NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

PREVALENCE OF ROTAVIRUSES, ADENOVIRUSES AND HEPATITIS A IN WATER SAMPLES COLLECTED FROM DOMESTIC WATER SOURCES IN WINDHOEK

By

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Thesis presented in fulfilment of the requirements for the degree of Master of Health Sciences, Faculty of Health and Applied Sciences, Namibia University of Science and Technology, Windhoek, Namibia

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October 2019

DECLARATION

I Sylvia Megameno Shilikomwenyo hereby declare that the work contained in the thesis entitled Prevalence of rotaviruses, adenoviruses and hepatitis a in water samples collected from domestic water sources in Windhoek is my own original work and that I have not previously in its entirety or in part submitted it at any university or other higher education institution for the award of a degree.

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ACKNOWLEDGEMENTS

I wish to thank:

- The Almighty lord for giving me this opportunity.
- My family for believing in me, being patient with me and for giving me support and encouraging me.
- I would also like to thank my Supervisor for always encouraging me and my friends that allowed me to sample from their household taps.
- I would also like to thank Ms Laura Ashipala and Mr Venus De Klerk at City of Windhoek Scientific services for allowing me to utilize their facility for filtration purposes.
- My lab assistants; Silvi Shilikomwenyo and Elia Muremi for availing themselves to assist me in the lab during testing.
- I would further like to thank my Colleagues at NIP who assisted me in any way especially during the sampling process as well as the carrying of water containers.
- I also appreciate the NUST team for the encouragement and being helpful concerning the availability of the laboratory for my testing processes as well as everyone that contributed in any way in making this possible

The financial assistance of The Namibia Institute of pathology (NIP) towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author, and are not necessarily to be attributed to the funding organisation.

DEDICATION

This thesis is dedicated to my family for their support and encouragement.

ABSTRACT

Background: A growing population contributes to increasing demand for water. Windhoek's water supply is based on the use of surface and groundwater. However, all potable water resources within a radius of 500 km have been fully exploited. The rainfall is uncertain and long spells of severe droughts are frequently encountered. There are four main sources of water supply to the central area of Windhoek: surface water obtained from the Von Bach, Swakoppoort and Omatako dams; groundwater abstracted from 50 municipal production boreholes; reclaimed water recovered by suitable treatment from both the New Goreangab Water Reclamation Plant (NGWRP) and the Old Goreangab Water Reclamation Plant (OGWRP).

Natural water sources which include rivers, lakes and ponds can be contaminated with microorganisms which inhabit the environment in the form of planktonic organisms and sessile biofilms. Microbial agents, associated with waterborne outbreaks, include bacterial organisms (*Salmonella, Campylobacter, Escherichia coli and Vibrio cholera*, amongst many others) as well as viruses (Hepatitis A virus, rotavirus and Norwalk virus), protozoa (*Giardia* and *Cryptosporidium*), helminths and fungi can contaminate water systems.

Methodology: Ten litres of water was collected per site per week in sterile Nalgene bottles containing 0.8 g of sodium thiosulfate that was added for de-chlorination of the water at the sampling sites and transported to the laboratory on ice. The 0.8 g sodium thiosulfate de-chlorinates up to 5 mg/l of free chlorine from water samples. The range of free chlorine in Windhoek's water is 0.1-5.0 mg/l. The water samples were collected from a house tap in Khomasdal (borehole and blended water), Central hospital line (blended water), Cimbebacia (borehole water), Von Bach dam (dam water) and Havana communal tap (surface water). The pH, temperature and specific gravity of each sample was measured using a portable pH meter (YSI pH 100 portable pH mV), pH strips, a mercury thermometer as well as dipstick for specific gravity. The water samples were then concentrated using the adsorption-elution method. The viral RNA and DNA were extracted using RNA/DNA extraction kits (Quick-DNA™Miniprep; Zymo Research, USA and Quick-RNA™ Miniprep; Zymo Research, Irvine, USA) which was followed by reverse-transcription. The amplification and the identification of rotavirus, adenovirus, hepatitis A, hepatitis E virus as well as *Escherichia coli* and Enterococcus organisms was done by using specific primers. The PCR products were photographed and visualized using a transiluminator.

Results: All sites had physiological determinant results that were acceptable and conductivity that was below 150 ms/M at 25°C, this however was not identified as cause for concern with regards to the safety of drinking water. Havana and Von Bach had the majority of turbidity readings that were less

than one NTU which is recommended by WHO. *E.coli* was found to be the most prevalent bacteria with 38% of the samples recording positive screening results using molecular techniques followed by *E. Faecium*, which had a positivity rate of 32% The combination of Rotavirus, Hepatitis A and Hepatitis E was found to be the most frequently occurring viruses in the water samples (90%) while Adenovirus was the least frequently distributed viral organism amongst the five sampling sites. Rotavirus, hepatitis A and hepatitis E were the most frequently distributed viruses accounting for 30 % each with the highest frequency recorded in Havana.

Conclusion: Although the concentration of viruses was not established in this study, it is worth noting that the actual isolated organisms can lead to infections of the residents in the long run.

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ABBREVIATIONS	Definition/Explanation
PCR	Polymerase Chain Reaction
NAMWATER	Namibia Water Co-operation
NGWRP	New Goreangab Water Reclamation Plant
DAF	Dissolved air flotation
BAC	Biologically active carbon
GAC	Granular active carbon
PAC	Powdered activated carbon
UF	Ultra-filtration
SCADA	Supervisory control and data acquisition
WHO	World Health Organisation
MoHSS	Ministry of Health and Social Services
cDNA	Complementary Deoxyribonucleic acid
qPCR	Quantitative PCR
NUST	Namibia University of Science and Technology
NIP	Namibia Institute of Pathology
CoW	City of Windhoek
NGWRP	New Goreangab Water Reclamation Plant
OGWRP	Old Goreangab Water Reclamation Plant
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
DAF	Dissolved Air Flotation
BAC	Biologically Active Carbon
GAC	Granular Active Carbon
PAC	Powdered Activated carbon
UF	Ultrafiltration
NAMWATER	Namibia Water Cooperation
SCADA	Supervisory Control and Data Acquisition
HAV	Hepatitis A Virus
HEV	Hepatitis E Virus
STEC	Shiga toxin-producing *E. coli*
UPEC	Uropathogenic *E. coli*

ETEC	Enterotoxigenic *E.coli*
NIST	National Institute for Standards Technology
USA	United States of America
NTU	Nephelometric Turbidity Unit
PCR	Polymerase Chain Reaction
(RT-) qPCR	Real Time Quantitative PCR
VIRADEL	Virus Adsorption and Elution
AdV	Adenovirus
NoV	Norovirus
JCPyV	JC Polyomavirus
RV	Rotavirus
PMMoV	Pepper Mild Mottle Virus
EOR	Efficiency of Recovery
EV	Enteroviruses
EM	Electron Microscope
IEM	Immuno- Electron Microscope
EIA	Enzyme-Linked Immunoassays
rt qRT-PCR/PCR	Real Time Reverse Transcriptase PCR
cDNA	Copy DNA
Cq	Quantification cycle
TE	Tris EDTA
gDNA	Genomic DNA
UNICEF	United Nations Children's Fund

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

One of the most essential elements within our ecosystem is water and covers a large portion of the earth. The area covered is approximated to be 71% in the form of oceans, rivers as well as lakes (Adekalu et al., 2002). Windhoek is the capital city of Namibia and is located in south-western Africa, situated approximately 1800m above sea level in a broad valley that opens to the north and is flanked by the Auas Mountains to the south, east and west (du Pisani, 2006). The surface area of the country is 825 000 km^2, and has five ephemeral rivers in the interior of the country having their origin around Windhoek. The perennial rivers are only on the northern and southern borders of the country and are 750 and 900 km from Windhoek (du Pisani., 2006). Windhoek has an average rainfall of approximately 360mm. Approximately 70% of the City's water is collected in three reservoirs which are built on ephemeral rivers that run only a few days after heavy rainfall.

Windhoek's water supply is based on the use of surface and groundwater. However, all potable water resources within a radius of 500 km have been fully exploited. The rainfall is uncertain and long spells of severe droughts are frequently encountered (Lahnsteiner & Lempert, 2007). There are four main sources of water supply to the central area of Windhoek: surface water obtained from the Von Bach, Swakoppoort and Omatako dams; groundwater abstracted from 50 municipal production boreholes; reclaimed water recovered by suitable treatment from both the New Goreangab Water Reclamation Plant (NGWRP) and the Old Goreangab Water Reclamation Plant (OGWRP). In years of average to good rainfall, surface water runoff from the catchment areas suffices to satisfy the potable water demand for Windhoek (Lahnsteiner & Lempert, 2007).

There are regular droughts in Namibia and a continuous shortage of potable water to Windhoek led to the municipality to investigate alternative sources of raw water supply. The most viable option proved to be reuse of municipal wastewater from the largest sewage treatment plant in Windhoek, the Gammams Water Care Works, with augmentation from a surface water source on the outskirts of the city, the Goreangab Dam. The original (now "Old") Goreangab Water Reclamation Plant was built in 1968 which is over 40 years ago to reclaim municipal effluent for potable water purposes. This plant

was upgraded and extended several times during the last 30 years but reached the end of its viable life span in the late 1990s (Lahnsteiner & Lempert, 2007) .

A new, larger reclamation plant, the New Goreangab Water Reclamation Plant (NGWRP) was built, using the "multiple barrier" approach. This plant was put into operation in mid-2002. The NGWRP produces 21,000m3/d of drinking water, safe for human consumption. A "multiple barrier" approach was taken during final selection of the process technology. This means that the treatment processes employed ensure that at least two unit processes are provided for removing each crucial contaminant that could be harmful to the human body or aesthetically objectionable. An example is the complete and/or partial barriers for one of the most resistant pathogens, Cryptosporidium, include ozonation, enhanced coagulation, dissolved air flotation (DAF), dual media filtration, ultrafiltration and chlorination. Similarly, five barriers have been included for organic substances, enhanced coagulation, ozonation, biologically active carbon (BAC), granular active carbon (GAC) adsorption and ultrafiltration. This ensures both micro pollutant removal and degradation. The following unit processes have been included in the final plant design; Powdered activated carbon (PAC) dosing, pre-oxidation and pre-ozonation, flash mixing, enhanced coagulation and flocculation, dissolved air flotation, dual media rapid gravity sand filtration, ozonation, BAC filtration, GAC filtration, ultra-filtration (UF), disinfection and stabilisation. Guarantee values that the final water produced by the plant must adhere to were based on WHO Guidelines (1993), Rand Water (South Africa) Potable Water Quality Criteria (1996) and the Namibian Guidelines for Group A water (NamWater, 1998).

Water samples are taken every 4 hours at various points throughout the plant and analysed in the plant laboratory for basic quality control purposes. Refrigerated composite samples are taken twice per week and used for extensive analyses of all major water quality parameters as defined for guarantee values. The process is fully automated based on a monitoring supervisory control and data acquisition (SCADA) system (Lahnsteiner & Lempert, 2007). Furthermore, Plant operation shows that the specified guarantee parameters can be easily met and a high quality drinking water is provided. Blending the reclaimed water with treated surface water and/or groundwater provides additional safety. The maximum portion of reclaimed water fed into the distribution system is 50% in times of water scarcity and low water demand. Originally, it was decided to limit the maximum percentage of reclaimed water to 35% of the total potable water released into the distribution network (Du Pisani, 2006).

Furthermore, river and fresh water sources are steadily becoming more contaminated and in some cases they are becoming toxic due to low rainfall patterns and increasing high temperature which

leads to the proliferation of microorganisms. This then leads to a significant increase in water borne illnesses (Gerba, 1996).

There have been huge advances in water and waste water treatment methods but water borne diseases remain a public health issue in the world especially in low income countries. Human enteric viruses which are shed in large numbers in the faeces by infected individuals are a major cause of water borne viral infections. Human enteric viruses are stable in the environment and can survive waste water treatment but the health impact of waterborne viruses is underestimated. Enteric viruses have been isolated in water which has been certified suitable for domestic consumption by conventional bacterial indicator methods. Adenoviruses are double-stranded DNA viruses that are members of the Adenoviridae family and are considered to be one of the most stable viruses in the environment (Ahmed et al., 2016). On the other hand, Rotaviruses belong to the Reoviridae family and are divided into 7 groups, A-G of which a majority of human infections are being caused by group A. Hepatitis A is a single-stranded RNA genome which has a positive-sense and belongs to the genus hepatovirus (Sibanda & Okoh, 2013).

The quality of ground and surface water may greatly be affected by pathogenic microorganisms such as enteric pathogens which are mostly derived from faecal contamination (Ahmed et al., 2016). There are a number of sources of faecal contamination which include; sewage treatment plant effluents, waste treatment discharges, land run-off from urban, agricultural and natural areas and leachates from sanitary landfills. It has been noted that millions of people throughout the world do not have access to microbiologically safe water for domestic, recreational and other general purpose use (Sibanda & Okoh, 2013).

Treated and untreated sewage and combined sewer overflows from urban areas are the major sources of environmental pollution from human enteric viruses in surface waters (Fong & Lipp, 2005: Rodriguez et al., 2012). It has been stated that human enteric viruses are not able to grow outside their host cells, it is thus important to note that the efficient removal and deactivation of these viruses at wastewater treatment plants can contribute greatly to reducing the amount of viruses that are discharged into an environment. The installation of wastewater treatment systems that are appropriate can aid in controlling the risk of viral infection that can result from various routes such as contact with recreational waters, ingestion of portable water, or consumption of virus contaminated shellfish. It is however very difficult to achieve the complete removal of viruses with conventional wastewater treatment processes (Sano et al., 2016).

A key challenge to scientists in the environmental field since the beginning of environmental virology in the mid-twentieth century has been how to collect, isolate and detect pathogenic viruses from water that is used for drinking and/ or recreational purposes. Early studies investigated different types of membrane filters, with more sophisticated technologies being developed more recently.

Many studies have so far focused on the detection of enteric viruses in different types of aquatic environments including, raw and treated wastewater, surface water, groundwater, seawater as well as treated drinking water (Fong and Lipp, 2005; Gerba et al, 2013). Human enteric viruses are present in environmental water samples in relatively low levels. Haramoto et al., 2018 stated that it is therefore, essential to start such studies by concentrating the viruses into smaller sample volumes to enhance the usefulness of detection assays.

We are now able to obtain quantitative information about viral genomes present in water through the rapid advancement of molecular biological techniques (Haramoto et al., 2018). It is also worth noting that although great efforts can be brought forth, viruses are sometimes not detected in tested water samples. These data are called “non-detects” and should be considered for more appropriate data treatments but studies done previously rarely considered this issue important.

Water quality monitoring and public health assurance is performed routinely by enumerating faecal indicator bacteria in drinking water. The faecal coliforms are being used as indicators due to the assumption that the fate and transport of faecal coliforms reflect that of the waterborne pathogens. Accumulating evidence on waterborne disease outbreaks however, shows that absence of faecal coliform indicators does not entirely eliminate possibilities of the presence of other pathogens with a potential to cause disease outbreaks. There is therefore a need to have multiple or supplementary indicators which include enteric viruses and to directly monitor the levels of viral pathogens in surface waters, irrigation water, sewage effluent as well as treated drinking water for public health safety and quality assessment (Ruhanya et al., 2016).

Most water quality standards including WHO guidelines do not currently specify the level of viruses considered acceptable for drinking water, irrigation water, recreational or treated waste water. This contributes to the burden of infectious diseases emanating from water polluted by enteric viruses, especially in resource limited settings. In some developing countries this contributing factor has not been documented, reported or properly investigated.

1.2 Literature review

Namibia is a sunny country that has 300 days of sunshine per year on average. The climate of Namibia is arid, hot and dry with fluctuations occurring during the different seasons. This contributes to the potential high evaporation rate that is seen to be higher than the precipitation. The different regions in Namibia experience climatic differences and this can be seen with the difference in rainfall that increases from an annual rainfall of 0 mm to 600 mm from the southwest of the country to the northeast (Internations, 2018).

The hottest period in Namibia is experienced from December to March with the main rainy season generally starting in January. From April to May there is usually still potential for rain and it might occur. The temperatures start to drop due to the winter season approaching. June to September are the winter months in Namibia where temperatures can drop to below 5 ^{o}C. During this season, there is no rain in throughout the majority of Namibia, except for the far south were light rain can be expected. During the months of October and November temperatures rise increasingly and light rain is usually expected (Internations, 2018).

The Northern regions receive the highest rainfall in the country, this can be up to 600 mm and can be as high as 800 mm annually in the far northeast. There are many rivers and swamps in these regions and these dominate the landscape. The capital Windhoek lies inland. The high altitude of 1800 metres is associated with the moderate temperatures and average rainfalls experienced in Windhoek. There is usually no rainfall occurring between June and September. Over the past 20 years, Windhoek has seen an annual precipitation average of 370 mm and of this, 296 mm of rain was received in the period January to May, 5.2 mm from June to September and 76 mm from October to December. In the Namib and Khalahari desert areas only little rainfall can be expected and the Kalahari usually receives higher rainfalls than the Namib (Internations, 2018).

1.2.1 Water consumption in Windhoek

An approximated volume of 25 million cubic meters of water is consumed yearly by the City of Windhoek's residents and industry, this is approximated to 70 000 cubic meters per day with a 3 per cent growth factor a year. The city’s water supply is mainly from the Von Bach Dam outside Okahandja,

the New Goreangab Water Reclamation Plant (NGWRC) situated in the city as well as the Windhoek aquifer (boreholes), these are the main sources that provide water to Windhoek. In normal circumstances the boreholes only contribute less than 10 per cent of the city's water consumption this however, can increase up to 40 per cent of the city's demand during emergencies. The Von Bach and NGWRP water sources are not able to supply the daily demand of the city during peak season. Therefore, water from the boreholes is then also required during these stages. The Swakoppoort Dam has been seen to be heavily polluted. This is mainly because of the fact that Windhoek and Okahandja both lie within the catchment of this dam. Treatment steps that are costly will be required in order to deem it ready for use. Furthermore, the increase in tourism and housing developments taking place is threatening the usage of the Von Bach Dam as a source of water as these activities are occurring within the dam basin. The increase in the level of reuse of the reclaimed water is causing the salinity levels to rise this would then require further treatment which might be costly. A long-term solution would therefore be the cleaning of the Swakoppoort Dam as well as piping water from other parts of the country, this might also be a challenging exercise as most towns in Namibia are located far from their primary sources of water, such as perennial rivers (Uhlendahl et al, 2010).

The high temperatures experienced in the country lead to a large amount of the water evaporating almost immediately during the rainy season. Reservoirs are the main source of fresh water in Windhoek. The high evaporation due to high temperatures, constantly blowing wind as well as the low average humidity of about 30% contributes to the challenge of saving water in reservoirs. Windhoek has three different main sources that provide the city with water with 17 Mio m^3 of the water demand being catered for by dams around Windhoek from NamWater. These are the von Bach dam which is being fed by the Swakoppoort dam and the Omatako dam and the second source is the Goreangab Reclamation Plant which is producing 5.4 Mio m^3. The third source is the boreholes in Windhoek that provide between 1 and 2 Mio m^3 of Windhoek's water demand (HRDC, 2007). The amount of water, available for consumption in Windhoek, is very limited. Different areas in Windhoek have different water access installed. Individual household water access points are available in every household in the high- and mid-income areas. In the formalised low-income areas, every plot has a private water connection set up. Non-payments of water bills due however lead to the discontinuation of water access to these areas. The informal settlements mainly have three different types of water access points. The first type is, an official tap linked to a pre-paid card system, this type is most common. The second type is also a tap that is similar but it is placed among a group of houses, this is called a housing group. This tap is only for a defined community. The third type of water access is the presence of communal taps, with theses people who pay rent to the City have access to water and the

payment thereof is recovered from the rental paid (Uhlendahl et al, 2010). Figure 1.1 shows the City of Windhoeks' water cycle, reclamation plants as well as the water sources as described above.

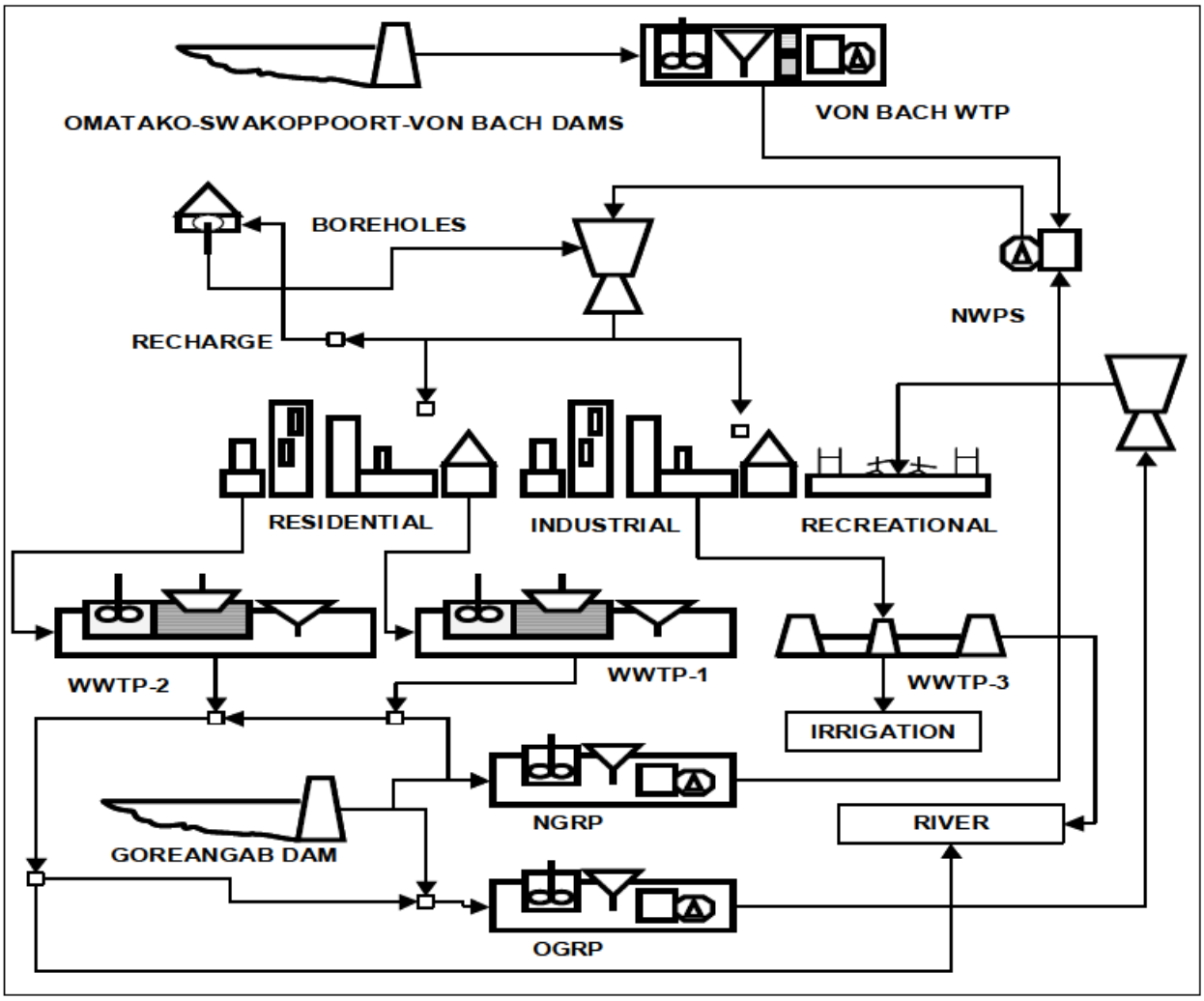

FIGURE 1.1: The city of Windhoek water cycle, reclamation and re-use scheme. Menge et al, 2009

The City of Windhoek's' estimated annual water demand is 22.6 Mio. m^3 yearly. The calculated water demand per capita per day based on data of City of Windhoek is approximately 200 litres with the inclusion of industrial water use as well as tourism (Uhlendahl et al, 2010).

1.2.2 Population dynamics in Windhoek

Windhoek is the administrative and economic capital of Namibia. People from different cultures migrate to Windhoek as they come to the city searching for jobs. Windhoek is a city that is continuously growing. The population of Windhoek before independence was 192 200 inhabitants in 1980 (Simon, 1983) but this number increased significantly. The 2011 Namibia Housing and Population Census reported that Windhoek's population increased from 233 529 to 325 858 inhabitants between 2001 and 2011 (Figure 1.2). This means 92 329 people were added to the city in 10 years, representing 39.5% increase in growth. People migrate from the different parts of Namibia as well as from other countries. Inadequately serviced informal settlements absorb the majority of the poor migrants and sanitation is a big concern in these informal areas. Since independence, Windhoek has increased significantly in size and number of inhabitants and rural-urban migration has been pointed out as the major contributing factor (Kasote, 2018). It has been estimated that there are around 51 informal settlements around Windhoek (City of Windhoek, 2006). About 20% of the municipal land is occupied by the informal settlements and others are on privately owned land that belong to self-help groups. Illegal squatting on peri-urban land where access has not been restricted led to the development of informal settlements (Newaya, 2010). The current updated statistics show that there are 308 informal settlements in Namibia with about 995 000 people being accommodated by 228 000 shacks in urban areas (New era Newspaper, October 2018).

Year	Urban Population (Capita)	Annual Growth Rate over 10 years	Number of Households	Household Size (Capita)
2001	233,529*	5.60%	53,258*	4.00*
2011	325,858*	3.90%	84,973*	3.50*
2021	442,135	3.83%	123,730	3.25
2031	570,270	3.41%	173,020	3.00
2041	690,690	2.00%	209,657	3.00
2050	790,540	1.40%	240,035	3.00

Figure 1.2: shows the summary of census figures and expected growth in population and households in the Windhoek area (Namibia Statistics Agency, 2011)

1.2.3 Informal settlements in Windhoek

The City of Windhoek is growing very fast, especially in the north-western part, where there is an increase in informal settlements. There is however, a great concern when it comes to water supply as a result of the increase in the population due to urbanisation. The northern part of Windhoek consists of the old township Katutura, Goreangab, Wanaheda and the north-western suburbs. It is in this section of Windhoek that informal settlements dominate. Approximately 100 000 people live in informal corrugated iron shacks, on land that has not been subdivided and is serviced by low density communal services and in some cases, no services are available. A growing population contributes to increasing demand for water. High and mid income areas consists of water access points in each house, taps are found in the kitchen, bathroom and sometimes more taps in the garden or at the pool area. This however is not the case in low-income areas, as less taps are installed and there are no private taps installed in some cases (Uhlendahl et al, 2010). Major social activities in Windhoek include drinking and this leads to possible unsanitary practices of defecating in open river beds into which rivers flow or is the catchment area to a dam.

Basic sanitation includes toilets, waste water systems and showers or other places where people can take care of body hygiene. Lack of access to waste water systems, leads to the wastewater coming from cooking, body hygiene, toilets and laundry water containing washing powder getting into the ground water system. Pouring waste water into rivers and surface waters is also a form of contamination with the Goreangab dam being an example. The Goreangab Dam area has a community occupying informal and formal settlements to the north of the dam within the Goreangab sub-catchment. Many of the informal settlements in that area lack sanitary facilities of acceptable standard (Ogumokun et al, 2000). A substantial portion of the settlements are also located very close to the dam shore. Figure 1.3 shows that the majority of Windhoek's population has access to flush toilets, these is however not the case in informal settlements. The informal settlements make up 36.76 % of the population of Windhoek (Uhlendahl et al, 2010).

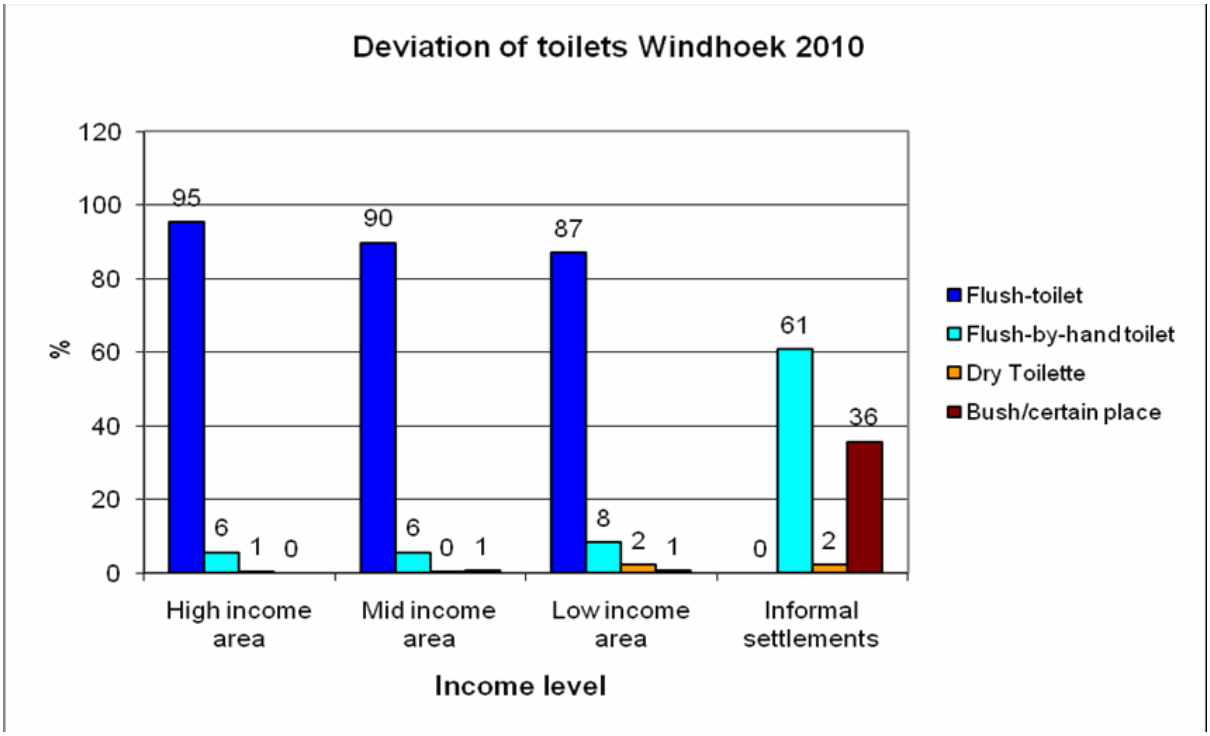

Figure 1.3: Distribution of toilets in Windhoek 2010 (Uhlendahl et al, 2010)

1.2.4 Recent outbreak in Windhoek

An outbreak of Hepatitis E was declared by the Ministry of Health and Social Services (MoHSS) on 14 November 2017 in Windhoek. Thereafter, cases have been reported in regions such as the Erongo, Omusati, Oshana, Ohangwena, Oshikoto as well as the Kavango East. It was also noted that more than 80 % of the reported cases were linked to the Havana and Goreangab informal settlements in Windhoek and these were declared as the epicentre of the outbreak. The most affected age group is between 20 and 39 years, representing 76 percent. More males at 314 are affected compared to 239 females (New era newspaper, 2018). The following were identified as the main drivers for the Windhoek Hepatitis E outbreak; open defecation, poor sanitation and hygiene practices. Previous epidemiological studies determined that the areas within the affected informal settlements with inadequate water and sanitation infrastructure such as communal taps, public toilets and latrines were the hardest hit by the outbreak (Ministry of Health and Social Services (MoHSS), 2018).

1.2.5 Waterborne pathogens

Rivers, lakes and ponds that are part of natural water sources can be contaminated with microorganisms which inhabit the environment in the form of planktonic organisms and sessile biofilms (Decho, 1990). Microbial agents, associated with waterborne outbreaks, include bacterial organisms (*Salmonella, Campylobacter, Esherichia coli and Vibrio cholera*, amongst many others (Gerba, 1996) as well as viruses (Hepatitis A virus, rotavirus and Norwalk virus), protozoa (*Giardia* and *Cryptosporidium*), helminths and fungi (WHO, 1996). Throughout the world, epidemics linked to waterborne pathogens occur frequently, with the largest reported outbreak of waterborne *E. coli* 0157:H7 occurring in New York, following a country fair in August 1999 and included 775 confirmed cases. Six pandemics of cholera have been recorded in history, which claimed the lives of millions of people across Europe, Africa and America (WHO, 2007). The last major outbreak of Cholera in Namibia was reported beginning 2014 where, 554 cases where reported and 18 people had died. This outbreak was reported in 6 regions across the country with the first case being recorded in Opuwo district which is located in the north-western part of Namibia in November 2013 and 493 cases were reported in the same town. The Ministry of Health and social services, in collaboration with WHO, UNICEF and other partners put in place immediate effective and coordinated action in order to contain the spread of the disease. Furthermore, the first confirmed case of cholera in Windhoek was recorded on 5 February 2014 in the informal settlements of Katutura, the population is put at risk here because of overcrowding, as well as poor hygiene and sanitation conditions put the population at risk and further increased the risk of transmission. By the beginning of March 2014, 39 suspected cases had been recorded in Windhoek with two confirmed cases and including two deaths (The Namibian newspaper, 2014). In January 2018, another cholera outbreak was declared in Windhoek where 4 cases were recorded (New era newspaper, 2018).

In September 2018, there was a Cholera outbreak reported in Zimbabwe. As early as the beginning of the following month, October 2018, 8535 cumulative cases, including 163 laboratory-confirmed cases, and 50 deaths were recorded with a case fatality rate of 0.6%. Moreover, of these 8535 cases, 98% were reported from the capital Harare which is densely populated. Of which, the most affected suburbs in Harare were, Glen View and Budiriro. The age was known for 8340 cases with the majority being aged between 5 and 35 years old. Both Males and females were equally affected by the outbreak. Furthermore, a multi-drug resistant strain was identified and confirmed to be in circulation. This however, did not affect the treatment of most cases that had supportive care such as rehydration

solutions being used. Wells and boreholes that are contaminated were suspected as the source of the outbreak (WHO, 2018).

Since the beginning of 2019 a total of 417 cases of cholera including 10 deaths have been reported in Zambia (Cholera platform, 2019). A cholera outbreak was declared The District Health Office was prompted by the continued increase in the number of diarrheal cases and deaths to request for epidemiological and laboratory support from the Ministry of Health and the Zambia National Public Health Institute through the Provincial Health Office. The continual increase was prominent even with negative laboratory results (WHO, 2019).

The excretion of human enteric viruses in high concentrations of about 10^5 to 10^{13}/g faeces by infected people has great potential in polluting water sources. It has been reported that approximately 30 to 90% of waterborne disease outbreaks worldwide are caused by human enteric viruses (Ahmed et al., 2016). Enteric viruses are mainly transmitted by the faecal-oral route which can occur directly from one person to the other or through consumption of contaminated food or water. Furthermore, there are more than 140 enteric viruses that are associated with human infections. These are considered to be emerging waterborne pathogens which is attributed to the fact that they are highly stable in the environment and are resistance to the current water treatment processes (Chigor & Okoh, 2012). Worldwide outbreaks of waterborne infections have been reported with enteric viruses being detected in water sources. In South Africa, there has been studies that have reported the detection of enteric RNA including the polio virus, non-polio enteroviruses, astroviruses, rotaviruses and hepatitis A virus (Ahmed et al., 2016). Studies that assessed South Africa's water sources for viral agents, have however only taken place in a limited number of locations as well as provinces and none have occurred in Namibia to date.

Enteric viruses are human viruses that replicate within the gastrointestinal tract and are shed into the surrounding environment containing water by the faecal-oral route. There are some common types of enteroviruses which include Rotaviruses, Adenoviruses as well as Hepatitis A. These viruses contain a non-enveloped structure that is highly restricted and can live and survive for many months in diverse environments (Ahmed et al., 2016).

Rotavirus A has been found to be one of the most common causes of acute gastroenteritis and is responsible for more than half a million deaths among young children under the age of 5 per annum (Ahmed *et al*., 2016). Adenoviruses have been seen to be associated with infections that cause gastroenteritis, conjunctivitis, respiratory diseases as well as chronic systematic infections in immuno-suppressed individuals (Osuolale & Okoh, 2015). Various types of water including, swimming pools,

oceans, river water and waste water globally have been found to contain Adenoviruses which are known to contaminate the surface waters when water body has been polluted (Osuolale & Okoh, 2015). Hepatitis A is known to be the principal cause of acute hepatitis and is primarily transmitted through the faecal-oral route (Ahmed *et al*., 2016).

1.2.5.1 Rotavirus

Rotaviruses are classified under the genus rotavirus and the family *Reoviridae.* They have an icosahedral symmetry and contain a characteristic wheel-like appearance when viewed by electron microscopy. Rotaviruses have a triple layered capsid that is composed of 11 segments of double-stranded, linear, non-enveloped RNA (Morris and Estes, 2001). There are three concentric protein layers that enclose the genome that is enclosed by the capsid (Prasad and Estes, 2000). Furthermore, rotaviruses infect the cells of the intestinal epithelium. There are two structural proteins (VP4 and VP7) in the outermost layer of the capsid, which is required for cell attachment, membrane penetration and cell entry. Viral proteins three (VP3) and six (VP6) encode for proteins required for RNA transcription and correct viral structure (Saayman, 2012).

1.2.5.1.1 Mode of Transmission

Rotaviruses are shed in high concentrations (>1012 particles/gram) and persist in the stools and vomit of infected individuals for many days (Staat et al., 2005). Symptoms appear approximately two to three days after infection, and this is the time period when children between the ages of three months and two years are most likely to display the symptoms. The virus spreads rapidly through person-to-person contact, airborne droplets, or possibly contact with contaminated toys. Symptoms include vomiting and watery diarrhoea, often with fever and abdominal pain. Oral rehydration therapy is recommended as no specific drug treatment exists. The WHO states that two rotavirus vaccines were licensed in 2006, which exhibited good safety and efficacy profiles in large clinical trials. The full potential of the current vaccines has however, not been confirmed in all the regions of the world, particularly Asia and Africa and the WHO therefore does not recommend that these vaccines be included into national immunisation programmes (WHO, 2007).

1.2.5.1.2 Disease Distribution

A three year longitudinal study conducted by Bishop et al. (1983) showed that neonatal rotavirus infection did not restrict re-infection but it did confer significant resistance to disease severity during re-infection. Bishop et al. (1983) also observed that rotavirus infection can occur at a very early age. The infection in neonates is usually asymptomatic, and neonatal infection may confer protection against severe rotavirus gastroenteritis. Parashar et al. (2003) reviewed studies on global illness and deaths caused by rotavirus disease in children from 1986 to 2000 in developing and developed countries. It was found that there are cases in excess of 100 million of gastroenteritis caused by rotavirus where home care is required each year. Out of these, 25 million will visit their local clinic, 2 million will be hospitalised and this includes about 440 000 children of which 36 children that are younger than five years old die. It is also estimated that nearly every child would have experienced rotavirus gastroenteritis by age 5 and 20% would have visited a clinic, one in 65 will be admitted to hospital, and about one in 293 will die (Parashar et al., 2003). The study by Parashar et al in 2003 also indicates that 82% of rotavirus deaths are from children in poverty stricken countries, with the WHO also estimating an annual average death rate as high as 527 500 due to rotavirus infections (Parashar et al., 2003). In Africa nearly 150 000 children younger than 5 years die annually of rotavirus disease (Page, 2006). In Sub-Saharan Africa, rotavirus causes approximately 25% of diarrhoeal deaths and on average rotavirus causes 25% of hospitalisations due to diarrhoeal disease (Cunliffe et al., 1998). In a study conducted by Steele et al., (2003), regarding the epidemiology and surveillance of rotavirus in South Africa, it was found that rotavirus accounts for approximately 25% of diarrhoeal hospitalisations. The most commonly identified strain was VP7 serotype G1, followed by the G2 strains. Furthermore, the G1 strain was found to be the most prevalent internationally in infecting children <5 years old (Steele et al., 2003). In another study conducted by Jere et al., (2011), on the characterisation of rotavirus strains in Sierra Leone, it was found that approximately 50% were G2 serotypes. It was reported by Reddy (2006) that the mortality rate from diarrhoea is 14% in Durban, moreover, 22% of the mortality cases in a local study of diarrhoea incidence at King Edward Hospital were ascribed to rotavirus infection.

1.2.5.2 Hepatitis A virus

Hepatitis A (HAV) viruses are also classified under the genus enteroviruses and the family *Picornaviridae*. The hepatitis A virus is non-enveloped and icosahedral in shape measuring about 28 nm in diameter. The genome consists of linear, single stranded positive-sense RNA of about 7.5 kb. It

has a large polyprotein which is expressed from a large open reading frame which extends through most of the genomic RNA. This polyprotein is cleaved by a viral protease (3Cpro) to form three and possibly four capsid proteins and several non-structural proteins (Lemon, 1994). The virus can survive denaturation by ether, acid (pH 3.0), drying, 56ºC, and freezing temperatures (-20ºC). Inactivation of HAV requires one minute contact time with household bleach, or exposing it to heat higher than 85ºC for at least one minute (Melnick, 1992; Nainan et al., 2006).

1.2.5.2.1 Mode of Transmission

Hepatitis A is transmitted via the faecal-oral route from person to person, and through contaminated water, food supplies and occasionally via blood transfusions. The virus spreads easily in areas where sanitation is poor and crowded living areas. The HAV can survive in the environment for long periods (Biziagos et al., 1988). The virus is excreted in high concentration one to three weeks before the onset of illness. In acute hepatitis A the anti-HAV IgM is detectable about three weeks after exposure, while its concentration peaks after about two months and declines to undetectable levels within six months of infection. Hepatitis A IgA and IgG antibodies are detected within a few days after the onset of symptoms and IgA antibodies decrease to undetectable levels in a few months while IgG antibodies remain in the patient's circulation for years and imparts lifelong immunity to the patient (WHO, 2000). Treatment is symptomatic as the HAV infection is self- limiting (Previsani & Lavanchi, 2000).

1.2.5.2.2 Disease Distribution

The hepatitis A virus is one of the major causes of acute hepatitis (Villar et al., 2006). A report published in 1994 indicated that the majority of black adults in South Africa below twenty years of age, tested positive for antibodies to HAV (Melnick, 1995) as compared to thirty to forty percent of white adults that had antibodies to HAV by the age twenty years. This increased to about sixty percent between forty and forty nine years of age (Taylor, 1997; Poovorawan et al., 2002). Hepatitis A virus infection often appears asymptomatic among children, while symptoms ae clearly visible with infected adults (Lemon, 1997). Overcrowding, poor sanitation, and lack of a reliable clean water supply are predisposing factors that lead to people acquiring hepatitis A infection (Nainan et al., 2006). Hepatitis A only replicates in the liver cells of humans. Once the virus has gained entry into the hepatocyte, the host ribosomes bind to the released viral RNA to form polysomes. Copies are made of the viral genome and shed into the biliary tree to be excreted into faeces. Transmission of the virus is highest 14 – 21

days after infection, corresponding to the period of highest shedding of the virus and after the development of jaundice (Lemon, 1997). A notable case of acute hepatitis A infection was recorded at a Pennsylvania restaurant where the source of the virus was traced to green onions that were used to make a mild salsa. Tracing the contamination to the onions before it arrived in the United States of America, illustrated the resilience of the virus to survive and spread (Gilroy et al., 2009).

Dual infection with hepatitis E virus can also occur as both viruses have a similar clinical presentation, and are transmitted via the same route. Inflammation of the liver (hepatitis) can be caused by a variety of viruses such as hepatitis A, B, C, D and E with a characteristic feature being jaundice. Testing the patient's serum for the presence of specific anti-viral antibodies will indicate the causative agent. A single serotype of hepatitis A virus exists in humans despite its genetic "heterogeneity" at the nucleotide level. This single serotype virus imparts immunity in its host (Melnick, 1992). Vaccines are available which may reduce disease incidence and potentially eliminate infection transmission (WHO, 2000). The South African Department of Health reported 1612 hepatitis A cases between January 2001 and December 2005. A decrease in the mortality rate from 1.1% in 2001 to zero in 2005 was also reported. The decrease in the fatality rate could, however, be ascribed to the vaccination program launched by the Department of Health (DOH, 2005).

1.2.5.3 Adenovirus

Adenovirus has been said to be the most common enteric virus in sewage and may survive longer than any other enteric virus, along with hepatitis A virus (Enriquez et al., 1995; Pina et al., 1998). Other DNA viruses also found in the water environment and causing gastroenteritis and other disease are parvovirus, polyomavirus and circovirus (Bosch et al., 2008). Adenoviruses are classified under the family *Adenoviridae.* They are divided into four genera, namely *Mastadenovirus, Aviadenovirus, Atadenovirus and Siadenovirus*. The majority of adenoviruses isolated from mammals are grouped under the family *Mastadenovirus*, while the family *Aviadenovirus* includes adenoviruses isolated from avian species. Human adenovirus serotypes 8, 19 and 37 belong to species D and may be the causative agents for nosocomial infections and cause sporadic cases and outbreaks of severe epidemic keratoconjunctivitis (Reddy et al., 2006). They are non-enveloped icosahedral viruses and their genome consists of double stranded, linear DNA. The genome of human adenovirus has about 35 000 bp and contains an inverted terminal repetition (ITR) of 103 bp. Its guanine and cytosine (G+C) content also varies between 34% and 60%. To date 51 human adenovirus serotypes have been identified and are classified into six species, A-F. Adenovirus classification is based on their hemagglutination

properties and 44 biophysical and biochemical criteria are also used (Shenk, 1996; De Jongh et al., 1999; Van Heerden et al., 2003).

1.2.5.3.1 Mode of Transmission

Adenoviruses are transmitted by direct contact, the faecal-oral route, and through waterborne transmission. Adenoviruses cause diarrhoea, eye infections, and respiratory disease in humans (Kapikian & Chanock, 1995; Horwitz, 1995; Kapikian, 1997; Foy, 1997; Van Regenmortel, et al., 2000). Adenovirus infections vary by serotype and some serotypes causes persistent asymptomatic infections in the tonsils, adenoids and the gut of infected persons (Russell, 2009). It has also been shown that insufficiently chlorinated swimming pools and small lakes are linked to epidemics of febrile disease and conjunctivitis as well as waterborne transmission of some adenovirus serotypes. The clinical manifestation of disease associated with the infection of certain adenovirus serotypes depend on the site of infection as well as the mode of entry into the body. Severe lower respiratory tract disease, for example, is associated with serotype 7 if acquired by inhalation, while oral transmission of the virus causes no or only mild disease. Adenovirus infections can occur throughout the year despite adenovirus-associated respiratory illness being more prevalent in late winter (Pickering et al., 2006). Enteric serotypes 40 and 41 of adenovirus cause gastroenteritis, mostly in children. Adenovirus can be excreted for prolonged periods and may cause infections throughout the year, however respiratory illness caused by adenovirus generally occur in winter, spring, and early summer. Outbreaks of adenovirus-associated diseases can also be prevented from spreading by good infection-control practices such as the regular washing of hands (Horwitz, 1995; Foy, 1997; Pickering et al., 2006).

1.2.5.3.2 Disease Distribution

The prevalence of adenovirus were reported to be the same throughout the world, with serotypes 40 and 41 constituting between 38% and 100% of adenovirus serotypes (Enriquez, 1995; Moore, et al., 2000). Adenovirus is regarded as a robust virus, found almost everywhere in human and animal populations. It is endemic throughout the year and can survive for long periods outside a host. This virus was first isolated in the 1950's in adenoid tissue-derived cell cultures. Adenovirus causes gastroenteritis, respiratory illness, conjunctivitis, cystitis, and various other illnesses depending on the infecting serotype. Respiratory infection caused by adenovirus may display as the common cold syndrome, pneumonia, croup or bronchitis. In addition, adenovirus infections may cause severe complications in patients whose immune system is compromised (Pickering et al., 2006).

1.2.5.4 Hepatitis E virus

Hepatitis E is a liver disease that is caused by the hepatitis E virus (HEV). The Hepatitis E virus is a small single-stranded RNA virus that has a positive-sense. There are at least 4 different genotypes of the hepatitis E virus these are; genotypes 1, 2, 3 and 4. Out of these genotypes, only 1 and 2 have only associated with humans whereas, genotypes 3 and 4 viruses have been found to be associated with a number of animals such as pigs, wild boars, and deer. These genotypes circulate in these animals without causing any disease, and occasionally infect humans (Perez-Gracia et al., 2015).

Infected persons shed the virus in their stool, thereby contaminating the environment. The virus is mainly transmitted from person to person through contaminated drinking water. The infection is usually self-limiting and resolves within 2–6 weeks. In rare cases a serious disease that is known as fulminant hepatitis or acute liver failure develops. This type of disease leads to a proportion of people dying (European Association, 2018).

Hepatitis E infection is found worldwide in both resource-poor areas with frequent water contamination as well as in areas with safe drinking water supplies (European Association, 2018).

Countries with limited access to essential water, sanitation, hygiene and health services are reported to be associated with increased Hepatitis E cases. The disease occurs as outbreaks and as sporadic cases in these countries. After periods of faecal contamination of drinking water supplies, outbreaks usually follow and may affect a minimum of several hundreds of people. Outbreaks have also been reported in areas of conflict and humanitarian emergencies including war zones, as well as in refugee camps or internally displaced populations. These are situations where challenges are experienced due to poor sanitation and safe water supply. The genotype 1 virus has been found to cause sporadic infection cases that are related to the contamination of food and water (European Association, 2018).

Areas that do not have major challenges with regards to sanitation and water supply, do not frequently hepatitis E disease outbreaks, sporadic cases due however occur. The genotype 3 virus has been associated with these sporadic cases and are caused by infection with virus that infects animals. This virus occurs through the ingestion of undercooked animal meat and is not related to the contamination of water or other foods (European Association, 2018). Evidence through serology of prior exposure to the virus has been found in most areas that have, higher seroprevalence rates. This has been seen in regions that have poor sanitation and this poses a

higher risk for transmission of the virus. The presence of these antibodies does however not mean that there is the presence of or increased risk of disease. Furthermore, the possible sub-optimal performance of available serological assays as well as the possible disappearance of the antibody with the passage of time among those exposed to the virus, limits the usefulness of such data for epidemiological purposes (European Association, 2018).

1.2.5.4.1 Mode of transmission

The transmission of the hepatitis E virus mainly occurs through the faecal-oral route. Most of the cases of the disease are transmitted through this route. As described above, the major risk factors associated with the spread of this virus are inadequate or poor sanitation as well the lack of access to water. These factors are related to the excretion of faeces by infected persons into the environment thereby contaminating water sources and thus enabling the spread of the virus. There are other routes that have been linked to a few cases, these are the ingestion of undercooked meat or other meat products that are animals that have been infected, transfusion of infected blood products from infected persons to other individuals as well as the maternal transmission from a pregnant woman to her foetus (European Association, 2018).

1.2.5.4.2 Disease distribution

The virus is excreted form infected persons from a period ranging from a few days to about 3 to 4 weeks after disease onset. It is believed that the incubation period of this virus is on average between 2 to 10 days and can be up to 5 to 6 weeks. The age group of adults between 15 and 40 years of age are the common group who usually have cases of symptomatic infection. Although the infection of children in endemic areas does occur, they tend to have no symptoms and in some cases, they present with a mild illness that usually goes undetected (European Association, 2018).

Infection with Hepatitis E initially presents anorexia, nausea and vomiting which only persists for a few days. Some people experience abdominal pain as well as itching in the absence of skin lesions, skin rash, or joint pain, jaundice, with dark urine and pale stools and a slightly enlarged, tender liver. It is however worth noting that these symptoms can be associated with other liver illnesses. These symptoms are however, often indistinguishable from those experienced during other liver illnesses and normally last between 1 to 6 weeks (European Association, 2018).

Fulminant hepatitis results when acute hepatitis is severe. This happens rarely in some cases it is frequently observed in pregnancy and can result in death. Pregnant women that are in the second or

third semester have a risk that is increases for acquiring acute liver failure and foetal loss and mortality. High case fatality rates have been recorded amongst pregnant women that are in the third trimester, these rates are between 20 % and 25%. Immunocompromised people have been identified to have the most cases of chronic hepatitis E infection. These people are usually those that organ transplant recipients that are on immunosuppressive drugs and have hepatitis E infections caused by the genotypes 3 and 4. Furthermore, the establishment of adequate and functional human faeces disposal systems as well as the provision of safe and adequate public water systems reduce the rate of transmission of hepatitis E infection (European Association, 2018).

1.2.5.5 *Escherichia coli*

Escherichia coli (*E.coli*) is a bacterial organism that belongs to the genus *Escherichia*. It has a rod shape that is 2.0 μm^3 long and 0.25–1.0 μm^3 in diameter and is known as a coliform bacteria. This bacteria has a cell volume of 0.6–0.7 μm^3. It stains as a gram-negative organism because of the thin peptidoglycan layer and an outer membrane that surrounds the cell wall and provides a barrier to some antibiotics like penicillin. It is a facultative anaerobic. *E.coli* is present in the lower intestine of warm blooded organisms including humans and does not cause disease in normal circumstances as most strains are considered to be harmless. There are however some serotypes that have been seen to cause serious food poisoning. *E. coli* like most organisms that colonize the gut, is excreted into the environment through feces and is mainly transmitted through the fecal oral route. The ability of the bacterial cells to survive outside of the body for some time, makes *E.*coli valuable in water quality checking mechanisms as it is considered as an indicator organism for fecal contamination. *E.coli* tends to multiply rapidly in fecal matter that is fresh and under aerobic conditions for 3 days, this number however stats to decline after 3 days (Jang, et al., 2017).

E.coli is serotyped based on its major surface antigens, the O antigen that is part of lipopolysaccharide layer, the H antigen that is the flagellin and the K antigen that is part of the capsule), e.g. O157:H7) and about 190 serogroups are known (Jang, et al., 2017) .

Disease causing E.coli has been found to cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease. Common signs and symptoms include severe abdominal cramps, diarrhoea, hemorrhagic colitis, vomiting, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to haemolytic-uremic syndrome, peritonitis, mastitis, sepsis, and

gram-negative pneumonia. Very young children are more susceptible to develop severe illness, such as haemolytic uremic syndrome; however, healthy individuals of all ages are at risk to the severe consequences that may arise as a result of being infected with *E. coli* (Poolman, 2017).

Some strains of *E. coli*, for example O157:H7, can produce Shiga toxin (classified as a bioterrorism agent). The Shiga toxin is produced by some strains of *E.coli*, this toxin is one of the causative agents of inflammation of the gut and thus causes diarrhoea that is bloody. This toxin is also known to cause haemolytic-uremic syndrome (HUS) especially in children and the elderly, HUS usually occurs when the first symptoms are improving and this is on average 7 days after the first symptoms. This syndrome is characterized by a decrease in the frequency of urination, lethargy as well as paleness of cheeks and paleness in the inside of the lower eyelids. Some complications involving the nervous system occur in 25% of patients with HUS, these lead to strokes. Oedema around the lungs, legs and arms is another outcome from infection with this strain, this oedema usually occurs due to the fluid build-up. Increased blood pressure is observed in patients with Shiga toxin producing *E.coli*, this is because of the fluid build-up that interferes with the hearts' function (Poolman, 2017).

There are two other strains of *E.coli* that are main causes of urinary tract infections as well as travellers' diarrhoea. These are the Uropathogenic *E. coli* (UPEC) and Enterotoxigenic *E.coli* (ETEC). The latter has been associated with 840 million cases worldwide of which occur in developing countries yearly (Jang, et al., 2017. *E.coli* is characteristically transmitted through food or water that has been contaminated. It then attaches itself to the lining of the intestines where it secretes two types of enterotoxins that cause diarrhoea that is watery. There are 380,000 deaths recorded annually among children that are under the age of five. The bacteria incubates in the host for a period that is usually between 1 to 10 days after infection. The symptoms include mild belly pain or diarrhoea that is not bloody but that worsens over a few days (Poolman, 2017).

1.2.5.6 Enterococcus

*Enterococc*i are cocci that commonly occur in pairs as diplococci. These organisms are gram-positive. There are two species are found in the intestines of humans, these do not cause disease in normal circumstances, these are; *Enterococcus faecalis* which is more common (90–95%) and Enterococcus *faecium* (5–10%). Other species such as *Enterococcus casseliflavus*, *Enterococcus gallinarum*, and *Enterococcus raffinosus* rarely cause infections (Fisher and Phillips, 2009).

Enterococcus causes urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, meningitis, and spontaneous bacterial peritonitis. Treatment using drugs such ampicillin, penicillin and vancomycin have been found to be effecting in eliminating sensitive strains of *Enterococci*. Urinary tract infections caused by *enterococci* are treatable a drug known as nitrofurantoin. (Fisher and Phillips, 2009).

The *Enterococci* species are said to be better indicators of fecal contamination of water than *E.coli* this was identified and adopted as the new USA federal standard for water quality at public saltwater beaches whereas *E. coli* was to be used at freshwater beaches instead. (Heimer et al, 2015).

1.2.5.6.1 *Enterococcus faecalis*

Although *E. faecalis* is found in the gut of normal individuals, it can be associated with causing life-threatening infections such as endocarditis, sepsis, urinary tract infections (UTIs) and meningitis . These usually occur in hospital environments and present themselves as nosocomial infections (Heimer et al, 2015).

The virulence of this bacteria is aggravated by the following factors such as the presence of a plasmid-encoded hemolysin that is called the cytolysin. The presence of the cytolysin combined with high-level gentamicin resistance is results in a five-fold increase in risk of death in human bacteremia patients (Heimer et al, 2015).

1.2.5.6.2 Enterococcus faecium

E. faecium is a non-hemolytic bacteria that forms part of the *Enterococcus* genus. It can also be found in the gut of humans and animals, but it can be pathogenic as well. It is associated with diseases such as neonatal meningitis (Heimer et al, 2015).

1.2.6 Water pollution

Water pollution mainly results from natural as well as contamination that is known as anthropogenic. Anthropogenic pollution is caused by agriculture developments, industrialisation, population increases and urbanisation. Furthermore, leaking sewage pipes as well as malfunctioning sewage treatment plants are the main source of pollution in developing countries and it has been established

that 90% of human waste flows untreated into rivers and streams (Hill., 2004). In Namibia, water pollution occurs because of toxic chemicals such as pesticides and organic material which is mostly sewage. Pesticides are largely used for the removal of weed more specifically in areas that are paved, car parks as well as in sports fields. Sewage leakages are also often observed from sewer pipes that cross the Arebush River which is one of the rivers that flow towards a water catchment source in Windhoek, Figure 1.4 shows the catchment area of the Goreangab dam (Environmental Impact Assessment for service provision for Goreangab extension 4 township, Windhoek, Khomas region October, 2015).

Figure 1.4: Map indicating the rivers that flow into the Goreangab dam and the catchment area around the dam (Environmental Impact Assessment for service provision for Goreangab extension 4 township, Windhoek, Khomas region October, 2015)

The Goreangab Dam catchment runs in a north-south direction, with the Auasberg range being the highest and most distant portion of the catchment whereas the Goreangab Dam is the lowest. The Catchment area is mainly dissected by the Gammams River. Most of the informal settlements that are mushrooming are situated at the source of runoff, storm water system connecting the catchment and the leaking sewerage system that is constructed within the river systems. Furthermore, these informal settlements have poor sanitation and the use of the dam for recreational purpose e.g. swimming can

lead to health problems on direct swallowing because of the presence of infectious microorganism. The dam water is polluted with untreated sewerage and show faecal and metal pollution. (Environmental Impact Assessment for service provision for Goreangab extension 4 township, Windhoek, Khomas region October, 2015).

A report by the Socio-Economic Rights institute of South Africa in 2018 states that because of the shortage that is being experienced with the provision of affordable housing, a large number of people that are poor and are from low income households, were faced with a challenge and had to resort to living in informal settlements. It also states that communal water sources have been identified to be the major means of water access in informal settlements. Furthermore, the report also indicates that sanitation remains a concern in the informal settlements which is attributed by the lack of proper infrastructure for the purpose of waste disposal and sewage. It also states that households that use the bucket system as their sole form of toilets account for 6.8 % of households in the informal settlements. This is higher than the 0.1% of households that are living in formal housing, informal settlements have been approximated to be five times more likely to engage in the practice defecating in open areas than those living in formal households (Socio-Economic Rights institute of South Africa, 2018).

The growing problem of informal settlements as outlined by Mukonoweshuro in 2014, was brought forth by the population growth in Zimbabwe as by 2012 29% of Zimbabweans were found to have been living in urban areas with urbanization increasing by 4% yearly. The major challenge that was outlined concerning the informal settlements was that of sanitation not being adequate and the lack thereof, this picture was not seen in the formal settlements. The lack of access to adequate sanitation in informal settlements was evidenced by the 2008-2009 cholera epidemic in Zimbabwe. Furthermore, the report noted that the informal settlements were epicentre of future diarrheal disease outbreaks and that this was because of the continuous increase in the populations in older informal settlements. It further stated that the combination of factors such as poor sanitation, contaminated water supply, crowded water supply. It was further noted that the unavailability of adequate drainage facilities contributed to the fatal outbreaks of cholera in 2008 and typhoid in 2010. It further outlined that an earlier study revealed that some residents started using plastic bags or buckets to dispose of their faeces in communal skips or open drains due to the fact that the communal facilities were overcrowded, unreliable as well as dangerous (Mukonoweshuro, 2014). Namibia is one of the African countries that is experiencing situations that are similar to those in South Africa and Zimbabwe as

described above. Table 1.1 below indicates the principle sources of water pollution in Africa. These factors contribute greatly to the situation in informal settlements in Namibia. Figure 1.5 shows an aerial photograph of the Goreangab dam as well as the settlements that lie next to the riverbeds of rivers that flow into the dam as well as in the catchment area of the dam. These settlements vastly contribute to the pollution the Goreangab dam.

Table 1.1: Principle sources of water pollution in Africa

Non-point sources	Point sources
Spills and washings: wastes/oil/gas/chemical spills, auto workshop spills, domestic washings and shop spills	Industrial discharges: petrochemicals, iron, steel, cement, power plants (gas/coal), pulp and paper, garment and rubber tyre
Urban runoff: waste and litter	Sewage disposal systems: sewage lagoons, septic systems and cesspool
Indiscriminate throw-aways: metal cans and scraps	Surface waste disposal: barnyard/feed lot, landfills(garbage dumps) and surface waste dumps
Buildings and utilities: rainfall wash-down	Underground waste disposal: storage tanks and pit latrines
Agriculture: cropland, pasture and grassland, woodland and feed lots	Mining and drilling: surface and underground
Construction: road building, highway, urban and building construction and logging	

Reference: Nriagu, 1992

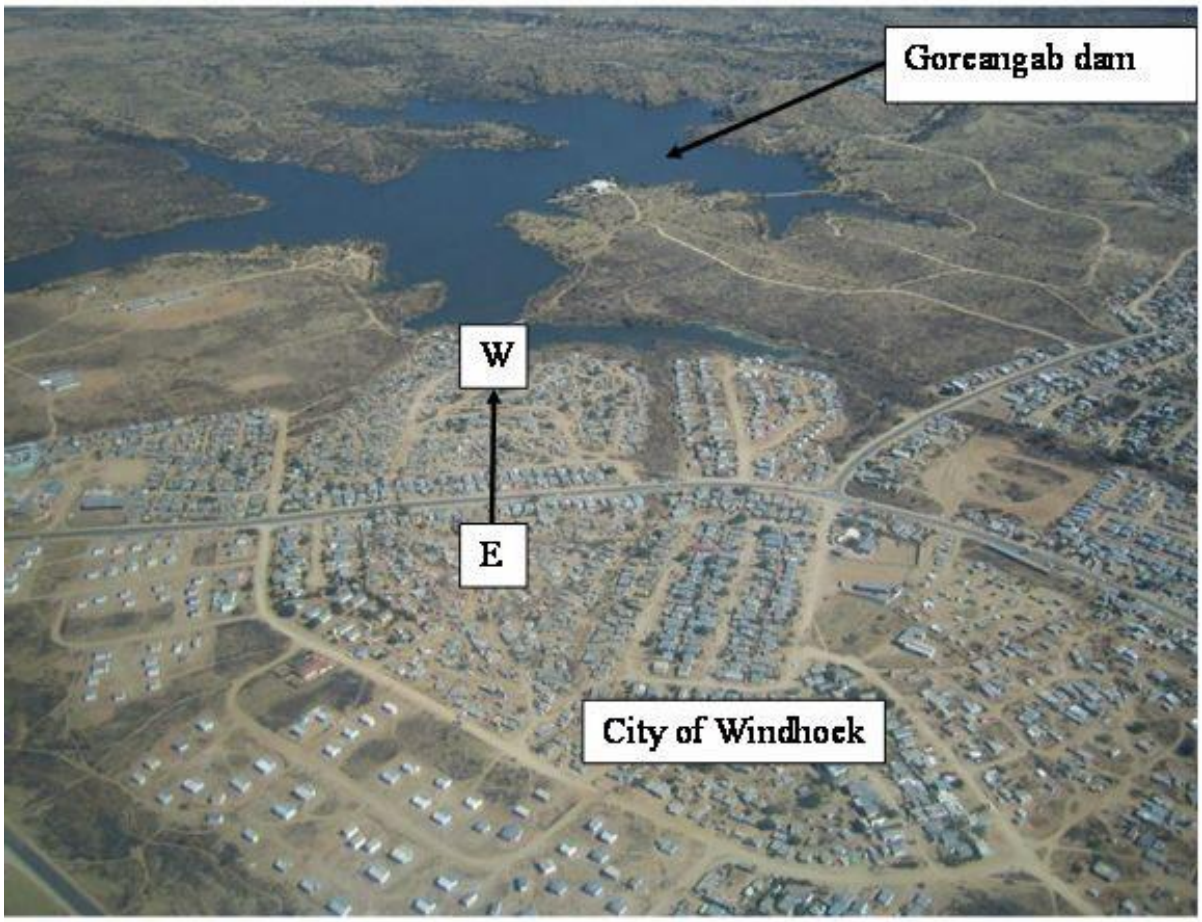

Figure 1.5: Aerial photograph of the Goreangab dam (Adopted from Chinsembu *et al.,* 2010)

1.2.7 Water quality guidelines for drinking water

Water quality standards and guidelines are established to sustain public health. The Ministry of Agriculture, Water and Forestry and the Namibian Water Co-operation (NAMWATER) are recognised for establishing and monitoring the quality of water sources. These bodies ensure that water used for domestic purposes comply with stipulated standards by the National Institute for Standards Technology (NIST), USA. Table 1.2 below shows the concentration of and limits for the aesthetic, physical and inorganic determinants of drinking water in Namibia.

Table 1.2: The concentration of, and limits for the aesthetic, physical and inorganic determinants of drinking water in Namibia

DETERMINANTS	UNITS	LIMITS FOR GROUPS	
		Water which is bacteriologically very safe	**Water which is bacteriologically still suitable for human consumption**
Colour	mg/l Platinum Units	20	-
Conductivity	mS/m 25^{0}C	150	300
Total hardness	mg/l $CaCO_3$	300	650
Turbidity	Nephelometricturbity units	1	5
Chloride	mg/l Cl	250	600
Chlorine (free)	mg/l Cl	0.1-5.0	0.1-5.0
Fluoride	mg/l F	1.5	2.0
Sulphate	mg/l SO_3	200	600
Copper	µg/l Cu	500	1000
Nitrate	mg/l N	10	20
Hydrogen Sulphide	µg/l H_2S	100	300
Iron	µg/l Fe	100	1000
Manganese	µg/l Mn	50	1000
Zinc	mg/l Zn	1	5
pH	pH-unit	6.0-9.0	5.5-9.5
Aluminium	µg/l Al	150	500
Cadmium	µg/l Cd	10	20
Chromium	µg/l Cr	100	200
Lead	µg/l Pb	50	100
Magnesium	mg/l Mg mg/l $CaCo_3$	70 290	100 420
Mercury	µg/l Hg	5	10
Nickel	µg/l Ni	250	500
Standard plate counts	per 1 ml	100	1000
Total coliforms counts	per 100 ml	0	10
Faecal coliform counts	per 100 ml	0	5
E.coli counts	per 100 ml	0	0

(www.dev.azafrica.com/namwater4/data/Knowledge_Centre.htm?pn=Knowledge_Centre, updated 2006)

1.2.7.1 Physical qualities of water

1.2.7.1.1 Turbidity

The colour of the drinking water has a physical characteristic that is associated with its colour and this is only visible when it is present in high concentrations. Furthermore, there certain elements such as

clays, silts, sand, algae as well as leaf particles that lead to turbidity of water. The presence of turbidity in water has been associated with shielding bacteria in such a way that disinfection with chemicals becomes ineffective. Trihalomethanes are harmful chemicals that arise when organic materials come in contact with chlorine. Turbidity of surface water sources is higher than groundwater sources and can vary from 1 to 200 Nephelometric Turbidity Units (NTU). Furthermore, the turbidity of a surface water sources. Turbidity is defined as a physical parameter and optical determination that measures the degree at which the transparency water is lost as a result of the presence of suspended particulates. When water is turbid, its appearance is cloudy or murky and this affects the physical appearance of the water. There is a direct link between the amount of total suspended solids in the water and the physical colour of the water. Murky water is indicative of an increase amount of total dissolved solids in the water. It has therefore been established that turbidity is considered as a good measure of the quality of water (WHO, 2017). Furthermore, factors such as inappropriate waste discharge as well as urban runoff also contribute to water turbidity. The WHO (World Health Organization), states that the turbidity of drinking water should be less than 5 NTU, and should ideally be below 1 NTU to be considered safe for consumption. The suspended particles in water absorb heat from the sun thus making the water warm thereby reducing the oxygen concentration in the water. This process is associated with an increase in the attachment of heavy metals and other organic compounds that are toxic as well as pesticides (Huey and Meyer, 2010). Turbid water in lakes and streams has been found to impact recreation and tourism negatively. An increase in water treatment costs is observed when water turbidity increases. Aquatic life can be affected by turbid water as reduces it food supplies due to the decrease oxygen concentration in the water. It also physically interferes with aquatic life through the degradation of spawning beds and affecting gill function (Huey and Meyer, 2010).

1.2.7.1.2 Conductivity

Conductivity is defined as a measure of the ability of an aqueous solution to carry an electrical current. The conductivity of water can be affected by factors such as the number of ions present in the water, mobility of the ions, the oxidation state as well as the temperature of the water. Conductivity is important in assessing water quality as it is used to identify methods that can be utilized during water treatment such as mineralization determination. Furthermore, the amount of total dissolved solids allows for the determination of the overall ionic effect in a water source as well as that of chemical reagents or treatment chemicals that are to be added to a water sample for treatment. When there is an increased level of dissolved solids in water it can cause drinking water to have a sort of mineral taste. Furthermore, metallic surfaces can be corroded by water that contain high levels of dissolved

solids and this affects industrial equipment such as boilers, domestic plumbing, hot water heaters, toilet flushing mechanisms, faucets, washing machines and dishwashers (Oyem at al., 2014).

1.2.7.1.3 pH

The measure of hydrogen ions in water is referred to as the pH of the water. Water is made up of both hydrogen ions as well as hydroxyl ions. When these ions are found in equal proportions, the water is said to be neutral. There is a direct link between the number of hydrogen ions and the acidity of the water, the more the hydrogen ions present in the water, the more the water becomes acidic. A similar link is observed with the hydroxyl ions, these however indicate the alkalinity and an increase in these ions leads, the water becoming more basic. A narrow pH tolerance range of 6.5 – 8.5 has been found to be the most prominent amongst many aquatic organisms. The release of toxic heavy metals can be aggravated by the high acidity of water. The pH of pure water is neutral, pH 7 (Oyem at al., 2014).

1.2.7.2 Microbial qualities of water

Disease causing bacteriaas well as viruses determine the microbial quality of water. These can cause contamination of water and usually spread through waste from both humans and animals.

Efforts to try and curb and reduce contamination of water that is for drinking purposes, multiple barriers are introduced throughout the route from the catchment to the consumer. The more barriers are used, the safer the water is considered to be. This in turn contributes to the protection of water resources, the proper selection and operation of steps within the treatment process and the management of piped and distribution systems that are not piped. Furthermore, it has been found that an increase in risk associated with the utilization as well as consumption of faeces contaminated water is notable. The detection of microbial contamination usually only occurs by the time the exposure of microbial organisms occurs. It is for these reasons that end-product testing cannot be utilised in isolation (WHO, 2017).

Although water can be a very significant source of infectious organisms, many of the diseases that may be waterborne may also be transmitted by other routes, such as person-to-person contact

as well as droplets and aerosols. These form part of the possible routes through which waterborne illness can be transmitted (WHO, 2017).

Microbial, chemical and physical properties are used to determine the quality of water. This can be done through assessing the odour or taste of the water. The presence of these properties in the water may lead to the unpleasant effects of consumers questioning the water as being unsafe and reject it, especially when it is turbid or has a smell. The consumers sometimes choose to reject aesthetically unacceptable drinking water that is safe and choose water that appears to be pleasant but may be from sources that are unsafe (WHO, 2017).

One challenge that may be experienced regarding the contamination of water by faeces is that distribution thereof may not be evenly distributed along the water distribution system. Systems with good water quality significantly lower the chances of detecting faecal indicator bacteria in the collected samples. Furthermore, frequent testing for the presence of faecal indicator bacteria may increase the chances of actually detecting the faecal indicator bacteria. This is called Presence/absence testing and this method is considered to be much easier, utilises less time and is less costly than quantitative methods (WHO, 2017).

Seasons, rainfall patterns as well as other conditions in an area play an important role in assessing possible water contamination. It is therefore ideal for sampling to be done randomly and be increased at times of epidemics, flooding or emergency operations or after there has been interruptions of supply or repair work within the piping system. There are certain substances that can be found and these do not change significantly in concentration over a period of time, these tend to require less sampling frequency than those that might vary significantly. Many water testing protocols therefore state that the water quality should be done at least once a year or less. Surface water tend to have Concentrations of naturally occurring substances that vary and therefore require more sample depending on contaminant type as well as the importance thereof (Aw, 2018).

Sampling sites are selected depending on the water quality characteristic that will be examined. It is ideal to sample at the treatment plant itself or at the head of the distribution system as well as near the extremities such as from taps that are connected to the main water line. Sampling at the

distribution head is more ideal for some of the constituents whose concentrations do not vary during delivery. Some constituents' concentrations tend to change during distribution, sampling for these constituents should then be carried out once the behaviour or source of the specific substance has been determined. Lead is one major element that should be sampled at taps of consumers, this is mainly because service as well as plumbing may contribute to the presence of lead in water (WHO, 2017).

1.2.7.3 Chemical qualities of water

Severe health effects can be caused by long spells of exposure to chemical constituents in water. These health effects are different from those associated with microbial contamination. A small number of chemical constituents have been linked to health problems after single exposure or after accidental contamination of a drinking-water supply. In these cases, the water tends to have a taste, odour and appearance that is unacceptable for human consumption. Although most health issues associated with water contamination are due to microbial contamination, adverse health problems have also been found to be as a result of contamination by chemicals (WHO, 2017).

Furthermore, certain chemicals have a positive effect in drinking water, this is because their presence in the water prevents diseases. An example is flouride which prevents dental caries. Guideline values show the constituents' concentration that does not cause and result into health risks of significance in the long run. Guidelines for many of the chemical constituents of drinking-water have been established. Water temperature has been linked to ammonia toxicity as well as the concentration of dissolved oxygen in the water as well as other tests (WHO, 2017).

1.2.7.4 Radiological qualities of water

Drinking-water may contain Radioactive substances and pose a risk to the health of humans. These risks are normally smaller than those that are caused by microorganisms as well as those caused by chemicals. In cases where radiation enters the human body through drinking water, the radiation is of a lower dose than that received from other sources (WHO, 2017).

Furthermore, higher radiation is usually from radionuclides that occur naturally in drinking-water through absorption from the soil as compared to those given off by artificially produced radionuclides

from activities such as mining and processing of mineral sands or phosphate fertilizer production. A radioactive gas known as radon may be present in some water sources such as those from groundwater. (WHO, 2017).

Radiation exposure through any form is considered to be associated with a level of risk especially during exposure for a long period of time. When there are radionuclides present in drinking water, the ingestion of these nucleotides over extended periods of time at doses above 100 mSv may increase the risk of cancer in humans (Brenner et al., 2003). Doses that are below this have not shown an increase in risk. There is therefore an assumption that there is a linear relationship between exposure and risk, with no threshold value below which there is no risk (WHO, 2017).

1.2.8 Methods for concentrating viruses

The presence and quantity of enteric viruses that are associated with humans can vary greatly. This variation is depended on the water types in which they are found. For instance, concentrations that are high can be found in small volumes <100 mL of wastewater or sludge samples while volumes that can be larger than 10-1000 L show lower concentration. This is particularly true for surface, recreational, and drinking waters. This contributed to many studies utilizing one or more in-series methods for concentrating viruses in water samples. Methods for concentration should be considered with the following factors in mind, the quality of water as well as the volume of the sample. These factors are important because they affect the efficiencies of virus concentration and downstream detection techniques with ease (Haramoto et al., 2018).

Primary and secondary virus concentration methods for environmental samples have previously been carried out in studies and many of these were developed for small volumes of water that are <1 L and these include virus adsorption and elution (VIRADEL), size-exclusion, and coagulation/flocculation methods (Haramoto et al., 2018). Some of the most earliest primary methods used for virus concentration included two-phase separation techniques, hydroextraction, soluble membranes, ultracentrifugation, and precipitation by salt addition (Ikner et al., 2012; Cashdollar & Wymer, 2013). Secondary methods included the use of celite and non-flocculating beef extract (McMinn et al., 2012). There has been challenges that have been experienced with the use of these methods in studies that are targeting larger volumes of water but due to the lack of methods that are specifically for volumes

of water, these methods are still used in many studies as primary or secondary concentration methods today (Haramoto et al., 2018).

1.2.8.1 Adsorption-elution techniques

The first virus concentration method known as a pad method for water was developed in the 1940's during this method a pad was dipped in water overnight, thereafter, the recovery of viruses adsorbed on the pad was performed. Although this method was considered as a practical way for virus detection in water, it lacked information about volume of water that is required. A virus concentration method using a negatively charged membrane was developed by Wallis et al. (1967). In this method viruses are adsorbed onto a negatively charged membrane that has been conditioned under 25-50mM Mg^{2+}, after which it was eluted with beef extract solution that is at pH 9.5. This negatively method was modified using acid in the place of Mg^{2+} (Sobsey et al., 1973). A Positively charged membrane method was developed by Sobsey et al. (1979). Here viruses are adsorbed onto positive charged membrane that was not conditioned as the negatively charged membrane. The viruses would then however also eluted with beef extract solution. An advantage of this method is that it does not require any pre-treatment before it is used for a large volume of fresh water. These method are all depended on the mechanism of virus adsorption to the elution from membranes. Other methods developed include the cellulose coagulation method (Yano et al., 1993), glass wool and glass powder methods used as the adsorbent of viruses. The elution of viruses in all these methods was done either by beef extract solution (3 % and 1%) at pH 9-11 or by glycine buffer. The elution process was based on the assumption that viruses are adsorbed due to hydrophobic interaction. These methods extract viruses by competition of adsorption sites with viruses. The use of beef extract is good for following cultivation with mammalian cell but has inhibitory effect on Polymerase Chain Reaction (PCR) detection of viruses. Inorganic eluent is preferred for following detection of PCR so that the electrostatic interaction should be considered. The electrostatic interaction was also working and considered in the adsorption step in these methods. Under neutral pH condition, viruses are negatively charged but are changed by multivalent cation. Viruses are positively charged under acid condition (Katayama & Shinichiro, 2009).

The VIRADEL technique is the most widespread and used technique for a number of water sample types. This technique uses electronegative membranes. The addition of a salt such as $MgCl_2$ is needed

prior to the concentration of the viruses, the purpose of a salt is to facilitate the binding of the negatively-charged viruses to the membrane required, with the exception of seawater which already has cations present in it (Wallis & Melnick, 1967). Katayama et al in 2002 demonstrated that the rinsing of filters with an acid such as H_2SO_4 to facilitate the removal of cationic salts before the final elution of viruses with NaOH which improves the recovery of the virus. Furthermore, the potential inhibition of DNA/RNA extractions or (RT-) qPCR that is seen with organic eluents such as beef extract has been reduced by this technique. The use of this method has been seen for types of water such as seawater, tap water, surface water, and wastewater (Katayama et al., 2002; Haramoto et al., 2009).

The filtration of larger samples such as 40 L for river water and 1000 L for tap water using a cartridge-type mixed-cellulose ester filter (Opticap XL; pore size, 0.5 mm; Merck Millipore, Billerica, USA) (Hata et al., 2015) is a modification that has been added to the elution method. This method has also been adopted for use for viral concentration in Treatment facilities for drinking water (Asami et al., 2016). There are other studies have add the cationic salt to the membrane prior to filtering water samples (Haramoto et al., 2004). The use of electropositive media and filters for virus concentration such as 1MDS filters have also been used (3M, Maplewood, USA) (Ikner et al., 2012; Cashdollar & Wymer, 2013). NanoCeram filters (Argonide, Sanford, USA) have recently been found to be to be more cost-effective than the 1MDS filters.

The adsorption-elution method is based on the ionic properties of the viruses as previously stated. Due to the virus surface properties that are unique, viruses, they can be absorbed by substances such as starch, minerals, fabrics alumina gel and a number of resins among others. Wallis and his colleagues in 1967 reported the first application of virus adsorbing filters. This was after they described a method for concentrating viruses from sewage. This methods' principle is based on the fact that when a virus containing sample is brought in contact with a solid matrix, the virus will be adsorb onto that surface under a specific pH and ionic strength. When the virus is adsorbed, the residual water is discarded as soon as the virus is absorbed. Afterwards, the virus is released from the matrix by elution into a smaller volume, usually one tenth of the original volume. The nature of the sample influences the choice of the adsorbing matrix, eluting fluid and processing conditions (Katayama et al., 2002; Haramoto et al., 2009).

The use of these filters for sewage samples has been proved to be a challenge as these types of samples clog the filters. Furthermore, amino acids and/or salts containing solutions have been proven to be successful at removing viruses absorbed by filters. The vast variation in the number of proteins

present in their capsid of enteric viruses, affects the size as well as the charge of the proteins that make up the capsid. Enteric viruses' size varies between about 30 nm for enterovirus to about 100 nm for adenovirus in diameter. Hepatitis A has isoelectric points that vary from 28 and about 80 isoelectric points for rotavirus (Michen & Graule 2010). Due to the fact that viruses in water have a net negative surface charges, the type of filter to be used or the actual virus needs to be conditioned before filtering in order to allow for adsorption to take place.

Virus separation from crude cell extracts allowed for the building of the first electronegative filters that were used for virus concentration (Wallis & Melnick, 1967). Furthermore, the use of membrane filters to concentrate viruses from crude cell lysates through the adjusting of the concentration of salt as well as the pH was made possible (Wallis & Melnick 1967). Viruses could then be concentrated with cartridges or flat membrane filters to allow for larger sampling volume. Farrah et al. (1976) used poliovirus and showed that >90% of the seeded virus samples filters that had been conditioned to a pH of 4 or less were adsorbed to the Filterite. Virus recoveries were found to vary from 40 to 67% in the tap water samples that were seeded. In 1978 Gerba et al. also used poliovirus and showed its recovery from seeded tap water samples to be at an average of 52%, while seawater recoveries averaged to 53%. Preston et al. (1988) used poliovirus 1, coxsackievirus B5, echovirus 1 and echovirus 5 and this resulted in 96% of viruses being adsorbed and 99% of the viruses were recovered from the cationic polymers that were added to the filter membrane as compared to 20% adsorption and only 18% virus recovery when the filters were not treated.

Recently, Haramoto et al. in 2009 developed a method allows for the usage of an electronegative filter together with aluminium or magnesium. This combination proved to be successful in recovering human norovirus from 250 to 500 ml of MilliQ water (186%), tap water (80%), bottled water (167%), river water (15%) and pond water (39%). Furthermore, Victoria et al. (2009) evaluated negatively charged membranes and the results found that noroviruses as well as human astroviruses had recovery rates of 18 and 64%, this was from mineral and river water, whereas tap water and sea water only had recoveries of noroviruses and astroviruses from 3 to 14%. One major advantage of using electronegative filters is that viruses such as common enteroviruses have high recoveries when these filters are used, they are also affordable and available. On the other hand, there have challenges when it comes to the conditioning a large volumes of water samples. This then makes the process longer as pH must be adjusted by either injecting acid into the water flow before filtering. This has to be done in a control environment and can thus not be performed in the field. This limits the volume of sample

that can be filtered because most laboratories do not have the equipment to handle large volumes of water than can be hundreds of litres of water. The Preconditioning of electronegative filters can however be done but it also has disadvantages. One of the major disadvantages is that the possibility of more variability in the filter surface charge, which could negatively affect routine monitoring (Haramoto et al., 2004).

NanoCeram filter are made up of nano-alumina fibers that are dispersed in a non-woven microglass fiber matrix VIRADEL primary concentration (Karim et al., 2009). Furthermore, there were comparisons done with the 1MDS filter which showed a high recovery of enteroviruses (EVs) in water samples from a tap and river water, these samples were both 10 L samples which was comparable to the recovery from 100 L samples (Karim et al., 2009). In 2010, Bennett et al. also investigated the use of the NanoCeram material but in a disposable capsule format. Their results showed greater recovery of the MS2 coliphage from deionized water as compared to the 1MDS, and from artificial seawater when compared to Opticap XL, here, no cationic salt was added. Ikner et al. in 2011 concluded that the virus retention of NanoCeram filters was high and can be up to and above 99.8%. They further found that elution methods improved the efficiency of virus recovery and thereby resulting in the highest known recovery of 86% for MS2 coliphage with a 1% NaPP/0.05M glycine solution (Cashdollar & Wymer, 2013).

Concentrating viruses with the use of NanoCeram filters has been demonstrated in many types of water such as drinking water, surface water as well as wastewater. The recovery of Adenoviruses through the use of Nanoceram filters has been demonstrated to be lower than those of other viruses. Studies showed 14% recovery from tap water 4.5% from seawater and 0.02% from lake water in 2013 recovery of adenoviruses was improved through the use of small disk filters by McMinn (Cashdollar & Wymer, 2013).

It has recently been shown that skimmed milk flocculation is a low-cost, virus concentration method that only has one-step. This method has been demonstrated using seawater as well as river water. The results showed recovery from 5 to 10 L samples of adenoviruses and rotaviruses, of approximately 50%. Volumes that are more than 10 L have not been studied using this method and efficiencies have therefore not been established as the process of sedimenting as well as the removing of supernatant from larger sample volumes is impractical. The most notable result from studies that have been

performed regarding virus concentration for enteroviruses is that complete effectiveness by a single method from different water types has not been achieved (Cashdollar & Wymer, 2013).

1.2.8.2 Ultrafiltration

Hollow-fibre ultrafiltration is a virus concentration method that is used large volumes of water. Water samples are passed through capillaries, hollow fibres or through flat sheets using tangential flow. These modes all have molecular weight cut-offs that are between 30 and 100 kDa. Size exclusion is important in this method. Water and other substances with low molecular weight pass through the fibres into the filtrate with ease, while substances that have larger molecular weights such as viruses and other micro-organisms, are trapped and retained in the retentate (Olszewski et al., 2005).

The use of a single filter concentration method for microorganisms in water samples will be of great value to researchers and water utility practitioners as this will enable them to save time as well as costs that are often increased with the use of multiple concentration methods. In 2011 and 2012, Haramoto et al. was successful in using electronegative mixed cellulose ester membranes that had pore sizes of 0.45 mm in diameter. This group of researchers used these membranes and concentrated viruses and protozoa from wastewater, river water, and groundwater simultaneously. The electronegative membranes used for the VIRADEL virus concentration method were used in the concentration of protozoan that are in the form of oocysts or cyst (Haramoto et al., 2012).
Different viruses have different properties and based on this, ultrafiltration, ultracentrifugation, and two-phase separation with polymers or flocculation, and adsorption-elution using filters, membranes, glass wool or glass powder are considered to be the four main principal approaches that are utilized in the process of recovering viruses as well as for the concentration of viruses (Ruhanya et al., 2016).

1.2.8.3 Membranes and filters

When using membranes and filters, the sample is passed through a cellulose nitrate membrane under positive pressure or vacuum. The viruses and filter materials both contain negative charges at neutral pH, the water samples therefore have to undergo the adjusting of the pH to 3.5, this preconditioning step is carried out by adding aluminium or magnesium ions to allow for electrostatic binding of the viruses to the filters. River water has shown recoveries of viruses of about 60% to 70%. Membranes and filters are affordable and can be packaged in sterile cartridges that are user friendly and

disposable. One major disadvantage to the use of membranes is that they tend to clog up when turbid water is used (Ruhanya et al., 2016).

The recovery of enteric viruses, rotaviruses and coliphages from water as well as other materials is mostly carried out with the use of electropositive membranes and cartridges without prior conditioning of the sample as most enteric viruses have a negative charge at an ambient pH. The other advantage of the electropositive membranes is the ability to recover Rotaviruses and coliphages are not recoverable with electronegative membranes because they are sensitive to the low pH that is needed for negatively charged membranes. Electropositive filters have viral recoveries that are comparable to those of the electronegative filters, but they have been shown to clog easily and they have lower recovery rates for viruses from marine water samples (Ruhanya et al., 2016.

1.2.8.4 Glass wool

Glass wool can be used as an alternative that is more economical than microporous filters and charged membranes. In 1993 in France Vilaginés and colleagues identified this technique and used it on samples from drinking (these need to be de-chlorinated), surface as well as wastewaters to concentrate a wide range of viruses. The glass wool is washed with the following soultions in this order, 1 M hydrochloric acid (HCl), sterile distilled water, 1 M sodium hydroxide (NaOH) and then with sterile distilled water once more to bring the glass wool to a neutral pH. Thereafter, the sample is passed through the filter. The viruses are eluted through the use buffered beef extract or skimmed milk solutions that are at pH 9.5 (Ruhanya et al., 2016).

glass wool is has a good efficiency of recovery (EOR) for enteric viruses such as namely 29% for norovirus from drinking water with a EOR of 29%, norovirus in treated waste water EOR of 56%, and an EOR of 3.4% for norovirus recovered from acidified fresh water. Enteroviruses are said through studies to have the highest recoveries in drinking water from the use of glass wool, with poliovirus having an EOR of 102.5%, an EOR of 75% for Coxsackie virus (Ruhanya et al., 2016). Studies that revealed this have all used different quantities of glass wool, water matrices, elution buffer volumes, as well as virus quantification methods that are different. The recovery of viruses with glass wool from large volumes of water is possible but this is however only the primary process and a secondary process is required to ensure adequate viral assays by reducing possible inhibitors (Ruhanya et al., 2016).

1.2.9 Virus detection methods

There are several methods that are used for the detection of viruses concentrated from water sample. These include, Electron microscope (EM), Immuno- Electron Microscopy (IEM) or Enzyme-linked Immunoassays (EIA). Molecular methods are a faster way of detecting viruses and have the highest sensitivity and a good specificity, this in turn allows for the detection of enteric viruses that appear in low numbers. Furthermore, PCR is now the preferred method that is used for easy monitoring of the presence of virus contamination in water samples. Quantitative real time polymerase chain reaction rt PCR, has been found to be faster, more reliable and efficient as compared to conventional PCR (Ruhanya et al., 2016). Introduced in 1992 initially by, real time reverse transcriptase (*rt* RT-PCR/PCR) was pioneered by Higuchi and co-workers in 1992 and it allows for the quantification of nucleic acids that is more precise despite a low initial concentration of nucleic acid. Furthermore, the amplicon amount that is produced at each cycle during amplification is measured as it is produced by fluorescence based technology in *rt* PCR. The amplicon measurement is done by labelling it with a fluroscent dye and is detected with a fluorescently tagged substrate during the procedure of amplification. The measuring of the amplicon occurs earlier in *rt* RT-PCR/PCR during the exponential phase of PCR when amplification is proceeding most efficiently.

rt PCR has fewer cycles and thus an increased speed and has higher sensitivity of fluorescent dyes that are used for the amplicon detection. One of the main uses of Real time quantitative (*rt* qRT-PCR/PCR) is that it provides data concerning the enteric viral genomes presence in samples, this is important in estimating the public health risks of low levels of enteric viruses in environmental samples (Ruhanya et al., 2016) .

Real time RT-PCR/PCR requires the following; high quality nucleic acid extraction/purification of RNA/DNA, the optimal conversion of RNA to cDNA as well as he accurate detection of PCR products. Inhibitory substances however reduce the effectiveness of PCR, this is one major disadvantage. The inhibitory substances that are co-concentrated with viruses, include, humic acids, polysaccharides, fulvic and tannic acid. These are frequently present in environmental water samples and should be rid of during the sample concentration and the nucleic acid extraction and purification step.

There is no method for the extraction of nucleic acid that is able to remove all the inhibitors in the environmental samples. There are methods that have been evaluated for the removal of *rt* RT-

PCR/PCR inhibitors of virus detection in water sample concentrates. Gel filtration resins such as Sephadex or Sepharose are considered to be effective in the removal of inhibitors but there is loss of nucleic acids during the process. Nucleic acid loss can however be avoided through binding resins. Another inhibitor removal agent is bovine serum albumin (BSA) that has been added to PCR mixtures in order to reduce inhibition by binding to inhibitors. The usage of internal or external controls is part of the methods that are available for the identification and monitoring of inhibition (Ruhanya et al., 2016.

Another method that is used in identifying inhibition involves analysing the shifts in the amplification efficiency of *rt* qRT-PCR/PCR reactions. A shift or increase in the quantification cycle (Cq) shows the presence of inhibitors. The cq is the cycle at which sufficient amplification of target nucleic acid has been done in order to reach a defined threshold. The identification of samples that contain inhibitors from their flat curves or from the complete absence of amplification products is considered to be adequate in some instances. The removal of inhibitors is important as it reduces the chances of difficulties in the interpretation of public health risk of enteric virus pathogen, and this can lead to the underestimating of exposure and consequently health risk (Ruhanya et al., 2016).

1.3 Quality control

Positive controls are prepared by spiking samples with a known concentration of viral DNA or cDNA and negative controls are made up of either nuclease free water or any PCR buffer. These controls formed part of PCR assays and were included in each PCR assay. The purpose of including quality controls into the process is to confirm the effectiveness of the sample concentrations steps, nucleic acid extractions, primer combinations as well as to ascertain the efficiency of qPCR assays. Quality control is also used in order to avoid false positive and false-negative results. Adefisoye et al. (2016) carried out testing of these processes, by spiking a known amount of the control viruses into sterile distilled water as well as into test wastewater effluent samples. They then took these samples through the whole processes. These samples were amplified successfully through the use of primer combinations. The DNA extraction and PCR assays were performed in different rooms in order to eliminate the possible cross contamination between the samples and the amplified PCR product. During this process the DNAZap^{TM} (Ambion_) solution was always used for the washing of micropipettes before every PCR assay in order to degrade all DNA and RNA completely, to reduce possible sample contamination. The use of pre-sterilised filtered racked micropipette tips throughout the assays also further reduced the risk of possible contamination (Adefisoye et al., 2016).

Process controls have been introduced for the monitoring of the critical steps that are involved in the recovery and molecular detection of enteric viruses found in water and other environmental samples. The features of an ideal process control should be similar to the target virus. The process control must also not be associated or naturally present in samples, or be infectious to human beings. Furthermore, the process control must be added to the sample prior to it being processed. The process control is then recovered, concentrated and extracted together with the target enteric virus and then detected in the same nucleic acid extract (Haramoto et al., 2018). Figure 1.6: framework of process controls used during a virus detection procedure in water.

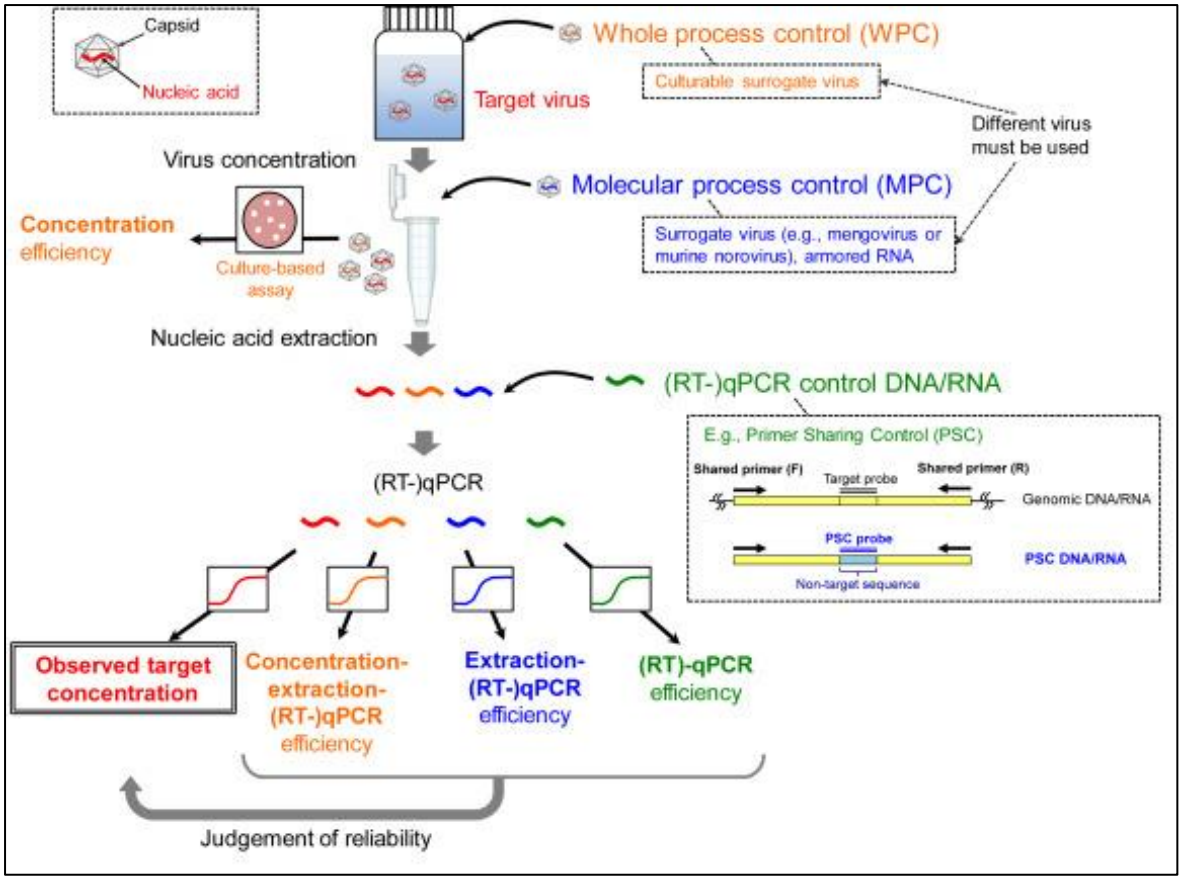

Figure 1.6: Framework of process controls used during a virus detection procedure in water Haramoto et al., 2018

The elimination of false positives and false negatives when running *rt* RT-PCR tests was done by carrying out quality control steps. Negative and positive controls were therefore included in each run. A negative that is made up of the master mix was added in order to confirm that the PCR reagents are not contaminated when the result is negative. When there are no template controls that become positive, it is usually due to one or more reagents being contaminated with template or previously amplified product. The presence of a positive control ascertains that all reagents are working properly. The positive control can either be quantified DNA or RNA containing the target sequence (Haramoto et al., 2018).

1.4 Statement of the research problem

Surface water contamination with enteric viruses is a concern for public health especially if these surface waters are used for purposes like recreational use and production of drinking water. Furthermore, in developing countries, many areas lack access to safe drinking water with about 70%

of the global population without improved drinking water sources residing in rural areas. Rural populations are said to commonly obtain water on an individual or household basis from nearby surface and ground water sources where the microbial quality is often unknown (Sibanda & Okoh, 2013). In Windhoek, more than 50% of the water reclaimed comes from treatment of sewage effluent. Although enteric viruses are a major cause of water borne infections world-wide, viral water contamination is currently not being monitored in Namibian water sources and there is potential risk of spreading viruses to the urban population which largely depends on recycled water for domestic use. This study seeks to determine if viral water contamination is a problem in Windhoek. An investigation into the prevalence of rotavirus, adenovirus and hepatitis A in water samples collected from sources in Windhoek will contribute greatly towards the ministry of Health and Social Services' (MoHSS) baseline information regarding the prevalence of viruses which can potentially cause water borne diseases.

1.5 Research question and objectives

Research questions:

1. What is the prevalence of Rotaviruses, Adenoviruses and Hepatitis A in water samples collected from domestic water sources in Windhoek?
2. Do the physiochemical properties of water samples from the different domestic water sources differ?
3. Is there viral contamination in surface and ground water sources?

The main objective of the study was to determine the prevalence rate of Rotaviruses, Adenoviruses and Hepatitis A in water samples collected from domestic water sources in Windhoek. The specific objectives were as follows:43

1. To determine the prevalence of Rotaviruses, Adenoviruses and Hepatitis A in water samples collected from domestic water sources in Windhoek.
2. To evaluate the physiochemical properties of water samples from domestic water sources.
3. To compare viral contamination between surface and ground water sources.

CHAPTER 2

METHODOLOGY

The amplification and the identification of rotavirus, adenovirus, hepatitis A, hepatitis E virus as well as *E.coli*, and Enterococcus was done by using species specific primers as outlined in Table 1.3. The PCR products were visualized with agarose gel electrophoresis.

2.1 Study design and setting: A Descriptive cross sectional study was conducted in Windhoek. The sampling sites are indicated in the map below (figure 2.1):

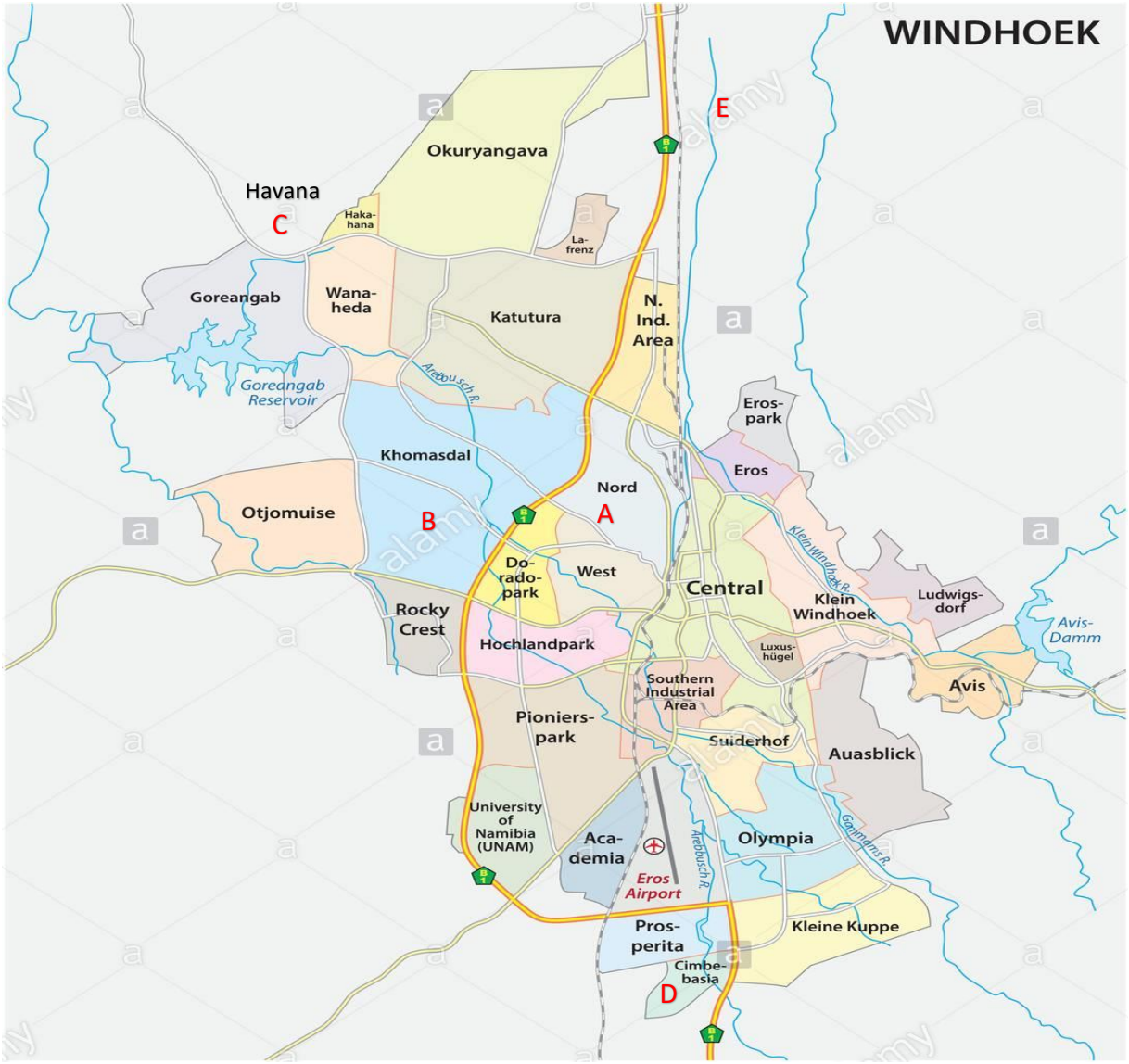

Figure 2.1: Map of the City of Windhoek with the sampling sites labelled A-E (Alamy, 2019)

Key: A=Windhoek central hospital complex; B=Khomasdal residential tap; C= Havana communal tap; D= Cimbebacia residential tap; E= Von Bach dam (approximately 70 km outside Windhoek)

2.2 Inclusion criteria: Water used for domestic purposes was sampled in Windhoek with the advice and direction of the City of Windhoek.

2.3 Exclusion criteria: Water sources that are not for domestic use were excluded from the study.

2.4 Sample size: The sample size was aligned as adopted from Chigor & Okoh (2012). A total of 8 samples were collected per site and 40 samples were available from all sites in total.

2.5 Sampling technique: A convenience (non-probability) sampling technique was used.

Ten litres of water was collected per site per week in sterile Nalgene bottles containing 1% weight out as 0.8 g sodium thiosulfate that was added for de-chlorination of the water at the sampling sites and transported to the laboratory on ice. The water samples were collected at different locations in a defined perimeter as was advised by the City of Windhoek.

There were 5 sample collection sites which were divided as follows:

- House tap in Khomasdal (borehole and blended water)
- Central hospital line (blended water)
- Cimbebacia (borehole water)
- Von Bach dam (dam water)
- Havana communal tap (surface water)

2.6 Analytical laboratory methods: Water samples were analysed using laboratory methods and techniques as described by Chigor & Okoh (2012) as well as those described by Ahmed *et al* (2016).

The pH, temperature and specific gravity of each sample was measured using a portable pH meter (YSI pH 100 portable pH mV) (Jackson *et al*., 2009), pH strips, a mercury thermometer as well as dipstick for specific gravity. The water samples were then concentrated by using the adsorption-elution method of Haramoto *et al* (Chigor & Okoh., 2012). The viral RNA and DNA were be extracted using RNA/DNA extraction kits (Quick-DNA™Miniprep; Zymo Research, USA and Quick-RNA™ Miniprep; Zymo Research, Irvine, USA) which was followed by reverse-transcription for RNA viruses. Detailed methods are provided below.

2.7 Methods of data analysis: Data was analysed and presented using graphs and frequency tables as shown in the results section.

2.8 Sample viral concentration: After collection, the samples were concentrated using the adsorption-elution method of Haramoto *et al*. This method is based on electrostatic interactions. As described by Chigor and Okoh in 2012, 5 ml of 250 mM $AlCl_3$ was passed through an HA filter that is 0.45 µm pore size and 47 mm diameter (Millipore) to form a cation (Al^{3+})-coated filter, and then 1L of the sampled

water was filtered through. The filter was then rinsed with 200 ml of 0.5 mM H_2SO_4 (pH 3.0), followed by elution of viruses with 10 ml of 1.0 Mm NaOH (pH 10.8). This acid rinse removed the Al^{3+} allowing the viruses and bacteria to attach directly to the negatively charged membrane. The eluate was recovered in a tube containing 50 µl of 100 mM H_2SO_4(pH 1.0) and 100 µl of 100 x Tris EDTA (TE) buffer (pH 8.0) for neutralization, followed by centrifugation using a Centriprep® centrifugal filter, Ultercel® YM-50 (Millipore), as per the manufacturer's protocol. Ultracentrifugation using a centrifuge was carried out at 2500 rpm for 10 minutes, this was followed by removal of the sample that passed through the ultrafiltration membrane and further centrifugation at 2500 rpm for 5 minutes was done to obtain a final volume of 700 µl. The final concentrates were aliquoted in 200 µl and stored in cryovials at -80^{o}C until further analysis could be done (Chigor & Okoh., 2012).

2.1 DNA/RNA extraction

Adenovirus, *E.coli* and *Enterococcus* DNA was extracted from 200 µl of the concentrated samples using DNA extraction kits (Quick-DNA™Miniprep; Zymo Research, (Irvine), USA) as per the manufacturer's instructions.

Genomic Lysis Buffer was added as 4 volumes to each volume of sample (4:1). 800 µl of Genomic Lysis Buffer was added to 200 µl of sample). This was briefly mixed by vortexing, then allowed to stand at room temperature for 5-10 minutes. The mixture was centrifuged at 10,000 x g for 5 minutes and then up to 1 ml supernatant transferred to the Zymo-Spin™ IIC Column. The mixture was transferred to a Zymo-Spin™ IIC Column in a Collection Tube and centrifuged at 10,000 x g for one minute. The Collection Tube with the flow through was discarded and the Zymo-Spin™ IIC Column was transferred to a new Collection Tube. Thereafter, two hundred microliters of DNA Pre-Wash Buffer was added to the spin column and it was centrifuged at 10,000 x g for one minute. Furthermore, five hundred microlitres of g-DNA Wash Buffer was added to the spin column after which, it was centrifuged at 10,000 x g for one minute. The spin column was transferred to a clean microcentrifuge tube and 60 µl DNA Elution Buffer was added to the spin column. It was then incubated for 2-5 minutes at room temperature and then centrifuged at 11,000 X g for 30 seconds to elute the DNA. The eluted DNA was immediately used for PCR.

The viral RNA of hepatitis A and rotavirus was extracted using RNA purification kits (Quick-RNA™ Miniprep; Zymo Research, Irvine, USA). One hundred microliters of the concentrated samples was

eluted in a final volume of 10-µl elution buffer (or RNase-free water) as per the manufacturer's instructions (Adefisoye *et al*., 2016). Prior to reverse-transcription, the rotavirus RNA was subjected to denaturation at 95^{o}C for 5 minutes followed by flash chilling on ice for 2 minutes in order to separate the double-stranded rotavirus RNA (Chigor & Okoh, 2012).

Sample lysis was obtained by adding RNA Lysis Buffer to each sample as 4 volumes which was 400 µl to 100 µl of sample (4:1) then mixed well. Thereafter, sample clearing and gDNA was carried out through clearing the lysate by centrifugation at 10,000 x g for 1 minute and then transferring the supernatant into a Spin-Away™ Filter (yellow) in a Collection Tube. This was then centrifuged at 10,000 x g for 1 minute to remove the majority of gDNA. The flow-through was used in the next step which was RNA Purification. One volume of ethanol (100%) was added to the sample in RNA Lysis Buffer (1:1) and mixed well. The mixture was then transferred to a Zymo-Spin™ IIICG Column1 (green) in a Collection Tube and centrifuged for 30 second the flow-through was thereafter discarded. Four hundred microliters of RNA Prep Buffer was added to the column and this was centrifuged for 30 seconds. The flow-through was discarded and 700 µl RNA Wash Buffer was added to the column and further gentrification was performed for 30 seconds and the flow-through was discarded. Four hundred microliters of RNA Wash Buffer was added and the column was centrifuged for 2 minutes to ensure complete removal of the wash buffer. The column was carefully transferred into an RNase-free tube and 100 µl DNase/RNase-Free Water was directly added to the column matrix which was then centrifuged for 30 seconds.

2.2 Reverse transcription of hepatitis A, hepatitis E and rotaviruses

Ten microliters of eluted RNA genomes were converted into complementary DNA (cDNA). The reverse transcription step included a final volume of 26.5 µl and this consisted of the 10 µl of the extracted RNA, 2 µl of 100 µM Random Hexamer primer and 2.5 µl nuclease-free water. This mix was incubated for 5 minutes at 65 ^{o}C followed by brief spinning and then placing the mixture on ice. Ten microliters of ProtoScript II reaction mix (2X) and 2 µl of ProtoScript II Enzyme mix (10X) was added to the mixture. The cDNA synthesis reaction was then incubated at 25 ^{o}C for 5 minutes followed by further incubation at 42 ^{o}C for one hour. The resulting 26µl of cDNA was kept at -20^{o}C until used for PCR (Quick-RNA™ Miniprep; Zymo Research, Irvine, USA.

2.3 Polymerase chain reaction

Twelve microliters of One Taq^R Master Mix with standard buffer (New England Biolabs, United Kingdom) containing: 20mM Tris-HCl, 1.8mM $MgCl_2$, 22mM NH_4Cl, 22mM KCl, 0.2mM dNTPs, 5% glycerol, 0.06% IGEPAL^R CA-630, 0.05% Tween^R 20 and 25units/mL One Taq DNA polymerase, was mixed with 10.5 µL of nucleic acid free water, I uL each of 10 pMol of reverse and forward primers for the respective capsular type, 5uL of DNA template to make a final reaction volume of 29.5uL.

The cycling conditions were as follows: 94˚C for 4 minutes as an initial denaturation followed by denaturation at 93˚C for 1 min, annealing at the respective annealing temperature as shown in Table 4 above for 1 minute followed by 45 cycles of extension at 72˚C for 1 minute with a final elongation step of 72˚C for 7 minutes followed by a hold at 4˚C. Amplification was verified in a 1% agarose gel electrophoresis at 110 volts for 45 minutes in a 0.5X TBE buffer and thereafter viewed in a transilluminator and photographed. ESBL *E. coli,* Rotavirus and Hepatitis E were used as a positive control.

All the primers and probes that were used in this study were designed by previous investigators and synthesized by Zymo research. Table 2.1 shows the oligonucleotide primers used for the detection of the viruses. Table 2.2 shows the thermocycling condition using cDNA of the respective RNA virus.

Table 2.1: Specific oligonucleotide primers for detection for viral genomes

Enteric virus	Primer sequence (5'-3')	Reference	Control strain
Adenovirus	JTVX (F): 5'-GGACGCCTCGGAGTACCTGAG-3' JTVX (R): 5'-ACIGTGGGGGTTTCTGAACTTGTT-3'	Xagoraraki et al 2007	ATCC VR-6
Hepatitis A	HAV68 (F):5'-TCACCGCCGTTTGCCTAG-3' HAV240 (R): 5'-GGAGAGCCCTGGAAGAAAG-3' HAV150	Pinto *et al* 2009	ATCC VR-1357; Strain PA21
Rotavirus	JVK (F): 5'-CGATGGTTGATGCTCAAGATGGA-3' JVK (R): 5'-TCATTGTAATCATATTGAATACCCA-3' JVK(	Jothikumar *et al* 2009	ATCC VR-2274; Strain 248

Reference: Xagoraraki et al 2007; Pinto *et al* 2009 and Jothikumar et al 2009

Table 2.2: Thermal cycling protocols for PCR, using DNA and cDNA of the respective DNA and RNA virus (Chigor & Okoh, 2012)

Virus	Taq activation	Denaturation	Annealing	Extension
Hepatitis A virus	4 min at 94°C	1 min at 95°C	1 min at 60°C	1 min at 72°C 7 min at 72°C
Rotavirus	4 min at 94°C	1 min at 95°C	1 min at 60°C	1 min at 72°C 7 min at 72°C
Hepatitis E virus	4 min at 94°C	1 min at 95°C	1 min at 60°C	1 min at 72°C 7 min at 72°C
E.coli	4 min at 94°C	1 min at 93°C	1 min at 58°C	1 min at 72°C 7 min at 72°C
Enterococcus (faecalis and faecium)	*4 min at 94°C*	1 min at 93°	1 min at 58°	1 min at 72°C 7 min at 72°C

Reference: Chigor & Okoh, 2012

2.4 Agarose gel preparation

Four grams of agarose powder was weight in a 250 ml volumetric flask and 0.5 X TBE buffer was added to make a final volume of 200 ml, the mixture is mixed and heated in the microwave until all the powder has melted and dissolved. Five microliters of ethidium bromide was added to the melted gel and mixed. The gel was then poured into a gel casting tray and a comb was placed in position to make wells into which samples will be pipetted. The gel was then allowed to cool and solidify after which the comb was removed. The gel was then placed into an electrophoresis tank and the electrophoresis buffer 0.5 X TBE was poured into the tank until the surface of the gel was covered (Mukesi et al., 2019). A dye was added to the samples of DNA before electrophoresis in order to increase the viscosity of the samples so as to prevent them from floating out of the wells and to allow for the visualization of the samples. Five microliters of the amplicons pipetted into the wells of the gel after which the lid was placed on the electrophoresis tank making sure that the orientation of the gel and positive and negative electrodes is correct. The Electrophoresis will be carried out at 110 V for 45 minutes. The bands were visualized under a ultraviolet transilluminator and the images captured.

2.5 Quality control

Positive controls were prepared by spiking samples of known viral DNA/cDNA concentrations and the negative controls were made of nuclease-free water and PCR buffer. These controls were included in all the PCR assays. In order to ascertain efficiencies of the sample concentrations steps, nucleic acid extractions and primer combinations and to avoid false-positive/false-negative results, initial testing of this process was carried out by spiking a known amount of the control viruses into sterile distilled water. These samples were then taken through the whole process to ensure successful amplification by using the specific primer combinations (Adefisoye et al., 2016).

CHAPTER 3

RESULTS

The samples were collected over a period of 8 weeks from five different sites. Eight samples of 10 litres were collected per site.

3.1 Physiological properties results

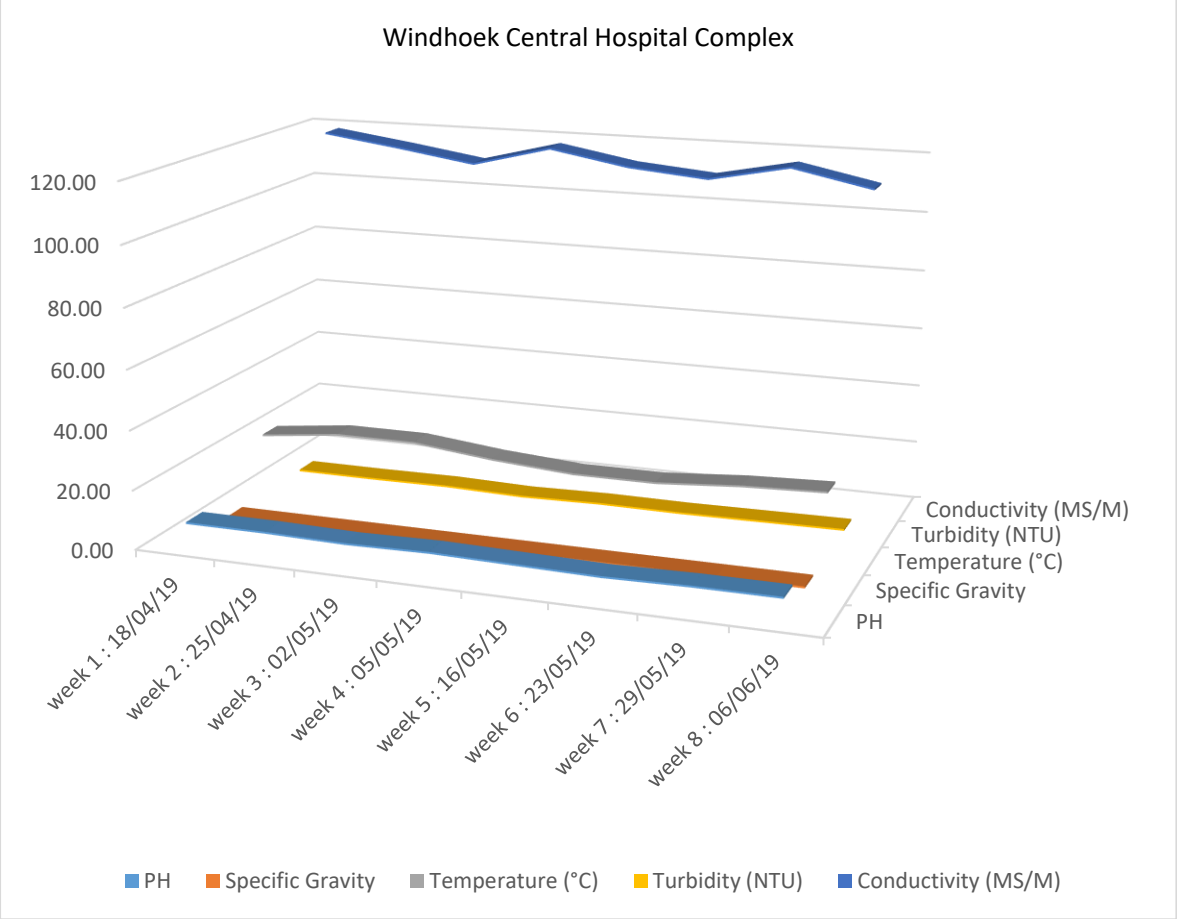

Figure 3.1: Physiochemical characterisation of Windhoek central water samples

The lowest pH recorded was 7.00 and the highest was 8.00 they were both within the acceptable range for water that is acceptable for human consumption. The turbidity reading for week 4 was 0.675 NTU and was the lowest throughout the sampling period. All results were within the acceptable limits.

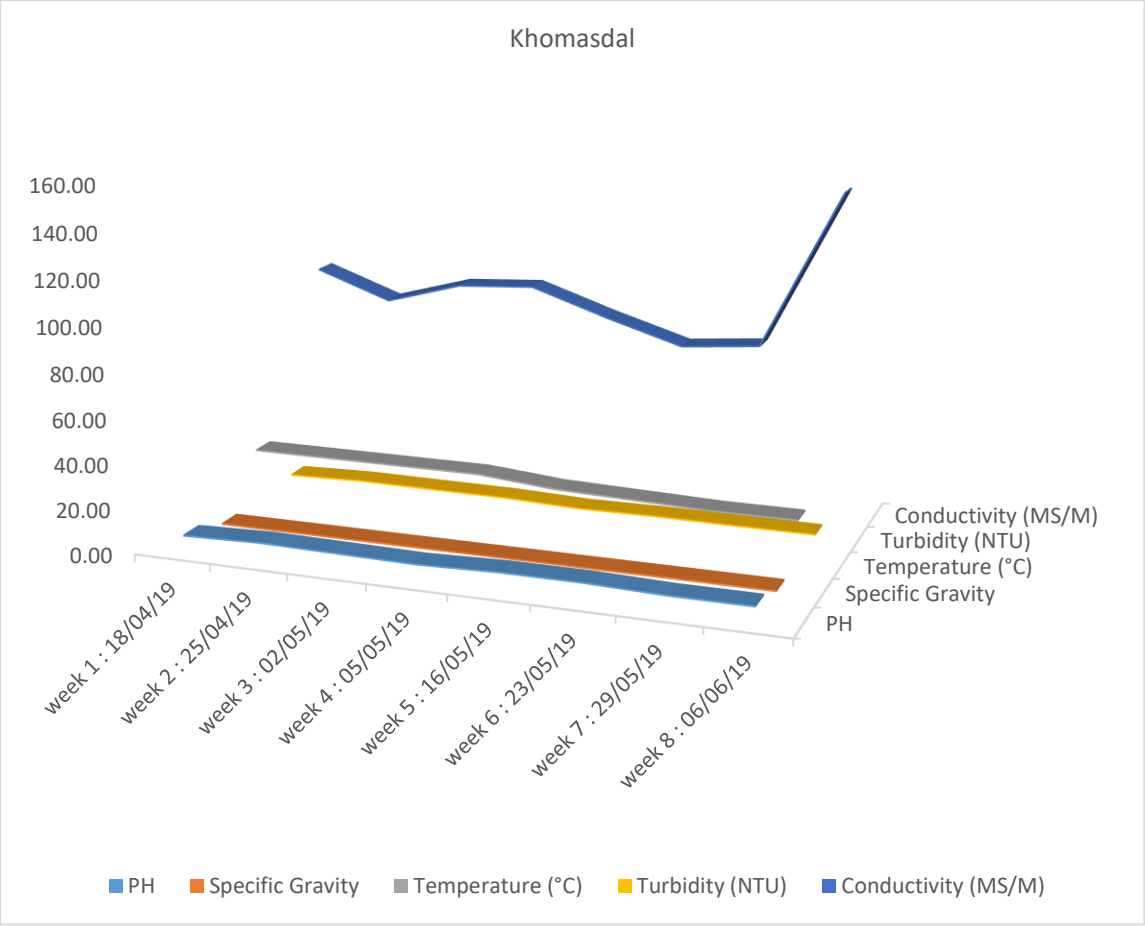

Figure 3.2: Physiochemical characterisation of Khomasdal water samples

The turbidity reading for this site were all above 2.000 NTU and the highest recorded was in week 2, which was 3.680 NTU. The conductivity for week 8 was the highest at 146.7 ms/m. This was close to the upper range that is for water that is acceptable for human consumption 150 ms/m. All results were within the acceptable limits.

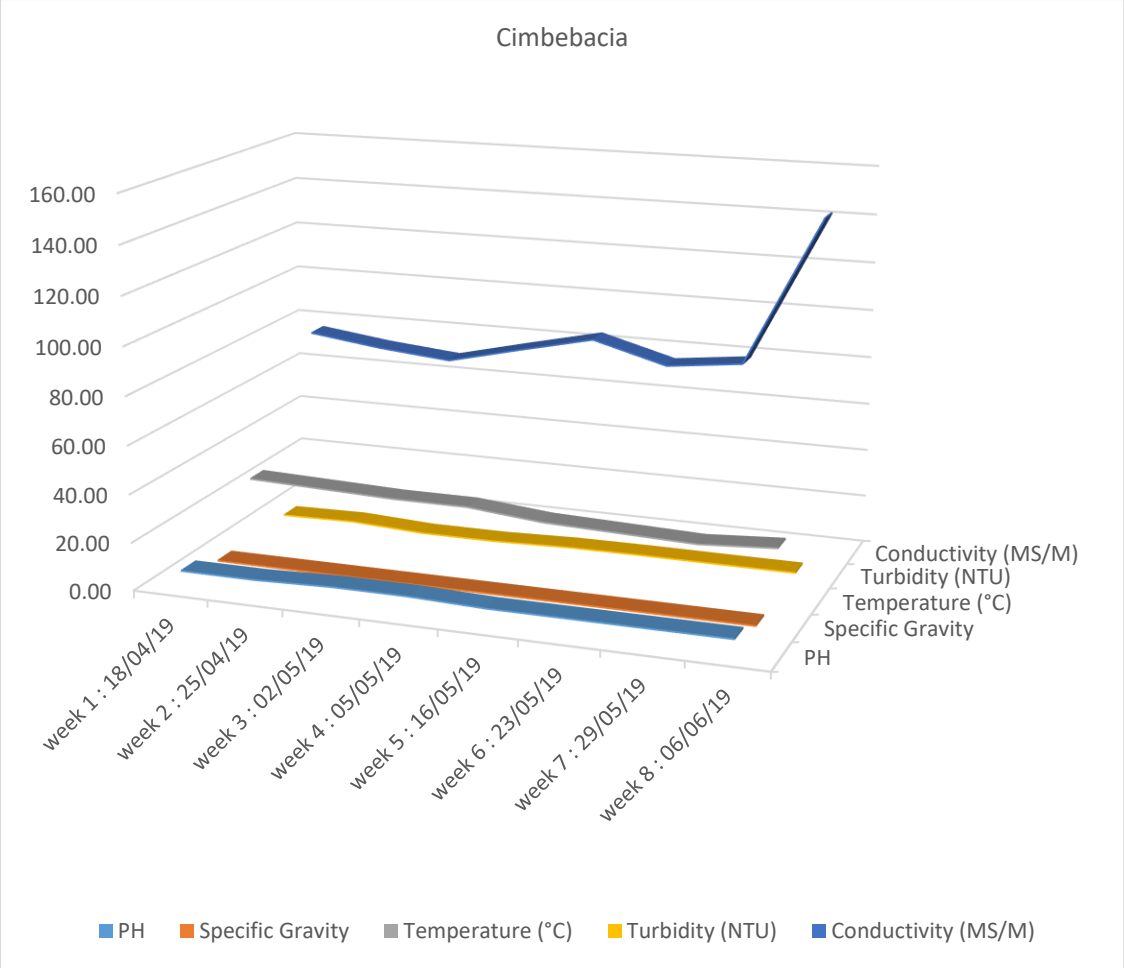

Figure 3.3: Physiochemical characterisation of Cimbebacia water samples

The highest recorded temperatures were 26 ^{o}C and 27 ^{o}C during week 1 and 2. The highest turbidity reading was 1.960 NTU whereas the highest conductivity was recorded during week 8 at 141.5 ms/m. All results were within the acceptable limits.

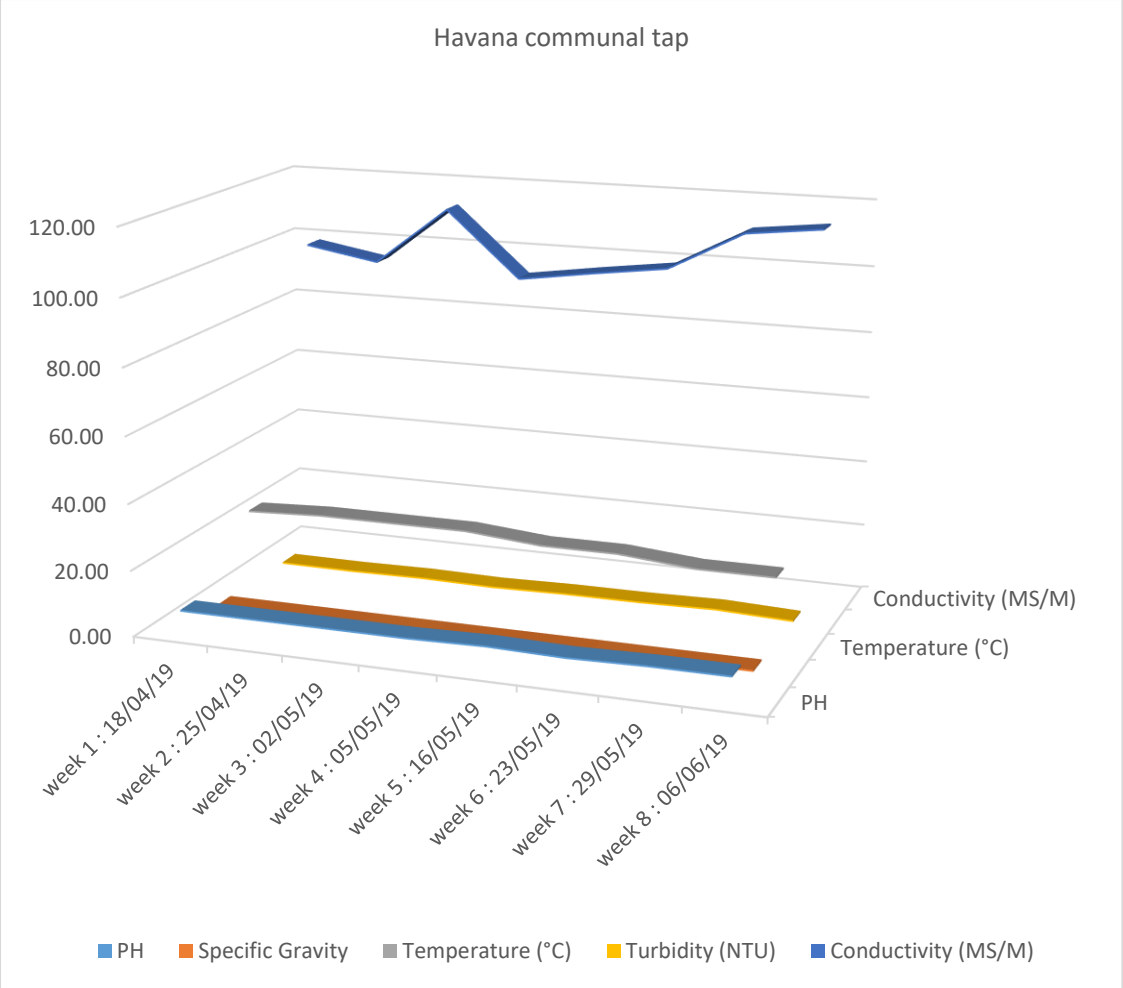

Figure 3.4: Physiochemical characterisation of Havana communal tap water samples

The pH readings were all >8.00 with the lowest being 7.00 for 3 weeks at the highest being 7.50 for 5 weeks. The specific gravity for all eight samples shows little variation with readings being 1.000 for 6 weeks and 1.005 for 2 weeks. Only week 7 recorded a turbidity reading of 1.250 NTU that is >1.000. All results were within acceptable limits.

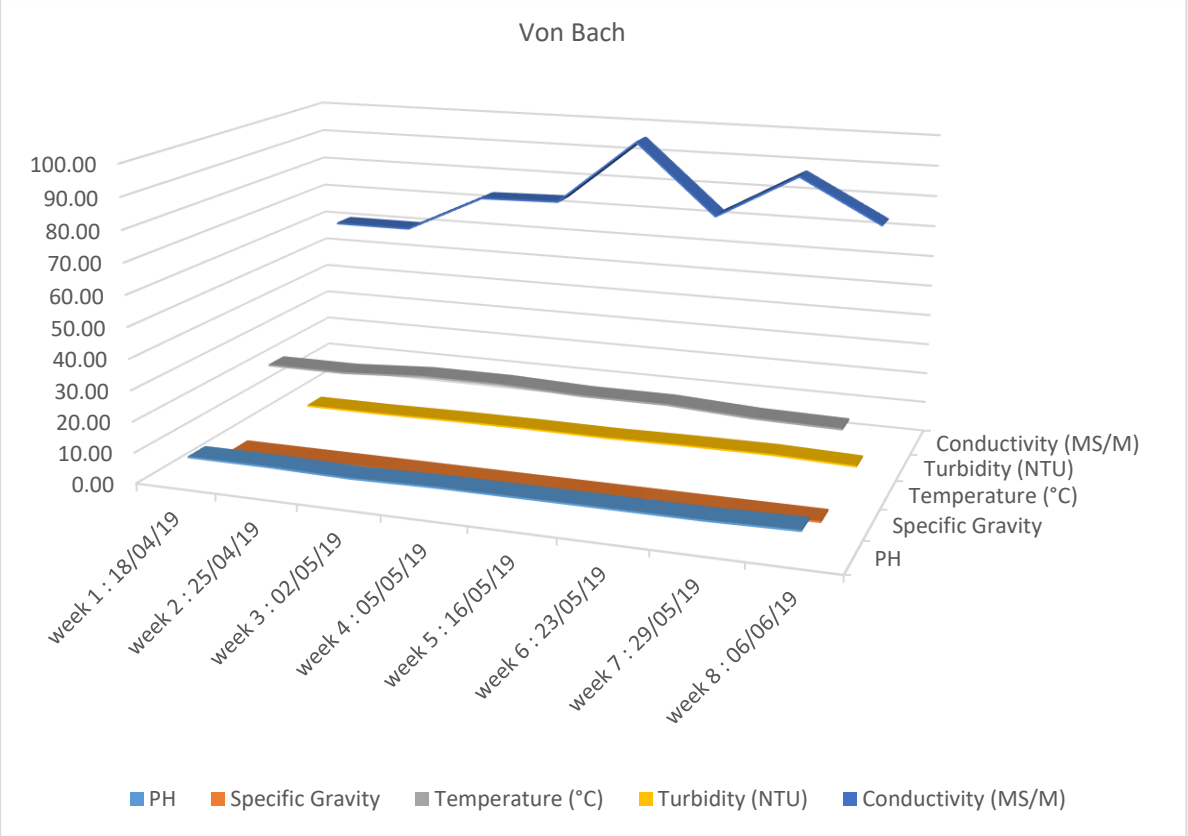

Figure 3.5: Physiochemical characterisation of Von Bach water samples

Turbidity results for weeks 6 and 7 were the only ones >1.000 NTU and all conductivity readings were <100 ms/m. All results were within acceptable limits.

3.2 Screening for microbes

Bacterial organisms were isolated a total of 37 times with *E.coli* being the most distributed bacterial organism amongst the sampling sites accounting for 38% and the least distributed was *E. faecium* with 30 %. This is illustrated in Table 3.1 below. The percentages presented in the table are calculated from the number of isolates per bacteria and the total number of isolates of all bacterial types.

Table 3.1: Frequency distribution of bacteria screened from water samples

Bacterial species	Frequency (n)	Percentage (%)
E. coli	14	38
E. faecalis	12	32
E. faecium	11	30
Cumulative total	37	100

Virial organisms were detected 50 times in the sampled water and the frequency of Rotavirus, Hepatitis A and hepatitis E was found to be the highest with a prevalence of 30 % with Adenovirus' frequency being 10 %. This is illustrated in Table 3.2 below. The percentages presented in the table are calculated from the number of isolates per virus and the total number of isolates of all virus types.

Table 3.2: Frequency distribution of viruses screened from water samples

Viral species	Frequency (n)	Percentage (%)
Adenovirus	5	10
Rotavirus	15	30
Hepatitis A	15	30
Hepatitis E	15	30
Cumulative total	50	100

Table 3.3 below illustrates the frequency distribution of bacteria screened from samples according to water sources. *E.coli* was the only bacterial organism isolated from Havana and Khomasdal sampling sites. Central hospital had *E.faecalis* as the most frequently distributed bacterial organisms with a distribution rate of 16.2 %. Cimbebacia had the highest frequency of *E. coli* with a distribution rate of 10.8 %. The percentages presented in the table are calculated from the number of isolates of bacteria per site and the total number of isolates of all bacteria types.

Table 3.3: Frequency distribution of bacteria screened from samples according to water sources

Water source	*E.coli*	*E. faecalis*	*E. faecium*	Negative	Total
			Frequency (%)		
Havana	2 (5.4)	0 (0)	0 (0)	35 (94.6)	37(100)
Cimbebacia	4 (10.8)	2 (5.4)	1 (2.7)	30 (81.1)	37 (100)
Khomasdal	2 (5.4)	0 (0)	0 (0)	35 (94.6)	37 (100)
Central hospital	1 (2.7)	6 (16.2)	5 (13.5)	25 (67.6)	37 (100)
Von Bach dam	1 (2.7)	4 (10.8)	5 (13.5)	27 (73.0)	37 (100)

Adenovirus was the least frequently distributed viral organism amongst the five sampling sites with 10 %. Whereas, Rotavirus, hepatitis A and hepatitis E were the most frequently distributed viruses accounting for 30 % each. The highest frequency of Rotavirus, hepatitis A and hepatitis E was found in Havana with 10% each and least distribution of the above-mentioned three viruses was noted in Khomasdal and Von Bach dam samples. The percentages presented in Table 3.4 below are calculated from the number of isolates of virus per site and the total number of isolates of all virus types.

Table 3.4: Frequency distribution of viruses screened from samples according to water sources

Water source	Adenovirus	Rotavirus	Hepatitis A	Hepatitis E	Negative	Total
			Frequency (%)			
Havana	1 (2)	5 (10)	5 (10)	5 (10)	34 (68)	50 (100)
Cimbebacia	1 (2)	3 (6)	3 (6)	3 (6)	40 (80)	50 (100)
Khomasdal	1 (2)	2 (4)	2 (4)	2 (4)	43 (86)	50 (100)
Central	0 (0)	3 (6)	3 (6)	3 (6)	41 (82)	50 (100)
Von Bach	2 (4)	2 (4)	2 (4)	2 (4)	42 (84)	50 (100)

Table 3.5 below illustrates that *E.coli* is the only bacterial organism that was isolated from samples collected in the morning. *E.faecalis* was isolated more frequently form samples collected in the afternoon than E. *faecium*. The percentages presented in the table below are calculated from the

number of isolates of bacteria per site per sampling site and the total number of isolates of bacteria per type per sampling site.

Table 3.5: Frequency distribution of bacteria according to sampling timeframe

Site	*E.coli*		*E. faecalis*		*E. faecium*	
	Frequency (%)					
	AM	**PM**	**AM**	**PM**	**AM**	**PM**
Havana	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Cimbebacia	2 (28.6)	2 (28.6)	0 (0)	2 (28.6)	0 (0)	1 (14.3)
Khomasdal	1 (50.0)	1 (50.0)	0 (0)	0 (0)	0 (0)	0 (0)
Central	0 (0)	1 (8.3)	0 (0)	6 (50.0)	0 (0)	5 (41.7)
Von Bach	0 (0)	1 (10.0)	0 (0)	4 (40.0)	0 (0)	5 (50.0)

Key: AM = sample collected in the morning between 08h00 hrs and 12h00 hrs. PM = sample collected in the afternoon between 13h00 hrs and 17h00 hrs.

Hepatitis A and hepatitis E viruses were not isolated from samples collected in the morning. Hepatitis A and hepatitis E were the most frequently distributed viruses from samples collected in the afternoon. This is illustrated in Table 3.6 below. The percentages presented are calculated from the number of isolates of virus per site per sampling site and the total number of isolates of virus per type per sampling site.

Table 3.6: Frequency distribution of viruses according to sampling timeframe

Site	Adenovirus		Rotavirus		Hepatitis A		Hepatitis E	
	Frequency (%)							
	AM	**PM**	**AM**	**PM**	**AM**	**PM**	**AM**	**PM**
Havana	0 (0)	1 (6.25)	0 (0)	5 (31.3)	0 (0)	5 (31.3)	0 (0)	5 (31.3)
Cimbebacia	1 (12.5)	0 (0)	1 (12.5)	2 (16.7)	0 (0)	3 (25.0)	0 (0)	3 (25.0)
Khomasdal	1 (14.3)	0 (0)	2 (28.6)	0 (0)	0 (0)	2 (28.6)	0 (0)	2 (28.6)
Central	0 (0)	0 (0)	0 (0)	3 (33.3)	0 (0)	3 (33.3)	0 (0)	3 (33.3)
Von Bach	0 (0)	2 (25.0)	0 (0)	2 (25.0)	0 (0)	2 (25.0)	0 (0)	2 (25.0)

Key: AM = sample collected in the morning between 08h00 hrs and 12h00 hrs. PM = sample collected in the afternoon between 13h00 hrs and 17h00 hrs.

CHAPTER 4

DISCUSSION AND CONCLUSION

4.1 Discussion

According to the WHO, the acceptable values for water that is still suitable for human consumption are as follows; pH between 5.5 and 9.5; Turbidity between 1 and 5 NTU; Conductivity between 150 and 300 ms/M at 25°C. Eighty litres of water was collected per site over the sampling period and this totals to 400 litres of water that was filtered.

According to this current study, physiological determinants were all found to be within the acceptable limits for samples from Windhoek central hospital complex (Figure 3.1). One notable observation was that the water temperature was 22°C over the sampling period but this is due to the winter season starting. The water from this site was also found to be considerably brown as compared to other sites. Purified water has a clear and colourless appearance. However, the brown appearance noted in water samples from this source could be due to pipe bursts that are allowing sand into the water or rust within the piping system. A study done by Wedgworth et al, in 2014 in the USA reported that the brown appearance of purified water could be due to contaminants such as rust and sediment. All physiological determinants for samples from Khomasdal were all within the acceptable limits (Figure 3.2). The turbidity for all samples was higher than that reported from samples collected in other sites. This is a cause for concern as it shows that there might be factors that are contributing to the high turbidity in the water such as old pipes or possibly pipes with cracks that are allowing sand or silt into the water might have contributed to the increase in turbidity (Huey and Meyer, 2010).

All physiological determinants for samples from Cimbebacia, Havana and Von Bach dam were all within the acceptable limits. It is worth noting that all sites had conductivity that was below 150 ms/M at 25°C. Havana and Von Bach had the majority of turbidity readings that were less than one NTU as recommended by WHO. When physiochemical properties of water are within limits it indicates that the water is suitable for human consumption. Although the physiochemical properties of water samples could be within the required limits, studies have shown that the water may not be suitable for human consumption due to bacterial, viral and other forms of contamination, which are not measured by physiochemical analysis. This has been reported in the WHO guidelines for drinking water and by studies done by Payment et al, in 2003.

E.coli was found to be the most prevalent bacteria in water samples with 38% of the samples recording positive screening results using molecular techniques followed by *E. Faecium*, which had a positivity rate of 32% as shown in Table 3.1. Furthermore, the Central area, which has blended water and the Von Bach dam, which is a source for surface water showed the highest number of bacterial contaminants with *E.faecalis* and *E.faecium* being the frequently isolated organism for both sources. The presence of bacteria in the water system may indicate that the testing methods employed by the City of Windhoek as well as the water treatment methods employed are not effective. The presence of these organisms may also lead to sporadic illnesses in individuals due to the consumption of contaminated water over a period of time. Furthermore, the absence of outbreaks does not guarantee the safety of drinking water, as some waterborne pathogens only tend to cause self-limiting diarrhoeal disease. The City of Windhoek uses bacterial count to assess the presence of possible bacterial contaminants in the water. This study used molecular techniques for assessing bacterial contamination in the water samples.

Similar studies by Edberg et al, in 2012 and Payment et al, in 2003 in (USA) reported that *E. coli* could be detected in water samples certified for human consumption using bacterial count methods. The presence of *E. coli* in water samples is an indicator of contamination by human waste and samples that test positive for E. coli are deemed not fit for human consumption. The testing of bacteria using molecular techniques has the following advantages: it is fast and has high sensitivity and specificity. It has the following disadvantages It is costly and can only be performed in countries that have the resources. A study done by Nurliyana et al, in 2018 in Malaysia reported that it is better to test water samples using bacterial count while a study by Maheux et al, in 2014 in Canada recommended testing using molecular techniques.

A similar study by Maheux et al, in 2011 in Canada reported that *Enterococci (Faecalis and Faecium)* could be detected in water samples certified for human consumption using bacterial count methods. The presence of these *Enterococci* in water samples is an indicator of contamination by both human and animal waste and samples that test positive for these *Enterococci* are deemed not fit for human consumption.

Rotavirus, Hepatitis A and Hepatitis E were found to be the most prevalent in the water samples, with 30% frequency distribution being found for each. Whereas, adenovirus was found to be the least frequently distributed virus with a distribution of 10 % amongst all the water samples as shown in Table 3.2. Similar studies by Lodder et al, in 2010 in the Netherlands and Wyn-Jones et al, in 2002 in the UK reported that Enteroviruses could be detected in water samples from different sources

certified for human consumption using molecular methods. The presence of these Adenovirus and Enteroviruses in water samples is an indicator of contamination by both human and animal waste and samples that test positive for these viruses are deemed not fit for human consumption. A study conducted by Osuolale and Okoh in 2015 in the Eastern Cape, also detected Adenoviruses but did not detect Hepatitis A in the final effluent of wastewater treatment, RT-PCR was also the method used for virus detection.

Table 3.3 illustrates the frequency distribution of bacteria screened from samples according to water sources. *E.coli* was the only bacterial organism isolated from Havana (Surface water) and Khomasdal (borehole and blended water) sampling sites. Central hospital (blended water) had E.faecalis as the most frequently distributed bacterial organisms with a distribution rate of 16.2 %. Cimbebacia (borehole water) had the highest frequency of *E. coli* with a distribution rate of 10.8 %. In 2017 in Nigeria, Obioma et al, conducted a similar study and fond that bacterial organisms (*E.coli and Enterococci*) were most isolated from surface water as is the case in this study. Sapkota et al, in 2007 in the USA also found the presence *E.coli* and *Enterococci* was higher in surface water than in ground water sources.

Adenovirus was the least frequently distributed viral organism amongst the five sampling sites with 10 %. Whereas, Rotavirus, hepatitis A and hepatitis E were the most frequently distributed viruses accounting for 30 % each as shown in Table 3.4. The highest frequency of Rotavirus, hepatitis A and hepatitis E was found in Havana with 10% each and least distribution of the above-mentioned three viruses was noted in Khomasdal and Von Bach dam samples. The same picture was seen in similar studies done by Lodder et al, in the Netherlands in 2010 as well as by Wyn-Jones et al, in the UK in 2002 as well as by Medema et al, in 2003 in the Netherlands as well.

Table 3.5 below illustrates that *E.coli* is the only bacterial organism that was isolated from samples collected in the morning. *E.faecalis* was isolated more frequently form samples collected in the afternoon than E. *faecium*. Richard et al, in 2004 in the US conducted a study on *E.coli* sampling reliability and found that E.coli densities were higher in samples collected in the morning than in the afternoon. This study's' results were similar to those of Richard et al, 2004.

Hepatitis A and hepatitis E viruses were not isolated from samples collected in the morning. Hepatitis A and hepatitis E were the most frequently distributed viruses from samples collected in the afternoon. Adenovirus and rotavirus were isolated from both the samples collected in the morning as well as those collected in the afternoon as shown in Table 3.6. Pinon and Vialette in 2018 in France stated that although virus survival in water is challenged by factors such as the increase in temperature, stronger sunlight, higher bacterial concentration, or the presence of disinfecting

substances, when the ability of viruses to form aggregates, to adhere to particles or surfaces, or to be internalized in living organisms is there, they are able to resist adverse environmental conditions. More similar studies stating the difference in virus distribution in relation to collection time could not be found.

4.2 Conclusion

This study aimed at determining the prevalence of rotaviruses, adenoviruses and hepatitis a in water samples collected from domestic water sources in Windhoek. *E. faecalis, E. Faecium*, Rotavirus, Hepatitis A and Hepatitis E being the most prevalent bacteria and viruses in water samples. Bacterial contamination was detected from all water sources using molecular techniques while viruses were most isolated from the informal settlement area of Havana where surface water is the source. There is a need to look into the sanitation with regards to the availability of toilet facilities in informal settlements as well as the close proximity of these facilities to drinking water taps. This will greatly reduce the risk of water contamination by contributing to the reduction in bacterial as well as viral organisms entering the drinking water system through faecal contamination. There was increased turbidity in some samples especially those collected from Khomasdal.

4.3 Recommendations

The City of Windhoek needs to start looking into the piping system to establish as to why the high turbidity (although still within the acceptable limits) to areas such as Khomasdal.

It would also be worth stating that there might be a need to look into different ways of eliminating the bacterial as well as viral organisms from water points as the current methods are leaving traces of bacteria and viruses in water.

Although the concentration of viruses was not established in this study, it is worth noting that the actual isolated organisms can lead to infections of the residents in the long run.

Further studies should be carried out to determine the concentration of these organisms as well as the virulence thereof.

The City of Windhoek is being applauded for being recognized as providing one of the best reclaimed water in the world.

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