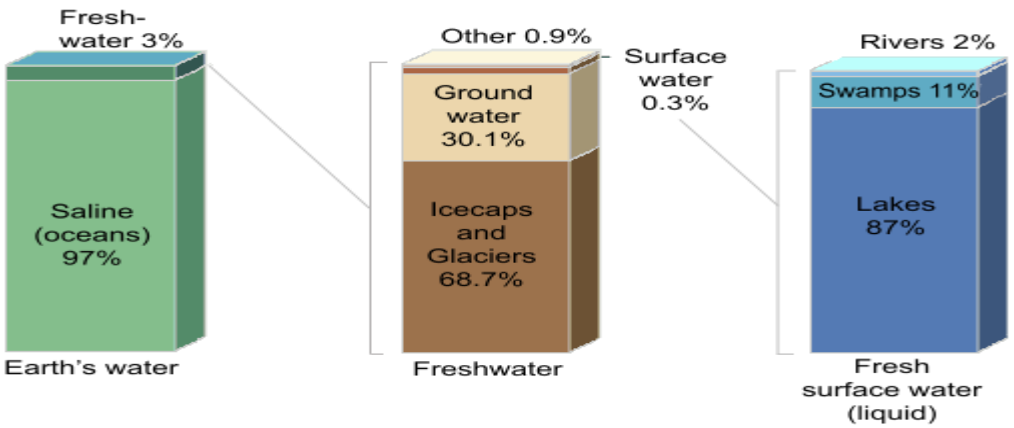


FIGURE1.1: Distribution of the Earth's water (Gleick, 1996)

2.1.2 National state of freshwater system and resources

South Africa is a semi-arid developing country with an average rainfall of 450 mm per year, which is about half of the world average of about 860 mm/year (Kongolo, 2011). In this country, fresh water has been reported to be the most limited water resource. Consequently, South Africa has been classified as a water-stressed country (United Nations, 2006; Kongolo, 2011). The nation's water supply is expected to become even further stretched in the coming years, moving from a situation of water stress to one of water scarcity. If the country continues to use the remaining water resources at its present rate of consumption, South Africa could run out of drinking water by 2025 (Rand Water, 2011). The degree of water stress and scarcity in South Africa's water resources has also been visible in the deterioration of the quality of its water resources (Muller *et al*., 2009).

In South Africa, surface water sources comprise dams and rivers which, in terms of the size of their contribution (77 %), rank first in the total supply of fresh water, while 14 % of fresh water is sourced from return flows (sewage and effluent purification). The quality of surface water sources is frequently subjected to change as a result of various activities on a watershed (Momba *et al*., 2008). One-third of the water resources is unusable because of current patterns of pollution from industries, domestic effluent discharges and acid mine drainage. In addition, it is increasingly difficult to estimate the magnitude of the country's pollution problem because of the wide geographical spread of pollution sources accross the country (DEAT, 1999).

Groundwater sources, which include wells and spring water, occupy the third position in the total supply of fresh water in South Africa and constitute 9 % of fresh water (Van Vuuren, 2009). Although groundwater has historically been thought to be free of microbial contamination, the pollution of groundwater sources is still the main challenge currently facing rural communities who depend almost exclusively on these water sources. These communities obtain their drinking water directly from uncovered or covered boreholes and wells.

The microbiological pollution of groundwater is caused by human and animal activities, which include on-site sanitation, cemeteries, waste disposal, feedlots and unsewered settlements (Engelbrecht & Tredoux, 2000). Studies have indicated that contaminated groundwater sources could result in waterborne diseases if consumed without prior treatment *(*Momba & Mnqumevu, 2000; Momba *et al*., 2006). In certain areas, natural groundwater complies neither with the limits set for salinity, nitrates, fluorides, iron, manganese and other trace elements such as arsenic and uranium, nor with potable standards for microbial indicators (Engelbrecht & Tredoux, 2000).

2.2 IMPACT OF WATER QUALITY ON HUMAN HEALTH

Safe drinking water is a basic human right. It is required for domestic purposes, including drinking, food preparation and personal hygiene (WHO, 2011). Water quality has long been regarded as the primary indicator of health and well-being and water of a good quality is crucial for the economic development of a country. However, the deterioration of drinking water quality has become a global issue of

concern as it affects human health and welfare. Polluted water not only has the potential to cause human suffering, but also reduces individual productivity. Families experience diminished disposable income due to payments for medical treatment, and valuable time and energy are spent on efforts to secure their water supply. Consequently, thousands of people, especially children, die due to a lack of clean and safe water (WHO, 2002). According to the WHO (2003a), an estimated 1.1 billion people globally drink unsafe water. Approximately 3.1 % of the annual deaths (1.7 million) and 3.7 % of the annual health burden (disability-adjusted life years [DALYS]) worldwide; 54.2 million) are attributable to unsafe water, and a lack of sanitation and poor hygiene (WHO, 2003b).

Waterborne pathogens are distributed worldwide and outbreaks have been reported in both developed and developing countries. In spite of this fact, studies have revealed that the vast majority of people who are affected by human health threats posed by a lack of access to safe water and poor water quality as a result of physical, chemical, microbial contaminants and subsequent diseases are members of developing countries (UN WWAP, 2003; UNEP, 2010). This implies that many people in the developing world still depend on contaminated water sources for their daily water needs.

2.2.1 Waterborne pathogens causing-diseases

Microbial contamination of drinking water is a major factor in spreading waterborne diseases (Franz, 2005). Waterborne pathogens include bacteria, viruses, and protozoan parasites that are transmitted to people by means of drinking water. They can exist in water naturally or as a result of contamination from human or

animal waste (LeChevallier *et al*., 1996). Their consumption can lead to waterborne diseases which can be life-threatening to human beings, especially to those with a low immune system. Some of the waterborne pathogens related to this investigation are described in subsections below.

2.2.1.1 Bacteria

Bacteria are unicellular microorganisms that lack a nucleus (prokaryotes) and exist as either free-living organisms or as parasites. They have various shapes: spherical (coccus), rod-shaped (bacillus), comma-shaped (vibrio), spiral (spirillum), or corkscrew-shaped (spirochete) (Rosen, 2000), and are members of the *Enterobacteriaceae* family. Microorganisms within this family are Gram-negative. Species within this family that are significant waterborne pathogens include *Escherichia coli, Salmonella typhi, Shigella spp., and Vibrio cholerae* (Franz, 2005).

Escherichia coli are facultative Gram-negative rods that inhabit the intestinal tract of humans and other animals (WHO, 2006b). They are members of the *Enterobacteriaceae* family, and their presence indicates faecal contamination of water sources (Edberg *et al*., 2000). Most *E.coli* strains are harmless commensal organisms, but some strains are pathogenic and often cause severe infections such as urinary tract infections (UTI), neonatal meningitis, and intestinal diseases (gastroenteritis) (Health Canada, 2006). Clarke (2001) identified seven classes of diarrheagenic *E.coli,* namely: enteropathogenic *E.coli* (EPEC), enterohaemorrhagic *E.coli* (EHEC), enteroinvasive *E.coli* (EIEC), enterotoxigenic *E.coli* (ETEC), enteroaggregative *E.coli* (EAggEC), diarrhea-associated hemolytic

E.coli (DHEC), and cytolethal distending toxin (CDT) - producing *E.coli.* Rump (2011) also stated that the EPEC and the ETEC are the most important pathogens responsible for diarrhoeal incidents worldwide. Recently EHEC has become the major cause of hemorrhagic colitis in humans (Rump, 2011). Enterohaemorrhagic *E.coli* (EHEC), with serotypes like *E.coli* 0157:H7 are pathogenic and can cause serious foodborne and waterborne illness in humans (Vogt & Dippold, 2005). *Escherichia coli* 0157:H7 leads to diarrhoea and abdominal cramps though sometimes the infection causes non-bloody diarrhoea and haemolytic uraemic syndrome (HUS) (Health Canada, 2006). This bacterium produces toxins which have cytotoxic on Vero cells (verotoxin) related to shiga toxin-producing *E.coli* (STEC) of the *Shigella dysenteriae* (Clark, 2001; Rump, 2011).

The *Salmonella* species are Gram-negative rod-shaped bacteria. The most common strains include *S. typhi, S. enteritidis, S. paratyphi, and S. typhimurium*, with *S. typhi* being the most pathogenic strain causing typhoid fever (Behardien, 2008). These organisms are also commonly present in the faeces of animals and can be transmitted when faecal contamination of a groundwater or surface water source occurs (Health Canada, 2006; Behardien, 2008). *Salmonella typhi* are responsible for a variety of symptoms after infection, including severe abdominal pain, diarrhoea, nausea, vomiting, and fever (Klein, 2002; Franz, 2005).

The *Shigella* species grow in aerobic or anaerobic conditions, and can cause severe intestinal disease such as bacillary dysentery (WHO, 2004). They inhabit the epithelial cells of their host's intestines, and are spread through contact with infected individuals or the consumption of contaminated food and water (Franz,

2005). *Shigella* spp. infection is termed shigellosis and the symptoms of the disease include abdominal cramps, bloody diarrhoea, fever, and dysentery (Frankel *et al*., 1989).

The *Vibrio* species are Gram-negative, curved, rod-shaped bacteria that belong to the genus *Vibrio.* Under this genus there are three species: *V. cholerae, V. parahaemolyticus* and *V. vulnificus* (Fort & Colwell, 1996). *Vibrio cholerae* causes a waterborne disease called cholera (Behardien, 2008). It is found in water sources as a result of faecal contamination and the symptoms associated with cholera include diarrhoea, abdominal pain, cramps, nausea, vomiting, and dizziness (Jay, 1992).

2.2.1.2 *Viruses*

Viruses are the smallest, non-cellular organisms that range in size from 0.02 µm to 0.3 µm. Viruses are composed of an outer shell or capsid, which consists of proteins (Madigan *et al.*, 2003). They use host cells for reproduction and are unable to reproduce outside their host (Arnone & Walling, 2007). When a virus enters a cell and starts reproducing itself, it is called a viral infection (Behardien, 2008). Viruses are known to infect almost all cells.

Waterborne viruses are human enteric viruses which multiply and infect the gastrointestinal tract of humans and animals before they are excreted in their faeces (Low, 2002). Disease-causing viruses include *Hepatitis A* causing diarrhoea and jaundice and result in liver damage, *Rotaviruses* causing gastroenteritis primarily in children, *Polioviruses* causing polio, and *Adenoviruses*

causing acute gastroenteritis. However, the detection of these human enteric viruses is not cost-effective and also time consuming (Hot *et al.*, 2003). Consequently, faecal bacteriophages – mainly somatic coliphages, RNA-F-specific phages, and *Bacteroides fragilis* phages are used as potential indicators for predicting the occurrence of enteric viruses in water and wastewater (Leclerc *et al*., 2000; Grabow, 2001).

2.2.1.3 Protozoan parasites

Protozoan parasites are unicellular microorganisms that have a nucleus (eukaryotes) ranging from 2 µm to 100 µm. Protozoa are characterised by their motility. They move by amoeboid action, cilia, or flagella (Madigan *et al*., 2003). Normally they survive in cysts (protective shells) when outside of an organism (Arnone & Walling, 2007).

Infectious waterborne protozoan species include *Acanthamoeba* spp., *Cryptosporidium parvum, Giardia lamblia,* and *Toxoplasma gondii.* The waterborne pathogenic protozoa of greatest concern in countries are *Cryptosporidium* and *Giardia*. *Cryptosporidium* oocysts and *Giardia* cysts occur in surface water, where their concentrations are positively associated with the level of faecal contamination (Rosen, 2000). Once in water, protozoa can survive for several weeks and even longer if frozen in ice (Fayer & Nerad, 1996).

Cryptosporidium parvum is an intracellular parasite ranging from 4 µm to 6 µm oocysts. It is an enteric pathogenic protozoa of the phylum, *Apicomplexa,* and is most commonly associated with waterborne diseases (Behardien, 2008).

Cryptosporidium parvum can be spread through contact with an infected individual or consumption of contaminated water and may cause a gastrointestinal disease termed cryptosporidiosis (Franz, 2005). *Cryptosporidium parvum* is the main species of *Cryptosporidium* responsible for human infections. Symptoms of these infections include fever, nausea, vomiting, and diarrhoea (Franz, 2005). Even though these oocysts are highly resistant to disinfection, they can be removed from the water source by filtration or boiling (Senior & Dege, 2005).

Giardia lamblia is a flagellated parasite of the intestinal tracts of humans and animals ranging from 8 µm to 12 µm cysts. It is also spread through contact with infected individuals, and faecally contaminated water (Franz, 2005). Once in the body, the *Giardia* causes giardiasis. Infected individuals will experience abdominal cramps, nausea, vomiting, bloating, and diarrhoea (Franz, 2005). The cyst can survive for weeks to months in cold water (Huang & White, 2006).

2.2.2 Burden of waterborne diseases

Sporadic outbreaks of waterborne diseases such as cholera, typhoid fever, and dysentery have been recorded and associated with polluted surface water and groundwater, with serious public health implications and risks for users (Banoeng-Yakubo *et al*., 2006; Nkhuwa, 2006; Montgomery & Elimelech, 2007). Diarrhoeal diseases due to contaminated drinking water result in 2.5 million childhood deaths yearly (Kosek *et al*., 2003).

In 2007, a cholera outbreak was reported in 53 countries which resulted in 177,963 cases of illness, including 4,031 deaths (case fatality rate of 2.3 %; WHO,

2008b). This represented a 46 % increase in the mean number of cases reported between 2002 and 2005 with 93.6 % of cases accounted to Africa. In addition, countries in Asia reported cases of cholera outbreaks the first time since 2000, accounting for 6.4 % of global cases. Between September 2004 and January 2005, there was an outbreak of typhoid fever in the Democratic Republic of Congo which resulted in 42 564 cases of illness, including 214 deaths and 696 cases of peritonitis and intestinal perforations (WHO, 2009).

A waterborne outbreak of *E.coli* O157:H7 took place in Scotland with 496 cases (272 laboratory-confirmed cases) and 19 deaths (Dev *et al*., 1991). In a small rural township of Missouri in the USA, an *E.coli* O157:H7 outbreak from an unchlorinated water supply resulted in 243 case patients, of whom 86 had bloody stools, 32 were hospitalised, four died and two had haemolytic uremic syndrome (Swerdlow *et al*., 1992). In Canada, Walkerton, an outbreak of *E.coli* O157:H7 and *Campylobacter jejuni* were reported during May 2000, where seven died and over 2 300 became ill from consuming groundwater (O'Connor, 2002).

In 1993 in Milwaukee, Wisconsin, over 400 000 people fell ill with a cryptosporidiosis outbreak as a result of consuming drinking water that was contaminated by *Cryptosporidium* cysts (MacKenzie *et al*., 1994; Hoxie *et al*., 1998; Naumova *et al*., 2003). During 2001, cryptosporidiosis was also responsible for 8 000 people becoming ill (WHO, 2003a). An outbreak of the viral disease, hepatitis A, was also reported in Shanghai, China during 1988 when 300 000 cases were recorded due to the consumption of clams harvested from a bay polluted with sewage (Halliday *et al*., 1991).

In South Africa, pathogenic bacteria such as *Escherichia coli, Vibrio cholerae, Aeromonas hydrophila, Shigella dysenteriae, Salmonella typhimurium, Pseudomonas* spp*., and Klebsiella* spp*.* have been reported in groundwater sources (Momba & Mnqumevu, 2000; Momba & Notshe, 2003; Momba *et al*., 2006; Gibson & Schwab, 2011; Ferguson *et al*., 2012). Outbreaks of cholera, typhoid fever, salmonellosis, shigellosis, gastroenteritis and hepatitis in some parts of South Africa have been linked to contaminated drinking water (Department of National Health and Population Development, 2001). Cholera and typhoid infections in the South African provinces of Mpumalanga, KwaZulu-Natal and the Eastern Cape have been reported since 2000 (Department of Health, 2005). By the end of 2003, the cholera outbreak had spread to eight of South Africa's nine provinces, with 106 389 reported cases of cholera and 229 reported deaths (Department of Health, 2003). The majority of the reported cases and deaths occurred in the rural communities of KwaZulu-Natal and the Eastern Cape. In September 2005, a typhoid and diarrhoea outbreak due to contaminated groundwater in Delmas in the Mpumalanga Province caused a month-long health crisis. A total of 3 000 people were diagnosed with diarrhoea and 561 with typhoid infections; five deaths occurred, according to official figures. However, the community claimed that more than 49 deaths were caused due to typhoid and diarrhoea (Groenewald & Dibetle, 2005; Masinga, 2005).

2.3 REACHING THE MILLENNIUM DEVELOPMENT GOAL TARGET FOR SAFE DRINKING WATER

2.3.1 Southern African Development Community (SADC) Region

The vital role that safe drinking water plays in human health and well-being has been globally recognised for decades. As a result, in 2002, the United Nation Millennium Development Goals (MDG) firmly established the issue of water and sanitation on their global agenda. The vision of the MDG is to halve the proportion of people without access to safe drinking water and sanitation by 2015 (WHO & UNICEF, 2010). Although tremendous progress has been made to date, the 2010 updated report by the WHO and the United Nations Children's Fund (UNICEF) has indicated that 884 million people in the world still lack access to drinking water from improved water sources. Almost all of these sources are located in developing regions; and Sub-Saharan Africa has been reported among the regions that continue to face the greatest challenge in increasing their use of improved drinking water sources. This region is currently lagging behind in its progress towards the MDG target, with only 60 % of the population using improved sources of drinking water, despite an increase of 11 % points since 1990 (WHO & UNICEF, 2010).

2.3.2 South Africa (SA)

Being one of the signatories of the MDG, the provision of safe drinking water is currently a high priority for the South African government. There has been a progressive increase (4 %) in the percentage of households with access to water supply from a safe source between 2002 (88.7 %) and 2007 (92.7 %), with a slight

decrease in 2008 (92.0 %), and then a rise in 2009 (92.4 %). The percentage of households with access to some form of water infrastructure above or equal to the Reconstruction and Development Programme standard increased from 61.7 % in 1994 to 91.8 % in March 2009. Based on these data, it is estimated that 93 % of the population had access to an improved drinking water supply in the year 2010 (Stats SA, 2010). In most cases, non improved drinking water supplies are currently found in rural communities that are widely dispersed and peri-urban informal communities that are continuously expanding.

It is crucial for developing countries to promote the eradication of waterborne diseases in communties. This will, however, only be possible if governments focus on accessing drinking water technnologies that are affordable and capable of providing potable water that complies with water quality regulations. The following section provides a survey of some water treatment technologies.

2.4 SUPPLY OF DRINKING WATER TO COMMUNITIES

To prevent waterborne diseases and deliver microbiologically safe drinking water to communities, two approaches have been considered. These include centralised drinking water supply and decentralised point-of-use (POU) systems. Point-of-use systems are particularly useful in geographically isolated areas where centralised water networks are not feasible. This section focuses on these two options of drinking water treatment.

2.4.1 Centralised drinking water supply

Centralised drinking water supply systems comprise treated water pipe systems that deliver drinking water to all the communities in the geographical area served by the treatment plant, thus requiring an extensive pipe network so as to reach even the most remote communities (Swartz, 2009). The centralised treatment plants are usually managed and operated by government employees, larger municipalities or by the Water Boards (Sansom *et al*., 2003; Swartz, 2009). They consist of unit treatment processes that treat large volumes of water intended for residential, business, and industrial uses. These treatment processes are characterised by high costs and generally require water source development, construction of infrastructure, and the adoption of a system to distribute the water to consumers.

2.4.1.1 Treatment processes in centralised systems

Water treatment processes are used to remove microbial and physicochemical contaminants from water so as to improve and protect water quality which will in turn improve human health by reducing waterborne diseases (WHO, 2003a). However, the treatment processes vary from system to system depending on the type of water intake quality. They include a combination of coagulation, flocculation, sedimentation, filtration, and disinfection. These processes are described in subsections below.

(1) Coagulation and flocculation

Coagulation is a process which involves the addition of chemicals such as iron or aluminium salts (i.e. aluminium sulphate, ferric sulphate, ferric chloride or

polymers) to the water with rapid mixing (WHO, 2003a) to enable small particles suspended in water to stick to each other (UNICEF, 2008). These chemicals are called coagulants, and have a positive charge that neutralises the negative charge of dissolved and suspended particles in the water (SDWF, 2011). Even though coagulation can remove particles and some dissolved matter, the water may still contain pathogens. Subsequent to coagulation, flocculation allows smaller particles to agglomerate into larger particles to form flocs by gentle mixing.

(2) Sedimentation

Water exiting the flocculation basin may enter the sedimentation basin, also called a clarifier or settling basin. Sedimentation allows flocs to settle to the bottom of the tank by gravity. The process usually takes place in horizontal-flow tanks, where water flows uniformly over the cross-section of the tank (UNICEF, 2008). The amount of floc that settles out of the water is dependent on the basin retention time and the basin depth as stated, but also most importantly, the load of dissolved and suspended matter in the water. Primary sedimentation tanks can remove bulk (50 % to 90 %) of flocs in water, depending on the nature of the flocs in suspension. However, most of the time a small amount of (broken) flocs or non-flocculated colloidal material remains in the water. It also lowers the load of pathogens prior to the main treatment phase because microorganisms are often attached to suspended material in water (UNICEF, 2008).

(3) Filtration

The filtration system consists of filters with pores of varying sizes, and is often comprised of sand, gravel and charcoal. It is commonly employed after

sedimentation to further reduce turbidity and remove pathogens. This process removes small remaining floc particles from water by forcing the water to pass through porous media (Schutte, 2006; UNICEF, 2008). There are two basic types of sand filtration: slow sand filtration and rapid sand filtration.

Slow sand filtration is a biological process that employs microrganisms (i.e. bacteria) to treat the water (SDWF, 2011). After the filter has been in operation for some time, a layer of microbes called “schmutzdecke” develops near the top of the sand bed (UNICEF, 2008). Rapid sand filtration operates at high flow rates and requires relatively little space (SDWF, 2011). This process alone can effectively remove large pathogens (i.e. helminths), protozoa (*Cryptosporidium* and *Giardia* cysts), and bacteria (50 % - 90 %), but viruses are small enough to pass through the filter beds (UNICEF, 2008). It has been reported that microbial communities are known to attach to slow sand filters, rather than to rapid sand filters which will increase the possibility of contamination (Brennan *et al*., 1990).

(4) Disinfection

The disinfection of water is the final and most important step in water treatment prior to the water being distributed to consumers. It necessitates the addition of a specific amount of chemical agent (disinfectant) to the water or physical agent. Contact between the water and the disinfectant is required for a pre-determined period of time so as to guarantee the success of the disinfection process with regard to the removal, deactivation or killing of any remaining pathogens resulting from the filtration process (Schutte, 2006).

The choice of the disinfectant depends on several factors which include: (1) efficacy against pathogens, (2) the ability to accurately monitor and control the methods during the disinfection process, (3) the ability to maintain a disinfectant residual within the distribution system, and (4) the ability to avoid a compromise of the aesthetic quality of the drinking water (Långmark, 2004). Among the disinfection methods, chlorination, ozonation, and ultraviolet radiation (UV) are commonly used worldwide. Some of the disinfection methods and problems associated with disinfecting water are discussed in the following subsections.

(i) Chlorination

Currently, chlorine is the most widely used drinking water disinfectant because of its relatively low running costs. This disinfectant has made an immense contribution to the safety of drinking water supplies, yet, the limitations of chlorine and several disadvantages linked to its use have been widely publicised (WHO, 2003a). Chlorine produces disinfection by-products (DBPs) such as trihalomethanes (THMs), which are known to be carcinogenic (WHO, 2003a; van der Walt *et al*., 2009). Although chlorine is effective against most vegetative bacteria and viruses when used at the normal concentration levels for treatment, it is unable to inactivate protozoan parasites such as *Cryptosporidium* and *Giardia* (Hambidge, 2001; Li *et al*., 2002; Arnold & Colford, 2007). Furthermore, sporeforming bacteria such as *Bacillus* or *Clostridium,* and acid-fast bacteria such as *Mycobacterium* and *Nocardia* are highly resistant to chlorine disinfection (WHO, 2004). Chlorine also has a very limited effect upon pathogens growing in biofilms. It has been reported that the bacterial survival after disinfection is a problem for potable water utilities (Momba *et al*., 2000; Simões *et al*., 2010; Armbruster *et al*.,

2012). Consequently, such bacteria will continue to contaminate piped water supplies. Chlorine gas dissolves in water to form free chlorine (hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) species), which reacts with various structures of bacterial cells (WHO, 2011). Free residual chlorine disrupts the metabolism and protein synthesis of the microorganisms so as to decrease its respiration (LeChevallier & Au, 2004).

(ii) Chlorine dioxide

Chlorine dioxide is an excellent disinfectant; its biocidal efficiency is equal or superior to those of chlorine and chloramines (Solsona & Méndez, 2003). It is effective over a wide range of pH and is five times more soluble in water than chlorine. Chlorine dioxide has limited residual properties (Solsona & Méndez, 2003). In water, it exists as ClO_2 (little or no dissociation) and, therefore, is able to permeate through bacterial cell membranes and destroy these cells. Its actions on viruses include the absorption and penetration of the protein coat of the viral capsid and reacting with the viral RNA, thus damaging the genetic capacity of the virus (Solsona & Méndez, 2003).

Finally, chlorine dioxide produces much lower total organic chlorine levels compared to those obtained with chlorination (Volk *et al*., 2002). However, chlorine dioxide is an unstable and extremely corrosive gas (Zuma, 2008). The chlorite ion (ClO_2^-) is a chlorine dioxide-initiated disinfection by-product. When chlorine dioxide is decomposed, chlorite is formed (Lee *et al*., 2004). Its costs are more than chlorine and trained workers are required for its operation and maintenance (Solsona & Méndez, 2003).

(iii) Ozone

Ozone (O_3) is a tri-atomic form of oxygen created by passing dry oxygen or air through a high voltage corona discharge in a controlled oxygen atmosphere (Morelli, 1994). It is a faintly blue, pungent-smelling and unstable gas with high oxidation potential and must be generated at the point-of-use (Zuma, 2008). Ozone is one of the most powerful and effective disinfectants employed in water treatment. The contact times and concentrations for inactivating or killing waterborne pathogens are much lower than those of free chlorine or any other disinfectant (Solsona & Méndez, 2003).

It has been determined that ozone is effective against bacteria, viruses, and protozoan parasites such as *Giardia* and *Cryptosporidium* (Behardien, 2008; Zuma, 2008). Ozone attacks various cellular components of microorganisms including proteins, unsaturated lipids, and respiratory enzymes in cell membranes (Zuma, 2008). Its main disadvantage is that it does not provide a stable residual and it can thus promote biological growth in distribution systems (Solsona & Méndez, 2003; Zuma, 2008). Therefore, the use of ozone has been suggested in combination with other disinfectants so as to provide a disinfectant residual for the protection of water against possible recontamination in the distribution system (Solsona & Méndez, 2003).

Ozone has also been reported to be capable of oxidising many organic and inorganic compounds under aqueous conditions. Ozone itself does not form halogenated DBPs: however, it can produce by-products such as bromates, bromoform, bromacetic acid, aldehydes, ketones and carboxylic acids (Solsona &

Méndez, 2003; Behardien, 2008; Zuma, 2008). Its cost is also higher than chlorine.

(iv) Ultraviolet radiation (UV)

Ultraviolet radiation is commonly employed as a nonchemical disinfectant treatment of water sources. The process involves water passing through a chamber where it is exposed to ultraviolet light at a wavelength of 254 nm (Margolin, 1997). When microorganisms are exposed to UV energy, the DNA in their cells is disrupted, effectively inactivating them and preventing reproduction (Behardien, 2008). Ultraviolet radiation is simple to operate, no chemicals are required, they are efficient in killing off microorganisms, and they can be managed by unskilled personnel. However, this disinfectant requires electric power which is costly to use when compared to a chlorine solution. Beside the observation that it provides no residual disinfectants, UV disinfection also requires a low turbidity in the water and a shallow depth during treatment (Solsona & Méndez, 2003).

2.4.1.2 Access to centralised systems in urban areas

The improvement and extension of water treatment systems is essential and important for the government and development agencies in many countries (Mintz *et al*., 2001). Rapid population growth in urban areas places excessive stress on the existing water and sanitation infrastructures, and creates enormous problems in the planning and construction of new infrastructures (EAWAG/SANDEC, 2002). Consequently, there is a growing demand for centralised systems of water supply in urban areas due to the continuing trend of population migration to larger cities. In these urban areas, government (or a privately contracted company) frequently

invest in the installation of sophisticated water treatment plants and distribution network infrastructures so as to provide a potable water source to all residents in a timely and convenient manner (EAWAG/SANDEC, 2002; Lee & Schwab, 2005). This potable water source is distributed to individual households, buildings and communal standpipes through a pressurised pipe network. The World Health Organization (WHO) and UNICEF (2010) estimate that, in the largest cities, those citizens with a household or yard connection range from only 35 % in Africa to 73 % in Asia, Latin America, and the Caribbean.

2.4.1.3 Access to centralised systems in rural areas

Communities in rural areas are usually scattered and in most cases they use non-improved drinking water supplies. Implementing centralised systems such as piped systems is complex and often too costly for the local authority. Therefore, the development of centralised systems in rural areas is often unaffordable due to the remote locations of the communities and a lack of financial resources (Peter-Varbanets *et al*., 2009). The unavailability of centralised systems in rural areas usually leads to frequent use of untreated natural water sources (rivers, lakes, groundwater or rain). In the rare cases where centralised systems exist, the systems fail due to insufficient financial resources, unprofessional maintenance and management, unavailability of chemicals and spare parts, and lack of trained operators which affect water quality and sustainability (Swartz, 2000; Lenton & Wright, 2004). Tap water from a supply network and a central water treatment facility is therefore generally unavailable in rural areas. The WHO and UNICEF (2010) also estimate that, in rural areas, those citizens with a household or yard connection range from only 5 % in Africa to 35 % in Asia, Latin America, and the

Caribbean. Consequently, the implementation of decentralised systems is needed to provide these rural communities with safe drinking-water sources.

2.4.1.4 Problems associated with centralised systems

(1) Deterioration of drinking water in distribution systems

The deterioration of drinking water quality in distribution systems remains one of the major challenges experienced worldwide by centralised systems for the supply of potable water to communities (Momba *et al*., 2000). Although water entering the distribution system may meet the regulatory standards, water quality may deteriorate during transportation within the distribution system before reaching the consumer (EPA, 2008). It has been pointed out that interrupted services, whereby water is provided to urban area residents for a restricted number of hours per day, result in the stagnancy of water and the growth of microorganisms.

Microbiological growth in the drinking water distribution system causes a deterioration of the water quality due to increased turbidity resulting from an increase in bacteria, as well as taste and odour problems (Hem, 2002). Lee and Schwab (2005) reported that negative hydraulic pressure can also cause deterioration of the water quality as it draws pathogens from faecally contaminated material surrounding water pipes into the water supply, through leakages in the network. Failure to maintain sufficient disinfection residuals, low water pressure, intermittent services, and ageing infrastructure can create conditions favourable to bacterial growth that could result in the deterioration of water quality in the distribution system (WHO & UNICEF, 2000; Lee & Schwab, 2005). Studies have revealed that distribution networks contribute to the deterioration of water quality

(Basualdo *et al*., 2000; Agard *et al*., 2002). In La Plata, Argentina, intestinal parasites were detected in tap water sampled from four regional zones, but they were not detected from samples taken in the immediate vicinity of the point of treatment (Basualdo *et al*., 2000). Also in a Trinidadan community, 80 % of household tap water samples tested positive for total coliforms, while no samples from the treated reservoir tested positive (Agard *et al*., 2002, Khabo-Mmekoa & Momba, 2010). The deterioration of drinking water in distribution systems may cause waterborne outbreaks that will result in diarrhoeal disease, a significant disease burden in developing countries.

(2) Biofilm formation in the distribution system

Biofilms are a layerlike aggregation of microorganisms attached to a solid surface of the piping material (Lu *et al*., 1999; Szewzyk *et al*., 2000). The process of biofilm formation is considered complex and occurs through a number of stages such as adherence (attachement), growth, and detachment (dispersal) (Percival *et al.,* 2000; Lindsay & von Holy, 2006). Figure 2.2 depicts the biofilm life cycle. The organisms excrete slimy sticky organic polymers called extracellular polymeric substances (EPS) that allow them to adhere to the piping or other water distribution system components (Behardien, 2008; EPA, 2008). During this stage of adhesion, bacteria in planktonic microorganisms adhere to the surface of the pipes. These are called secondary colonisers and result in the aggregation of bacterial cells on the surface (Dunne, 2002; Nikolaev & Plakunov, 2007). After adhesion, when microorganisms exist under favourable conditions, the biofilms continue to grow for a long time (Nikolaev & Plakunov, 2007). Detachment is the removal of individual or groups of cells from a biofilm and it involves five different

processes which include erosion, sloughing, abrasion, human intervention, and predator grazing (Percival *et al.,* 2000; McLean & Decho, 2002).

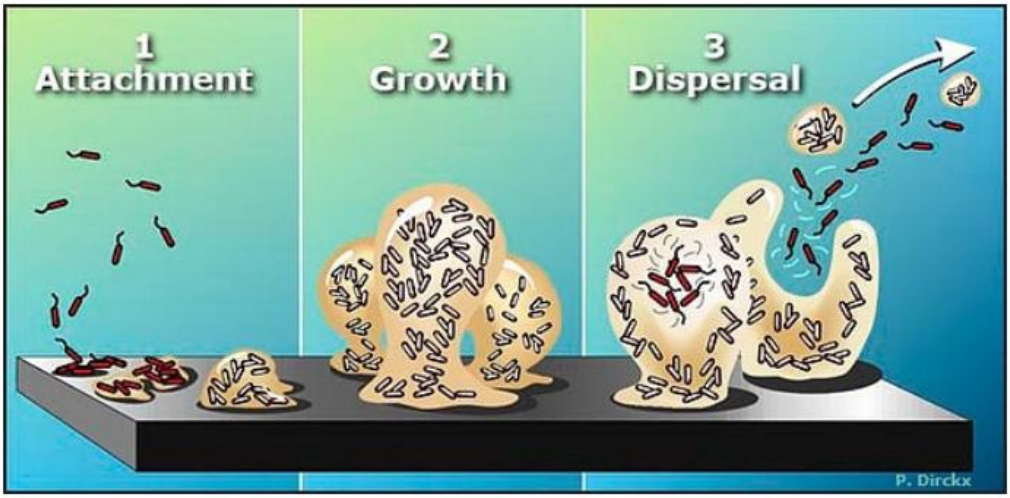


FIGURE 2.2: Biofilm life cycle (CBE, 2003)

The biofilm formed in water distribution systems predominantly consist of bacteria, while protozoa, fungi, algae, and viruses may be present in numbers (Szewzyk *et al*., 2000; Kerr *et al*., 2003, EPA, 2008). For example, biofilms can shield disease-causing microorganisms such as mycobacteria, aeromonads, and *Legionella* from residual disinfectants (EPA, 2008). Biofilms create an environment that enhances antimicrobial resistance (Chaw *et al*., 2005). In biofilms, the cells can tolerate much higher concentrations of biocides in suspension (Flemming, 2008).

(3) Factors contributing to regrowth or aftergrowth

Regrowth is the most important phenomenon that occurs when bacteria, injured during the treatment process, start to multiply after recovering from a form of reversible injury. Aftergrowth consequently indicates the growth of microorganisms

that are native to a water distribution system (Momba *et al*., 2002). The regrowth or aftergrowth of bacteria in water distribution systems has been reported by various investigators (WHO, 2002; Van der Kooij, 2002; Simões *et al*., 2010). This bacterial regrowth or aftergrowth is affected by many different factors such as the types of piping material used from a water treatment plant to the consumers, the temperature, the disinfectant concentration, the types and amount of nutrients present, and the rate of flow of the water (WHO, 2003a).

(i) Piping materials

Drinking water distribution systems are important to the quality and safety of drinking water. Water entering the distribution system must be microbiologically safe and ideally biologically stable as well. Distribution systems consist of different kinds of materials, which generally include copper, cement, stainless steel, galvanised steel, lead, iron, and plastic (polyvinyl chloride-PVC, high density polyethylene-HDPE) (Parizzi *et al*., 2004; Beech & Sunner, 2006; EPA, 2008). However, the pipes used for domestic drinking water are generally made from copper and galvanised steel pipes (Percival *et al*., 1998), and can be subjected to microbial colonisation (Beech & Sunner, 2006).

Studies have reported that other piping materials such as plastic and copper may contribute to microbial regrowth by releasing chemical compounds such as copper, iron, phosphorus ions, and organic compounds (Lehtola *et al*., 2004; Yu *et al*., 2010). It has also been found that the growth of biofilms are lower in plastic materials than that found in iron, steel or cement materials (Camper *et al*., 1996; Niquette *et al*., 2000; Mains, 2008).

(ii) Temperature

Temperature plays a major role in the adherence and formation of biofilm by many bacteria. Studies have revealed that temperatures in distribution systems change as a result of seasonal changes (Donlan, 2002; Momba *et al*., 2002; Rao, 2010). Geldreich (1996) and Donlan (2002) reported that warmer water (15 °C to 25 °C) contributes to the growth of microorganisms on pipe surfaces in the distribution system. This growth may ultimately lead to biofilm formation (Donlan, 2002).

(iii) Disinfectant residual

Disinfecting drinking water is considered to be important for the maintenance of water quality in transmission and distribution systems. Treated water is disinfected before it enters the distribution system (Clark & Coyle, 1990). However, most bacteria survive in disinfected drinking water by finding or creating environments where they are protected from the disinfectant residual (WHO, 2006b). Because bacterial contamination of water can be expected in the transmission and distribution system, a detectable disinfectant residual should remain in the water so that the potential for waterborne diseases and biofilm growth is minimised. The distribution system itself must provide a secure barrier to post-treatment contamination as the water is transported to the consumer. Residual disinfection will provide partial protection against recontamination, but may also act to disguise the presence of such contamination.

(iv) Nutrient Availability

The growth of microorganisms in biofilm depends on the availability of nutrient sources such as organic carbon, phosphorous, and nitrogen (Behardien, 2008).

The multiplication of bacteria in a piped distribution system is driven by the availability of organic and inorganic nutrients in the conveyed water and in surface deposits (WHO, 2004). Total Organic Carbon (TOC) has usually been reported to be the key nutrient that impacts biofilm growth in drinking water systems (EPA, 2008).

Biofilms can form under diverse nutrient concentrations, ranging from high to almost non-detectable (Prakash *et al.,* 2003). The authors reported that an increase in nutrient concentrations correlated with an increase in the number of attached bacterial cells. High levels of nutrients appear to produce an open structure in the biofilm, which facilitates the diffusion of nutrients to the bacteria that are remote from the aqueous phase, while lower concentrations tend to provide a more compact structure (Melo & Bott, 1997; Allison, 2003). Consequently, the structure of the biofilm has an effect on the availability of nutrients to the constituent cells. Nutrient availability is known to have an effect on biofilm formation and can encourage changes in cell physiology and composition (Allan *et al.,* 2002).

(v) Surface condition

An important factor that results in the initial adhesion of bacteria to pipe and other surfaces is surface conditioning (Kolari, 2003; Trachoo, 2007). The attachment of microorganisms to surfaces is a complex process with many variables affecting the outcome. Attachment will occur most readily on surfaces that are rougher, more hydrophobic and coated by surface conditioning films (Zacheus *et al.,* 2000; Dunne, 2002).

A material surface exposed in an aqueous medium will become conditioned or coated by polymers from that medium and the resulting chemical modification will affect the rate and extent of microbial attachment (Prakash *et al*., 2003). Surfaces cannot be colonised by biofilms unless they have been exposed to organic material from the surrounding environment (Allison *et al*., 2000). However, the effect of surface characteristics such as charge, hydrophobicity, roughness, and elasticity on microbial attachment cannot be ignored (Allison, 2000).

2.4.2 Decentralised drinking water supply

Taking into account the lack of skills, costs linked to operations and maintenance, and especially the distance between scattered villages and farms in rural areas, new approaches to treat and deliver microbiologically safe drinking water to rural communities at household level have to be considered so as to prevent waterborne diseases in developing countries.

Decentralised point-of-use (POU) water treatment systems are devices or methods employed for the purposes of treating water in the home or at POU settings known as household water treatment (HWT). These systems are possible options for improving the water quality for rural communities and could be very beneficial to individuals or families who treat their own water. POU water treatment systems are particularly useful in geographically isolated areas, where centralised water networks are not feasible (Brady-Estévez, 2009). The systems not only provide a positive barrier against pathogenic infection in homes, but can also be more cost-effective than large-scale projects in allowing individuals a selection between multiple technologies so as to meet their specific needs. These POU

treatment systems are also much smaller as well as simpler to use, maintain, and supply than larger scale treatment systems.

Different treatments can be selected with regard to the environmental hazards that become present in local sources of water over time (Brady-Estévez, 2009). Studies have indicated that simple and inexpensive POU systems are capable of reducing diarrhoeal disease and deaths caused by pathogenic organisms found in drinking water (Mintz *et al*., 2001; Sobsey, 2002; Clasen *et al*., 2004; Mwabi *et al*., 2011, 2012).

2.4.2.1 Point-of-use (POU) water treatment technologies

Many POU water treatment technologies exist and have been implemented worldwide in an effort to reduce the incidence of diarrhoeal disease. Physical methods for POU water treatment include boiling, heating (using fuel and solar), filtering, settling, and ultraviolet (UV) radiation (solar or ultra violet lamps). Chemical methods, however, include coagulation-flocculation, precipitation, ion exchange, chemical disinfection with germicidal agents (primarily chlorine), and adsorption. Many of these technologies such as boiling, chlorination, bio-sand filtration, ceramic filters, solar disinfection (SODIS), and combined coagulation-disinfection systems have been widely studied and are discussed below. The benefits and drawbacks of each system are summarised in Table 2.1.

(1) Household chlorination

Chlorination was first used to disinfect public water supplies in the early 1900s, and to reduce waterborne diseases in Europe and the United States (Gordon *et*

al., 1987; Cutler, 2005). This disinfection process has been reported to be effective against most pathogenic organisms (but is less effective at treating *Cryptosporidium* cysts) and it produces a residual disinfectant which can prevent recontamination of water for days or weeks at a time (Barstow, 2010). Although different types of chlorine are used and available, the majority of household chlorination research has been conducted using liquid sodium hypochlorite (bleach) (Figure 2.3) and calcium hypochlorite (pellets) for the chlorine supply.

FIGURE 2.3: Bleach bottle (Cater Warehouse, 2011)

To use sodium hypochlorite, it has been recommended that families add one full bottle cap of the solution to clear water (or 2 caps to turbid water) in a standard sized storage container, stir the solution and wait 30 minutes before consumption (Lantagne *et al*., 2006). However, South Africa recommends a dosage of 1 teaspoon of bleach ("Jik") per 25 L of water and the solution must be allowed to stand for at least 2 h or preferably overnight before use (DWAF, 2005).

(2) Boiling

Boiling is the oldest and most common POU household water treatment technology, and it has been widely promoted for decades (Sobsey, 2002; Rosa &

Clasen, 2010). Although boiling time recommendations vary significantly up to 20 minutes, waterborne microbes that are pathogenic to humans are killed or inactivated even before the water reaches 100 °C. The World Health Organization (WHO) thus recommends that water be heated until it reaches boiling point (WHO, 2005). Water should be stored in the same container in which it was boiled (Figure 2.4), handled carefully, and consumed within 24 h to minimise recontamination. Boiling is highly effective at inactivating pathogenic organisms, including bacteria, viruses, and protozoan parasites that cause diarrhoeal diseases (Clasen, 2008; Bartow, 2010).

FIGURE 2.4: A pot of boiling water (Magnus, 2011)

(3) SODIS

Solar disinfection (SODIS) was initially employed for centuries to disinfect water needed for oral rehydration solutions which aid in the treatment of diarrhoeal diseases (Acra *et al*., 1984; Reed, 2004). It is a simple and cost-effective household treatment option in which clear plastic bottles of 0.3 L - 2.0 L are filled

with low-turbidity (<30 NTU) or contaminated water, shaken vigorously for oxygenation and then placed outside in the sun from 6 h to several days, depending on the available sunlight (EAWAG/SANDEC, 2008; Barstow, 2010). SODIS has been proven to inactivate bacteria and viruses (Sommer *et al*., 1997) while protozoa (*Cryptosporidium* and *Giardia*) are sensitive to solar irradiation (Méndez-Hermida *et al*., 2007). Figure 2.5 depicts 1 L - 2 L volumes of plastic bottles placed on their sides in the sunlight.

FIGURE 2.5: The SODIS method (EAWAG/SANDEC, 2002)

(4) BioSand Filtration

The BioSand Filter (BSF) is a modified slow-sand filter adapted for POU water disinfection. Most BSFs consist of a container with a layer each of fine sand, coarse sand, and gravel which is stored in an approximately 0.9 metres high and 0.3 metres square (Lantagne *et al*., 2006; Barstow, 2010). The water level is maintained at 5 cm - 6 cm above the sand layer by setting the height of the outlet pipe (EAWAG/SANDEC, 2008). Biosand filters operate like a slow sand filter with

the formation of a bioactive layer called the “Schmutzdecke” which is used to reduce pathogenic organisms (Yung, 2003). A plate with holes in it is placed on top of the sand to prevent the disruption of the bioactive layer when water is added to the system. A cross section of the biosand filter is presented in Figure 2.6.

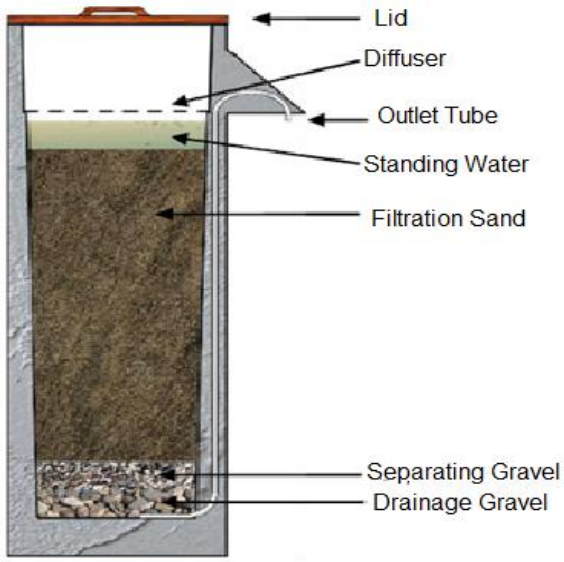


FIGURE 2.6: Cross section of a concrete biosand filter (CAWST, 2011)

(5) Ceramic filtration

Ceramic filters have traditionally been employed as POU water treatment systems throughout the world. These ceramic filters are made from clay and other combustible material such as sawdust (Barstow, 2010). When fired, the sawdust will burn away to create micro-pores within the structure, which is usually shaped like a flower pot and impregnated with colloidal silver by painting or dipping the pot in the silver (Barstow, 2010). The clay pot is then inserted into a 5 gallon plastic bucket with a spigot so that water can be stored therein. While there are several mechanisms for how ceramic filters work, the main mechanisms are size exclusion by the small pores and inactivation of organisms by the colloidal silver. However,

the size exclusion is usually only effective down to bacteria-sized microorganisms (Lantagne *et al*., 2006). Smaller microorganisms such as viruses can be inactivated by the silver, but the silver will often leach out of the filter within less than a year and needs to be reapplied. Additionally low flow rates of 1 to 3 L/h will continue to decrease if the filter is not properly cleaned and maintained (Sobsey *et al*., 2008). Currently, the most widely distributed ceramic filter is the Potters for Peace (PFP) filter. A flat bottom ceramic pot filter and container with a cross section of the said filter is depicted in Figure 2.7.

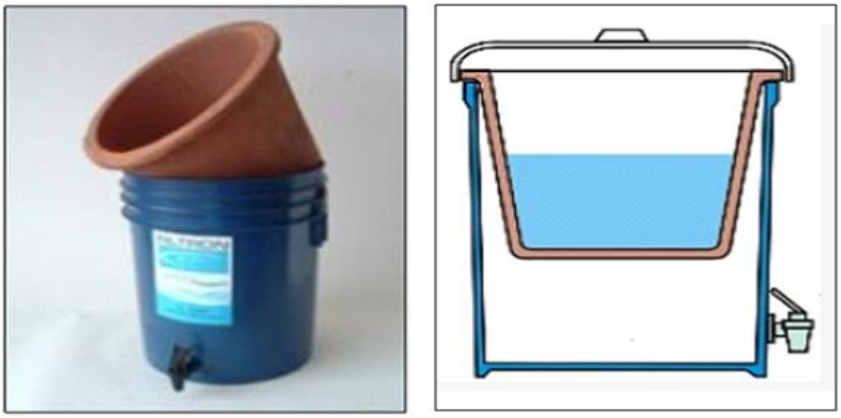


FIGURE 2.7: Flat bottom ceramic pot filter and container with a cross section of ceramic pot filter (Potters for Peace, 2006)

(6) Combined coagulation and disinfection systems

Combined coagulation and disinfection systems (also called PUR) are one of the more widely distributed water purification sachets, developed by Procter and Gamble (P&G). It incorporates both a chemical coagulation step for particle removal (flocculation) and a chlorination step for disinfection (Lantagne *et al*., 2006). The sachet includes both powdered ferrous sulfate (a flocculant) and

calcium hypochlorite (a disinfectant) (Lantagne *et al*., 2006; Barstow, 2010; Jeffreys, 2012).

To use PUR effectively, add the contents of the sachet to a 10 L bucket of water, then stir for five minutes, allowing the solids to settle at the bottom of the bucket. Thereafter, filter the water through a cloth into a second container and wait for 20 minutes to allow for disinfection. The PUR system has demonstrated high removal rates of bacteria, viruses, and protozoa in a variety of water qualities, including highly turbid waters and water containing heavy metals (Souter *et al*., 2003; Barstow, 2010). The system has proven to be effective in the reduction of diarrhoeal diseases in both the developing world and in disaster relief applications (Lantagne *et al*., 2006). Figure 2.8 demonstrates the effect of a combined coagulation-flocculation disinfection sachet on turbid water.

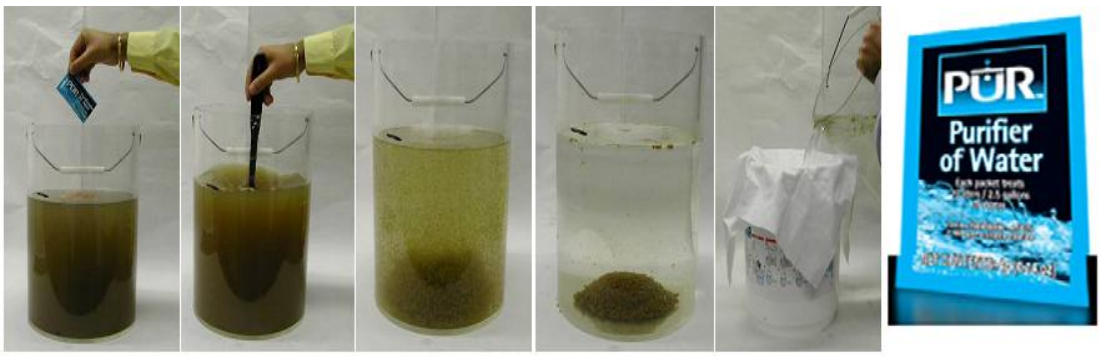


FIGURE 2.8: The effect of a combined coagulation-flocculation disinfection sachet on turbid water (UNICEF, 2008)

TABLE 2.1: Summary of benefits and drawbacks of POU systems

POU SYSTEMS	BENEFITS	DRAWBACKS	REFERENCES
Household chlorination	• Proven reduction of bacteria and most viruses; • Residual protection against contamination; • Ease of use and thus acceptability to users; • Treats water quickly (less than 1 hour); • Low cost.	• Relatively low protection against parasitic cysts; • Lower disinfection effectiveness in turbid waters contaminated with organic and some inorganic compounds; • Potential user taste and odour objections; • Effects of chlorination by-products	Lantagne *et al*.(2006); Jeffreys (2012)
Boiling	• Existing presence in many households of materials needed to boil water; • Documented inactivation of bacteria, viruses and protozoa, even in turbid or contaminated water; • Socio-cultural acceptance of boiling for water treatment in some cultures; and • Needed “hardware” (e.g. heat source and pot) already in place in most homes.	• Lack of residual protection against contamination; • Not usually able to produce large quantities of water for a family; • Potential for burn injuries and increased risk of respiratory infections from indoor stoves or fires; • Potentially high cost of carbon-based fuel source (with concurrent deforestation risk); • Takes time to bring water to a boil and then let it cool to drinking temperature; • Can be labour and time-intensive to collect wood, biomass, charcoal, etc., most of which typically fall upon women and children.	Lantagne *et al*.(2006); Clasen (2009); Jeffreys (2012)

Solar disinfection (SODIS)	• Documented reduction of viruses, bacteria, and protozoa in water; • Documented reduction of diarrhoeal disease in users; • Acceptability to some users because of the simplicity of use; • Uses locally available bottles and sunlight; • Minimal change in taste of the water; and, • Minimal likelihood of recontamination due to safe storage; • Low cost.	• Ineffective on turbid or heavily contaminated water; • Produces a relatively small amount of water: the amount of water treated is limited to the number of bottles a family owns; • Lack of visual improvement in water aesthetics to reinforce benefits of treatment; • Long time required to treat water; • Many plastic bottles required; • Time consuming.	Lantagne *et al*.(2006); EAWAG/SANDEC (2008); Jeffreys (2012)
BioSand Filtration	• Documented removal of protozoa and bacteria; • Acceptability to users because of high flow rate (~20 litres/hour), ease-of-use, and visual improvement in the water; • Local production; • One-time installation with low maintenance requirements; and • Long life span.	• Comparatively low inactivation of viruses; • Lack of residual protection so that if water is filtered into an open or unclean bucket there is potential for contamination; • Difficulty in producing and transporting a 100-350 pound filter, housing, and the high initial cost that make scalability more challenging; • Takes time before effective filtration is achieved.	Lantagne *et al*.(2006); EAWAG/SANDEC (2008).

Ceramic filtration	• Documented reduction of bacteria and protozoa in water; • Acceptability to users because of the simplicity of use and the aesthetic improvement in treated water; • Documented reduction of diarrhoeal diseases among users; • Potentially long life if the filter remains • unbroken; and • One-time cost.	• Low effectiveness against viruses; • Lack of residual protection can lead to recontamination if treated water is stored unsafely; • Filter breakage and need for spare parts; • Filters and receptacles need to be regularly cleaned, especially when using turbid source waters; and • A low flow rate of 1-3 litres per hour.	Lantagne *et al*.(2006); EAWAG/SANDEC (2008); Jeffreys (2012)
PUR	• Removal or inactivation of viruses, bacteria, parasites, heavy metals, and pesticides, even in highly turbid waters; • Residual protection; User acceptability due to water's visual improvement; • Ease of scalability or use in an emergency because the sachets are centrally produced; and • Reduced concern about carcinogenic effects of chlorination because organic materials are removed in the treatment process.	• Multistep process requiring demonstrations for new users and a time commitment for water treatment from the users; • High relative cost per litre of water treated.	Lantagne *et al*.(2006); EAWAG/SANDEC (2008); Jeffreys (2012)

2.4.2.2 Access to decentralised systems

Most of the POU technologies are already being explored or employed in developing countries to some extent (Sobsey, 2002). Some of these methods, such as boiling water with fuel, are traditionally and widely used, although they may not always be the optimal solution. However, in areas where wood, and other biomass fuels or fossil fuels are in limited supply and must be purchased, the costs of boiling water are unaffordable. Therefore, boiling household water is considered unrealistic and inaccessible to many of the world's poorest people, due to the scarcity and high cost of fuels and the lack of sustainability of biomass or fossil fuels in the community or region (Sobsey, 2002). Other methods such as SODIS, which have a high potential for application, then become more appealing. It can also be noted that most of the other methods are commercially available, even though challenges are encountered in ensuring the effectiveness of the POU products.

2.4.2.3 Selection criteria of POU technologies

There are large numbers of household water treatment systems (HWTS) available in the market today. Due to this diversity, it is critical to decide which device or devices would be most suitable for communities living in rural areas. The following points were considered by Mwabi *et al*. (2012) in order to help the communities to select a suitable device for their POU water treatment system (Table 2.2):

TABLE 2.2: Selection criteria for HWTS and criteria for evaluation (Mwabi *et al*., 2012)

Selection Criteria - to choose devices to evaluate in the lab / field.	**Evaluation Criteria - characteristics to be tested during lab / field work.**
1. Can members of rural communities afford obtaining the unit? Construction and operation costs must not exceed earnings	1.Cost (capital/running)
2. Representative of a number of similar systems	2. Final water quality must comply with SANS 241
3. Systems already extensively evaluated	3.Turbidity of treated water must comply with SANS 241, <1NTU
4. Pressure requirement, maximum two metres	4. Ease of operation
5. Power requirement does not exceed equitable share	5. Storage ability and ability to deliver enough water
6. Robustness durability of filter	6.Robustness (test)
7. Minimum required volume for basic human needs 25 ℓ/p/d, for drinking,	7. Social acceptance.

2.5 ADVANCED WATER TREATMENT TECHNOLOGIES

The use of conventional water treatment processes becomes increasingly challenged with the identification of more and more contaminants, rapid population growth, and consequent industrial activities as well as the diminishing availability of water resources. Consequently, there is a need for advanced water treatment technologies to improve the quality of drinking water. There are a number of these technologies that can be employed for the purification of water for domestic and other uses. They include membrane processes, activated carbon adsorption, ion exchange, chemical precipitation, oxidation processes, and nanotechnology for

water purification. The two emerging treatment technologies, membrane filtration and nanotechnology, are discussed below.

2.5.1 Membrane technology

Membrane is a thin permeable or semi-permeable solid phase (polymer, inorganic or metal), which is used as a selective barrier to remove contaminants from water. It works by selectively allowing some constituents to pass through the membrane while blocking the passage of others (Baker, 2000). For this to occur, the movement of material across a membrane requires a driving force. Membrane processes offer an attractive alternative for the primary disinfection of water regarding the removal of viruses and bacteria from water systems (Momba *et al*., 2008).

Membrane filtration removes microbial pathogens mainly by size exclusion; that is, microbes that are larger than the membrane pores are removed (LeChevallier & Au, 2004). Based on pore size, the order of effectiveness of microbial removal is reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF), and microfiltration (MF), with RO being the most effective. The advantage of membrane processes are that no chemicals are required (Momba *et al*., 2008). However, its main limitations are membrane fouling and the fact that it does not provide any residuals, which could promote biological growth during storage or in distribution systems. Therefore, it is necessary to employ membrane processes in combination with other disinfecting systems so as to provide disinfectant residuals that will result in the protection of drinking water against possible contamination during storage and in the distribution system. Currently, the four categories of

pressure-driven membrane processes that are utilised to remove microbes include: reverse osmosis, nanofiltration, microfiltration, and ultrafiltration.

2.5.1.1 Reverse osmosis (RO)

Reverse osmosis membranes generally refer to high-pressure membranes. The membrane has smaller pore sizes of approximately 0.1 nm (0.0001 µm) (Schutte, 2006). In RO, the two solutions are separated by a semi-permeable membrane, where pressure is applied to reverse the natural flow of the water. This forces the water to move from the more concentrated to the weaker solution. Consequently, the contaminants end up on one side of the semi-permeable membrane and the pure water is on the other side. RO membranes are the most commercially available POU water treatment systems (Peter-Varbanets *et al*., 2009). These systems can effectively remove all particulate matter, including all bacteria, protozoa and viruses (Schutte, 2006).

2.5.1.2 Nanofiltration (NF)

Nanofiltration is also one of the high-pressure membrane filtration processes with a smaller pore size around 0.001 µm (Schutte, 2006). Because of the small size of the pores, the membrane could easily be fouled by very small substances. These systems can remove most organic molecules, nearly all viruses and cysts, most of the natural organic matter, and a range of salts. NF membranes also remove alkalinity; therefore, the final water can be corrosive (Tech brief, 1999). NF removes divalent ions (which make water hard), consequently NF is often used to soften hard water.

2.5.1.3 Microfiltration (MF)

Microfiltration membranes have pore sizes larger than 50 nm and operating pressures around 100 kPa (Schutte, 2006). Theoretically, MF can remove protozoa, algae, and most bacteria very effectively (LeChevallier & Au, 2004). A Study conducted by Jacangelo (1995) has shown the removal of *Giardia* and *Cryptosporidium* by using MF membranes. However, viruses, which are 0.01 µm – 0.1 µm in size, can generally pass through MF membranes, but may be removed by the membrane if they are associated with larger particles. Findings from the same author revealed that the removal of MS2 bacteriophage by these MF membranes was less than 1 log, because the phage is 0.025 µm and the pore size of the membranes is 0.08 µm – 0.22 µm. Another application for the technology is the removal of natural synthetic organic matter to reduce the fouling potential (Tech brief, 1999). MF can be used as a pretreatment to RO or NF in order to reduce the fouling potential.

2.5.1.4 Ultrafiltration (UF)

An ultrafiltration filter is a low-pressure membrane with a pore size around 0.01 µm and an operating pressure of approximately 200 kPa to 700 kPa (30 to 100 psi) (Schutte, 2006). UF membranes can be made from both organic (polymer) and inorganic materials. It has been reported that the system is capable of removing all microbiological species (partial removal of bacteria), some viruses (not an absolute barrier to viruses), and humic materials (Laîné *et al*., 2000). In water applications, UF could be the main process or a pretreatment, as for example in the RO system. UF technology has been found to exceed current water regulations for turbidity, *Giardi*a, and also for virus removal (Laîné *et al*., 2000).

2.5.2 Nanotechnology

Nanotechnology is an emerging technology that covers a wide range of disciplines, including design, synthesis, characterisation, manipulation, and exploitation of materials at a nanoscale with at least 1 nm -100 nm (Schutte & Focke, 2007; Ashe, 2011). Due to their small size, nanoparticles have a larger surface area than macro-sized materials (Ashe, 2011). This technology has unveiled a huge potential in various areas such as drug development, information and communication technologies, production of stronger and lighter materials, human health care, and water treatment (Koch, 2002; Cross *et al*., 2009).

Nanotechnology can be useful in resolving current shortcomings in water treatment (Savage & Diallo, 2005; Bottero *et al*., 2006). It offers the potential of new nanomaterials for the treatment of surface water, groundwater and wastewater contaminated by toxic metal ions, organic and inorganic solutes, and microorganisms (Theron *et al*., 2008). Researchers are currently developing different kinds of nanomaterials due to their flexibility and application in the treatment of various water contaminants. Various forms of nanotechnology such as nanoadsorbents, nonocatalysts, nanostructured and reactive membranes, and nanobiocides are used in water treatment (Schutte & Focke, 2007).

2.5.2.1 Nanosorbents/Nanoadsorbents

Nanosorbents/Nanoadsorbents are widely used as separation media in water purification to remove inorganic and organic pollutants from contaminated water (Savage & Diallo, 2005). They can contribute to improving the drinking water quality, recover heavy metals and other materials from wastewater streams, and

remove trace contaminants. Several water sorbents have been discovered to remove microorganisms; they are classified in three main categories:

i. Inorganic materials (zeolites), in the form of silver zeolite, have been reported as antibacterial agents against coliform microorganisms from wastewater in a column system (De la Rosa-Gómez *et al.,* 2008).

ii. Carbon-based adsorbents (carbon nanotubes) have demonstrated antimicrobial characteristics on microorganisms, including bacteria such as *E.coli* (Kang *et al*.,2008) and *Salmonella* spp. (Arias & Yang, 2008), and viruses such as MS2 bacteriophage (Brady-Estevez *et al*., 2008).

iii. Organic polymers (dendrimers) such as silver- based complexes and nanocomposites have been reported to be effective antimicrobial agents *in vitro* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Balogh *et al*., 2001).

2.5.2.2 Nanocatalysts

Nanoscale catalysts have great potential for treating particularly challenging contaminants in water that must be removed down to a very low level. Obare and Meyer (2004) have reported that the potential of these catalysts as water-purification catalysts and redox active media is due to their large surface areas and their size and shape dependent optical, electronic, and catalytic properties. The catalytic particles may either be dispersed homogeneously in a solution, or deposited on membrane or filter structures (Schutte & Focke, 2007).

During the last decade, titanium dioxide (TiO_2) nanoparticles have emerged as promising photocatalysts for water purification (Adesina, 2004). Titanium dioxide nanoparticles are very versatile; they can serve both as oxidative and reductive catalysts for organic and inorganic pollutants (Savage & Diallo, 2005). The utilisation of photocatalysts in the treatment of water contaminated by organic and inorganic pollutants was reported by Kabra *et al*. (2004). They also documented the successful use of TiO_2 nanoparticles to (1) degrade organic compounds (e.g. chlorinated alkanes and benzenes, dioxins, furans, PCBs, etc.) and (2) reduce toxic metal ions (e.g., Cr(VI), Ag(I) and Pt(II)) in aqueous solutions under UV light.

Titanium dioxide nanoparticles greatly enhanced the removal of total organic carbon (TOC) from water sources contaminated by organic wastes (Chitose *et al*., 2003). It has been reported that these nanoparticles can inactivate bacteria and viruses, especially in the presence of UV light (Savage & Diallo, 2005; Alrousan *et al*., 2009; Giraldo *et al*., 2010). The study by Tsuang *et al*. (2008) demonstrated the bactericidal effects of TiO_2 nanoparticles against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus hirae,* and *Bacteroides fragilis.*

2.5.2.3 Nanostructured membranes

Membranes are an important passive element in water treatment systems that concentrate on and remove contaminants from water. Nanotechnology can aid the tailoring of membrane thickness, pore size distribution, permeability, and surface chemistry (Schutte & Focke, 2007). Nanostructured membrane filters are used for water treatment and desalination (Srivastava *et al*., 2004). Substantial

nanomaterials are providing novel opportunities to develop more efficient and cost-effective nanostructured and reactive membranes for water purification and desalination. An example is a reactive nanostructured ceramic membrane for the remediation of organic waste in water (Schutte & Focke, 2007). Silver-loaded acetate hollow fibers have demonstrated an antibacterial activity against *E.coli* and S. *aureus* (Chou *et al*., 2005). Don *et al*. (2005) also reported that composite membranes containing a 50 nm thick chitosan layer exhibit strong antibacterial activity towards Gram-positive and Gram-negative bacteria.

2.5.2.4 Nanobiocides

The use of nanobiocides for water disinfection is another key area in nanotechnology which is applied in water purification. Nanobiocides are antimicrobial nanoparticles that include metals and metal oxides, of which silver and gold, copper, zinc, and titanium oxides are most widely used (Du Plessis, 2011). Silver nanoparticles, particularly, are ranked first among the noble metal nanoparticles used in water purification (Botes & Cloete, 2010). Nanobiocides present alternative opportunities for the chlorination of drinking water; they are discussed in the sections below:

(1) Metal oxide nanoparticles

(i) Iron oxide

Iron oxide nanoparticles are particles with diameters ranging approximately between 1 nm and 100 nm. The three main forms are magnetite (Fe_3O_4), hematite (α-Fe_2O_3) and its oxidised form maghemite (γ-Fe_2O_3) (Teja & Koh, 2009). Iron oxide nanoparticles are naturally present in high concentrations in the environment

(Ju-Nam & Lead, 2008). Researchers have demonstrated the effectiveness of iron oxide nanoparticles impregnated with fibreglass for the removal of bacteria and viruses in water (Li, 2010; Nangmenyi *et al*., 2011). Viruses are attached to various positively charged particles and surfaces with metal oxides and positively charged media such as clays that are rich in ferric oxides (Chu *et al*., 2001; Brown & Sobsey, 2009). Zero-valent iron nanoparticles have been reported to deactivate Gram-negative *E.coli* (Lee *et al*., 2008), Gram-positive *Bacillus subtilis,* and fungus *Aspergillus versicolor* (Diao & Yao, 2009). Columns packed with zero-valent iron nanoparticle or sand coated with ferric hydroxide has been suggested as an efficient means for the removal of viruses (Lukasik *et al*., 1999).

(ii) Zinc oxide

Zinc oxide usually appears as a white powder and is nearly insoluble in water (Ashe, 2011). It is usually used in sunscreens and special textiles due to its large ultraviolet (UV-A and UV-B) blocking properties and low toxicity to human beings (Becheri *et al*., 2008; Ju-Nam & Lead, 2008; Newman *et al*., 2009). Zinc oxide nanoparticles have proven to be a powerful antibacterial agent in the formulation of the microscale and nanoscale systems for therapeutic applications (Ashe, 2011). It has been reported that they exhibit the antibacterial activity, with the Gram-positive *B. subtilis* being more sensitive to its effects than the Gram-negative *E.coli* (Sawai *et al.,* 1995; Ashe, 2011). It has been also pointed out that ZnO nanopartilces with a truncated triangular shape had the strongest biocidal action on *E.coli,* followed by spherical and rod-shaped nanoparticles, based on test concentrations of 10, 125 and 1000 mg/L (Pal *et al*., 2007).

(iii) Titanium dioxide

Titanium dioxide (TiO_2) nanoparticles have been revealed to be the most promising technology for the inactivation of viruses and other microorganisms in recent years (Theron *et al*., 2008). The inhibitory activity of TiO_2 is due to the photocatalytic generation of strong oxidising power when illuminated with UV light (Chorianopoulos *et al*., 2011). Titanium dioxide nanoparticles are also considered to be an excellent band-gap semiconductor (Ju-Nam & Lead, 2008). They have been demonstrated to be effective in deactivating both Gram-negative and Gram-positive bacteria, although Gram-positive bacteria are less sensitive due to their ability to form spores (Wei *et al*., 1994). A broad spectrum of viruses and bacteria, have been successfully inactivated using nanosized TiO_2 (Cho *et al*., 2005; Zan *et al*., 2007). Investigators have demonstrated the antiviral properties of TiO_2 and its potential for inactivating viruses even though the inactivation rates obtained in most of these studies were extremely low (1 log removal) (Koizumi & Taya, 2002; Cho *et al*., 2005).

(2) Copper nanoparticles

Copper oxide (CuO) is a semiconducting compound having direct bandgap with a monoclinic crystal structure. It exhibits a range of potentially useful physical properties such as high temperature, superconductivity, electron correlation effects, and spin dynamics (Ravishankar & Jamuna, 2011). High concentrations of CuO nanoparticles are required to achieve a bactericidal effect (Ren *et al*., 2009). Ren and co-workers (2009) reported that copper oxide nanoparticles cause an antimicrobial activity against a range of Gram-positive and Gram-negative bacteria. Its effectiveness in killing a range of bacterial pathogens has been

demonstrated in hospital-acquired infections (Ravishankar & Jamuna, 2011). Lin *et al.* (1998) and Stout *et al.* (1998) reported that a combination of copper and silver ions resulted in the disinfection of cold and hot water systems at hospitals against *Legionella*, with most applications on recirculating hot water systems. It maintains a high antimicrobial activity against *B. subtilis*. Top and Ulku (2004) reported that copper embedded in natural zeolite was found to be the most effective disinfectant agent for all the identified bacteria.

(3) Silver nanoparticles

Silver nanoparticles are one of the promising products in the nanotechnology industry today. The development of various silver nanomaterials is an important aspect of current nanotechnology research. In general, silver nanoparticles can be obtained from commercial sources (Morones *et al*., 2005) or newly synthesised in a laboratory (You, 2010) by chemical, physical, and biological methods.

The chemical methods are based on the reduction of silver salts using a reduction agent such as sodium borohydride, N,Ndimethylformamide (DMF), ethylene glycol, or ascorbate in the presence of appropriate surfactants like sodium citrate, poly(vinylpyrrolidone) (PVP) or polyvinyl Alcohol (PVA) in order to prevent aggregation processes (Nath *et al.,* 2005, Lok *et al*., 2007, Choi *et al*., 2008; Lukhele *et al*., 2010). The particle size, shape, and morphology of silver nanoparticles depend on the solvents, reducers, and surfactants used in the reaction. Physical methods are based on a gas phase for the synthesis of silver nanoparticles with supersaturated metal vapour. The study by Nangmenyi *et al*. (2009) has demonstrated the synthesis of silver nanoparticles using a thermal

reduction method. The biological method employs microorganisms to reduce silver salts. For example, bacteria such as *Pseudomonas stutzeri* isolated from silver mine materials have been found to reduce Ag+ ions to silver nanoparticles (Klaus *et al.,* 1999).

With the advancement in materials development, silver nanoparticles can easily be deposited on solid materials for the deactivation of microorganisms in water treatment (Nair & Pradeep, 2007). Several studies have shown the incorporation, coating or impregnation of silver nanoparticles in various materials or substrates such as fibreglass (Nangmenyi *et al*., 2009; Li, 2010), polysulfone ultrafiltration membranes (Zodrow *et al*., 2009), polyurethane foams (Jain & Pradeep, 2005), ceramic filters (Oyanedel-Craver & Smith, 2008), sand (Mahmood *et al*., 1993), and zeolite (Matsumura *et al*., 2003). Silver nanoparticles can be applied in biomedicine (dressing and bandages, biological implants, disease treatments), household items (nano-silver lined refrigerators, air conditioners, washing machines), clothing (footwear, socks, home textiles), food storage containers, cosmetics and personal care products, and water purification (Maynard & Michelson, 2006; Woodrow Wilson Centre, 2011).

(i) Silver nanoparticles as an antimicrobial agent

Silver ion (Ag^+) has long been known as a potential antimicrobial agent. It is used in wound dressings to prevent infections in burn patients, to treat blindness in newborns, for severe chronic osteomyelitis and urinary infection, to control *Legionella* bacteria in hospitals, and to enhance the performance of drinking-water filters (Klasen, 2000; Bosetti *et al*., 2002; Richard *et al*., 2002; Niven, 2005). Silver

in the form of nanoparticles that release silver ions more effectively has proven to be a more effective bactericidal activity due to its high surface-area-to-volume ratio (Kumar *et al*., 2008; Duran *et al*., 2010). Recent studies have indicated that distinctively prepared silver nanoparticles display good antibacterial activity (Matsumura *et al*., 2003, Sondi & Salopek-Sondi, 2004). As a result, researchers have considered the use of silver nanoparticles for drinking-water treatment due to their strong and broad spectrum of antimicrobial activities (Stoimenov *et al*., 2002; Cho *et al*., 2005; Jain & Pradeep, 2005).

Silver nanoparticles are an effective killing agent for a broad spectrum of Gram-negative and Gram-positive bacteria (Burrell *et al*., 1999; Wijnhoven *et al*., 2009), including multi-resistant strains (Wright *et al*., 1998; Shrivastava *et al*., 2007). Gram-negative bacteria include genera such as *Acinetobacter*, *Escherichia*, *Pseudomonas*, *Salmonella*, and *Vibrio*. *Acinetobacter* species are associated with nosocomial infections, that is, infections that are the result of treatment in a hospital or a healthcare service unit, but secondary to the patient's original condition. Gram-positive bacteria include many well-known genera such as *Bacillus*. Silver nanoparticles have also been demonstrated to be effective in viral removal (antiviral activity) (Elechiguerra *et al*., 2005; Rogers *et al*., 2008; Zodrow *et al*., 2009). Other investigators have pointed out that silver nanoparticles are effective in the inactivation of coliphages (You, 2010), monkeypox virus (Rogers *et al*., 2008), and HIV-1 virus (Elechiguerra *et al*., 2005). The inactivation of viruses by silver nanoparticles impregnated into materials or substrates such as fibreglass (Li, 2010) and polysulfone UF membranes (Zodrow *et al*., 2009) have also been

reported. Researchers have also demonstrated that silver nanoparticles are capable of inhibiting planktonic and biofilm bacteria (Choi, 2009; Climent, 2009).

(ii) Mechanisms of antimicrobial action of silver nanoparticles

The mechanisms by which silver nanoparticles deactivate microorganisms are largely, but not fully understood. However, researchers have reported that silver nanoparticles inhibit microbial growth which include particle attachment to or penetration of the cell membranes accompanied with a slow release of silver ions, causing changes to the membrane permeability and redox cycle in the cytosol intracellular radical accumulation process, as well as dissipation of the proton motive force for ATP synthesis (Sondi & Salopek-Sondi, 2004; Morones *et al*., 2005; Lok *et al*., 2006).

Silver nanoparticles can bind to bacterial cells and enzymes (proteins) at multiple sites, damaging them and preventing them from performing their functions, resulting in cell death through penetration at specific bacterial DNA and RNA (Klassen, 2000; Ovington, 2004; Rai, *et al*., 2009). It has also been reported that bacterial membranes include sulphur-containing proteins and that the silver nanoparticles interact with these proteins in the cell as well as with the phosphorus-containing compounds like DNA (Doyle *et al.*, 1980; Feng *et al.*, 2000; Morones *et al.*, 2005). Other studies have indicated that Ag nanoparticles bind to the outer membrane of *E.coli,* causing the inhibition of active transport, dehydrogenase and periplasmic enzyme activity, and eventually the inhibition of RNA, DNA and a decrease in the cell permeability, which finally results in cell lysis (Russell & Hugo, 1994; Sondi & Salopek-Sondi, 2004; Morones *et al*., 2005;

Zhang & Chen, 2009). While microorganisms carry a negative charge, the Ag ions carry a positive charge, which is crucial for its antimicrobial activity through the electrostatic attraction between the negatively charged cell membrane of microorganisms and the positively charged nanoparticles (Dragieva *et al*., 1999; Hamouda *et al*., 2000; Dibrov *et al*., 2002).

2.6 COST-EFFECTIVE NANOTECHNOLOGY FOR WATER SUPPLY IN DEVELOPING COUNTRIES

Researchers in general and the water sector in particular can apply nanotechnology to develop more cost-effective and high-performance water treatment systems so as to improve not only water quality, but also quality of life in developing countries. Due to financial constraints in developing countries, there is a need to develop cost-effective water treatment technologies. The idea behind this innovative technology is to decontaminate water required for water supply in a cost-effective manner that is readily implementable and requires minimal infrastructure and very limited maintenance. Cost-effective materials or substrates coated with silver nanoparticles is an alternative technology that could assist developing countries in meeting the MDG, and South Africa in particular, in providing a safe drinking-water supply to all scattered rural areas and informal settlements. Some of the locally available materials that could be employed for the deposition of silver nanoparticles are presented in Figure 2.9.

FIGURE 2.9: Images of cost-effective materials found in South Africa

2.6.1 Zeolite

The use of natural zeolites (e.g. clinoptilolite) as cation exchangers in water treatment has increased in recent years due to their availability, low cost, high surface area and sorptive capacity, negative surface charge, chemical inertness, and low or null toxicity for humans (Rivera-Garza, 2000; Top & Ülkü, 2004). Zeolites are hydrated aluminosilicates that consist of symmetrically stacked alumina and silica tetrahedra (Curkovic *et al.*, 1997). They provide a very good combination of ion exchange and molecular sieve properties, which can be modified with ease (Cincotti, 2006). Zeolite can be modified by incorporating silver nanoparticles. Inoue *et al.* (2002) demonstrated the loading of silver ion into zeolite by the ion-exchange method, which resulted in a strong bactericidal substrate. Matsumura *et al.* (2003) reported that the mechanisms for bactericidal action of

silver zeolite might be due to the intake of silver ion by bacterial cells, which damages the cells.

2.6.2 Fibreglass

Fibreglass is a fibre reinforced polymer made of a plastic matrix reinforced by fine fibres of glass. It is a lightweight, extremely strong, and robust material (Trimo, 2011). Most kinds of fibreglass are used for thermal and acoustic insulation in building construction, shipbuilding, and filtration applications. Fibreglass-reinforced plastics (FRPs) have been utilised for various types of process equipment in the chemical industry, pulp and paper industry, power and mining industries, municipal sewer treatment, and water treatment as well as many other associated industries handling corrosive equipment (SMACNA, 1997).

Fibreglass can be modified by incorporating silver nanoparticles. Nangmenyi *et al*. (2009) demonstrated the impregnation of silver ions by employing the thermal reduction method and thereafter performing dynamic flow tests to evaluate the antimicrobial ability of the fibreglass impregnated with silver nanoparticles. Recent work by Li (2010) and Nangmenyi *et al*. (2011) have indicated that a new material system composed of silver-modified iron oxide (Fe_2O_3) nanoparticles, loaded onto a fibreglass support, displayed excellent antiviral properties against the model virus, MS2 phage, but it has been proven to be ineffective against bacteria, specifically *E.coli*. Medina-Valtierraa *et al*. (2004) utilised a chemical deposition of copper complex solution to prepare CuO thin films on commercial fibreglass.

2.6.3 Sand

Sand is a naturally occurring granular material composed of finely divided rock and mineral particles. Sand filtrations have been used in water purification for over 150 years in order to control microbiological contamination (Logsdon *et al*., 2002). Sand filters are a less expensive and more effective method of water treatment that could be self-constructed by using local skills. Modifying sand filters can result in improved water quality by incorporating silver nanoparticles with sand. Mahmood *et al.* (1993) have described the coating of silver on sand by employing the chemical method, and subsequently the coated silver-coated sand was utilised for the removal of *E.coli* from contaminated water in a column experiment.

2.6.4 Resins

Resins are very small plastic beads with a diameter of approximately 0.6 mm. These beads are porous and contain invisible water, measured as “humidity” or “moisture content”. The structure of the resin is a polymer (like all plastics) to which a fixed ion has been permanently attached. Cation resins are created by attaching negatively charged functional groups to the copolymer structure (Gottlieb, 2005). The fixed ions of this cation exchange resin are sulphonates (SO_3^-) that are attached to the skeleton. Resins have been used in ion exchange and are reported to be a very powerful technology in the removal of impurities from water and other solutions (Gottlieb, 2005). Many industries, including nuclear and thermal power stations, semiconductors, computer chip and display panel production, and removal systems for toxic contaminants from drinking water use ion exchange resins. There is no health risk with the use of resins, as many industries use resins for multiple purposes, including dental and pharmaceutical

applications, and drinking-water treatment for the removal of toxic contaminants (Purolite International LTD, 2004; Rohm & Haas, 2008).

Antimicrobial resins can be fabricated by incorporating silver nanoparticles into the resin through chemical reduction methods. The synthesis of resins containing silver nanoparticles has been well researched (Nath *et al*., 2005; Jana *et al*., 2006). Recently, some investigations were carried out regarding the use of resins containing silver or silver nanoparticles for oral and dental applications (Bürgers *et al*., 2009; Fan *et al*., 2011). Researchers have also investigated the use of polymer composites (Jain & Pradeep, 2005) and polymer microspheres, employing plate and test tube batch methods (Gangadharan, 2010) for water disinfection.

2.7 MONITORING TECHNIQUES OF DRINKING WATER SAFETY

2.7.1 Microbial parameters used for the evaluation of drinking water safety

The assessment of the microbiological quality of drinking water aims to protect consumers from illness due to the consumption of water that may contain pathogens such as bacteria, viruses, and protozoa, thus preventing drinking-water related illness outbreaks. In South Africa, the quality of drinking water must comply with the limits set by the South Africa National Standard (SANS 241, 2006; 2011) and *Water Quality Guidelines for Domestic Use* (DWAF, 1996) as the official specification for assessing the quality of drinking water.

Water quality monitoring techniques for drinking water safety are usually based on testing for indicator microorganisms. Primarily, bacterial indicator organisms are used for the routine monitoring of a potential presence of pathogens in water. These pathogens are mainly of human and animal origin. Faecal indicator bacteria are utilised to assess the microbiological quality of water. However, bacterial indicators are not suited to the monitoring of more resistant microbial pathogens, that is, enteric viral pathogens and protozoan parasites. These pathogens are often more persistent than bacteria in water environments and are not easily removed by water treatment processes. The most widely used indicators are total coliforms, faecal coliforms, *E.coli,* and bacteriophages, which are found in the intestinal tracts of warm blooded animals. However, protozoan parasites like *Giardia* and *Cryptosporidium spp.* are also transmitted through the faecal-oral route.This section focuses on these bacterial and viral indicators.

2.7.1.1 Total coliforms

Total coliform bacteria are aerobic or facultative anaerobic, Gram-negative, non-spore-forming rod-shaped bacteria. They ferment lactose with gas and acid production within 48 h at 35 ºC (APHA, 1998; Environment Agency, 2002). They belong to the family of *Enterobacteriaceae* that include *Escherichia, Enterobacter, Klebsiella* and *Citrobacter,* and some members of the genus *Serratia*. They are commonly used as indicator organisms to signify the presence of faecal contamination in water; they are possibly the disease-causing microorganisms, such as bacteria, viruses or parasites, which may give rise to gastro-intestinal diseases (DWAF, 1996; WRC, 1998).

2.7.1.2 Faecal coliforms

Faecal coliform bacteria are Gram-negative bacteria, also known as thermotolerant coliforms (APHA, 1998). Thermotolerant coliforms are defined as the group of total coliforms that are capable to ferment lactose at 44 °C - 45 °C and to grow in the presence of bile salts (deoxycholate). This subgroup includes the genus *Escherichia,* and to a lesser extent some species of *Klebsiella, Enterobacter,* and *Citrobacter* (Stevens *et al*., 2003). Faecal coliform bacteria are present in water whenever it is contaminated with faecal waste of human or animal origin. They are also commonly used as indicator organisms to signify faecal pollution in water (DWAF, 1996).

2.7.1.3 *Escherichia coli*

Escherichia coli are a member of the total coliform group of bacteria and the only member that is present exclusively in the faeces of humans and other animals. It is a taxonomically well-defined member of the family *Enterobacteriaceae*, and is characterised by its possession of the enzymes, β-galactosidase and β-glucuronidase (WHO, 2003a). It grows at 44 ºC - 45 ºC on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan (APHA, 1998). *Escherichia coli* is also commonly used as indicator organisms to signify the origin of faecal contamination in water (WRC, 1998).

2.7.1.4 Bacteriophages

Bacteriophage groups have also been classically utilised as faecal and viral indicators (Leclerc *et al*., 2000). They are viruses, which specifically infect bacteria

(Grabow, 2001). There are several bacteriophages that can be used as indicator organisms, including the somatic coliphages and male specific F-RNA coliphages (Grabow, 2001).

(1) Somatic coliphages

Somatic coliphages are a heterogeneous group of organisms which could originate from faecal sources (Calci *et al*., 1998). They occur in large numbers in sewage and polluted water environments and are easy to detect, but they may be replicated by host bacteria in certain water environments (DWAF, 1996). Somatic coliphages infect *E.coli* host strains WG5 by adsorbing to viral receptors situated on the cell wall (DWAF, 1996; Leclerc *et al*., 2000). The double layer plaque assay is generally used to detect somatic bacteriophages (APHA, 1998; Mooijman *et al*., 2001). Somatic coliphages are, therefore, useful indicators of the potential presence of faecal pollution and enteric viruses in water environments (DWAF, 1996; Grabow, 2001).

(2) Male-specific F-RNA coliphages

Male-specific (F-RNA) coliphages are *Enterobacteriaceae* viruses of the *leviviridae* family that are physically and genomically analogous to human enteric viral pathogens found in sewage (CEFAS, 2003). They are highly specific to sewage pollution and cannot be replicated in water environments, but detection methods are more complicated (DWAF, 1996). There are two groups of F-specific coliphages; those containing RNA and those containing DNA, and both groups are found in human and animal faecal wastes (WHO, 2003a). Male-specific (F-RNA)

coliphages are also indicators of faecal pollution in water sources and the presence of pathogenic viruses, predominantly enteric viruses (DWAF, 1996).

Male specific F-RNA coliphages are similar in size, shape, and basic composition to many human enteric viruses in water treatment such as *Enteroviruses*, *hepatitis A,* and *E* viruses (Leclerc *et al*., 2000; Grabow, 2001; CEFAS, 2003). These bacteriophages infect bacteria through the F- or sex-pili. *Salmonella typhimurium* strain (WG49) contains a plasmid coding for F-pili production making it a suitable host strain for detecting male-specific RNA bacteriophages (CEFAS, 2003). The F-plasmid with its F-pili occurs in *E.coli* as well and is most often worked with this bacterium.

2.7.2 Detection techniques of bacterial and viral indicators and protozoan parasites in water

2.7.2.1 Test for coliforms bacteria

Coliform bacteria can be detected by several procedures, including the membrane filtration (MF) test, most probably number (MPN) test, Presence/Absence (P/A) test and molecular techniques. In the MF test, a water sample is filtered through a filter with a pore size of 0.45 µm and the filtrate incubated with growth media at 35 °C - 37 °C for 24 h (APHA, 1998), after which.colonies are counted. Total coliforms and *E .coli* can be assessed by Chromocult® Coliform Agar resulting in a salmon to red colouration in colonies counted as total coliforms, and a dark-blue to violet colouration in colonies counted as *E .coli* (Merck, 1996).

Faecal coliforms can be assessed by M-FC agar and incubated at 44.5 °C for 24 h so as to produce blue coloured colonies. In the MPN test, selective growth media and different dilutions of the water sample are placed in separate tests tubes. After incubation at 35 °C - 37 °C for 48 h, the tubes are examined for bacteria (Rompré *et al.*, 2002). Estimations for coliform numbers are then presented, based on the positive results of different dilutions. Tubes containing coliform bacteria are subsequently used to inoculate agar. Growth of colonies on the selected agar reveals the presence of coliforms in the sample. In the presence or absence (P/A) test, broth and water samples are added to a test tube, along with certain salts and enzymes (Rompré *et al.*, 2002). If coliform bacteria are present in the water sample, the liquid in the test tube will change colour.

Molecular techniques provide sensitive, rapid, and quantitative analytical tools for detecting specific pathogens, including new emergent strains and indicators (Girones *et al*., 2010). They are used to evaluate the microbiological quality of water. Through the molecular technique the detection of culturable and non-culturable bacteria can be achieved within hours, instead of days as compared to traditional methods (Rompré *et al.*, 2002). The most frequently used nucleic-acid-based methods are the polymerase chain reaction (PCR) method.

2.7.2.2 Test for viruses

Tests for coliphages include the P/A test and double agar layer plaque assays. In plaque assays, a water sample is mixed with the nalidixic acid resistant *Escherichia coli* WG5 strain host bacteria and agar at temperatures above 30 °C and incubated at 37 °C for 24 h (ISO, 1998; USEPA, 2001). Coliphages that infect

the host cells will be present on the plates as plaques (lysis zones), which can be counted and used to estimate the number of coliphages present in the original sample (ISO, 1998; USEPA, 2001).

2.7.2.3 Test for protozoan parasites

Detecting protozoan parasites (Giardia cysts and Cryptosporidium oocysts) involves the filtration of large volumes of water as these protozoa are usually present in very low numbers. Methods 1623 developed by USEPA, employ filtration and immuno-based techniques with monoclonal antibodies for separation (immunomagnetic separation, IMS) and detection (immunofluoresecence assay, IFA) in order to determine concentrations of (oo)cysts, confirming their presence through vital dye staining (DAPI) and differential interference contrast (DIC) microscopy (USEPA, 2001). However, the recovery success of this process can be variable monoclonals which may vary in their avidity and specificity to (oo)cysts or cross-react with other animal species and the methods, costly.

2.8 CONCLUSION

The presence of pathogenic microorganisms in groundwater poses a serious health risk to consumers. Groundwater, which is considered to be the main water source supply in many disadvantaged communities, is consumed without any prior treatment.

Therefore, to supply drinking water that is safe and free from the aforementioned pathogens to rural areas, treatment is required. POU treatment can provide

numerous benefits to the individual or family who treats their own water. This is especially helpful in geographically isolated and scatted areas where centralised water networks are not feasible. These small-scale systems can permit individual household to receive clean and safe drinking water. POU systems are generally affordable and are low-cost investment.

The following chapters focus on the assessment of groundwater quality in rural areas in two provinces of South Africa; the development and evaluation of POU filter systems, and the effectiveness of POU filter systems with silver nanoparticle-coated susbtrates for the removal of pathogenic microorganisms from groundwater. The impact of silver-resin nanoparticle filters in inhibiting bacterial regrowth and biofilm formation in potable water distribution systems using a laboratory-scale unit remains one of the most important aspects of this investigation.

CHAPTER 3

ASSESSMENT OF GROUNDWATER QUALITY IN RURAL AREAS OF NORTH WEST AND MPUMALANGA PROVINCES IN SOUTH AFRICA

3.1 ABSTRACT

A survey of 200 boreholes in rural areas of North West and Mpumalanga Provinces was conducted so as to investigate whether the quality of the groundwater supply to communities poses a possible threat to public health as defined by the regulations of the South African National Standard. Culture- based methods were employed to isolate faecal and total coliform bacteria, while molecular techniques were applied for the identification of coliform isolates. Selected physicochemical parameters were also determined by means of standard methods. The results revealed that pH, temperature, and concentrations of sulphates, potassium, sodium, and choride were within the limits set by the national guidelines for domestic purposes in both provinces. However, some of the boreholes in both provinces did not comply in terms of fluoride limit (0 to 1 mg/L), the magnesium limit (0 to 30 mg/L as Mg), calcium limit (0 to 32 mg/L as Ca), TDS limit (0-500 mg/L), and turbidity limit (<1 NTU). Twenty-three percent (23 %) and 86 % of the boreholes in North West and 78 % and 81 % of the boreholes in Mpumalanga did not comply with the limits set by the national guidelines (SANS 241 and DWAF) in terms of faecal (0 cfu/100 mL) and total coliforms (0 to 5 cfu/100 mL), respectively. The results of the molecular study revealed that out of 200 boreholes, 51 % in North West and 35 % in Mpumalanga tested positive for

Citrobacter freundii, 28 % (North West) and 19 % (Mpumalanga) for *Serratia marcescens,* 12 % (North West) for *Morganella morganii,* 11 % (Mpumalanga) and 5 % (North West) for *Bacillus cereus*, 9 % (Mpumalanga) and 2 % (North West) for *Enterobacter cloacae,* 8 % (North West) and 7 % (Mpumalanga) for *Salmonella enterica,* 7 % (North West) for *Aeromonas veronii,* 7 % (Mpumalanga) for *Pseudomonas maltophili and* 1 % (North West) for *Escherichia coli* and *Shigella dysenteriae,* and 1 % (Mpumalanga) for *Escherichia coli* O157:H7*, Shigella flexineri*, *Klebsiella oxytoca,* and *Cronobacter sakazakii.* The findings of this study revealed convincing evidence that some groundwater supplies in rural areas of North West pose a serious health risk to consumers.

3.2 INTRODUCTION

An adequate supply of fresh and clean drinking water is a basic need of the entire human race, yet it has been observed that millions of people worldwide are deprived of access thereto. Approximately 1.2 billion people across the world do not have access to safe drinking water (WHO, 2002). A report by the World Health Organisation (WHO) (2003a) revealed that approximately 1.6 million deaths were attributable to unsafe water and sanitation, including a lack of hygiene.

South Africa has a population of 49.4 million people, 52 % of whom are estimated to be living in the rural areas (DEAT, 2008). Of this percentage of the population, 6 million still do not have access to a reliable source of drinking water supply (DWAF, 2008), even though the South African constitution states that every citizen has the right to be supplied with clean, safe drinking water (Constitution, 1996).

This implies that a large number of communities in rural areas depend on untreated surface and groundwater sources for their daily water needs. Water from these sources is faecally contaminated and untreated (Momba & Kaleni, 2002; Momba & Notshe, 2003).

Groundwater is a vital natural resource for the provision of water to South African communities for domestic, industrial and agricultural purposes. It contributes to between 13 % and 15 % of the total available water in South Africa (Pietersen, 2005). Approximately 400 rural communities in South Africa depend on groundwater sources for domestic purposes (Pietersen, 2005). In provinces such as Mpumalanga and North West, the rural communities, which include villages, farms, and formalised towns, rely mostly on groundwater for their daily needs, and it is used directly without any prior treatment. However, the groundwater sources can be contaminated by chemical and microbiological pollutants originating from natural sources, human activities, and on-site sanitation systems used in the rural areas.

Chemical pollution of groundwater sources can include nitrate, fluoride, and trace metals, especially arsenic, sulphate or chloride, which could have detrimental effects on the health of consumers (Ansa-Asare *et al*., 2009). Microbiological pollution is caused by bacteria, viruses, and protozoa. The major health risk associated with groundwater is from the microbial pathogens derived from human and animal faeces (Lehloesa & Muyima, 2000). Pathogenic organisms found in groundwater with high counts of faecal coliforms include especially *Escherichia coli*, and other pathogenic microorganisms such as *Vibrio cholerae, Aeromonas*

hydrophila, Shigella dysenteria, Salmonella typhimurium, Pseudomonas, and *Klebsiella* spp. (Momba & Mnqumevu, 2000; Momba & Notshe, 2003; Momba *et al*., 2006) and these organisms contribute to waterborne diseases.

The transmission of diseases by polluted water has a long history and remains a worldwide problem to this day. Diarrhoea is a symptom of a waterborne disease, although not all cases of diarrhoea are related to water (Gundry *et al*., 2004; Jensen *et al*., 2004). Diarrhoeal diseases due to contaminated drinking water result in 2.5 million childhood deaths yearly (Kosek *et al*., 2003). Sporadic outbreaks of waterborne diseases such as cholera, typhoid fever, and dysentery due to polluted groundwater have been recorded, with serious public health implications and risks for consumers (Banoeng-Yakubo *et al*., 2006; Nkhuwa, 2006; Montgomery & Elimelech, 2007).

As mentioned earlier the outbreaks of cholera and typhoid infections in the South African provinces of Mpumalanga, KwaZulu-Natal and the Eastern Cape have been reported since 2000 (Department of Health, 2005). By the end of 2003, the cholera outbreak had spread to eight of South Africa's nine provinces, with 106 389 reported cases of cholera and 229 reported deaths (Department of Health, 2003). The majority of the reported cases and deaths occurred in the rural communities of KwaZulu-Natal and the Eastern Cape. In September 2005, a typhoid and diarrhoea outbreak due to contaminated groundwater in Delmas in the Mpumalanga Province caused a month-long health crisis. A total of 3 000 people were diagnosed with diarrhoea and 561 with typhoid infections, and according to official figures five deaths occurred. However, the community claimed that more

than 49 deaths occured due to typhoid and diarrhoea (Groenewald & Dibetle, 2005; Masinga, 2005). In April 2009, a cholera outbreak was reported in North West Province with 91 reported cases that resulted in four deaths (Department of Health, 2009).

The risk posed by microbial pathogens in water necessitates the monitoring of water for various types of microbial pathogens. Coliform bacteria have been widely used as indicators of water contamination (APHA, 1998) and particular attention has been afforded to the survival of faecal indicator bacteria in drinking water for sanitary reasons. Faecal coliforms have been included in water quality standards in different parts of the world, including South Africa [USEPA (1986); DWAF (1996); WHO (2004); SANS 241 (2006, 2011)]. The WHO and SANS 241 recommend the measurement of *E.coli* in drinking water samples as the best indicator of water quality, stating that potable water must contain less than one *E.coli* per 100 ml of drinking water (WHO, 2004; SANS 241, 2006, 2011).

In most developing countries, the detection and identification of a microbial species in water is conventionally based on the isolation of the microorganisms in pure culture and the examination of their morphological and physiological properties (Beneduce *et al*., 2007). The limits of conventional culture-dependent and phenotypic characterisation are nowadays seen as a severe constraint for precise and reliable diagnostics of microbial pathogens. With the scientific progress in the field of genetic and molecular biology, a variety of DNA, RNA, and protein-based methods have been developed (Beneduce *et al*., 2007). The employment of molecule-based technologies in microbial diagnostics has greatly

enhanced the ability of researchers to detect and quantify pathogenic bacteria in water within hours, as compared to days with traditional methods (Rompré *et al*, 2002; Beneduce *et al*., 2007).

In this study, conventional and molecule-based techniques were applied to determine whether the microbiological quality of groundwater supply to the rural communities of the North West and Mpumalanga Provinces poses a possible threat to the health of the consumers by referring to the limits set by the South Africa guidelines. The physical and chemical quality of this water supply was also determined. The intention of this study was to provide information that could assist water authorities in addressing problems with the management of groundwater systems in South Africa.

3.3 MATERIALS AND METHODS

3.3.1 Study area and sampling points

3.3.1.1 North West Province

The North West Province of South Africa shares an international border with Botswana in the north. The climate of this province is characterised by hot summers and cool and sunny winter seasons. The annual rainfall varies from 500 mm to 700 mm in the eastern part, 400 mm to 600 mm in the central regions and 100 mm to 400 mm in the western part of the province (NWPG, 2002). The province has a total population of 3.4 million, 60 % of whom live in rural areas. More than 80 % of the rural population depend on groundwater as their main drinking water source (NWPG, 2002). The aquifers that serve as water sources

are composed of limestone, carbonated shale, and dolomite. Groundwater samples were collected from 100 boreholes located in the villages of the Bojanala district municipality (which includes the local municipalities of Moretele, Madibeng and Moses Kotane), the central district municipality (Ramotshere Moiloa, Mafikeng and Tswaing) and the Bophirima district municipality (Kagisano and Greater Taung) (Table 3.1 and Figure 3.1). Forty (40) of the boreholes are privately owned, and the remaining 60 are communal boreholes in the North West Province. The communities in these villages obtain underground water from boreholes by using a rotary hand pump that is connected to a standpipe, or directly from standpipes that are connected to the boreholes.

3.3.1.2 Mpumalanga Province

The Mpumalanga Province is located in South Africa, bordered by Mozambique and Swaziland in the east and Gauteng in the west. It is situated mainly on the high plateau grasslands of the Middleveld, which roll eastwards for hundreds of kilometres. The best-performing sectors in the province are mining, manufacturing, and agriculture. The province has a total population of more than 3.5 million, 42 % of whom live in rural areas and depend on groundwater as their main source of drinking water (MPG, 2007). The bulk (65 %) of the water available in Mpumalanga comes from surface water resources. Water transfers into the province constitute 19 % of the total water available, groundwater contributes to 6 % of the available water, and the return flows from mining, industries, irrigation, and urban sectors contribute to 10 % (MPG, 2007). Groundwater sources are surrounded by weathered rock, dolomitic or karst, and alluvial aquifers. Groundwater samples were collected from 100 boreholes located in the villages of

the Nkangala district municipality (which includes the Delmas, eMalahleni and Emakhazeni municipalities); the Gert Sibande district municipality (Mkhondo, Pixle Ka Seme and Albert Luthuli), and the Enhlanzeni district municipality (Mbombela and Nkomazi) (Table 3.2 and Figure 3.2). The EMalahleni communities obtain underground water from boreholes using a rotary hand pump connected to a standpipe, while the Delmas, Emakhazeni, Mkhondo, Pixle Ka Seme, Albert Luthuli, Mbombela, and Nkomazi communities obtain their drinking water directly from standpipes connected to boreholes. Seventy-six of the boreholes are privately owned and the remaining 24 are communal boreholes.

3.3.2 Collection of water samples

Water samples from the abovementioned sources were collected between September and November 2008 in a once-off sampling exercise. The standpipes or the taps in the operation room were flushed for approximately 5 min before collecting samples using 2 L sterile glass bottles. All the bottles were sealed and properly labelled. A mobile laboratory containing all the necessary equipment (a membrane filtration unit, a vacuum pump, kettles, portable incubators, sterile Petri dishes containing selective cultural media, sterile membrane filters, a pH meter, thermometer, turbidity meter, conductivity meter, etc.) was used for the on-site analysis of water quality. The plates containing coliform isolates and samples for chemical analysis were subsequently placed in ice bags and transported to the Tshwane University of Technology, Water Research Group laboratory for analysis.

TABLE 3.1: List and locations of the boreholes surveyed in North West

Municipalities surveyed	Number of boreholes	Locations of boreholes
Moretele	8	Dikebu (1), Makapanstad (2), Noroki (3), Moretele Clinic (4), Kgomo-Kgomo (5), Mashilomatsho 1 (6) and 2 (7) and Swartdam (8).
Madibeng	7	Shakung 1 (9) and 2 (10), Dipompong (11), Maboloka (12), Rabokale (13), Jerico (14) and Moiletswane (15).
Moses Kotane	15	Ratau (16), Mabalstad (17), Makgope (18), Khayakhulu (19), Letlhakeng (20), Voordonker (21), Lefaratlhatlha (22), Matau (23), Masekoloane (24), Molorwe (25), Motlhabe (26), Tweelaagte 2 (27), Manamela (28), Bapong 2 (29) and Mantsho (30).
Ramotshere Moiloa	14	Luthern Mission (31), Malebelele P.S (32), Braklaagte 1 (33), 2 (34) and 3 (35), Leeufontein 1 (36), 2 (37) and 3 (38), Supingstad 1 (39) and 2 (40), Lekgopung 1 (41) and 2 (42) and Mushane 1 (43) and 2 (44).
Mafikeng	26	Signal Hill 1 (45) and 2 (46), Lonely Park 1 (47) and 2 (48), Moshewane (49), Megokgoane (50), Dimorogwane 1 (51) and 2 (52), Miga 1 (53) and 2 (54), Ikopeleng 1 (55) and 2 (56), Six Hundred 1 (57) and 2 (58), Dihatshwane 1 (59) and 2 (60), Majemantsho 1 (61) and 2 (62), Setlopo 1 (63), 2 (64) and 3 (65), Motloung (66), Mokgokoe(67), Lekoko 1 (68) and 2 (69) and Morwatshethla (70).
Kagisano	8	Mophohung 1 (71) and 2 (72), Ganyesa 1 (73) and 2 (74), Moswane 1 (75) and 2 (76), Selosesha (77) and Thokoza (78).
Greater Taung	12	Dryharts 1 (79) and 2 (80), Moretele (81), Ntswanahatshe (82), Choseng (83), Pudimoe (84), Matlapeng 1 (85) and 2 (86), Leshobo (87), Myra (88) and Amalia 1 (89) and 2 (90).
Tswaing	10	Groeteland (91), Rapoeli (92), Delareyville (93), Bamberg Farm (94), Eclipse Farm (95), Vriegevagte 1 (96) and 2 (97), Atamelang (98) and Setlagole 1 (99) and 2 (100).

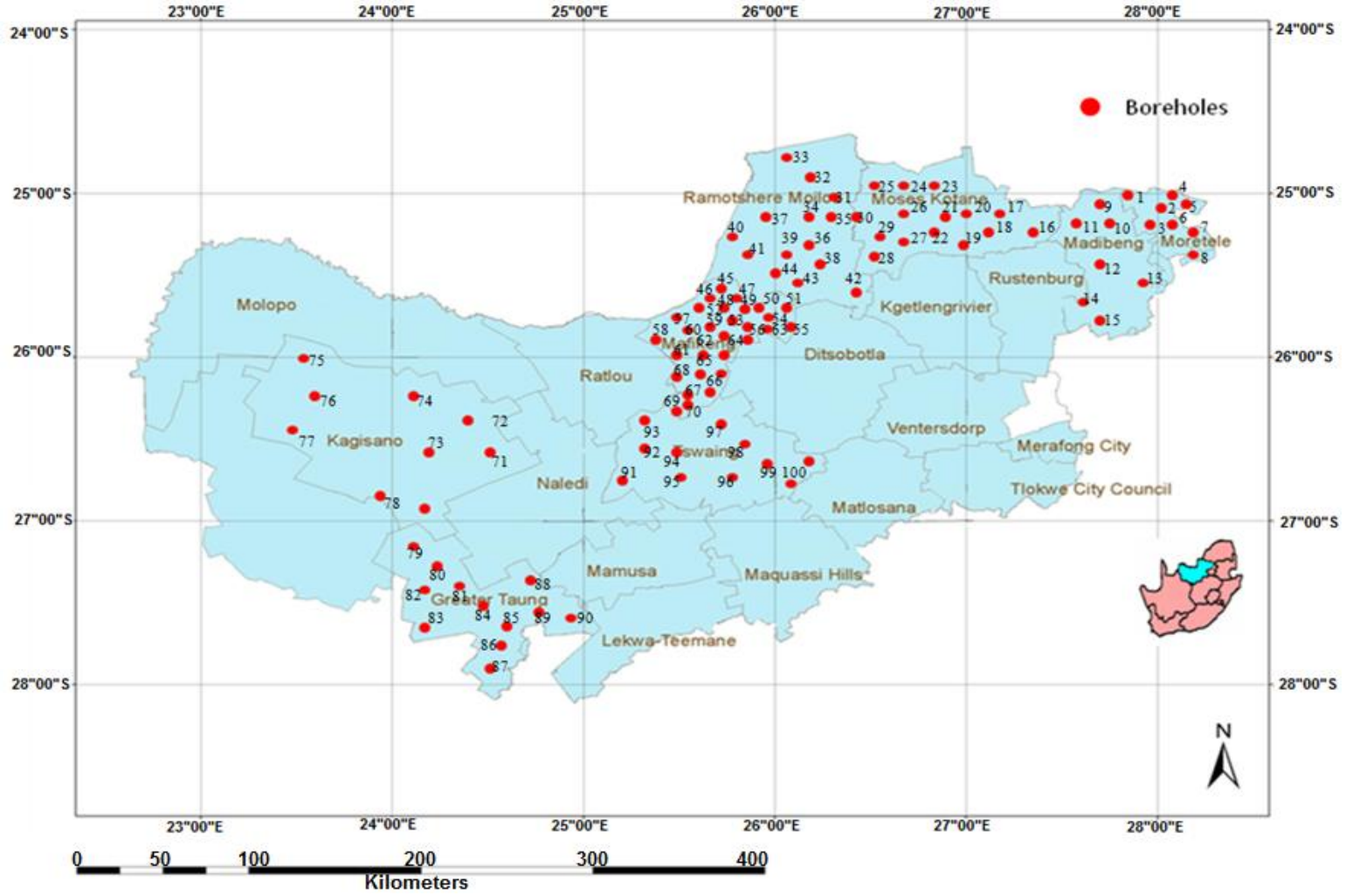


FIGURE 3.1: Map of North West Province indicating location of borehole sites in the local municipal areas of Moretele, Madibeng, Moses Kotane, Ramotshere Moiloa, Mafikeng, Kagisano, Tswaing and Greater Taung

TABLE 3.2: List and locations of the boreholes surveyed in Mpumalanga

Municipalities surveyed	Number of boreholes	Locations of boreholes
Delmas	10	C1 (1), C2 (2), C3 (3), C4 (4), A4 (5), A7 (6), A3 (7), BOT6 (8), BOT 4 (9) and D10 (10)
Emalahleni	6	Leeufontein (11), Kendal (12), Emakhosi (13), Borax 1 (14) & 2 (15), Legdaar Farm 78 (16)
Emakhazeni	6	Mooifontein (17), Nhlupheko P.S. (18), Sanbery (19) and Belfast 1 (20), 2 (21) & 3 (22)
Mbombela	18	Katoen (23), Mphatheni Mahushu (24), Mphatheni Guesthouse (25), White River 73B (26), White River 73A (27), White River 53 (28), White River 60 (29), Heide Eggs (30), Mtimba B (31), Malapa Farm (32), Heidelberg Farm 7 (33), Heidelberg Farm 25 (34), Heidelberg Tevrede (35), Moegeploeg Plot 36 (36), Moegeploeg Plot 37 (37), Weltevreden Plot 455A (38), Weltevreden Plot 455B (39) and Weltevreden Plot 43 (40)
Nkomazi	15	River View P.S. (41), Beyers Farm 1 (42) & 2 (43), Baundi Farm (44), Mpapani Farm (45), Bambinyati Farm Plot 19 (46), Bambinyati Farmhouse (47), Boland Farm (48), Malelani Farm (49), Eskom Distribution (50), Lilly Pond (51), Voorspoed Farm (52), Voorspoed Commonage 1 (53) & 2 (54) and Elenberg Komatipoort Farm (55)
Albert Luthuli	11	Travelpoort Garage Badplaas (56), Rooihoogte Farm B16 (57), Elstone Farm (58), Mosley Farm 13 (59), Mosley Farm 17 (60), Mosley Farm 27 (61), Natal Drift Plot 13 (62), Pullenshope (63), Drankenstein (64) and Bosmansfontein 1 (65) & 2 (66)
Mkhondo	19	Groenvlei-Buhleni Farm (67), Vezinyaho 1 (68), 2 (69) & 3 (70), Weeber Farm Piet Retief (71), Goedetrouw 1 (72) & 2 (73), Driehoek (74), Anyspruit (75), Ematafeleni (76), Kwa-Ngema 1 (77) & 2 (78), Zeelie Farm (79), Dirkiesdorp (80), Dirkiesdorp Farm (81), St Helena Farm (82), Themba Trust School (83), Injabulo Combined School (84) and Dirkiesdorp Police Station (85)
Pixle Ka Seme	15	Goededorp Amersfoort (86), Piet Zyn Drift Farm (87), Langspoort Volksrust (88), Wenber Wakkerstrroom (89), Pietskop Wakkersttroom 1 (90) & 2 (91), Wakkerstroom plot 40 (92), Uithenden Wakkerstroom (93), Kranspoort (94), Heins Farm Resort (95), Drinkwater 1 (96) & 2 (97), Tafelkop (98), Rietvlei (99) and Winkelhaak (100)

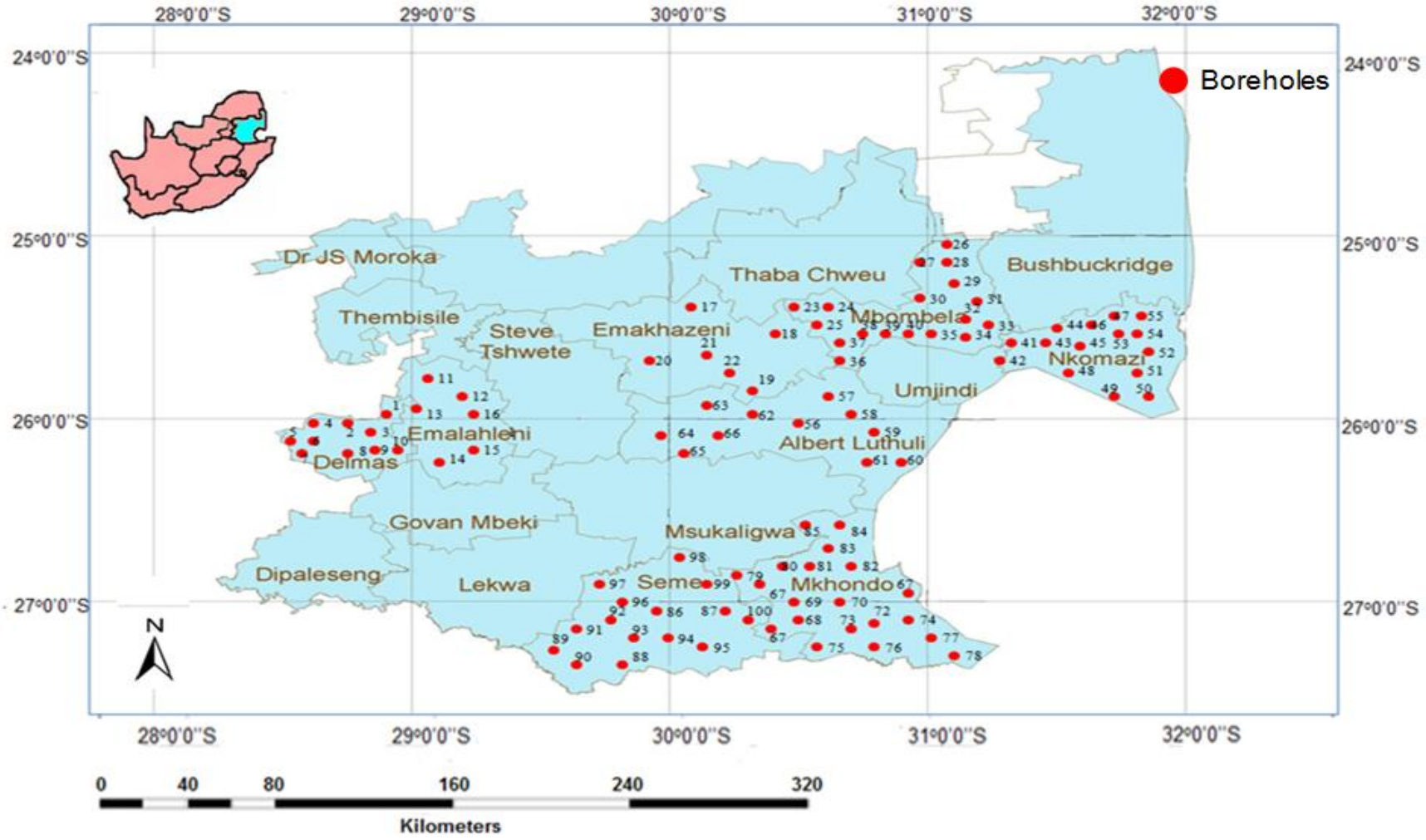


FIGURE 3.2: Map of Mpumalanga Province indicating location of borehole sites in the local municipal areas of Delmas, Emalahleni, Emakhazeni, Mbombela, Nkomazi, Albert Luthuli, Mkhondo and Pixle Ka Seme

3.3.3 Water quality variables

The water quality tools used to measure the environmental health risk in this study complied with SANS 241 (2006) and the *South African Water Quality Guidelines for Domestic Use* (DWAF, 1996). Molecular identification of the coliform isolates was also used as a proxy measure in order to confirm the possible threat that the microbial quality of groundwater poses to public health in the rural communities of the North West and Mpumalanga Provinces.

The pH and turbidity were measured on site using a pH meter (Metrohm Co. Model 713) and a microprocessor turbidity meter (Eutech Instrument Turbidimeter TN-100), while the temperature and the TDS (total dissolved solids) of the water samples were determined using a conductivity meter (Hach Co. Sension7). The concentrations of nitrates, fluoride, sulphate and chloride were determined in the laboratory using the Spectroquant Nova 400 manual water analyser (Merck) and the photometric test kits (Merck), while the concentrations of magnesium, sodium, calcium and potassium in water samples were determined by atomic absorption spectrophotometry (SpectrAA 220FS), according to the standard method (APHA, 1998).

The initial microbiological analysis of water samples performed on site was limited to total and faecal coliforms. The membrane filtration technique, the Chromocult coliform agar (Merck) and the M-FC agar (BioLab) were used for the enumeration of coliforms. Water samples were analysed for this group of indicator bacteria applying internationally accepted techniques and principles. The physicochemical and microbiological water quality parameters were then compared with the

standards set by SANS 241 (2006) and the *Water Quality Guidelines for Domestic Use* (DWAF, 1996).

3.3.4 Molecular identification of coliform isolates

For the identification of bacterial isolates, individual coliform colonies from water samples were randomly selected from different plates based on their size, shape and colour. They were transferred onto chromocult coliform agar (Merck) by the streak plate technique and incubated at 35 ± 2 °C for 24 h. The colonies were further purified at least three times employing the same methods and medium (Biolab) before Gram staining. Subsequently, oxidase tests were conducted on those colonies that were Gram- negative. Thereafter, the oxidase-negative colonies were transferred onto nutrient agar slants, incubated at 35 ± 2 °C for 24 h and kept at 4 °C until further use.

Extraction of the total genomic DNA – A total of 60 oxidase-negative isolates were used for the molecular study. Individual isolates were grown in nutrient broths, followed by an incubation period at 35 ± 2 ºC for 24 h. The inoculated broths (1 mL) were centrifuged at 13 300 g for 5 min. The pellets were then washed twice with sterile molecular graded water. The total genomic DNA from the bacterial pellets was subsequently extracted using the DNeasy DNA purification kit (QIAGEN) in accordance with the manufacturer's instructions. The quality and quantity of the isolated nucleic acids were determined using the NanoDrop^{TM} 2000 spectrophotometer (Thermo scientific) and agarose electrophoresis (BioRad).

Amplification of the 16S rRNA gene – Eubacterial universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') (Lane, 1991) and 1507R (5'-TACCTTGTTACGACTTCACCCCA-3') (Heyndrickx *et al*., 1996) were used in PCR reactions for the amplification of the 16S rRNA gene of each of the isolates. The PCR reaction mixtures contained 12.5 µL DreamTaq Master mix (2x) (Fermentas, 140 St. Leon-Rot, Germany), 0.5 µL of each primer (10 pmoL), 8.5 µl of nuclease-free water (Fermentas, 140 St. Leon-Rot, Germany), and 5 µL of DNA template. The PCR reaction mixtures were placed in an MJ MINI thermal cycler (BIORAD), and the following thermal cycling conditions were used: pre-denaturation for 10 min., followed by 35 amplification cycles of denaturation at 94 °C for 30 s, annealing of primers with template DNA at 55 °C for 30 s and primer extension at 72 °C for 30 s. This was followed by a final extension at 72 °C for 7 min. The PCR amplicons were resolved through electrophoresis of 1 % (w/v) agarose gel stained with ethidium bromide, followed by visualisation under ultraviolet light. The low-range Fast Ruler (Fermentas) was included in all the gels as a size marker. All results were captured using a gel documentation system (Syngene, Cambridge, U.K.).

Restriction analysis of PCR amplicons – In order to select representative isolates for sequencing, all PCR amplicons were subjected to restriction fragment length polymorphism (RFLP) analysis. For this purpose, 10 µL of the 16S rRNA amplicons was digested with *Taq1* and *Cs6pI* (Fermentas) according to the manufacturer's instructions. The restriction digests were resolved through electrophoresis of conventional 1.5 % (w/v) agarose gel stained with ethidium bromide, followed by visualisation under ultraviolet light. The hyperladder 1

(marker) 100 lines (Bioline) was included in all the gels as a size marker. All results were captured using a gel documentation system (Syngene, Cambridge, U.K.). The restriction patterns were determined manually and for every five similar profiles, the selected isolate was then ready for sequencing.

Sanger sequencing of the 16S rRNA gene – After grouping the isolates using the PCR-RFLP, the genomic DNA from 32 representative water samples was amplified using the existing 27 F and 1507 R primers as described above. The 1500 PCR amplicons were further studied by conventional Sanger (dideoxy) sequencing in both directions using 27 F and 1507 R primers. For this purpose, BigDye for ABI 3130XL was used according to the manufacturer's instructions and the gel was run on a 3130XL sequencer. All the sequences were inspected and manually corrected using Bioedit v.5.0.9 (33) software. For the preliminary identification of the bacterial isolates, the corrected sequences were then compared to those in the National Centre for Biotechnology Information (NCBI); using BLASTn.

Discrepant analysis (BLASTn) – Identification of the unknown isolates was assigned to the species level defined as a 16S rDNA sequence yielded a similarity score of ≥ 99 % with that of the prototype strain sequence in GenBank. However, when the score was < 99 % and ≥ 97 %, the unknown isolate was assigned to the corresponding genus.

3.4 RESULTS

3.4.1 Physical and chemical characteristics of groundwater samples

The physicochemical parameters of the groundwater samples from the North West and Mpumalanga Provinces collected during September and October, 2008 are presented in Table 3.3. The average pH value of the water samples from the selected boreholes in local municipalities of both North West and Mpumalanga Provinces were observed to be near neutral, ranging from 7.2 to 8.5, with the highest values of 9.1 observed in Moses Kotane (North West) and Mkhondo (Mpumalanga) municipal areas. In addition, the average temperatures of the groundwater samples were observed to range from 20.3 °C to 25.8 °C for North West Province and from 19.0 °C to 25.8 °C for Mpumalanga Province. The average levels of TDS in the water samples ranged between 270.84 mg/L to 568.75 mg/L for North West and from 107.15 mg/L to 439.00 mg/L for Mpumalanga. The highest level of 639.03 mg/L TDS was observed in the Greater Taung (North West) and 528.10 mg/L TDS in Emalahleni (Mpumalanga) municipal areas. Turbidity levels of all the groundwater samples ranged between 0.93 NTU and 7.63 NTU in North West and between 0.79 NTU and 6.64 NTU in Mpumalanga. The highest recorded levels of turbidity were 7.89 NTU in the Ramotshere Moiloa (North West) and 8.30 NTU in Emakhazeni (Mpumalanga) municipal areas.

The results of the chemical analysis, as exhibited in Table 3.4, revealed that the average chloride concentrations of all the groundwater samples ranged from 19.23 mg/L to 87.67 mg/L for North West and from 22.83 mg/L to 55.93 mg/L for

Mpumalanga, with the highest concentration of 98.89 mg/L observed for Moretele local municipality in North West and 98.50 mg/L in Emalahleni local municipality in Mpumalanga. The average sulphate concentrations ranged between 14.47 mg/L and 120.33 mg/L for both provinces. The highest sulphate concentrations were observed in Moretele local municipality (159.38 mg/L), North West and in Pixle Ka Seme local municipality (159.44 mg/L), Mpumalanga. With regard to nitrates and fluoride concentrations in the water samples, the average concentrations were observed to range from 0.44 mg/L to 11.05 mg/L nitrates and from 0.40 mg/L to 29.56 mg/L fluorides for North West and Mpumalanga Provinces, respectively. The highest nitrate concentration of 14.80 mg/L as N was found in the Moses Kotane (North West) and 16.11 mg/L as N in Emalahleni (Mpumalanga) local municipal areas. The highest fluoride concentration of 41.40 mg/L was found in the Moretele (North West) and 5.91 mg/L in Mbombela (Mpumalanga) local municipal areas.

In addition, sodium, potassium, magnesium and calcium concentrations were also measured and evaluated from borehole samples in different local municipal areas of the two provinces (Table 3.5). The average sodium concentrations ranged from 4.77 mg/L to 80.62 mg/L for North West and from 5.59 mg/L to 27.56 mg/L for Mpumalanga. The average potassium concentrations were between 2.68 mg/L and 17.28 mg/L for North West, and between 0.72 mg/L and 3.56 mg/L for Mpumalanga. The Madibeng municipal area in North West had the highest recorded sodium concentration (98.87 mg/L), while the highest in Mpumalanga was recorded in the Nkomazi municipal area (36.95 mg/L). The average values recorded for magnesium concentrations in the water samples ranged from 15.35 mg/L to 51.44 mg/L for North West and from 15.99 mg/L to 76.86 mg/L for

Mpumalanga, while the average calcium concentrations ranged from 14.77 mg/L to 72.57 mg/L for North West and from 12.52 mg/L to 45.26 mg/L for Mpumalanga. The highest magnesium concentration was observed in the Greater Taung municipal area (89.64 mg/L) in North West and Emalahleni municipal area (155.24 mg/L) in Mpumalanga. The highest calcium concentration was observed in the Kagisano municipal area (145.80 mg/L) in North West and Emalahleni municipal area (58.96 mg/L) in Mpumalanga.

3.4.2 Microbiological characteristics of groundwater samples

The analyses of the coliform isolates detected from various borehole samples in the different local municipal areas of North West and Mpumalanga Provinces are exhibited in Table 3.6.

The bacterial counts of all the water samples ranged between 0 cfu/100 mL and 460 cfu/100 mL with regard to total coliforms and between 0 cfu/100 mL and 150 cfu/100 mL with regard to faecal coliforms for the two provinces. The highest total (460 cfu/100 mL) and faecal (58 cfu/100 mL) coliform counts were recorded in the Moses Kotane municipal area of the North West Province; while, in the Mpumalanga Province the highest total (426 cfu/100 mL) and faecal (150 cfu/100 mL) coliform counts were recorded in the Delmas municipal area.

TABLE 3.3: Physicochemical quality of borehole samples analysed in eight local municipal areas of North West and Mpumalanga Provinces during the study period (n=3 per borehole)

Local Municipalities	pH value			Temperature (^{o}C)			Turbidity (NTU)			TDS (mg/L)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max
NORTH WEST PROVINCE												
Ramotshere Moiloa	7.2	6.5	7.7	24.3 (± 0.89)	22.2	25.5	7.63 (± 3.78)	0.33	7.89	270.84 (± 35.35)	85.51	502.00
Tswaing	7.4	6.9	7.8	24.2 (± 0.40)	23.3	24.7	0.93 (± 0.46)	0.20	1.60	461.60 (± 71.09)	354.20	575.07
Mafikeng	7.5	6.9	8.2	24.1 (± 1.06)	21.1	25.8	1.94 (± 0.66)	0.33	4.40	486.21 (± 84.41)	289.29	630.61
Kagisano	7.4	7.1	7.9	24.4 (± 0.29)	23.9	24.8	3.51 (± 1.73)	0.19	4.80	403.90 (± 44.15)	267.20	635.10
Greater Taung	7.5	7.2	7.9	21.2 (± 0.86)	20.3	21.7	1.37 (± 0.13)	0.30	2.68	568.75 (± 89.49)	376.00	639.03
Moretele	7.7	7.3	8.5	22.5 (± 0.47)	22.0	23.1	2.54 (± 1.22)	1.15	5.17	567.76 (± 79.57)	123.74	578.12
Madibeng	8.1	7.7	8.5	24.1 (± 0.60)	23.3	25.1	2.61 (± 0.33)	0.37	3.03	508.79 (± 74.00)	186.01	530.00
Moses Kotane	8.5	7.7	9.1	23.5 (± 0.60)	22.6	24.7	0.73 (± 0.72)	0.21	3.21	325.24 (± 64.06)	51.53	478.08
MPUMALANGA PROVINCE												
Delmas	7.3	6.6	8.0	19.8 (± 0.44)	19.0	20.3	1.22 (± 1.42)	0.10	4.48	224.53 (± 73.26)	127.70	324.00
Emalahleni	7.4	6.5	7.9	21.6 (± 2.79)	20.9	23.8	3.86 (± 3.58)	0.10	5.50	439.00 (± 73.20)	351.30	528.10
Emakhazeni	7.3	6.6	7.9	20.1 (± 2.12)	21.0	24.5	6.64 (± 4.00)	0.10	8.30	158.02 (± 52.26)	85.12	230.11
Mbombela	7.6	7.1	8.1	23.8 (± 2.00)	21.1	25.8	5.10 (± 0.48)	0.10	7.80	116.98 (± 75.08)	23.00	372.00
Nkomazi	7.9	7.5	8.3	23.2 (± 1.25)	20.3	25.0	0.79 (± 0.31)	0.29	1.36	393.61 (± 95.34)	147.70	510.20
Albert Luthuli	7.5	7.3	7.7	25.4 (± 2.65)	2210	24.6	1.95 (± 0.24)	0.23	2.93	171.17 (± 79.49)	80.30	319.63
Mkhondo	7.5	7.0	9.1	24.0 (± 1.17)	22.0	25.0	2.21 (± 0.83)	0.25	4.66	107.15 (± 56.46)	21.00	230.00
Pixle Ka Seme	7.6	7.1	8.0	24.9 (± 0.95)	23.0	25.3	1.73 (± 0.13)	0.19	2.21	284.44 (± 96.67)	51.20	321.08
SANS 241 (2006)	**5-9.5**			**≤ 25**			**0-1**			**0-500**		

TABLE 3.4: Chemical quality of borehole samples analysed in eight local municipal areas of North West and Mpumalanga Provinces during the study period (n=3 per borehole)

Local Municipalities	Chloride (mg/L)			Sulphate (mg/L)			Nitrate (mg/L)			Fluoride (mg/L)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max
NORTH WEST PROVINCE												
Ramotshere Moiloa	19.23 (± 2.73)	11.00	50.98	14.47 (± 1.98)	11.53	18.04	5.00 (± 1.68)	2.88	8.00	2.80 (± 1.68)	0.64	6.00
Tswaing	59.60 (± 11.02)	16.20	66.95	31.31 (± 7.21)	12.31	77.54	7.25 (± 2.56)	3.61	9.99	3.07 (± 0.15)	0.98	8.25
Mafikeng	55.02 (± 5.62)	15.98	27.78	31.11 (± 8.50)	11.68	128.30	4.62 (± 2.89)	0.44	9.16	2.40 (± 0.33)	0.10	5.52
Kagisano	89.10 (± 11.09)	44.99	73.94	15.65 (± 1.58)	12.36	25.81	1.71 (± 0.97)	0.48	2.98	1.02 (± 0.73)	0.12	2.10
Greater Taung	82.30 (± 9.78)	37.99	51.95	120.33 (± 9.72)	21.20	152.58	6.52 (± 0.51)	3.28	8.59	5.79 (± 0.94)	1.00	9.66
Moretele	87.76 (± 12.57)	19.43	98.89	98.83 (± 15.76)	14.59	159.38	3.16 (± 0.88)	0.34	7.91	29.56 (± 3.26)	9.89	41.40
Madibeng	71.67 (± 12.73)	30.00	90.11	91.24 (± 14.08)	25.47	110.33	0.78 (± 0.18)	0.10	1.28	12.60 (± 1.28)	5.70	16.00
Moses Kotane	28.25 (± 3.12)	11.65	35.99	17.23 (± 1.60)	10.42	89.97	11.05 (± 0.56)	4.73	14.8	28.35 (± 1.43)	17.8	33.48
MPUMALANGA PROVINCE												
Delmas	53.68 (± 10.72)	20.49	70.46	47.58 (± 6.61)	28.76	55.74	0.88 (± 0.44)	0.04	3.87	1.11 (± 0.19)	0.40	2.20
Emalahleni	55.93 (± 13.34)	11.49	98.50	45.71 (± 7.00)	11.34	68.00	7.27 (± 3.67)	0.20	16.11	0.85 (± 0.13)	0.90	1.81
Emakhazeni	55.02 (± 15.22)	12.58	85.12	40.12 (± 6.35)	11.68	70.30	3.50 (± 0.89)	0.10	1.54	0.40 (± 0.07)	0.20	1.32
Mbombela	33.93 (± 8.38)	22.49	49.98	25.29 (± 5.77)	10.51	45.46	1.38 (± 0.16)	0.10	2.58	1.55 (± 0.31)	0.40	5.91
Nkomazi	55.15 (± 13.52)	32.49	82.47	52.06 (± 7.59)	11.92	70.51	1.76 (± 0.35)	0.04	5.00	1.40 (± 0.32)	0.30	3.70
Albert Luthuli	26.71 (± 3.61)	8.99	42.48	31.79 (± 5.85)	11.05	67.34	1.09 (± 0.20)	0.20	2.00	0.74 (± 0.10)	0.56	1.17
Mkhondo	22.83 (± 4.36)	9.98	84.97	21.05 (± 3.81)	11.10	34.09	0.44 (± 0.12)	0.01	1.51	0.55 (± 1.28)	0.13	1.08
Pixle Ka Seme	34.41 (± 6.15)	5.93	61.23	84.30 (± 9.60)	12.07	159.44	0.46 (± 0.27)	0.00	4.20	0.45 (± 0.26)	0.06	0.83
SANS 241 (2006)	**0-100**			**0-200**			**0-6**			**0-1**		

TABLE 3.5: Chemical quality of borehole samples analysed in eight local municipal areas of North West and Mpumalanga Provinces during the study period (n=3 per borehole)

Local Municipalities	Sodium (mg/L)			Potassium (mg/L)			Magnesium (mg/L)			Calcium (mg/L)		
	Average	Min	Max	Average	Min	Max	Average	Min	Max	Average	Min	Max
NORTH WEST PROVINCE												
Ramotshere Moiloa	4.77 (± 1.27)	1.38	16.16	10.61 (± 2.60)	3.86	32.73	24.55 (± 4.38)	5.31	62.28	14.77 (± 2.54)	5.11	38.16
Tswaing	18.48 (± 4.05)	6.05	43.38	17.28 (± 3.38)	2.74	38.49	22.22 (± 3.49)	3.98	39.17	41.12 (± 5.87)	12.82	73.94
Mafikeng	17.04 (± 1.41)	4.25	29.18	9.74 (± 1.59)	0.40	25.56	32.53 (± 2.48)	17.33	65.29	26.02 (± 3.06)	5.30	57.69
Kagisano	13.96 (± 2.12)	7.69	26.23	8.97 (± 1.81)	0.11	13.53	21.17 (± 4.34)	0.22	40.06	72.57 (± 9.11)	41.04	145.80
Greater Taung	17.30 (± 4.39)	0.61	55.83	10.59 (± 2.83)	1.38	37.42	51.44 (± 5.76)	21.23	89.64	34.60 (± 5.63)	17.10	82.12
Moretele	80.62 (± 6.04)	25.97	90.92	9.21 (± 2.43)	2.64	22.05	20 57 (± 6.46)	1.63	45.78	61.62 (± 8.02)	20.06	136.22
Madibeng	91.01 (± 5.97)	33.14	98.87	5.07 (± 0.78)	2.89	9.06	15.35 (± 3.62)	1.86	26.60	52.22 (± 9.91)	15.23	104.25
Moses Kotane	67.18 (± 3.14)	23.29	80.12	2.68 (± 0.91)	0.09	14.04	48.44 (± 5.11)	20.87	82.78	30.46 (± 5.00)	9.61	68.93
MPUMALANGA PROVINCE												
Delmas	23.48 (± 1.92)	14.72	32.76	1.12 (± 0.20)	0.26	2.44	17.30 (± 1.90)	10.45	26.85	45.26 (± 3.77)	23.42	65.12
Emalahleni	22.46 (± 1.33)	1.14	28.55	2.41 (± 0.50)	0.04	5.42	76.86 (± 14.3)	3.33	155.24	32.30 (± 6.03)	11.50	58.96
Emakhazeni	15.23 (± 0.89)	1.00	20.42	3.56 (± 0.66)	0.02	7.89	20.23 (± 5.89)	0.15	35.99	25.00 (± 4.89)	6.12	33.12
Mbombela	7.92 (± 3.27)	0.30	11.56	1.39 (± 0.15)	0.35	2.99	18.96 (± 2.61)	0.76	32.37	21.87 (± 3.39)	0.20	48.52
Nkomazi	27.56 (± 4.29)	1.79	36.95	0.72 (± 0.13)	0.34	2.13	45.08 (± 7.94)	7.26	75.92	36.61 (± 4.54)	10.61	51.89
Albert Luthuli	10.50 (± 2.35)	0.73	26.83	2.07 (± 0.26)	0.85	3.55	15.99 (± 2.71)	3.01	33.35	17.69 (± 3.54)	3.02	40.85
Mkhondo	5.59 (± 1.13)	0.19	15.82	1.29 (± 0.14)	0.26	2.45	19.23 (± 3.72)	1.88	35.24	12.52 (± 1.98)	0.20	25.62
Pixle Ka Seme	20.59 (± 6.02)	3.19	36.28	3.23 (± 0.80)	0.24	8.23	34.40 (± 9.16)	6.18	49.70	38.66 (± 6.88)	9.26	50.16
SANS 241 (2006)	**0-100**			**0-50**			**0-30**			**0-32**		

TABLE 3.6: Microbial quality of borehole samples analysed in eight local municipal areas of North West and Mpumalanga Provinces during the study period (n=3 per borehole)

Local Municipalities	Total coliforms (cfu/100 mL)			Faecal coliforms (cfu/100 mL		
	Average	Min	Max	Average	Min	Max
NORTH WEST PROVINCE						
Ramotshere Moiloa	73 (± 11)	1	240	4 (± 2)	0	25
Tswaing	116 (± 6)	1	365	12 (± 5)	0	45
Mafikeng	105 (± 10)	1	440	7 (± 1)	0	42
Kagisano	95 (± 9)	3	185	5 (± 2)	0	12
Greater Taung	126 (± 10)	24	328	7 (± 3)	0	34
Moretele	76 (± 8)	0	320	3 (± 1)	0	12
Madibeng	81 (± 8)	1	400	8 (± 1)	0	50
Moses Kotane	118 (± 6)	4	460	11 (± 4)	0	58
MPUMALANGA PROVINCE						
Delmas	150 (± 21)	4	426	32 (± 7)	0	150
Emalahleni	137 (± 9)	6	360	27 (± 5)	0	29
Emakhazeni	47 (± 5)	0	79	15 (± 3)	0	39
Mbombela	107 (± 3)	0	128	30 (± 2)	0	15
Nkomazi	115 (± 7)	4	225	26 (± 2)	0	45
Albert Luthuli	99 (± 6)	0	335	30 (± 2)	0	16
Mkhondo	27 (± 5)	0	425	12 (± 2)	0	32
Pixle Ka Seme	60 (± 9)	1	210	17 (± 2)	0	25
SANS 241 (2006)		**0-5**			**0**	

Of the 200 borehole samples tested, a total of 72 isolates were oxidase-negative for North West Province and 68 isolates for Mpumalanga Province. Consequently, 64 isolates from North West and 60 isolates from Mpumalanga were processed for genomic DNA extraction. The concentrations of the quantified DNA ranged from 2.0 ng/µL to 80.0 ng/µL for North West and from 2.0 ng/µL to 120.0 ng/µL for Mpumalanga Province. To identify the possible pathogenic bacteria in the groundwater samples, their DNA was amplified using universal eubacterial primers27F and 1507R.

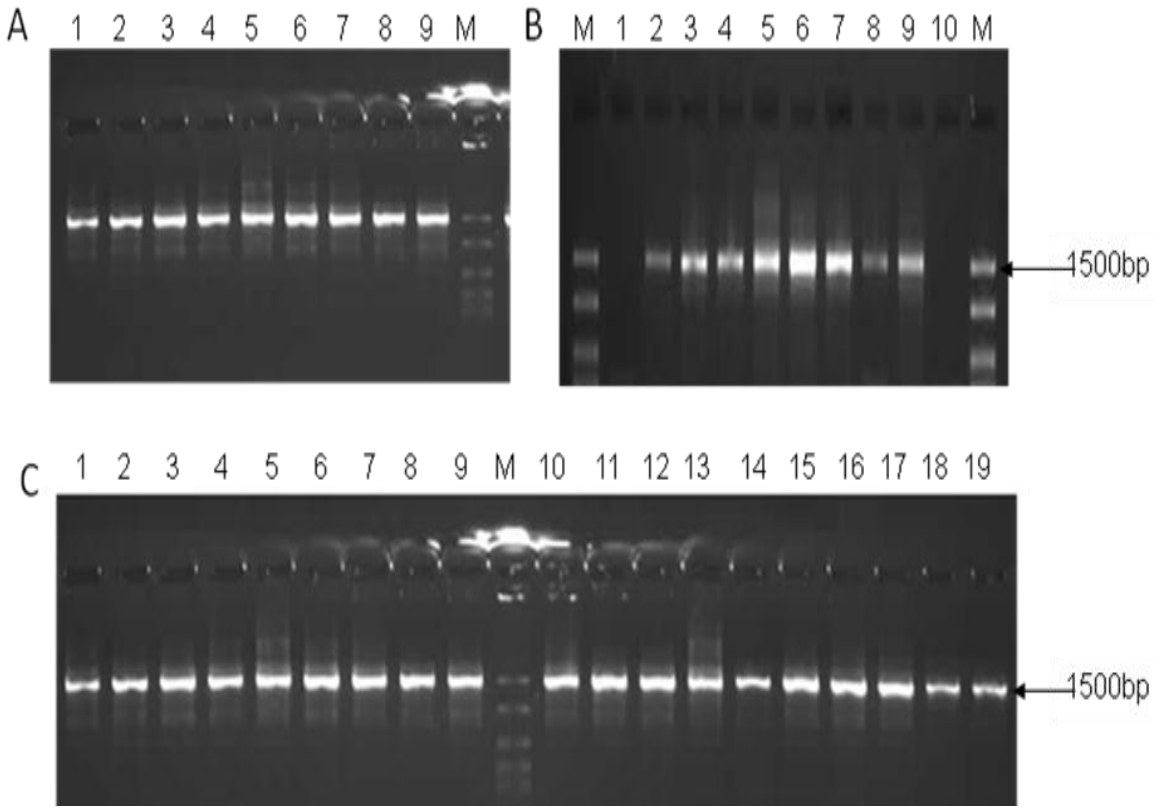


FIGURE 3.3: An example of an agarose gel electrophoresis for the amplified PCR product isolates from North West and Mpumalanga Provinces. Lane M represents molecular weight marker (1500 bp DNA size ladder). (A) Lanes 1 to 9 represents isolates from Pixle Ka Seme Municipality in Mpumalanga, (B) Lanes 1 and 10 are negative controls, Lane 6: positive control (*E.coli* ATCC 25922 (10 µℓ)), Lanes 2 to 5 and 7 to 9 are isolates from Albert Luthuli Municipality in Mpumalanga, (C) Lane 1 to 9 represents isolates from Ramotshere Moiloa Municipality and 10 to 19 Mafikeng Municipality in North West Province

Figure 3.3 indicates an example of the PCR amplicons of the 16S rRNA fragment for water microorganisms in North West and Mpumalanga Provinces. All the samples displayed a single band of 1 500 bp in agarose gel, indicating the successful amplification of the targeted gene of 16S rRNA from the isolates. The

gel represents an example of the amplified PCR product of the 36 isolates for both provinces, where Lanes 1 and 10 in gel B represent a positive control and Lane M represents a molecular weight marker (1 500 bp ladder). These products were further subjected to restriction fragment analysis by using *Cs6pI* enzymes that distinguished two different species. With the use of *Taq1* enzymes, 9 different species were identified in North West Province and 10 in Mpumalanga Province. These PCR-RFLP types were collapsed into groups based on similarity (Figure 3.4).

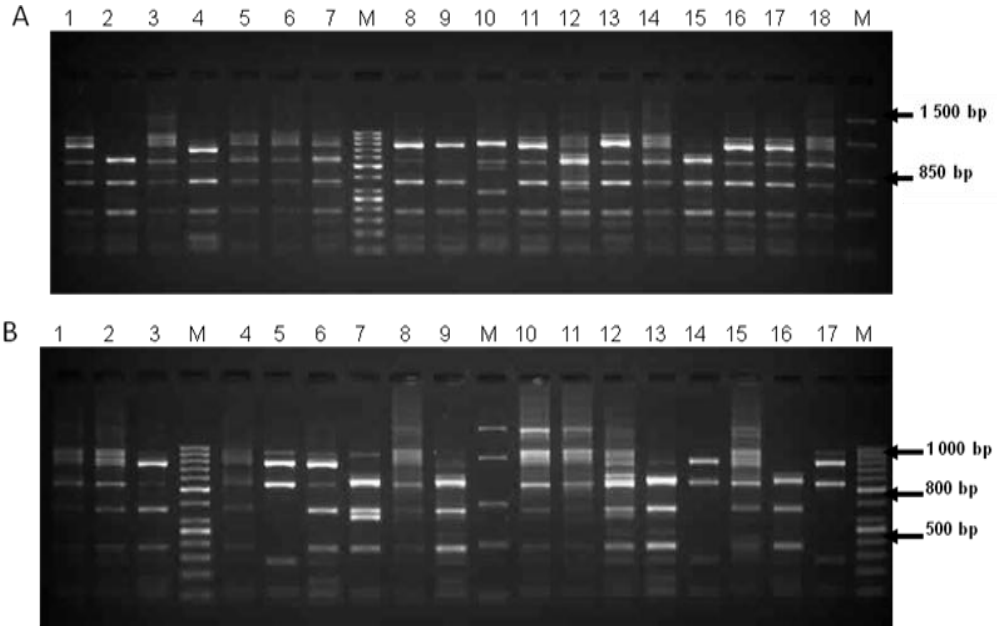


FIGURE 3.4: Example of the agarose gel electrophoresis for the restriction fragment profiles of the group-specific PCR products of North West and Mpumalanga Provinces after digestion with (A) *Cs6pI* enzyme and (B) *Taq1* enzyme. Lane M represents molecular weight marker (1500 bp and 1000 bp DNA size ladder)

The 28 (North West) and 32 (Mpumalanga) representative PCR amplicons were subjected to DNA sequencing with the original primers 27F and 1507R in both directions. These complete sequences were then probed using the NCBI BLASTn program. The sequences retrieved from the NCBI database furnishing the closest match in pair-wise BLASTn were identified as *Serratia marcescens, Aeromonas veronii, Citrobacter freundii, Salmonella enterica, Morganella morganii, Bacillus cereus, E.coli, Shigella dysenteriae and Enterobacter cloacae* in North West Province as indicated in figure 3.5. In the Mpumalanga Province, the following organisms were identified: *Serratia marcescens, Citrobacter freundii, Salmonella enterica, Bacillus cereus, Escherichia coli* O157:H7*, Shigella flexineri, Pseudomonas maltophilia, Enterobacter cloacae, Klebsiella oxytoca* and *Cronobacter sakazakii* as indicated in figure 3.6.

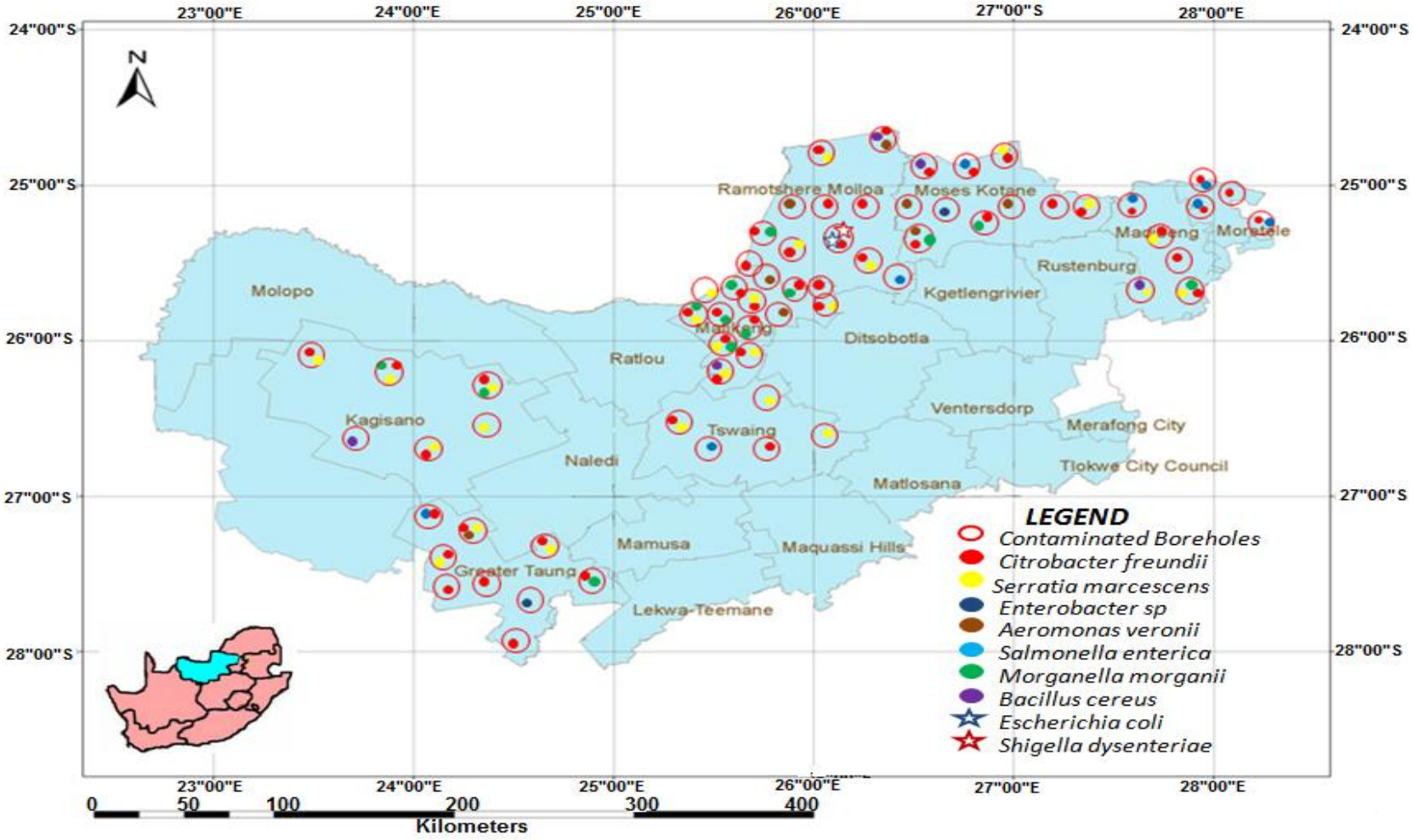


FIGURE 3.5: Map of North West Province indicating contaminated boreholes with microorganisms identified in the local municipal areas of Moretele, Madibeng, Moses Kotane, Ramotshere Moiloa, Mafikeng, Kagisano, Tswaing and Greater Taung

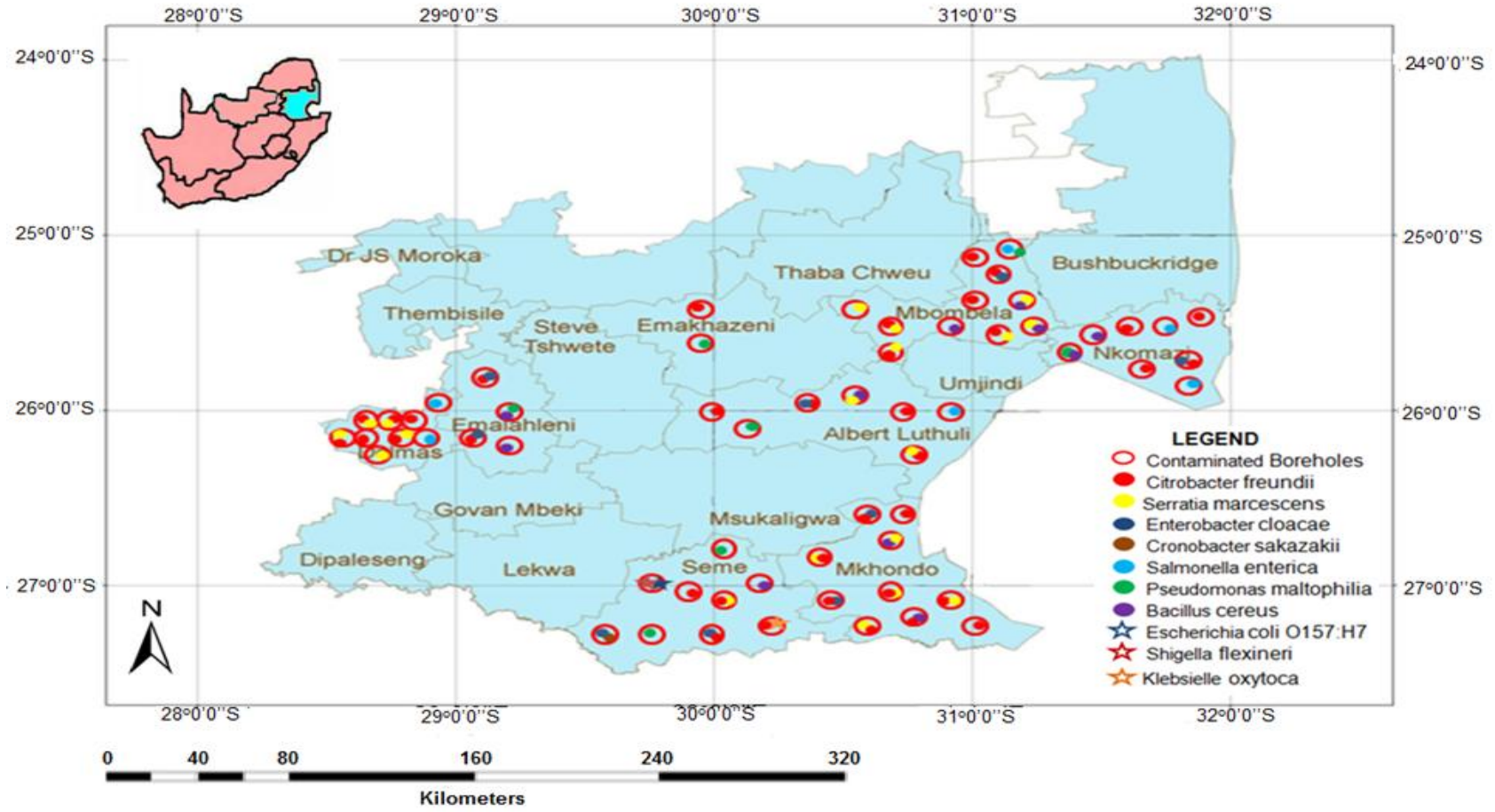


FIGURE 3.6: Map of Mpumalanga Province indicating contaminated boreholes with microorganisms identified in the local municipalities of Delmas, eMalahleni and Emakhazeni, Mbombela, Nkomazi, Albert Luthuli, Mkhondo and Pixle Ka Seme

3.5 DISCUSSION

An adequate safe potable water supply in rural communities is essential in order to satisfy basic needs. Consequently, efficient monitoring and management of groundwater is vital for reducing the number of diarrhoeal infections that might pose a public health risk to consumers. This study demonstrated (Table 3.3) that the pH and temperature values of all the water samples from the different local municipal areas were within the recommended limit of the no-risk parameter for drinking and domestic purposes of 5 to 9.5 and ≤ 25 °C (DWAF, 1996; SANS 241, 2006). The TDS levels of the water samples revealed that 31 % (10 % in Mafikeng and Greater Taung, 6 % in Moretele and 5 % in Madibeng) of the boreholes in North West and 3 % in Mpumalanga (1 % in Emalahleni and 2 % in Nkomazi) were above the recommended limit of 500 mg/L TDS for no-risk (DWAF, 1996; SANS 241, 2006). However, these lower concentrations usually pose no health threats to humans unless the values exceed 3 000 mg/L which may lead to long-term health effects (DWAF, 1996). A study conducted by NPWG (2000) also recorded high values of TDS of 580, 850 and 1 360 mg/L for Mafikeng and Greater Taung, Madibeng and Moretele, respectively. These high TDS concentrations may be due to high amounts of inorganic substances like Ca^{2+} and Mg^{2+} salts occurring in the dolomitic waters of the two provinces.

In addition, the turbidity results also indicated that 53 % of the borehole samples in North West and 52 % in Mpumalanga were within the recommended limits (0 NTU to 1 NTU) for potable water (DWAF, 1996; SANS 241, 2006). However, it was observed that turbid groundwater was found in all local municipal areas of North

West (13 % in Mafikeng, 10 % in Ramotshere Moiloa, 8 % in Moretele, 5 % in Madibeng, 4 % in Greater Taung and 3 % in Tswaing and Kagisano), except in one borehole in Moses Kotane. In Mpumalanga, the groundwater samples collected in eight areas (10 % in Nkomazi, 8 % in Mkhondo and Pixle Ka Seme, 6 % in Albert Luthuli, 5 % in Delmas and Mbombela, 4 % in Emalahleni and 2 % in Emakhazeni) cannot be considered safe for drinking purposes in terms of turbidity. A borehole in Braaklagte 3 in Ramotshere Moiloa local municipality (North West) and Nhlupheko P.S borehole in Emakhazeni local municipality (Mpumalanga) were observed to have a high turbidity level of 7.63 NTU and 8.30 NTU, which might have been due to silt, as the water sample appeared to be brown in colour. Turbidity is caused by the presence of suspended clay, silt, organic matter, inorganic matter, plankton and other microscopic organisms (DWAF, 1996). The measurement of turbidity provides only an indication of the extent of the pollution (Momba *et al*., 2006). High turbidity levels are associated with poor water quality and promote the survival of microorganisms (DWAF, 1996).

Although variations were found in the chemical characteristics of the groundwater samples of North West and Mpumalanga Provinces**,** the concentrations of chloride, sulphate, sodium and potassium fell within the limits (0-100 mg/L Cl^-, 0-200 mg/L SO_4^{2-}, 0-100 mg/L Na^+ and 0-50 mg/L K^+) set by the national guidelines for domestic purposes (DWAF, 1996; SANS 241, 2006) for all water samples, while fluoride, nitrate, magnesium and calcium concentrations in some boreholes exceeded the recommended limits (Tables 3.4 and 3.5).

With regard to nitrates, the results revealed that 40 % of the borehole samples in North West and 2 % in Mpumalanga were above the recommended limits (0 mg/L to 6 mg/L as N) for potable water (DWAF, 1996; SANS 241, 2006). These water samples were collected from Moses Kotane (14 %), Mafikeng (8 %), Greater Taung (8 %), Tswaing (5 %), Ramotshere Moiloa (4 %) and Moretele (1 %) in North West and from Emalahleni (Leeufontein and Emakhosini) in Mpumalanga. Most of the boreholes are privately owned and situated next to on-site unsanitary systems. Consequently, the presence of high nitrate-nitrogen concentrations in groundwater might occur due to agricultural activity or leaching effluent from on-site sanitation (WHO, 2007). However, NPWG (2000) reports that nitrates occur naturally in the Moretele district as inorganic nitrates due to geological formations such as basalt rocks. High nitrate levels in groundwater sources have been noted in urban and rural areas of other provinces of South Africa as well, namely the Northern Cape, and the North West and Limpopo Provinces (Tredoux *et al*., 2009). A nitrate concentration above the recommended level of 6.0 mg/L has been reported to be dangerous to pregnant women and poses a serious health threat to infants younger than three to six months of age, because of its ability to cause methamoglobin or “blue baby syndrome” in which blood loses its ability to carry sufficient oxygen; it is also reported to be carcinogenic (DWAF, 1996; WHO, 2007; Tredoux *et al*., 2009). Furthermore, high nitrate levels can be used as a crude indicator of faecal pollution where microbiological data are unavailable due to the fact that nitrate is used by microorganisms as a food resource (Cave & Kolsky, 1999). The fluoride concentration results indicated that 83 % of borehole samples collected from North West (20 % in Mafikeng, 15 % in Moses Kotane, 11 % in Ramotshere Moiloa and Greater Taung, 8 % in Moretele, 7 % in Tswaing and

Madibeng and 4 % in Kagisano) and 33 % from Mpumalanga (11 % in Mbombela, 6 % in Nkomazi, 5 % in Delmas, 3 % in Albert Luthuli, EMalahleni and Emakhazeni and 2 % Mkhondo) were above the recommended limit of 0 mg/L to 1 mg/L (DWAF, 1996; SANS 241, 2006). Health problems associated with the condition known as fluorosis may occur when fluoride concentrations in groundwater exceed 1.5 mg/L and staining of tooth enamel may become apparent (dental fluorosis) (Ncube & Schutte, 2005). With continued exposure, teeth may become extremely brittle (DWAF, 1996). The incidence and severity of dental fluorosis, and the much more serious skeletal fluorosis, depend on a range of factors including the quantity of water consumed and exposure to fluoride from other sources such as high-fluoride coal, as was noted in China (WHO, 2006b). Fluoride has also been implicated in higher rates of bone cancer (Osteosarcoma) (McCaffrey & Willis, 2001).

Forty-five percent (45 %) of borehole samples in North West and 15 % in Mpumalanga were above the recommended limits of 0 mg/L to 30 mg/L (DWAF, 1996) with regard to magnesium. It is important to note that the 45 % of boreholes in North West with high concentrations of magnesium included 13 boreholes located in Moses Kotane, 12 in Greater Taung, 10 in Mafikeng, 4 in Ramotshere Moiloa, 3 in Moretele, 2 in Kagisano and 1 in Tswaing. The 15 % in Mpumalanga included 6 borehole samples located in Nkomazi, 3 located in Pixle Ka Seme, 2 located in Delmas and eMalahleni, and 1 in Emakhazeni and Mbombela. Furthermore, with regard to calcium 43 % of borehole samples in North West and 32 % in Mpumalanga were above the recommended limits of 0 mg/L to 32 mg/L (DWAF, 1996). Of the 43 % in North West, 10 of the boreholes were located in

Mafikeng, 8 in Kagisano, 6 in Moretele, 5 in Madibeng, 5 in Moses Kotane, 4 in Tswaing, 4 in Greater Taung and 1 in Ramotshere Moiloa, while in Mpumalanga, 9 boreholes were located in Nkomazi, 8 in Delmas, 6 in Pixle Ka Seme, 4 in Mbombela, 3 in EMalahleni and 2 in Emakhazeni. The presence of high calcium and magnesium in water contributes to water hardness. Groundwater in the dolomitic areas like North West and Mpumalanga in the northern parts of the country tends to be very hard. This usually has no health implications, except where concentrations of magnesium are extremely high. Magnesium has a bitter taste and may have a laxative effect on people not accustomed to the water (WHO, 2007). Magnesium, together with calcium, is responsible for scaling problems in appliances using heating elements and plumbing (DWAF, 1996; WRC, 1998). On the other hand, high concentrations of calcium impair the lathering of soap (DWAF, 1996; WRC, 1998; WHO, 2007).

Table 3.6 depicts the coliform counts of the water samples analysed in different local municipalities of the North West and Mpumalanga Provinces. In general, both the average total and faecal coliform counts in all the local municipalities were above the South African recommended limits for drinking water, that is, water intended for domestic purposes. According to the South African guidelines, the total number of coliforms in drinking water should range between 0 cfu/100 ml to 5 cfu/100 ml, while the number of faecal coliforms should be 0 per 100 ml water sample and less than one *E.coli* per 100 ml (DWAF, 1996; SANS 241, 2006; 2011).

The results indicated that, of the 200 boreholes, 86 % in North West and 78 % in Mpumalanga had more than 5 cfu/100 ml total coliform counts. Total coliforms comprise a heterogeneous group that includes bacteria from the genera *Escherichia, Citrobacter, Enterobacter, Klebsiella, Serratia* and *Rahnella*. Although most of these bacteria belong to the family of *Enterobacteriaceae*, there is an indication of the possible presence of bacterial pathogens such as *Salmonella* spp. and *Shigella* spp., especially when detected in conjunction with other faecal coliforms (DWAF, 1996; WHO, 2006). Regarding this group of coliforms, the results of this study indicated that 77 % of the boreholes in North West and 81 % in Mpumalanga had less than one faecal coliform per 100 ml of drinking water, while 23 % and 19 % of the boreholes were faecally contaminated and were not within the recommended limits (Table 3.6). High concentrations of faecal coliforms in water indicate a risk for waterborne diseases, even if small amounts of water are consumed (DWAF, 1996). Faecal pollution of groundwater sources in certain areas of the Mpumalanga Province, especially in Delmas, is a matter of concern, as this area has a history of waterborne disease outbreaks which resulted in 3 000 cases of diarrhoea, 561 cases of typhoid infection and five deaths, according to the official figures, while the community claimed that 49 deaths were caused by typhoid and diarrhoea (Groenewald & Dibetle, 2005; Masinga, 2005).

The culture-based techniques applied in this study revealed only the general hygienic quality and faecal pollution of groundwater sources based on the detection of both total coliforms and faecal coliforms. Moreover, the presence of coliform bacteria in water samples might only indicate a possible presence of bacterial pathogens such as *Salmonella* spp., *Shigella* spp., *V. cholerae,*

Campilobacter jejuni, Campilobacter coli, Yersinia enterocolitica and pathogenic *E.coli* (DWAF, 1996). Consequently, the range of bacterial pathogens that might result in diseases and sicknesses in the province should be accurately proved. For this purpose, selected coliform isolates were subjected to a subsequent molecular analysis, since molecular tools for microbial diagnosis rely on the *in vitro* amplification of a DNA fragment and offer a higher level of the specificity of strain detection (Rompré *et al*., 2002; Beneduce *et al*., 2007). The results of the molecular study revealed that of 100 boreholes in North West 51 % tested positively for *C. freundii,* 28 % for *S. marcescens,* 12 % for *M. morganii,* 8 % for *S. enterica,* 7 % for *A. veronii,* 5 % for *B. cereus*, 2 % for *E. cloacae, and* 1 % for *E.coli* and for *S. dysenteriae* (Figure 3.5). However, for the 100 boreholes in Mpumalanga, the molecular study indicated findings of 35 % for *Citrobacter freundii*, 19 % for *Serratia marcescens*, 11 % for *Bacillus cereus*, 9 % for *Enterobacter cloacae*, 7 % for *Salmonella enterica* and *Pseudomonas maltophili,* and 1 % in *Escherichia coli* O157:H7*, Shigella flexineri*, *Klebsiella oxytoca* and *Cronobacter sakazakii* (Figure 3.6).

The presence of the above opportunistic pathogens in the groundwater samples indicates that communities in the North West and Mpumalanga Provinces, especially immuno-compromised individuals such as infants and the elderly, are at a potential risk of contracting infections and waterborne diseases such as bacillary dysentery, respiratory infections, urinary tract infections and gastroenteritis during exposure to or consumption of groundwater from these sources (Payment *et al*., 1991; Bartram *et al.,* 2003). It has been reported that enteric pathogens such as *E.coli, S. dysenteriae, S. enteric* and *B. cereus* are major causes of diarrhoea and

bacillary dysentery everywhere in the world (Gray, 1995; EFSA, 2007; WHO, 2006b). In South Africa, diarrhoeal diseases have been reported to be responsible for approximately 20 % of all deaths of one to five year-old children (MacKintosh & Colvin, 2003). A waterborne outbreak of *E.coli* O157:H7 occurred in Scotland with 496 cases (272 laboratory-confirmed cases) and 19 deaths (Dev *et al*., 1991). Abongo and Momba (2008) they also discovered that water sources with *E.coli* O157:H7 are potentially capable of causing diarrhoea in humans especially HIV／AIDS patients.

In addition, *Morganella morganii* causes a disease known as “Summer Diarrhoea”, which is also often encountered in postoperative patients and is mainly associated with urinary tract infections (Cox, 1985; Senior & Voros, 1990). *Citrobacter freundii, Serratia marcescens, Cronobacter sakazakii, Aeromonas veronii and Enterobacter cloacae* are known to cause a wide variety of nosocomial infections of the respiratory tract and urinary tract (Hejazi, 1997; Keller *et al*.**,** 1998; Coignard *et al*., 2006; Whalen *et al*., 2007). *Aeromonas veronii* can cause infections in humans, including septicaemia, particularly in immuno-compromised patients, and in patients with wound infections and respiratory tract infections. There have been some claims that *A. veronii* can cause gastrointestinal illness, but epidemiological evidence thereof is not consistent (Song *et al*., 2004). *Pseudomonas maltophilia* is a species of *Stenotrophomonas*, formerly named *Xanthomonas maltophilia*, which reduces nitrate. It is a cause of hospital-acquired ocular and lung infections, especially in patients with cystic fibrosis and those who are immunosuppressed (Cunha, 2005). *Klebsiella oxytoca* can cause systemic infections such as meningitis, adrenal haemorrhage, haemorrhagic colitis and septic shock (Tang *et*

al., 1995; Kashiwagi *et al*., 2007). Outbreaks of *K. oxytoca* infections have been reported in newborn babies following colonisation of their digestive tracts; in oncology patients following contamination of intravenous fluids; and in cardiac patients following contamination of invasive blood pressure monitoring devices (Ransjo *et al*., 1992; Berthelot *et al*., 2001; Watson *et al*., 2005). The findings of this study, therefore, predict a possible threat to public health because of the quality of the groundwater supply in some rural communities of North West and Mpumalanga Provinces.

3.6 CONCLUSIONS AND RECOMMENDATIONS

Based on the outcomes of the study, it could be ascertained that there is evidence of physicochemical and microbiological pollution of the groundwater supplied to communities living in the rural areas of the North West and Mpumalanga Provinces. High concentrations of magnesium, calcium, fluoride, nitrate, TDS as well as high turbidity levels and especially poor bacteriological quality of this water source may pose a serious threat to consumers. The detection of various opportunistic pathogens and pathogenic strains such as *Serratia marcescens, Citrobacter freundii, Salmonella enterica, Bacillus cereus, Escherichia coli* O157:H7*, Shigella flexineri, Pseudomonas maltophilia, Enterobacter cloacae, Klebsiella oxytoca,Cronobacter sakazakii, Aeromonas veronii, Morganella morganii and Shigella dysenteriae* indicates that communities in rural areas of the North West and Mpumalanga Provinces are at a constant risk of contracting waterborne diseases. Consequently, this study calls for the urgent involvement by the government to provide for the protection of groundwater sources and drinking

water treatment barriers so as to ensure the safe distribution of potable water in these two provinces.

CHAPTER 4

COST-EFFECTIVE FILTER MATERIALS COATED WITH SILVER NANOPARTICLES FOR THE REMOVAL OF PATHOGENIC BACTERIA IN GROUNDWATER

4.1 ABSTRACT

Contamination of groundwater sources by pathogenic bacteria poses a public health concern to communities who depend totally on this water supply. In the present study, potentially low-cost filter materials coated with silver nanoparticles were developed for the disinfection of groundwater. Silver nanoparticles were deposited on zeolite, sand, fibreglass, anion and cation resin substrates in various concentrations (0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM) of $AgNO_3$. These substrates were characterised by SEM, EDS, TEM, particle size distribution and XRD analyses. In the first phase, the five substrates coated with various concentrations of $AgNO_3$ were tested against *E.coli* spiked in synthetic groundwater to determine the best loading concentration that could remove pathogenic bacteria completely from test water. The results revealed that all filters were able to decrease the concentration of *E.coli* from synthetic groundwater, with a higher removal efficiency achieved at 0.1 mM (21 %–99.9 %) and a lower efficiency at 0.01 mM (7 %–50 %) concentrations. The cation resin-silver nanoparticle filter was found to remove this pathogenic bacterium at the highest rate, namely 99.9 %. In the second phase, only the best performing concentration of 0.1 mM was considered and tested against presumptive *E.coli*, *S. typhimurium,*

S. dysenteriae and *V. cholerae* from groundwater. The results revealed the highest bacteria removal efficiency by the Ag/cation resin filter with complete (99.9 %) removal of all targeted bacteria and the lowest by the Ag/zeolite filter with an 8 % to 67 % removal rate. This study, therefore, suggests that the filter system with Ag/cation resin substrate can be used as a potential alternative cost-effective filter for the disinfection of groundwater and production of safe drinking water.

4.2 INTRODUCTION

The World Health Organisation (WHO) has indicated that approximately 1.8 million deaths and 61.9 million disability-adjusted life years (DALYs) worldwide are attributable to unsafe water, sanitation and poor hygiene. An estimated 99.8 % of such deaths occur in developing countries, with children ranking (90 %) as the first victims (WHO, 2004). Consumption of groundwater and surface water sources contaminated with pathogenic bacteria such as *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Shigella dysenteriae* and *Vibrio cholera* continues to be one the major causes of diarrhoeal diseases and gastrointestinal infections (Momba & Kaleni, 2000; Edberg *et al*., 2000; Enriquez *et al*., 2001; Momba & Notshe, 2003; Momba *et al*., 2006). This implies that safe drinking water plays a significant role in human health and well-being.

In 2002, the United Nation Millennium Development Goals (MDG) firmly established the issue of water and sanitation on the global agenda. The vision of the MDG is to halve the number of people without access to safe drinking water and sanitation by 2015 (WHO/UNICEF, 2010). Although tremendous progress has

been made to date, the 2010 updated report by the WHO and the United Nations Children's Fund (UNICEF) has indicated that 884 million people in the world still lack access to drinking water from improved sources.

The provision of safe drinking water is currently a high priority for the South African government, one of the signatories of the MDG. The percentage of households with access to water infrastructure above or equal to the Reconstruction and Development Programme (RDP) standard increased from 61.7 % in 1994 to 91.8 % in March 2009. Based on these data, it is estimated that 93 % of the population had access to an improved drinking-water supply in 2010 (Stats SA, 2010). However, non-improved drinking water supplies are currently still found in rural communities that are widely dispersed as well as in informal peri-urban communities that are continuously expanding. It is therefore difficult to implement centralised systems such as piped systems, which not only require substantial financial support, but also highly skilled personnel to manage and maintain them. The implementation of decentralised systems is therefore needed to provide these rural communities with safe drinking-water sources.

Cost-effective filter materials coated with silver nanoparticles is an alternative technology that could assist developing countries in meeting the MDG, and South Africa, in particular, in providing a safe drinking-water supply to all scattered rural areas and informal settlements. Silver ion (Ag^{+}) has long been known as a potential antimicrobial agent and is used in wound dressings to prevent infections in burn patients, to prevent blindness in newborns, for severe chronic osteomyelitis and urinary infection, to control *Legionella* bacteria in hospitals and to enhance the

performance of drinking-water filters (Klasen, 2000; Bosetti *et al*., 2002; Richard *et al*., 2002; Niven, 2005). It can bind to bacterial cells and enzymes (proteins) at multiple sites, damaging them and preventing them from performing their functions, thus resulting in cell death by penetrating specific bacterial DNA and RNA (Klassen, 2000; Ovington, 2004; Rai, *et al*., 2009).

Silver in the form of nanoparticles that release silver ions more effectively has a better bactericidal activity due to its high surface-area-to-volume ratio (Kumar *et al*., 2008; Duran *et al*., 2010). Recent studies have shown that various procedures for preparation of silver nanoparticles have constantly displayed good antibacterial activity (Matsumura *et al*., 2003; Sondi *et al*., 2004). As a result, researchers have considered silver nanoparticles for drinking-water treatment due to its strong and broad spectrum of antimicrobial activities (Stoimenov *et al*., 2002; Cho *et al*., 2005; Jain & Pradeep, 2005). With the advancement of materials development, silver nanoparticles can be easily deposited on solid materials for the deactivation of microorganisms in water treatment (Nair *et al*., 2007). In the case of drinking-water treatment, various forms of silver nanoparticles coated on materials or substrates have been used such as Ag/sand (Mahmood *et al*., 1993), Ag/zeolite (Matsumura *et al*., 2003) and Ag/fibreglass (Nangmenyi *et al*., 2009). Sand filtrations have been used in water purification to control microbiological contamination for over 150 years (Logsdon *at al*., 2002). Sand filters offer a less expensive, more effective method of water treatment, can be self-constructed, and may be constructed by using local skills. Natural zeolites as cation exchangers in water treatment have increased due to their availability, low cost, high surface area and sorptive capacity, negative surface charge, chemical inertness and low or null

toxicity for humans (Rivera-Garza *et al*., 2000; Top & Ülkü, 2004). Most kinds of fibreglass are used for thermal and acoustic insulation in building construction, shipbuilding and filtration applications. Fibreglass-reinforced plastics (FRPs) have been used for various types of process equipment in the chemical industry, pulp and paper industry, power and mining industries, municipal sewer treatment and water treatment, as well as many other associated industries handling corrosive equipment (SMACNA, 1997). A number of investigations have been carried out regarding the use of resins containing silver or silver nanoparticles for oral and dental applications (Bürgers *et al*., 2009; Fan *et al*., 2011). Resins are used in ion exchange and constitute a very powerful technology for removing impurities from water and other solutions. Resins pose no health risks, as many industries use resins for multiple purposes (nuclear and thermal power stations, semiconductors, computers), including dental and pharmaceutical applications and drinking-water treatment for the removal of toxic contaminants (Purolite International Ltd, 2004; Rohm & Haas, 2008).

Even though a number of studies have been conducted on bacterial removal with Ag/zeolite, Ag/sand, Ag/fibreglass and Ag/resin nanoparticle substrates, there is no information on comparative studies related to the use of these technologies for the removal of pathogenic bacteria from drinking-water sources. This study, therefore, concentrated on the development of these substrates modified with silver nanoparticles and compared their effectiveness in removing pathogenic bacteria (*Escherichia coli*, *Vibrio cholerae*, *Shigella dysenterae and Salmonella typhimurium*) from polluted groundwater sources. Our main intention was to find an alternative cost-effective technology with the best concentration of silver

nanoparticles loaded onto the substrates, which could completely remove pathogenic bacteria from water samples and result in the production of safe drinking water for rural communities.

4.3 EXPERIMENTAL METHODOLOGY

4.3.1 Preparation of substrates

Locally available materials for silver deposition were utilised in the present study. Silver was coated on natural zeolite, sand, fibreglass, anion resin and cation resin. Natural clinoptilolite zeolite purchased from Ajax Industries CC (Cape Town, South Africa) was conditioned in a 500 mL solution of 2 M NaCl (Merck, South Africa), followed by a process of stirring at room temperature (between 20 °C and 25 °C) for 36 h. The solid-liquid mixture was separated by centrifugation at 3 000 rpm for 15 min. Liquids were discarded and the solids were washed with deionised water three times, and then oven dried at 105 °C for 8 h. Silica sand purchased from Eggo Sand (Pty) Ltd (Pretoria, South Africa) was submitted to a cleaning process by stirring 200 g of sand in a litre of 30 % nitric acid (Merck, South Africa) solution with a reciprocating shaker at 210 rpm at room temperature for 24 h. The sand was allowed to settle, separated from the solution by decantation, and then rinsed three times with deionised water and oven dried at 105 °C for 24 h. Fibreglass chopped-strand mat was purchased from Collins Fibreglass Plastics (Johannesburg, South Africa) and cleaned by immersion in an ultrasonic bath containing isopropanol (Sigma, South Africa) for 2 h. The substrate was finally rinsed three times with deionised water and oven dried at 105 °C for 24 h.

4.3.2 Synthesis of silver nanoparticle-coated substrates

5.3.2.1 Coating of zeolite, sand and fibreglass substrates

Silver nitrate ($AgNO_3$) (Merck, South Africa) stock solution (1 mM) was prepared by adding 169.87 mg of silver nitrate to a litre of deionised water. Thereafter, concentrations of 0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM silver nitrate (250 mL) were prepared by diluting the silver stock solution. The substrates (20 g) were then separately immersed in an aqueous solution containing concentrations of 0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM silver nitrate for 24 h. Thereafter, they were incubated, in the dark and at room temperature for 24 h, in a thermostatic shaker at a speed of 250 rpm. Substrates containing silver were separated from the mixture by centrifugation at 3 000 rpm for 15 min, then washed with deionised water, and finally oven dried for 24 h at 105 °C. For the reduction of silver ions to silver nanoparticles, the substrates containing silver were heated in an N_2 furnace (Lenntech, South Africa) at a flow rate of 400 mL/min for 1 h at 120 °C and then the furnace was ramped up to 350 °C for 3 h.

4.3.2.2 Coating of anion resin beads substrate

Amberlite-IRA-458 anion exchange resin (in chloride form) was purchased from Lenntech (Johannesburg, South Africa). The silver was coated onto the anion resin beads by modifying the method previously described by Jana *et al.* (2006). Briefly, the silver nanoparticle-resins were prepared following a two-step procedure. A known amount (20 g) of anion exchange resin was used. Firstly, 30 mL of 1 M HCl (Merck, South Africa) solution was added dropwise to 200 mL of stirred, freshly prepared aqueous solution of concentrations of 0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM $AgNO_3$ to form white precipitates of silver chloride. These

precipitates were washed three times with deionised water in order to remove HNO_3 and then immersed in a water bath at 65 °C for 2 h. A silver precursor $[AgCl_2]^-$ complex was subsequently prepared by dissolving 0.3 g of solid AgCl in a concentrated HCl solution and the mixture was then placed in an ultrasonic bath for dissolution. Thereafter, the silver precursor ions were allowed to exchange with Cl^- ions of the neat chloride form of anion-exchange resin beads (R^+Cl^-) and the mixture was stored overnight. The resin beads, on which silver precursor ions were immobilised, were then washed three times with water to drain out the liberated HCl and un-exchanged $[AgCl_2]^-$ and then reduced with a freshly prepared ice-cold aqueous solution of 0.01 M sodium borohydride. Finally, the prepared shining reddish-black silver-coated beads $[R(Ag)^0]^+Cl^-$ were washed thoroughly with deionised water and oven dried at 150 ^oC for 3 h.

4.3.2.3 Coating of cation resin beads substrate

The methods described by Nath *et al*. (2005) were also modified and employed for the coating of cation resin. A silver amine complex, $[Ag(NH_3)_2]^+$, was prepared by adding 10 mL of 25 % ammonia solution dropwise to each of 200 mL aqueous solution of concentrations of 0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM $AgNO_3$. A known amount (20 g) of cation exchange resin (R^-H^+) was then added to each of these mixtures, followed by a mixing process for 3 h using a magnetic stirrer. The resin silver amine moiety $[R–Ag(NH_3)_2]$ was subsequently washed three times with deionised water and then heated in an oven at 150 °C for 1 h. During this process, the yellow colour of the resin beads changed to black due to the formation of a resin silver oxide composite $[R(Ag_2O)]^-H^+$. Thereafter, the complex was reduced with an aqueous solution of freshly prepared 0.01 M sodium

borohydride to form silver nanoparticle-coated resin beads $[R(Ag^0)]^-H^+$, with a white colour. The silver nanoparticle-resin beads were then washed three times with deionised water and finally dried in an oven at 65 °C for 3 h to obtain dry silver-coated resin beads.

4.3.3 Characterisation of substrates coated with silver nanoparticles

The surface morphology of the silver nanoparticles-coated substrates was examined with a scanning electron microscope (SEM) (JEOL JSM-5800LV, JEOL Ltd, Tokyo, Japan), coupled with energy-dispersive spectroscopy (EDS) in order to confirm the chemical content on the substrates. Transmission electron microscope (TEM) analysis was performed with a JEOL 2100F (JEOL Ltd, Tokyo, Japan) that operated at 100 kV in order to examine the morphology and particle size distribution of the silver nanoparticle substrates. X-ray diffraction (XRD) was used to determine the crystal phase of the substrates. The patterns of the silver nanoparticles were recorded with a Bruker D8 Advance using Cu Kα radiation with 1.5416 Å wavelengths. The structure of the silver on the substrates was studied by scanning the media in 2θ ranges from 30 °C to 80 °C in a continuous scan mode. The crystallite size of the silver substrates was determined from X-ray line broadening using the Debye-Scherrer equation as follows:

$$D = \frac{K\lambda}{\beta \cos\theta} \qquad \text{(Eq. 4.1)}$$

where D = Crystallite size, A (Angstroms), K = Crystallite-shape factor = 0.9, λ = X-ray wavelength, 1.5416 Å for CuKα, θ = Observed peak angle, degree, β = X-ray diffraction broadening, radian.

4.3.4 Production of combined substrate-silver nanoparticle filter systems

The filter systems consisted of a polyvinyl chloride (PVC) column of 2 cm diameter and 20 cm length (Figure 4.1). Each column was packed with one type of the substrate coated with silver nanoparticles at a depth of 10 cm. With reference to the various substrates (sand, zeolite, fibreglass, anion resin and cation resin), five filters were used during the study period. Glass beads (2 cm) and glass wool (2 cm) were placed in the upper and bottom ends of each column. Glass beads were positioned in such a manner as to prevent the substrates from aggregating at one end. A 10 L bucket served as storage container for contaminated influent water, which was fed into the filter system with a 1 m length of 8 mm diameter latex tubing connected to a Rainin Dynamax peristaltic pump (Rainin Instrument Co., Woburn, MA, USA). The effluent sample (treated water) was collected at the top of the filter with a 1 m length of 8 mm diameter latex tubing into a 10 L bucket container. Figure 4.1 illustrates the schematic diagram of a laboratory-scale setup with an example of a combined substrate-Ag nanoparticle filter system.

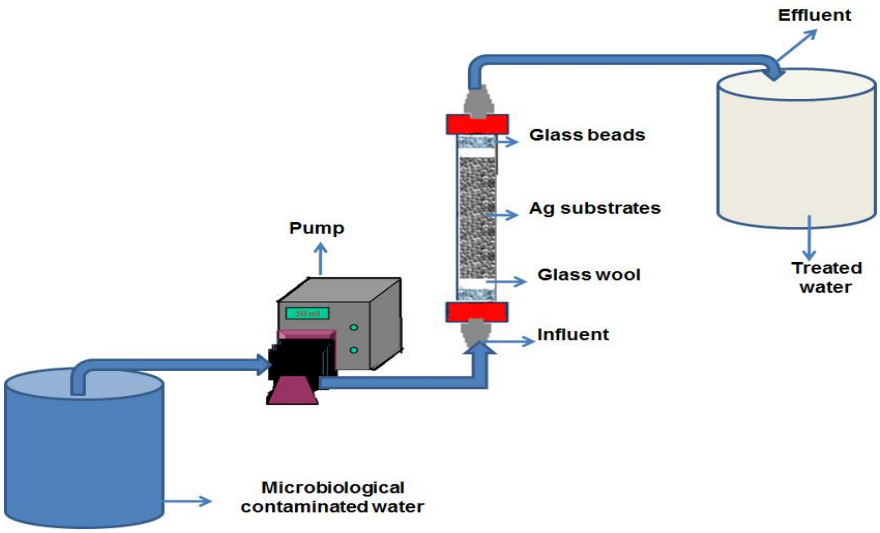


FIGURE 4.1: Schematic diagram of laboratory-scale setup to evaluate the antibacterial efficiency of Ag nanoparticle-coated substrates

4.3.5 Testing the efficiency of filter systems in removing pathogenic bacteria

The performance of the combined substrates-silver nanoparticle filter systems in removing pathogenic bacteria was studied in two phases. In the first phase, the five substrates coated with various concentrations of $AgNO_3$ were tested against *Escherichia coli* (ATCC 43895) spiked in synthetic groundwater samples. The main objective of this part of the study was to determine the best loading concentration of $AgNO_3$ that could result in the total removal of pathogenic bacteria from a water sample source. In the second phase, the performance of the five combined substrate-silver nanoparticle filter systems was tested using groundwater, and only the best concentration of silver nanoparticles loaded onto the substrates was investigated against the four different pathogenic bacteria - *E.coli*, *S. typhimurium*, *S. dysenteriae* and *V. cholerae*. In cases where these pathogens were not present in the intake groundwater source, the water was spiked with the pathogens. This was performed mainly to evaluate the efficiency of each filter in reaching the allowable recommended limits set by the *South African National Standards for Domestic Use* (DWAF, 1996; SANS 241, 2006). The bacterial removal efficiency was obtained by comparing the concentrations (Log_{10} cfu/100 mL) of target organisms before and after treatment. In each series of the experimental study, a control filter constituted of the substrate without silver nanoparticles was included. The experimental study for each combined substrate-silver nanoparticle filter system was performed in three different trials.

4.3.5.1 Preparation of bacterial stock suspensions

The microbial strains used in the experimental study included *Escherichia coli* (ATCC 43895) and *Salmonella typhimurium* (ATCC 14028) obtained from the American Type Culture Collection (Rockville, MD, USA), as well as *Vibrio cholera* and *Shigella dysenteriae* obtained from the Council for Scientific and Industrial Research (CSIR, Pretoria, South Africa) bacterial stock cultures. Prior to use, these bacterial strains were confirmed by cultural tests using selective agar media (Chromocult agar for *E.coli*, XLD agar for *S. typhimurium* and *S. dysenteriae*, TCBS agar for *V. cholera*) according to the Standard Method (APHA, 1998). One loop full of each bacterial culture was separately inoculated in 100 mL sterile nutrient broth (Merck, South Africa) medium and incubated aerobically at 37 °C in a shaking incubator (Scientific Model 353, Lasec, South Africa) at 120 rpm for 24 h. The bacteria were harvested by centrifugation at 4000 rpm for 10 min and the pellets were washed twice with 50 mL of sterile 0.01 M phosphate-buffered saline (PBS, pH 7.2).

The stock suspensions of *E.coli*, *S. typhimurium*, *S. dysenteriae* and *V. cholera* were prepared by re-suspending the final pellets in 10 mL of 0.01 M PBS solution. The initial concentrations of bacterial cells harvested were determined with the spread-plate technique, after the serial dilution of each culture in a sterile saline solution (0.9 % w/v NaCl). The plates were incubated at 37 °C for 24 h. The resulting colonies were counted and expressed as cfu/mL.

4.3.5.2 Preparation of synthetic contaminated groundwater

For each filter system, an aliquot of the stock suspension of *E.coli* (ATCC 43895) corresponding to 6 log cfu/100 mL was inoculated into 10 L final volumes of sterile saline water (8.5 % NaCl). The spiked water samples were shaken vigorously several times and 1 mL thereof was used to determine the initial concentration of the target organism before passing the remaining contaminated water through the filter systems.

4.3.5.3 Collection and analysis of the quality of groundwater samples

Groundwater samples were collected from a borehole at Delmas (A7) in the Mpumalanga Province of South Africa. The study was conducted between June 2010 and July 2010 and water samples were collected three times during this period. It is important to note that, during the study period, this groundwater supply was used by the community without prior treatment. The water samples were collected in sterile 50 L plastic buckets. Samples were also collected in sterile 1 L glass bottles in order to detect and enumerate the initial concentrations of target bacteria and selected physicochemical parameters prior to treatment. The samples were then transported to the laboratory and the quality of the water was determined for microbiological contamination and selected physicochemical parameters within 6 h (APHA, 1998).

Escherichia coli, *S. typhimurium*, *S. dysenteriae* and *V. cholerae* were detected and enumerated from groundwater samples according to the *Standard methods for examination of water and wastewater* set by the American Public Health Association (APHA, 1998). As mentioned above, in cases where these organisms

were not detected in groundwater samples, they were spiked with 10^2 cfu/mL stock suspension in the water sample sources using the same method as described for synthetic groundwater samples. All the tests were conducted under aseptic conditions.

The pH and turbidity were measured on site using a pH meter (Metrohm Co. Model 713) and a microprocessor turbidity meter (Eutech Instrument Turbidimeter TN-100), respectively. Nitrates and fluoride concentrations were determined in the laboratory using the Spectroquant Nova 400 manual water analyser (Merck) and photometric test kits (Merck), while the concentrations of magnesium and calcium in the water sample were determined by atomic absorption spectrophotometry (SpectrAA 220FS), according to the *Standard methods for examination of water and wastewater* (APHA, 1998).

4.3.5.4 Operating conditions and testing the performance of the filter systems

Prior to the start of each run, the packed columns were pumped upward with sterile deionised water in order to adjust and achieve a steady-state flow condition. Thereafter, each type of water sample source, which was contaminated with enteric pathogenic bacteria, was pumped continuously through the column in the up-flow mode with a Rainin Dynamax peristaltic pump (Rainin Instrument Co.) that operated at a flow rate of 0.12 L/h. Treated water samples were collected in sterile conical flasks at 10 min intervals at a volume of 50 mL. Prior to use, 1 mL 15 % sodium thiosulphate was added to the flasks to stop further disinfection of drinking water prior to determining the bacterial concentrations. The latter were determined

by using a serial dilution in a sterile saline solution and plating them onto a selective medium by applying the spread-plate technique according to the *Standard methods for examination of water and wastewater* (APHA, 1998).

4.3.5.5 Elution of silver ions from silver nanoparticle substrate filter systems

The degree of elution of silver from each silver nanoparticle substrate filter systems used in the first phase with spiked synthetic groundwater was measured. The silver content in the treated water samples was determined with atomic absorption spectroscopy (AAS) by using a Spectra AA-220FFS (Varian Medical Systems, Inc., Palo Alto, CA, USA).

4.3.6 Statistical analysis

All data were analysed statistically using the SPSS computer software, version 11.0. Significance testing was conducted by performing one-way analysis of variance (ANOVA) at a 95 % confidence interval. Comparisons were made between the treatment means of each filter system to determine whether there were any significant differences in treatments.

4.4 RESULTS

4.4.1 Characterisation of silver nanoparticle coatings on substrates

Figure 4.2 depicts the morphologies and the elemental composition of the silver nanoparticle coatings on substrates (Ag/zeolite, Ag/sand, Ag/fibreglass, Ag/anion resin, Ag/cation resin) examined with SEM micrograph and EDS analyses. The

silver nanoparticles were observed as small white spherical particles on the surface of the sand, fibreglass and anion resin. In cation resin, the silver nanoparticle coating completely covered the resin bead and the film revealed a homogeneous rough surface. The structure of the silver nanoparticles in zeolite was complicated due to sample charging. Zeolite sample charging could indicate a low electrical conductivity of the medium even though carbon coating had been applied to avoid charging. The EDS spectra also confirmed the elemental composition of silver nanoparticles by the presence of Ag peaks in the synthesised substrates. In the spectra, two peaks of Ag located between 3.0 kV and 3.5 kV were observed in zeolite, four peaks for sand located between 2.5 kV and 3.5 kV, four peaks for fibreglass between 1.0 kV and 4.0 kV, five peaks for anion resin between 2.0 kV and 4.0 kV and six peaks for cation resin between 1.0 kV and 4.0 kV. The EDS spectra also revealed the presence of other major elements found in zeolite (Na, Mg, Al, Si, Ca, K, Ti), sand (C, Fe, Al, Si), fibreglass (Al, Si, Ca), anion resin (C, N, O, Al, Cl) and cation resin (C, O, Al, S). The carbon and oxygen peaks exhibited in the EDS analyses, however, could have been due to the carbon tape used for the SEM stub preparation.

Figure 4.3 depicts the results of the TEM image and particle size distribution histogram of silver nanoparticle substrates (zeolite, sand, fibreglass, anion and cation resin beads). The morphology of silver nanoparticles deposited on substrates exhibited an aggregation of spherical-shaped particles. According to the particle-size distribution histogram, the silver nanoparticles have exhibited the majority of particle sizes distributed; from 40 nm to 90 nm for zeolite and sand, and 5 nm to 30 nm for fibreglass and resin beads.

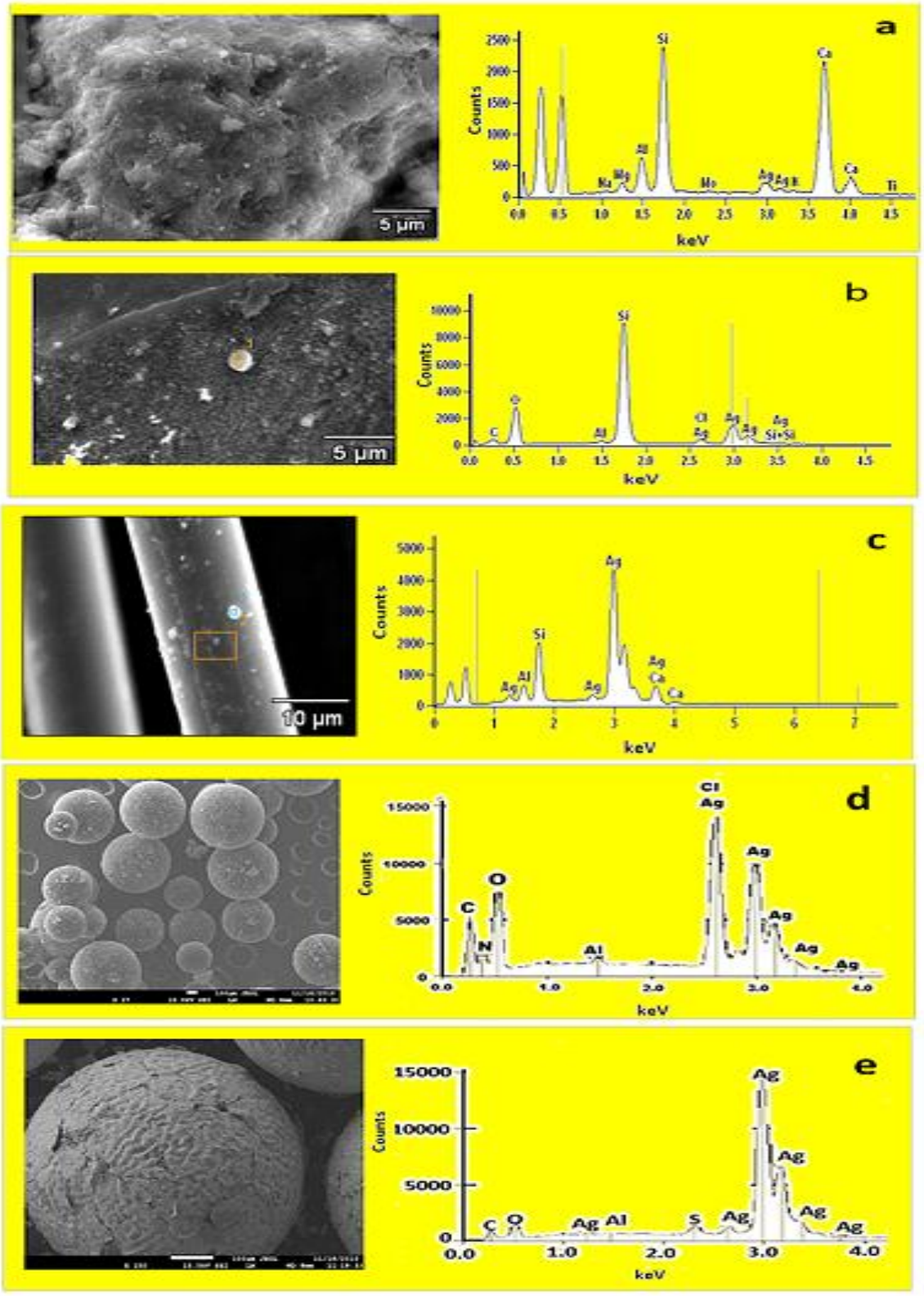


FIGURE 4.2: SEM image and EDS spectrum of (**a**) zeolite, (**b**) sand, (**c**) fibreglass, (**d**) anion resin, and (**e**) cation resin coated with silver nanoparticles

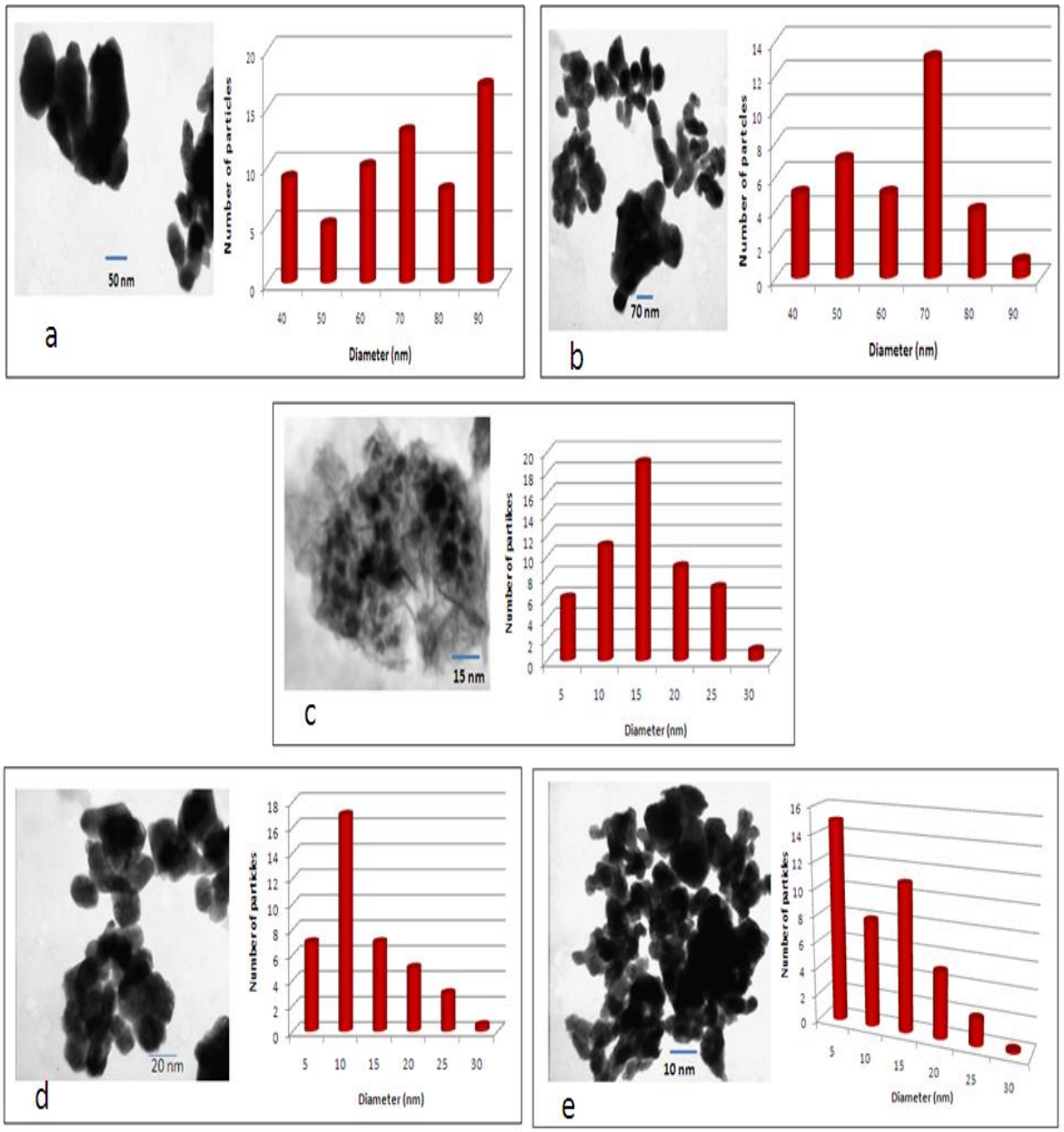


FIGURE 4.3: TEM image and particle size distribution of **(a)** zeolite, **(b)** sand, **(c)** fibreglass, **(d)** anion resin, and **(e)** cation resin coated with silver nanoparticles

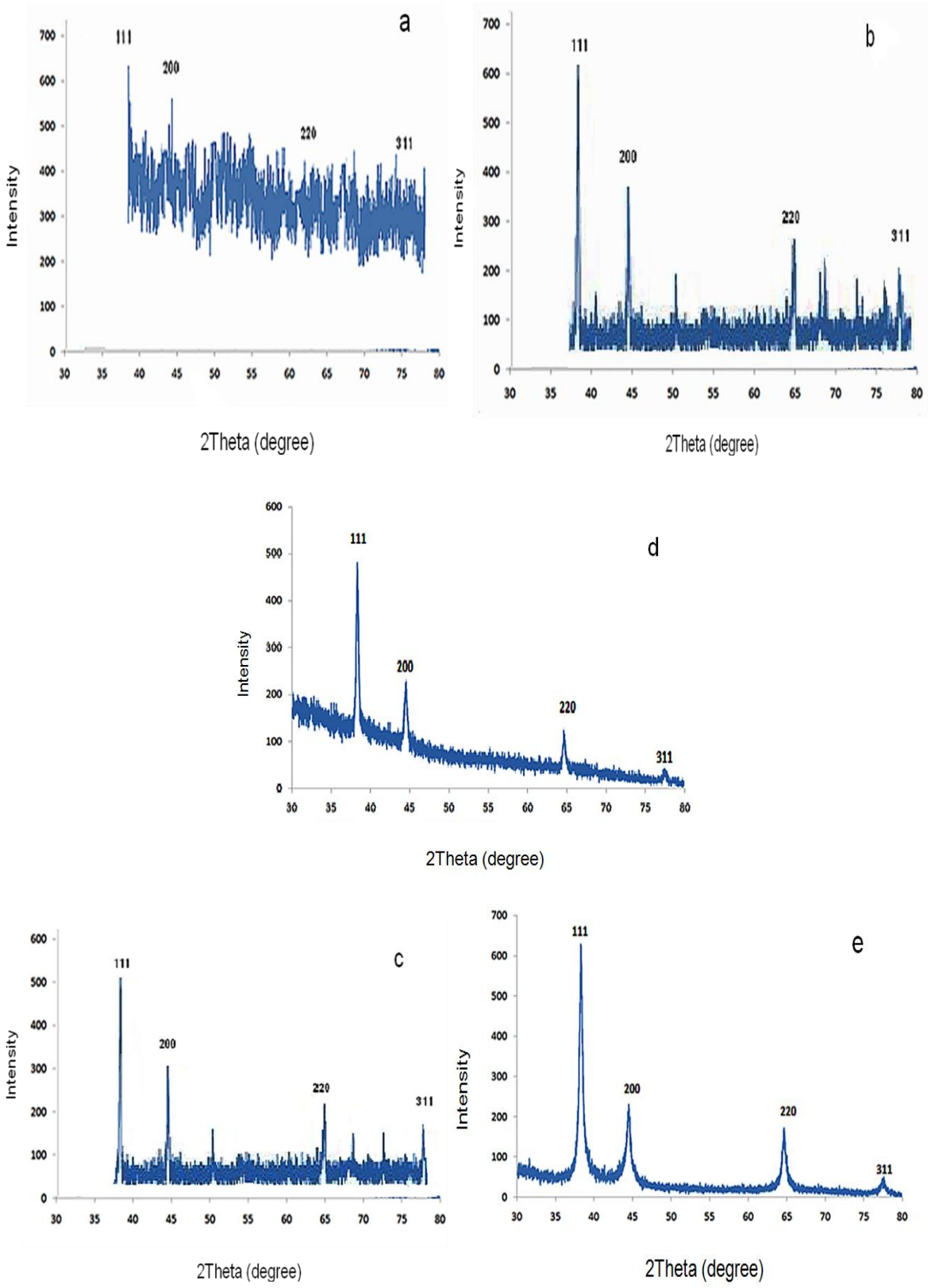


FIGURE 4.4: XRD patterns of the silver nanoparticles coated onto **(a)** zeolite, **(b)** sand, **(c)** fibreglass, **(d)** anion resin, and **(e)** cation resin

The diffraction pattern of the synthesised silver nanoparticle substrates depicted in Figure 4.4 matched the face-centered cubic (fcc) structure of silver observed at 2θ angle 37.7°, 44.0°, 64.2° and 77.1°. These corresponded to the four diffraction peaks above 30° (111), (200), (220) and (311) crystal planes, respectively, clearly indicating that the silver nanoparticles are crystalline in nature. The XRD patterns were analysed to determine the average crystallite size obtained by classical Scherrer equation 4.1 indicated in Section 4.3.3. The typical XRD pattern was consistent with average nanocrystallite sizes of 85 nm for zeolite, 80 nm for sand, 28 nm for fibreglass, 20 nm for anion resin and 13 nm for cation resin derived from full-width at half-maximum (FWHM) of peak, corresponding to a (111) crystal plane with a cubic shape. The zeolite substrate (Figure 4.4(a)) did not exhibit any well-defined peaks, which indicates the amorphous nature of the sample. All the substrates exhibited low-intensity peaks of silver at 2θ = 77.1°. It is evident from Figure 4.4(a–c) that, in addition to the four peaks of the crystal plane, other unidentified peaks also appeared in the XRD pattern.

4.4.2 Characteristics of water sample sources prior to treatment

4.4.2.1 Microbiological quality of water sample sources

Table 4.1 illustrates the initial concentrations of *E.coli,* spiked with sterile synthetic groundwater and groundwater, with targeted bacteria prior to treatment. The initial count of the suspension for *E.coli* spiked with synthetic groundwater was approximately 6 log cfu/100 mL. Therefore, after mixing thoroughly, the average count of *E.coli* was found to be 7 log cfu/100 mL. Initial presumptive *E.coli* in groundwater had an average count of 3 log cfu/100 mL. *Salmonella typhimurium*, *S. dysenteriae* and *V. cholerae* were not originally detected in the test groundwater

samples. Consequently, the said samples were spiked with initial concentrations of approximately 3 log cfu/100 mL for each target organism. However, after thoroughly mixing the spiked groundwater samples, the average counts for *S. typhimurium*, *S. dysenteriae* and *V. cholerae* as presented in Table 1 were approximately 3 log cfu/100 mL.

TABLE 4.1: Microbiological quality of spiked water sources prior to treatment

	Bacterial concentrations (cfu/100 mL) before filtration			
	Targeted organisms			
Water sources	***Presumtive E.coli***	***S. typhimurium***	***S. dystenteriae***	***V. cholerae***
Synthetic groundwater	3.21×10^7	ND	ND	ND
Groundwater	3.15×10^3	1.20×10^3	2.71×10^3	2.01×10^3

ND—Not done

4.4.2.2 Physicochemical and microbiological quality of groundwater sources

The characteristics of groundwater samples collected from a Delmas borehole (A7) are illustrated in Table 4.2. The average physicochemical values of the groundwater were 7.22 for pH; 1.59 NTU for turbidity; 0.46 mg/L for fluorides; 1.59 mg/L for nitrates; 98.25 mg/L as Ca for calcium; and 26.36 mg/L as Mg for magnesium, respectively. Regarding the microbiological quality, only presumptive *E.coli* (average count: 2.99×10^3 cfu/100 mL) was present in the groundwater samples.

TABLE 4.2: Characteristics of the groundwater sample

Parameters	Units	Concentration	SANS 241
pH	-	7.22 ± 0.14	5-9.5
Turbidity	NTU	1.59 ± 0.11	<1
Fluorides	mg/L	0.46 ± 0.18	<1
Nitrates	mg/L as N	1.59 ± 0.02	<10
Calcium	mg/L as Ca	98.25 ± 1.12	<150
Magnesium	mg/L as Mg	26.36 ± 7.18	<70
E.coli	cfu/100 mL	2.99×10^3	0

4.4.2.3 Performance of silver nanoparticles substrates in removing pathogenic bacteria from synthetic groundwater

Figure 4.5 illustrates the effect of different concentrations of $AgNO_3$ (0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM) deposited on substrates for the deactivation of *E.coli*. It is evident from Figure 4.5(a) that the filter system with uncoated zeolite (control) did not have any antibactericidal effects on *E.coli*. However, Ag/zeolite nanoparticle filter systems at 0.01 mM and 0.03 mM exhibited 0.5 log cfu/100 mL reduction of *E.coli*, while 0.05 mM and 0.1 mM indicated a removal of l.0 log and 1.5 log cfu/100 mL within the first 30 min, respectively, and then an increase of bacterial counts resulting in a survival of more than 6 log cfu/100 mL occurred in the filtered water after the 90 min trial. The results revealed a significant difference in the reduction of the *E.coli* count with 0.03 mM, 0.05 mM and 0.1 mM concentrations ($p < 0.05$), as compared to the control.

As illustrated in Figure 4.5(b), the uncoated sand (control) filter system did not have any antibacterial effect on *E.coli*. Filter systems with Ag/sand nanoparticles reduced the *E.coli* count in a 0.01 mM by 1.0 log cfu/100 mL within the first 10 min, and in a 0.03 mM within the first 30 min, with an increase in the *E.coli* count of more than 7 log cfu/100 mL in the filtered water after the 90 min trial. However, with 0.05 mM and 0.1 mM, a 2 log and 2.5 log cfu/100 mL reduction of the *E.coli* count was observed respectively within the first 10 min, and subsequently the *E.coli* counts increased again in the filtered water. The results indicated a significant difference in the reduction of the *E.coli* count with filters containing 0.03 mM, 0.05 mM and 0.1 mM ($P < 0.05$), as compared to the control.

Figure 4.5(c) illustrated that no *E.coli* deactivation was observed with uncoated fibreglass (control). The filter systems with Ag/fibreglass nanoparticles indicated a reduction of 1.2 log cfu/100 mL *E.coli* within the first 10 min with a 0.01 mM concentration, and 2.5 log cfu/100 mL with a 0.03 mM during the same period. Thereafter, the growth of *E.coli* increased between 6 cfu/100 mL and 7 cfu/100 mL in the filtered water after the 90 min trial. A high performance of the Ag/fibreglass nanoparticle filter system was observed when 0.05 mM and 0.1 mM concentrations were used. The results indicated a 3.5 log cfu/100 mL reduction in *E.coli* within the first 10 min at 0.05 mM, and a 4.3 log cfu/100 mL reduction within the first 30 min at 0.1 mM. Thereafter, the growth of *E .coli* in the filtered water was noticeable. There was a significant difference in the reduction of *E.coli* counts with these filter systems containing 0.03 mM, 0.05 mM and 0.1 mM ($P < 0.05$), as compared to the control.

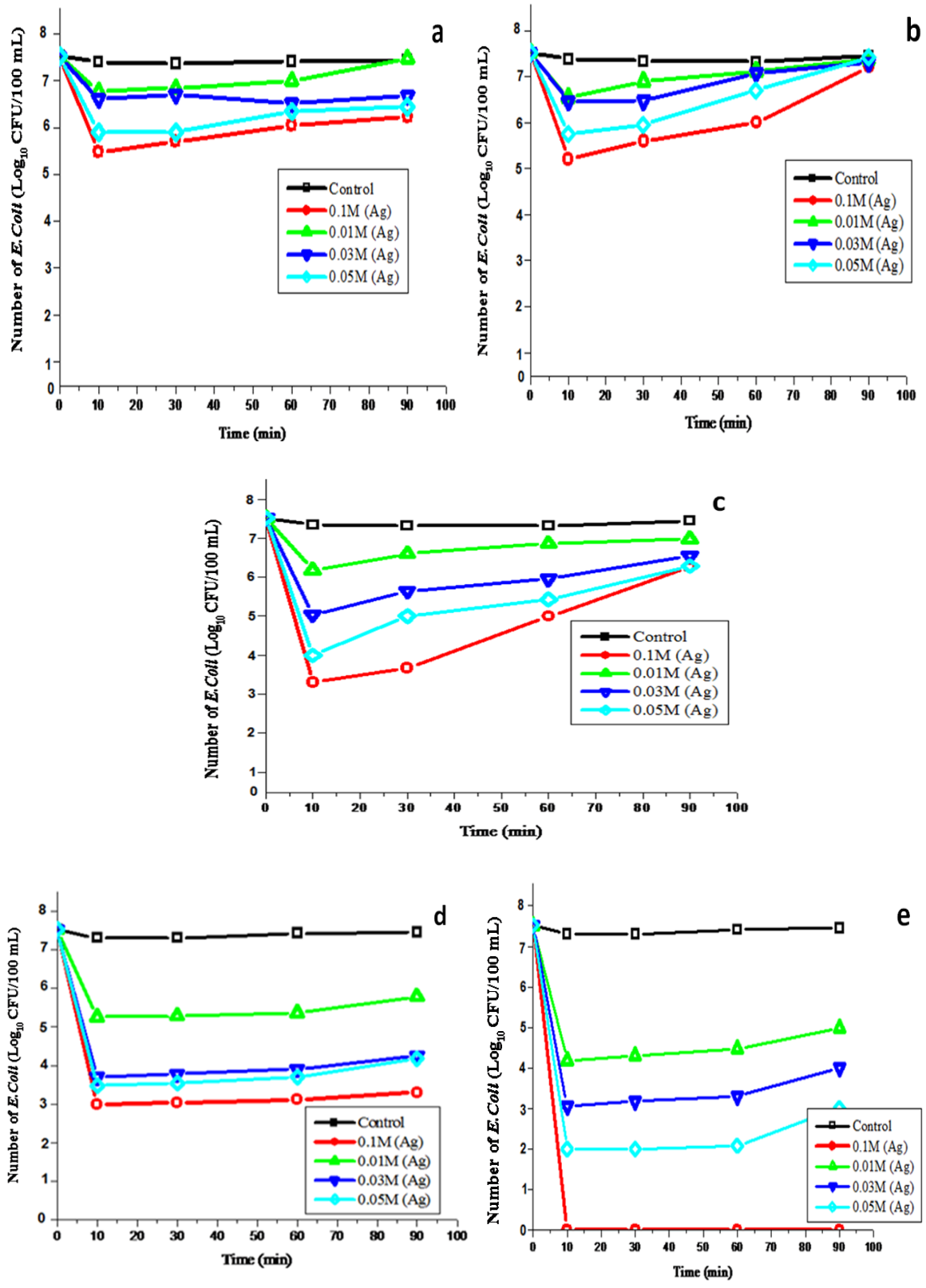


FIGURE 4.5: Antibacterial activity of various Ag nanoparticle-coated substrates against *E.coli* at different concentrations in synthetic groundwater: **(a)** zeolite, **(b)** sand, **(c)** fibreglass, **(d)** anion resin, and **(e)** cation resin

As illustrated in Figure 4.5(d), the uncoated anion resin (control) filter system did not reveal any antibacterial effects against *E.coli*. Filter systems with Ag/anion nanoparticles reduced the *E.coli* count in a 0.01 mM by 2.0 log cfu/100 mL within the first 10 min and exhibited an increase in the *E.coli* count in the filtered water. However, with 0.03 and 0.05 mM, a 4 log cfu/100 mL reduction of *E.coli* count was observed within the first 60 min, while with a 0.1 mM a 4.5 log cfu/100 mL reduction of *E.coli* was also observed during the same period. Thereafter, the growth of *E.coli* increased between 3 cfu/100 mL and 5.6 cfu/100 mL in the filtered water after the 90 min trial. The results indicated a significant difference in the reduction of the *E.coli* count with filters containing 0.03 mM, 0.05 mM and 0.1 mM ($p < 0.05$), as compared to the control.

The results of Ag/cation resin filter systems and uncoated cation resin (control) are illustrated in Figure 4.5(e). The results indicate that the uncoated resin filter did not exhibit any antibacterial activity against *E.coli*. The filter systems with Ag/cation resin reduced *E.coli* by 3.5 logs, 4.5 logs and 5.5 logs cfu/100 mL with 0.01 mM, 0.03 mM and 0.05 mM in the first 60 min of the filter run, respectively. Thereafter, a growth of between 2.5 cfu/100 mL and 4.8 cfu/100 mL *E.coli* was noticed in the filtered water after the 90 min trial. A complete removal (>7 log cfu/100 mL) of *E.coli* was achieved in 0.1 mM during the entire filter run (90 minutes) without a further reappearance in the treated water. There was a significant difference in the reduction of *E.coli* between filter systems containing 0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM ($p < 0.05$), as compared to the control. However, the antibacterial efficiency of the substrates with different concentrations was found not to be

significantly different from one another (p > 0.05), except for the 0.1 mM of Ag/cation resin (p < 0.05).

4.4.3 Performance of 0.1 mM silver nanoparticles substrates in removing pathogenic bacteria from groundwater

Silver nanoparticle-coated substrates with 0.1 mM $AgNO_3$ were selected for further investigation because of their performance efficiency. In general, the bactericidal activity of Ag nanoparticle substrates depended on the individual microorganism. For this reason, a test was carried out with Ag nanoparticle substrates against four bacterial strains by using groundwater containing presumptive *E.coli,* with 3 log cfu/100 mL seeded with 3 log cfu/mL of *S. typhimurium, S. dysenteriae* and *V. cholerae*.

Figure 4.6 indicates the reduction of pathogenic bacteria in the treated water by using five silver nanoparticle substrates filter systems. The filter systems containing Ag/zeolite nanoparticles (Figure 4.6(a)) completely removed presumptive *E.coli*, *S. typhimurium, S. dysenteriae* and *V. cholerae* within the first 10 minutes of the filter run. Thereafter, these pathogenic bacteria reappeared in the treated water samples with counts ranging between 0.25 log and 2 log removal. There was no significant difference (p > 0.05) in the regrowth of these pathogenic bacteria, except for the regrowth of *E.coli* and *S. typhimurium*, where a significant difference (p > 0.05) was observed with the Ag/zeolite nanoparticle filter system in the treated water.

The silver nanoparticles sand filter system (Figure 4.6(b)) indicated a complete removal (3 log) of presumptive *E.coli* and *S. dysenteriae* during the first 10 min of the filter run, while *S. typhimurium* and *V. cholerae* were completely removed within the first 20 min. Thereafter, *E.coli* and *S. dysenteriae* reappeared in the treated water samples for the rest of the study period and their counts ranged between 0.25 log cfu/100 mL and 2.4 log cfu/100 mL, respectively. *Salmonella typhimurium* and *V. cholerae* reappeared in the treated water for the last 100 min of the filter run and their counts ranged between 0.25 log cfu/100 mL and 2.5 log cfu/100 mL. There was no significant difference ($p > 0.05$) in the regrowth of these pathogenic bacteria when using the silver nanoparticles sand filter system in the treated water.

The filter system with silver nanoparticles fibreglaas filter system (Figure 4.6(c)) completely removed (3 log cfu/100 mL) the presumptive *E.coli within* the first 10 min, *S. dysenteriae* within the first 20 min, *S. typhimurium* within the first 30 min and *V. cholerae* within the first 60 min of the filter run. Subsequently, they reappeared in the treated water for the remaining time of the filter run and their counts ranged between 0.3 log cfu/100 mL and 2.5 log cfu/100 mL. There was no significant difference ($p > 0.05$) in the regrowth of *E.coli*, *S. typhimurium* and *S. dysenteriae* when using the silver nanoparticles fibreglaas filter system, except for *E.coli* and *V. cholerae,* where there was a significant difference ($p < 0.05$) in the treated water.

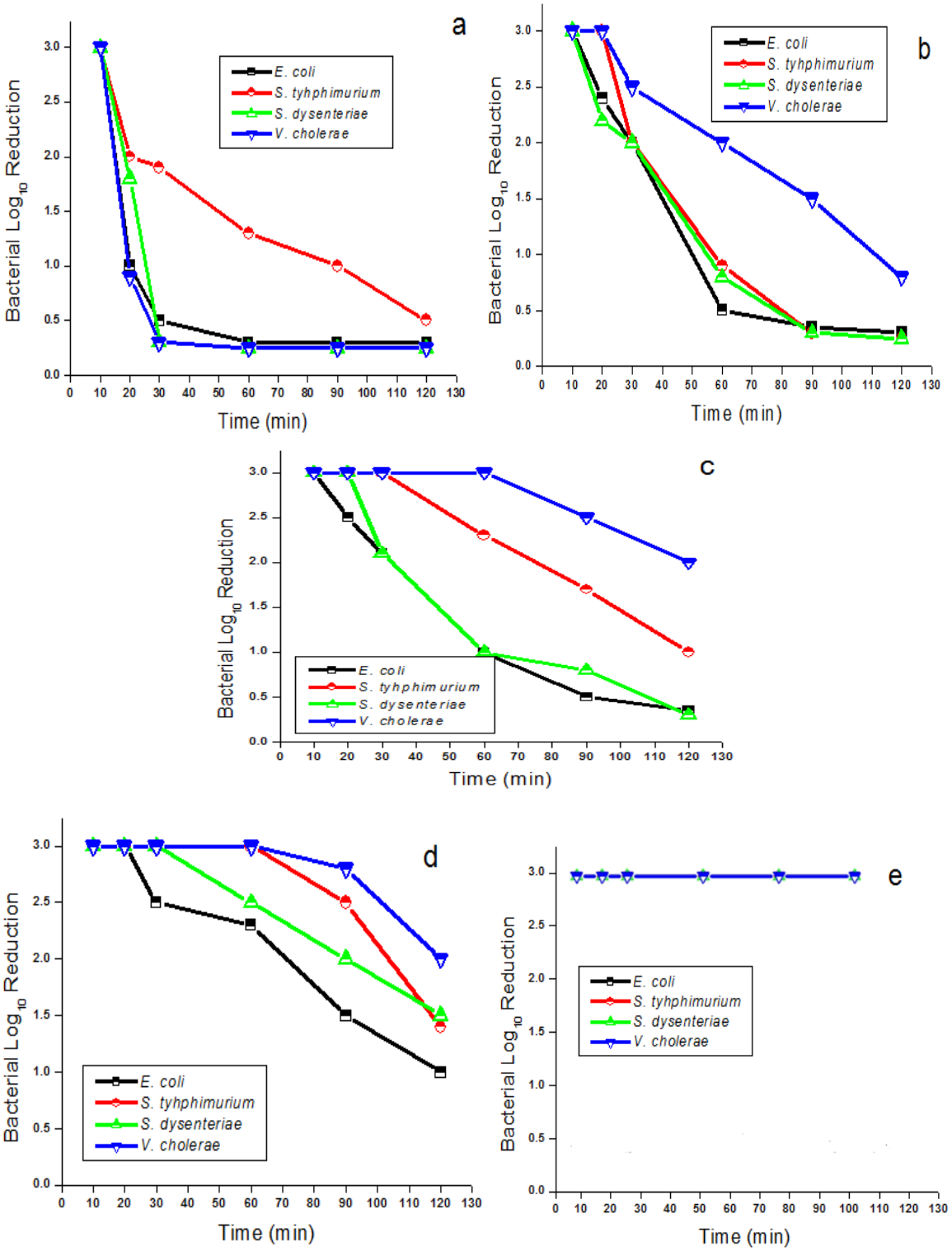


FIGURE 4.6: Antibacterial activity of various Ag nanoparticles-coated substrates in a 0.01 mM concentration against E.coli, S. typhimurium, S. dysenteriae and V. cholerae in groundwater: **(a)** zeolite, **(b)** sand, **(c)** fibreglass, **(d)** anion resin, and **(e)** cation resin

The silver nanoparticle anion resins filter system completely removed 3 log cfu/100 mL of presumptive *E.coli* within the first 20 min, *S. dysenteriae* within the first 30 min, and *S. typhimurium* and *V. cholerae* within the first 60 min of the filter run (Figure 4.6(d)). Subsequently, these organisms progressively reappeared in the treated water with counts ranging between 1 log cfu/100 mL and 2.8 log cfu/100 mL. There was no significant difference ($p > 0.05$) in the regrowth of these pathogenic bacteria when using the silver nanoparticle anion resins filter system in the treated water.

The use of the silver nanoparticle cation resins filter system (Figure 4.6(e)) resulted in a complete removal (3 log cfu/100 mL) of the target pathogenic bacteria and there was no bacteria observed during the entire 120 min of the filter run. Statistically, the performance of silver nanoparticle cation resins filter system in removing all targeted pathogenic bacteria from groundwater was found to be significantly higher compared to the other filters when considering the phenomenon of bacterial regrowth that characterised the aforementioned filters.

4.4.4 Elution of silver ions from silver nanoparticle substrates

Figure 4.7 shows the Ag^+ ions eluted from filter materials coated with silver nanoparticles. A high concentration of silver was eluted from Ag/zeolite, Ag/sand, Ag/fibreglass and Ag/anion resin substrates within the first 10 min of the filter run. The content of silver released from these filters ranged between 1.0 mg/L and 1.8 mg/L for 0.1 mM Ag, between 0.7 mg/ and 0.8 mg/L for 0.05 mM Ag, between 0.5 mg/L and 0.6 mg/L for 0.03 mM Ag, and between 0.1 mg/L and 0.2 mg/L for 0.01 mM Ag. Consequently, after the 90 min trial there was no silver ion, resulting

from zeolite, sand, fibreglass, or anion resin substrates, detected in the filtered water. However, the amount of silver released from the Ag/cation resin was below 0.1 mg/L for all four the different concentrations investigated during the study period.

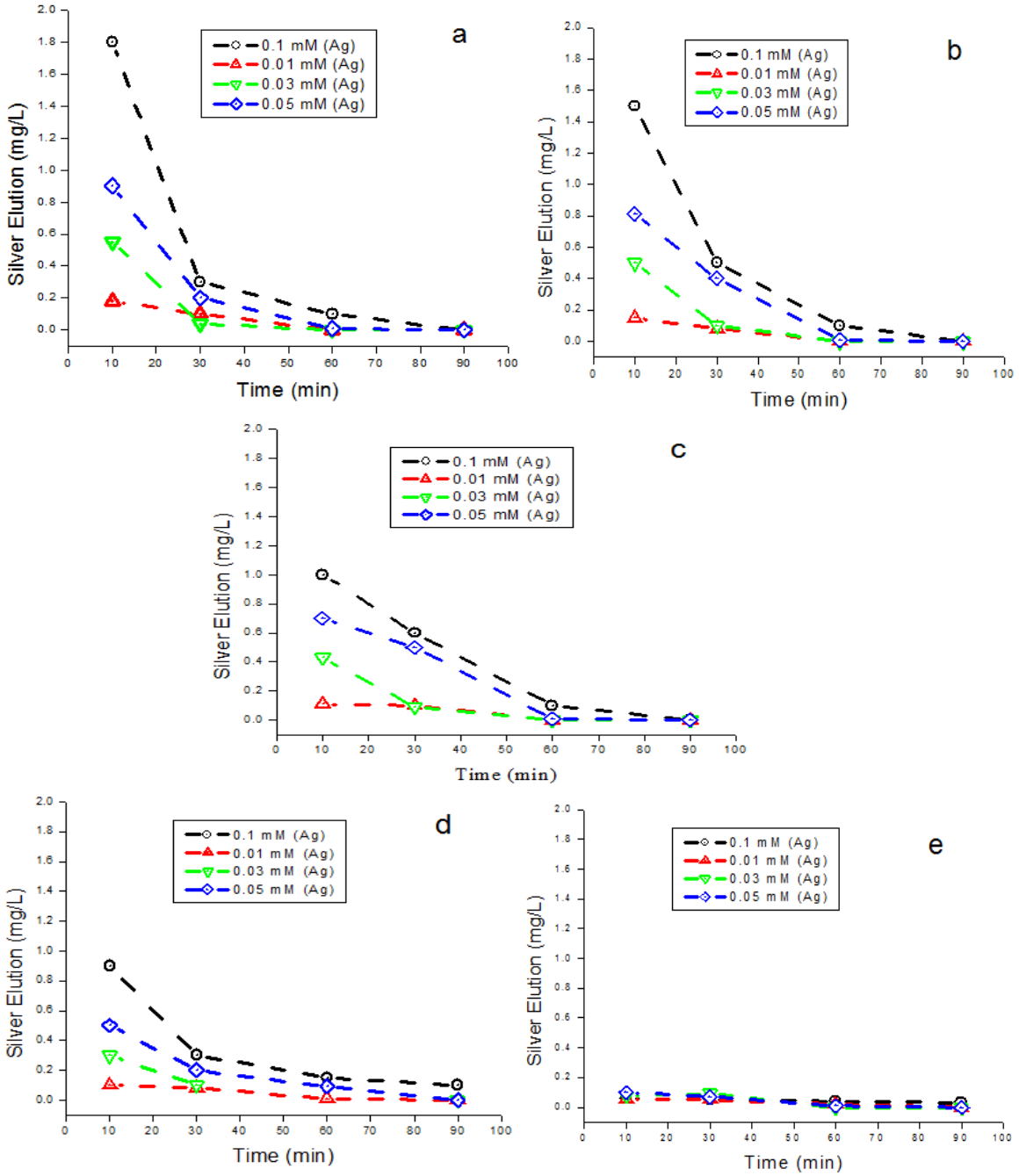


FIGURE 4.7: Amount of silver eluted from Ag nanoparticle-coated substrates in synthetic groundwater: **(a)** zeolite, **(b)** sand, **(c)** fibreglass, **(d)** anion resin, and **(e)** cation resin

4.5 DISCUSSION

The increasing demand for access to safe drinking water and the problems associated with centralised systems in developing countries have made decentralised systems vital for the development of new technologies so as to address these challenges, especially in scattered communities who depend totally on groundwater supplies. Therefore, this study explored the use of nanosized silver impregnated into cost-effective materials locally available in South Africa for its possible use in drinking water disinfection. Using hydrothermal and chemical methods, it was revealed that silver nanoparticles were successfully deposited on sand, zeolite, fibreglass as well as anion and cation resin substrates. However, in the case of the silver coatings, the antibacterial effect was found to reduce with time and the coatings had minimal antibacterial properties after 30 min. The ion elution studies (Figure 4.7) indicated that between 60 % and 90 % of silver loaded onto the Ag/zeolite, Ag/sand, Ag/fibreglass and Ag/anion resin substrates eluted into the treated samples. Consequently the low level of Ag ions remaining in the surface substrates after an elution of 30 min is responsible for the decrease of bacterial removal at this point in time. It was also discovered by Inoue *et al*. (2002) that silver eluted into water when using Ag-zeolite. The elution of silver from the substrates might be due to the weak attachment of silver nanoparticles to the surface substrates. High concentrations of silver ions eluted in water can be toxic to human cells and potentially cause adverse effects in the case of long-term implants. The amount of silver ions eluted from Ag/zeolite, Ag/sand, Ag/fibreglass and Ag/anion resin by far exceeded the recommended limit set by the WHO and US Environmental Protection Agency (USEPA), which is less than 0.10 mg/l in

drinking water used for human consumption. The amount of silver ions eluted from Ag/cation resin complied with the WHO and USEPA limits (WHO, 2006; USEPA, 2006). Conversely, the high levels of ions (95 %) still present in the Ag/cation resin surface after 30 min, would explain the retention of the antimicrobial properties of that surface over the 90 min of the trial. Scanning electron microsopic images revealed that the Ag nanoparticles on the substrates were predominantly small and spherical with EDS confirming the presence of silver peaks, as illustrated in Figure 4.2. The TEM images also revealed spherical-shaped particles that aggregated on the silver nanoparticle substrates, which indicated a particle size distribution ranging between 5 nm and 90 nm. This result was in accordance with the results obtained from the XRD pattern lattice measurement corresponding to the (111) silver plane (Figure 4.4). The crystalline nature of Ag nanoparticles was confirmed by the XRD experimental study. The diffraction pattern observed from the XRD matches the face-centered cubic (fcc) structure of silver as described by previous investigators (Dong *et al*., 2002; Jiang *et al*., 2005). Jana *et al*. (2006) report that the two broad reflection peaks corresponding to (111) and (200) planes indicate that the silver particles are nanocrystals with a cubic symmetry. A similar observation was reported by Pal *et al*. (2007). According to these authors, both the nanoscale size and the presence of a (111) plane of Ag nanoparticles promote the biocidal property of *E.coli* (Jana *et al*., 2006; Pal *et al*., 2007).

The physicochemical analyses of the tested groundwater source fell within the recommended limits of no-risk limit for drinking, except for turbidity which was above the SANS 241 limit (SANS 241, 2006). High turbidity levels are associated with poor water quality and promote the survival of microorganisms (DWAF, 1996). Turbidity

can also protect microorganisms from the effects of disinfection, stimulate the growth of bacteria and give rise to a significant demand for disinfection (WHO, 2006).

Escherichia coli was found to be present at the highest concentration of 3 log cfu/100 mL in groundwater samples collected from Delmas, while the limit recommended by SANS 241 is 0 cfu/100 mL for drinking water intended for human consumption. Although *S. typhimurium*, *S. dysenteriae* and *V. cholerae* were not detected in the groundwater samples, these pathogenic bacteria were seeded into the groundwater samples at a concentration of approximately 3 log cfu/100 mL in order to determine the removal efficiency of the filters. In the first part of this study, the antibacterial efficiency of the silver nanoparticle substrates filter systems was determined, using various concentrations of silver nitrate against *E.coli* as the test organism. The aim of this part of the study was to determine the concentration of silver that would have the most effective antibacterial property against *E.coli*. The results indicated that all the filter systems containing uncoated substrates (Figure 4.5(a–e)) were unable to deactivate *E.coli* from synthetic groundwater when compared to the silver nanoparticle substrates filter systems. The bactericidal effect of silver nanoparticle substrates depended on the concentrations of silver nitrate as well as on the type of substrates. The higher the concentration of Ag added to modify the substrates, the greater was the removal of *E.coli*. In analyses regarding the effect of silver nanoparticles in a size range of 5 nm –90 nm, significant reductions in the *E.coli* population were noted when using filter materials coated with 0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM silver concentrations as compared to the control ($p < 0.05$). The overall results indicated

a significantly higher bactericidal efficiency of 0.1 mM $AgNO_3$ ($p < 0.05$) compared to other silver concentrations, despite the phenomenon of bacterial reappearance that resulted in a progressive increase of bacterial counts in the treated water during the subsequent operation of all the filters, except for the Ag/cation resin filter (Figure 4.5(a–e)). This filter indicated the best performance, which resulted in the complete removal of *E.coli* from synthetic groundwater without the occurrence of bacterial reappearance when the cation resin was loaded with 0.1 mM silver (Figure 4.5(e)). The particle sizes of silver ranging between 1 nm and 100 nm have been reported to have an effect on the antibacterial properties of nanoparticles (Rai *et al*., 2008). Silver nanoparticles cause irreversible damage to the cellular membrane (Sondi & Salopek-Sondi, 2004; Gorgoi *et al*., 2006; Pal *et al*., 2007), which enables the accumulation of nanoparticles in the cytoplasm. The action of silver nanoparticles is due to this damage and not to its toxicity (Pal *et al*., 2007). Previous investigators have pointed out that Ag nanoparticles bind to the outer membrane of *E.coli,* causing the inhibition of active transport, dehydrogenase and periplasmic enzyme activity and eventually the inhibition of RNA, DNA and a decrease in cell permeability, which finally results in cell lysis (Russell & Hugo, 1994; Sondi & Salopek-Sondi, 2004; Morones *et al*., 2005; Zhang & Chen, 2009). While microorganisms carry a negative charge, the Ag ions carry a positive charge, which is crucial for its antimicrobial activity through the electrostatic attraction between the negatively charged cell membrane of microorganisms and positively charged nanoparticles (Dragieva *et al*., 1999; Hamouda *et al*., 2000; Dibrov *et al*., 2002). Yamamoto *et al*. (2002) and Jung *et al*. (2008) indicate that the higher the concentration, the better the antibacterial activity will be. The percentage removal of *E.coli* from synthetic groundwater was

also in accordance with findings by previous investigations using a silver-coated ceramic water filter and silver-coated cylindrical polypropylene filters (Oyanedel & Smith, 2008; Heidarpour *et al*., 2010).

In the second part of the study, the antibacterial activities of silver nanoparticle substrates filter systems prepared from 0.1 mM $AgNO_3$ were investigated against *E.coli*, *S. typhimurium*, *S. dysenteriae* and *V. cholerae* found in or spiked with groundwater samples. The results of this part of the study also indicated a decrease in bacterial concentrations from groundwater samples by all filters (Figure 4.6). While the reappearance of targeted pathogenic bacteria occurred in water treated by Ag/zeolite, Ag/sand, Ag/fibreglass and Ag/anion resin, this phenomenon did not occur in drinking water treated by the Ag/cation resin nanoparticle filter system. This filter produced drinking water that complied with the limit of 0 cfu /100 mL *E.coli* as set by the South African guidelines (DWAF, 1996; SANS 241, 2006). The silver cation resin nanoparticle filter system achieved a 99.9 % removal of all the targeted pathogenic bacteria during the entire 120 min of filter operation. This performance of the Ag/cation resin nanoparticle filter system, namely removing 99.9 % *E.coli*, was also reported by other researchers who used silver nanoparticle filters (Phong *et al*., 2009; Heidarpour *et al*., 2011). The results achieved during the first 10 min of the filter operation with an Ag/fibreglass nanoparticle filter system in removing *E.coli* confirmed those reported by Nangmenyi *et al*. (2009) when these authors used a similar filter system for the purification of drinking water. Taking into account the performance of silver nanoparticle cation resins filter system in removing pathogenic bacteria and their level of silver ion elution in the drinking water, the silver nanoparticle cation resins

filter system can be recommended to be use as an alternative decentralisation technology for the production of safe drinking water for communities depending on groundwater supplies.

4.6 CONCLUSIONS AND RECOMMENDATIONS

Silver nanoparticle-coated substrates were prepared successfully by employing the chemical reduction and hydrothermal synthesis method. Detailed characterisation of the nanoparticles was carried out using SEM, EDS, TEM, Particle Size Distribution and XRD analyses, which confirmed the presence of silver loading onto the substrates. The performance of the substrates as antibacterial water filter systems was checked and no bacteria were detected in the output water when the Ag/cation resin substrate was used as a filter system. Low bacterial removal by Ag/zeolite, Ag/sand, Ag/fibrelass and Ag/anion resin filter systems was observed, which led to the conclusion that these systems are not ideal systems for the disinfection of drinking water. Consequently, silver nanoparticle cation-resin system is the sole drinking-water purification system suggested by this study. This technology can offer complete anti-microbial solutions to rural communities. Further studies will be conducted on the lifespan of the silver nanoparticle cation resins filter system in order for the communities to be informed about the period for which the said system will be valid.

CHAPTER 5

THE EFFECTS OF MATERIAL LOADING AND FLOW RATE ON THE DISINFECTION OF PATHOGENIC MICROORGANISMS USING A SILVER NANOPARTICLE-CATION RESIN FILTER SYSTEM

5.1 ABSTRACT

Waterborne diseases have a negative impact on public health in instances where the available drinking water is of poor quality. Decentralised systems are therefore needed to provide safe drinking water to rural communities. Filters which are appropriate for POU treatment systems, are usually easy to operate and small enough to be used in individual households. In this study, silver nanoparticles deposited on cation resin were prepared and further characterised by using FT-IR and BET. The performance of the silver nanoparticles cation resins filter system was determined by studying the effect of filter bed mass (10 g, 15 g, 20 g) and flow rate (2 mL/min, 5mL/min, 10 mL/min) on breakthrough curves for the removal efficiency of presumptive *Escherichia coli, Shigella dysenteriae, Salmonella typhimurium* and *Vibrio cholerae* from spiked groundwater samples. The results revealed that, as the bed mass increased from 10 g to 20 g, the breakthrough time increased from 29 h to 92 h for *E.coli*, 38 h to 96 h for *S. typhimurium*, 43 h to 98 h for *S. dysenteriae* and 47 h to 101 h for *V. cholerae*. However, when the flow rate increased from 2 mL/min to 10 mℓ/min, the breakthrough time decreased from 60 h to 24 h with regard to *E.coli* removal, 64 h to 38 h in the case of *S. typhimurium*, 65 h to 42 h for *S. dysenteriae* and 70 h to 55 h for *V. cholerae*. The silver

nanoparticles cation resins filter system retained a high removal efficiency of 99.9 % for presumptive *Escherichia coli, Shigella dysenteriae, Salmonella typhimurium* and *Vibrio cholerae* with filter bed mass (10 g, 15 g, 20 g) and flow rate (2 mL/min, 5mL/min, 10 mL/min) before a breakthrough time was achieved. The filter system, packed with 20 g silver nanoparticles cation resins that operated at a flow rate of 10 mL/min, demonstrated a capability to produce 15 L/day at 99.9 % removal rate of all the targeted bacteria. Consequently, the bed mass of the filter system should increase for the filter to produce sufficient water to meet the daily demands of a family.

5.2 INTRODUCTION

According to the WHO/UNICEF (2010), an estimated 672 million people around the world still lack access to safe drinking water. The problem is particularly acute in peri-urban and rural areas where the large majority of the people are typically low-income earners. It appears unlikely that these communities, especially those in rural areas, will receive a potable, piped water supply in the near future. Due to the lack of access to a basic water supply, communities living in rural areas depend on groundwater sources as their main source of water. The WHO (2007) reported that groundwater is contaminated with faecal pathogenic organisms. These pathogens contribute to illness and death from waterborne diseases such as diarrhoea. Children, the elderly and immuno-compromised individuals are those who are the most susceptible to diarrhoeal and other waterborne infectious diseases (Brown, 2007).

To prevent waterborne diseases in developing countries, new approaches to treat and deliver microbiologically safe drinking water to rural communities at household level have to be considered. Decentralised POU systems are possible options for improving water quality for rural communities and could be very beneficial to individuals or families who treat their own water. POU systems are particularly useful in geographically isolated areas where centralised water networks are not feasible. Studies have shown that simple and inexpensive POU systems are capable of reducing diarrhoeal disease and deaths caused by pathogenic organisms found in drinking water (Mintz *et al*., 2001; Sobsey, 2002; Clasen *et al*., 2004).

Filter units, which are appropriate POU treatment systems, are usually easy to operate and small enough to be used in individual households. In filtration systems, sand is the most commonly used filter medium. The contaminant removal effectiveness of the filtration process depends on the type of medium, size, porosity, pore size and available surface area (Sagara, 2000). Filters with small pores will perform better in reducing turbidity and microbiological contaminants to the required standards, but may have very slow flow rates (Mwabi *et al*., 2011). On the other hand, filters with a larger surface area will have a greater flow rate, as there is more space for water to flow through, while filters with small surface areas will have slower flow rates (Franz, 2005). POU filtration technologies include cloth or fibre filters, membrane filters, porous ceramic filters and granular media filters (Brown, 2007). These filters effectively reduce microbes. A number of studies have pointed out that the porous ceramic filtration system is the most promising technology in that it provides an effective barrier against microbial pathogens in

water (Lantagne, 2001; Clasen *et al.*, 2004). However, a recent study that compared a ceramic candle filter with a Silver Impregnated Porous Pot (SIPP) indicated that the latter was more effective than the former, as it produced drinking water that complied with the recommended limits set by the South African National Standard (SANS) 241 Drinking Water Specifications in terms of turbidity and indicator coliform bacteria, regardless of the type of water source (Mwabi & Momba, 2012). Nano-filtration is therefore a typical example of the fact that advanced physical barriers can, by virtue of their small pore size, prevent the passage of bacteria and allow only water to pass through.

Resin materials have been identified for use in a wide range of POU and other water treatment options, usually in the form of ion exchange and reverse osmosis (Janda, 2009; Water Science & Marketing, 2012). Resins are very small plastic beads with a diameter of about 0.6 mm. These beads are porous and contain invisible water, measured as “humidity” or “moisture content”. The structure of the resin is a polymer (like all plastics) to which a fixed ion has been permanently attached. Cation resins are created by attaching negatively charged functional groups to the copolymer structure (Gottlieb, 2000). The fixed ions of this cation exchange resin are sulphonates (SO_3^-) that are attached to the skeleton. Resins have been used in ion exchange and are reported to be very powerful technology that removes impurities from water and other solutions (Gottlieb, 2000). Many industries (nuclear and thermal power stations, semiconductors, computer chip and display panel production, purification plants that remove toxic contaminants from drinking water) use ion exchange resins.

Antimicrobial resins can be fabricated by incorporating silver nanoparticles into the resin. The synthesis of resins containing silver nanoparticles has been well researched (Nath *et al*., 2005; Jana *et al*., 2006). Silver nanoparticles can be incorporated into the resin by employing chemical reduction methods. Recently, some investigations were carried out on the use of resins containing silver or silver nanoparticles for oral and dental applications (Bürgers *et al*., 2009; Fan *et al*., 2011). Researchers have also investigated the usage of polymer composites (Jain & Pradeep, 2005) and polymer microspheres, employing plate and test tube batch methods (Gangadharan, 2010) for water disinfection.

Although a number of studies have been conducted on the removal of bacteria by using resin-silver nanoparticle substrates, there is a paucity of information in the literature on the use of resin-silver nanoparticles in filter systems for removing pathogenic bacteria from drinking water sources. Therefore, this study concentrated on the development of this substrate modified with silver nanoparticles and compared the efficiency of different filter bed masses and flow rates in removing pathogenic bacteria (*Escherichia coli, Vibrio cholerae, Shigella dysenterae and Salmonella typhimurium*) from a polluted groundwater source. The main intention was to dicover if the filter system was capable of producing adequate and safe drinking water so that it could be developed into a POU water treatment system for rural communities.

5.3 EXPERIMENTAL METHODOLOGY

5.3.1 Synthesis and characterisation of silver nanoparticles cation resin substrate

The synthesis and characterisation of silver nanoparticles cation resins are described in Chapter 3. However, in this study the silver nanoparticles cation resins were further characterised by using Fourier transformed infra-red (FT-IR) and Brunauer-Emmett-Teller (BET). The ALPHA Fourier transformed infra-red (FT-IR) spectrometer (Bruker Optics GmbH, Ettlingen, Germany) was also used to identify the surface functional groups on the substrate. The mid-infrared spectra of the samples were dispersed on the attenuated total reflection (ATR) diamond crystal and recorded with a detector at a 4 cm^{-1} resolution of 4000 cm^{-1} -500 cm^{-1} with 32 scans per sample for the identification of bands. Conversely, the Brunauer-Emmett-Teller (BET) (Micromeritics ASAP 2020 V3.00H, Norcross, USA) technique was applied to analyse the surface area and porosity of uncoated resin and resin coated with silver nanoparticles. The samples were automatically degased with nitrogen gas at 150 ^{o}C for 10 h at a flow rate of 60 mL/min, prior to analysis.

5.3.2 Laboratory-scale of silver nanoparticles cationresins filter system

The filter system described in Chapter 4 was also used for this part of the study. Filter systems were packed separately with a known quantity (10 g, 15 g and 20 g) of silver nanoparticles cation resins substrate to evaluate their performance in removing pathogenic bacteria (*E.coli, S. typhimurium, S. dysenteriae* and *V. cholera*) from a groundwater source.

5.3.3 Collection and analysis of the quality of groundwater samples

Groundwater samples from a borehole at Delmas (A7) in the Mpumalanga Province of South Africa were collected between August and October, 2010. Similar procedures described in Chapter 4 for the collection and analysis of water samples was also followed in this part of the study.

In this study *Salmonella spp., Shigella spp. and Vibrio spp*. were not detected in the groundwater samples. As mentioned in Chapter 4, in cases where these organisms were not detected in groundwater samples, the samples were spiked with aliquots of an overnight culture of *S. typhimurium, S. dysenteriae* and *V. cholerae* corresponding to 10^3 cfu/mL of each bacterium being inoculated into 10 L final volumes of groundwater samples following the same procedure described in Chapter 4. The pH, turbidity, temperature and conductivity were measured on site using a pH meter (Metrohm Co. Model 713), a microprocessor turbidity meter (Eutech Instrument Turbidimeter TN-100), thermometer and conductivity meter (HACH, Model 1320), respectively.

5.3.4 Testing the efficiency of the filter system in removing pathogenic bacteria from water

5.3.4.1 Effect of material loading (bed mass) on bacterial removal

The performance of the filter system in removing pathogenic bacteria from groundwater was studied against four different pathogenic bacteria – presumptive *E.coli,* spiked *S. typhimurium, S. dysenteriae* and *V. cholerae* – so as to determine the effect of the filter bed mass on the removal of these pathogenic bacteria. The bed mass of the silver nanoparticles cation resins in the filter system was set at 10

g, 15 g and 20 g. A flow rate of 2 mL/min and an initial influent concentration of 3 log cfu/mL were maintained for the targeted bacteria. The overall performance of the filter system was monitored through breakthrough curves (S-shaped), where the effluent concentration and breakthrough time indicated the removal efficiency of the pathogenic bacteria.

Before the commencement of each run, the filter column was pumped upward with sterile deionised water so as to adjust it and achieve a steady-state flow condition. Thereafter, each type of test-water source contaminated with enteric pathogenic bacteria was pumped continuously through the column in the up-flow mode with a Rainin Dynamax peristaltic pump (Rainin Instrument Co., United States of America). Thereafter, a volume of 50 mL of each of the treated water samples was collected in sterile conical flasks at 1 h intervals. One mL of 15 % sodium thiosulphate was added to stop further disinfection of the samples before determining the bacterial concentration.

Finally, the bacterial concentrations were determined by using serial dilutions in a sterile saline solution and then plating them on a selective medium by applying the spread-plate technique according to the Standard Methods (APHA, 1998). Triplicate plates were used for counting viable cells from each diluted suspension and the average values were utilised.

5.3.4.2 Effect of flow rate on bacterial removal

Flow rates of 2 mL/min, 5 mL/min and 10 mL/min were used to evaluate the performance of the filter system in the removal of pathogenic bacteria from

groundwater. The initial influent concentration and filter bed mass for presumptive *E.coli,* spiked *S. typhimurium, S. dysenteriae* and *V. cholerae* were held constant at 3 log cfu/mL and 20 g, respectively. The experimental procedure and bacterial concentration determination are similar to those described in Section 5.3.4.1.

5.4 RESULTS

5.4.1 Characterisation of silver nanoparticles cation resins

The FT-IR spectra (Figure 5.1) of uncoated and silver nanoparticles cation resins were used to identify functional groups present on the resin beads. The spectra of the substrates were measured within the range of 4000 cm^{-1} -500 cm^{-1} wave numbers. The peaks identified in the FT-IR spectra of uncoated resin were observed at 3373 cm^{-1}, 3248 cm^{-1}, 2927 cm^{-1}, 1638 cm^{-1}, 1558 cm^{-1} and 971 cm^{-1}, while in the coated resin the spectra were observed at 3373 cm^{-1}, 3248 cm^{-1}, 2946 cm^{-1}, 1638 cm^{-1}, 1558 cm^{-1}, 1051 cm^{-1} and 971 cm^{-1}. The BET experimental results of uncoated resin and silver nanoparticles cation resin beads are illustrated in Table 5.1. The analysis was conducted to determine the surface area, pore volume and particle size of the resin. In particular, the surface area and pore volume for uncoated resin were 1.53 m^2/g and 0.11 cm^3/g, respectively, while for resin-silver nanoparticles they were 0.50 m^2/g and 0.021 cm^3/g, respectively.

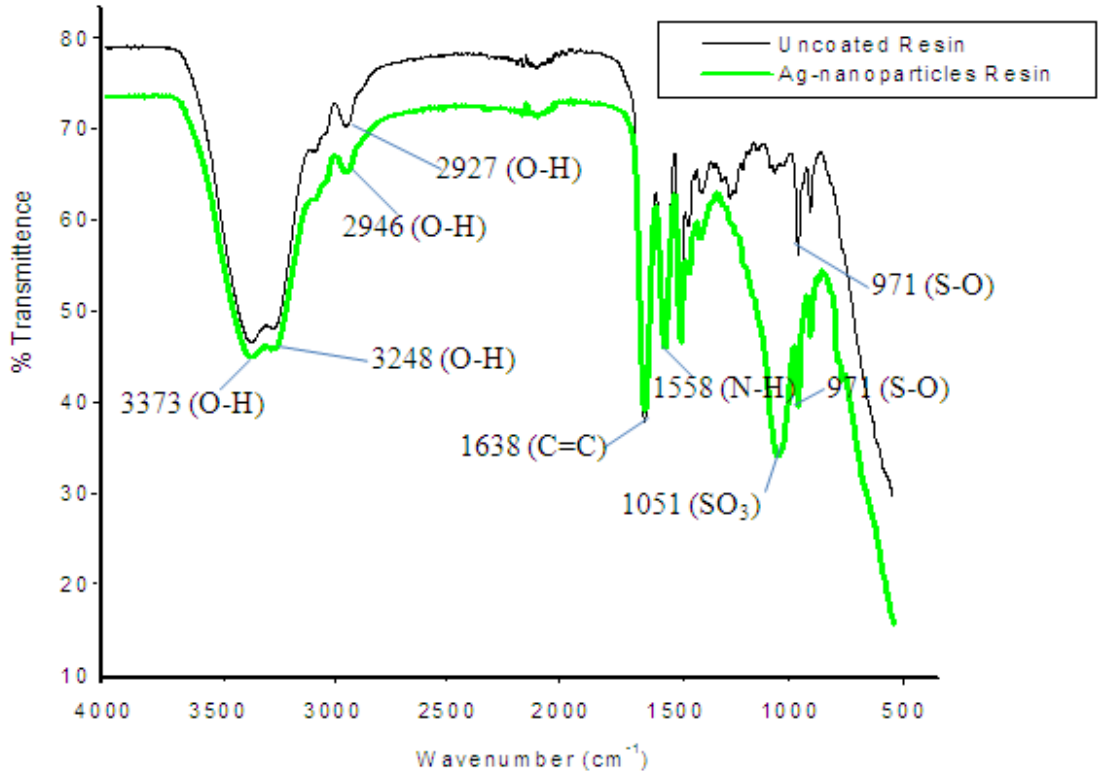


FIGURE 5.1: FT-IR spectrum of uncoated cation resins and cation resins with silver nanoparticles

TABLE 5.1: BET characterisation of resins substrates

Sample	BET Surface Area (m^2/g)	Pore Volume (cm^3/g)	Particle Size
Uncoated Resins	1.53	0.11	0.62 mm
Silver Nanoparticles Resins	3.30	0.00253	20.1 nm

5.4.2 Physicochemical and microbiological quality of groundwater source

The characteristics of groundwater samples collected from a Delmas borehole (A7) are illustrated in Table 5.2. The average physicochemical values of groundwater were 7.47 for pH; 1.86 NTU for turbidity; 23.0 ^{o}C for temperature and 15.17 mS/m for conductivity. Regarding the microbiological quality, only

presumptive *E.coli* (average count: 1.32 × 10^3 cfu/100 mL) was present in those groundwater samples. After spiking, the average *E.coli* concentration in groundwater was found to be 2.15 × 10^3 cfu/100 mL, whereas the average concentrations of spiked *S. typhimurium, S. dysenteriae* and *V. cholerae* were found to be >3.71 × 10^3 cfu/100 mL) prior to treatment.

TABLE 5.2: Characteristics of groundwater sample (average values n=3)

Parameters	Units	Concentration	SANS 241
pH		7.47 ± 0.19	5–9.5
Turbidity	NTU	1.86 ± 0.02	<1
Temperature	^oC	23.0 ± 0.10	15-25
Conductivity	mS/m	15.19 ± 6.23	<70
Presumptive *E.coli*	cfu/100mL	1.32×10^3	0
Concentrations of pathogenic bacteria spiked in groundwater sample			
S. typhimurium	cfu/mL	2.14×10^3	-
S. dysenterae	cfu/mL	3.53×10^3	-
V. cholerae	cfu/mL	5.28×10^3	-

5.4.3 The performance of the silver nanoparticles resin filter

5.4.3.1 Effect of filter bed mass on microbial removal

The effect of filter bed mass (10 g, 15 g and 20 g) on the breakthrough performance at a constant flow rate and initial influent concentrations for presumptive *E.coli, S. typhimurium, S. dysenteriae* and *V. cholerae* maintained at 2 mL/min and 3 log cfu/mL, respectively, was investigated. The results are presented in Figure 5.2. It is evident from this figure that, as the mass substrate increased from 10 g to 20 g, the breakthrough time and the volume throughput

increased from 29 h to 92 h and from 3.5 L to 11.1 L with regard to *E.coli;* from 38 h to 96 h and from 4.6 L to 11.5 L with regard to *S. typhimurium;* from 43 h to 98 h and from 5.2 L to 11.8 L with regard to *S. dysenteriae;* and from 47 h to 101 h and from 5.6 L to 12.1 L with regard to *V. cholerae*. Further particulars of the disinfection performance at various bed masses are summarised in Table 5.3. The results also indicate that, at a filter bed mass of 10 g, 15 g and 20 g, the bacterial concentrations at the breakthrough time of 30 h, 61 h and 93 h were 3 cfu/mL, 9 cfu/mL and 20 cfu/mL with regard to presumptive *E.coli;* 39 h, 64 h and 97 h were 4 cfu/mL, 6 cfu/mL and 13 cfu/mL with regard to *S. typhimurium;* 44 h, 65 h and 99 h were 2 cfu/mL, 3 cfu/mL and 2 cfu/mL with regard to *S. dysenteriae;* and 48h, 71 h and 101 h were 5 cfu/mL, 2 cfu/mL and 6 cfu/mL with regard to *V. cholerae.* After the breakthrough time had been reached, each bacterial count increased in all three filter bed masses (10 g, 15 g and 20 g) until they reached a steady state of approximately 3 log cfu/mL. The rate of removal of presumptive *E.coli*, *S. typhimurium, S. dysenteriae* and *V. cholerae* was 99.9 % in all three filter bed masses until the breakthrough time was reached. However, the removal rate decreased to 20 % when the steady state was reached.

5.4.3.2 Effect of influent flow rate on microbial removal

The effect of flow rate on bacterial removal with a silver nanoparticles cation resin filter was studied by varying the influent flow rate of 2 mL/min, 5 mL/min and 10 mL/min, while the substrate mass packed in the filter and the initial influent concentrations for presumptive *E.coli, S. typhimurium, S. dysenteriae* and *V. cholerae* were maintained at 20 g and 3 log cfu/mL, respectively. Figure 5.3 illustrates the breakthrough curves, which indicate that the removal of pathogenic

bacteria by a silver nanoparticles cation resin filter depends on the influent flow rate. The results indicate that, as the flow rate increased from 2 mL/min to 10 mL/min, the breakthrough time decreased and the volume throughput increased from 61 h to 25 h and from 7.3 L to 15.0 L with regard to *E.coli;* from 64 h to 39 h and from 7.7 L to 23.4 L with regard to *S. typhimurium;* from 65 to 42 h and from 7.8 L to 25.2 L with regard to *S. dysenteriae;* and from 71 h to 56 h and from 8.5 L to 33.6 L with regard to *V. cholerae*. Further particulars of the disinfection performance at various flow rates are summarised in Table 5.3. From the results it was also observed that, at flow rates of 2.5 mL/min and 10 mL/min, the bacterial concentrations at breakthrough time of 61 h, 35 h and 25 h were 9 cfu/mL, 2 cfu/mL and 4 cfu/mL with regard to presumptive *E.coli;* 64 h, 53 h and 39 h were 6 cfu/mL, 2 cfu/mL and 5 cfu/mL with regard to *S. typhimurium;* 65 h, 55 h and 42 h were 3 cfu/mL, 4 cfu/mL and 9 cfu/mL with regard to *S. dysenteriae;* and 71 h, 64 h and 56 h were 2 cfu/mL, 6 cfu/mL and 2 cfu/mL with regard to *V. cholerae.* After the breakthrough time had been reached, each bacterial count increased in all three flow rates (2 mL/min, 5 mL/min and 10 mL/min) until they reached a steady state of approximately 3 log cfu/mL. The rate of removal of presumptive *E.coli*, *S. typhimurium, S. dysenteriae* and *V. cholerae* was 99.9 % at all three filtration rates until breakthrough time was reached. However, the removal rate decreased to 29 % when asteady state was reached.

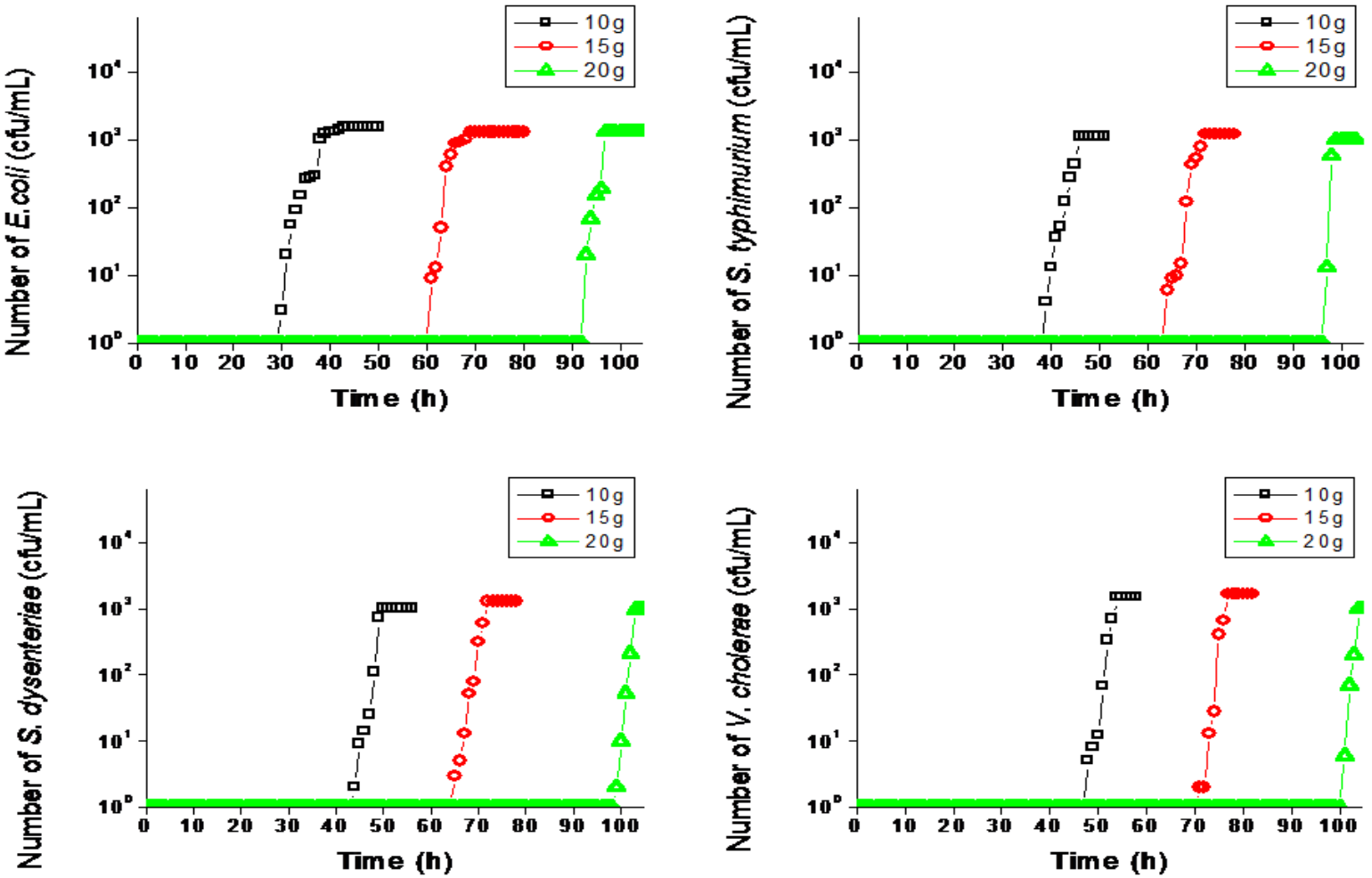


FIGURE 5.2: The effect of various bed masses on the breakthrough performance of pathogenic microorganisms disinfection

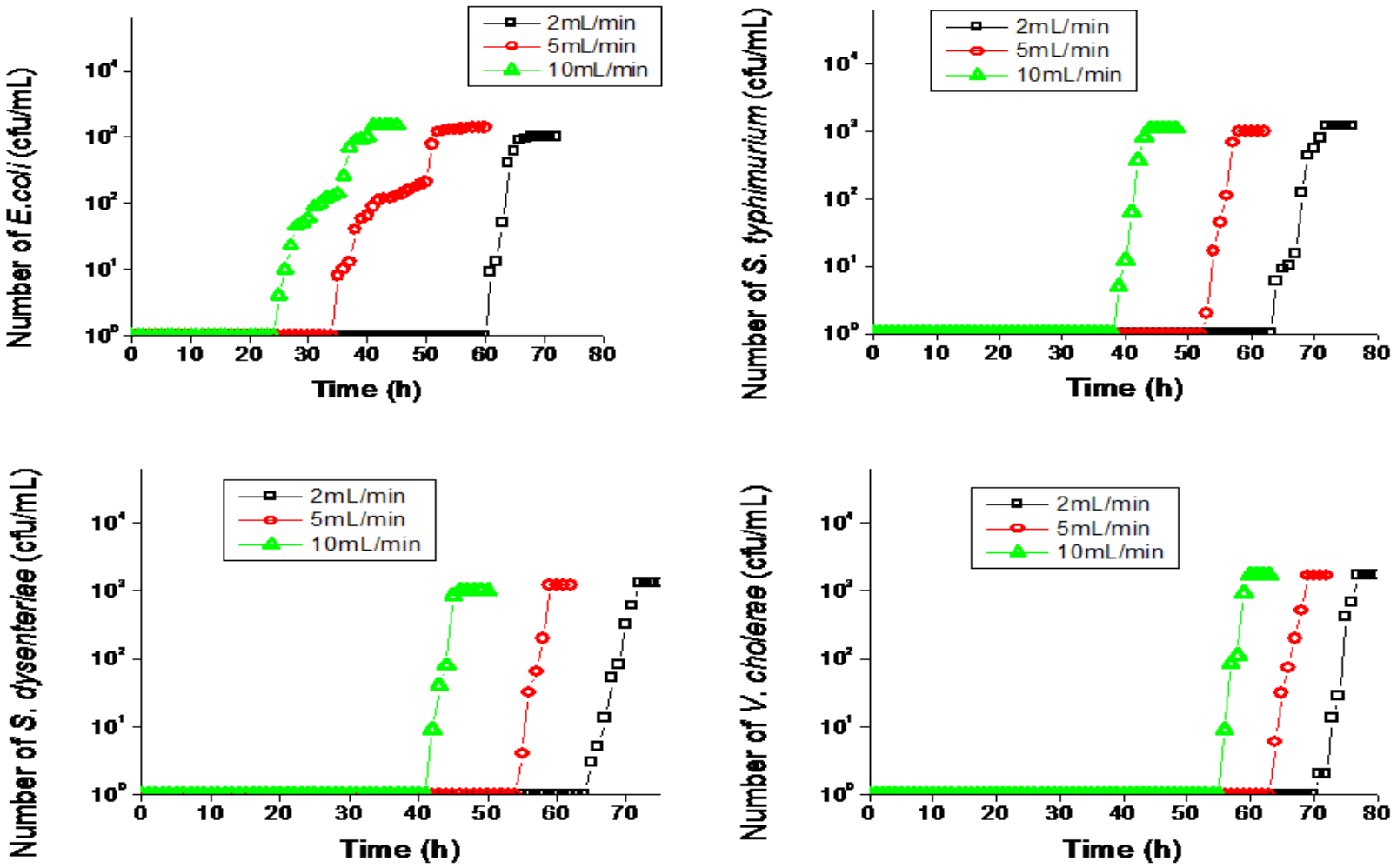


FIGURE 5.3: The effect of various flow rates on the breakthrough performance of pathogenic microorganisms disinfection

TABLE 5.3: Summary of results from a silver nanoparticles resin filter system at breakthrough point for groundwater disinfection

	E.coli			*S. typhimurium*			*S. dysenterae*			*V. cholerae*		
	B_t (h)	**V_t (L)**	**B_c (cfu/mL)**	**B_t (h)**	**V_t (L)**	**B_c (cfu/mL)**	**B_t (h)**	**V_t (L)**	**B_c (cfu/mL)**	**B_t (h)**	**V_t (L)**	**B_c (cfu/mL)**
	Bed mass (g)											
10	29	3.5	3	38	4.6	4	43	5.2	2	47	5.6	5
15	60	7.2	9	63	7.7	6	65	7.8	2	71	8.5	2
20	92	11.1	20	96	11.5	13	98	11.8	2	101	12.1	6
	Flow rate (mL/min)											
2	61	7.3	9	64	7.7	6	65	7.8	3	71	8.5	2
5	35	10.5	2	53	15.9	2	55	16.5	4	64	19.2	6
10	25	15	4	39	23.4	5	42	25.2	9	56	33.6	2

B_t: Breakthrough time (h); V_t: Volume throughput (L); B_c: Bacterial concentration (cfu/mL)

5.5 DISCUSSION

Access to a safe drinking-water supply (improved where necessary) is a basic human right. However, the provision of adequate water services to developing countries currently presents a serious challenge. The problem is especially severe in rural areas where there are limited services available for implementing centralised water treatment systems that require complicated technologies (Peter-Varbanets *et al*., 2009). Consequently, the development of decentralised systems for rural areas is required so as to provide safe drinking water using a POU system. These systems also need to provide at least 25 L per person perhousehold per day. Therefore, this study explored the use of a silver nanoparticles resin filter for the possible treatment of drinking water at the household level.

The results revealed that silver nanoparticles were successfully deposited on cation resin substrates. The peaks identified in the FT-IR spectra of uncoated resin are the O-H stretching vibration of carboxylic acids at 3373 cm^{-1}, 3248 cm^{-1} and 2927 cm^{-1}, the C=C stretching vibration of aromatic bonds observed at 1638 cm^{1}, the N-H bend mode of C-NH_3^+ observed at 1558 cm^{-1}, and the S-O stretching vibration of sulphonates observed at 971 cm^{-1} (Figure 5.1). Such functional groups were also observed by Smith (1999) and Özacar *et al*. (2008). There were some similarities between the peaks observed in the uncoated and the silver nanoparticle substrates (Figure 5.1). However, the O-H stretching vibration of carboxylic acids shifted by 19 cm^{-1} from 2927 cm^{-1} (uncoated resin) to 2946 cm^{-1} (silver-nanoparticle resin), indicating an interaction of the silver nanoparticles with

the resin beads. A similar observation was noted by Jana *et al*. (2006). One new peak at 1051 cm^{-1} was observed in the resin-silver nanoparticle sample, but not in the uncoated resin spectra, representing the sulfonates (-SO_3-) group.

BET analyses revealed a slight increase of the surface area and a decrease in particle size after the addition of silver nanoparticles to the resins (Table 5.1). In particular, the surface area and particle size of uncoated resin were totally different from those of silver nanoparticles cation resins. Larger surface areas and smaller particle sizes were observed with silver nanoparticles resins (3.30 m^2/g and 20.1 nm) as compared with smaller surface areas and larger particle sizes of the uncoated resins (1.53 m^2/g and 0.62 mm). An increase in surface area therefore leads to an increase in reactivity. It was also noticed that the results for the particle sizes of silver nanoparticles resins obtained by BET (20.1 nm) corresponds to the TEM (5-30 nm) and XRD (13 nm) results obtained in Chapter 4.

The physicochemical characteristics of the groundwater source revealed that most of the selected parameters (Table 5.2) were within the recommended limits set by the South African National Standards for the no-risk limit for public health regarding drinking water intended for domestic purposes (SANS 241, 2006). However, the turbidity of the groundwater samples was above the SANS 241 limit, which is < 1 NTU. A high level of turbidity in water is often associated with the possibility of microbiological contamination and makes the disinfection process ineffective in terms of producing safe drinking water (DWAF, 1996; WHO, 2006).

Breakthrough curves for the disinfection of targeted bacteria at a bed mass of 10 g, 15 g and 20 g (Figure 5.2) indicated that resin-silver nanoparticles material is an effective media with an antibacterial property. Consequently, a significant increase in the volume of water treated (volume throughput) and the breakthrough time was observed when the bed mass in the filter increased from 10 g to 20 g (Table 5.3). The increase in breakthrough time and volume throughput with an increase in bed mass could be due to the availability of more active sites for microbial disinfection. Apparently, the number of active sites is proportional to the mass of the disinfecting media, which leads to a delay in the breakthrough time and allows for the processing of a large volume of water (Mohan, 2006; Sivakumar & Palanisamy, 2009). Furthermore, it was noted that the filter bed was exhausted in a shorter period when the filter bed mass was lower (Figure 5.2). The performance of the filter bed in disinfecting microbes depends on the type of microorganisms, which confirms the results of Kawabata *et al*. (1992). These authors pointed out that *E.coli* is more resistant to filtration compared to *Staphylococcus aureus* and *Pseudomonas aeruginosa,* which were removed efficiently by filtration through unwoven cloth coated with a pyridinium-type polymer. However, the amount of water produced with a lower bed mass was approximately the same as the amount produced with a high bed mass (Table 5.3).

The curves indicate that the removal of pathogenic bacteria by silver nanoparticles resin filter depended on the influent flow rate (Figure 5.3). Regardless of bacterial species, the results of this study indicate that as the flow rate increased, the breakthrough time decreased and the volume throughput increased (Table 5.3). It is also important to note that the flow rate had an influence on the disinfection of

targeted bacteria in the filter (Figure 5.3). It was observed that a lower flow rate delayed the breakthrough time and the exhaustion of silver nanoparticle resin filter. Kananpanah *et al*. (2009) reported that, with lower flow rates, more favourable conditions are achieved in the disinfection process, as they provide enough contact time. In their study, which compared the efficiency of five household filtration units (conventional biosand filter, biosand filter with zeolite, bucket filter, ceramic candle filter and SIPP), Mwabi and co-workers (2012) found that the performance of the SIPP filter in removing *E.coli* and faecal coliforms (>5 log10, 99.9 %) was significantly higher than that of the other household water treatment systems ($p < 0.05$). The authors stated that the high performance of SIPP was related to its lower flow rates compared to the other filters (from 0.05 L/h to 2.49 L/h for SIPP, 1 L/h to 4 L/h for CCF, 0.81 L/h to 6.84 L/h for BSF-S, 1.74 L/h to 19.2 L/h and 106.5 L/h to 160.5 L/h for BF). The contact time of pathogenic bacteria with the resin-silver nanoparticle filter was very limited at the higher flow rate, causing a reduction in the removal efficiency of the filter. The results of this study corroborate findings by previous investigators who reported similar observations (Goel, 2005; Vijayaraghavan & Yun 2008; Sivakumar and Palanissamy, 2009). The volume of treated water produced through various flow rates (Table 6.3) indicated that by increasing the flow rate to 10 mL/min the volume throughput also increased. With a lower flow rate of 2 mL/min, it took longer to produce a similar volume of water to that produced when the flow rate increased to 10 mL/min. The highest volume throughput of 15 L, 23 L, 25 L and 34 L was observed at the breakthrough time of 25 h, 39 h, 42 h and 56 h with regard to *E.coli*, *S. typhimurium*, *S. dysenteriae* and *V. cholerae*. However, when using the water samples that were contaminated with *E.coli*, one day was required to

produce only 15 L of safe drinking water with the resin-silver nanoparticle filter. The Department of Water Affairs (DWAF) (2002) in South Africa states that 25 L per person per day is required as a basic water supply for cooking, bathing, washing and for sanitation purposes. According to Howard and Bartram (2003), the minimum quantity of water required for human consumption (drinking and food preparation) is 7.5 L per person per day. *Escherichia coli* was the sole bacterial species found in the intake groundwater source during the study period and if this organism is considered to be the main decisive factor that determines the volume throughput of the resin-silver nanoparticle filter, it appears that the amount of disinfected water (15 L/day) produced by the resin-coated silver nanoparticle filter would be too low to meet the basic requirements of the South African water authorities. However, this volume of water might be sufficient for the consumption of one person at a POU system if the water is used only for drinking and food preparation, as argued by Howard and Bartram (2003).

6.6 CONCLUSIONS AND RECOMMENDATIONS

Further characterisation results using FT-IR and BET confirmed that silver was successfully deposited on the cation resin. Consequently, the silver nanoparticles resins substrate inside the filter was successfully applied in the disinfection of water. The performance of the media in a fixed-bed configuration was mass and flow rate dependent. More than 3 log (99.9 %) of presumptive *E.coli*, *S. typhimurium*, *S. dysenteriae* and *V. cholerae* removal was achieved using a 10 g, 15 g and 20 g filter bed mass by passing contaminated water through, ranging from 3.5 L to 5.6 L at the breakthrough time ranging from 29 h to 47 h with regard

to 10 g bed mass, 7.2 L to 8.5 L at the breakthrough time ranging between 60 h and 71 h with regard to 15 g bed mass and 11.1 L and 12.1 L at the breakthrough time ranging between 92 h and 101 h with regard to 20 g bed mass. After the breakthrough time, the performance of the filter regarding bacterial removal decreased to 20 % when it reached an exhaustion state. On the other hand, a removal rate of 99.9 % for presumptive *E.coli*, *S. typhimurium*, *S. dysenteriae* and *V. cholerae* was achieved using a 2 mL/min, 5 mL/min and 10 mL/min flow rate by passing contaminated water through, ranging from 7.3 L to 8.5 L at the breakthrough time ranging from 61 to 71 h with regard to a flow rate of 2 mL/min, 10.5 L to 19.2 L at the breakthrough time ranging from 35 h to 64 h with regard to a flow rate of 5 mL/min, and 15 L to 33.6 L at the breakthrough time ranging from 25 h to 56 h with regard to a flow rate of 10 mL/min. By using a 20 g filter bed mass at a flow rate of 10 mL/min, 15 litres of clean water was produced per day. This amount of water may be sufficient for only one person per day. Accordingly, a larger scale is required to develop a POU system that can serve an entire family or a small community.

CHAPTER 6

SILVER NANOPARTICLES-RESIN FILTER SYSTEM FOR THE PRODUCTION OF DRINKING WATER FREE FROM VIRUSES AND PROTOZOAN PARASITES

6.1 ABSTRACT

The presence of enteric viruses and protozoan parasites (*Cryptosporidium and Giardia spp.*) in potable water is a potential health risk. In this study, the effectiveness of a silver nanoparticles resin filter system in removing turbidity and inactivating viral indicators and protozoan parasites from contaminated groundwater was assessed. The study was conducted in a flow-through laboratory-scale unit that consisted of resin-silver nanoparticles. Standard methods were employed to measure the level of turbidity and detect the presence of these pathogens before and after treatment. The turbidity of the raw groundwater samples ranged from 1.36 NTU to 2.99 NTU. The results indicated that the overall turbidity reduction performance by the uncoated resin filter ranged from 0.02 NTU to 1.74 NTU (41.8 % and 99.0 %), and from 0.01 NTU to 0.93 NTU (68.9 % and 99.3 %) for the silver nanoparticles resin filter system. During the study period, none of these pathogens were present in the intake water samples collected between October and November 2010 from Delmas (Mpumalanga Province) boreholes. Consequently, groundwater samples were spiked with up to 2 log pfu/mL somatic coliphages, 100 *Cryptosporidium* oocysts and 100 *Giardia* cysts. A comparison between the two filter systems indicated that the silver nanoparticles resin filter system had the highest performance (99.9 %) from the

first to the fifth runs, while the uncoated-resin filter system removed only 1 % of the somatic coliphages from the first to the second cycle of the filter. With protozoan parasites, the highest performance removal (99.9 %) of 2 log oocysts and cysts was observed from the first to the third cycle of the filter runs in the silver nanoparticles resin filter system. With the uncoated-resin filter, only 5 %-12 % of oocysts and 7 %-15 % of cysts were removed during those cycles. Statistical analysis confirmed that the effectiveness of the silver nanoparticles resin filter system in removing the somatic coliphages, *Cryptosporidium* oocysts and *Giardia* cysts, was significantly higher than that of the uncoated-resin filter system ($p < 0.05$). The results of this study revealed that there is a direct relationship between turbidity and viral removal as well as between turbidity and protozoan parasite removal. Strong positive correlations were recorded between the turbidity levels of the treated water and the viral concentrations after the fifth cycle of this filter run ($r = 0.775$) and also between the turbidity levels of the treated water and the protozoan concentrations after the third cycle of this filter run ($r = 0.952$ for oocysts; $r = 0.977$ for cysts). The findings of this study indicate that the high performance of the silver nanoparticles resin filter system can be attributed to the bacteriostatic properties of the silver nanoparticles when compared to the uncoated-resin filter. Therefore, the silver nanoparticles resin filter system has demonstrated that it has the potential to be used as an alternative POU disinfection system.

6.2 INTRODUCTION

Inadequate sanitation services are one of the major problems in developing countries. Approximately 2.6 billion people worldwide do not have access to proper sanitation systems, 565 million of them living in sub-Saharan Africa (WHO/UNICEF, 2010). The majority of these people are members of communities in rural areas, who use poorly constructed pit-latrines, septic tanks and soakways. It has been confirmed that poor sanitation impacts negatively on the quality of groundwater sources (Lehloesa & Muyima, 2000). Groundwater resources are usually exposed to pollution caused by human and animal activities, which include on-site sanitation, cemeteries, waste disposal, feedlots and unsewered settlements (Engelbrecht & Tredoux, 2000). The main health risk associated with groundwater is caused by the microbial pathogens contained in human and animal faeces (Lehloesa & Muyima, 2000; Schijven, 2001; USEPA, 2006). These pathogens are bacteria, viruses and protozoa that originate from faecal pollution; disease-causing organisms that are transmitted through drinking water (Ashbolt *et al*., 2001; Hunter *et al*., 2002). Contaminated groundwater is responsible for diarrhoeal diseases that result in approximately 2.5 million childhood deaths annually in developing countries (WHO, 2002).

Viruses are recognised as agents that contribute to the majority of waterborne disease outbreaks (Szewzyk *et al*., 2000; Ryan *et al*., 2002) and diarrhoeal illness worldwide (Brown, 2007). A report by the United States Centers for Disease Control and Prevention (2008) indicated that between 2003 and 2005, 282 people were affected by waterborne disease outbreaks attributed to viruses in drinking

water in the United States. Somatic coliphages are considered to be heterogenous (Calci *et al*., 1998) and are a suitable tool for monitoring enteric viral indicators from water sources, as they normally originate from faecally contaminated water sources (Havelaar *et al*., 1993; Calci *et al*., 1998; Grabow, 2001; Lucena *et al*., 2004).

Cryptosporidium and *Giardia* are also organisms that are characterised by their ability to survive in polluted water. Both organisms are reported as intestinal parasites that cause gastroenteritis in healthy people, but severe and often fatal disease in individuals living with the human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS) (EPA, 1999**;** Mor & Tzipori, 2008). Many waterborne outbreaks of cryptosporidiosis and giardiasis have been reported (Fox & Lytle, 1996; Craun *et al*., 1998). The largest outbreak caused by *Cryptosporidium* occurred in Brush Creek, Texas, USA, in 1998, because of the use of untreated groundwater (Bergmire-Sweat *et al.*, 1999).

To prevent or reduce the risk of waterborne diseases, many water utilities use disinfection processes, which include chlorination, ozonation and UV. However, studies by Lazarova *et al*. (1999) and Mujeriego and Asano (1999) have indicated that the effectiveness of chlorination and UV in viral inactivation is reduced by turbidity and suspended solids. Yates *et al*. (2006) have reported that adenoviruses are highly resistant to UV disinfection. The use of chlorination and ozone has been linked to harmful disinfection by-products (DBPs) such as trihalomethanes (THMs) that cause cancer (Minear & Amy, 1996; WHO, 2003a; Duncan, 2005). It has been suggested that ozone does not provide a stable

residual, while UV does not have any residual (Solsona & Méndez, 2003). Consequently, these two disinfectants need a secondary disinfectant to provide a residual. Studies have shown *Cryptosporidium* and *Giardia* to be resistant to chemical (chlorine) disinfection (Hambidge, 2001; Li *et al*., 2002; Gitis *et al*., 2005; Arnold & Colford, 2007). However, they can be inactivated by using UV and ozone (DeLoyde, 2007). The removal of *Cryptosporidium* and *Giardia* from water sources in order to provide safe drinking water can also be achieved by filtration (Logan, 2001; Cleary, 2005; DeLoyde, 2007).

Recent studies have indicated that there is an interest in using nanotechnology for water disinfection (Cho *et al*., 2005; Jain & Pradeep, 2005; Li *et al*., 2008). Silver in the form of nanoparticles has been demonstrated to be an effective antiviral agent (Elichiguerra *et al*., 2005; Rogers *et al*., 2008; Zodrow *et al*., 2009). Previous investigators have acknowledged the inactivation of coliphages (You, 2010), monkeypox virus (Rogers *et al*., 2008) and HIV-1 virus (Elechiguerra *et al*., 2005) by silver nanoparticles. The inactivation of viruses by silver nanoparticles impregnated into materials or substrates such as fibreglass (Li, 2010) and polysulfone UF membranes (Zodrow *et al*., 2009) has also been reported.

Silver nanoparticles resins filter system can be employed as an alternative disinfectant method for viral indicators and protozoan parasites. Recently, investigations were carried out on the use of resins containing silver or silver nanoparticles for oral and dental applications (Bürgers *et al*., 2009; Fan *et al*., 2011). To date, no studies have focused on the removal of *somatic coliphages*, *Cryptosporidium* and *Giardia spp*., by means of silver nanoparticles resin filter

systems. Therefore, the present study aimed to determine the effectiveness of a silver nanoparticles resin filter system in removing turbidity, viral indicators (somatic coliphages) and protozoa (*Cryptosporidium* and *Giardia spp*.) from groundwater with a flow-through laboratory-scale system. An uncoated-resin filter system was also included in this study in order to determine whether only silver nanoparticles play a role in removing these pathogens from water, or not.

6.3 EXPERIMENT AND METHODOLOGY

The synthesis and characterisation of silver nanoparticles resins were described in Chapter 4.

6.3.1 Laboratory-scale of silver nanoparticles-resin filter system

The filter systems used in this study consisted of a polyvinyl chloride (PVC) column with a diameter of 2 cm and a length of 20 cm as depicted in Chapter 4 (Figure 4.1). The first filter system was packed with 20 g of silver nanoparticles coated resins and the second filter was packed with 20 g of uncoated resins served as control. The two filter systems were used during this part of the study period to remove turbidity, viral indicators (somatic coliphages) and protozoa (*Cryptosporidium* and *Giardia spp*.) from the groundwater source.

6.3.2 Collection and analysis of the quality of the groundwater samples

Groundwater samples from a borehole at Delmas (A7) in the Mpumalanga Province of South Africa were collected between October and November, 2010. Similar procedures, as described in Chapter 4 for the collection and analysis of

water samples, were also followed; however, in this part of the study, the water samples were collected in a 50 L sterile plastic bucket and transported to the laboratory. The pH, turbidity, temperature, and conductivity were measured on site using a pH meter (Metrohm Co. Model 713), microprocessor turbidity meter (Eutech Instrument Turbidimeter TN-100), thermometer, and conductivity meter (HACH, Model 1320), respectively. The concentrations of the somatic coliphages and protozoan parasites in the intake water sources were determined according to South African National Standard (SANS) 10705-1 (2002) and USEPA Methods 1623 (2001), respectively. In cases where the target organisms were not detected in the groundwater samples, the water sample sources were spiked with these organisms. This was carried out mainly to evaluate the efficiency of the filter systems in removing the targeted pathogens.

The stock culture for the target organism, somatic coliphages, with which the water samples were spiked, was obtained from the Daspoort Wastewater Treatment Plant located in Pretoria, South Africa. The wastewater samples were collected from the influent raw sewage prior to treatment, using sterile 5 L glass bottles. The preparation of the water samples, media and inoculum cultures were carried out according to the ISO (1998). The enumeration of somatic coliphages was completed using a double-agar-layer technique and plaque assay with an *Escherichia coli* strain C (ATCC 700078) nalidixic acid-resistant mutant WG5. Briefly, 1 mL of the inoculum culture and 1 mL of the water sample were successively added to the test tube containing liquefied top-agar. The top-agar was mixed and poured onto solidified bottom agar plates. The plates were then inverted and incubated at 37 ^{o}C overnight. This procedure was completed in

triplicate for each water sample. As a result, plaques were observed as clearly visible circular disc-like clearings through the lawn of bacteria, phages were counted as plaque-forming units (pfu) and the phage concentration was expressed as pfu/mL. Water sample analysis was then performed in triplicate during which the average initial concentration of 1.2 x 10^2 pfu/mL somatic coliphages was spiked into groundwater samples.

During the study period *Cryptosporidium* and *Giardia spp*. were not detected in the groundwater source. Thus, protozoan parasite seeds (oocysts and cysts) were obtained from Wisconsin State Laboratory of Hygiene (USA) and these seeds contained 100 oocysts and 100 cysts which were spiked into 3 L of groundwater sample. Finally, the spiked water samples were thoroughly mixed prior to passing through each filter.

6.3.3 Testing the efficiency of the filter systems

6.3.3.1 Operating conditions of the filter systems

Similar operating conditions as described for the filter systems in Chapter 4 were also used in this part of the study. Each type of groundwater source contaminated with viral indicators and protozoa was conducted separately. The performance of the silver nanoparticles resin filter systems in removing somatic coliphages and protozoan parasites from water sources was evaluated in two phases. In the first phase, silver nanoparticles coated resin and uncoated resin filter systems were tested against somatic coliphages in groundwater samples. In the second phase, the filter systems were replaced with new media of silver nanoparticles coated resin and uncoated-resin for the removal of protozoan parasites (*Cryptosporidium*

parvum and Giardia lamblia) in groundwater samples. For *somatic coliphages,* 100 mL of treated water samples were collected every cycle (3 L sample filtered) in sterile conical flasks. For *Cryptosporidium* and *Giardia spp,* each filter system was fed with a 3 L sample seeded with 100 oocysts and 100 cysts and afterwards the 3 L treated samples were collected in sterile buckets in phases of nine cycles during the filter runs. The removal efficiency was determined by comparing the concentrations of target pathogens before and after treatment.

6.3.3.2 Testing the efficiency of filter systems in reducing turbidity

The performance of the combined silver-resin nanoparticle filter system and uncoated-resin filter system (control) in removing turbidity from groundwater samples was evaluated. The filters were run in phases of nine cycles (filtering 3 L per cycle), monitoring the influent and effluent of the water samples without changing the filter media. A portable turbidity meter (2100P Hach) was used to determine the level of turbidity in water samples before and after filtration. The percentage turbidity reduction achieved by each of the filter systems was calculated using the following equation:

$$\% \text{ Turbidity reduction} = [(\text{turbidity}_{\text{unfiltered}} - \text{turbidity}_{\text{filtered}}) / (\text{turbidity}_{\text{unfiltered}})] \times 100 \quad \text{(Eq. 6.1)}$$

6.3.3.3 Testing the efficiency of filter systems in removing target organisms

The indicator viral concentrations were determined in treated water samples by using a serial dilution in a sterile saline solution, and the enumeration of somatic

coliphages was completed by applying the double-agar-layer technique and plaque assay with an *Escherichia coli* strain C (ATCC 700078) nalidixic acid-resistant mutant WG5. The analyses were performed as described above. For *Cryptosporidium spp.* and *Giardia spp*., USEPA Methods 1623 (USEPA, 2001) were used to detect the presence or absence of (oo) cysts from the treated water samples. After each cycle, the filtrates were concentrated by filtering them through cellulose-acetate filters of 0.8 µm pore size and 142 mm diameter (Sartorius Stedim biotech, South Africa) to capture oocysts (4 µm -7 µm) and cysts (8 µm - 4 µm). The membrane capsule filters were scraped and washed using 50 ml 0.1 % Tween 80 followed by centrifugation at 2000 x *g* to pellet the (oo)cysts. The supernatants were aspirated to 10 mL above the pellet according to the USEPA 1623 method (USEPA, 2001). Briefly, the cysts and oocysts were captured from the remaining 10 mL of the supernatant using Dynalbead anti-*Giardia* and anti-*Cryptosporidium* immunomagnetic antibodies (DEHTEQ, RSA). A 50 µL aliquot of the purified suspension containing the captured oocysts was then air-dried on a well-slide and stained with anti-*G. lamblia* and anti-*C. parvum* monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) (Aqua-Glo G/C Kit, Invitrogen USA). The slides were then examined at 1000 x magnification using an Axio Carl Zeiss epifluorescence microscope (Carl Zeiss, RSA). Subsequently, *Giardia* cysts were identified based on their size and shape, and the intensity of immunofluorescent assay staining (bright green fluorescence of the cyst wall). *Cryptosporidium* oocysts were also identified based on their size and shape. Thereafter, the number of oocysts and cysts were counted in duplicate for each sample. In each series of the experimental study, a control filter that consisted of resin without silver nanoparticles was included. Finally, the percentage removal of

protozoan parasites was calculated according to Brözel and Cloete (1991), using the following equation:

$$\text{The removal \%} = 100 - \frac{\text{Survivor count}}{\text{Initial count}} \times 100 \qquad \text{(Eq. 6.2)}$$

6.3.4 Statistical analysis

All data were analysed statistically using the SPSS computer software, version 11.0. Significance testing was conducted by using one-way analysis of variance (ANOVA) at a 95 % confidence interval. Comparisons were made between the treatment means of each filter system to determine whether there were significant differences in treatments. The tests for relationships between turbidity and somatic coliphages or turbidity and protozoan parasite removal were conducted by using the Pearson Correlation index.

6.4 RESULTS

6.4.1 Characteristics of groundwater source prior to treatment

The physical characteristics of the groundwater samples collected from the Delmas borehole (A7) are presented in Table 6.1. The average values of groundwater sampled were 7.16 for pH, 2.17 NTU for turbidity, 24.0 ^{o}C for temperature and 38.10 mS/m for conductivity. Regarding the microbiological quality, the targeted pathogens (*Somatic coliphages*, *Cryptosporidium* and *Giardia spp*.) were not detected in the groundwater during the study period. In order to conduct this study, the three target pathogenic microoganisms were seeded into the groundwater samples at a concentration of approximately 2 log pfu/mL for somatic coliphages, 100 *Cryptosporidium* oocysts and 100 *Giardia* cycts.

6.4.2 The efficiency of the filter systems in removing turbidity

Table 6.2 illustrates the efficiency of continuous uncoated-resin and silver nanoparticles resin filter systems in removing turbidity. The results of this study revealed a decrease of turbidity in the treated water after passing through the uncoated-resin filter and the silver nanoparticle resin filter. The systems produced drinking water that had water turbidities ranging from 0.02 NTU to 1.74 NTU for the uncoated-resin filter and from 0.01 NTU to 0.93 NTU for the silver nanoparticles resin filter system. The highest and lowest turbidity reduction efficiency for uncoated resin ranged between 41.8 % and 99.0 %, while for the silver nanoparticles resin filter system, the ranges were between 68.9 % and 99.3 %, respectively (Table 6.2). An extended use of the systems up to six cycles resulted in the deterioration of the performance of these systems and a gradual increase in turbidity levels occurred between the third and the sixth cycle. This increase was prominent in the case of the uncoated-resin filter compared to the silver nanoparticles resin filter system. However, statistically the results showed a significant difference ($p < 0.05$) between the two filter systems in the removal of turbidity.

TABLE 6.1: Characteristics of a groundwater sample

Parameters	Units	Concentration	SANS 241
pH		7.16 ± 0.12	5–9.5
Turbidity	NTU	2.17 ± 0.05	<1
Temperature	^{o}C	24.0 ± 0.08	15-25
Conductivity	mS/m	38.10 ± 5.17	<70
somatic coliphages	pfu/mL	0	0
Cryptosporidium spp.		0	0
Giardia spp.		0	0

TABLE 6.2: Continuous turbidity removal efficiency by resin filter systems

Number of filter runs (cycles)	Turbidity (NTU) of groundwater samples before filtration	Turbidity (NTU) and percentage removal after filtration	
		Uncoated resin	Coated resin
1	1.36 ± 0.11	0.02 ± 0.06 **(99.0 %)**	0.01 ± 0.01 **(99.3 %)**
2	1.57 ± 0.07	0.16 ± 0.03 **(89.8 %)**	0.10 ± 0.02 **(93.6 %)**
3	1.64 ± 0.10	0.23± 0.04 **(85.9 %)**	0.17 ± 0.05 **(89.6 %)**
4	1.88 ± 0.05	0.39 ± 0.08 **(79.3 %)**	0.26 ± 0.02 **(86.2 %)**
5	1.95 ± 0.03	0.73 ± 0.10 **(62.6 %)**	0.39 ± 0.09 **(80.0 %)**
6	2.17 ± 0.05	1.00 ± 0.16 **(53.9 %)**	0.57 ± 0.07 **(73.7 %)**
7	2.95 ± 0.23	1.40 ± 0.21 **(52.5 %)**	0.80 ± 0.14 **(72.9 %)**
8	2.98 ± 0.15	1.55 ± 1.87 **(48.0 %)**	0.88 ± 0.10 **(70.5 %)**
9	2.99 ± 0.31	1.74 ± 0.96 **(41.8 %)**	0.93 ± 0.08 **(68.9 %)**

6.4.3 The efficiency of silver nanoparticles resin filter systems in removing the viral indicator (somatic coliphages)

The results of the disinfection assay on somatic coliphages with regard to the use of the silver-resin nanoparticle filter system and the uncoated-resin system (control) are presented in Table 6.3. The initial influent somatic coliphages was approximately 120 pfu/mL counts. The filter system with uncoated resin (control) was inefficient in the removal of somatic coliphages. Consequently, the viral indicators were detected in all the filtered water samples throughout the study period with counts ranging between 118 pfu/mL and 120 pfu/mL. However, the silver nanoparticles resin filter system revealed a complete (99.9 %) removal of the viral indicator for the first 5 h of the filter run, and then a decrease in the removal efficiency occurred for the remaining hours of the study period. Somatic coliphage counts in the treated water, therefore, ranged from 3 pfu/mL to 39 pfu/mL during

the 6 h to 9 h of the filter run. The results demonstrated a significant difference in the reduction of the somatic coliphages count ($p < 0.05$) in the silver nanoparticles resin filter system, as compared to the control.

The turbidity and viral reduction were correlated in order to determine the relationship between the turbidity level and the concentration of somatic coliphages in the treated water samples (Figure 6.1). The results indicated that there was a weak positive correlation between turbidity and somatic coliphage removal by using the uncoated resin filter system (r = 0.601), while moderate positive correlations were recorded for silver nanoparticles resin filter system (r = 0.764).

TABLE 6.3: Average percentage somatic coliphage removal efficiency of resin filter systems in groundwater

Number of filter runs (cycles)	Somatic coliphage (pfu/mL) and removal efficiency (%) after filtration	
	Coated resin	**Uncoated resin**
Groundwater samples spiked with 120 pfu/mL (2 log) before filtration		
1	0 ± 0.01 **(99.9 %)**	118 ± 0.78 **(1 %)**
2	0 ± 0.16 **(99.9 %)**	119 ± 0.66 **(1 %)**
3	0 ± 1.02 **(99.9 %)**	120 ± 0.32 **(0 %)**
4	0 ± 0.91 **(99.9 %)**	120 ± 0.36 **(0 %)**
5	0 ± 0.45 **(99.9 %)**	120 ± 0.41 **(0 %)**
6	3 ± 0.75 **(97 %)**	120 ± 1.08 **(0 %)**
7	8 ± 0.99 **(93 %)**	120 ± 1.25 **(0 %)**
8	15 ± 0.03 **(87 %)**	120 ± 0.89 **(0 %)**
9	39 ± 0.09 **(68 %)**	120 ± 0.42 **(0 %)**

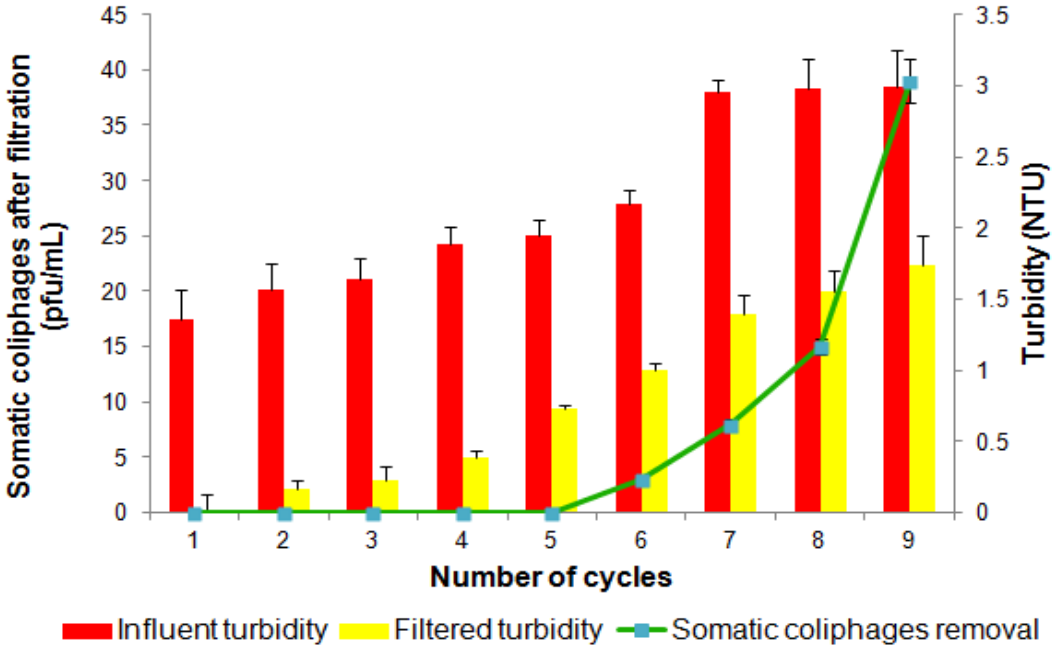


FIGURE 6.1: Effect of turbidity of on the removal of somatic coliphages with silver nanoparticles resin filter system

6.4.4 The efficiency of the silver nanoparticles resin filter system in the removal of protozoan parasites

The efficiency of the silver nanoparticles resin filter system and the uncoated-resin filter system in removing (oo)cysts spiked in the groundwater source during the study period was investigated (Table 6.4). The performance rate of the silver nanoparticles resin filter system in oocysts removal ranged between 64 % and 99.9 %, while the uncoated-resin filter only achieved removal efficiency rates ranging from 0 % to 12 %. In terms of cyst removal, the silver nanoparticles resin filter system was able to achieve a removal efficiency ranging between 75 % and 99.9 % and the uncoated-resin filter system indicated a removal efficiency ranging between 1 % and 15 %. The results revealed that the performance of the resin silver nanoparticles filter system was significantly higher ($p < 0.05$) than that of the uncoated-resin filter system in removing *Cryptosporidium* and *Giardia*.

The turbidity reduction and protozoan parasite reduction were correlated to determine the relationship between the turbidity level and the concentration of oocysts and cysts in the treated water samples (Figure 6.2). The results indicated strong positive correlations between turbidity removal and oocyst reduction (r = 0.956) with silver nanoparticles resin filter and moderate correlation (r = 0.789) with uncoated resin filter. Furthermore, a strong correlations were also found between the turbidity levels and the cyst removal (uncoated filter: r = 0.869; silver nanoparticles resin filter: r = 0.965).

TABLE 6.4: Protozoan removal efficiency in groundwater by using resin filter systems

Number of filter runs (cycles)	Coated resin		Uncoated resin	
	Cryptosporidiumsp p. (oocysts)	*Giardia spp.* (cysts)	*Cryptosporidium spp.* (oocysts)	*Giardia spp.* (cysts)
	After filtration	**After filtration**	**After filtration**	**After filtration**
Groundwater samples spiked with 100 seeds oocycts and cycts/3L before filtration				
1	0 ± 0.05 **(99.9 %)**	0 ± 0.03 **(99.9 %)**	88 ± 0.11 **(12 %)**	85± 0.21 **(15 %)**
2	0 ± 0.09 **(99.9 %)**	0 ± 0.33 **(99.9 %)**	91 ± 0.19 **(9 %)**	89 ± 0.42 **(11 %)**
3	0 ± 0.11 **(99.9 %)**	0 ± 0.15 **(99.9 %)**	95 ± 0.08 **(5 %)**	93 ± 0.21 **(7 %)**
4	4 ± 0.85 **(96 %)**	1 ± 0.61 **(99 %)**	98 ± 0.52 **(2 %)**	94 ± 0.33 **(6 %)**
5	7 ± 1.23 **(93 %)**	5 ± 0.63 **(95 %)**	99 ± 10.01 **(1 %)**	97 ± 9.05 **(3 %)**
6	11 ± 2.31 **(89 %)**	10 ± 3.41 **(90 %)**	99 ± 11.21 **(1 %)**	98 ± 9.08 **(2 %)**
7	20 ± 5.15 **(80 %)**	15 ± 1.66 **(85 %)**	99± 12.09 **(1 %)**	98 ± 10.31 **(2 %)**
8	28 ± 7.42 **(72 %)**	23 ± 4.016 **(77 %)**	99 ± 10.16 **(1 %)**	99 ± 9.21 **(1 %)**
9	36 ± 5.23 **(64 %)**	25 ± 4.07 **(75 %)**	100 ± 13.44 **(1 %)**	99 ± 8.24 **(1%)**

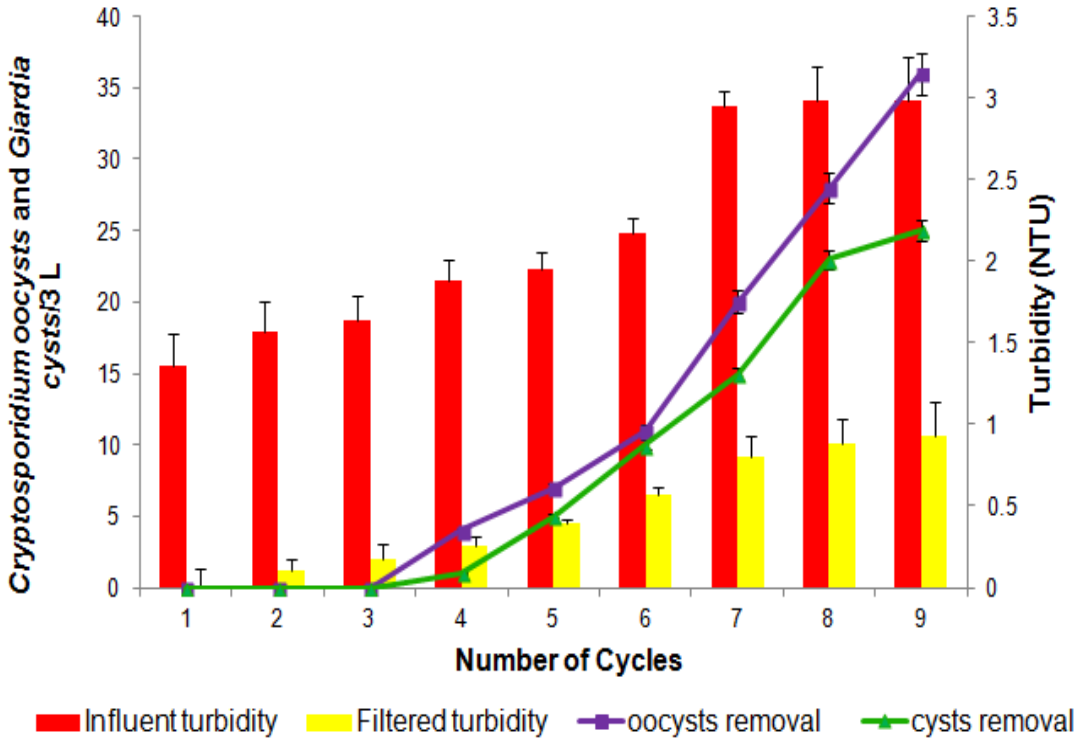


FIGURE 6.2: Effect of turbidity of on the removal of oocysts and cysts with silver nanoparticles resin filter system

6.5 DISCUSSION

It is of vital importance to provide adequate and safe drinking water for rural communities in developing countries, as safe drinking water plays a significant role in preventing a health risk due to waterborne pathogens. With the exception of the turbidity values, which were found to be above the limits (0-1 NTU) set by South African National Standard 241, the physical characteristics of the groundwater samples collected from the Delmas borehole revealed that the quality of this type of water source supply was within the recommended limits for no-risk in terms of pH (5–9.5), temperature (≤ 25 °C), and conductivity (<70 mS/m) (DWAF, 1996; SANS 241, 2006) (Table 6.1). High levels of turbidity enhance the survival of

microorganisms in water (DWAF, 1996) and consequently reduce the effectiveness of the disinfection process (WHO, 2006). During the study period, the Delmas borehole did not contain the targeted pathogens (somatic coliphages, *Cryptosporidium* and *Giardia spp*.). This was good news for the community living in this area, where a number of outbreaks of waterborne illnesses such as cholera and diarrhoea have occurred during rainy seasons in the past (Groenewald & Dibetle, 2005; Masinga, 2005)

The evaluation of the filters' performance in removing turbidity from groundwater is very important, as turbidity is a vital parameter when assessing water quality and determining an appropriate treatment process (Page *et al*., 2006). Although the turbidity of the intake water was not excessively high, it has been reported that a turbidity level above 1 NTU impacts negatively on the performance of the disinfection process (WHO, 2006a). The results of the present study revealed that both the uncoated-resin filter system and the silver nanoparticles resin filter system were able to remove turbidity from groundwater samples up to the recommended limits during the first hours of the study period (Table 6.2). However, extended filtration runs (up to six cycles = 18 L) of raw water source samples reduced the performance of the filters. It is well known that the turbidity of water can also protect microorganisms from the effects of disinfection, stimulate the growth of bacteria and give rise to a significant demand for disinfection (Jiang *et al*., 2005). The silver nanoparticles resin filter system was found to be more efficient than the uncoated-resin filter system in turbidity reduction by producing treated water with less than 1 NTU throughout the filter runs (filtering 27 L). This could be related to its physical retention capability of the smaller particle sizes of

resin-silver nanoparticles (20.1 nm) compared to the larger particle sizes (0.62 mm) of the filter media found in uncoated resins. The present study corroborates findings by previous investigators who found that filter media (such as coarse media) of more than 0.45 mm were less efficient than finer media with a smaller particle size in removing bacteria, turbidity and colour (<1.7 mm) (Muhammad *et al*., 1996; Jenkins *et al*., 2011).

The larger particle size of the uncoated-resin filter also explains the inability of this filter to achieve the total removal of somatic coliphages from groundwater samples during the experimental study (Table 6.3). A comparison of the two filter systems indicated that the silver nanoparticles resin filter system had the highest removal performance (99.9 %) from the first to the fifth runs (filtering15 L) while the uncoated-resin filter only removed 1 % of somatic coliphages from the first to the second cycles of the filter (filtering 6 L) (Table 6.3). Statistical analysis confirmed that the effectiveness of the resin-silver filter system in removing somatic coliphages was significantly higher ($p < 0.05$) than that of the uncoated-resin filter system. A removal of 2 log of viruses has been reported by previous investigators when using nanofiltration membrane in their studies (Madaeni *et al*., 1995). Zodrow *et al*. (2009) pointed out that silver nanoparticle substrates improved the removal of viruses by filtration. Other investigators also demonstrated that silver nanoparticles completely inactivated viruses (Elichiguerra *et al*., 2005; Kim *et al*., 2008; Rogers *et al*., 2008). The present study also indicated that the enhanced performance of the silver nanoparticles resin filter system was limited to the fifth cycle of the filter runs. Beyond these runs, this filter became incapable of producing water that complied with the recommended limits (zero coliphages). The

results of our study identified a direct relationship between turbidity reduction and viral removal (Figure 6.2). This clearly explains the strong positive correlations found between the turbidity level of the treated water and the viral concentrations after the fifth cycle of these filter runs (r = 0.775). The inefficiency of the silver nanoparticles resin filter system beyond the fifth cycle resulted in viral concentrations ranging from 3 pfu/mL to 39 pfu/mL in the filtered water, indicating a reduction of only between 68 % and 97 %, respectively. The poor performance of the silver nanoparticles resin filter system from the fifth cycle onwards might be due to the depletion of silver ions from the resin substrates over time (Mpenyana-Monyatsi *et al*., 2012). It has been revealed that an increase in the turbidity of water can also protect microorganisms from the effects of the disinfection process (Jiang *et al*., 2005, Mann *et al*., 2007). Health Canada (2011) reported that an increase in the turbidity of filter effluent can signal the potential for increasing the passage of unwanted organisms, even if the turbidity in the effluent is less than 1.0 NTU.

A lower efficiency of the uncoated-resin filter was also noted during the removal of protozoan parasites (Table 6.4). The results of the silver nanoparticles resin filter system exhibited the highest performance (99.9 %) of 2 log oocysts and cysts removal from the first to the third cycle of the filter runs (filtering 9 L), while the uncoated-resin filter removed only 5 % to 12 % of oocysts and 7 % to 15 % of cysts during the same cycles (Table 6.4). Statistical analysis also confirmed the significant effectiveness of the silver nanoparticles resin filter system in removing protozoan parasites as compared to the uncoated-resin filter system ($p < 0.05$). The results of this study agreed with findings by Lantagne (2001), who reported a

reduction of more than 2 log of *Cryptosporidium* oocysts and *Giardia* cysts by silver-impregnated ceramic filters. However, the detection of oocysts and cysts beyond the first three runs indicated the incapability of the silver nanoparticles resin filter system to produce a final water sample that complied with the recommended limits (zero protozoan parasites). This also elucidated the strong positive correlations between the turbidity level of the treated water and the protozoan concentrations that occurred after the third cycles of these filter runs (r = 0.952 for oocysts) and (r = 0.977 for cysts). LeChevallier and Norton (1992) found that for every log removal of turbidity, a 0.89 log removal *Giardia* and *Cryptosporidium* was achieved in the relationship between turbidity and parasites. Nevertheless, in the present study, the reduction of protozoan parasites was higher than the reduction reported by Fogel *et al*. (1993), demonstrating a removal of 93 % of *Giardia* cysts and 48 % of *Cryptosporidium* cysts using a full-scale operational slow-sand filter. Previous researchers also reported that an increase in water turbidity level from 0.2 NTU also increased the concentration of *Giardia* cysts in water (Cleasby, 1983; Logsdon *et al*., 1985). These findings also confirm the results of the present study.

6.6 CONCLUSSION AND RECOMMENDATION

During the course of this study, the silver nanoparticles resin filter system and the uncoated-resin filter system were assessed based on turbidity, somatic coliphages, *Cryptosporidium* oocysts and *Giardia* cysts removals. The performance of silver nanoparticles resin filter system in turbidity reduction was higher as compared with uncoated-resin filter system by producing treated water

with less than 1 NTU throughout the filter runs (filtering 27 L). The results of this study also demonstrated the superiority of the silver nanoparticles resin filter system in removing the somatic coliphages, *Cryptosporidium* and *Giardia spp*. from contaminated groundwater sources. No viral indicators and protozoan parasites were noted in the treated water during the first five (filtering 15 L) and the first three cycles (filtering 9 L) of the filter runs, respectively. Beyond these runs, this filter was found to be incapable of producing safe drinking water. This study therefore recommends the treatment of drinking water up to three runs. It is also recommended that the silver nanoparticles resin filter system be developed on a larger scale and its performance in removing pathogenic organisms also be investigated before the community uses it.

CHAPTER 7

PERFORMANCE OF SILVER NANOPARTICLES-CATION RESIN FILTER IN INHIBITING BACTERIAL REGROWTH AND BIOFILM FORMATION IN DRINKING WATER SUPPLY SYSTEMS

7.1 ABSTRACT

Drinking water contamination caused by bacterial regrowth and biofilm formation during storage or in distribution systems remains one of the difficulties experienced with water supplies. In this study, a groundwater source was used to evaluate the impact of a silver nanoparticle cation resin filter system in inhibiting bacterial regrowth and biofilm formation in potable water distribution systems using a laboratory-scale unit. The effectiveness of the cation resin-silver was compared to that of the chlorine. Plastic-based pipe materials (polyvinyl chloride-PVC) and metallic-based pipe materials (galvanised steel-GS) which are generally used in drinking water distribution systems were used for the purpose of this study. The disinfection process was carried out using *ca* 2.5 mg/L free chlorine and *ca* 0.030 mg/L free silver ions in resin-silver nanoparticles. Non-disinfected water was added as a control water system. The evaluation of the process relied on coliforms and heterotrophic plate count bacteria. Scanning electron microscopy was utilised to view the regrowth and formation of biofilm on PVC and GS piping material. Results indicated the potential for bacterial regrowth as, within the first day, the adhesion of the heterotrophic plate count bacteria on both piping materials was apparent in untreated and chlorinated water systems, with approximately an

average count of 1 log cfu/cm^2 of heterotrophic bacteria attached to the piping materials. The use of silver nanoparticle cation resin water system resulted in the inhibition of the growth of heterotrophic bacteria on pipe materials. However, the biofilm formation occurred on the surface of the pipe materials between day 28 and day 30, due to the depletion of silver ion residual. In conclusion, silver nanoparticles resin s preserve the integrity of potable water distribution systems by inhibiting the phenomon of bacterial regrowth and bacterial adhesion on plastic-based and metallic-based pipe materials. This study therefore recommends the use of silver nanoparticles resins to control bacterial regrowth and biofilm formation in drinking water systems.

7.2 INTRODUCTION

The distribution system is referred to as a complex network of piping materials that are used to deliver potable water from water treatment plants to the consumers. Pipes used in the distribution systems are generally made of cement, asbestos cement, plastic (polyvinyl chloride-PVC, high density polyethylene-HDPE), steel, iron (EPA, 2008), copper and galvanised steel (Percival *et al*., 1998). The survival and regrowth of microorganisms in the distribution system has been reported to depend on biological and physicochemical factors such as nutrients, carbon, phosphorus, pipe material, water temperature and disinfection residual (LeChevallier *et al*., 1996; Momba & Binda, 2002; Payment & Robertson, 2004). Previous investigators have reported that piping materials such as plastic and copper may contribute to microbial regrowth by releasing chemical compounds such as copper, iron, phosphorus ions, and organic compounds (Lehtola *et al*.,

2004; Yu *et al*., 2010). Studies have also indicated that the growths of biofilms are lower in plastic materials than those found in iron, steel or cement materials (Camper *et al*., 1996; Niquette *et al*., 2000; Mains, 2008).

It is well known that the water that enters the distribution system must be microbiologically safe. This goal can be achieved by using disinfection processes such as chlorination, chloramination, ozonation and UV irradiation. Despite the progress made in science and engineering to render drinking water free from waterborne pathogens, various studies have repeatedly reported the problem of bacterial regrowth and biofilm formation in potable water supplies (LeChevalier *et al*., 1996; Momba & Binda, 2002, Momba & Makala, 2004). It has been alleged that microorganisms embedded in matrices (extracellular polymer substances) in biofilms are protected from the disinfectants in the distribution system (Momba & Binda, 2002; Payment & Robertson, 2004). These organisms predominantly consist of bacteria, while protozoa, fungi, algae and viruses may be present in significant numbers (Szewzyk *et al*., 2000; Kerr *et al*., 2003). The presence of biofilms in distribution systems has been reported to cause the deterioration of drinking water quality and also contribute to bacterial regrowth, an increase in the demand for disinfectants and corrosion in the piping materials, resulting in taste and odour problems (Astier *et al*., 1995; Camper *et al*., 1999; Hu *et al*., 1999; Lu *et al*., 1999; Critchley & Fallowfield, 2001). These limitations, therefore, motivate the need to explore alternative methods for controlling biofilms and microbial regrowth in the distribution system.

Silver ion is known to demonstrate antimicrobial efficiency in controlling *Legionella* bacteria in the water distribution systems of hospitals (Liu *et al*., 1994; Lin *et al*., 1996; Biurrun *et al*., 1999). Silver binds to the bacterial cells, damaging and preventing them from performing their function and as a result the cells die due to specific bacterial DNA and RNA being penetrated (Klassen, 2000; Ovington, 2004; Rai *et al*., 2009). Silver has also been used to control biofilm formation in medical catheters (Gabriel *et al*., 1996; Cicalini *et al*., 2004; Gentry & Cope, 2005) and also in water distribution systems (Silvestry-Rodriguez *et al*., 2008). Recently, researchers have demonstrated that silver nanoparticles are capable of inhibiting planktonic and biofilm bacteria (Choi, 2009; Climent, 2009). However, technical requirements may compel nanoparticles to be loaded onto a substrate for use in distribution systems in order to control biofilms.

Resins provide a good matrix on which to load nanoparticles. A study conducted by Beyth *et al*. (2010) used cross-linked quaternary ammonium polyethylenimine nanoparticles incorporated in a resin composite to prevent biofilm in dental orals. Mpenyana-Monyatsi *et al*. (2012) demonstrated the use of resins to load silver nanoparticles and the prepared material exhibited good antimicrobial properties. There is a paucity of information in open literature regarding the application of silver-resin nanoparticles in bacterial regrowth and biofilm inhibition in drinking water distribution systems. This study therefore investigated the application of silver nanoparticles cation resin as an alternative disinfectant in inhibiting bacterial regrowth and biofilm formation in the drinking water system. Piping materials such as PVC and GS were used to explore the impact of silver nanoparticles cation resin on bacterial regrowth and biofilm formation in a laboratory water supply scale

unit. Since the chlorination process is the most common and effective disinfectant method used in the treatment of drinking water, it was compared to that of silver nanoparticles cation resin filter system for the inhibition of biofilm.

7.3 EXPERIMENTAL AND METHODOLOGY

The synthesis and characterisation of silver nanoparticles cation resins were described in Chapter 4.

7.3.1 Groundwater collection

Groundwater samples were collected in 3 L ×50 L sterile polyethylene drums from the Delmas borehole (A7) in the Mpumalanga Province of South Africa. The study was conducted between January and March, 2011 and the water samples were collected three times during this period. It is important to note that during the study period, this groundwater supply was used by the community without prior treatment. The water did not comply with the drinking water standards set by SANS: 241 (2006) and DWAF (1996).

7.3.2 Laboratory-scale test unit

The filter system was made of a polyvinyl chloride (PVC) column, with a diameter of 2 cm and a length of 30 cm. Each filter was packed with 40 g of silver nanoparticle-coated resins as illustrated in Chapter 4. The biofilm study was performed by using a modified Pedersen device described by Momba and co-workers (1998, 1999, 2000, and 2002). Figure 7.1 depicts the laboratory-scale system used in the study. Three laboratory-scale systems were used during the

study period (raw intake water, chlorinated water and resin-silver nanoparticle disinfected water). The system for the raw intake water and chlorinated water consisted of 1 L × 50 L polyethylene drum, which was attached to a peristaltic pump, a Pedersen device (26 cm x 10 cm x 3 cm) and a tap, while the system for the resin-silver nanoparticle water consisted of 1 L × 50 L polyethylene drum, which was attached to a peristaltic pump, a silver nanoparticles resin filter system, a Pedersen device (26 cm x 10 cm x 3 cm) and a tap. All components were connected using latex tubing (8 mm diameter, 4 m length), which allowed for a continuous circulation of the water into the system. The flow rate of all the water samples through the system was 300 mL/h.

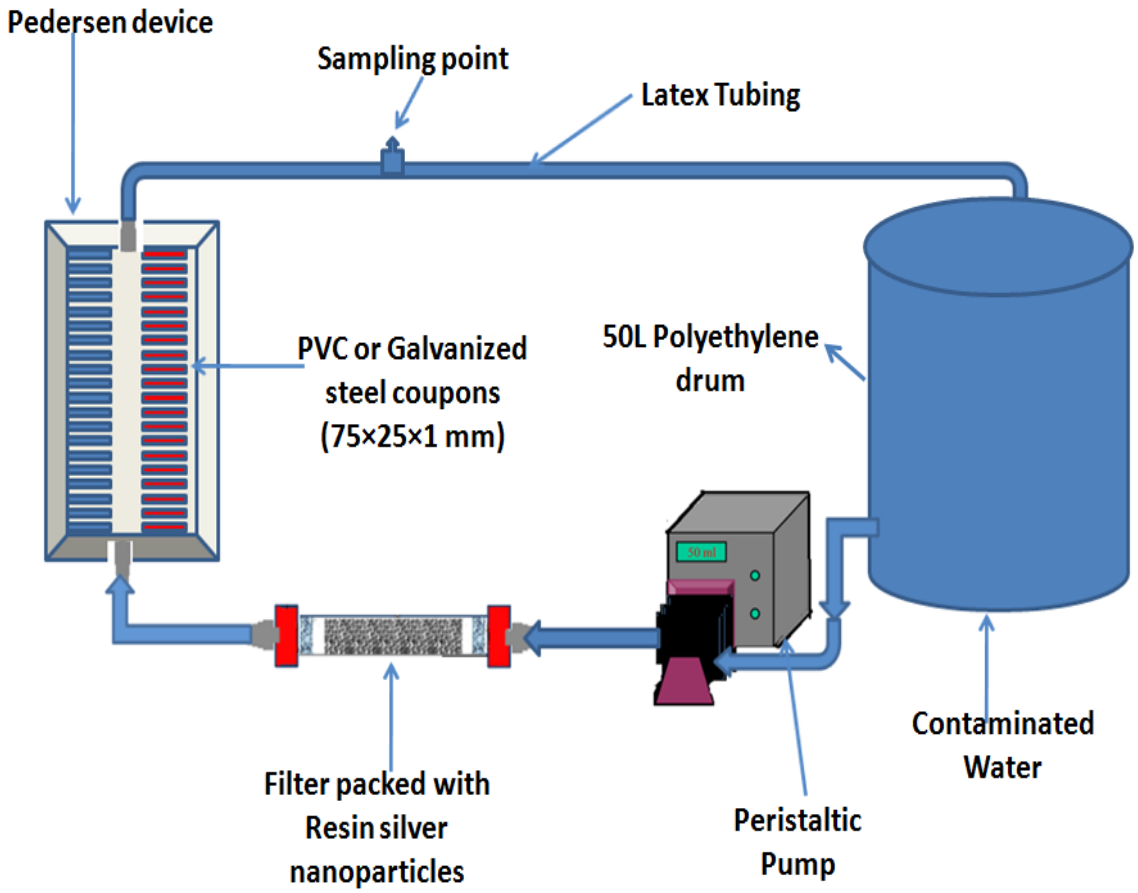


FIGURE 7.1: Schematic diagram of laboratory-scale unit for biofilm formation

Plastic-based material (polyvinyl chloride-PVC) and steel-based material (galvanised steel-GS) generally used for drinking water distribution systems were considered in order to study bacterial regrowth and biofilm formation. Twenty coupons of each piping material (PVC and GS with the size of a microscope slide of 75 mm x 25 mm x 1 mm) were vertically inserted into the Pedersen device. Prior to use, all three systems were thoroughly washed with sterile distilled water. The methods previously used by Momba and co-workers were applied for the treatment of the coupons (Momba *et al*., 1998). Briefly, the coupons were cleaned in a detergent solution, rinsed first in tap water and then disinfected with chorine at 5 mg/L for 24 h. Thereafter, the coupon was dechlorinated with sodium thiosulfate (± 17.5 mg/L) and flushed with sterile distilled water to remove any residues (Momba *et al*., 1998).

7.3.3 Treatment of groundwater

7.3.3.1 Preparation of disinfectant stock solution

A chlorine stock solution was prepared by using sodium hypochlorite (commercial bleach). Two millimetres of sodium hypochlorite were diluted into 1 L distilled water, in order to obtain 100 mg/L of free chlorine. The stock solution was then standardised with N,N-diethyl-pphenylenediamine (DPD powder pillows, Hach) by using a chlorine colorimeter C401 (Eutech instruments) to determine the correct concentration of chlorine. The chlorine concentration of the stock solution was found to be 2.5 mg/L free of chlorine.

7.3.2.2 Disinfection of water source

Chlorine and silver nanoparticles resin filter systems were used to disinfect the groundwater collected from the Delmas borehole. Prior to the circulation of the groundwater through the chlorinated system, the water was subjected to a chlorination process. The chlorinated water was produced by adding 2.5 mg/L free chlorine stock solution into a 1 L×50 L drum that contained the groundwater water sample. For the resin-silver nanoparticle system, the raw intake groundwater from the 50 L drum was pumped continuously into the silver nanoparticles resin filter system for disinfection before entering the Pedersen device as depicted in Figure 7.1.

7.3.4 Physicochemical analyses

The concentration of residual free chlorine was measured by N, N-diethyl-p-phenylenediamine (DPD powder pillows, Hach) using the chlorine colorimeter C401 (Eutech instrument), and the residual silver ion was measured by atomic absorption spectrophotometry (SpectrAA 220FS), according to the standard method (APHA, 1998). The temperature, pH and turbidity of the water samples were also measured using the standard method (APHA, 1998). Nitrates (NO_3^-), sulphates (SO_4^{2-}) and total organic carbon (TOC) concentrations were determined by using the Spectroquant Nova 400 manual water analyser (Merck, South Africa) and the photometric test kits (Merck, South Africa). Table 7.1 summarises the physicochemical parameters.

TABLE 7.1: Physicochemical characteristics of tested water during the study

Parameters	Units	Raw Water	Chlorinated Water	Resin-Ag nano	SANS 241 (2006)
pH		7.38 ± 0.05	7.21 ± 0.10	7.36 ± 0.09	5–9.5
Temperature	^{o}C	24 ± 0.14	24 ± 0.11	24 ± 0.12	<25
Turbidity	NTU	3.28 ± 0.11	3.26 ± 0.15	3.28 ± 0.10	<1
SO_4^{2-}	mg/L	26.36 ± 1.18	29.05 ± 0.91	26.36 ± 1.18	<250
NO_3^{2-}	mg/L as N	1.04 ± 1.02	1.06 ± 1.04	1.04 ± 1.03	<10
TOC	mg/L	2.30 ± 0.18	2.36 ± 0.16	2.30 ± 0.18	<10
Free chlorine	mg/L	0	2.5 ± 0.02	N/A	0.5
Silver ions	mg/L	N/A	N/A	0.03	-

7.3.5 Microbiological analyses

7.3.5.1 Water samples

For the microbiological analysis of the quality of groundwater in the non-disinfected water system, water samples were collected in sterile glass bottles, while for the treated water systems, the collection was done using sterile bottles, which contained 1 mL of 15 % sodium thiosulphate to neutralise the disinfection process. Total coliforms, faecal coliforms and presumptive *E.coli* were detected by using membrane filtration techniques incorporating chromocult coliform and mFC agar (Merck, South Africa). The plates were incubated at 37 ºC for 24 h. Heterorophic plate count (HPC) bacteria were enumerated by using a standard spread plate procedure incorporating nutrient agar (Merck), according to the *Standard Methods for Examination of Water and Wastewater* (APHA, 1998). Analyses were carried out in triplicate.

7.3.5.2 Biofilm formation

Attached viable counts- For each piping material (PVC and GS-75 mm x 25 mm x l mm), two coupons were removed from the Pedersen devices after 24 h, 48 h and 72 h during the continuous circulation of untreated, chlorinated and resin-silver nanoparticle disinfected waters in separate systems. Thereafter, the coupons were removed once a week for a period of three weeks with the exception of the fourth week where coupons were removed twice. The removed coupons were subsequently immersed in 50 mL sterile centrifuge tubes with 30 mL sterile saline water. The tubes were then vortexed for 2 min using a vortex mixer to detach the biofilms from the coupon surface. The resultant suspensions were then serially diluted in a sterile saline solution (0.9 % w/v NaCl). For the enumeration of attached heterotrophic plate count bacteria, 100 µL of diluted biofilm suspension were then placed on spread plates in triplicate onto nutrient agar plates and finally incubated at 35 °C for 48 h. The following equation was used to calculate the number of HPC bacteria (cfu/cm^2):

$$\text{HPC (cfu/cm}^2\text{)} = \text{N x D/Surface area of slides} \qquad \text{(Eq. 7.1)}$$

Where N= average number of colonies and D=dilution factor (Momba *et al*., 2002).

Visualisation of biofllm formation – One coupon of each material (25 mm x 25 mm x l mm) was removed from the Pedersen devices after 24, 336 and 720 h of continuous circulation of the non disinfectant, chlorinated and resin-silver nanoparticle disinfectant water. The removed coupons were then rinsed with sterile MilliQ water for 30 s to remove any unattached cells. Subsequently, the coupons were fixed and treated sequentially with 20 mL of 2.5 % gluteraldehyde

(30 min); 0.15 M phosphate-buffer (3 min x 15 min); 50 % ethanol (1 min x 15 min); 70 % ethanol (1 min x 15 min); 90 % ethanol (1 min x 15 min) and 99.9 % ethanol (3 min x 15 min). Thereafter, the coupons were dried in a critical point dryer for 3 h. Finally, the dried samples were coated with gold plasma so as to make them visible under the scanning electron microscope (SEM) (JEOL JSM 840).

7.3.6 Statistical analyses

All data were analysed statistically using the SPSS computer software, version 11.0. Significance testing was conducted by using one-way analysis of variance (ANOVA) at a 95 % confidence interval. Comparisons were made between the treatment means of each filter system to determine whether there were significant differences in the treatments. The tests for relationships between attached heterotrophic plate count and physicochemical properties of the water samples were conducted by using the Pearson Correlation index.

7.4 RESULTS

7.4.1 Physicochemical characteristics of the water samples

Characteristics of the tested water samples used in this study are presented in Table 7.1. The average values of the physical parameters ranged between 7.21 and 7.38 for pH; 3.26 NTU and 3.28 NTU for turbidity and 24.0 ^{o}C for temperature, while those for the chemical parameters ranged from 26.36 to 29.05 for SO_4^{2-}, 1.04 to 1.06 for NO_3^{2-} and 2.30 to 2.36 for TOC.

7.4.2 Disinfectant residual feature in treated water systems

The free chlorine concentration was gradually depleted from 2.5 mg/L to 0.7 mg/L in the chlorinated water system after the first day of disinfection (Figure 7.2). On the third day, no residual chlorine was detected in the chlorinated water system. However, the Ag residual concentration gradually decreased from 0.03 mg/L to 0.01 mg/L in the silver nanoparticles resin filter system between the first day and the 14th day after the disinfection process (Figure 7.3). A complete depletion of the Ag occurred only on the 21st day of the experimental study.

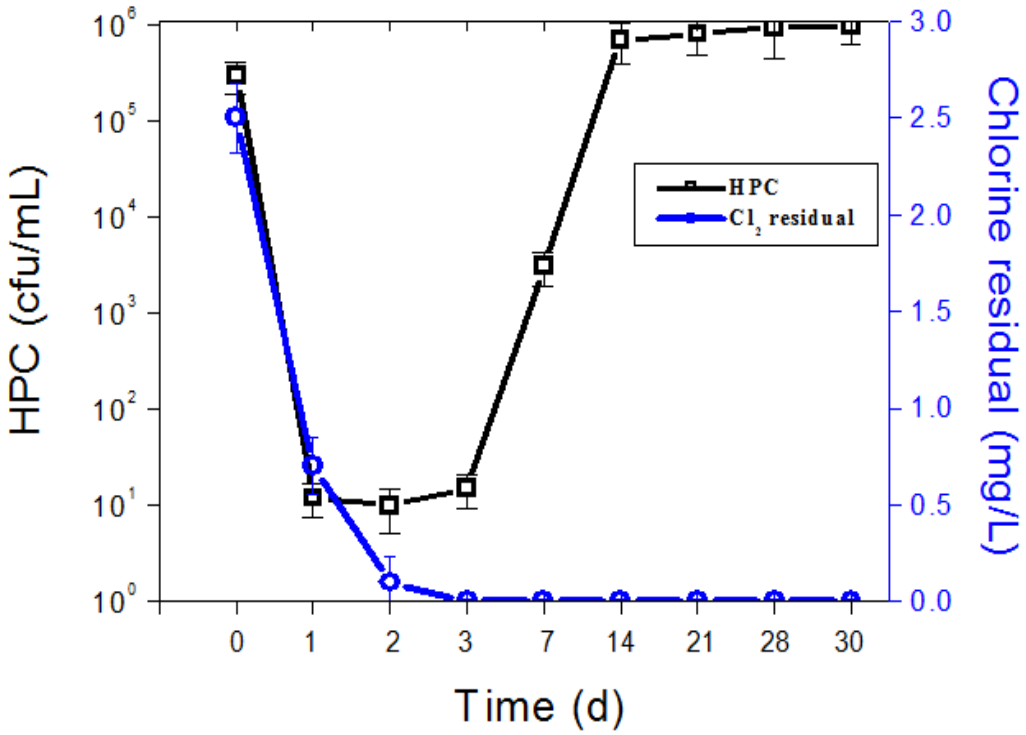


FIGURE 7.2: Regrowth of heterotrophic plate count during the depletion of chlorine residual in a chlorinated groundwater system

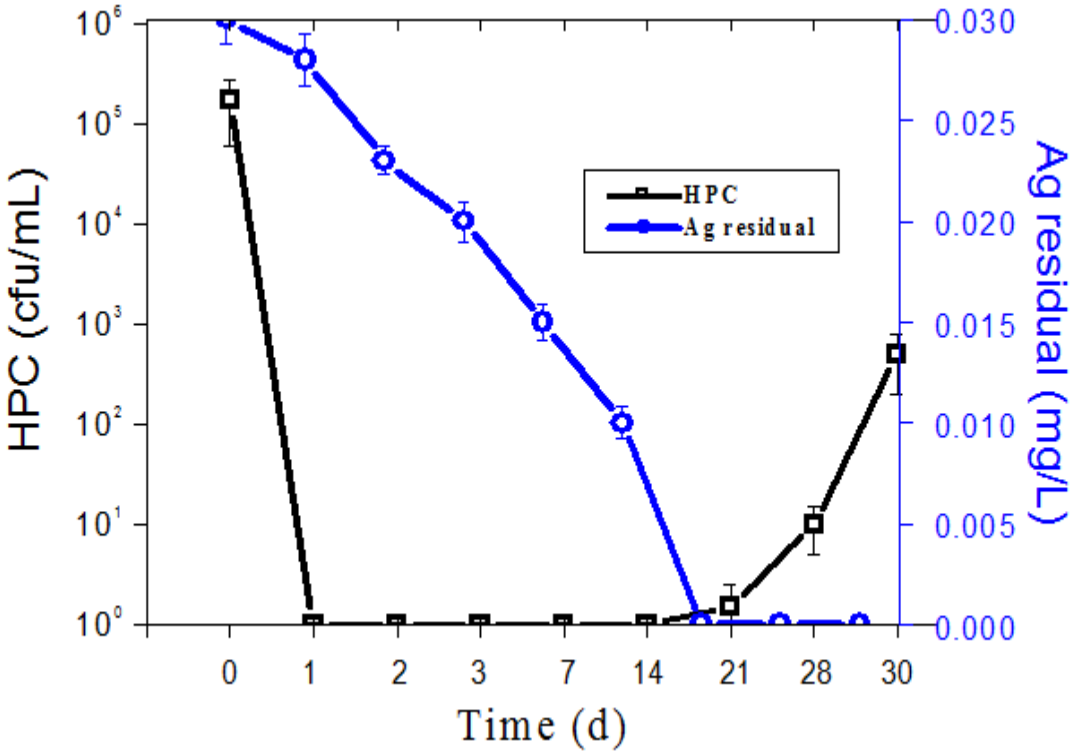


FIGURE 7.3: Effect of silver nanoparticles resin filter system on the inhibition of heterotrophic plate count bacteria groundwater system

7.4.3 Microbial characteristics of water samples

7.4.3.1 Coliform bacteria

The average coliform bacterial counts of groundwater in the control system and disinfected (chlorinated and groundwater treated with silver nanoparticles resin filter systems are illustrated in Table 7.2. All the systems were supplied with groundwater that contained a similar amount of initial coliform counts. In the untreated groundwater system, there was a gradual decrease in the coliform group from the first day of the experimental study. At the end of day 28, the total colifom counts in this system had decreased from 1.4×10^{3} to 53 cfu/100 mL, while a complete disappearance of faecal coliforms and *E.coli* occurred during the 21st and 28th day, respectively. In the chlorinated groundwater system, a gradual

decrease was also noticed in the coliform group from the first day of the experimental study. At the end of the 28th day, the total colifom counts in this system decreased to 2 cfu/100 mL. A complete disappearance of faecal coliforms and *E.coli* occurred during the 3rd and 28th day, respectively. However, the groundwater samples subjected to a silver nanoparticles resin filter disinfection process resulted in a drastic decrease of total coliforms and complete disappearance of faecal coliforms and *E.coli* during the first 30 min. Thereafter, none of the coliform groups reappeared in the final water sample up to the 28th day of the experimental study (Table 7.2).

7.4.3.2 Heterotrophic plate count bacteria

Figures 7.2-7.4 indicate the general quality of the treated and untreated groundwater systems during the study period. While a similar initial count for HPC bacteria (5 log cfu/mL) was found in the intake groundwater before its circulation in various laboratory water supply systems, a slight increase to 5.5 log cfu/mL was observed within the 1st day when using the control system (Figure 7.4). No specific growth of HPC bacteria was noted between 1st and 21st days. Thereafter, HPC bacterial counts decreased to a similar average count than that initially noted before the circulation of the water through the control system.

The system with the chlorinated groundwater reduced HPC bacterial counts by 4 log cfu/mL within the 1st day (Figure 7.2). Thereafter, the HPC bacterial counts increased gradually to a level greater than the initial counts noted before the circulation of the water through the system. The HPC bacterial counts increased to 6 log cfu/mL between the 2nd day and the 30th day. Contrary to the chlorinated

system, there was a total removal of HPC bacteria from the first day of the circulation of the water through the silver nanoparticles resin filter system (Figure 7.3). The reappearance of HPC bacteria occurred after the 14th day and the bacterial counts increased from 0.5 log to 2.8 log cfu/mL between 21st and 30th day.

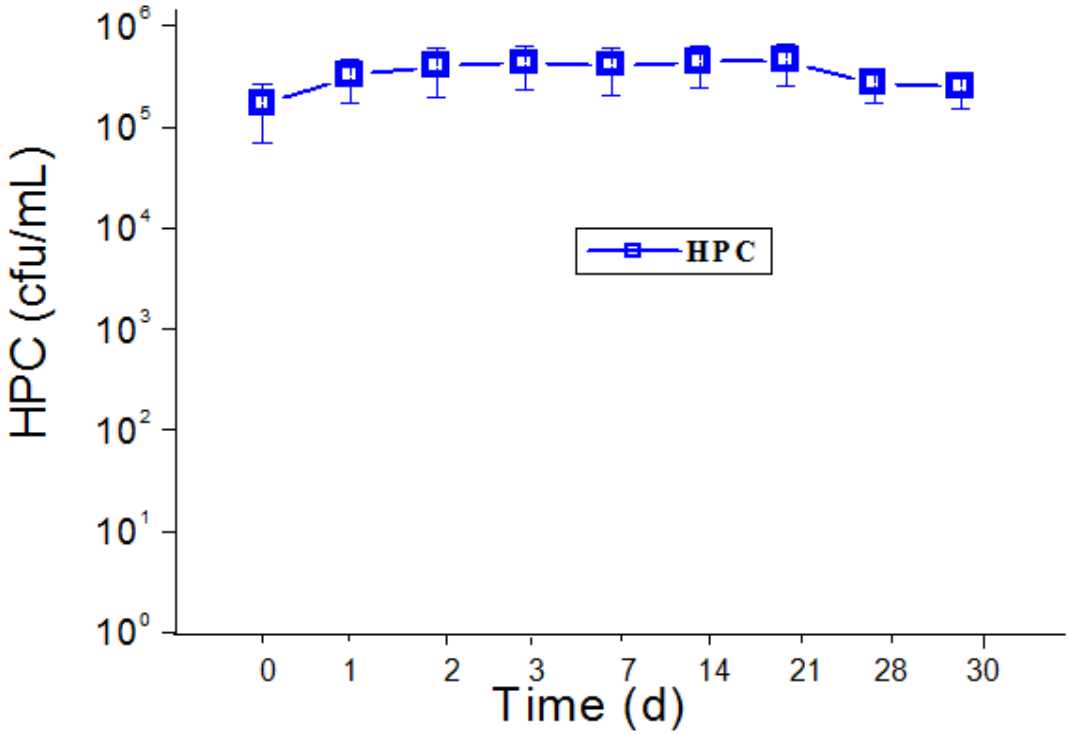


FIGURE 7.4: Growth of heterotrophic plate count in the untreated groundwater system during the experimental study

TABLE 7.2: Average coliform bacterial counts in different water systems during the experimental study

Time (d)	Test Water/Coliforms bacteria (cfu.10^{-2}mL)								
	Control system (untreated groundwater)			Chlorinated system			Resin-Ag nanoparticles system		
	TC	FC	Ec	TC	FC	Ec	TC	FC	Ec
0	1.4×10^{3}	7.8×10^{1}	3.0×10^{2}	1.4×10^{3}	7.8×10^{1}	3.0×10^{2}	1.4×10^{3}	7.8×10^{1}	3.0×10^{2}
30 min	N/A	N/A	N/A	6.3×10^{1}	2.2×10^{1}	3	9	0	0
1	5.3×10^{2}	5.1×10^{1}	15 ± 5.45	4.7×10^{1}	1.6×10^{1}	0	0	0	0
2	3.2×10^{2}	4.2×10^{1}	1.1×10^{1}	2.9×10^{1}	4	2	0	0	0
3	3.0×10^{2}	3.0×10^{1}	7	1.3×10^{1}	0	0	0	0	0
7	1.0×10^{2}	1.8×10^{1}	4	9 ± 5.45	0	0	0	0	0
14	1.2×10^{2}	6	9	1.5×10^{1}	0	0	0	0	0
21	7.7×10^{1}	0	1	6	0	0	0	0	0
28	5.3×10^{1}	0 ± 0.00	0	2	0	0	0	0	0

TC: Total coliforms, FC: Faecal coliforms, Ec: *Escherichia coli,* N/A: not applicable, Target limits: TC 5 cfu/100mL, FC 0 cfu/100mL, Ec 0 cfu/100mL (DWAF, 1996; SANS: 241, 2006).

7.4.4 Microbiological characteristics of piping materials

The adhesion of bacteria on the surface of PVC and GS pipes was monitored for 30 days. In general, a gradual increase in HPC bacterial counts was observed on the surface of the PVC and GS coupons inserted in the control system and in the chlorinated water system. Within the first day, an average of 0.8 log and 1 log cfu/cm^2 of HPC colonised the PVC and GS coupons inserted in the untreated system, respectively (Figure 7.5). After 30th day, the average HPC bacterial counts on these coupons reached 4.8 log and 5.9 log cfu/cm^2 for PVC and GS coupons, respectively. There was a significant difference ($p < 0.05$) in the viable counts attached to the surface of both coupons throughout the study period.

In the chlorinated water system (Figure 7.6), the average viable bacterial counts that colonised the surface of the PVC and GS coupons within the first day were 0.5 log and 1 log cfu/cm^2 HPC, respectively. Between the 1st and 30th day, the viable bacterial counts on these piping materials progressed to a maximum average of 4.9 log and 6 log cfu/cm^2 HPC for PVC and GS coupons, respectively. There was a significant difference ($p < 0.05$) in the viable counts attached to both coupons throughout the trial. Statistically temperature, sulphates, nitrates and total organic carbon revealed weak negative correlations with heterotrophic plate count bacteria attached to plastic-based and galvanised steel-based piping materials, while the pH and the turbidity showed weak positive correlations (Table 7.3).

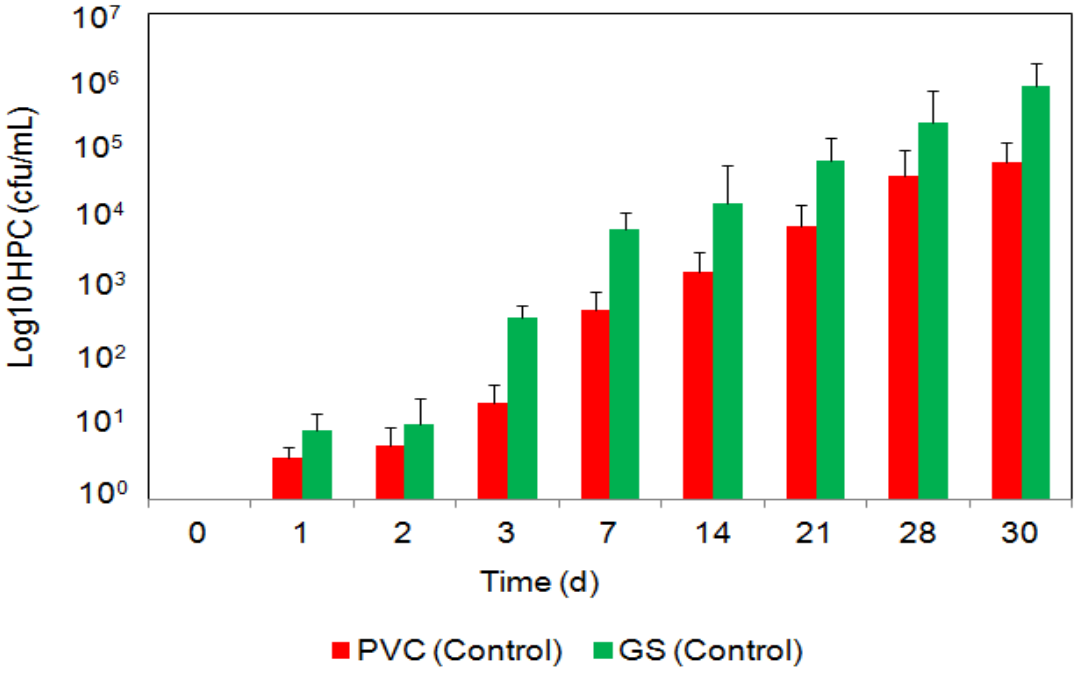


FIGURE 7.5: Average counts of viable bacteria attached to the surface of PVC and GS coupons exposed to the untreated groundwater system (control)

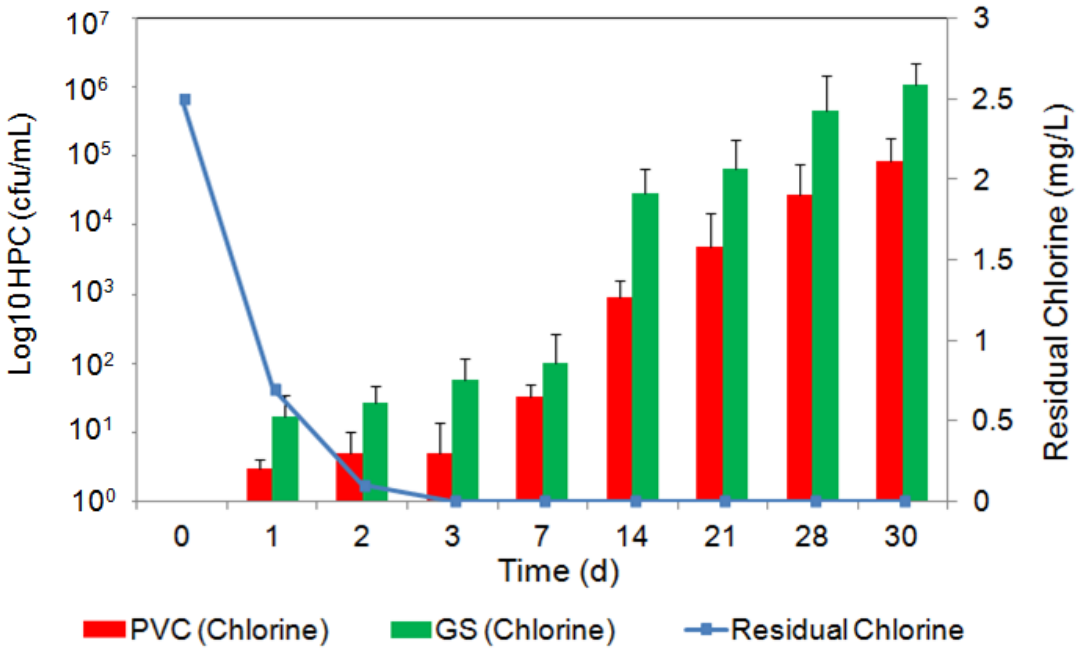


FIGURE 7.6: Average counts of viable bacteria attached to the surface of PVC and GS coupons exposed to chlorinated groundwater system

TABLE 7.3: Correlations between attached heterotrophic bacterial counts (mean log cfu/cm^2) and physicochemical values (mean) in treated water systems (between the 2nd and 28th day at p < 0.05)

Parameters	Water sample							
	Chlorinated water				Silver nanoparticles resin filter			
	PVC pipe material		GS pipe material		PVC pipe material		GS pipe material	
	R	P	r	p	R	p	R	p
Temp (^{o}C)	-0.267	0.089	-0.232	0.075	-0.378	0.067	-0.345	0.431
pH	0.269	0.090	0.229	0.079	0.377	0.223	0.354	0.211
Turbidity (NTU)	0.230	0.040	0.235	0.051	0.190	0.031	0.170	0.033
SO_4^{2-} (mg/L)	-0.276	0.190	-0.283	0.193	-0.281	0.194	-0.300	0.498
NO_3^{2-} (mg/L)	-0.349	0.051	-0.365	0.059	-0.388	0.283	-.395	0.250
TOC (mg/L)	-0.237	0.072	-0.122	0.061	-0.244	0.252	-.284	0.161

r: correlation, p < 0.05

In the silver nanoparticles resin filter groundwater system (Figure 7.7), no viable bacteria were detected on the surfaces of both types of piping materials under this investigation from the 1st day to the 21st day. However, the adhesion of viable bacteria was observed on GS coupons on 28th day, while no bacteria attached to the PVC coupons. Between the 28th and 30th days, there was a gradual increase of attached viable bacteria from an average count of 1.2 log to 2.2 log cfu/cm^2 HPC on GS coupons. The attachment of viable bacteria to PVC coupons was apparent only on day 30 and the average count was 1.4 log cfu/cm^2 of HPC colonised on this piping material. There was a significant difference (p > 0.05) in the formation of biofilm between (p < 0.05) PVC and GS coupons on the 30th day. Statistically, temperature, sulphates, nitrates and total organic carbon

demonstrated weak negative correlations with heterotrophic plate count bacteria attached to plastic-based and galvanised steel-based piping materials, while the pH and turbidity indicating weak positive correlations (Table 7.3).

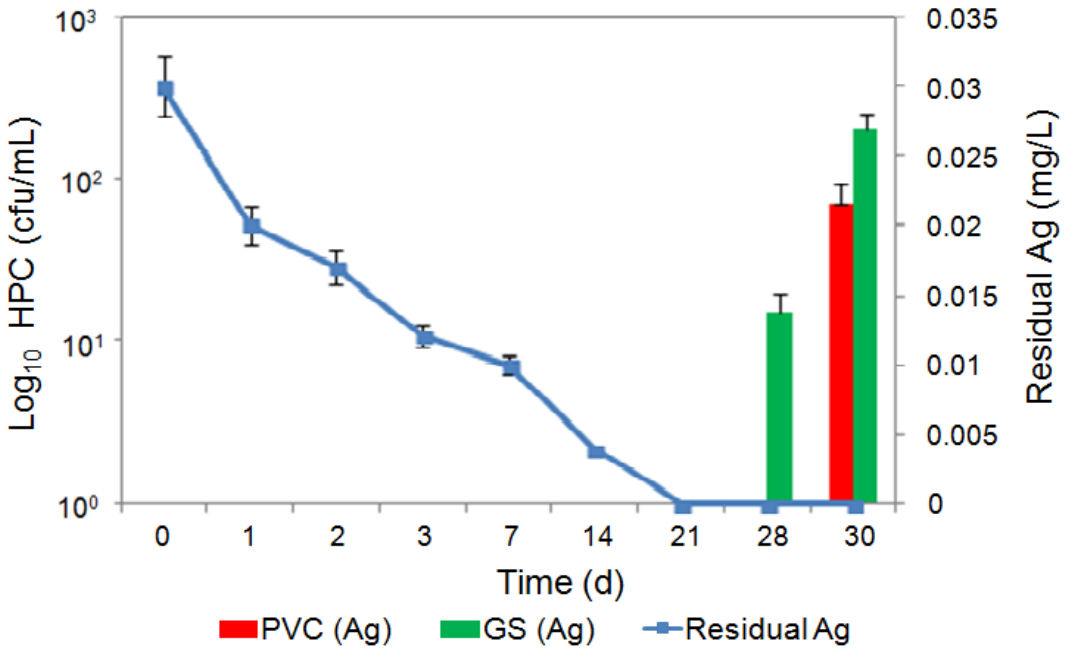
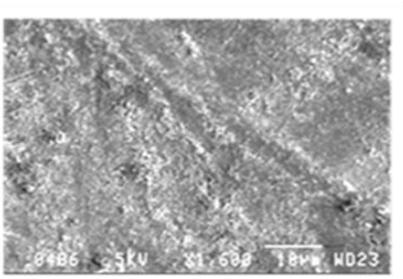


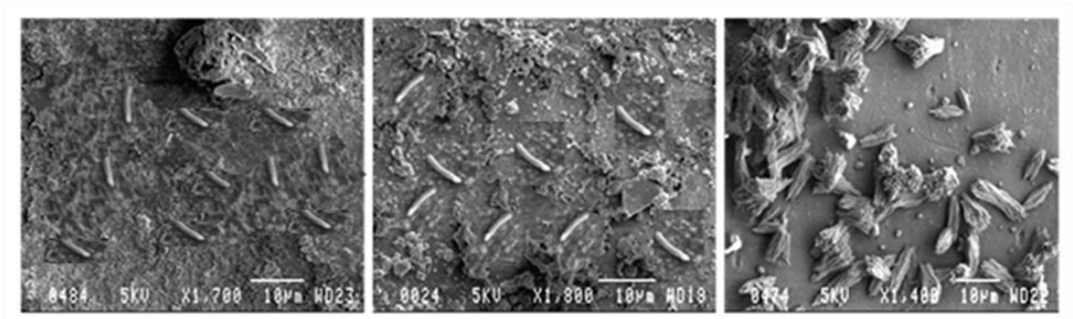
FIGURE 7.7: Average counts of viable bacteria attached to the surface of PVC and GS coupons exposed to the silver nanoparticle resin filter system

7.4.5 Visualisation of cells on coupons (SEM)

Scanning electron microscopic observation confirmed the presence of biofilm cells on the surface of GS and PVC coupons exposed to the control groundwater system and the chlorinated groundwater system from the 1st day to the 30th day (Figures 7.8-7.13). This technique revealed no presence of attached bacterial cells on the piping during the first 7days of their exposure to the resin-silver nanoparticle system (Figs. 7.8-7.9). However, the formation of biofilm was obvious on the surface of both coupons (PVC and GS) exposed to this system, on the 30th day after disinfection (Figures 7.10 and 7.13).

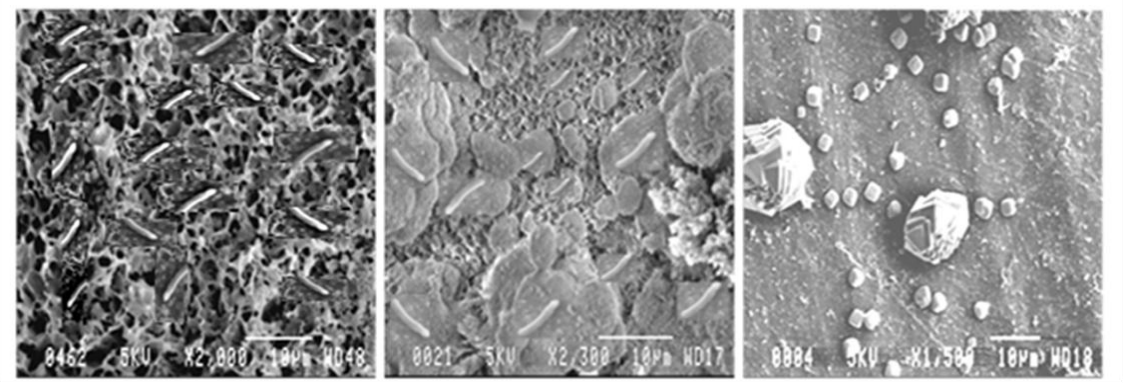


Galvanised steel coupon



Untreated groundwater system | Chlorinated groundwater system | Silver nanoparticles resin filter system

FIGURE 7.8: SEM images depicting microbiological features of GS coupons before and after a 1st day exposure to untreated groundwater, chlorinated groundwater and a silver nanoparticles resin filter system



Untreated groundwater system | Chlorinated groundwater system | Silver nanoparticles resin filter system

FIGURE 7.9: SEM images depicting microbiological features of GS coupons after the 7th day exposure to untreated groundwater, chlorinated groundwater and a silver nanoparticles resin filter system

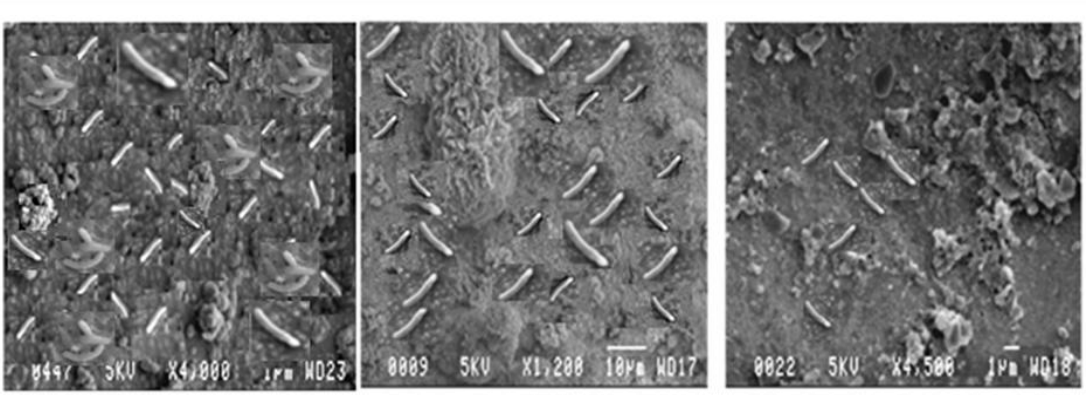


FIGURE 7.10: SEM images depicting microbiological features of GS coupons after the 30th day exposure to untreated groundwater, chlorinated groundwater and a silver nanoparticles resin filter system

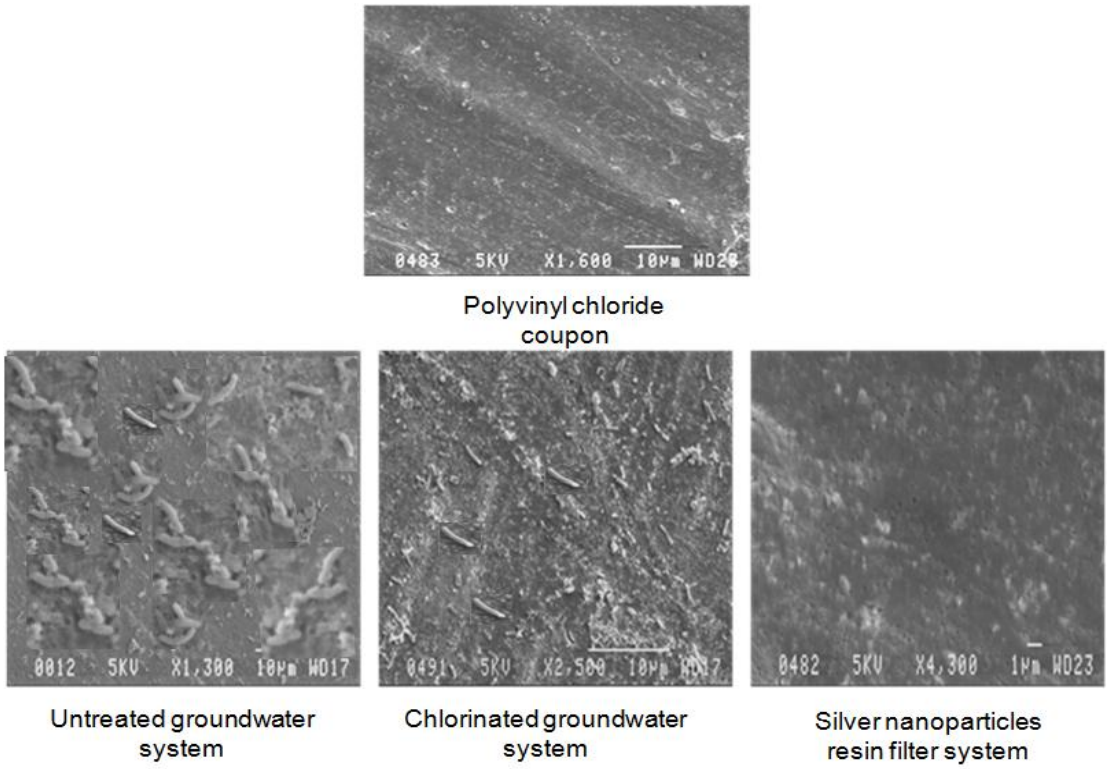


FIGURE 7.11: SEM images depicting microbiological features of PVC coupons before and after the 1st day exposure to untreated groundwater, chlorinated groundwater and a silver nanoparticles resin filter system

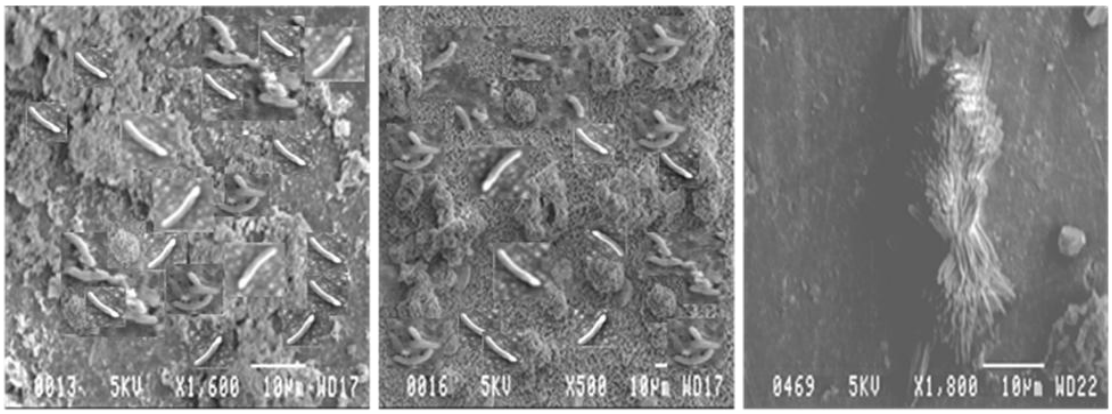


FIGURE 7.12: SEM images depicting microbiological features of PVC coupons before and after 7th day exposure to untreated groundwater, chlorinated groundwater and a silver nanoparticles resin filter system

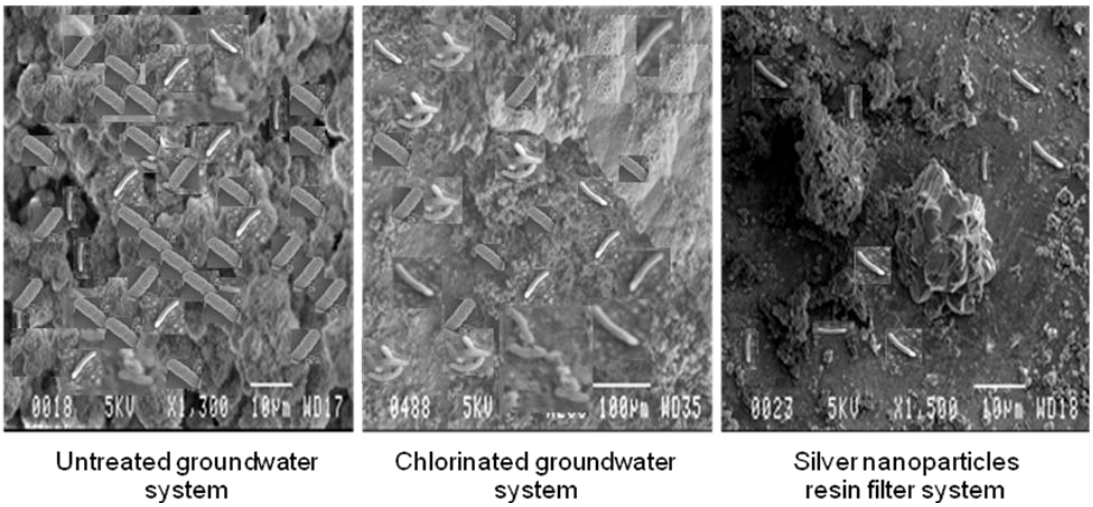


FIGURE 7.13: SEM images depicting microbiological features of PVC coupons before and after 30th day exposure to untreated groundwater, chlorinated groundwater and a silver nanoparticles resin filter system

7.5 DISCUSSION

The microbial flora found in the treatedwater mostly originates from the raw water source. Although the disinfection processes play an important role in producing safe drinking water, low numbers of culturable heterotrophic bacteria can still increase considerably in distribution systems (Momba & Binda, 2002). This phenomenon has been reported to result in the formation and presence of biofilms in drinking water distribution systems (Momba & Makala, 2004; September *et al*., 2007). A large variety of different heterotrophic bacteria ranging from potentially pathogenic bacteria to coliform bacteria have been isolated from treated water as drinking water distribution systems provide an oligotrophic environment (September *et al*., 2007). Researchers have reported that the majority of waterborne disease outbreaks are related to quality deterioration in distribution systems (Craun & Calderon, 2001). Post-treatment recovery and growth of bacteria in drinking water distribution systems are therefore a matter of great concern because of their negative effect on public health (Craun & Calderon, 2001). Consequently, alternative technologies are required to control the attachment of cells to, and the formation of biofilms on the surfaces of piping materials.

With the exception of turbidity levels that were above the recommended limit (< 1 NTU) (Table 7.1), the physicochemical characteristics of the intake groundwater complied with the limits set by the South African water quality guidelines (DWAF, 1996 and SANS 241, 2006) and the WHO (WHO, 2006) for drinking water. It is well known that high levels of turbidity enhance the survival of microorganisms in

water (DWAF, 1996) and renders the disinfection process ineffective (WHO, 2006). Although the temperature of the intake water was within the SANS 241/WHO recommended limits, it has been affirmed that temperatures above 15 ^{o}C contribute to the formation of biofilm in drinking water systems (Kaye & Nagy, 1999; Zacheus *et al*., 2000). This study therefore suggests that both turbidity and temperature could influence bacterial regrowth and biofilm formation in treated water systems.

Results of this study also revealed that the microbiological quality of the Delmas borehole did not comply with the recommended limits (Table 7.2). The average counts for heterotrophic culturable bacteria, total coliforms, faecal coliforms and *E.coli* by far exceeded the limits that were set for water intended for human consumption (Table 7.2). Such water sources may increase the risks of infectious disease transmission if it is utilised for domestic use without prior effective treatment. These results corroborated the findings of early studies that reported an the outbreak of waterborne diseases within the Delmas community who totally depended on boreholes as their unique source of water supply in 2005. Typhoid and diarrheoal diseases resulted in 49 deaths within this community due to the consumption of water from these water sources (Groenewald & Dibetle, 2005; Masinga, 2005).

Although an appropriate concentration of free chlorine (2.5 mg/L) was used to treat the Delmas borehole water during the study period, the treated water that contained the residual chlorine of 1.7 mg/L could not produce safe drinking water within the first 30 min of contact. Heterotrophic bacteria and coliform bacteria

including *E.coli* were still present in chlorinated water even in the presence of the free chlorine residual (0.7 mg/L). Virto *et al*. (2005) discovered that two Gram-positive organisms, *Listeria monocytogenes* and *Bacillus subtilis*, and two Gram-negative organisms, *Yersinia enterocolitica* and *Escherichia coli* were more resistant to chlorine concentrations of 0.3 mg/L to 0.7 mg/L and 0.8 mg/L to 1 mg/L. During the present study, the potential for bacterial regrowth was observed between the 2nd and 30th days, which were accompanied by an increase in the bacterial counts from 2 log to 6 log cfu/mL in the chlorinated water system (Figure 7.2). This confirms the report by Momba (1997) and Williams and Braun-Howland (2003) that microorganisms can regrow in water even in the presence of residual chlorine. Contrary to the chlorination process, the bacterial regrowth in water treated with silver nanoparticle resin filter system was only observed between day the 21st and 30th days with a bacterial count increase of up to 3 log cfu/mL (Figure 7.3). This system provided a longer silver residual of 0.03 mg/L to 0.01 mg/L that inhibited the bacterial regrowth, and this residual concentration was by far less than the limit set by the WHO, which is 0.1 mg/L (WHO, 2006). Similarly, Lin *et al*. (1996) achieved a complete inhibition of *Legionella pneumophila* from hospital hot water recirculating systems with residuals of silver ion between 0.04 mg/L and 0.08 mg/L for a period of 5 days. The bacteriostatic properties of the silver ion has been reported by previous investigators, who found that this chemical disinfectant inactivates bacteria by disrupting the disulphide bonds of proteins in their cell membrane or by inhibiting DNA replication (Oyandele-Craver *et al*., 2008; Sharma *et al*., 2009). The results of the present study suggest that the bacterial regrowth in a resin-silver nanoparticle filter system was due to the depletion of free residual disinfectants. The maintenance of a sufficient and stable residual of silver ion may

therefore be considered to be a prerequisite in preserving the integrity of this system. But, it is important to note that the concentration of the silver in drinking water must be within the recommended limit.

Although the formation of biofilms on PVC and GS coupons was observed in all the water systems, the attached viable bacterial counts were noticeable on the surfaces of both piping materials exposed to untreated and chlorinated water systems within the first day of their exposure (Figures 7.5 and 7.6). Similar observations were also reported by Momba *et al*. (2002) in terms of bacterial adhesion on the piping materials exposed to untreated and chlorinated water systems. Various researchers also observed the formation of biofilm on plastic and galvanised steel surfaces (Lethola *et al*., 2004; İlhan-Sungur *et al*., 2007; Dogruoz *et al*., 2009). Although, in the present study, the formation of biofilm on PVC and GS were quite similar, the results indicated that the HPC values were slightly higher on GS than on PVC pipes. Niquette *et al*. (2000) and Tsvetanova *et al*. (2006) also reported higher bacterial counts in steel pipes than in plastic pipes. The attachment of the heterotrophic plate count bacteria to the surface of test piping materials exposed to chlorinated water is supported by negative weak correlations of free chlorine residual concentrations with bacterial numbers (r = - 233 for PVC and r = - 245 for GS).

With the resin-silver nanoparticle system, the results indicate that this system inhibited biofilm formation due to the antibacterial activity of the silver ion, even though this activity decreases over time (Figure 7.7). A complete inhibition of biofilm formation was achieved in both PVC and GS piping materials for a period

of 27 days with a residual silver ion ranging between 0.030 mg/L and 0.010 mg/L. Findings by Lui *et al*. (1998) indicated that a small concentration of silver ion can be used to inhibit bacterial counts in water systems. Negative correlations were found between silver ion residual concentrations and attached bacterial numbers under the silver nanoparticles resin filter treatment ($r = -0.326$ for PVC and $r = -0.351$ for GS). Compared to the chlorine treatment, these negative correlations were found to be much stronger and more significant (at $p < 0.05$) in the silver nanoparticles resin filter groundwater system.

The scanning electron microscope clearly exhibited the inhibition and the presence of biofilms on the surfaces of both piping materials with different water system (Figures 7.8-7.13). The assessment of uncontaminated GS and PVC pipes through SEM demonstrated a clear difference between the two piping materials, where the GS pipe appeared to a have a rough surface and PVC appeared to have a smooth surface (Figures 7.8 and 7.11). However, after 30 days, large amounts of biofilms had been deposited at the surface of both piping materials in untreated and chlorinated water systems. Moderately, different concentrations of microorganisms with various morphologies were clearly observed depending on the type of pipe material. A relatively smaller amount of microorganisms were observed on the PVC pipes, which seemed to have smooth surfaces, while the GS pipes with their rough surfaces, were covered with large numbers of microorganisms. According to Chang *et al*. (2003) pipes made of rough surface materials such as galvanised steel (GS) has a greater biofilm regrowth capability than those that have smooth surfaces such as polyvinyl chloride material. Yu *et al*. (2010) also reported that the surface structure of piping materials appears to be

the main reason for biofilm formation on metal pipes. However, the crystalline structures of silver nanoparticles were observed on the surface of GS piping material between 1 and 7 days, while in PVC they were observed even after 7 days. Biofilm formation on both pipes were observed after 30 days with lower numbers of attached cells on GS piping, while PVC piping material also exhibited a lower number of cells with silver nanoparticle crystal structures. Results of this experimental study revealed the effectiveness of the silver nanoparticles resin filter system in controlling the growth of attached heterotrophic plate count bacteria in a laboratory-scale unit.

7.6 CONCLUSIONS AND RECOMMENDATIONS

The study revealed colonisation of heterotrophic plate count bacteria in both plastic (PVC) and steel (GS) piping materials within the 1st day in untreated groundwater and chlorinated water systems. However, in the silver nanoparticles resin filter system the colonisation was only observed after 28 and 30 days. From this observation it is evident that the depletion of residual disinfectant increases the growth of bacteria in the distribution system. The results indicated high HPC counts on GS pipes when compared to PVC pipes. Statistically, there was a significant difference in attached bacterial counts between the two types of piping materials. In conclusion, the resin-silver nanoparticle water system was found to preserve the integrity of the water distribution system for a long period and consequently reduce bacterial adhesion and inhibit biofilm formation. Therefore, the study recommends the use of a silver nanoparticles resin filter system with plastic pipes for the distribution systems.

CHAPTER 8

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The need for improved water supply systems in rural communities constitutes an essential component of social and economic development. Safe water is essential for the protection of community health by limiting the transmission of waterborne diseases. However, the monitoring and treatment of groundwater in order to make it safe for human consumption is a great concern in developing countries.

Conventional disinfection methods in water treatment often include the use of chlorine, which is the most widely used drinking water disinfectant. This disinfectant has made an immense contribution to the safety of drinking water supplies. Chlorine destroys microorganisms by chlorinating the lipid protein substance, as it reacts with bacterial cell wall to form toxic chloro compounds (Venkobachar, *et al*., 1976; Hass & Engelbrecht, 1980). It also induces the leakage of macromolecules from the cells indicating the permeability changes of the membrane (.Venkobachar, et al., 1976). However, it produces disinfection by-products (DBPs) such as trihalomethanes (THMs), which are known to be carcinogenic (WHO, 2003a; van der Walt *et al*., 2009). Nanobiocides, such as silver nanoparticles in particular, offer an alternative method of disinfection without reacting with the water itself. Silver nanoparticles can bind to bacterial cells and enzymes (proteins) at multiple sites, damaging them and preventing them from performing their functions, resulting in cell death through penetration at specific bacterial DNA and RNA (Klassen, 2000; Ovington, 2004; Rai, *et al*., 2009). It also

inhibit microbial growth which include particle attachment to or penetration of the cell membranes accompanied with a slow release of silver ions, causing changes to the membrane permeability and redox cycle in the cytosol intracellular radical accumulation process, as well as dissipation of the proton motive force for ATP synthesis (Sondi & Salopek-Sondi, 2004; Morones *et al*., 2005; Lok *et al*., 2006). The only currently known health consequence of excessive silver intake is a condition known as argyria in which skin and hair become discoloured by silver accumulation. However, it has estimated that 0.1 mg/ℓ silver ion in drinking water should have no adverse effects over a lifetime of consumption based on half the of a total dose over 70 years for NOAEL (no-observed-adverse-effect-level) of 10 g in human could then be tolerated without risk to health or to cause argyria (WHO, 2003b).

The present study was initiated with the aim of developing and evaluating the effectiveness of cost- effective filter materials coated with silver nanoparticles for the removal of pathogenic microorganisms from groundwater and the inhibition of biofilm formation in drinking water systems. The study was divided into three phases. The first phase assessed the groundwater quality of 200 boreholes around the two Provinces of North West and Mpumalanga in South Africa so as to identify whether this water source was safe for drinking water consumption. The second phase focused on the development and evaluation of the effectiveness of filter materials coated with silver nanoparticles for the removal of pathogenic microorganisms from groundwater. In the last phase, a groundwater source was used to evaluate the impact of silver nanoparticles cation resin filter in inhibiting

bacterial regrowth and biofilm formation in potable water distribution systems using a laboratory-scale unit.

In the first phase of the study, groundwater samples were analysed for their physicochemical and microbial quality (which included culture-based methods and molecular techniques). The results obtained in this part of the study have determined that some of the boreholes used in these two provinces are chemically and microbiologically contaminated. The physicho-chemical findings revealed high concentrations of magnesium, calcium, fluoride, nitrate, TDS and turbidity, which by far exceeded the limits recommended by the South African guidelines (SANS 241 and DWAF). The microbiological assessment indicated that these water sources were faecally contaminated and contained unacceptable high counts of total and faecal coliform bacteria. Various opportunistic pathogens and pathogenic strains such as *Serratia marcescens*, *Citrobacter freundii*, *Salmonella enteric, Bacillus cereus*, *Escherichia coli* O157:H7, *Shigella flexineri* and *dysenteriae*, *Pseudomonas maltophilia*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Morganella morganii, Aeromonas veronii* and *Cronobacter sakazakii* were also found in some of the boreholes by applying molecular techniques. These findings presented convincing evidence that groundwater supplies in these rural areas may pose a serious health risk to consumers. Consequently, groundwater should be subjected to treatment prior to consumption by communities.

In the second phase of the study, five different types of substrates (zeolite, sand, fibreglass, anion resins and cation resins) were used in the development of antibacterial silver nanoparticles coated substrates. These five substrates with

various concentrations (0.01 mM, 0.03 mM, 0.05 mM and 0.1 mM) of $AgNO_3$ were successfully synthesised by employing hydrothermal reduction and chemical reduction methods. The coated substrates were confirmed by applying various characterisation techniques. Scanning electron microsopic images revealed that the Ag nanoparticles on the substrates were predominantly small and spherical with EDS confirming the presence of silver peaks. The TEM images also revealed spherical-shaped particles that aggregated on the silver nanoparticle substrates, which indicated a particle size distribution ranging between 5 nm and 90 nm. The crystalline nature of Ag nanoparticles was confirmed by conducting an XRD experimental study. The XRD Bragg pattern of these particles indicated the presence of reflection peaks typical of an *fcc* structure of silver.

At varying concentrations of silver nanoparticles substrates, laboratory-scale filter systems packed with silver nanoparticle substrates demonstrated that 0.1 mM of silver nanoparticles in all the substrates was effective in decreasing the concentration of *E.coli* from synthetic groundwater. Moreover, the investigation revealed that when a 0.1 mM concentration of silver nanoparticles substrates were tested against four different bacterial species (*E.coli*, *S. typhimurium, S. dysenteriae* and *V. cholerae*) found in the groundwater sources, the cation resin-silver nanoaprticle filter completely (99.9 %) removed all the tested bacterial species. Furthermore¸ it was also discovered that silver ion was eluting at a rate of 60 % and 90 % from Ag/zeolite, Ag/sand, Ag/fibreglass and Ag/anion resin substrates within 30 min of commencing the investigation, while high levels of ions (95 %) were still present in the Ag/cation resin substrate within that same period.

Breakthrough studies were conducted in order to evaluate the optimum operating parameters of the cation resin-silver nanoparticle filter system. The results demonstrated that the performance of this filter system depended on the amount of bed mass used as well as the flow rate. It was found that the breakthrough curves obtained at different bed mass indicate an increase in breakthrough time with an increase in bed mass. The higher flow rate decreased the performance of the filter, while the lower fow rate increased the contact time between bacteria and bed mass material which subsequently increased the disinfection activity of the filter. Once the effectiveness of silver nanopartilces resin filter has been exhausted, the filter requires regeneration. Silver nanopartilces resin filter could be regenerated by the addition of a solution containing $AgNO_3$ and $NaBH_4$ to the resins. It was also determined that the filter system, packed with 20 g of silver nanoparticles resin and operating at a flow rate of 10 mL/min, was capable of producing 15 litres of clean water per day. This amount of water may, however, only be sufficient for one person per day. This study, therefore, suggests the manufacturing of a larger system that can provide the required volume of safe drinking water per person per day so as to serve an entire family or a small community.

The cation resin-silver nanoparticle filter system also demonstrated its effectiveness by completely (99.9 %) removing somatic coliphages during the first five runs of filtering 600 mL of contaminated groundwater. Regarding protozoan parasites, the complete removal (99.9 %) of 2 log oocysts (*Cryptosporidium spp.*) and 2 log cysts (*Giardia spp.)* was achieved during the first three cycles of the filter runs, by filtering 15 L of contaminated groundwater.

In the last phase, the study demonstrated the performance of the cation resin-silver nanoparticle filter in inhibiting bacterial regrowth and biofilm formation in drinking water supply systems. It was found that chlorine residuals were not sustainable in protecting the distribution system against bacterial recontamination. From this observation, it was evident that the depletion of residual disinfectant increased the growth of bacteria in the distribution system. The use of silver nanoparticle filter systems provided an effective barrier for the inhibition of bacterial regrowth and biofilm formation in the laboratory-scale unit. The study also revealed that the colonisation of bacteria depends on the type of piping materials used.

This study clearly indicated that filter systems with Ag/cation resin substrates have the capability to produce safe drinking water and preserve the integrity of potable water distribution systems by inhibiting the phenomenon of bacterial regrowth and bacterial adhesion on plastic-based and metallic-based pipe materials. Silver nanoparticles resin filter systems can be considered as a potential alternative cost-effective filters for the disinfection of groundwater. The silver nanoparticles resin filter systems can be used as POU water treatment for rural communities.

Futher studies need to be conducted in order to investigate the mechanisms of removal or inactivation of pathogens using silver nanopartilces resins. Methods for the regeneration of the resin-silver nanoparticle filter after exhaustion need to be developed in order to sustain the filter material. Finally, there is a need to operate

the filter system on a larger scale in order to investigate its performance *in-situ* for the production of safe drinking water at the quantities required per person per day.

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APPENDIX A – NORTH WEST AND MPUMALANGA PROVINCES DATA

(All values are average of triplicate sample)

Date- September and November 2008

North West Province Coordinates

Local Municipalities	Locations of Boreholes	GPS Coordinates	
Moretele	Dikebu	S 25^0 12′ 24.4″	E 27^0 58′ 20.2″
	Makapanstad	S 25^0 14′ 55.2″	E 28^0 07′ 53.4″
	Noroki	S 25^0 16′ 34.6″	E 28^0 01′ 49.2″
	Moretele Clinic	S 25^0 08′ 46.0″	E 27^0 57′ 34.2″
	Kgomo-Kgomo	S 25^0 09′ 26.4″	E 28^0 04′ 14.9″
	Mashilomatsho 1	S 25^0 05′ 11.9″	E 27^0 55′ 49.1″
	Mashilomatsho 2	S 25^0 05′ 10.4″	E 27^0 55′ 49.1″
	Swartdam	S 25^0 21′ 31.0″	E 28^0 04′ 08.0″
Madibeng	Shakung 1	S 25^0 21′ 05.4″	E 27^0 55′ 37.1″
	Shakung 2	S 25^0 21′ 24.0″	E 27^0 55′ 06.1″
	Dipompong	S 25^0 24′ 22.0″	E 27^0 57′ 11.1″
	Maboloka	S 25^0 26′ 26.0″	E 27^0 51′ 23.2″
	Rabokale	S 25^0 26′ 00.1″	E 27^0 50′ 03.2″
	Jericho	S 25^0 19′ 01.0″	E 27^0 48′ 10.4″
	Moiletswane	S 25^0 23′ 37.0″	E 27^0 56′ 14.4″
Moses Kotane	Ratau	S 25^0 10′ 16.1″	E 26^0 43′ 43.3″
	Mabalstad	S 25^0 18′ 37.2″	E 26^0 45′ 19.7″
	Makgope	S 25^0 06′ 53.5″	E 26^0 53′ 27.1″
	Khayakhulu	S 25^0 06′ 26.3″	E 26^0 39′ 19.5″
	Letlhakeng	S 25^0 08′ 49.5″	E 26^0 43′ 29.6″
	Voordonker	S 25^0 08′ 20.2″	E 26^0 39′ 37.2″
	Lefaratlhatlha	S 25^0 16′ 41.0″	E 26^0 44′ 01.2″
	Matau	S 25^0 14′ 25.6″	E 26^0 39′ 30.9″
	Masekoloane	S 25^0 14′ 23.2″	E 26^0 39′ 37.9″
	Molorwe	S 25^0 05′ 25.7″	E 26^0 53′ 50.2″
	Motlhabe	S 25^0 04′ 05.2″	E 26^0 56′ 41.7″
	Tweelagte 2	S 25^0 17′ 46.6″	E 26^0 49′ 22.3″
	Manamela	S 25^0 10′ 28.7″	E 26^0 40′ 30.9″
	Bapong 2	S 25^0 18′ 02.2″	E 26^0 51′ 06.1″
	Mantsho	S 25^0 04′ 24.1″	E 26^0 44′ 03.7″

Ramotshere Moiloa	Luthern Mission	S 25^0 23$'$ 17.9$''$	E 26^0 05$'$ 23.8$''$
	Malebelele P.S	S 25^0 23$'$ 25.0$''$	E 26^0 05$'$ 57.9$''$
	Braklaagte 1	S 25^0 18$'$ 03.0$''$	E 26^0 06$'$ 25.1$''$
	Braklaagte 2	S 25^0 23$'$ 13.2$''$	E 26^0 06$'$ 20.1$''$
	Braklaagte 3	S 25^0 23$'$ 02.2$''$	E 26^0 06$'$ 09.2$''$
	Leeufontein 1	S 25^0 17$'$ 31.0$''$	E 26^0 05$'$ 53.4$''$
	Leeufontein 2	S 25^0 17$'$ 23.3$''$	E 26^0 05$'$ 45.7$''$
	Leeufontein 3	S 25^0 20$'$ 02.0$''$	E 26^0 07$'$ 47.6$''$
	Supingstad 1	S 24^0 46$'$ 51.0$''$	E 26^0 06$'$ 04.5$''$
	Supingstad 2	S 24^0 46$'$ 50.6$''$	E 26^0 06$'$ 02.5$''$
	Lekgopung 1	S 24^0 48$'$ 07.0$''$	E 26^0 00$'$ 54.1$''$
	Lekgopung 2	S 24^0 48$'$ 02.2$''$	E 26^0 00$'$ 49.2$''$
	Mushane 1	S 24^0 57$'$ 57.7$''$	E 25^0 53$'$ 47.7$''$
	Mushane 2	S 24^0 58$'$ 55.3$''$	E 25^0 53$'$ 46.2$''$
Tswaing	Groeteland	S 26^0 48$'$ 27.5$''$	E 25^0 15$'$ 04.6$''$
	Rapoeli	S 26^0 45$'$ 13.4$''$	E 25^0 20$'$ 20.0$''$
	Dellaryville	S 26^0 39$'$ 13.3$''$	E 25^0 27$'$ 14.1$''$
	Bamberg Farm	S 26^0 37$'$ 50.6$''$	E 25^0 26$'$ 26.4$''$
	Eclipse Farm	S 26^0 35$'$ 21.8$''$	E 25^0 24$'$ 30.0$''$
	Vriegevagte 1	S 26^0 32$'$ 20.7$''$	E 25^0 24$'$ 30.1$''$
	Vriegevagte 2	S 26^0 32$'$ 01.1$''$	E 25^0 24$'$ 03.1$''$
	Atamelang.	S 26^0 30$'$ 17.4$''$	E 25^0 21$'$ 54.8$''$
	Setlagole 1	S 26^0 15$'$ 45.9$''$	E 25^0 07$'$ 50.9$''$
	Setlagole 2	S 26^0 15$'$ 37.4$''$	E 25^0 07$'$ 32.5$''$
Mafikeng	Signal Hill 1	S 25^0 49$'$ 11.2$''$	E 25^0 38$'$ 23.9$''$
	Signal Hill 2	S 25^0 49$'$ 04.7$''$	E 25^0 38$'$ 34.5$''$
	Lonely Park 1	S 25^0 49$'$ 33.9$''$	E 25^0 38$'$ 27.9$''$
	Lonely Park 2	S 25^0 49$'$ 44.2$''$	E 25^0 39$'$ 07.4$''$
	Moshewane	S 25^0 48$'$ 00.5$''$	E 25^0 37$'$ 54.2$''$
	Megokgoane	S 25^0 43$'$ 06.0$''$	E 25^0 37$'$ 03.9$''$
	Dimorogwane 1	S 25^0 40$'$ 16.9$''$	E 25^0 34$'$ 53.4$''$
	Dimorogwane 2	S 25^0 40$'$ 15.6$''$	E 25^0 34$'$ 51.9$''$
	Miga 1	S 25^0 39$'$ 17.7$''$	E 25^0 34$'$ 21.4$''$
	Miga 2	S 25^0 39$'$ 16.5$''$	E 25^0 33$'$ 49.8$''$
	Ikopeleng 1	S 25^0 41$'$ 07.3$''$	E 25^0 36$'$ 07.6$''$
	Ikopeleng 2	S 25^0 40$'$ 56.5$''$	E 25^0 36$'$ 06.9$''$
	Six hundred 1	S 25^0 37$'$ 38.7$''$	E 25^0 37$'$ 38.7$''$
	Six hundred 2	S 25^0 42$'$ 42.1$''$	E 25^0 37$'$ 08.5$''$

	Dihatshwane 1	S 25^0 54$'$ 30.6$''$	E 25^0 45$'$ 17.6$''$
	Dihatshwane 2	S 25^0 54$'$ 28.0$''$	E 25^0 45$'$ 12.0$''$
	Majemantsho 1	S 25^0 53$'$ 17.7$''$	E 25^0 39$'$ 28.1$''$
	Majemantsho 2	S 25^0 53$'$ 25.5$''$	E 25^0 39$'$ 37.4$''$
	Setlopo 1	S 25^0 54$'$ 15.3$''$	E 25^0 38$'$ 22.0$''$
	Setlopo 2	S 25^0 54$'$ 07.3$''$	E 25^0 38$'$ 20.4$''$
	Setlopo 3	S 25^0 54$'$ 15.6$''$	E 25^0 38$'$ 20.5$''$
	Motloung	S 25^0 52$'$ 47.0$''$	E 25^0 36$'$ 39.3$''$
	Mokgokoe	S 25^0 53$'$ 30.1$''$	E 25^0 35$'$ 07.2$''$
	Lekoko 1	S 25^0 55$'$ 31.2$''$	E 25^0 30$'$ 49.6$''$
	Lekoko Clinic	S 25^0 55$'$ 48.4$''$	E 25^0 30$'$ 10.5$''$
	Morwatshethla	S 25^0 56$'$ 26.1$''$	E 25^0 28$'$ 35.1$''$
Kagisano	Mophohung 1	S 26^0 37$'$ 18.3$''$	E 24^0 11$'$ 46.9$''$
	Mophohung 2	S 26^0 36$'$ 55.5$''$	E 24^0 11$'$ 27.1$''$
	Ganyesa 1	S 26^0 35$'$ 22.2$''$	E 24^0 10$'$ 26.1$''$
	Ganyesa 2	S 26^0 35$'$ 05.3$''$	E 24^0 10$'$ 26.1$''$
	Moswane 1	S 26^0 32$'$ 14.0$''$	E 24^0 14$'$ 22.8$''$
	Moswane 2	S 26^0 32$'$ 05.5$''$	E 24^0 14$'$ 14.5$''$
	Selosesha	S 26^0 34$'$ 37.9$''$	E 24^0 09$'$ 14.3$''$
	Thokoza	S 26^0 34$'$ 47.0$''$	E 24^0 09$'$ 23.6$''$
Greater Taung	Dryharts 1	S 27^0 19$'$ 39.8$''$	E 24^0 43$'$ 34.8$''$
	Dryharts Clinic	S 27^0 20$'$ 01.6$''$	E 24^0 43$'$ 10.6$''$
	Moretele	S 27^0 19$'$ 26.2$''$	E 24^0 41$'$ 29.5$''$
	Ntswanahatshe	S 27^0 18$'$ 59.9$''$	E 24^0 41$'$ 26.6$''$
	Choseng	S 27^0 22$'$ 51.2$''$	E 24^0 41$'$ 17.1$''$
	Pudimoe	S 27^0 23$'$ 34.8$''$	E 24^0 42$'$ 51.7$''$
	Matlapeng	S 27^0 24$'$ 50.5$''$	E 24^0 41$'$ 06.1$''$
	Matlapeng Clinic	S 27^0 25$'$ 23.0$''$	E 24^0 41$'$ 05.3$''$
	Leshobo	S 27^0 28$'$ 37.3$''$	E 24^0 42$'$ 06.3$''$
	Myra (Thakung P.S)	S 27^0 24$'$ 02.1$''$	E 24^0 46$'$ 03.6$''$
	Amalia 1	S 27^0 14$'$ 51.7$''$	E 25^0 01$'$ 50.5$''$
	Amalia Clinic	S 27^0 15$'$ 01.1$''$	E 25^0 01$'$ 50.6$''$

Mpumalanga Province Coordinates

Local Municipalities	Locations of Boreholes	GPS Coordinates	
Delmas	C1	S 26^0 08$^'$ 54.9$^"$	E 28^0 39$^'$ 55.8$^"$
	C2	S 26^0 09$^'$ 09.3$^"$	E 28^0 39$^'$ 49.7$^"$
	C3	S 26^0 09$^'$ 20.8$^"$	E 28^0 39$^'$ 59.7$^"$
	C4	S 26^0 09$^'$ 17.4$^"$	E 28^0 39$^'$ 56.0$^"$
	A3	S 26^0 07$^'$ 09.9$^"$	E 28^0 42$^'$ 22.1$^"$
	A4	S 26^0 07$^'$ 15.1$^"$	E 28^0 42$^'$ 27.3$^"$
	A7	S 26^0 07$^'$ 10.7$^"$	E 28^0 42$^'$ 15.4$^"$
	BOT6	S 26^0 07$^'$ 02.8$^"$	E 28^0 41$^'$ 34.2$^"$
	BOT9	S 26^0 05$^'$ 51.7$^"$	E 28^0 40$^'$ 54.8$^"$
	D10	S 26^0 08$^'$ 45.0$^"$	E 28^0 40$^'$ 24.1$^"$
Emalahleni	Leeufontein	S 26^0 08$^'$ 21.1$^"$	E 28^0 57$^'$ 20.5$^"$
	Kendal	S 26^0 04$^'$ 00.9$^"$	E 28^0 57$^'$ 02.0$^"$
	Emakhosi	S 26^0 03$^'$ 36.9$^"$	E 28^0 58$^'$ 07.0$^"$
	Borax	S 26^0 16$^'$ 05.2$^"$	E 28^0 14$^'$ 13.1$^"$
	Bethal	S 26^0 28$^'$ 26.7$^"$	E 30^0 24$^'$ 15.8$^"$
	Legdaar Farm 78	S 26^0 19$^'$ 45.8$^"$	E 29^0 27$^'$ 38.6$^"$
Emakhazeni	Nhlupheko P.S	S 25^0 45$^'$ 52.0$^"$	E 29^0 57$^'$ 18.2$^"$
	Sanbery	S 25^0 45$^'$ 44.3$^"$	E 29^0 57$^'$ 21.0$^"$
	Belfast 1	S 25^0 45$^'$ 46.8$^"$	E 29^0 57$^'$ 20.9$^"$
	Belfast 2	S 25^0 41$^'$ 31.0$^"$	E 30^0 02$^'$ 20.6$^"$
	Belfast 3	S 25^0 41$^'$ 31.8$^"$	E 30^0 02$^'$ 21.3$^"$
	Mooifontein	S 26^0 23$^'$ 34.0$^"$	E 29^0 27$^'$ 31.9$^"$
Mkhondo	Groenvlei-Buhleni Farm	S 26^0 03$^'$ 04.8$^"$	E 30^0 23$^'$ 09.6$^"$
	Vezinyaho 1	S 26^0 16$^'$ 18.7$^"$	E 30^0 35$^'$ 36.5$^"$
	Vezinyaho 2	S 26^0 35$^'$ 06.5$^"$	E 30^0 43$^'$ 14.4$^"$
	Vezinyaho 3	S 26^0 35$^'$ 29.4$^"$	E 30^0 43$^'$ 30.5$^"$
	Weeber Farm Piet R.	S 27^0 02$^'$ 44.2$^"$	E 30^0 42$^'$ 35.3$^"$
	Goedetrouw 1	S 27^0 02$^'$ 36.3$^"$	E 30^0 39$^'$ 48.7$^"$
	Goedetrouw 2	S 27^0 02$^'$ 42.5$^"$	E 30^0 40$^'$ 49.3$^"$
	Driehoek	S 27^0 03$^'$ 11.7$^"$	E 30^0 37$^'$ 10.3$^"$
	Anyspruit	S 27^0 03$^'$ 24.3$^"$	E 30^0 36$^'$ 42.5$^"$
	Ematafeleni	S 27^0 04$^'$ 35.7$^"$	E 30^0 32$^'$ 48.6$^"$
	Kwa-Ngema 1	S 27^0 04$^'$ 44.4$^"$	E 30^0 31$^'$ 20.2$^"$
	Kwa-Ngema 2	S 27^0 04$^'$ 54.2$^"$	E 30^0 30$^'$ 48.0$^"$
	Zeelie Farm	S 27^0 05$^'$ 53.3$^"$	E 30^0 29$^'$ 47.2$^"$

	Dirkiesdorp	S 27^0 10$^{'}$ 02.0$^{''}$	E 30^0 24$^{'}$ 26.3$^{''}$
	Dirkiesdorp Farm	S 27^0 07$^{'}$ 38.8$^{''}$	E 30^0 27$^{'}$ 23.2$^{''}$
	Dirkiesdorp Police S.	S 27^0 10$^{'}$ 26.1$^{''}$	E 30^0 24$^{'}$ 26.2$^{''}$
	Injabulo Combined S.	S 27^0 10$^{'}$ 27.0$^{''}$	E 30^0 24$^{'}$ 15.8$^{''}$
	St Helena Farm	S 27^0 08$^{'}$ 29.9$^{''}$	E 30^0 27$^{'}$ 04.7$^{''}$
	Themba Trust School	S 27^0 09$^{'}$ 18.9$^{''}$	E 30^0 25$^{'}$ 38.0$^{''}$
Pixle Ka Seme	Kranspoort.	S 26^0 20$^{'}$ 09.1$^{''}$	E 29^0 52$^{'}$ 25.0$^{''}$
	Heins Farm resort	S 26^0 25$^{'}$ 31.2$^{''}$	E 29^0 56$^{'}$ 26.2$^{''}$
	Drinkwater 1	S 26^0 40$^{'}$ 17.1$^{''}$	E 29^0 57$^{'}$ 10.7$^{''}$
	Drinkwater 2	S 26^0 42$^{'}$ 17.3$^{''}$	E 29^0 56$^{'}$ 50.2$^{''}$
	Tafelkop	S 26^0 30$^{'}$ 02.0$^{''}$	E 29^0 52$^{'}$ 46.7$^{''}$
	Rietvlei	S 26^0 30$^{'}$ 27.3$^{''}$	E 29^0 51$^{'}$ 14.6$^{''}$
	Winkellaak	S 26^0 31$^{'}$ 00.7$^{''}$	E 29^0 49$^{'}$ 38.4$^{''}$
	Goededorp Amersfoort	S 26^0 43$^{'}$ 47.4$^{''}$	E 29^0 56$^{'}$ 29.4$^{''}$
	Piet Zyn drift Farm	S 26^0 47$^{'}$ 52.9$^{''}$	E 29^0 55$^{'}$ 30.3$^{''}$
	Langspoort Volksrust	S 27^0 08$^{'}$ 48.5$^{''}$	E 29^0 52$^{'}$ 29.3$^{''}$
	Wenber Wakkerstrroom	S 27^0 21$^{'}$ 25.0$^{''}$	E 30^0 00$^{'}$ 09.8$^{''}$
	Pietskop Wakkersttroom 1	S 27^0 21$^{'}$ 00.7$^{''}$	E 30^0 05$^{'}$ 09.9$^{''}$
	Pietskop Wakkersttroom 2	S 27^0 21$^{'}$ 00.1$^{''}$	E 30^0 04$^{'}$ 23.6$^{''}$
	Wakkerstroom plot 40	S 27^0 21$^{'}$ 33.2$^{''}$	E 30^0 06$^{'}$ 34.5$^{''}$
	Uithenden Wakkerstroom	S 27^0 21$^{'}$ 53.0$^{''}$	E 30^0 07$^{'}$ 41.8$^{''}$
Albert Luthuli	Travelpoort Garage	S 25^0 45$^{'}$ 05.7$^{''}$	E 30^0 58$^{'}$ 13.0$^{''}$
	Rooihoogte Farm B16	S 25^0 55$^{'}$ 04.9$^{''}$	E 30^0 35$^{'}$ 58.1$^{''}$
	Elstone Farm	S 25^0 32$^{'}$ 54.5$^{''}$	E 30^0 57$^{'}$ 53.4$^{''}$
	Mosley Farm 13	S 25^0 41$^{'}$ 42.3$^{''}$	E 30^0 58$^{'}$ 10.7$^{''}$
	Mosley Farm 17	S 25^0 41$^{'}$ 42.1$^{''}$	E 30^0 58$^{'}$ 10.7$^{''}$
	Mosley Farm 27	S 25^0 41$^{'}$ 55.3$^{''}$	E 30^0 58$^{'}$ 19.4$^{''}$
	Natal drift plot 13	S 25^0 42$^{'}$ 46.1$^{''}$	E 30^0 58$^{'}$ 53.5$^{''}$
	Pullenshope	S 26^0 00$^{'}$ 26.6$^{''}$	E 29^0 39$^{'}$ 02.5$^{''}$
	Drankenstein	S 26^0 04$^{'}$ 10.5$^{''}$	E 29^0 41$^{'}$ 11.2$^{''}$
	Bosmansfontein 1	S 26^0 04$^{'}$ 28.8$^{''}$	E 29^0 40$^{'}$ 55.0$^{''}$
	Bosmansfontein 2	S 26^0 06$^{'}$ 27.5$^{''}$	E 29^0 41$^{'}$ 45.7$^{''}$
Mbombela	Katoen Farm	S 25^0 41$^{'}$ 45.8$^{''}$	E 30^0 02$^{'}$ 31.5$^{''}$
	Mphatheni Mahushu	S 25^0 06$^{'}$ 38.7$^{''}$	E 31^0 08$^{'}$ 13.6$^{''}$
	Mphatheni Guest house	S 25^0 06$^{'}$ 58.7$^{''}$	E 31^0 08$^{'}$ 13.3$^{''}$
	White River 73A	S 25^0 20$^{'}$ 51.9$^{''}$	E 30^0 59$^{'}$ 46.8$^{''}$
	White River 73B	S 25^0 20$^{'}$ 52.0$^{''}$	E 30^0 59$^{'}$ 47.2$^{''}$
	White River 53	S 25^0 20$^{'}$ 58.4$^{''}$	E 31^0 00$^{'}$ 05.6$^{''}$

	White River 60	S 25^{0} 20$^{'}$ 58.5$^{''}$	E 30^{0} 59$^{'}$ 47.2$^{''}$
	Heide Eggs	S 25^{0} 19$^{'}$ 32.0$^{''}$	E 30^{0} 56$^{'}$ 38.4$^{''}$
	Mtimba B	S 25^{0} 19$^{'}$ 30.8$^{''}$	E 30^{0} 56$^{'}$ 33.7$^{''}$
	Malapa Farm	S 25^{0} 21$^{'}$ 14.6$^{''}$	E 30^{0} 59$^{'}$ 39.5$^{''}$
	Heidelberg Farm 7	S 25^{0} 20$^{'}$ 58.9$^{''}$	E 31^{0} 00$^{'}$ 04.8$^{''}$
	Heidelberg Farm 25	S 25^{0} 20$^{'}$ 55.3$^{''}$	E 31^{0} 00$^{'}$ 05.6$^{''}$
	Heidelberg Tevrede	S 25^{0} 20$^{'}$ 57.8$^{''}$	E 31^{0} 00$^{'}$ 04.0$^{''}$
	Moegeploeg Plot 36	S 25^{0} 24$^{'}$ 38.4$^{''}$	E 31^{0} 56$^{'}$ 05.0$^{''}$
	Moegeploeg Plot 37	S 25^{0} 24$^{'}$ 38.6$^{''}$	E 31^{0} 56$^{'}$ 05.9$^{''}$
	Weltevreden Plot 455A	S 25^{0} 33$^{'}$ 03.4$^{''}$	E 30^{0} 57$^{'}$ 13.3$^{''}$
	Weltevreden Plot 455B	S 25^{0} 32$^{'}$ 53.5$^{''}$	E 30^{0} 57$^{'}$ 46.0$^{''}$
	Weltevreden Plot 43	S 25^{0} 32$^{'}$ 53.3$^{''}$	E 30^{0} 57$^{'}$ 45.8$^{''}$
Nkomazi	River View P.S	S 25^{0} 07$^{'}$ 19.9$^{''}$	E 31^{0} 08$^{'}$ 05.0$^{''}$
	Beyers Farm 1	S 25^{0} 31$^{'}$ 07.0$^{''}$	E 31^{0} 29$^{'}$ 58.1$^{''}$
	Beyers Farm 2	S 25^{0} 30$^{'}$ 57.9$^{''}$	E 31^{0} 29$^{'}$ 20.6$^{''}$
	Baundi Farm	S 25^{0} 31$^{'}$ 15.5$^{''}$	E 31^{0} 29$^{'}$ 32.6$^{''}$
	Mpapani Farm	S 25^{0} 30$^{'}$ 37.4$^{''}$	E 31^{0} 29$^{'}$ 27.1$^{''}$
	Bambinyati Farm 19	S 25^{0} 30$^{'}$ 35.2$^{''}$	E 31^{0} 30$^{'}$ 03.8$^{''}$
	Bambinyati Farm house	S 25^{0} 30$^{'}$ 35.3$^{''}$	E 31^{0} 30$^{'}$ 03.5$^{''}$
	Boland Farm	S 25^{0} 30$^{'}$ 50.3$^{''}$	E 31^{0} 30$^{'}$ 25.2$^{''}$
	Malelani Farm	S 25^{0} 29$^{'}$ 59.8$^{''}$	E 31^{0} 30$^{'}$ 24.7$^{''}$
	Eskom Distribution	S 25^{0} 29$^{'}$ 49.8$^{''}$	E 31^{0} 31$^{'}$ 21.9$^{''}$
	Lilly Pond	S 25^{0} 29$^{'}$ 42.7$^{''}$	E 31^{0} 31$^{'}$ 21.3$^{''}$
	Voorspoet Farm	S 25^{0} 24$^{'}$ 43.0$^{''}$	E 31^{0} 56$^{'}$ 47.7$^{''}$
	Voorspoet commonage 1	S 25^{0} 22$^{'}$ 54.5$^{''}$	E 31^{0} 56$^{'}$ 39.1$^{''}$
	Voorspoet commonage 2	S 25^{0} 22$^{'}$ 55.7$^{''}$	E 31^{0} 56$^{'}$ 39.7$^{''}$
	Elenberg Komatipoort Farm	S 25^{0} 22$^{'}$ 35.7$^{''}$	E 31^{0} 55$^{'}$ 16.8$^{''}$

Groundwater quality of North West Province

BOJANALA DISTRICT			°C	mg/ℓ	NTU	mg/ℓ Cl	mg/ℓ SO_4	mg/ℓ NO_3	mg/ℓ F	mg/ℓ Mg	mg/ℓ Na	mg/ℓ Ca	mg/ℓ K	count/100mℓ	count/100mℓ
Local Municipalities	**Locations of Boreholes**	**pH Value**	**Temperature**	**TDS**	**Turbidity**	**Chloride**	**Sulphate**	**Nitrates**	**Fluoride**	**Magnesium**	**Sodium**	**Calcium**	**Potassium**	**Faecal coliforms**	**Total coliforms**
Moretele	Dikebu	7.89	22.0	395.0	1.83	87.42	117.60	5.08	41.40	64.66	17.84	44.08	12.30	1	46
	Makapanstad	7.78	22.5	570.0	2.18	84.51	259.40	0.34	37.70	41.49	45.78	100.2	12.00	12	50
	Noroki	7.60	22.0	614.0	1.82	86.44	16.54	2.33	26.91	179.7	14.78	64.92	3.85	2	21
	Moretele Clinic	7.61	23.1	147.7	2.39	32.05	17.36	4.50	26.90	27.24	3.35	26.45	4.65	4	0
	Kgomo-Kgomo	8.47	22.9	105.2	3.17	327.3	240.40	7.91	34.84	290.1	40.51	64.92	22.1	0	6
	Mashilomatsho 1	7.26	23.0	168.1	5.17	19.43	14.59	1.67	28.10	25.97	1.63	20.04	2.64	4	148
	Mashilomatsho 2	7.27	22.1	123.7	2.57	33.00	28.68	1.98	29.83	28.89	2.95	36.07	2.90	2	320
	Swartdam	7.94	22.6	146.4	1.15	511.9	96.09	1.49	11.00	346.9	37.71	136.3	13.3	1	20
Madibeng	Shakung 1	7.70	24.0	683.0	5.80	233.60	95.41	0.11	15.00	126.6	22.47	104.2	9.06	50	400
	Shakung 2	8.28	25.1	478.2	2.21	71.39	84.44	0.99	16.00	100.7	17.84	62.52	5.28	1	80
	Dipompong	8.33	23.9	930.0	1.15	386.10	44.51	1.01	5.70	386.9	1.86	15.23	3.38	0	1
	Maboloka	7.92	24.4	321.0	0.98	44.19	210.30	1.00	11.30	40.54	22.07	40.88	2.89	0	10
	Rabokale	7.69	24.0	186.5	0.37	30.00	25.47	0.10	12.80	33.14	3.72	27.25	3.88	1	30
	Jericho	7.98	23.3	511.0	1.72	109.30	131.70	0.99	13.11	82.32	12.91	55.31	5.56	2	41
	Moiletswane	8.49	24.8	452.0	6.03	166.10	46.89	1.28	14.20	76.92	26.60	60.12	5.44	1	3
Moses Kotane	Ratau	8.29	23.8	446.1	0.39	14.57	10.90	4.73	32.80	90.16	34.62	9.61	1.69	2	217
	Mabalstad	8.19	22.6	441.1	0.27	13.02	16.24	10.60	22.41	63.00	45.90	11.22	1.17	5	12
	Makgope	8.68	24.7	151.9	0.33	13.60	13.77	10.60	17.70	100.1	21.25	12.82	3.37	16	17
	Khayakhulu	8.39	23.2	486.0	0.66	40.80	13.14	11.31	32.62	34.99	81.00	26.45	0.09	0	24
	Letlhakeng	8.40	22.9	878.0	0.78	22.34	11.92	11.10	32.90	26.32	62.71	28.05	0.98	6	4

	Voordonker	8.44	23.0	402.2	0.76	34.00	11.00	14.80	33.40	41.54	37.92	50.50	2.75	5	36
	Lefaratlhatlha	8.83	23.5	257.0	0.82	11.65	13.19	10.41	24.90	160.1	53.13	31.26	1.80	1	78
	Matau	8.81	23.7	187.4	0.68	34.00	11.34	12.70	28.00	55.42	25.88	40.88	1.88	1	17
	Masekoloane	8.80	24.1	68.2	0.91	17.48	10.66	11.00	33.20	66.20	82.78	44.08	0.68	6	105
	Molorwe	8.43	23.9	471.3	0.32	18.45	10.51	11.40	32.60	73.21	36.29	68.93	0.16	5	10
	Motlhabe	7.65	23.6	426.0	0.58	14.57	11.11	11.00	23.80	38.90	55.74	14.42	14.00	36	147
	Tweelagte 2	9.11	22.8	130.4	0.21	23.31	12.31	9.96	23.81	88.56	69.01	64.92	1.17	58	153
	Manamela	8.63	23.7	413.2	0.29	11.65	89.97	11.40	20.70	25.21	20.87	21.64	5.31	14	167
	Bapong 2	8.19	24.0	68.7	0.74	18.45	10.42	11.10	33.30	120.8	43.25	17.63	0.48	4	318
	Mantsho	8.49	22.6	51.5	3.21	136.00	11.92	13.80	33.20	23.29	56.31	14.42	4.64	1	460

CENTRAL DISTRICT			**°C**	**mg/ℓ**	**NTU**	**mg/ℓ Cl**	**mg/ℓ SO_4**	**mg/ℓ NO_3**	**mg/ℓ F**	**mg/ℓ Mg**	**mg/ℓ Na**	**mg/ℓ Ca**	**mg/ℓ K**	**count/100mℓ**	**count/100mℓ**
Local Municipalities	**Locations of Boreholes**	**pH Value**	**Temperature**	**TDS**	**Turbidity**	**Chloride**	**Sulphate**	**Nitrates**	**Fluoride**	**Magnesium**	**Sodium**	**Calcium**	**Potassium**	**Faecal coliforms**	**Total coliforms**
Ramotshere Moiloa	Luthern Mission	6.60	25.0	124.0	2.18	15.00	12.90	4.23	2.61	2.04	12.16	11.47	4.90	1	4
	Malebelele P.S	6.81	25.0	85.5	13.80	16.99	11.51	8.00	3.11	2.12	5.31	5.85	3.86	0	1
	Braklaagte 1	6.50	24.1	96.7	1.32	11.01	12.20	3.33	4.21	1.94	7.49	10.49	6.48	2	45
	Braklaagte 2	6.83	25.0	93.3	0.92	25.99	14.33	4.00	2.42	1.38	8.14	9.65	5.52	1	33
	Braklaagte 3	6.62	24.1	156.0	20.60	17.98	14.40	4.76	2.98	3.43	17.98	13.52	5.23	2	10
	Leeufontein 1	7.40	25.1	219.2	19.70	13.10	15.50	3.98	1.00	4.67	17.27	20.05	32.70	38	94
	Leeufontein 2	7.51	24.0	220.0	40.00	12.21	16.11	2.88	0.95	2.79	17.55	25.59	31.40	0	2
	Leeufontein 3	7.71	25.0	312.5	2.97	11.16	14.80	4.52	3.98	2.66	18.72	27.19	16.60	4	13

	Supingstad 1	7.10	25.0	502.0	1.03	50.98	12.84	7.54	1.22	8.63	46.25	11.42	9.78	5	84
	Supingstad 2	7.51	25.0	500.0	0.72	20.99	16.80	3.99	3.96	3.28	62.28	5.11	3.93	7	31
	Lekgopung 1	7.33	23.4	285.9	1.27	16.90	17.00	6.57	6.00	2.03	28.05	11.48	10.30	62	240
	Lekgopung 2	7.30	24.2	285.5	0.92	13.00	13.20	7.58	1.34	1.43	28.36	8.37	9.36	150	180
	Mushane 1	7.60	22.3	458.7	1.21	21.99	18.00	4.25	5.18	14.15	37.05	38.16	4.27	0	130
	Mushane 2	7.65	26.0	456.5	0.33	21.99	13.00	4.56	0.64	16.16	37.06	8.39	4.16	0	159
Tswaing	Groeteland	7.50	25.0	575.0	1.05	42.98	12.32	5.50	2.54	13.55	20.66	32.15	17.50	36	190
	Rapoeli	6.90	23.0	465.5	0.78	16.20	16.00	9.03	8.25	6.05	3.98	32.48	7.32	1	118
	Dellaryville	7.10	25.0	492.0	0.56	100.0	77.50	9.55	1.72	40.79	21.8	12.88	27.00	1	5
	Bamberg Farm	7.81	24.2	382.0	1.34	167.0	68.90	10.00	0.98	43.38	39.17	41.1	38.50	1	1
	Eclipse Farm	7.40	24.3	354.6	0.20	20.99	23.22	3.94	2.67	16.9	17.18	25.42	22.70	17	141
	Vriegevagte 1	7.53	25.0	490.0	0.88	34.97	19.41	3.61	2.17	12.55	27.25	66.18	19.50	0	29
	Vriegevagte 2	7.50	24.0	454.0	1.56	94.97	20.60	9.59	4.66	11.75	21.56	52.96	22.10	45	82
	Atamelang.	7.60	24.0	483.0	0.45	28.99	20.50	9.39	4.06	13.09	9.14	40.61	8.34	2	22
	Setlagole 1	7.42	24.2	381.0.	1.60	32.94	21.40	7.00	1.23	10.59	23.21	73.94	2.74	14	205
	Setlagole 2	7.51	24.2	539.0	0.84	56.98	33.20	4.88	2.45	16.1	38.24	33.48	7.22	2	365
Mafikeng	Signal Hill 1	7.39	25.8	567.0	0.40	38.90	11.71	4.40	2.7	15.9	64.52	5.3	7.23	1	6
	Signal Hill 2	7.78	24.6	584.2	0.36	32.90	16.00	5.57	5.52	24.3	65.29	7.02	7.43	1	58
	Lonely Park 1	7.44	24.4	412.0	0.80	16.00	14.30	5.61	3.12	6.01	38.48	6.68	2.90	32	65
	Lonely Park 2	8.17	24.5	382.0	0.66	40.91	21.20	8.31	1.22	4.25	50.7	15.81	1.88	42	76
	Moshewane	8.00	24.7	343.3	3.83	30.90	14.00	1.50	0.4	12.1	26.04	40.48	17.00	2	1
	Megokgoane	7.38	23.6	465.3	0.82	37.92	17.70	1.70	3.7	11.6	43.28	46.51	8.17	2	40
	Dimorogwane 1	7.96	23.5	468.0	1.18	54.90	12.80	7.13	2.64	22.9	29.42	7.36	20.40	19	57
	Dimorogwane 2	7.89	24.4	460.0	0.64	53.90	228.0	5.57	0.42	16.5	36.28	28.41	19.80	4	86
	Miga 1	7.42	22.9	575.0	0.53	51.00	33.40	3.90	2.8	19.4	24.32	20.67	18.60	1	1
	Miga 2	7.41	23.1	492.1	1.44	40.90	12.41	9.11	0.12	29.2	29.17	20.11	20.00	1	75
	Ikopeleng 1	7.21	23.7	631.0	0.95	104.0	15.30	3.30	2.6	21.3	39.27	12.81	23.40	1	210
	Ikopeleng 2	7.17	24.1	613.0	0.33	116.0	60.40	8.51	2.33	17.4	43.13	11.56	8.17	2	27
	Six hundred 1	7.32	24.5	505.0	3.15	62.90	16.72	2.50	4.7	27.6	28.93	57.69	12.30	6	35
	Six hundred 2	7.79	24.6	454.1	2.05	36.90	12.90	5.57	0.51	4.27	28.7	41.47	21.10	2	265
	Dihatshwane 1	7.04	22.5	425.0	1.34	34.90	18.20	0.60	2.4	18.4	19.78	19.99	1.81	0	33
	Dihatshwane 2	7.08	23.6	463.0	2.45	40.90	13.50	1.80	4.71	8.14	17.33	24.43	2.11	0	2

	Majemantsho 1	7.54	22.4	541.1	0.56	118.00	48.80	6.00	3.12	23.9	20.02	29.07	6.39	1	85
	Majemantsho 2	7.62	24.9	483.0	0.50	99.80	12.31	3.60	3.3	25.8	27.26	43.04	3.87	1	127
	Setlopo 1	7.28	25.1	533.0	1.51	590.00	42.30	5.25	5.1	18.7	27.33	18.23	2.39	0	365
	Setlopo 2	7.43	25.0	485.1	1.45	44.90	18.51	9.16	2.2	16.4	21.83	24.91	2.26	2	275
	Setlopo 3	7.28	24.8	522.0	0.99	57.90	74.30	9.00	4.43	16.9	30.02	24.71	1.87	3	94
	Motloung	7.52	25.7	585.0	0.84	94.81	13.50	0.44	2.73	26.6	26.6	24.56	0.40	3	43
	Mokgokoe	7.97	24.6	490.0	0.67	39.90	13.00	1.20	0.4	21.70	23.79	8.29	6.13	0	440
	Lekoko 1	7.69	24.4	516.0	2.11	35.91	22.80	7.50	0.6	15.10	38.18	53.61	25.60	1	3
	Lekoko Clinic	7.39	24.1	289.0	1.62	17.00	14.40	0.61	0.1	9.63	19.39	32.74	1.72	36	124
	Morwatshethla	6.88	21.1	354.0	19.4	69.90	30.20	2.4	0.4	9.40	26.73	51.17	10.50	7	144

BOPHIRIMA DISTRICT			**°C**	**mg/ℓ**	**NTU**	**mg/ℓ Cl**	**mg/ℓ SO_4**	**mg/ℓ NO_3**	**mg/ℓ F**	**mg/ℓ Mg**	**mg/ℓ Na**	**mg/ℓ Ca**	**mg/ℓ K**	**count/100mℓ**	**count/100mℓ**
Local Municipalities	**Locations of Boreholes**	**pH Value**	**Temperature**	**TDS**	**Turbidity**	**Chloride**	**Sulphate**	**Nitrates**	**Fluoride**	**Magnesium**	**Sodium**	**Calcium**	**Potassium**	**Faecal coliforms**	**Total coliforms**
Kagisano	Mophohung 1	7.16	24.0	315.1	19.80	57.98	15.03	1.51	1.70	11.30	17.40	46.97	1.64	12	185
	Mophohung 2	7.87	24.1	390.0	1.35	66.97	13.04	2.73	0.12	15.90	19.00	52.94	11.50	45	135
	Ganyesa 1	7.15	24.0	366.3	0.34	91.97	12.89	1.10	1.54	12.30	20.30	56.60	10.70	1	22
	Ganyesa 2	7.45	24.3	624.1	0.19	103.0	25.81	2.74	0.12	26.20	36.10	145.8	13.50	0	62
	Moswane 1	7.26	25.0	267.2	4.43	44.99	15.95	0.48	2.10	7.69	0.22	41.00	0.00	6	91
	Moswane 2	7.07	24.5	307.3	0.67	55.98	12.36	0.76	0.72	9.71	17.30	63.02	11.40	1	3
	Selosesha	7.38	25.0	326.1	0.34	77.98	12.70	1.35	1.24	10.30	18.90	48.42	10.30	1	97
	Thokoza	7.87	25.0	635.0	0.95	213.9	17.41	2.98	0.65	18.20	40.10	125.7	12.50	10	165

Greater Taung	Dryharts 1	7.15	21.9	739.1	0.30	152.0	252.6	5.56	5.00	32.60	46.90	38.79	14.20	3	31
	Dryharts Clinic	7.51	20.5	696.0	1.19	102.0	83.13	6.15	7.98	55.80	47.30	28.17	9.77	3	328
	Moretele	7.61	21.8	476.5	1.67	66.98	199.2	3.28	2.26	15.80	44.60	65.00	1.38	2	24
	Ntswanahatshe	7.29	20.6	502.0	7.68	37.99	121.5	6.05	9.54	8.98	53.30	30.75	12.70	34	111
	Choseng	7.38	21.2	690.3	2.90	75.98	82.69	8.59	7.77	11.00	89.60	32.84	9.23	14	165
	Pudimoe	7.56	21.5	472.1	0.48	54.98	22.56	7.58	6.32	25.00	36.10	21.92	2.77	1	128
	Matlapeng	7.84	22.2	716.4	0.47	128.0	74.29	8.00	1.78	8.39	82.80	82.12	12.20	6	100
	Matlapeng Clinic	7.87	22.2	577.2	0.42	102.0	138.9	5.78	1.00	7.21	66.70	17.04	15.70	14	138
	Leshobo	7.38	21.0	442.8	0.33	39.98	21.20	6.03	1.96	3.48	55.70	25.72	6.42	1	140
	Myra (Thakung P.S)	7.38	21.1	376.3	0.62	78.98	235.6	4.10	7.21	0.61	21.20	22.10	37.40	0	137
	Amalia 1	7.40	23.5	568.7	0.19	75.97	95.85	8.56	9.66	19.50	38.40	20.04	2.61	1	102
	Amalia Clinic	7.45	23.6	567.5	0.24	72.98	116.6	8.54	9.00	19.30	34.60	30.73	2.75	0	113

Groundwater quality of Mpumalanga Province

NKANGALA DISTRICT			**°C**	**mg/ℓ**	**NTU**	**mg/ℓ Cl**	**mg/ℓ SO_4**	**mg/ℓ NO_3**	**mg/ℓ F**	**mg/ℓ Mg**	**mg/ℓ Na**	**mg/ℓ Ca**	**mg/ℓ K**	**count/100mℓ**	**count/100mℓ**
Local Municipalities	**Locations of Boreholes**	**pH Value**	**Temperature**	**TDS**	**Turbidity**	**Chloride**	**Sulphate**	**Nitrates**	**Fluoride**	**Magnesium**	**Sodium**	**Calcium**	**Potassium**	**Faecal coliforms**	**Total coliforms**
Delmas	C1	7.90	19.1	145.0	0.10	20.49	28.76	0.04	0.4	19.7	15.61	40.34	2.44	5	77
	C2	7.95	19.5	187.1	4.48	37.98	67.14	0.20	1.7	19.5	10.86	37.88	1.68	3	96
	C3	7.69	20.3	312.0	1.18	122.5	95.74	0.15	0.9	32.8	11.24	48.53	0.26	8	350
	C4	7.41	19.8	250.3	0.21	40.48	31.08	3.87	1.0	30.3	22.43	40.12	1.23	6	12
	A4	6.68	19.3	198.0	0.91	58.48	30.57	1.04	1.8	23.5	18.01	65.12	1.27	34	88

	A7	7.16	20.0	291.0	2.75	80.47	44.15	0.12	2.2	24.7	25.74	53.2	1.31	2	42
	A3	7.27	20.1	324.5	1.51	90.97	46.68	0.22	0.8	25.4	10.45	49.76	0.78	150	425
	BOT6	7.50	20.3	128.2	0.24	22.49	49.64	0.05	1.3	14.7	26.85	31.03	1.23	1	376
	BOT4	7.30	20.2	145.0	0.35	29.99	51.41	0.08	0.6	15.6	17.82	23.42	0.67	1	34
	D10	6.99	19.9	264.0	0.21	32.99	30.70	3.03	0.4	28.6	14.07	36.23	0.34	2	4
Emalahleni	Leeufontein	7.12	18.0	292.5	0.10	13.49	57.14	13.4	1.5	1.14	15.00	24.28	0.04	7	60
	Kendal	6.56	18.6	446.6	20.3	11.49	32.09	0.28	0.7	12.4	152.1	16.59	0.33	4	6
	Emakhosi	6.88	18.9	435.1	1.26	88.47	28.80	46.1	0.6	17.7	49.46	34.22	0.12	1	360
	Borax	7.50	17.9	349.1	0.56	158.5	32.09	5.20	1.8	1.18	17.03	20.00	1.66	29	333
	Bethal	7.24	27.0	620.0	0.26	85.97	142.3	5.51	0.6	55.9	52.87	78.96	2.12	11	50
	Legdaar Farm 78	7.41	22.0	494.0	0.19	17.99	149.1	2.90	0.9	39.7	34.68	69.24	2.5	0	14
Emakhazeni	Nhlupheko P.S	7.84	22.3	88.0	20.0	22.49	12.07	2.00	0.5	4.97	7.39	23.85	3.95	0	0
	Sanbery	7.50	23.4	79.0	4.31	22.49	12.51	1.30	1.4	3.49	3.33	22.63	4.03	9	4
	Belfast 1	7.86	21.6	189.0	6.67	49.98	20.71	4.00	0.7	19.5	5.14	32.26	2.53	39	10
	Belfast 2	7.90	22.6	186.6	2.07	42.48	11.34	2.70	0.8	16.8	7.75	35.86	4.25	14	30
	Belfast 3	7.85	23.4	134.5	5.54	44.98	13.33	3.70	0.1	7.96	19.41	18.25	1.98	2	79
	Mooifontein	7.58	24.0	274.2	0.78	113.0	37.08	0.20	0.7	88.8	558.2	11.5	5.52	61	157

GERT SIBANDA DISTRICT			°C	mg/ℓ	NTU	mg/ℓ Cl	mg/ℓ SO_4	mg/ℓ NO_3	mg/ℓ F	mg/ℓ Mg	mg/ℓ Na	mg/ℓ Ca	mg/ℓ K	count/100mℓ	count/100mℓ
Local Municipalities	**Locations of Boreholes**	**pH Value**	**Temperature**	**TDS**	**Turbidity**	**Chloride**	**Sulphate**	**Nitrates**	**Fluoride**	**Magnesium**	**Sodium**	**Calcium**	**Potassium**	**Faecal coliforms**	**Total coliforms**
Mkhondo	Groenvlei-Buhleni Farm	7.73	24.0	21.0	2.07	19.99	11.44	1.4	1.05	0.78	1.91	3.01	2.45	1	3
	Vezinyaho 1	7.48	25.1	34.1	1.73	19.99	13.14	1.5	0.70	0.67	2.75	5.54	2.04	2	6
	Vezinyaho 2	7.44	25.5	22.4	0.25	24.99	13.72	0.5	1.08	8.47	37.39	0.20	1.01	1	1
	Vezinyaho 3	7.04	23.0	58.8	10.0	37.48	14.00	1.2	0.98	4.00	40.01	6.75	2.37	1	425
	Weeber Farm Piet R.	7.48	24.2	142.3	1.89	20.90	15.42	0.0	0.40	8.35	15.61	21.2	0.91	2	65
	Goedetrouw 1	7.04	24.4	73.8	1.07	9.98	12.55	0.6	0.37	3.29	9.86	11.10	2.14	0	52
	Goedetrouw 2	7.05	23.9	51.2	0.72	17.96	12.99	0.1	0.17	1.45	9.86	7.15	1.32	0	102
	Driehoek	7.39	23.2	160.1	2.97	16.91	74.09	0.1	0.38	7.86	20.56	23.20	0.99	19	46
	Anyspruit	7.27	22.0	83.2	1.60	11.00	16.49	0.2	0.49	3.02	11.88	13.91	0.62	0	1
	Ematafeleni	9.11	24.0	138.3	1.38	14.95	19.26	0.2	0.35	0.19	55.24	1.48	0.43	2	338
	Kwa-Ngema 1	7.92	26.1	156.2	4.33	15.99	25.47	1.2	0.50	11.9	10.45	22.10	1.66	32	207
	Kwa-Ngema 2	8.00	26.2	98.3	0.51	11.00	30.82	0.1	0.24	0.96	1.88	3.60	0.26	0	0
	Zeelie Farm	7.53	25.1	153.0	0.30	14.95	11.58	1.0	0.13	15.3	7.95	24.80	1.23	0	30
	Dirkiesdorp	7.61	22.0	180.5	1.78	11.96	11.10	0.2	0.35	15.8	14.07	25.62	1.27	6	108
	Dirkiesdorp Farm	7.38	24.0	117.8	9.43	84.97	12.46	0.1	0.83	5.83	7.81	16.00	1.31	0	196
	Dirkiesdorp Police S.	7.35	23.0	100.8	0.44	11.01	55.63	0.0	0.94	2.00	27.10	12.80	0.78	0	1
	Injabulo Combined S.	7.52	24.5	100.7	0.26	9.98	13.38	0.1	0.22	5.77	7.54	15.41	1.30	0	105
	St Helena Farm	7.43	24.0	230.0	0.96	58.98	24.11	0.0	0.69	9.95	41.74	21.10	1.33	0	335
	Themba Trust School	7.35	23.1	111.8	0.56	20.90	12.46	0.0	0.60	0.76	41.76	3.17	1.13	0	5
Pixle Ka Seme	Kranspoort.	7.50	24.0	821.0	0.57	211.9	359.4	0.2	0.11	96.3	23.79	135.4	6.24	2	5
	Heins Farm resort	7.70	25.0	329.0	0.31	31.99	330.3	0.6	0.64	32.60	20.84	41.24	4.79	7	88
	Drinkwater 1	7.72	25.0	203.0	0.26	18.92	89.73	0.0	0.27	19.40	11.29	28.61	0.84	15	115

	Drinkwater 2	8.00	25.0	264.0	0.96	17.89	66.03	0.6	0.38	17.80	52.06	22.82	1.39	4	265
	Tafelkop	7.70	23.0	307.0	1.12	30.90	12.65	0.0	0.69	20.70	33.03	45.11	12.40	1	184
	Rietvlei	7.40	25.0	396.0	0.63	25.92	173.4	4.2	0.18	36.20	22.59	57.78	3.26	8	79
	Winkellaak	7.80	25.0	51.2	8.16	9.90	13.87	0.0	0.48	3.35	6.18	89.07	1.77	4	335
	Goededorp Amersfoort	7.80	23.1	209.1	1.54	17.99	12.41	0.0	0.06	18.30	24.68	25.69	2.85	2	210
	Piet Zyn drift Farm	8.00	25.0	214.2	1.22	17.99	36.40	0.2	0.70	5.21	58.15	16.34	2.22	18	95
	Langspoort Volksrust	7.70	25.0	270.0	2.47	53.98	82.15	0.2	0.73	22.60	18.55	26.74	6.11	14	110
	Wenber Wakkerstrroom	7.80	26.0	239.0	0.45	26.95	34.31	0.0	0.58	8.51	47.07	25.81	2.56	2	4
	Pietskop Wakkersttroom 1	7.60	26.0	186.3	2.66	5.93	15.56	0.0	0.20	12.50	26.46	21.89	0.99	25	140
	Pietskop Wakkersttroom 2	7.40	25.0	574.1	4.46	12.90	12.07	0.1	0.83	3.19	149.70	11.94	2.04	0	1
	Wakkerstroom plot 40	7.30	25.0	143.0	0.5	16.00	13.23	0.5	0.51	7.81	14.2	22.36	0.90	1	92
	Uithenden Wakkerstroom	7.10	26.1	59.1	0.19	16.99	13.04	0.3	0.50	4.33	7.49	9.26	0.24	0	8
Albert Luthuli	Travelpoort Garage	7.60	27.1	148.0	0.25	29.99	26.83	2.0	1.01	13.50	14.42	22.92	0.85	16	36
	Rooihoogte Farm B16	7.50	27.0	120.0	0.79	24.99	11.05	1.7	0.69	8.06	13.40	20.03	0.99	1	6
	Elstone Farm	7.72	27.0	272.0	0.78	40.00	29.50	1.5	0.80	20.80	25.20	40.90	2.40	9	128
	Mosley Farm 13	7.69	27.3	214.5	0.27	42.50	62.34	0.9	1.20	13.80	26.90	33.70	1.70	13	31
	Mosley Farm 17	7.47	27.5	232.0	0.32	35.00	43.54	0.3	0.81	8.76	17.31	3.02	1.81	2	77
	Mosley Farm 27	7.70	27.3	319.0	3.50	25.00	67.34	1.7	1.00	26.80	33.42	7.23	1.50	21	147
	Natal drift plot 13	7.59	28.1	172.3	0.23	32.50	30.28	1.7	0.80	0.73	3.01	6.58	1.50	5	81
	Pullenshope	7.34	23.2	90.8	4.32	18.99	14.69	0.5	0.60	7.09	9.00	11.03	2.68	2	49
	Drankenstein	7.64	21.0	86.3	2.96	8.99	11.73	0.4	0.60	4.77	9.50	13.20	3.22	1	24
	Bosmansfontein 1	7.35	22.3	148.0	0.32	26.00	36.74	1.1	0.50	8.50	13.01	22.84	2.61	1	140
	Bosmansfontein 2	7.56	22.5	80.3	6.93	9.98	15.71	0.2	0.20	2.67	10.89	13.23	3.55	0	365

ENHLANZENI DISTRICT			°C	mg/ℓ	NTU	mg/ℓ Cl	mg/ℓ SO_4	mg/ℓ NO_3	mg/ℓ F	mg/ℓ Mg	mg/ℓ Na	mg/ℓ Ca	mg/ℓ K	count/100mℓ	count/100mℓ
Local Municipalities	**Locations of Boreholes**	**pH Value**	**Temperature**	**TDS**	**Turbidity**	**Chloride**	**Sulphate**	**Nitrates**	**Fluoride**	**Magnesium**	**Sodium**	**Calcium**	**Potassium**	**Faecal coliforms**	**Total coliforms**
Mbombela	Katoen	7.27	21.1	91.7	10.2	27.50	11.15	1.8	0.9	0.30	15.00	15.50	1.60	2	5
	Mphatheni Mahushu	7.82	24.3	97.0	13.2	30.00	27.85	0.3	2.0	3.38	0.76	7.43	0.50	4	31
	Mphatheni Guest house	7.71	25.9	99.3	3.49	50.01	12.07	1.3	1.1	3.49	16.70	21.20	1.61	2	0
	White River 73B	7.38	23.3	91.2	3.46	42.50	12.17	1.7	1.3	57.20	36.91	37.10	0.62	9	17
	White River 73A	7.10	22.3	62.9	18.10	27.51	11.87	2.1	1.0	3.52	13.20	21.70	1.80	0	35
	White River 53	7.55	23.3	91.9	2.41	32.50	11.83	0.9	2.5	5.54	11.22	10.80	2.30	0	3
	White River 60	7.74	22.7	39.3	13.10	47.50	95.46	1.1	0.6	0.58	7.39	5.43	2.00	1	3
	Heide Eggs	7.40	23.6	91.8	4.03	30.01	11.49	2.5	1.1	2.28	17.61	17.30	3.02	1	3
	Mtimba B	8.07	25.9	23.2	2.20	42.50	71.76	0.1	5.9	6.28	51.40	44.91	1.30	1	20
	Malapa Farm	8.00	21.3	81.7	9.09	35.00	11.29	1.4	0.4	4.56	11.60	14.21	1.30	15	76
	Heidelberg Farm 7	7.61	21.9	106.0	10.70	25.00	72.88	1.1	2.1	3.94	15.81	22.10	1.10	1	6
	Heidelberg Farm 25	7.81	21.2	116.1	0.52	25.01	10.51	0.9	2.3	3.24	14.40	36.90	1.10	3	8
	Heidelberg Tevrede	7.61	22.7	80.8	0.90	32.50	11.68	2.0	4.1	2.21	18.10	13.81	1.21	1	8
	Moegeploeg Plot 36	7.25	23.3	118.1	2.36	35.00	17.36	0.7	0.5	0.43	19.20	43.30	1.11	1	2
	Moegeploeg Plot 37	7.42	23.3	124.0	0.11	35.30	15.90	1.7	0.8	3.47	20.81	30.00	2.40	1	1
	Weltevreden Plot 455A	7.74	26.0	161.0	0.10	32.50	17.36	2.2	0.9	2.55	21.20	0.20	1.71	2	3
	Weltevreden Plot 455B	7.43	26.8	209.2	0.17	22.50	24.16	2.1	0.6	2.69	18.90	0.61	1.00	2	5
	Weltevreden Plot 43	7.87	27.1	165.3	0.82	25.00	19.79	0.3	1.1	8.64	14.61	24.50	0.60	9	57
Nkomazi	River View P.S	7.87	26.8	372.0	1.96	47.51	14.06	2.0	0.8	36.20	35.50	48.50	0.35	45	225
	Beyers Farm 1	8.26	26.3	483.0	0.53	65.01	85.65	1.1	0.7	2.77	8.97	10.60	2.13	0	4
	Beyers Farm 2	8.21	28.2	510.0	0.73	55.02	12.60	5.0	0.8	62.90	31.60	44.60	0.68	8	24
	Baundi Farm	7.89	27.6	148.0	0.94	35.00	50.39	0.0	0.9	12.20	10.51	23.40	0.44	4	24

	Mpapani Farm	8.25	25.7	357.1	0.35	47.50	11.92	1.9	0.9	26.81	53.40	36.20	0.59	25	163
	Bambinyati Farm 19	8.33	27.6	437.2	0.88	60.30	12.85	2.1	3.7	29.70	91.70	24.31	0.58	4	51
	Bambinyati Farm house	8.17	25.0	292.2	1.04	50.00	43.74	1.7	0.3	33.42	23.33	16.60	0.64	3	54
	Boland Farm	8.23	28.6	446.0	0.54	52.50	90.51	3.6	2.8	36.30	61.10	40.00	0.53	12	26
	Malelani Farm	7.87	28.1	435.3	1.36	55.01	59.03	2.7	0.5	43.45	41.71	62.50	0.34	0	18
	Eskom Distribution	8.11	28.8	349.0	1.09	50.00	70.64	0.8	1.4	33.30	32.72	50.30	0.44	1	9
	Lilly Pond	7.67	28.9	389.1	0.53	75.02	82.88	2.1	2.7	29.80	33.80	71.84	0.39	15	64
	Voorspoet Farm	7.87	29.2	462.0	0.93	32.50	38.73	0.2	2.5	20.81	82.70	25.10	0.75	5	175
	Voorspoet commonage 1	7.54	27.3	507.1	0.40	50.00	65.15	1.0	0.8	17.90	95.84	40.40	0.64	8	30
	Voorspoet commonage 2	7.65	27.0	246.2	0.76	62.50	25.86	1.4	1.1	34.90	56.60	36.00	0.40	9	24
	Elenberg Komatipoort Farm	7.57	28.4	448.0	0.29	82.54	78.95	0.9	0.6	1.79	7.26	30.81	1.54	25	175

APPENDIX B – COST EFFECTIVE FILTER MATERIALS COATED WITH SILVER NANOPARTICLES DATA

(All values are average of triplicate sample)

Date- June 2010 to July 2010

Antibacterial activity of various Ag nanoparticle-coated substrates against *E. coli* at different concentrations in synthetic groundwater

Zeolite flow through test against

Time (min)	Control	Silver Concentrations			
		0.1 M	0.01 M	0.03 M	0.05 M
	Bacterial concentrations (cfu/100mL)				
0	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7
10	2.9×10^7	3.0×10^3	6.0×10^6	4.0×10^6	8.0×10^4
30	2.8×10^7	5.0×10^4	7.0×10^6	5.0×10^6	8.0×10^5
60	2.8×10^7	1.1×10^6	1.0×10^7	3.3×10^6	2.2×10^6
90	2.8×10^7	1.7×10^6	3.0×10^7	4.8×10^6	2.8×10^6

Sand flow through test

Time (min)	Control	Silver Concentrations			
		0.1 M	0.01 M	0.03 M	0.05 M
	Bacterial concentrations (cfu/100mL)				
0	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7
10	1.8×10^7	1.6×10^5	5.6×10^6	3.5×10^6	2.9×10^6
30	2.1×10^7	4.0×10^5	9.0×10^6	7.8×10^6	3.6×10^6
60	2.2×10^7	1.1×10^6	1.3×10^7	1.2×10^7	5.0×10^6
90	2.1×10^7	1.6×10^7	2.4×10^7	2.0×10^7	2.6×10^7

Fiberglass flow through test

Time (min)	Control	Silver Concentrations			
		0.1 M	0.01 M	0.03 M	0.05 M
	Bacterial concentrations (cfu/100mL)				
0	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7
10	2.0×10^7	2.0×10^3	1.5×10^6	1.1×10^5	8.5×10^3
30	2.1×10^7	4.7×10^3	4.0×10^6	4.4×10^5	2.9×10^4
60	2.1×10^7	9.9×10^4	7.2×10^6	9.0×10^5	2.7×10^5
90	2.8×10^7	1.8×10^6	9.6×10^6	3.6×10^6	1.9×10^6

Anion Resins flow through test

Time (min)	Control	Silver Concentrations			
		0.1 M	0.01 M	0.03 M	0.05 M
	Bacterial concentrations (cfu/100mL)				
0	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7
10	2.0×10^7	2.9×10^2	1.8×10^5	1.1×10^3	3.0×10^3
30	2.0×10^7	2.5×10^2	1.9×10^5	4.4×10^3	3.5×10^3
60	2.6×10^7	1.3×10^3	2.3×10^5	9.0×10^3	5.0×10^3
90	2.8×10^7	2.0×10^3	6.1×10^5	3.6×10^4	1.5×10^4

Cation Resins flow through test

Time (min)	Control	Silver Concentrations			
		0.1 M	0.01 M	0.03 M	0.05 M
	Bacterial concentrations (cfu/100mL)				
0	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7	3.3×10^7
10	2.0×10^7	0	1.5×10^4	1.1×10^3	1.0×10^2
30	2.0×10^7	0	2.0×10^4	1.5×10^3	1.0×10^2
60	2.6×10^7	0	3.0×10^4	2.0×10^3	1.2×10^2
90	2.8×10^7	0	9.6×10^4	1.0×10^4	1.0×10^3

Antibacterial activity of various Ag nanoparticles-coated substrates at 0.01 mM concentration against *E. coli, S. typhimurium, S. dysenteriae* and *V. cholerae* in groundwater

Zeolite flow through test

Time (min)	Bacterial concentration Log_{10} Removal (cfu/100mL)			
	E. coli	***S.typhimurium***	***S.dysenteriae***	***V.cholerae***
10	3.00	3.00	3.00	3.00
20	1.00	2.00	1.80	0.90
30	0.50	1.90	0.30	0.30
60	0.30	1.30	0.25	0.25
90	0.30	1.00	0.25	0.25
120	0.30	0.50	0.25	0.25

Sand flow through test

Time (min)	Bacterial concentration Log_{10} Removal (cfu/100mL)			
	E. coli	***S.typhimurium***	***S.dysenteriae***	***V.cholerae***
10	3.00	3.00	3.00	3.00
20	2.40	3.00	2.20	3.00
30	2.00	2.00	2.00	2.50
60	0.50	0.90	0.80	2.00
90	0.35	0.30	0.30	1.50
120	0.30	0.25	0.25	0.80

Fiberglass flow through test

Time (min)	Bacterial concentration Log_{10} Removal (cfu/100mL)			
	E. coli	***S.typhimurium***	***S.dysenteriae***	***V.cholerae***
10	3.00	3.00	3.00	3.00
20	2.50	3.00	3.00	3.00
30	2.10	3.00	2.10	3.00
60	1.00	2.30	1.00	3.00
90	0.50	1.70	0.80	2.50
120	0.35	1.00	0.30	2.00

Anion Resins flow through test

Time (min)	Bacterial concentration Log_{10} Removal (cfu/100mL)			
	E. coli	***S.typhimurium***	***S.dysenteriae***	***V.cholerae***
10	3.00	3.00	3.00	3.00
20	3.00	3.00	3.00	3.00
30	2.50	3.00	3.00	3.00
60	2.30	3.00	2.50	3.00
90	1.50	2.50	2.00	2.80
120	1.00	1.40	1.50	2.00

Cation Resins flow through test

Time (min)	Bacterial concentration Log_{10} Removal (cfu/100mL)			
	E. coli	***S.typhimurium***	***S.dysenteriae***	***V.cholerae***
10	3.00	3.00	3.00	3.00
20	3.00	3.00	3.00	3.00
30	3.00	3.00	3.00	3.00
60	3.00	3.00	3.00	3.00
90	3.00	3.00	3.00	3.00
120	3.00	3.00	3.00	3.00

Amount of silver eluted from Ag nanoparticle-coated substrates in synthetic groundwater

Zeolite flow through test

Time (min)	Silver Concentrations			
	0.1 M	**0.01 M**	**0.03 M**	**0.05 M**
10	1.80	0.18	0.55	0.90
30	0.30	0.10	0.04	0.20
60	0.10	0	0	0.01
90	0	0	0	0

Sand flow through test

Time (min)	Silver Concentrations			
	0.1 M	0.01 M	0.03 M	0.05 M
10	1.50	0.15	0.50	0.81
30	0.50	0.08	0.10	0.40
60	0.10	0	0	0.01
90	0	0	0	0

Fiberglass flow through test

Time (min)	Silver Concentrations			
	0.1 M	0.01 M	0.03 M	0.05 M
10	1.00	0.11	0.43	0.70
30	0.60	0.10	0.09	0.50
60	0.10	0	0	0.01
90	0	0	0	0

Anion Resins flow through test

Time (min)	Silver Concentrations			
	0.1 M	0.01 M	0.03 M	0.05 M
10	0.90	0.10	0.30	0.50
30	0.30	0.08	0.10	0.20
60	0.15	0.01	0.04	0.09
90	0.10	0	0	0

Cation Resins flow through test

Time (min)	Silver Concentrations			
	0.1 M	0.01 M	0.03 M	0.05 M
10	0.06	0.05	0.08	0.10
30	0.05	0.05	0.09	0.07
60	0.04	0.02	0	0.01
90	0.03	0	0	0

APPENDIX C – THE EFFECTS OF MATERIAL LOADING AND FLOW RATE ON THE DISINFECTION OF PATHOGENIC MICROORGANISMS USING SILVER NANOPARTICLE-CATION RESIN FILTER SYSTEM DATA

(All values are average of triplicate sample)

Date- August to October 2010

The effect of various bed masses on breakthrough performance for pathogenic microorganisms disinfection

	Material Loading (g)											
	10	15	20	10	15	20	10	15	20	10	15	20
Time (h)	*E. coli*			*S. typhimurium*			*S. dysenteriae*			*V. cholerae*		
	Bacterial concentrations for breakthrough curves (cfu/100mL)											
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0

10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0	0	0	0
29	0	0	0	0	0	0	0	0	0	0	0	0
30	3	0	0	0	0	0	0	0	0	0	0	0
31	2.0×10^{1}	0	0	0	0	0	0	0	0	0	0	0
32	5.5×10^{1}	0	0	0	0	0	0	0	0	0	0	0
33	9.0×10^{1}	0	0	0	0	0	0	0	0	0	0	0
34	1.5×10^{2}	0	0	0	0	0	0	0	0	0	0	0
35	2.6×10^{2}	0	0	0	0	0	0	0	0	0	0	0
36	2.7×10^{2}	0	0	0	0	0	0	0	0	0	0	0
37	2.9×10^{2}	0	0	0	0	0	0	0	0	0	0	0
38	1.0×10^{3}	0	0	0	0	0	0	0	0	0	0	0

39	1.2×10^{3}	0	0	4	0	0	0	0	0	0	0	0
40	1.3×10^{3}	0	0	1.3×10^{1}	0	0	0	0	0	0	0	0
41	1.3×10^{3}	0	0	3.6×10^{1}	0	0	0	0	0	0	0	0
42	1.4×10^{3}	0	0	5.1×10^{1}	0	0	0	0	0	0	0	0
43	1.5×10^{3}	0	0	1.2×10^{2}	0	0	0	0	0	0	0	0
44	1.5×10^{3}	0	0	2.7×10^{2}	0	0	2	0	0	0	0	0
45	1.5×10^{3}	0	0	4.3×10^{2}	0	0	9	0	0	0	0	0
46	2.0×10^{3}	0	0	1.1×10^{3}	0	0	1.4×10^{1}	0	0	0	0	0
47	2.7×10^{3}	0	0	3.9×10^{3}	0	0	2.5×10^{1}	0	0	0	0	0
48	3.7×10^{3}	0	0	3.9×10^{3}	0	0	1.1×10^{2}	0	0	5	0	0
49	4.8×10^{3}	0	0	3.9×10^{3}	0	0	7.2×10^{2}	0	0	8	0	0
50	5.5×10^{3}	0	0	-	0	0	1.0×10^{3}	0	0	1.2×10^{1}	0	0
51	5.5×10^{3}	0	0	-	0	0	1.9×10^{3}	0	0	6.7×10^{1}	0	0
52	5.5×10^{3}	0	0	-	0	0	3.0×10^{3}	0	0	3.3×10^{2}	0	0
53	5.5×10^{3}	0	0	-	0	0	5.7×10^{3}	0	0	6.9×10^{2}	0	0
54	5.5×10^{3}	0	0	-	0	0	6.1×10^{3}	0	0	1.5×10^{3}	0	0
55	-	0	0	-	0	0	6.2×10^{3}	0	0	2.3×10^{3}	0	0
56	-	0	0	-	0	0	-	0	0	5.9×10^{3}	0	0
57	-	0	0	-	0	0	-	0	0	5.9×10^{3}	0	0
58	-	0	0	-	0	0	-	0	0	5.9×10^{3}	0	0
59	-	0	0	-	0	0	-	0	0	5.9×10^{3}	0	0
60	-	0	0	-	0	0	-	0	0	5.9×10^{3}	0	0
61	-	9	0	-	0	0	-	0	0	-	0	0
62	-	1.3×10^{1}	0	-	0	0	-	0	0	-	0	0
63	-	5.0×10^{1}	0	-	0	0	-	0	0	-	0	0
64	-	4.0×10^{2}	0	-	6	0	-	0	0	-	0	0
65	-	6.0×10^{2}	0	-	9	0	-	3	0	-	0	0
66	-	8.9×10^{2}	0	-	1.0×10^{1}	0	-	5	0	-	0	0
67	-	9.3×10^{2}	0	-	1.5×10^{1}	0	-	1.3×10^{1}	0	-	0	0

68	-	1.0×10^{3}	0	-	1.2×10^{2}	0	-	5.2×10^{1}	0	-	0	0
69	-	1.3×10^{3}	0	-	4.3×10^{2}	0	-	8.0×10^{1}	0	-	0	0
70	-	1.4×10^{3}	0	-	5.4×10^{2}	0	-	3.2×10^{2}	0	-	0	0
71	-	1.7×10^{3}	0	-	7.9×10^{2}	0	-	6.0×10^{2}	0	-	2	0
72	-	1.7×10^{3}	0	-	1.2×10^{3}	0	-	2.3×10^{3}	0	-	2	0
73	-	1.7×10^{3}	0	-	3.3×10^{3}	0	-	3.9×10^{3}	0	-	1.3×10^{1}	0
74	-	1.7×10^{3}	0	-	4.2×10^{3}	0	-	5.0×10^{3}	0	-	2.8×10^{1}	0
75	-	1.7×10^{3}	0	-	4.2×10^{3}	0	-	5.8×10^{3}	0	-	4.1×10^{2}	0
76	-	1.7×10^{3}	0	-	4.2×10^{3}	0	-	6.0×10^{3}	0	-	6.7×10^{2}	0
77	-	1.7×10^{3}	0	-	-	0	-	6.0×10^{3}	0	-	1.7×10^{3}	0
78	-	1.7×10^{3}	0	-	-	0	-	6.0×10^{3}	0	-	2.4×10^{3}	0
79	-	1.7×10^{3}	0	-	-	0	-	6.0×10^{3}	0	-	4.9×10^{3}	0
80	-	1.7×10^{3}	0	-	-	0	-	-	0	-	4.9×10^{3}	0
81	-	-	0	-	-	0	-	-	0	-	4.9×10^{3}	0
82	-	-	0	-	-	0	-	-	0	-	4.9×10^{3}	0
83	-	-	0	-	-	0	-	-	0	-	-	0
84	-	-	0	-	-	0	-	-	0	-	-	0
85	-	-	0	-	-	0	-	-	0	-	-	0
86	-	-	0	-	-	0	-	-	0	-	-	0
87	-	-	0	-	-	0	-	-	0	-	-	0
88	-	-	0	-	-	0	-	-	0	-	-	0
89	-	-	0	-	-	0	-	-	0	-	-	0
90	-	-	0	-	-	0	-	-	0	-	-	0
91	-	-	0	-	-	0	-	-	0	-	-	0
92	-	-	0	-	-	0	-	-	0	-	-	0
93	-	-	2.0×10^{1}	-	-	0	-	-	0	-	-	0
94	-	-	6.9×10^{1}	-	-	0	-	-	0	-	-	0
95	-	-	1.5×10^{2}	-	-	0	-	-	0	-	-	0
96	-	-	1.9×10^{2}	-	-	0	-	-	0	-	-	0

97	-	-	1.3×10^3	-	-	1.3×10^1	-	-	0	-	-	0
98	-	-	2.1×10^3	-	-	5.8×10^2	-	-	0	-	-	0
99	-	-	2.8×10^3	-	-	1.0×10^3	-	-	2	-	-	0
100	-	-	3.0×10^3	-	-	3.3×10^3	-	-	1.0×10^1	-	-	0
101	-	-	3.4×10^3	-	-	5.0×10^3	-	-	5.3×10^1	-	-	6
102	-	-	4.3×10^3	-	-	5.0×10^3	-	-	2.1×10^2	-	-	7.0×10^1
103	-	-	5.0×10^3	-	-	5.0×10^3	-	-	9.8×10^2	-	-	2.0×10^2
104	-	-	5.3×10^3	-	-	5.0×10^3	-	-	2.9×10^3	-	-	3.2×10^3

The effect of various flow rates on breakthrough performance for pathogenic microorganisms disinfection

	Flow Rates (mL/min)											
	2	**5**	**10**	**2**	**5**	**10**	**2**	**5**	**10**	**2**	**5**	**10**
Time (h)	***E. coli***			***S. typhimurium***			***S. dysenteriae***			***V. cholerae***		
	Bacterial concentrations for breakthrough curves (cfu/100mL)											
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0

8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	0	0
25	0	0	4	0	0	0	0	0	0	0	0	0
26	0	0	1.0×10^{1}	0	0	0	0	0	0	0	0	0
27	0	0	2.3×10^{1}	0	0	0	0	0	0	0	0	0
28	0	0	4.5×10^{1}	0	0	0	0	0	0	0	0	0
29	0	0	5.1×10^{1}	0	0	0	0	0	0	0	0	0
30	0	0	6.0×10^{1}	0	0	0	0	0	0	0	0	0
31	0	0	8.9×10^{1}	0	0	0	0	0	0	0	0	0
32	0	0	1.0×10^{2}	0	0	0	0	0	0	0	0	0
33	0	0	1.2×10^{2}	0	0	0	0	0	0	0	0	0
34	0	0	1.3×10^{2}	0	0	0	0	0	0	0	0	0
35	0	0	1.4×10^{2}	0	0	0	0	0	0	0	0	0
36	0	0	2.6×10^{2}	0	0	0	0	0	0	0	0	0

37	0	0	7.0×10^{2}	0	0	0	0	0	0	0	0	0
38	0	8	8.9×10^{2}	0	0	0	0	0	0	0	0	0
39	0	1.0×10^{1}	9.2×10^{2}	0	0	5	0	0	0	0	0	0
40	0	1.3×10^{1}	1.0×10^{3}	0	0	1.2×10^{1}	0	0	0	0	0	0
41	0	4.0×10^{1}	1.5×10^{3}	0	0	6.2×10^{1}	0	0	0	0	0	0
42	0	5.7×10^{1}	1.8×10^{3}	0	0	3.7×10^{2}	0	0	9	0	0	0
43	0	6.5×10^{1}	2.0×10^{3}	0	0	8.1×10^{2}	0	0	4.0×10^{1}	0	0	0
44	0	8.9×10^{1}	2.5×10^{3}	0	0	2.1×10^{3}	0	0	7.9×10^{1}	0	0	0
45	0	1.1×10^{2}	2.7×10^{3}	0	0	4.9×10^{3}	0	0	8.3×10^{2}	0	0	0
46	0	1.2×10^{2}	2.7×10^{3}	0	0	6.1×10^{3}	0	0	1.0×10^{3}	0	0	0
47	0	1.2×10^{2}	2.7×10^{3}	0	0	6.1×10^{3}	0	0	4.8×10^{3}	0	0	0
48	0	1.3×10^{2}	2.7×10^{3}	0	0	6.1×10^{3}	0	0	5.9×10^{3}	0	0	0
49	0	1.4×10^{2}	-	0	0	-	0	0	5.9×10^{3}	0	0	0
50	0	1.6×10^{2}	-	0	0	-	0	0	5.9×10^{3}	0	0	0
51	0	1.7×10^{2}	-	0	0	-	0	0	5.9×10^{3}	0	0	0
52	0	1.9×10^{2}	-	0	0	-	0	0	-	0	0	0
53	0	2.1×10^{2}	-	0	2	-	0	0	-	0	0	0
54	0	7.8×10^{2}	-	0	1.7×10^{1}	-	0	0	-	0	0	0
55	0	1.2×10^{3}	-	0	4.5×10^{1}	-	0	4	-	0	0	0
56	0	1.2×10^{3}	-	0	1.1×10^{2}	-	0	3.2×10^{1}	-	0	0	6
57	0	1.3×10^{3}	-	0	6.8×10^{3}	-	0	6.5×10^{1}	-	0	0	3.1×10^{1}
58	0	1.3×10^{3}	-	0	1.0×10^{3}	-	0	2.0×10^{2}	-	0	0	7.4×10^{1}
59	0	1.3×10^{3}	-	0	4.4×10^{3}	-	0	1.2×10^{3}	-	0	0	2.0×10^{2}
60	0	1.3×10^{3}	-	0	5.9×10^{3}	-	0	4.1×10^{3}	-	0	0	5.1×10^{2}
61	9	-	-	0	5.9×10^{3}	-	0	6.1×10^{3}	-	0	0	1.7×10^{3}
62	1.3×10^{1}	-	-	0	5.9×10^{3}	-	0	6.1×10^{3}	-	0	0	4.4×10^{3}
63	5.0×10^{1}	-	-	0	5.9×10^{3}	-	0	6.1×10^{3}	-	0	0	4.4×10^{3}
64	4.0×10^{2}	-	-	6	5.9×10^{3}	-	0	6.1×10^{3}	-	0	0	4.4×10^{3}
65	6.0×10^{2}	-	-	9	-	-	3	-	-	0	6	4.4×10^{3}

66	8.9×10^{2}	-	-	1.0×10^{1}	-	-	5	-	-	0	3.1×10^{1}	-
67	9.3×10^{2}	-	-	1.5×10^{1}	-	-	1.3×10^{1}	-	-	0	7.4×10^{1}	-
68	1.0×10^{3}	-	-	1.2×10^{2}	-	-	5.2×10^{1}	-	-	0	2.0×10^{2}	-
69	1.2×10^{3}	-	-	4.3×10^{2}	-	-	8.0×10^{1}	-	-	0	5.1×10^{2}	-
70	1.3×10^{3}	-	-	5.4×10^{2}	-	-	3.2×10^{2}	-	-	0	1.7×10^{3}	-
71	1.4×10^{3}	-	-	7.9×10^{2}	-	-	6.0×10^{2}	-	-	2	4.4×10^{3}	-
72	1.4×10^{3}	-	-	1.2×10^{3}	-	-	2.3×10^{3}	-	-	3	4.4×10^{3}	-
73	1.4×10^{3}	-	-	3.3×10^{3}	-	-	3.9×10^{3}	-	-	1.3×10^{1}	4.4×10^{3}	-
74	-	-	-	4.2×10^{3}	-	-	5.0×10^{3}	-	-	2.8×10^{1}	-	-
75	-	-	-	5.7×10^{3}	-	-	5.8×10^{3}	-	-	4.1×10^{2}	-	-
76	-	-	-	6.0×10^{3}	-	-	5.8×10^{3}	-	-	6.7×10^{2}	-	-
77	-	-	-	6.0×10^{3}	-	-	5.8×10^{3}	-	-	1.7×10^{3}	-	-
78	-	-	-	6.0×10^{3}	-	-	-	-	-	2.4×10^{3}	-	-
79	-	-	-	-	-	-	-	-	-	4.9×10^{3}	-	-
80	-	-	-	-	-	-	-	-	-	4.9×10^{3}	-	-

APPENDIX D – PERFORMANCE OF SILVER NANOPARTICLES-CATION RESIN FILTER IN INHIBITING BACTERIAL REGROWTH AND BIOFILM FORMATION IN DRINKING WATER SUPPLY SYSTEMS DATA

(All values are average of triplicate sample)

Date- August to October 2010

Microbial characteristics (Heterotrophic plate count bacteria) of test waters

Time (d)	Raw Groundwater HPC (cfu/100mL)	Chlorinated water HPC (cfu/100mL)	Residual Cl_2 (mg/L)	Ag-Resin nanoparticles HPC (cfu/100mL)	Residual Ag (mg/L)
0	1.7×10^5	1.7×10^5	2.5	1.7×10^5	0.03
2	3.2×10^5	1.2×10^2	0.7	0	0.028
4	4.0×10^5	1.5×10^2	0.1	0	0.023
6	4.3×10^5	1.7×10^2	0	0	0.02
8	4.1×10^5	3.1×10^3	0	0	0.015
10	4.4×10^5	7.1×10^5	0	0	0.01
12	4.6×10^5	8.1×10^5	0	0	0
14	2.7×10^5	9.5×10^5	0	1.0×10^1	0
16	2.5×10^5	9.8×10^5	0	5.0×10^2	0

Counts of viable bacteria attached to the surface of PVC and GS coupons exposed different systems

	Raw Water		Chlorinated Water			Ag-Resin nanoparticles		
Time (d)	**PVC**	**GS**	**PVC**	**GS**	**Residual Cl_2**	**PVC**	**GS**	**Residual Ag**
0	-	-	-	-	2.5	-	-	2.5
1	4	1.0×10^1	3	1.7×10^1	0.7	0	0	2
2	6	1.2×10^1	5	2.7×10^1	0.1	0	0	1.3
3	2.5×10^1	4.3×10^2	7	5.7×10^1	0	0	0	0.9
7	5.3×10^2	8.0×10^3	3.3×10^1	1.0×10^2	0	0	0	0.5
14	1.9×10^3	1.9×10^4	8.9×10^2	2.9×10^4	0	0	0	0.1
21	8.9×10^3	7.6×10^4	4.9×10^3	6.6×10^4	0	0	0	0
28	4.7×10^4	2.8×10^5	2.8×10^4	4.6×10^5	0	0	1.5×10^1	0
30	7.3×10^4	9.1×10^5	8.3×10^4	1.1×10^6	0	7.0×10^1	2.0×10^2	0