BACTERIOLOGICAL QUALITY OF BOTTLED DRINKING WATER VERSUS MUNICIPALITY TAP WATER IN BULAWAYO, ZIMBABWE

By

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APPROVAL FORM

This is to certify that the dissertation entitled "Bacteriological quality of bottled drinking water versus municipality tap water in Bulawayo, Zimbabwe", submitted in partial fulfillment of the requirements for Bachelor of Science Honors Degree in applied Biosciences and Biotechnology at Midlands State University, is a record of the original research carried out by Unathi Mpofu R141139Q under my supervision and no part of the dissertation has been submitted for any other degree or diploma. The assistance and the help received during the course of this research have been duly acknowledged. Therefore, I recommend, that it be accepted as fulfilling the dissertation requirements.

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ABSTRACT

The most important characteristics of drinking water that have to be assured, monitored and conserved are its safety. Water safety is a growing concern as its quality is not thoroughly monitored especially in third world countries due to lack of proper labs and funding. Unsafe water contains a lot of microorganisms that are a threat to health, most of which contain faecal coliforms that cause serious illnesses like gastrointestinal diseases. Due to the present Zimbabwe water status, there has been an increase in the use of bottled water over municipality tap water. The purpose of this study was to assess the bacteriological quality of bottled water compared to municipality tap water in Bulawayo, Zimbabwe. Six bottled water brands and six samples of municipality tap water were randomly collected. These were subjected to the Total Plate counts, Total Coliform counts and faecal coliform counts. Eight different organisms were observed and these were subjected to gram staining and biochemical tests for identification to genus level. The most prevalent bacteria in municipality tap water included *Staphylococcus* spp (41,6%) and *E. coli* (41,6%). Other bacteria isolated from municipality tap water were *Citobacter* spp (8,3 %) and *Entrobacter* spp (8.3 %). However, the most prevalent bacteria in bottled water was *Staphylococcus* spp (58,3%) followed *E.coli (*20%). Other bacteria found in the bottled water were *Streptococcus* spp (6,6%), *Proteus* spp *(*6,6%) , *Entrobacter* spp (5%), and *Pseudomonas* spp (1,6%) and *Enterococcus* spp (1.6%). The bacterial count means for municipality tap water were lower than these of bottled water. The presence of *E. coli* in municipality tap water was probably due to water pipe bursts that had occurred recently in some of the areas. Chlorination had low bacterial counts as compared to the other types of water treatment. Most of the faecal coliforms isolated were found in bottled water and this might have been due to ineffective disinfection methods used and also due to the fact that some of the brands were not registered with the Standards Association of Zimbabwe (SAZ) to be selling their product to consumers. It was established that chlorination was the most effective water treatment method therefore making municipality tap water of Bulawayo safer than bottled water. Municipality tap water was able to meet the WHO and SAZ standards as compared to bottled water. This could be fixed by proper monitoring of the disinfection methods and making sure they are registered with SAZ in order to regulate these brands and make sure their products meet the set standards before being sold to the public.

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ACRONYMS AND ABBREVIATIONS

BCC – Bulawayo City Council

DOH – Department of Health

ESFA – European Food Security Authority

ESR – Environmental Science and Research

HPC – Heterotrophic plate count

IBWA – International Bottled Water Association

MR-VP – methyl red-voges proskauer

PET – polyethylene terephthalate

PVC – polyvinyl chloride

SAZ – Standards Association of Zimbabwe

spp – species

TCC – Total Coliform Count

TFCC – Total Faecal Coliform Count

THMs – trilalomethanes

TPC – Total Plate Count

UN – United Nations

UNICEF – United Nations Children's Fund

VP – voges proskauer

WHO – World Health Organisation

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Chapter 1

1.1 Background

1.1.1 Importance of Water

Water is a crucial natural resource in the world and life cannot occur without it (Gleick, 2002). It is needed for the upkeep of metabolic processes, for example, homeostasis. The body of a human being is composed of 60% water in male adults, 50% in female adults and lastly 70% in newly born babies (Svagzidiene *et al.*, 2010). The European Food Safety Authority (ESFA, 2010), states that the human dietary requirement for water should be approximately two liters a day for an average adult.

The most important characteristic of drinking water that has to be assured, monitored and conserved is its safety and quality to make sure it is safe for human use (Sadeghi *et al*., 2007). This means that drinking water should not contain pathogens, harmful metals, toxic substances and lastly undesirable organoleptic properties like odour, colour and taste.

Supplies of drinking water have a long history of being contaminated by a wide range of microbes including faecal coliforms (Sadeghi *et al*., 2007) thus the quality of drinking water is of great worry to mankind. Contaminated water can cause a wide range of diseases, from self-limiting gastrointestinal disturbances to severe life-threatening infections (Akoto, 2007). According to World Health Organization (WHO), 80 % of the diseases in developing countries including Zimbabwe are either water or sanitation related.

Microorganisms play a major role in water quality and the microorganisms that are concerned with water borne diseases are *Salmonella* spp., *Shigella* spp. and *Escherichia coli (*Adetunde and Glover, 2010). These microorganisms cause typhoid fever, diarrhoea, dysentery and gastroenteritis. It has been estimated that the mortality of water associated diseases exceeds

five million people per year around the world (Gleick, 2002). Diarrhoea is the world's second leading killer of children under the age of five after pneumonia, claiming about one and a half million children a year, more than AIDS and measles combined (Gleick, 2002). Mankind is at risk of these diseases, which if proper monitoring of all our sources of drinking water be it municipality tap water or bottled water is not done properly they can be affected by these diseases. Many studies have reported the presence of heterotrophic bacteria along with coliforms in bottled water in counts, exceeding national and international standards (Semerijian, 2011).

1.1.2 The growing industry of bottled water

Claim for safe and high quality drinking water by the world's growing population has dramatically increased (Herath *et al.*, 2012). This is clearly true for bottled water which has gained a lot of admiration over the years due to its assumed safety and quality. Bottled water is drinking water packaged in plastic or glass bottles and this can be well water, distilled water, mineral water or spring water. International studies reveal that customers choose bottled water because of the postulation of it being safer and of better quality than municipality tap water sources (Kassenga, 2007). The main consequence of this observation, is the increase in the consumption of processed and bottled water (Raj, 2007). Many uncredited suppliers are flocking this industry thereby causing a threat to the supply of safe water.

The World Health Organization reported that about 30,000 people and children die every day from water-related diseases (WHO, 2000). The Standards Association of Zimbabwe (SAZ) recently warned citizens on use of bottled water on the market with broken seals and do not have the SAZ stamp as this may be bottled water belonging to companies that are not registered with SAZ and might be selling water that is unsafe for drinking (SAZ, 2017). This might pose

a health risk as people continuously buy the water. It is thus necessary to assess the quality of drinking water to guarantee its safety for human drinking.

During 2008 when Zimbabwe faced economic challenges as a lot of companies shut down only to re-emerge after "dollarizing" the economy. These bottled water companies were greatly affected and some still struggle to operate on full capacity thus the quality of their products is highly questionable. The ministry of Health and Child Care of Zimbabwe (2015) stated that there are 67 registered local brands of bottled water. These were approved as the Ministry of Health and Child Care was satisfied with chemical and microbiological sample test results. The microbiological assessment of the bottled water plays an important role as the water must be monitored closely for indicator organisms in order to avoid the outbreak of water borne diseases.

1.1.3 Bulawayo municipal water supply

Municipal water systems are basic utility services delivered to the public by local governments in most countries. Many safety apprehensions have been raised, for example, the chemical treatment such as chlorination that is applied to the municipal tap water and the effect of pipe materials on the organoleptic quality of the waters are disliked by consumers (Ahmad *et al*., 2009). However the most imperative is the fact that they are growing worries on the human health effects of chlorination by products such as trihalomethanes (THMs) present in treated municipal drinking water (Ahmad and Bajahlan 2009). Studies have shown that these substances have carcinogenic potential to humans when they are exposed to high levels in drinking water.

On the other hand ineffectively chlorinated municipality water supply can lead to growth of disease causing microorganisms (Payment *et al.,* 1997). Taken together these two factors can

pose as a health risk, in the absenteeism of water safety management practices in the municipal water systems.

Increase in the number of brands of bottled water in Bulawayo is mostly due to water rationing by the local municipality. Upon reconnection after rationing the water has a muddy appearance thus people/ residents prefer bottled water (Ivanova, 2013).

1.1.4 Water quality monitoring and prevention of waterborne illnesses

Many health problems are posed by ingestion of contaminated drinking water. These include gastro-intestinal illnesses with vomiting, diarrhoea and nausea depending on the type of pathogen and health condition of a person (ESR, 2011). Worst case scenario are when symptoms heighten to bloody diarrhoea, sepsis, renal failure and even death, therefore national studies and regular monitoring of drinking water for the presence of pathogen and indicator organisms are needed. In Bulawayo the water quality is monitored every week by the Bulawayo city council. This is done at Criterion laboratory and Waterworks. The standards followed are their own laboratory's standards, WHO and SAZ.

In addition to regular monitoring and end product testing for microbiological hazard in drinking water, several researchers have emphasized the importance of employing good manufacturing practices and compulsory control points (Pant *et al.,* 2016: Moyo *et al.,* 2014). Studies further show that regular compliance with national and international standards by bottled water manufactures and water service providers require stricter regulatory obligation and increased monitoring by health authorities (Varga, 2011).

1.2 Problem Statement

Many water cuts occur in Bulawayo and the water is reconnected is appears muddy water. At times the municipality tap water has a strong smell and taste of chlorine. These have led

residents to prefer drinking bottled water over municipality tap water. They even choose bottled water with broken seals and those without the SAZ stamps, which might not have met the WHO and SAZ requirements. Consumers assume that bottled drinking water is safer than municipality tap water which is not always the case. Some of the bottled water companies do not even follow the recommended standards thereby posing as a health threat as compared to the municipality tap water, which is frequently treated and monitored (SAZ, 2017). Great measures are taken by the Bulawayo City Council to maintain water quality standards according to SAZ and WHO. Therefore there is need for the microbial diversity of the water to be known for the safety of the public.

With increase in sales and consumption of bottled drinking water there should be thorough monitoring of these products. Notably some vendors now reuse the water bottles and refill them with water from different sources like boreholes, well, tap water and sell it to unsuspecting people on street corners as a faster way of making money. This is a serious health threat because once the seal is broken microorganisms are introduced into the bottle, which poses as another health threat as microorganisms such as *E. coli* are introduced. This can lead to water borne diseases.

1.3 Justification

Bottled drinking water is often neglected as one of the least recognized health problems with many unauthorised companies flocking the market to sell their products. People continue to use and term municipality water unsafe. This may be due to lack of awareness because some bottled water companies are not monitored regularly to see if their product is fit for human consumption. It is acknowledged that the major threat to public health from drinking water is from microbiological contamination (WHO, 2008). WHO has reported that about 30, 000

people die every day from water-related diseases, more critically in Least Developed Countries (Pant *et al*., 2016).

It is important to ascertain the safety of drinking water because safe drinking water is fundamental to the protection of public health. Evaluation of water quality is of importance as it helps to achieve the United Nations Millennium Development Goal 7 of decreasing the proportion of people without sustainable access to safe drinking water. Access to clean safe drinking water is a declared human right (U.N, 2006).

Some of the sources of these bottled water are not purely clean and even as they undergo treatment their main aim is to remove minerals and chemicals present in the water but never the bacteria that is present in the water (www.freedrinkingwater.com). The water treatment process like reverse osmosis only target on reducing minerals not bacteria in the water.

The results of the study will serve as baseline information on bottled drinking water and municipality tap water in terms of some selected microbiological parameters. The data obtained may also assist in advising the citizens of Bulawayo regarding on bottled drinking water and municipality tap water.

1.4 Objectives

The main objective of the study was to assess the bacteriological quality of six brands of bottled drinking water and Bulawayo municipality drinking water.

The specific objectives of the study were:

1. to detect bacterial quality of bottled drinking water and municipality tap water using three methods (i.e. total plate count method (TPC), the total coliform count (TCC) and the total faecal coliforms count (TFCC).

2. to isolate and characterise bacteria found in bottled drinking water and municipality tap water using biochemical tests (i.e. gram staining, catalase test, oxidase test, motility test, Kliger's iron test, Lysine Iron test, Citrate Utilisation Test, Indole test, MR-VP test and sugar fermentations test).
3. to determine which water type is safe to drink according to the SAZ and WHO standards.
4. to determine which method of water treatment between reverse osmosis, filtration and chlorination is effective.
5. to assess compliance of the results obtained from both municipality tap water and bottled water with the standards of WHO and SAZ.

Chapter 2

Literature review

2.1 Types of drinking water

Water can be categorised into many different types though the most common way is based on the delivery and treatment method. Treated water is defined as drinking water that goes through various standard processing steps, including one or a combination of the following physical and chemical treatments: filtration, ozonation, reverse osmosis, distillation (Senior and Dege, 2005). Treated water includes many bottled brands and municipality tap water systems. Municipal drinking water systems usually apply chlorination to the water as the main treatment method. Untreated water however includes many natural mineral waters and spring waters (Seniour and Dege, 2005).

The treatment of drinking water is obligatory due to the presence of undesirable physical, chemical and microbiological constituents, which are harmful to public health. Technically, when none of these factors occur, there should be no need to apply any treatment.

Constant maintenance of the bacteriological safety of drinking water is challenging due to the possibility of pollution any time and inadequate monitoring (WHO, 2002). Maintenance of quality is especially difficult in highly developed areas where contamination from industrial and domestic areas is widespread. Some areas are naturally short of water supply and continuous pumping out of water can lead to intrusion of undesirable microbiological and chemical contaminants to the groundwater (McGlynn, 2011). Some of the major microbiological contaminates are strains of *E.coli*, *Salmonella*, *Shigella* and parasites such as *Crytosporidium parvum* and *Giardia lamblia* (Odonkor and Ampofo, 2013). Therefore necessary treatment methods are applied to the drinking water supply, specifically on municipal

water systems and bottled water brands, to ensure the removal or inactivation of harmful micro-organisms.

There are a number of treatment methods available to produce drinking water that is free from pathogens. These involve the application of sequential multiple barriers aimed at inactivating different kinds of pathogens (Edberg, 2005). The first stages of water treatment involve coagulation and flocculation. Here chemicals with a positive charge are added to the water. The positive charge of these chemicals neutralizes the negative charge of dirt and other dissolved particles in the water. When this occurs, the particles bind with the chemicals and form larger particles, called floc. This is followed by sedimentation and floc settles to the bottom of the water supply, due to its weight (http://www.bottledwater.org).

The next stage is disinfection and six methods are involved here**.** The most powerful disinfection treatments for water include: (1) filtration; (2) reverse osmosis (RO); (3) distillation; (4) ozonation; (5) chlorination and (6) UV radiation (Edberg, 2005). Table 1 shows the effectiveness of each water treatment types on different groups of pathogens.

Table 2.1 Effectiveness of water treatment types on pathogen groups.

	Treatment Effectiveness					
Pathogen Group	Filtration	Reverse Osmosis	Distillation	Ozonation	Chlorination	UV Radiation
Bacteria	Low	Good	High	Good	High	Good
Protozoa	High	High	High	Fair	Fair	Good
Viruses	Low	Good	High	Good	Good	Good

Source: Adapted from Edberg (2005), Percival *et al.*, (2000) and Senior and Dege (2005).

Filtration is one of the most commonly used treatment methods for drinking water. It employs filters, screens, and granular material or membranes to trap materials including

microorganisms. The size of the particles accumulating in the filter are usually between 0.001 and 100 µm in diameter, and the drop in water pressure is an important monitoring parameter to check the efficiency of the method (Seniour and Dege, 2005). This method however is mostly efficient for removal of protozoan parasites such as *Cryptosporidium* and *Giardia lamblia* (Edberg, 2005).

Reverse osmosis is another treatment method and is applied to alter the water's mineral content, but also results in the removal of pathogens. Reverse osmosis is similar to the membrane process, however reverse osmosis employs a controlled diffusion mechanism using pumps that deliver the required pressure and flow velocity across the membranes (Edberg, 2005). Proper maintenance of the membranes is one of the major challenges of this method because of its usual spiral, winding configuration, resulting in cleaning difficulty. Therefore in adequate cleaning can lead to a build-up of bacteria in the membranes that serve as intrusion points for contamination. Nonetheless, the implementation of the effective preventive maintenance and monitoring systems can help prevent these problems (Senior and Dege, 2005).

Distillation is a treatment method whereby water is boiled and the hot vapours produced are cooled, condensed and collected (APHA *et al*., 2012). This method usually results in sterile and pathogen free water. However once the water passes through tubes and pipes after leaving the distiller it may again be contaminated with microorganisms.

Ozonation is treatment of water with the chemical oxidant known as ozone. Ozone is a high energy, short acting, and powerful disinfecting agent (Varga, 2011). It is mostly employed in bottled water industry because of its strong oxidizing capacity that damages cell membranes of bacteria resulting in bacterial cell death whilst oxidising minerals especially dissolved manganese and iron present in water. Ozone is usually effective against viruses and bacteria, but not much on parasites.

Chlorination uses the chemical chlorine which is also an oxidant. It is effective in most economical water treatments plants and chlorination is used in the municipality of Bulawayo. Chlorine kills microorganisms in water. However it also produces undesirable by-products after reacting with natural organic contaminates in the water (Rosenfeldt *et al*., 2009). Excessive chlorine residuals, which are harmful to health, can also occur in water thus subsequent processes, such as the adsorption with activated carbon, are applied to neutralise or remove these contaminants. The disinfection potential of a disinfectant is related to its activity concentration and contact time with a pathogen. Compared with ozone, chlorine has a higher disinfection power because it is low energy and slow acting and not being easily dissipated characteristics. Thus chlorine produces a higher and effective disinfection residual throughout the municipal piped distribution system (Percival *et al*, 2000).

Ultraviolet Radiation is aimed at inactivating microorganisms. A microbicidal activity is achieved through the action of the radiation energy at around a 260nm wavelength on a microbial cell, causing the destruction of nucleic acids bases adenine and thymine and eventually resulting in bacterial cell death. The advantages of UV radiation in water is the absence of chemical by products after the process.

Combined application of more than one of these treatments can compensate for each methods limited disinfection capacity and improve the quality of the final water product (Montemayor *et al*., 2008). Moreover, the effectiveness of each treatment system or treatment combination is also influenced by the quality of the source water (Percival *et al.*, 2000). To ensure the quality and safety of bottled drinking water and municipal tap water the regular implementation of a multiple barrier system, including the protection of the water source, source monitoring, effective disinfection treatment methods and good sanitation and manufacturing programs must be adopted.

2.2 Municipal tap water and bottled water brand categories in the study area

In the residential and commercial urban areas the dominant source of drinking water supply usually originate from municipal taps provided by local water districts. The municipality drinking water system is defined as a basic public service utility normally administered by local government, semi government or government controlled authorities and companies (Francisco, 2014). On the other hand, bottled water is usually marketed by private manufacturers (Rodwan, 2011). In Bulawayo, while there is a growing apprehension about the reliability of municipality water supplies leading to the increased use of bottled water, strong efforts by local authorities are still needed to ensure the provision of safe and high quality drinking water to consumers (Husayan, 2013). It is required by the law of Zimbabwe that city authorities provide clean drinking water to their residents. With all this being done it is up to the consumer to make the final choice as to which drinking water type to use. The decision is however influenced by environmental and personal factors such as lifestyle, health risk information, personal values and economic considerations. The major reason for treatment is to disinfect the water to ensure it is free from pathogens hence, safe for human consumption.

Municipal tap water supplies are usually tapped from underground or surface water sources (Francisco, 2014). Most of the water in Bulawayo comes from dams and reservoirs were water collects during the rainy season. These sources should undergo disinfection treatment. This treated water is distributed in pipes utility networks and then this accessed through taps.

Bottled drinking water is often taken from groundwater sources similar to municipal tap waters. The major differences is treatment type and the fact that they are bottled unlike tap water. The categories of bottled water vary greatly between countries and are largely influenced by national and local regulations. Many characteristics are used commonly (1) type of water used, (2) source of water, (3) treatment type and (4) packaging formats (Seniour and Dege, 2005).

Based on water type, water can be carbonated or still. The water source can be grouped as natural mineral water, spring water and other waters (DOH, 2011). Bottled water can also be categorised based on the type of packaging material used (glass, plastic packaging). Glass packaging can be of returnable or non-returnable types. Likewise, plastic packaging for botted cab be made of polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyethylene and multi-layered PET and returnable polycarbonate bottles (Seniour and Dege, 2005). Whilst many differences occur when categorising bottled water, there could be similarities and differences in the naming and definition of different bottled water types among countries (Codex, 2001). Nevertheless, it is important to understand some of the most conventional types of bottled water as defined by IBWA (2011) and widely used in the bottled water industry to provide consumers with informed choice on which type to purchase.

Types of bottled water

(1) Mineral water: is natural water that contains less than 250 parts per million of total dissolved solids. The water is distinct from other types because of the constant level and proportions of minerals and trace elements at the point of emergence from the source and cannot be remineralised. Mineral water is similar to spring water in the fact that it contains minerals and other dissolved substances. Mineral water comes from natural, spring sources, and is typically bottled at the source (IBWA, 2011).

(2) Spring water: is extracted from an underground formation from which water flows naturally to the earth’s surface. This can be collected only at the spring or through a borehole tapping the underground formation feeding the spring. Spring water is probably one of the most recognized forms of bottled water available. When a manufacturer calls its water, "spring water", he or she is making the claim that the source of its water is from an underground formation where water naturally flows to the earth's surface (DOH, 2011). Not all bottled water should be considered spring

water. Spring water may also contain beneficial nutrients like calcium, potassium and fluoride, which also affect the taste.

(3) Purified water: is produced by deionization, reverse osmosis and distillation. Other suitable product names for bottled water treated by one of the above processes include "distilled water" if it is produced by distillation, "deionized water" if it is produced by deionization, or "reverse osmosis water" if the process used is reverse osmosis (Rodwan, 2011).

(4) Well water: is from a hole bored, drilled or constructed which tapes water from an aquifer or any source that penetrates the water table. The water table is a level in the ground below which all pore spaces are filled with water. These are then collected ad bottled (European Council, 1998).

(5) Artesian water: taps water from a confined aquifer in which the level stands at some height above the top of the aquifer which is under pressure from surrounding upper layers of rock or clay. When tapped, the pressure in the aquifer, commonly called artesian pressure, pushes the water above the level of the aquifer, sometimes to the surface. Other means may be used to help bring the water to the surface (DOH, 2011).

(6) Sparkling bottled water: after treatment and possible replacement of carbon dioxide contains the same amount of carbon dioxide that it contained as it emerged from the source and can be labelled as sparkling mineral water (IBWA, 2011). Sparkling water is often recognized as the main ingredient for sodas. Tonic water is a type of sparkling water, with an added ingredient of quinine, and is often used in mixed drinks. Sparkling water can have the same attributes as other types of water, like spring or mineral, depending on the water's source, and it is common to see various types of sparkling water such as sparkling spring water, sparkling drinking water or sparkling mineral water (Senior and Dege, 2005).

2.3 Water quality standards and regulations

The bacteriological quality of drinking water refers to the level of occurrence of microorganisms in the final product (WHO, 2012). It is furthermore defined by parameters which separate safe from unsafe water (Codex, 2001), based on measurements of indicator species of microorganisms present in a water sample. Due to issues relating to complexity, cost and timeliness of obtaining results, testing for specific pathogens is generally limited to validation, where monitoring is used to determine whether a treatment or other process is effective in removing target organisms. In many countries, the regulatory requirements for bottled and municipal waters are different because of the significant differences between the two types (municipality tap water and bottled water). In Zimbabwe these two are regulated by SAZ and WHO. Bottled water companies should ensure that they meet with the standards and are registered with SAZ. To the consumer this is shown by the SAZ stamp on the packaging. For municipality tap water this is checked once a month by comparing results obtained from a certain sampling round to check if both organisations have the same results (SAZ and Bulawayo City Council).

There are differences in each country's potable water regulation, hence, there is no single standard to categorise water across countries. To date there are many bodies at international and national level, each one dealing with different aspects of bottled water regulation.

The standards set by WHO and SAZ for both municipality tap water and bottled water are shown below (Table 2.2 and Table 2.3).

Table 2.2: Standards of WHO for municipality tap water and bottled water

PARAMETER	WHO standards
E. coli or thermotolerant coliforms	0/100ml
Total coliforms	0/100ml
Colony count at 37°C	0/100ml

Source: Adapted from WHO (2012).

Table 2.3: Standards of SAZ for municipality tap water and bottled water.

PARAMETER	SAZ standards
Total coliforms	0/100ml
Faecal coliforms	0/100ml
Faecal *Streptococcus*	0/100ml
Salmonella	0/100ml
Yeast and mould	0/100ml
Sulfide reducing clostridia	0/100ml
*Total plate counts should not exceed 10 cfu/1ml after bottling.	

Source: Adapted from SAZ (2015).

The SAZ and WHO standards should be followed strictly in order to ensure that safe water is provided to the community at large and avoid an outbreak of diseases.

2.4 Bacteriological quality of the drinking water

A positive in any bacteriological test result on water is always a potential health risk to consumers. The health risk posed by the presence of indicator organisms and pathogens in

drinking water samples is determined based on the number of bacteria present, the virulence of the bacterium and the condition of the host (Edberg and Allen, 2004). These virulent factors may be released by bacteria in water or inside the body of the host when the water is ingested. These can then damage the host system and the trigger a series of chain reaction such as, bloody diahorrea, kidney failure, paralysis, and toxic shock syndrome.

In bottled water the level of microflora can be altered because of the difference in the water's microenvironment compared with the original underground source. Studies have shown that placing water in bottles increases the surface area of the water environment compared to the water's interstitial underground source and disrupts the natural dynamics of metabolite and nutritional exchange between the bacterial cells and the "in-situ" environment (Zobell and Anderson, 1936).

The integrity of bottled water in private shops can be compromised by poor storage conditions, for instance, those in high relative humidity in non-air-conditioned or inadequately ventilated rooms. These conditions may result in water condensation, which can lead to the development of biofilms penetrating the internal surface of the bottle with inconsistent sealing systems after boiling (Zobell and Anderson, 1936).

Municipality tap water systems also contain naturally occurring microorganism overwhelmingly dominated by bacteria. The water is distributed in pipes and is usually inhabited by heterotrophic microorganisms. The pipe works are usually made up of metal material mostly iron and copper. Water is transported from the source (dam or reservoir) to the consumer and it is usually accompanied with microflora. More specifically the level of bacteria in municipality tap water and bottled water are not the same. The dominant bacteria types in municipal water systems include many acid-fast bacilli, Gram negatives and Gram positives (Geldreich, 1996).

Kumpel and Nelson (2014) carried out a study that showed that low water pressure in distribution pipes increases the levels of coliforms even in the presence of chlorine residuals. In contrast high pressure and chlorine residuals reduce the level of coliforms and no *E. coli* was detected. Possible explanations for these contaminations during low pressure flow are the occurrence of external intrusion of contaminants into the pipes, internal backflow, internal pipe wall particulate release and sloughing of bacteria from attached biofilms as a consequence of low flow. Problems with plumbing systems, ineffective disinfection, and low water pressure and flow interruption are factors that could significantly contribute to the proliferation of biofilms and the periphery of long pipes, making treatment systems ineffective and unsuitable.

Both water types must not contain harmful bacteria or pathogens and must be safe for human consumption regardless of the presence of microflora (Senior and Dege, 2005). Therefore microbiological testing are similar in both types.

Very occasionally, pathogen testing may be performed to verify that a specific treatment or process has been effective. However, microbial testing included as part of operational and verification monitoring is usually limited to that for indicator organisms, either to measure the effectiveness of control measures or as an index of faecal contamination. The concept of using indicator organisms as signals of faecal pollution is a well-established practice in the assessment of drinking-water quality (WHO, 1976).

2.4. 1 Total coliform bacteria

Total coliform bacteria includes a wide range of aerobic and anaerobic, Gram-negative, non-spore-forming bacilli capable of growing in the presence of relatively high concentrations of bile salts with the fermentation of lactose and production of acid or aldehyde within 24 hours at 35–37 °C. *Escherichia coli* and thermotolerant coliforms are a subset of the total coliform group that can ferment lactose at higher temperatures (Ashbolt, 2004). Traditionally, coliform

bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Klebsiella* and *Enterobacter*, but the group is more heterogeneous and includes a wider range of genera, such as *Serratia* and *Hafnia*. The total coliform group includes both faecal and environmental species.

Total coliforms organisms are organisms that can survive and grow in water. Hence, they are not useful as an index of faecal pathogens, but they can be used as an indicator of treatment effectiveness and to assess the cleanliness and integrity of distribution systems and the potential presence of biofilms. They are generally measured in 100 ml samples of water. A variety of relatively simple procedures are available based on the production of acid from lactose or the production of the enzyme b-galactosidase. The procedures include membrane filtration followed by incubation of the membranes on selective media at 35–37 °C and counting of colonies after 24 hours. The presence of total coliforms in distribution systems and stored water supplies can reveal regrowth and possible biofilm formation or contamination through ingress of foreign material, including soil or plants (Sueiro *et al*., 2001).

2.4.2 *Escherichia coli* and thermotolerant coliform bacteria

Total coliform bacteria that are able to ferment lactose at 44 – 45 °C are known as thermotolerant coliforms. In most waters, the predominant genus is *Escherichia*, but some types of *Citrobacter, Klebsiella* and *Enterobacter* are also thermotolerant. *Escherichia coli* can be differentiated from the other thermotolerant coliforms by the ability to produce indole from tryptophan or by the production of the enzyme β-glucuronidase (George *et al.*, 2001). *Escherichia coli* is present in very high numbers in human and animal faeces and is rarely found in the absence of faecal pollution, although there is some evidence for growth in tropical soils. *Escherichia coli* is considered the most suitable index of faecal contamination.

Escherichia coli (or, alternatively, thermotolerant coliforms) are generally measured in 100 ml samples of water. A variety of relatively simple procedures are available based on the production of acid and gas from lactose or the production of the enzyme β-glucuronidase. The procedures include membrane filtration followed by incubation of the membranes on selective media at 44 – 45 °C and counting of colonies after 24 hours.

2.4.3 Heterotrophic plate counts

Heterotrophic plate counts (HPC) measurement detects a wide spectrum of heterotrophic microorganisms, including bacteria and fungi, based on the ability of the organisms to grow on rich growth media, without inhibitory or selective agents, over a specified incubation period and at a defined temperature (Ashbolt, 2004). Heterotrophic plate counts test detects organisms sensitive to disinfection processes, such as coliform bacteria; organisms resistant to disinfection, such as spore formers; and organisms that rapidly proliferate in treated water in the absence of residual disinfectants. The actual organisms detected by HPC tests vary widely between locations and between consecutive samples. Some drinking-water treatment processes, such as coagulation and sedimentation, reduce the number of HPC organisms in water. However, the organisms proliferate in other treatment processes, such as biologically active carbon and sand filtration. Numbers of HPC organisms are reduced significantly by disinfection practices, such as chlorination, ozonation and UV light irradiation (Bartram *et al*., 2003).

2.4.4 Intestinal *Enterococci*

Intestinal enterococci are a subgroup of the larger group of organisms defined as faecal streptococci, comprising species of the genus *Streptococcus*. These bacteria are Gram-positive and relatively tolerant of sodium chloride and alkaline pH levels (Bartram *et al*., 2003). They are facultatively anaerobic and occur singly, in pairs or as short chains. The intestinal

enterococci group can be used as an index of faecal pollution. Most species do not multiply in water environments. The numbers of intestinal enterococci in human faeces are generally about an order of magnitude lower than those of *E. coli.* Important advantages of this group are that they tend to survive longer in water environments than *E. coli* (or thermotolerant coliforms).

Enterococci are detectable by simple, inexpensive cultural methods that require basic bacteriology laboratory facilities. Commonly used methods include membrane filtration with incubation of membranes on selective media and counting of colonies after incubation at 35–37 °C for 48 hours. The presence of intestinal enterococci provides evidence of recent faecal contamination, and detection should lead to consideration of further action, which could include further sampling and investigation of potential sources such as inadequate treatment or breaches in distribution system integrity (Junco *et al*., 2001).

2.5 Impact of water quality on public health

Many cases have been reported on the non-compliance with the microbiological requisites for drinking water during monitoring in some countries. For example, in New Zealand 6% of the population was supplied with drinking water that had failed the bacteriological quality criteria for the period July 2009 to June 2010 (ESR, 2011). Moreover a study in the republic of Yemen showed high levels of bacteria from faecal and non-faecal sources although it is believed that bottled water is of better quality (Dawson and Sartory, 2000). In Zimbabwe, an outbreak in 2008 was due to faecal contamination in water bodies and in adequate monitoring of these sources (WHO, 2008).

These events demonstrate the importance of good processing and maintenance programs in water treatment plants. It shows the need for stricter monitoring systems, control measure and regulations, to ensure good quality of drinking water (Herath *et al.*, 2012).

2.6 Environmental impacts associated with water supply to residential urban areas

Freshwater demand has increased worldwide (Yu *et al.*, 2014). Zimbabwe is a semi-arid country. Rain falls in one season from November to April, most rivers, especially in the drier parts of the country, are not perennial. Because mean annual rainfall is generally low in Zimbabwe, it is necessary for water received during the main rainy season to be stored for use during the dry season. An extensive network of dams has been constructed throughout the country. These range from small dams on commercial farms and in rural areas, to large dams for the purpose of supplying water to major cities and for irrigation. Current utilisation is only 22% of mean annual run-off (www.ema.com). However, if all this water were to be stored, the flow of international rivers such as Zambezi would be affected. Thus, there is a need to balance the amount of inter-country dam storage with the need to maintain certain minimum flow level in the international rivers (www.ema.com).

Besides surface water storage, Zimbabwe also relies on underground water. Numerous boreholes and wells have been drilled throughout the country. Small, shallow, low yielding wells and boreholes in communal areas supply villages with drinking water, especially during the dry season and dry years. Deeper, high yielding wells are used for irrigation on commercial farms (UNICEF, 2012).

One major issue affecting water quality is the pollution of various drinking water sources, such as the underground and surface water sources (Barell *et al.*, 2000). Intensive agriculture and the increased use of pesticides and fertilisers have significantly affected the water quality of surface water bodies such as rivers and streams. In addition, pollution coming from industrial manufacturing sectors is also affecting various ground water and surface bodies (Perk, 2006).

Poor drinking water has resulted from pollution and has caused various infectious water borne diseases (DOH, 2011). Hence efforts to improve and maintain drinking water quality have been

made through the various techniques (Besic *et al.*, 2011). Studies have shown that bottled water can have greater environmental impacts than municipality tap water (Bonto *et al.*, 2012). Due to the relatively intense use of resources, such as water, energy, extra chemicals, longer transport and more waste disposal these contribute to resource depletion, global warming, eutrophication, acidification and ozone depletion compared with conventional municipal treatment methods and delivery systems (Crettaz *et al.*, 1999).

Considering water treatment, boiled water employs more processes and techniques, including addition of chemicals to ensure quality of the product. The use of energy and material resources and the associated emissions have resulted in varying degrees of water pollution and other negative environmental impacts (Jungbluth, 2005). With regards to the delivery mechanisms, bottled water usually uses various packaging forms, most notably bottles made of PET, to ensure product safety, integrity, safety as well as consumer convenience. This involves the transportation for both raw and finished product, resulting in high energy use and gas emissions. Furthermore, when the water has been consumed, the packaging material become unwanted waste and ends up in landfills, repressing an additional environmental problem (Ferrier, 2001).

Chapter 3

Materials and Methods

3.1 Study Area

The study was performed in the residential and urban area of Bulawayo City (Figure 3.1). The residential area is defined in this case as where people stay and the urban area where businesses including industries are located. Bulawayo is the second largest city in Zimbabwe after the county's capital, Harare. It is located in southwest Zimbabwe with geographic coordinates of 20.1325° south and 28.6265° east and has a population of 653,337 as of 2012 census. The total land area of the city is 1,707 km² (www.bcc.org).

Bulawayo has a moderately high altitude, it experiences a subtropical type of weather despite lying within the tropics. The city experiences a typical annual rainfall of 594 mm. Most rain falls in the December to February period, while June to August is usually rainless. Being close to the Kalahari Desert, it is vulnerable to famines and rainfall differs abruptly from one year to another. This therefore compromises the supply of water. Thereby a trial is faced when providing safe drinking water. The urban zone area hosts the biggest commercial and trading facilities of the city. Along with growth in the city there is an increasing demand for infrastructure and basic commodities particularly drinking water.

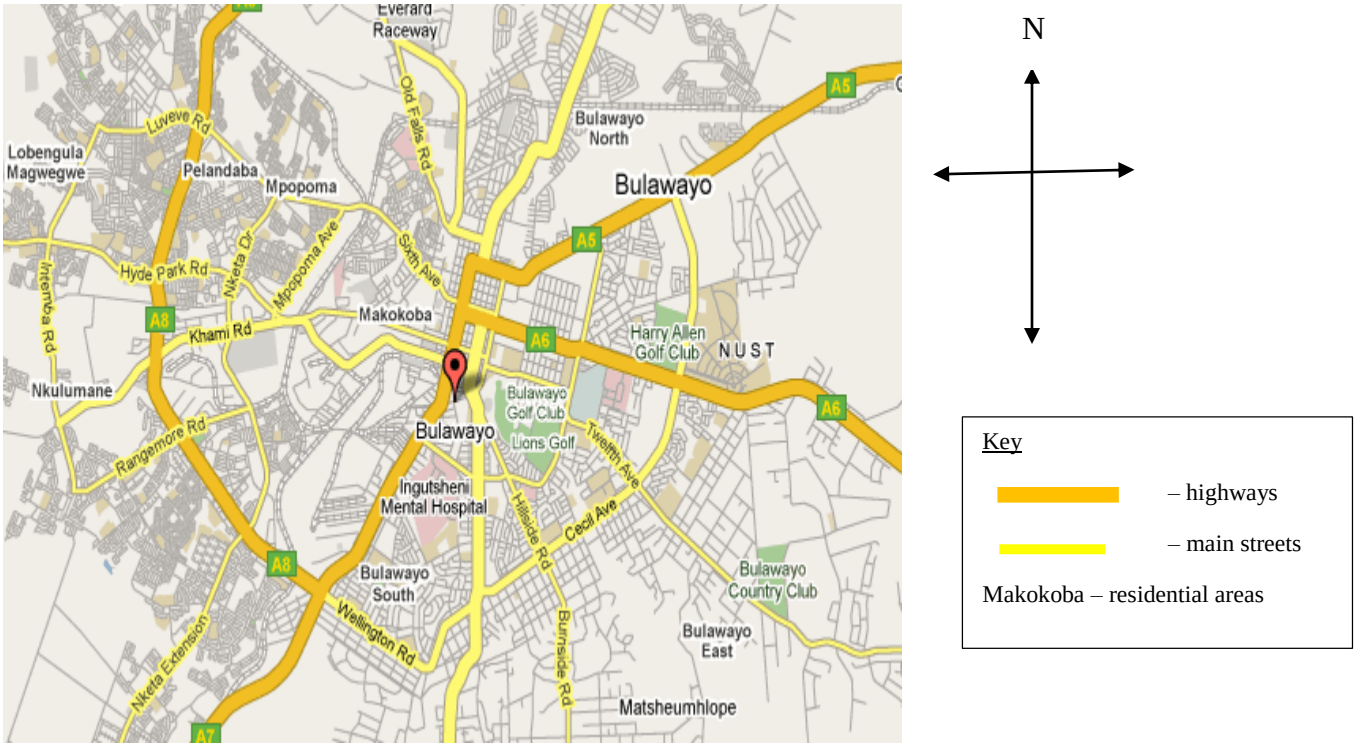

Fig 3.1: Map of Bulawayo area (www.googlemaps.com)

3.1.2 Water infrastructure

The water supply in the Bulawayo metropolitan undergoes various methods of treatment, transmission and distribution to consumers. The main dam that provides water to Bulawayo is Ncema dam. This dam then dispenses the water into three major reservoirs which are Criterion, Magwegwe and Tuli. These three reservoirs supply water to different parts of the city. Criterion supplies water to the low density suburbs, some parts of the medium density suburbs of Bulawayo, for example, Thorngrove and the industrial area of the city. Magwegwe reservoir supplies water to the high density suburbs, for example, Magwegwe and Ntumbane. Lastly the Tuli reservoir supplies water to the urban area of the city, and parts of the medium density suburbs, for example, Mahatshula (www.bcc.org).

3.1.3 Sampling

Bottled water and municipality tap water collected from the urban area, industrial and residential area were used. The names of brands, stores and areas of municipality water collection were not mentioned in order to protect involved parties. Only codes were used. For municipality tap water, labels M1 – M6 were used and for bottled water B1 – B6 were used. Six different brands of bottled drinking water were randomly collected in the central business district of Bulawayo. In order to account for variability due to batches produced each brand was replicated six times. These private shops were selected based on the investigators knowledge on location and study area, and according to the following selection criteria: (1) identification of all six pre-identified bottled water brands in these private shops during preliminary visit to the private shops (not all brands are present in all private shops) and (2) location within study area. The brands used in the study accounted for approximately 80% of the market share (SAZ, 2016) as sales suggested that.

For municipality tap water, a total of six samples was collected from six different homes that are supplied by the main reservoirs of Bulawayo city. These samples were replicated six times. The locations were randomly selected so that all areas in the city were covered. It is important to note that all these samples collected were replicated to reduce errors.

3.1.4 Sampling procedure

3.1.4.1 Municipality tap water

Water samples for microbiological examination were collected in stabilizable and non-reactive glass bottles with a dechlorinating agent (1 ml of 0.3 % sodium thiosulphate). Brown colored bottles were used to prevent the UV rays from penetrating into the water. The chlorine was present in order to deactivate the residual chlorine. The samples were collected in the morning

to ensure that they were taken to the lab and analysed as the time from sample collection to initiation of analysis should not be longer than 24 hours (Collins and Lyne, 1989).

Hands were washed prior to sampling. Taps used for sampling were free of aerators, strainers, hose attachments, mixing type faucets and purification devices. The sampling bottles used were at least 200ml to enable enough sample for the experiment. Leaking taps were avoided. The dirty taps were cleaned with sodium hypochlorite solution and water was allowed to run for an additional 2 to 3 minutes. Samples were collected from cold water taps. The taps were flashed using running water for 2 to 3 minutes. The tap was then closed. A gas burner was lit to the blue flame and was used on the mouth of the tap for 5 minutes to ensure that all the microorganisms were killed. The tap was then allowed to run again for a short time. The sampling bottle was also burnt at the opening to sterilize it and kill any bacteria that may have been left during handling. The lid of the sample container (150ml) was opened with one hand. While holding lid with one hand, the bottled was filled with the other hand. The bottle was held as far away from its neck as possible when the sample was collected. Care was taken so that the water would not come into contact with the hand since this will prevent contamination of the sample. Aseptic techniques were used. After the sample was collected, ample air space was left at the bottle (at least 2.5 cm) to facilitate mixing by shaking prior to analysis. The stopper was then secured and the bottle was labelled. Samples were placed on ice packs in a cooler box during transit to laboratory to maintain temperature below 10 ºC.

3.1.4.2 Bottled drinking water

Bottled drinking water was collected from refrigerators in the private shops and placed in cooler boxes on ice packs during transit to the lab.

3.2 Bacteriological analysis of water samples

All the media was prepared prior to the experiments following Manufacturer's instructions. All the media and apparatus used were autoclaved at 121 ºC at one atm for 15 minutes. All the tests were carried out aseptically in order to avoid contamination. The use of a control in every test was employed. Labelling was done by adding the date of the experiment, test done and name of student.

3.2.1 Total Plate Counts

The water samples including replicates were cultured on nutrient agar. An amount of one ml was inoculated using a pipette on nutrient agar using the spread plate method. These were then dried in a 44 ºC incubator. After drying they were then incubated at 37 ºC for 48 hours. The results were recorded.

3.2.2 Total Coliform Counts

A membrane filtration pump was used together with cellulose membrane filter papers of diameter 47mm (pore size 0.45mm) that were placed onto filter disks and filter cups over the membrane. Using a vacuum pump attached to a filter funnel (shown in Fig 3.2) an amount of 100 ml of the water sample was filtered through the membrane filter. The cellulose membrane paper was then removed using sterile forceps and placed on Membrane Filtration Agar (MFA). Samples were incubated for 48 hours at 37 ºC. Result were recorded. Coliform count is considered positive if the count is three and above.

Fig 3.2: Membrane Filtration Pump.

3.2.3 Total Faecal Coliform Counts

In this test, positive samples for Total Coliform Count Test after 48 hours were sub cultured in brilliant green broth and tryptone water for 24 hours at 41 ºC. Results were recorded. Positive is denoted by gas formation in Brilliant green and after addition of 3 – 4 drops of Kovac's reagent in Tryptone water a red ring at the top.

3.3 Macroscopy and microscopy

Macroscopy: Colony Morphology

Plates used in the Plate count method were used in this section. Bacteria that had grown on nutrient agar were used for further studies. Firstly colonies were observed to see their morphology in the plates. The following parameters were noted; form, size, elevation, margin/boarder, surface, opacity and colour.

3.4 Procedures for Biochemical tests

Catalase Test

The different isolated colonies were positioned on different slides and hydrogen peroxide (3%) was added. Bubble formation was observed for a positive result.

Oxidase Test

Newly prepared oxidase strips were used. These were placed on isolated colonies from the nutrient agar. A positive is noted by change to a blue color. Results were recorded.

Motility Test

Motility test was carried out by picking up an isolated colony using an inoculating loop and stabbing in the semi solid agar, straight down in the center. This was then incubated at 37 ºC for 24 hours with caps being loosely closed. Results were logged.

Kliger's Iron Test

Kliger Iron Agar was prepared slants were made. When carrying out test the isolated colonies were stabbed into the Kliger Iron agar to the base of the tube and this is considered as the butt. The same loop is streaked on the slant. This was then incubated at 37 ºC for 24 hours.

Lysine Iron Test

Lysine Iron Agar media was prepared and slants were made. An inoculum was placed aseptically in a sterile tube with the agar on the butt and slant. The inoculated tube were incubated at 37 ºC for 24 hours and the results were recorded. It was taken back into the incubator for another 24 hours and results were recorded.

Citrate Utilisation Test

The inoculum was streaked on Simmons Citrate agar using an inoculating loop. The plates were incubated at 37 ºC for 24 hours and the results were recorded.

Indole Test

Indole uses tryptone water and Kovac's reagent. In this test the inoculum was aseptically placed into the media tubes using an inoculating loop and incubated at 44 ºC for 24 hours. After this incubation period a few drops of Kovac's reagent was added. Positives were recorded

Sugar Fermentations Test

Three sugars were tested lactose, glucose and sucrose. These were prepared with sterilized bottles containing durham tubes. The inoculum was placed into the bottles using an inoculating loop and incubated at 37 ºC. Positives were noted by gas formation that collected in durham tubes and color change.

MR-VP Test

Each colony was incubated MR-VP both for 24 hours at 37 ºC. After 24 hours a one ml of this mixture was added to alpha naphthanol (VP pill plus 95% alcohol). A drop of KOH was added. Results were recorded after an hour positives were noted by a red ring. MR-VP broth was then taken back to the incubator and incubated for a further five days. After five days methyl red was added and results were recorded positives were denoted by a red ring at the top.

3.4 Data Analysis

Data collected of coliforms and faecal coliforms found in bottled water and municipality tap water subjected to One -Way Anova using a computer package SPSS version 2.1. This was to

establish which treatment method was better in the disinfection of both types of water. Three main methods were established (filtration, reverse osmosis) for bottled water and chlorination for municipality tap water. Since all data conformed to normality and homogeneity of variance the test was then carried out and results were obtained.

Chapter 4

RESULTS

4.1 BACTERIOLOGICAL RESULTS

4.1.1 Bottled Water

4.1.1.1 Bacterial counts for bottled drinking water

All the brands had bacterial growths for the Total Plate count, Total coliform test and the total faecal coliform test according to the WHO and SAZ standards. Brand 5 had high total bacterial count in all the test performed whereas Brand 4 had low bacterial counts in the tests performed including the lowest number for the total faecal coliform count test (Table 4.1).

Table 4.1: Bottled water bacterial counts (means)

		BACTERIAL COUNTS (means) (cfu/ml)	
Bottled Water brands	Plate Count	Total Coliform Count	Total Faecal Coliform Count
B1	66	20	5
B2	17	3	2
B3	16	4	2
B4	7	3	1
B5	101	89	8
B6	68	10	2
*SAZ standards	(0 – 300) is good	0	0
	More than 300 bad	0	0
*WHO standards	(0 – 300) is good	0	0
	More than 300 bad	0	0

B: Brands

4.1.1.2 Macroscopic and microscopic results for bottled drinking water

Seven colonies were isolated from bottled drinking water. Most colonies observed were circular and white in colour for the macroscopic results. Gram positive cocci and gram negative rods were observed for most colonies (Table 4.2).

Table 4.2: Macroscopic and microscopic results for bottled drinking water

Colony	Macroscopic	Microscopic
1	Circular, orange, convex, entire, smooth	+ cocci in pairs
2	Circular, white, flat, entire, smooth	- rod in strips
3	Irregular, white, flat, entire, waxy	+ rod in pairs
4	Circular, yellow, raised, entire, smooth	+ cocci in clumps
5	Irregular, white, craterform, lobate, rough	+ cocci in pairs
6	Green, flat, irregular, flat, entire, granular	- rod in pairs
7	Orange, irregular, waxy, flat, undulate	- rod in strips

+: gram positive, -: gram negative

4.1.1.3 Biochemical test results for the bottled water

Colonies isolated from bottled water underwent biochemical tests and the results below were obtained (Table 4.3).

Table 4.3: Biochemical test results for bottled water

Col	Mot	Kli	Lys	Suc	Lac	Glu	Sim	Ind	VP	Met	Cat	Oxi
1	+	-	+acid slunt, -butt	-	-	-	-	-	+	+	+	+
2	+	+ slunt	-	+	+	+	-	+	+	-	-	+
3	+	-	-	+	-	+	-	+	-	+	-	+
4	+	-	-	-	-	-	-	-	-	+	-	+
5	-	-	+ acid slunt, - butt	-	-	-	-	-	-	+	-	-
6	+	-	+	-	-	+	-	+	-	+	+	+
7	-	-	+acid slunt, -butt	-	-	+	+	-	-	+	-	+

Col: colony, Mot: motility, Kli: kliger, Suc: sucrose, Lac: lactose, Glu: glucose, Sim: simmon's citrate, Ind: indole, VP: voges proskauer, Met: methy red, Cat: catalase, Oxi: oxidase, +: positive, -: negative

4.1.2 Municipality Tap Water Results

4.1.2.1 Bacterial counts

Sample M1 had the high plate count whereas M4 had the low plate count (Table 4.4). Sample M2 had the highest coliform count of 5, whereas sample M4 and M6 did not have any coliforms (Table 4.4). Faecal coliform were present in samples M1, M2 and M3 whereas samples M4, M5 and M6 did not have any faecal coliforms.

Table 4.4: Municipality Tap Water bacterial counts (means)

		BACTERIAL COUNTS (means) (cfu/ml)	
Municipality Tap Water	Plate Count	Total Coliform Count	Total Faecal Coliform Count
M1	67	2	1
M2	6	5	1
M3	29	2	1
M4	2	0	0
M5	17	2	0
M6	6	0	0
*SAZ standards	(0 – 300) is good	0	0
	More than 300 bad	0	0
*WHO standards	(0 – 300) is good	0	0
	More than 300 bad	0	0

M: Municipality Tap Water

4.1.2.2 Macroscopic and microscopic results for municipality tap water

Four colonies were isolated from municipality tap water. Different morphologies were observed, for example, circular, white and flat. Most of the colonies were rod shaped (Table 4.5).

Table 4.5: Macroscopic and microscopic results

Colony	Macroscopic	Microscopic
1	Circular, orange, convex, entire, smooth	+ cocci in pairs
2	Circular, white, flat, entire, smooth	- rod in strips
3	Filamentous, irregular, white, craterform, filiform, rough	- rod in pairs
4	Irregular, white, flat, entire, waxy	+ rod in pairs

+: gram positive, -: gram negative

4.1.2.3 Biochemical test results for municipality tap water

Colonies isolated from municipality tap water underwent biochemical tests and the results below were obtained (Table 4.6).

Table 4.6: Biochemical test results for municipality tap water

Col	Mot	Kli	Ly	Suc	Lac	Glu	Sim	Ind	VP	Met	Cat	Oxi
1	+	-	+acid slunt, -butt	-	-	-	-	-	+	+	+	+
2	+	+slunt	-	+	+	+	-	+	+	-	-	+
3	+	-	-	-	+	+	-	-	+	-	-	+
4	+	-	-	+	-	+	-	+	-	+	-	+

Col: colony, Mot: motility, Kli: kliger, Suc: sucrose, Lac: lactose, Glu: glucose, Sim: simmon's citrate, Ind: indole, VP: voges proskauer, Met: methy red, Cat: catalase, Oxi: oxidase, +: positive, -: negative

4.2 Frequency of different bacteria in water

The frequency of the different bacteria in the samples of water is shown shown in Figure 4.1. The most prevalent bacteria in municipality tap water were *Staphylococcus* spp (41,6%) and *E. coli* (41,6%). Other bacteria isolated from municipality tap water were *Citobacter* spp, and *Entrobacter* spp (8.3 %) for both. However the most prevalent bacteria in bottled water was *Staphylococcus* spp (58,3%) followed *E.coli* (20%). Other bacteria found in the samples of the bottled water were *Streptococcus* spp (6,6%), *Proteus* spp *(*6,6%) , *Entrobacter* spp (5%), and *Pseudomonas* spp (1,6%) and lastly *Enterococcus* spp (1.6%).

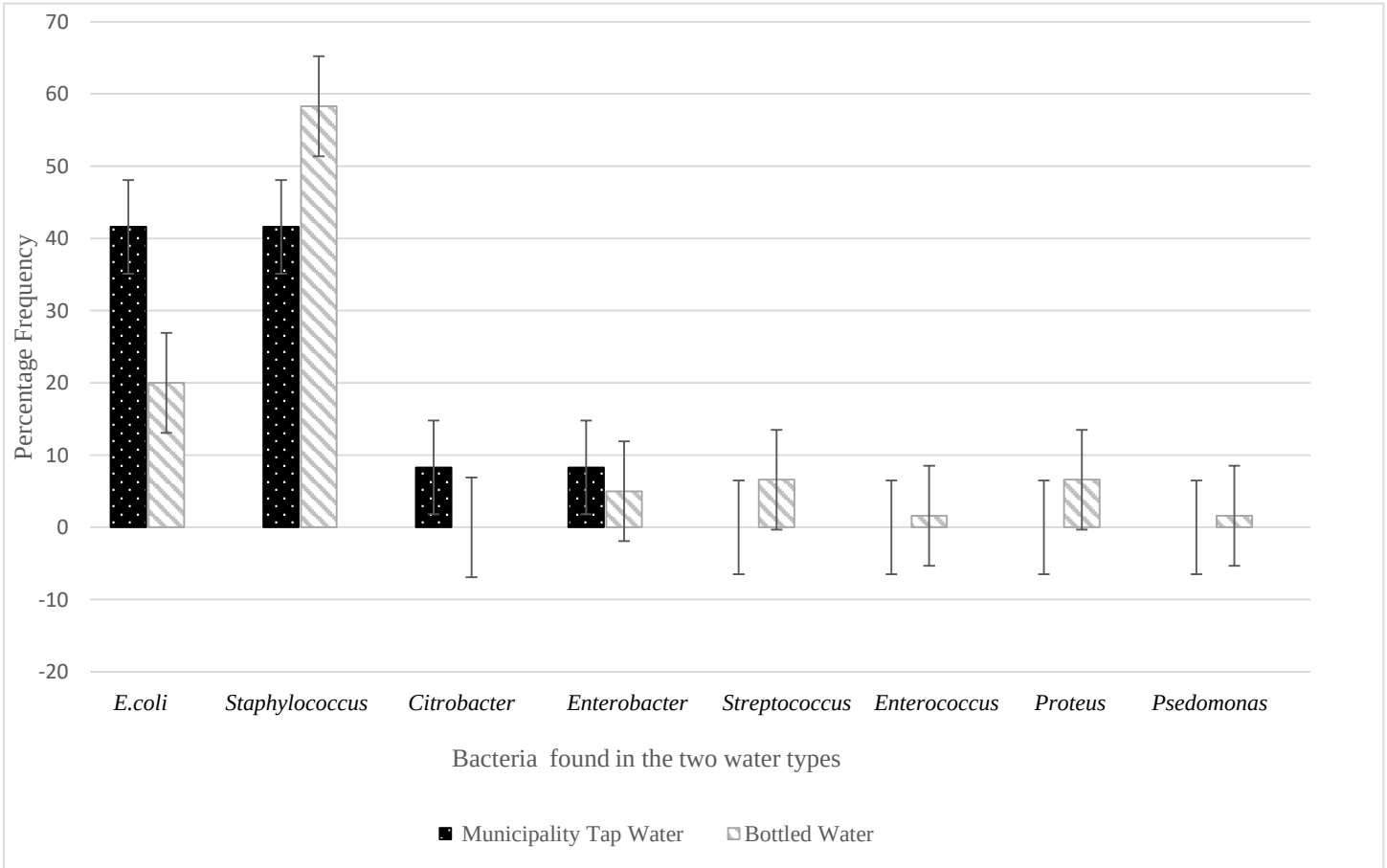

Fig 4.1: Percentage frequency of bacteria in the water

4.3 Bacterial comparison between municipality tap water and bottled water

The means for municipality tap water were lower than those of bottled water for all the three tests done (Figure 4.2). Under the plate count test the results for bottled water are around 45 and for municipality tap water around 25. For total coliform count bottled water had a mean close to 20 and municipality tap water has a mean close to zero and lastly for total faecal coliform count the differences for the two water types are close together although bottled water still has a higher mean (Figure 4.2).

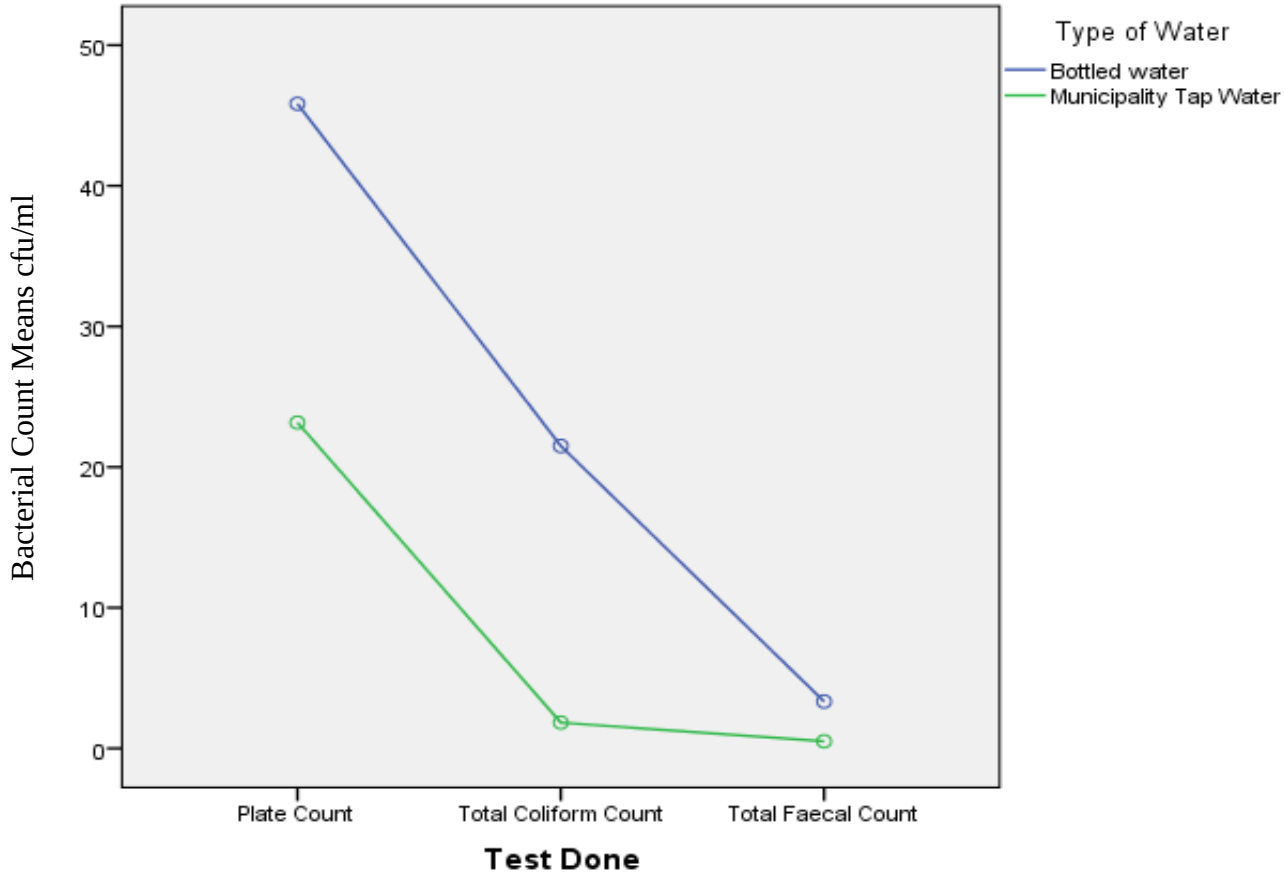

Fig 4.2: Bacterial contamination between the two water types (municipality tap water and bottled water).

4.4 Effective water treatment method used on the water types

Coliform Count

Three treatment methods (filtration, reverse osmosis and chlorination) were used in treatment of the two types of water (municipality tap water and bottled water). Chlorination had the lowest number of coliforms around 2, followed by filtration around 8 and lastly reverse osmosis around 17 (Figure 4.3).

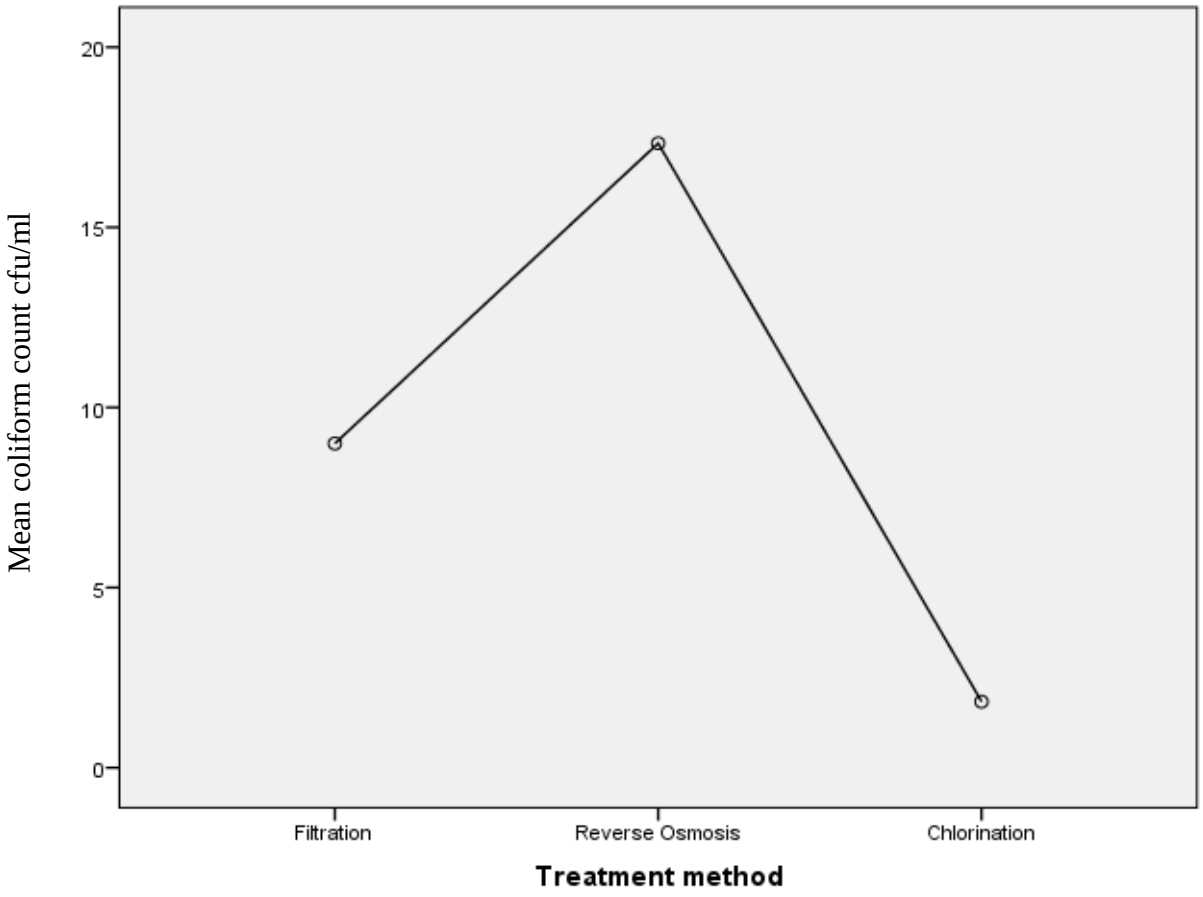

Fig 4.3: Bacterial quality (coliform count) between the three treatment methods.

Faecal Coliform Count

Three treatment methods (filtration, reverse osmosis and chlorination) were used in treatment of the two types of water (municipality tap water and bottled water). Chlorination had the lowest number of coliforms around 1, followed by filtration around 3 and lastly reverse osmosis around 3.5 (Figure 4.4).

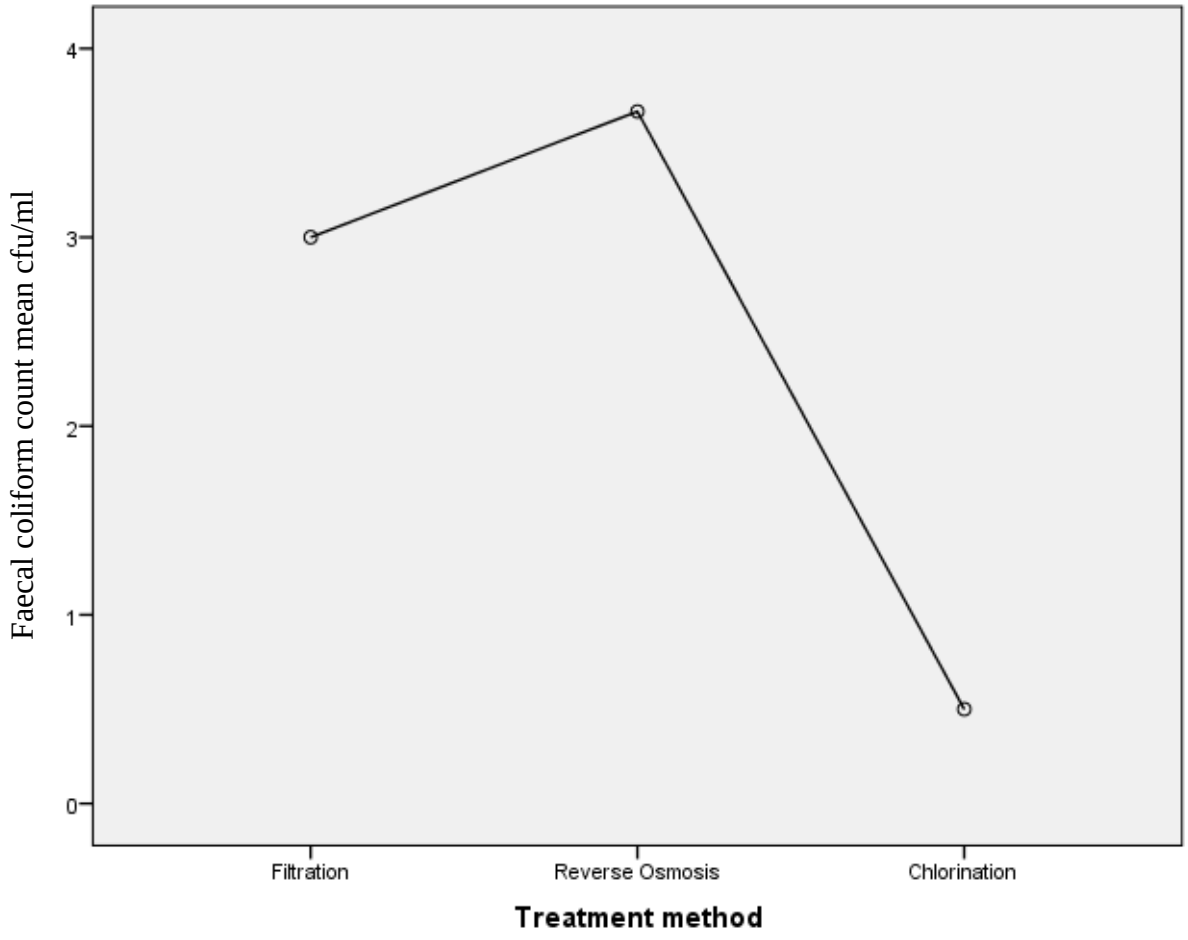

Fig 4.4: Bacterial quality (faecal coliform count) between the three treatment methods.

Chapter 5

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

In this study, the bacteriological quality of bottled and tap water was assessed in the urban area of Bulawayo. The main objective was to capture the variability at the point of sale. For the bottled water, the assumption was that the consumer would check the shelf life of the bottle. Therefore only bottles within their shelf life were sampled. The production batch number was not used as a blocking factor for the sampling of bottled water as this parameter is not likely to be considered at purchase by the average consumer. Moreover, each tap water is an independent production unit as there are no batches for taps.

Bacteriological Results

All the bottled water brands showed a positive result for all tests as compared to the standards of SAZ and WHO (Table 4.1). The positives for Total Coliform count were noted by of yellow colonies (lactose fermenters) with a count more than three (Collins and Lyne, 1989).

In view of its intended use as a safe drinking water type for human consumption, it was initially hypothesized that bottled water will have better bacteriological characteristics however as the results show municipality tap water of Bulawayo has a better quality as compared to bottled water (Fig 4.2). This showed that bottled water is more contaminated than municipality tap water. Municipality tap water has a lower number of coliforms mean (1) as compared to bottled water (20), (Fig 4.2) which causes a bit of concern as coliforms are the main cause of many gastro-intestinal diseases. However this hypothesis was derived from the fact that bottled water undergoes a lot of processing and treatment as compared to municipality tap water. However

production of under strict control programs including good manufacturing practises could improve the bacteriological quality of the finished product (Jagals and Jagals, 2004).

Figure 4.3 and 4.4 show that the most effective method in water disinfection is chlorination as compared to reverse osmosis and filtration used in some of the brands. Reverse osmosis has a higher number of both coliforms (17) and faecal coliforms (3) making it the least reliable method. Both figures show that chlorination is the best method in this case for water purification thereby making municipality tap of Bulawayo city safer to drink than bottled water.

The combined results of Plate count, TCC and TFC analysis indicated the presence of bacteria in both types of water. Differences were also noted in the type of contamination between the two water types. The contaminated bottled water brands was positive for Plate Counts, Coliforms and Faecal Coliforms, moreover municipality tap water has less positives as compared to bottled water (Fig 4.2) according to WHO and SAZ standards although it has a higher number of *Staphylococcus* spp (Figure 4.1). One explanation for the presence of less *Staphylococcus* spp in bottled water as compared to municipality tap water could be the effect of multiple barrier treatments applied by most bottled water manufactures, which included at least two of the following treatment methods: filtration, distillation, ozonation, ultraviolet radiation and ionization (Perk, 2006).

In the case of the municipality tap water samples where chlorination was the only treatment applied, significant reduction in disinfection residual at any point in the distribution loop could have resulted in regrowth of some of these species, for instance from a biofilm. Carter *et al.*, (2000) showed that bacterial levels in distribution pipes increases with distance from the treatment plant as a results of reduction in effectiveness of disinfection residuals. In the study carried out there are indication that growth could have happened due to backflow, low flow at a point in the reticulation system of the city (Kumpel and Nelson, 2014) because there were

pipe bursts in some of the areas the previous day before sampling. Increase in level of bacteria due to backflow or low flow have been reported in most drinking water distribution system in the world because water supplied to consumers relies on disinfection residuals to maintain the bacteriological quality of water in the pipes. This can be avoided by pumping water out at high pressure therefore meaning a low bacterial level will be detected. Figure 4.3 and 4.4 show that chlorination is the most effective method of treatment as number of coliforms and faecal coliforms where lower than those found in bottled water.

Underground sources of water contain naturally occurring microorganism that are considered harmless to human (Allen *et al.,* 2004). This is supported by a study done by Ducluzeau (1976) that showed the inability of heterotrophic bacteria found in mineral waters to colonize a human gastrointestinal tract. Therefore when the source of water can be guaranteed, these drinking waters can be bottled without treatment.

Bottled water, in this study, can be categorised as treated water based on the information present in the bottle labels. In contrast to untreated drinking water, treated water is expected to contain no or only low levels of microorganisms because of the disinfection treatments applied (Fig 4.3, Fig 4.4) shows that even though bottled water is treated it still contains a high number of coliforms and faecal coliforms making the water unsafe to drink. Bottled water did not conform to the standards set by WHO and SAZ for the number of total coliforms and faecal coliforms. Brand 5 had a high number of coliforms and faecal coliforms this may have been due to the fact that they did not contain a SAZ stamp meaning this water was being packaged without the necessary requirements and not meeting health standards. The water brands containing high counts may have originated from a poor quality of a water source or due to the effectiveness of the water treatments applied (Fig 4.3 and 4.4). Moreover, contamination during processing could have occurred due to inadequate sanitary facilities and procedures and improperly implemented quality control programs.

In municipality tap water (Fig 4.1) there are only four types of bacteria found being *Staphylococcus* spp, *E. coli*, *Citrobacter* and *Enterobacter*. Moreover in bottled water there is a greater variety of bacteria and most of them are bacteria found in faecal matter showing faecal contamination like *E .coli*, *Streptococcus* spp, *Enterococcus*, and *Pseudomonas* and some *Proteus* spp mostly found in soil were present. These are all indicators for faecal contamination meaning the methods used for disinfection were not effective. Brand 5 is the only brand of bottled water that had *Streptococcus* spp, which causes great concern as this bacteria causes gastro-intestinal infections. A study by Althaus (1982), also shows the presence of these types of bacteria in drinking water. *Staphylococcus* spp was also found in a study done by LeChevallier (1980) in underground water. Diseases that are caused by faecal coliform contamination are ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis and cases of Hepatitis (Fresno, 2008). Most faecal coliforms found are indicators of faecal contamination.

An interesting aspect of this study was the high diversity of the bottled water production batches found on the store shelve. This suggested lack of proper stock rotation, which could increase the risk of selling expired batches. Good commercial practice (GCP) such as the application of First-to-expire, First-Out (FEFO) policy require an effective rotation of the food item to prevent the storage of expired goods (Codex, 2009). One of the assumption of this study was that storage conditions could influence the results. Thus different stores and occasions were sampled. It is important to note that the bottled water sampled in this study were within its quality shelf life period, hence, were expected to contain low or no microbial count.

Another point to consider was the temperature of the bottled water sample on the shelves, which varied from store to store as some stores did not have proper air conditioning and properly working refrigerators. Some of the water was obtained from a refrigerator that was

malfunctioning and was not cold enough. The one obtained from properly working refrigerators in which the store had proper air conditioning had a low plate count.

Contaminated tap water samples were positive for *Staphylococcus* and *E. coli*. There was also the presence of *Citrobacter* and *Enterobacter* species. The presence of coliforms indicates unsanitary condition of the water at point of sampling. This could have been due to the bursts that had occurred the day before. The fact that some of the other taps that were collected from areas that did not have any bursts were negative of indicator organisms shows that the overall disinfection treatment applied on the municipality tap water is effective, but no treatment can completely eliminate the risk in the event of an interruption of the water supply. Compared to bottled water Coliform count was low which might have been due to the chlorination treatment's high disinfection residual maintained throughout the distribution network as suggested in numerous studies (Carter *et al.*, 2000).

Based on SAZ and WHO regulatory requirement, water showing results beyond standard limits is regarded as unsafe for humans. Drinking water that contains pathogens may not necessarily cause any illness to healthy individuals and the probability of an adverse health effect depend on the interaction of the organism with the immune system of the host (Payment, 1995). For instance, some bacteria which are not normally pathogenic, such a *Pseudomonas aeruginosa,* are capable of causing diseases in individuals that have suppressed immune systems. Nonetheless, many virulent pathogens such as *Shigella* spp*, Salmonella* spp, *Vibrio cholera* and *E. coli,* can cause serious waterborne diseases also in immunocompetent individuals (Soller *et al.,* 2010).

5.2 Conclusion and Recommendations

Despite the limitations encountered, important conclusions were drawn about the quality of the water. The results indicated that the bottled water industry is dominated by manufacturers that

apply water treatment and quality systems able to safeguard the consumer health. However brand 5 should be closely monitored by health authorities, as this product consistently failed to meet the set standards. There is also variability in batches in the bacteriological quality. The occurrence of different bacteria may cause a health risk as faecal coliforms were found. The bottled water companies should try and employ two or more treatment methods in order to effectively disinfect the water.

For tap water, most of the samples were bacteriologically negative indicating effective disinfection regime. However the occurance of *E. coli* was due to the bursts that had occurred the previous day. Other points of water collection had clean water meaning the city's disinfection system is good.

Limitations in this study were funds to buy a larger number of samples for bottled water and the media to use due to the country's current economical state.

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Appendices

Appendix 1: SPSS output

ANOVA

Coliform_Count

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	489.500	2	244.750	2.375	.149
Within Groups	927.500	9	103.056		
Total	1417.000	11			

Multiple Comparisons

Dependent Variable: Coliform_Count

Tukey HSD

(I) Treatment method	(J) Treatment method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Filtration	Reverse Osmosis	-8.333	8.289	.592	-31.48	14.81
	Chlorination	7.167	7.178	.596	-12.88	27.21
Reverse Osmosis	Filtration	8.333	8.289	.592	-14.81	31.48
	Chlorination	15.500	7.178	.132	-4.54	35.54
Chlorination	Filtration	-7.167	7.178	.596	-27.21	12.88
	Reverse Osmosis	-15.500	7.178	.132	-35.54	4.54

Coliform_Count

Tukey HSD[a,b]

Treatment method	N	Subset for alpha = 0.05
		1
Chlorination	6	1.83
Filtration	3	9.00
Reverse Osmosis	3	17.33
Sig.		.156

ANOVA

Faecal_Coliform_Count

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24.750	2	12.375	3.079	.096
Within Groups	36.167	9	4.019		
Total	60.917	11			

Multiple Comparisons

Dependent Variable: Faecal_Coliform_Count

Tukey HSD

(I) Treatment method	(J) Treatment method	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Filtration	Reverse Osmosis	-.667	1.637	.913	-5.24	3.90
	Chlorination	2.500	1.417	.236	-1.46	6.46
Reverse Osmosis	Filtration	.667	1.637	.913	-3.90	5.24
	Chlorination	3.167	1.417	.118	-.79	7.12
Chlorination	Filtration	-2.500	1.417	.236	-6.46	1.46
	Reverse Osmosis	-3.167	1.417	.118	-7.12	.79

Faecal_Coliform_Count

Tukey HSD[a,b]

Treatment method	N	Subset for alpha = 0.05
		1
Chlorination	6	.50
Filtration	3	3.00
Reverse Osmosis	3	3.67
Sig.		.141

Appendix 2: Gram Staining

In this test a slide containing the cell sample was to be stained. The sample was heat fixed to the slide by carefully passing the slide with a drop of distilled water and a small piece of sample on it through a Bunsen burner three times. The primary stain (crystal violet) was added to the heated fixed sample for one minute. This was rinsed gently with water for a maximum of five seconds to remove unbound crystal violet. Next Gram's iodine was added for a one minute. This was rinsed with alcohol (95%) for approximately three seconds. Safranin a secondary stain was added to the slide for one minute. This was washed gently with water for a maximum of five seconds. This was then viewed under the microscope under the oil immersion lenses.