KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI –GHANA

COLLEGE OF SCIENCE

FACULTY OF BIOSCIENCES

DEPARTMENT OF THEORITICAL AND APPLIED BIOLOGY

EFFECT OF VARYING STORAGE TEMPERATURES AND VENDOR HANDLING PRACTICES ON THE MICROBIOLOGICAL QUALITY OF STREET SOLD WATER IN KUMASI METROPOLIS, GHANA.

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A THESIS PRESENTED TO THE DEPARTMENT OF THEORITICAL AND APPLIED BIOLOGY, COLLEGE OF SCIENCE, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (ENVIRONMENTAL SCIENCE)

OCTOBER 2010

DECLARATION

I hereby declare that this work presented to the Department of Theoretical and Applied Biology in partial fulfillment for the award of MSc. Degree, is a true account of my own work except where particularly all sources of information have been acknowledged by means of references.

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DEDICATION

This work is dedicated to the Woods, and Thomas families, my beloved husband and children.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to God Almighty for the many blessings He has conferred upon me. Without His help this study would not have been possible. May the name of the Lord forever be praised, Amen.

I also wish to express my sincere and kind gratitude to my supervisor, Prof. K. Obiri-Danso, Dean of International Programmes, Kwame Nkrumah University of Science and Technology (KNUST) for his many valuable suggestions, time and encouragement to see this work through successfully. I feel very happy to have such a wonderful supervisor for this project.

To my parents, Mr. & Mrs. K. William Woods, Mr. & Mrs. O. Natty B. Davis and Mcfrances Mikki Ireland, I am very much grateful for the immense support, motivation, and sacrifices. I say thank you.

My heartfelt thanks and appreciation to my beloved husband, Mr. Alphonso T. Mansarray, who has been there for me from the very beginning. His physical, moral and spiritual supports have brought me this far. I praise God for having such a loving husband by my side.

Special thanks to Dr. John A. Larbi of the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology (KNUST) for his assistance during the course of this research.

Again special thanks to my colleague Emelda A. Adii and friend Maxwell Akplae for their vital contributions and love. My heartfelt thanks and appreciation to Mr. and Mrs. Lincoln Gankpala and family for their moral and spiritual support. They have been my guardian during my stay here in Ghana.

Last, but not the least, my sincere appreciation goes to my sponsor, and also the National Oil Company of Liberia for financially and morally supporting my studies in Ghana.

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ABSTRACT

The study assessed the effect of varying storage temperatures and handling practices on the microbiological quality of street sold plastic-bagged sachet drinking water by vendors in the Kumasi Metropolis. Ten different brands of factory-bagged sachet water samples (thirty pieces each), purchased from distributors and vendors were stored at refrigerator (4^{o}C), normal atmospheric (30^{o}C) and room (26.1^{o}C) temperatures over a six month period. Factory-bagged sachet water samples were also bought at random from vendors and the overall hygiene of the unopened bag assessed. Total coliforms, faecal coliforms, *Escherichia coli*, enterococci, and *Salmonella* numbers were determined as an index of quality. Bacterial indicator counts (geometric mean per 100ml) varied from 9.00 to $5.56x10^{2}$ for total coliforms, 4.00 to $3.92x10^{2}$ for faecal coliforms, 3.00 to $1.75x10^{1}$ for *E. coli*, 2.00 to $3.71x10^{2}$ for enterococci and 3.0 to 5.45 for *Salmonella*. The microbiological quality of most of the factory-bagged sachet drinking water tested deteriorates if stored at temperatures higher than refrigeration temperatures. Total coliform numbers in sachet water stored over the six month period increased by between 118-182% at normal atmospheric temperatures, 112-154% at room temperatures and decreased by 74% to 92% at refrigeration temperatures. Faecal coliform numbers followed the same trend; increasing by 128-193% at normal, 114-165% at room and decreasing by 79-82% at refrigeration temperatures. *Escherichia coli* increased by between 102 and 112% and decreased by 59- 93% at normal atmospheric, increased by 33-78% at room and decreased by -25-20% at refrigeration temperatures. Enterococci numbers increased by between 112- 180% at normal atmospheric, 104-147% at room and decreased by 35-96% at refrigeration temperatures. *Salmonella* decreased by between -28-47% at normal, room and refrigeration temperatures. Vendors handling of plastic-bagged sachet drinking water should be improved in order to avoid potential risk to human health. The numbers were sufficient to affect the WHO Guidelines Standard on drinking water quality and the Ghana EPA guidelines.

CHAPTER ONE

1.0 INTRODUCTION

The sale and consumption of plastic bagged drinking water has grown tremendously over the years in many developing countries, including Ghana. Plastic bagged drinking water has outnumbered the bottled water because of its easy accessibility and affordability; and therefore, its production and sale is economical compared to bottled water. The plastic bagged drinking water was introduced into the Ghanaian market as a less expensive means of accessing drinking water than bottled water. It also acts as an improvement over the former types of drinking water packaged for sale to consumers in hand-filled-hand-tied polythene bags or a plastic cup in a bucket of water with ice blocks. Today, the easy accessibility to drinking water in packaged forms has resulted in a big and flourishing water production enterprise with hundreds of million liters of these water products consumed every year by the populace (Ogundipe, 2008).

The standards of hygiene at the various stages of production of plastic bagged sachet water vary among various manufacturers. While some employ sophisticated techniques such as ozonization and reverse osmosis, most use ordinary boiling of well-water sources and exclusion of particles by use of unsterilized filtration materials. Several studies on the microbial quality of plastic bagged sachet water have reported violations of international quality standards (Obiri-Danso *et al.*, 2003; Bharath *et al*., 2003; Warburton *et al*., 1998). The high frequency of diseases such as diarrhea, typhoid fever, cholera and bacillary dysentery among the populace has been traced to the consumption of unsafe water and unhygienic drinking water production practices (Mead *et al.,* 1999). Water borne diseases continue to be one of the major health problems in developing nations especially on the issue of safe drinking water quality (Mead *et al.,* 1999). The number of outbreaks that have been reported throughout the world demonstrates that transmission of pathogens by drinking water remains a significant cause of illness. However, estimate of illness based solely on detected outbreaks is likely to underestimate the problem. This is so because a significant proportion of water-borne illnesses are likely to go undetected by the communicable diseases surveillance reporting systems. The symptoms of gastrointestinal illness (nausea, diarrhea, vomiting and abdominal pain) are usually mild and generally last a few days to a week and only a small percentage of those affected will visit a health facility (Dufour *et al*., 2003).

The potential of drinking water to transmit microbial pathogens to a vast majority of people and its consequent illness is well documented in many countries at all levels of economic development. Chlorination of drinking water has become a preferred option in ensuring its safety and reliability (Dufour *et al*., 2003; WHO, 2003). Therefore, the concern of drinking water safety to consumers, water suppliers, regulators and public health authorities is vital, especially in the Kumasi Metropolis.

The Kumasi Metropolis is the most popular and fastest growing metropolis in Ghana with a population of about 1.8 million (Ghana Statistical Services, 2009). In relation to its growing population, the production, sale and consumption of plastic bagged sachet drinking water have increased immensely over the years. The sale of the bulk of the plastic bagged sachet water within the metropolis is in the heart of its trading suburb, Kejetia, which is the busiest centre in central Kumasi.

The introduction of plastic bagged sachet water in Ghana was to provide safe, hygienic and affordable instant drinking water to the public and to curb the magnitude of water related infections in the country, but studies suggest that this innovative idea is not risk free (Obiri-Danso *et al*., 2003).

Varying storage devices and methods of plastic bagged water production along with unhygienic handling practices by those selling the product have compromised the microbial quality of the product. The line of distribution from public distributors using wired storage fences to street vendors that use unclean containers and improper handling by street vendors encourage the introduction of varying microbial populations onto the plastic bags.

Fundamentally, most of the persons involved in the sale of the product are from poor backgrounds and exhibit a low level of personal hygiene. Consequently, these vendors contaminate the plastic bags with their unclean hands. Most of the street vendors are children of school going age. Plastic bagged drinking water is consumed by the majority of the population, and hence there is a need to ascertain its quality in order to safeguard the health of consumers. Many common and widespread health risks have been found to be associated with drinking water in developing countries, a large percentage of which are of microbiological origin (Suthar *et al*., 2008).

Unsafe water, poor sanitation and hygiene have been reported to rank third among the 20 leading risk factors for health burden in developing countries, including Ghana (WHO, 2003). Contamination of water, either directly or indirectly, by human or animal activities is known to contribute to acquisition of disease by consumers. If the contamination is recent and those contributing to it include carriers of communicable enteric diseases, some of the micro-organisms responsible for causing these diseases may be present in the water, thereby questioning its safety. Drinking such contaminated water may lead to new cases of infection (Suthar *et al.*, 2008).

Ghana has many small and large scale industries that produce plastic bagged and bottled drinking water. The level of treatment given drinking water generally depends on the source of water and therefore, it would be expected that factory-filled water would be better treated than hand-filled hand tied drinking water. For water to be sold out to the public as "pure" drinking water, it is required under regulation that the water source should be further treated and clearance obtained from the Foods and Drugs Board of Ghana (Food and Drugs Administration, 2005), but this is not always the case.

Microbiological quality is the most important aspect of drinking water in relation to waterborne diseases. Detection of bacterial indicators in drinking water implies the presence of pathogenic organisms that are the source of waterborne diseases which could be fatal (Yassin *et al.*, 2006).

Lack of information on pathogenic parasitic organisms associated with drinking water in our markets creates some uncertainties in our understanding of the overall quality of drinking water. To clarify this, there is an urgent need for the determination of microorganisms associated with drinking water in our communities (Kwakye-Nuako *et al*., 2007).

1.1 RESEARCH QUESTIONS

1. What is the quality of plastic bag drinking water sold on the streets of Kumasi?

2. Could unhygienic handling practices by producers and vendors affect its microbial quality?

1.2 General Objective

The main objective of this study is to assess the microbial quality of Ghanaian plastic bagged drinking water and to ascertain the effect of poor handling practices by street vendors on the overall quality of sachet drinking water in Kumasi, Ghana.

1.2.1 Specific Objectives

1. To determine the extent to which varying temperature storage conditions alter the microbial quality of factory plastic bagged drinking water by the enumeration of microbial indicators (total coliforms, faecal coliforms, *Escherichia coli*, enterococci), and pathogenic salmonella.

2. To determine the numbers of microorganisms on the plastic bags through poor handling by the enumeration of microbial indicators and *salmonella*.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MICROBILOGICAL QUALITY OF DRINKING WATER

The determination of the microbiological quality of water is essential in testing for the overall quality of water, which often involves the enumeration of bacteria of faecal origin (Luksamijarulkul, 1994). The contamination of water with infected faecal material is common in areas with poor standards of hygiene and sanitation (Luksamijarulkul *et al.*, 1994).

Microbial contamination of drinking water also remains a concern in several regions of Europe. In Central and Eastern Europe and Western Asia, it is estimated that greater than 5% of all childhood deaths are attributable to diarrheal disease, which is often a result of poor-quality drinking water, inadequate sanitation, or improper personal hygiene (Valent *et al*. 2004).

Although there is concern over the microbial contamination in drinking water in some areas, the presence of metals in drinking water is also a significant health threat. Contaminants in surface and groundwater may come from anthropogenic sources, runoff from agricultural activities (Chapin *et al*. 2005), or controlled or uncontrolled discharges from sewage treatment facilities or leaking landfill sites (Kolpin *et al*. 2002).

Arsenic contamination of groundwater is a problem in many parts of the world. Specific to Central and Eastern Europe, elevated levels of arsenic in drinking water have been detected in Slovakia, Hungary, and Romania (Lindberg *et al*. 2006).

Good quality water is odorless, colorless, tasteless, and free from faecal pollution (Shilklomanov, 2000). Lamentably, a substantial portion of the population of the world, especially in Sub-Saharan Africa, is without water that fits this qualification. This means that a lot of people probably settle for unwholesome water, water that pose a serious health threat by way of water-borne infections. It is for this reason that the need for having potable water is considered a great public health issue.

In a bid to remedy the problem of unwholesome water consumption, plastic bagged drinking water production was introduced. This, however, has not meant that the problem has gone away. According to the Food and Drugs Board of Ghana, majority of plastic bagged drinking water are produced under questionable hygienic environmental conditions and they have had cause to impose a ban on some producers. Besides, some products do not bear the stamp of approval of the Food and Drugs Board. Even those who have registered do not always meet the standard required of them. Because of this, an investigation was undertaken to analyze some of these plastic bags drinking water samples in order to ascertain their potability (Shilklomanov, 2000).

Securing the microbial safety of drinking-water supplies is based on the use of multiple barriers, from catchment to consumer, to prevent the contamination of drinking-water or to reduce contamination to levels not injurious to health. Safety is increased if multiple barriers are in place, including protection of water resources, proper selection and operation of a series of treatment steps and management of distribution systems (piped or otherwise) to maintain and protect treated water quality. The preferred strategy is a management approach that places the primary emphasis on preventing or reducing the entry of pathogens into water sources and reducing reliance on treatment processes for removal of pathogens (WHO, 2004).

2.2 The Food and Drugs Board of Ghana and the Ghana Standards Board

The Ghana Standards Board (GSB) and the Food and Drugs Board of Ghana (FDB), established in 1965 and in 1992 respectively, are both responsible for ensuring that products being marketed in Ghana are of required quality. While the GSB generally develops and regulates standards for varying products that range from foods, drinks, and drugs to electrical and other engineered products, the FDB regulates and certifies only food, drinks, drugs, cosmetics, and other products which have health implications for the consuming public (GSB, 2004).

Both the FDB and the GSB regulate and certify sachet-water production and therefore there is some duplication of functions by the two authorities. However, while it is optional to have factory-produced sachet water registered with the GSB, it is mandatory to have the products approved and registered with the FDB. The main advantage of being registered by the GSB is to build product reputation.

2.3 Water Quality Testing Methodology for Sachet Water

The GSB (GSB, 1998) specify that the appropriate number of samples considered for water quality analysis, obtained for a lot that contains up to 1000 units of packaged water should at least be 15 units per lot.

2.4 WHO Drinking Water Guidelines

World Health Organization (WHO) guidelines are generally followed throughout the world for drinking water quality requirements. In addition to the WHO guidelines, each country or territory or water supply body can have their own guidelines in order for consumers to have access to safe drinking water.

The World Health Organization (WHO) Guidelines for Drinking Water (2004) describes the quality of drinking-water as a controlled process through a combination of protection of water sources, control of treatment processes and management of the distribution and handling of the water. Guidelines must be appropriate for national, regional and local circumstances, which require adaptation to environmental, social, economic and cultural circumstances and priority setting. WHO (2008) suggests that it may be useful to classify drinking water systems into categories that are predefined depending on the risks associated with the drinking water, the order of priorities placed, and the local circumstance, by using the percentage of samples tested negative for *E.coli* and also all water directly intended for drinking *E. coli* or thermotolerant coliform bacteria must not be detectable in any 100-ml sample (WHO, 2008).

2.4.1 Drinking Water Standards

The standard recommended by the Public Health Service specified the maximum permissible limits of bacteriological impurity (Zoeteman, 1980).

The water is to be clear, colourless, odourless and pleasant to taste. The water supply system should properly be operated under the supervision of qualified personnel.

2.4.2 Drinking Water Directive

The primary aim of the directive for drinking water quality is the protection of Public Health. Directives are intended to be used as a basis for the development of national standards that if properly implemented, will ensure the safety of drinking water supplies through the elimination or reduction to a minimum concentration of constituents of water that are known to be hazardous to health (Anon, 1993).

2.5 Production of Plastic Bagged Drinking Water

Tap water is collected into a reservoir and is treated with chlorine tablet. The water is then pumped into an overhead tank through four sets of filters with pore sizes of 5 microns each. The water descends or flows with force into four other sets of filters, two with pore size of 1 micron and the other two with pore size of 0.5 micron. The water then passes through carbon into a stainless steel ultra violet machine before finally passing through a packaging machine where it is automatically packed into sachets (500ml). In built in the machine is an ultra violet light that casts on the roll of the rubber for packaging. The bags used for packaging factory produced plastic bag drinking water are made of high-density polyethylene (HDPE), which is very strong and has higher tensile strength difficult to elongate, and can withstand higher temperatures (Polyprint, 2007).

2.6 Water Treatment Requirements

The water treatment requirements in sachet water production are filtration and UV disinfection. At least five filters and one UV disinfection unit are required for each sachet machine. The filter cartridges are required to be changed at least once every three months according to a short interview with one of the Food and Drugs Board staff.

Ultraviolet light is very effective at inactivating cysts, as long as the water has a low level of colour so the UV can pass through without being absorbed. The main disadvantage to the use of UV radiation is that, like ozone treatment, it leaves no residual disinfectant in the water. Because neither ozone nor UV radiation leaves a residual disinfectant in the water, it is sometimes necessary to add a residual disinfectant after they are used. This is often done through the addition of chloramines which is a primary disinfectant. When used in this manner, chloramines provide an effective residual disinfectant with very little of the negative aspects of chlorination (http://en.wikipedia.org/wiki/Water_purification).

Membrane filters are widely used for filtering drinking water. Membrane filters can remove virtually all particles larger than 0.2 um—including G*iardia* and *Cryptosporidium.* Membrane filters are an effective form of tertiary treatment when it is desired to reuse the water for industry, for limited domestic purposes, or before discharging the water into a river that is used by towns further downstream. They are widely used in industry, particularly for beverage preparation (including bottled water) (http://en.wikipedia.org/wiki/Water_purification).

2.7 Poverty, Child Labor and Hygienic Handling of Plastic Bagged Drinking Water

Poverty and the absence of jobs in Ghana have increased the number of "street children" in the urban cities who have to care for themselves. Child labor is a major problem that is affecting our society and the world today. Child labor can be termed as children below the working age being forced or compelled due to circumstances to work at the expense of enjoying basic privileges such as education, good health and protection. Poverty and over population have been identified as the two main causes of child labor. Parents are forced to send little children into hazardous jobs for reasons of survival, even when they know it is wrong. Child labor in present times has increased tremendously in the developing countries including Ghana. In the Ghanaian society a lot of factors contributes to the problem of child labor with the major one being poverty (http://www.iearn.org.au/clp/archive/write82.htm). Children between the ages of 12 years and even younger than that are on the streets of Kumasi selling plastic bagged drinking water which is now called “pure” water. In Amakom and Oforikrom, suburbs within the Kumasi metropolis, children below the working age are often seen selling to support their parents. This is because of irresponsible parenthood and poverty. High rates of illiteracy and lack of hygiene education have had some negative impact on the hygiene practices of the people in Africa. The poor hygienic practices by children can lead to plastic bagged drinking water contamination.

2.8 Unhygienic Handling of Plastic Bagged Drinking Water

In Ghana most consumers get water supply from sources other than the Ghana Water Company Limited (GWCL) via their taps because only 40% of the total urban population is directly covered by the GWCL’s networks (Anon, 2006).

Investigation conducted on the safety of drinking water in Ghana has shown that bottled water on the Ghanaian market is of good microbiological quality while the quality of some factory bagged drinking water and hand-filled/hand-tied polythene-bagged drinking water was noted to be doubtful (Obiri-Danso *et al*., 2003). This observation was based on studies carried out on water samples to ascertain the presence of heterotrophic bacteria, indicators of faecal contamination (total coliforms, faecal coliforms and enterococci) and for lead, manganese and iron. Lack of information on pathogenic parasitic organisms associated with drinking water on our markets creates some uncertainties in our understanding of the overall quality of drinking water on our markets. To clarify this, there is an urgent need for the determination of protozoan and helminthes organisms associated with drinking water in our communities (Kwakye-Nuako *et al*., 2007).

The presence of faecal indicators, *P. aeruginosa* and *Aeromonas spp*. in bagged waters has been reported to be due to poor hygienic practices of producers, failure to wash hands, illiteracy and sheep and goats in the vicinity of factories (Coroler *et al*., 1996).

In the Greater Accra Region, the quality of 'ice-water' sold in the streets was analyzed by the Stockholm Environment Institute (SEI). Tests were conducted to obtain the numbers of total coliforms, faecal coliforms and enterococci. Although no faecal coliforms were detected, 78% of total coliforms were found in the range of 11-100 CFU/100ml, and enterococci, 33% in the range of 11-100CFU/100ml and 67% in the range of 101-1000CFU/100ml, were found confirming the presence of faecal contamination (SEI, 1993).

2.8.1 Microorganisms Associated with Storage Conditions and Unhygienic Handling of Plastic Bagged Drinking Water

One of the major characteristics of microorganisms is that they are ubiquitous. Because of this, there are so many possible routes leading to the contamination of factory-bagged drinking water including unhygienic handling by streets vendors. Studies mostly carried out on water samples are to ascertain the presence of heterotrophic bacteria, microbial indicators (total coliforms, faecal coliforms and enterococci). Examples of parasitic protozoa often encountered in water include *Giardia lamblia, Entamoeba histolytica,* and *Cryptosporidium parvum* (Kwakye-Nuako *et al*., 2007).

It is difficult with the epidemiological knowledge currently available to assess the risk to health presented by any particular level of pathogens in water, since this risk will depend equally on the infectivity and invasiveness of the pathogen and on the innate and acquired immunity of the individuals consuming the water. It is only prudent to assume, therefore, that no water in which pathogenic micro-organisms can be detected can be regarded as safe, however low the concentration. Furthermore, only certain waterborne pathogens can be detected reliably and easily in water, and some cannot be detected at all (WHO, 1996).

2.8.2 Coliforms (total and faecal coliforms)

Coliforms are a group of bacteria that can be associated with unhygienic handling of food and water. Coliforms are a broad class of bacteria found in the environment, including the faeces of man and other warm-blooded animals. The presence of coliform bacteria in drinking water may indicate a possible presence of harmful disease-causing organisms. Drinking water must be free of disease-causing organisms called pathogens. Pathogens can be bacteria, protozoa or viruses. Waterborne pathogens can cause diseases like giardiasis, dysentery and hepatitis. The analysis of drinking water for coliforms is relatively simple, economical, and efficient (http://www.bfhd.wa.gov/info/coliform.php).

Coliform bacteria live in soil or vegetation and in the gastrointestinal tract of animals. Coliforms enter water supplies from the direct disposal of waste into streams or lakes or from runoff from wooded areas, pastures, feedlots, septic tanks, and sewage plants into streams or groundwater. In addition, coliforms can enter an individual house via backflow of water from a contaminated source, carbon filters, or leaking well caps that allow dirt and dead organisms to fall into the water (Craun, 1986).

Coliforms are not a single type of bacteria, but a grouping of bacteria that includes many strains, such as E. coli. They are ubiquitous in nature, and many types are harmless. Therefore, it is not definitive that coliform bacteria will cause sickness. Many variables such as the specific type of bacteria present, and your own immune system's effectiveness will determine if you will get sick.

In fact, many people become immune to bacteria that are present in their own water **(**Craun, 1986).

Total coliforms and faecal coliforms are types of bacteria that are able to utilize lactose sugar for their growth. Coliforms indicate the presence of pathogens. Total coliform is organisms that exist in the human or from the environment. As a source and occurrence, total coliform bacteria (excluding *E. coli*) occur in both sewage and natural waters. Some of these bacteria are excreted in the faeces of humans and animals, but many coliforms are heterotrophic and able to multiply in water and soil environment. Total coliforms can also survive and grow in water distribution systems, particularly in the presence of biofilms (Craun *et al*., 1997).

Faecal coliform is more tolerant of high temperature that is 40°C and above and are bacteria that are associated with human or animal wastes. They usually live in human or animal intestinal tracts, and their presence in drinking water is a strong indication of recent sewage or animal waste contamination (Food and Drugs Administration, 1995). Detection and identification of these organisms as faecal organisms or presumptive *Escherichia coli* is considered to provide sufficient information to assess the faecal nature of pollution (Geldreich, 1980).

2.8.3 *Escherichia coli*

Escherichia coli (*E. coli*) are Gram-negative, non-spore-forming, rod shaped bacteria which are capable of aerobic and facultative anaerobic growth in the presence of bile-salts or other surface-active agents with similar growth-inhibiting properties. They usually ferment lactose at 37 °C within 48 hours, possess the enzyme β-galactosidase and are oxidase-negative (Anon, 1992).

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 (Grabow, 1996).

2.8.4 Enterococci

Enterococci are gram-positive bacteria that grow at pH 9.6, 10°C and 45°C, resistance to 60°C for 30 minutes and in 6.5% sodium chloride (NaCl), and the ability to reduce 0.1% methylene blue. Enterococci, a term used in the USA, include all species described as members of the genus *Enterococcus*. Since the most common environmental species fulfill these criteria, in practice the terms faecal streptococci, enterococci, intestinal enterococci and Enterococcus group may refer to the same bacteria (WHO, 2003). Intestinal enterococci group can be used as an index of faecal pollution. 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*, they are more resistant to chlorination. Intestinal enterococci have been used in testing raw water as an index of faecal pathogens that survive longer than *E.coli* and in drinking-water to augment testing for *E. coli*. In addition they have been used to test water quality after repairs of distribution systems or after new mains have been laid (Ashbolt *et al*., 2001).

2.6.5 *Salmonella*

Salmonella is a group of bacteria that cause typhoid fever, food poisoning, gastroenteritis, enteric fever and other illnesses. People become infected mostly through contaminated water or foods, especially meat, poultry and eggs. *Salmonella* is a genus of rod-shaped Gram-negative enterobacteria which remains threat in developing world affecting millions of people annually.

Salmonella infection, or *salmonellosis*, is a bacterial disease of the intestinal tract. Most salmonella species are motile and produce hydrogen sulfide (Ryan *et al*., 2004).

2.8.6 *Bacillus*

Bacillus is a genus of rod-shaped bacteria and a member of the division Firmicutes. *Bacillus* species are either obligate or facultative aerobes, and test positive for enzyme catalase. Unbiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera (Turnbull, 1996).

Bacillus cereus has been recognized agent of food poisoning since 1995. There are only a few outbreaks a year reported by the Center for Disease Control and Prevention (CDC). Between 1972 and 1986, 52 outbreaks of food-borne disease associated with *B. cereus* were reported to the CDC. In 2003, there were two but this is thought to represent only 2% of the total cases which have occurred during these periods. It is not a reportable disease, and usually goes undiagnosed. *B. cereus* causes two types of food-borne illnesses. One type is characterized by nausea and vomiting and abdominal cramps and has an incubation period of 1 to 6 hours. The second type is manifested primarily by abdominal cramps and diarrhea following incubation period of 8 to 16 hours. In either type, the illness usually lasts less than 24 hours after onset (Todar, 2008).

2.8.7 *Giardia lamblia*

Giardia is a flagellated protozoa that are parasitic in the intestines of humans and animals. They have two stages, one of which is a cyst form that can be ingested from contaminated water. Once the cyst enters the stomach, the organism is released into the gastrointestinal tract where it will adhere to the intestinal wall. Eventually the protozoa will move into the large intestine where they encyst again and are excreted in the feces and back into the environment (http://www.cdc.gov/ncidod/diseases/giardia/sources.htm).

Giardia lamblia is a well known cause of diarrhea and infection. It is acquired through person to person contact and waterborne sources. The ingestion of as few as 10 cysts is enough to cause infection in humans. The *Giardia* parasite attaches to the epithelium by a ventral adhesive disc, and reproduces via binary fission. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastro-intestinal tract, but remains confined to the lumen of the small intestine (Huang *et al*., 2006).

2.8.8 *Cryptosporidium parvum*

Cryptosporidium parvum is a protozoan parasite that causes cryptosporidiosis, which has gained notoriety in the past five years. In 1993, over 400,000 people in Milwaukee, Wisconsin became ill with it after drinking contaminated water (http://www.h2O-ngwa.org/pubaff/bacq_a.html).

Cryptosporidium parvum has been acknowledged as a human pathogen since 1976 and is noted to be associated with various forms of waterborne outbreaks among several categories of people. Potential sources of infection include the ingestion of contaminated food and water. Studies indicate that the infective dose is about 132 oocysts; the ingestion of only one oocyst is noted to have caused disease in people (Kwakye-Nuako *et al*., 2007).

Cryptosporidium parvum is considered to be the most important waterborne pathogen in developed countries. It is resistant to all practical levels of chlorination, surviving for 24 hours at 1000mg/L free chlorine (Dupont *et al*., 1995).

2.8.9 *Entamoeba histolytica*

Entamoeba histolytica is a major cause of morbidity and mortality worldwide and contaminated water is a prime source of infection in many areas. *Entamoeba histolytica* is an anaerobic parasitic protozoan, part of the genus *Entamoeba*. Predominantly infecting humans and other primates, *Entamoeba histolytica* is estimated to infect about 50 million people worldwide. Many textbooks state that 10% of the world population is infected, but these figures predate the recognition that at least 90% of these infections were due to a second species, *Entamoeba dispar* (Anon, 1997).

2.8.10 *Micrococcus*

Micrococcus is a genus of bacteria in the micrococcaceae family. Micrococcus occurs in a wide range of environment. Micrococci have Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. Micrococci have been isolated from human skin, animal and diary products, and beer. They are found in many other places in the environment, including water, dust, and soil (Greenblatt *et al.*, 2004).

Micrococcus is generally thought to be a commensal organism, though it can be an opportunistic pathogen, particularly in host with compromised immune systems, such as HIV patients.

Micrococcus can be difficult to identify as the cause of an infection since the organism is normally present in skin micro flora, and the genus is seldom linked to disease. In rare cases, death of immunocompromised patients has occurred from pulmonary infections caused by M*icrococcus*. *Micrococci* may be involved in other infections, including septic arthritis, endocarditis, meningitis, and septic shock (Smith *et al.*, 1999).

2.8.11 Hepatitis A

Hepatitis A is an enteric virus that is very small. It can be transferred through contaminated water, causing outbreaks (John DeZyane, 1990). The virus is excreted by a person carrying it, and if the sewage contaminates the water supply, then the virus is carried in the water until it is consumed by a host. Symptoms such as an inflamed liver, accompanied by lassitude, anorexia, weakness, nausea, fever and jaundice are common. A mild case may only require a week or two of rest, while a severe case can result in liver damage and possible death (WHO, 1996). Generally, water systems utilize chlorination, preceded by coagulation, flocculation, settling and filtration to remove the virus (John DeZyane, 1990).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The Kumasi metropolis is the most populous district in the Ashanti Region and also the second largest city in Ghana with a growing population of about 1,889,934 based on a growth rate of 5.47% per annum (Ghana Statistical Service, 2009). Kumasi is the regional capital of the Ashanti region and the most commercialized centre in the region (Ghana Statistical Service, 2009). Kejetia, a suburb at the heart of Kumasi is located at 5°9'0"N - 5°9'30"North latitude and 1°35'30"W - 1°35'0"West longitude above sea level.

Kejetia has the largest market within the Kumasi Metropolitan Area. It is always choked with traders and shoppers offering various goods and services.

3.1.2 Sampling Area

Five sampling sites were selected in the central Kejetia area for the study. These sites were labeled Site 1 to Site 5. Site 1 was located in the northern part of Kejetia which houses a number of local sachet water distributors and vendors. Additionally, there were a lot of local chop bar operators. Site 2 was located at the Southern portion of Kejetia and was close to an open public toilet; Site 3 was at the eastern part of Kejetia and samples were exposed to the scorching sun; Site 4 was to the West of Kejetia; with a large urban bus terminal; Site 5 was at the centre of Kejetia with sheep and goats wandering in the vicinity.

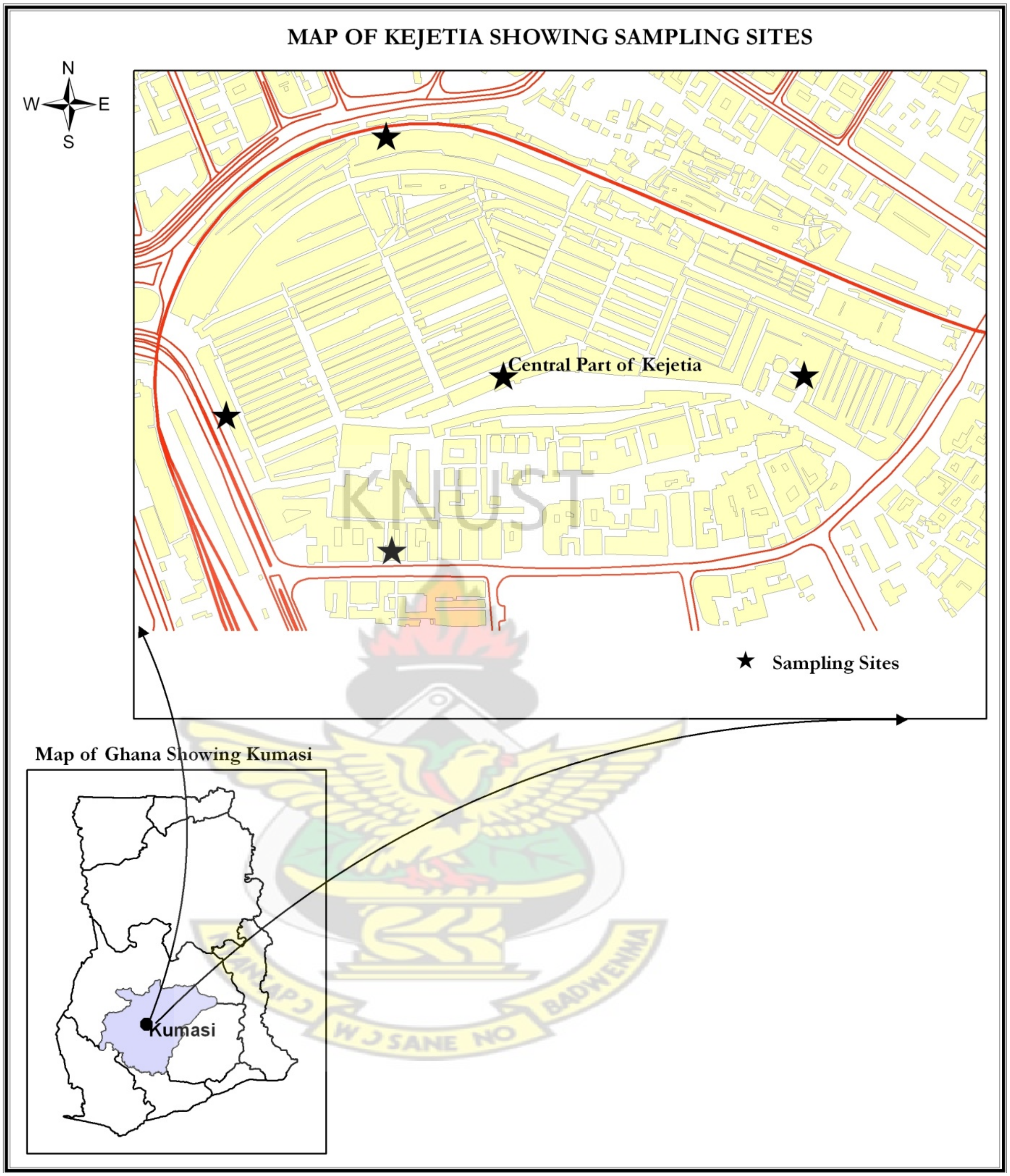

Figure 1: Map of Kejetia showing sampling sites
Courtesy: Friends of Geomatic Engineering Department; March 2010

Site 1

Sampling site 1 was the Northern part of Kejetia which houses a number of local sachet water distributors and vendors.

Plate 1: Site1- A street vendor with plastic bag drinking water

Plate2: Site 1-Distributor with plastic bag drinking water

Site 2

Sampling site 2 was the Southern part of Kejetia close to open public toilets.

Plate3: Site 2-Street vendors selling plastic bag drinking water

Plate 4:** Site 2-plastic bag drinking water in metal fences **by distributor

Site 3

Sampling site 3 was the Eastern part of Kejetia and samples were exposed to the scorching sun.

Plate 5: Site 3-purchasing plastic bag drinking water from vendor

Plate 6: Site 3-purchasing plastic bag drinking water by sacs from distributor

Site 4

Sampling site 4 was the Western part of Kejetia with a large urban bus terminal.

Plate 7: site 4-plastic bag drinking water sold by vendor at Kejetia

Plate 8:** Site 4-Sacs of plastic bag drinking water sold by **distributors

Site 5

Sampling site 5 was the Central part of Kejetia with sheep and goats wandering in the vicinity.

Plate 9: ***Site 5- sacs of plastic bag drinking water***

3.1.3 SAMPLING

Ten different brands of 500ml plastic bagged sachet water samples were purchased using purposive sampling from different vendors and distributors in Kejetia. The sachet water samples purchased were placed in sterilized food bags by the vendors and sent to the laboratory in an iced packed cooled box. These water samples were purchased directly from the vendors who often sell in aluminum pans, metal baskets, and plastic bowls on their heads under the scorching sun.

Using an observational check list, hundred Sachet Water Street vendors were carefully observed on their general hygiene and surrounding environment where the sale and distribution of the plastic bagged drinking water was carried out.

The 500ml plastic bagged sachet waters are often packed in 30s (i.e. 30 bags each of 500ml quantity). Samples of all the ten brands of the plastic bagged sachet water samples were purchased from different distributors and transported to the laboratory in ice packed cool box for storage under three different temperature conditions and was analyzed from November 2009 to April 2010.

The ten brands packed in 30s were stored under Normal atmospheric conditions, Room temperature and in a Refrigerator. Before storage one of each brand sample was analyzed.

3.2 ENUMERATION

3.2.1 Enumeration of Total coliforms

Total coliforms were estimated using the Membrane Filtration Technique (MF) according to standard procedures (Anon, 1992). Lauryl sulphate agar was prepared by measuring 9.2g in 250ml of distilled water in a flask, allowed to dissolve and autoclaved at 121°C for 15 minutes. Hundred milliliters of different brands of sachet water was filtered in triplicate through white, grid marked 47mm diameter, Millipore HA-type cellulose filters with pore size of 0.45 μm. Samples were filtered using a vacuum pump at a pressure of 65kpa (500mmHg) and a triple glass filtration unit (Millipore, Bedford, UK). The filters were placed on Lauryl sulphate agar in Petri dishes using flame sterilized forceps. Petri dishes were inverted and incubated at 37°C for 24 hours. Filter papers on Petri dishes showing golden sheen (yellowish) colonies were identified as positive and counted using a colony counter and expressed as Colony Forming Units (cfu) per 100ml.

3.2.2 Enumeration of Faecal coliforms

Faecal coliforms were estimated using the Membrane Filtration Technique (MF) according to standard procedures (Anon, 1992). The procedure was as described for Total Coliforms but Petri dishes were inverted and incubated at 44°C for 24 hours. Filter papers on Petri dishes showing golden sheen (yellowish) colonies were identified as positive and counted using a colony counter and expressed as Colony Forming Units (cfu) per 100ml.

3.2.3 Enumeration of *Escherichia coli*

MacConkey broth was prepared by measuring 20g in 500ml distilled water, allowed to dissolve and distributed in 5ml quantities into test tubes and autoclaved at 121°C for 15 minutes. Tryptophan broth was also prepared by measuring 4g in 250ml of distilled water, allowed to dissolve and distributed in 5ml quantities into test tubes and autoclaved at 121°C for 15 minutes. Dilutions of 10ml were prepared in 90ml sterile distilled water and 1ml of each dilution inoculated into 5ml of MacConkey broth in test tubes and incubated at 44°C for 24 hours. Positive tubes showing yellow color were inoculated into 5ml tryptophan broth at 35°C for 24 hours. Afterwards a drop of Kovac's reagent was then added to test tubes of tryptophan broth. All tubes showing a red ring color were used as confirmation of the presence of *E. coli.*

3.2.4 Enumeration of Enterococci

A Membrane Filtration Technique was used in the detection and isolation of enterococci (Anon, 1994). Hundred milliliters of water samples were filtered through 47 mm diameter Corning membrane filter with the pore size of 0.45 μm. Slanetz and Bartley agar were prepared by measuring 10.9g in 250ml distilled water and brought to boil and then poured into sterile Petri dishes and allowed to cool to solidify. It needed no autoclaving. Filters were placed on Slanetz and Bartley agar on Petri dishes using flamed sterilized forceps. The Petri dishes were incubated at 37°C for 4 hours and then transferred to 44°C for 44 hours. Red, maroon or pink colonies were counted using a colony counter and expressed as Colony Forming Units (cfu) per 100ml.

3.2.5 Enumeration of *Salmonella*

Salmonella was enumerated using Buffered Peptone Water (BPW-Oxoid) as a pre-enrichment medium. Selenite broth was prepared by measuring 5.8g in 250ml of distilled water and allowed to dissolve. Triplicate 1, 10^{-1} 10^{-2} 10^{-3} ml samples of sachet water were directly inoculated into 10ml volumes of Buffered Peptone water in universal bottles as pre-enrichment medium and incubated at 37°C for 24 hours. Aliquots of 0.1ml were inoculated into 10ml Selenite broth and incubated for 48 hours at 44°C. Salmonella-Shigella agar (SS agar) was prepared by measuring 12g in 200ml distilled water, boiled and then poured into Petri dishes and allowed to cool to solidify. Plates were loop streaked from Selenite broth bottles that had turned red in color and incubated for 24 hours at 37°C. Results were recorded as the number of streaks, out of three, showing positive *Salmonella* with dark black growth.

3.2.6 Enumeration of Total Heterotrophic Bacteria or Total Viable Count (TVC)

The total heterotrophic bacteria plate counts (HPC) in the water samples were obtained using the pour plate technique according to Anon (1994). Dilutions of 10^{-1} – 10^{-6} of water samples were prepared in 0.1% buffered peptone water and triplicate 1ml aliquots of each dilution inoculated into 10 ml molten standard plate count agar in universal bottles. After thorough mixing, these were poured into sterile Petri dishes and incubated for 48 hours at 35°C. Discrete colonies were counted and the results expressed as the numbers of bacteria colonies per milliliter.

3.3 STATISTICAL ANALYSIS

For microbiological analysis graphical presentation of values was done using Microsoft Excel 2003. A two-way randomized analysis of variance (ANOVA) was used to analyze the data performing GenStat version 7.22 software. One-way randomized analysis of variance (ANOVA) was also used to analyze the data with one parameter using Duncan's multiple range test.

CHAPTER FOUR

4.0 RESULTS

4.1 MICROBIAL QUALITY OF PLASTIC BAGGED SACHET DRINKING WATER

4.1.1 Geometric mean indicator bacterial numbers in factory-bagged plastic sachet drinking water stored at different temperatures

Total coliforms

Mean indicator bacterial numbers and salmonella counts contained in the ten different brands of plastic bagged sachet water stored under varying storage temperatures are presented in Tables 4.1 –4.9. The results indicated that the microbial quality of plastic bagged sachet drinking water differed under varying storage temperature regimes (Table 4.1-4.9).

Irrespective of the brand of plastic bagged sachet water tested, initial geometric mean total coliform numbers varied between 9.00 and 1.50 x 10^1. Geometric mean total coliform numbers increased steadily by between 118-182% in all the brands during storage at normal atmospheric temperatures. These increases were statistically ($p<0.001$) significant in the Mobile, Boadwoo, Everkool, S&M, and Gofex brands. However, samples stored at 4^oC in the refrigerator had four of the brands decreasing (Mobile, Davis, Everkool, and Cobb-ji) from between 74% to 92% (Table 4.1-4.4). There were no statistically significant differences between all the brands stored in the refrigerator (Table 4.1-4.4). Total coliform numbers in water samples stored at room temperature increased by between 112-154% from the initial numbers of 9.00 and 1.50 x 10^1 to1.20×10^2 and 3.60×10^2 after six weeks in storage. However, there were statistically significance differences ($p<0.001$) between the brands Mobile, Boadwoo, Everkool, S&M, Cobb-ji, St. Hubert and Gofex (Table 4.1-4.4).

Faecal coliforms

Geometric mean faecal coliform numbers increased from the initial 4.00 to 9.00 to 2.00 $\times10^2$ and 5.00×10^2 in all the brands recording the lowest in Cobb-ji and the highest in Mobile (Table 4.1).

Faecal coliform numbers followed the same trend as recorded for the total coliform with all the brands increasing by between 128-193% after storage at normal atmospheric temperature (Table 4.4). With samples stored at refrigeration temperatures, faecal coliform numbers rather decreased by between 79-82% during the six weeks of storage. Comparatively, samples stored at room temperature over the six weeks recorded increasing faecal coliforms numbers by between 114-165%. However, there were statistically ($p<0.001$) significant differences in the brands at normal atmospheric temperature and no statistically significance differences in Rocky, Boadwoo, Everkool, S&M, at room temperatures (Table 4.5).

Escherichia coli

Geometric mean *Escherichia coli* numbers varied from the initial 3.00 to between 7.00 and 2.00×10^1in all the brands after six weeks of storage at normal atmospheric temperature (Table 4.1) The increases of between 102 and 112% were recorded in Mobile, Rocky, St. Hubert and Dominion. However, six of the brands decreased by 93% in Gofex, 88% in Boadwoo, 62% in

Cobb-ji, 59% in Davis, 50% in Everkool, and 39% in S&M brands (Table 4.6). For samples stored at refrigeration temperatures *Escherichia coli* decreased in all the samples by between -25-20% (Table 4.6). *Escherichia coli* counts in samples stored at room temperature all decreased by between 33-78% (Table 4.6). Statistically significance differences ($p<0.001$) in *E. coli* numbers at normal atmospheric temperature were recorded in Mobile, Davis, and Dominion. At refrigeration temperatures all the brands showed no statistically significant differences. With the exception of the statistically significant differences in the St. Hubert's brand, all of the other brands showed no statistically significant differences at room temperature (Table 4.6).

Enterococci

At normal atmospheric temperatures mean enterococci numbers in all the brands of factory bagged sachet water increased by between 112- 180% except in the Cobb-ji brand which decreased by 93%. However, with the exception of one brand that showed no statistically significant differences (St. Hubert), there were statistically significant differences in all the brands at normal atmospheric temperatures (Table 4.7). Enterococci numbers decreased by 96% in Gofex, 84% in Cobb-ji, 56% in Mobile, 47% in Rocky and 35% in Davis after the six weeks in the refrigerator and there were no significant variations in all the brands in the refrigerator (Table 4.7). At room temperatures after six weeks of storage, enterococci numbers decreased in two of the brands by 98% in Mobile, and 90% in Davis and eight of the brands had increased by between 104-147%. However, there were statistically significant differences ($p<0.001$) in enterococci numbers between the brands at room temperature (Table 4.7).

4.1.2 Geometric mean *Salmonella* numbers in factory-bagged plastic sachet drinking water stored at different temperatures

Geometric mean *Salmonella* numbers varied from the initial 3.00 to between 4.00 and 1.20×10^1 in all the brands after six weeks of storage at normal atmospheric temperatures (Table 4.1). After six weeks of storage at normal atmospheric temperatures, in the refrigerator and at room temperatures, *Salmonella* numbers decreased by between -28-47% in all the brands (Table 4.8). Statistically significant differences were observed in the brands at normal atmospheric temperatures, no statistically significant differences between all the brands in the refrigerator and also there were statistically significant differences in Everkool and St. Hubert at room temperature (Table 4.8).

4.1.3 Geometric mean heterotrophic bacteria total viable numbers in factory-bagged plastic sachet drinking water stored at different temperatures

Initial geometric mean total viable counts varied from 4.30×10^4 and 4.30×10^5 (Table 4.1). Heterotrophic bacteria counts decreased by between 9-38% in all the brands at normal atmospheric temperatures. Statistically significant differences were observed between the brands stored at normal atmospheric temperatures (Table 4.9). However, samples stored at 4^oC in the refrigerator had all the brands decreasing by between -11-31%. There were no statistically significant differences in all the brands stored in the refrigerator (Table 4.9). Heterotrophic bacteria total viable counts in water samples stored at room temperature decreased by between 8-

38%. However, there were statistically significant differences ($p<0.001$) between the brands stored at room temperature (Table 4.9).

Table 4.1 a: Geometric mean indicator bacterial numbers in factory bagged sachet water stored at normal atmospheric temperatures for six weeks

BRANDS	INITIAL	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	%↑
TOTAL COLIFORM								
Mobile	1.50×10^{1}	5.50×10^{2}	5.00×10^{2}	5.50×10^{2}	5.50×10^{2}	5.80×10^{2}	6.60×10^{2}	100
Davis	1.00×10^{1}	3.50×10^{2}	3.80×10^{2}	4.00×10^{2}	4.50×10^{2}	4.50×10^{2}	5.00×10^{2}	100
Rocky	1.10×10^{1}	2.80×10^{2}	2.80×10^{2}	2.90×10^{2}	3.00×10^{2}	3.20×10^{2}	4.50×10^{2}	100
Boadwoo	9.00	2.20×10^{2}	3.00×10^{2}	4.00×10^{2}	3.50×10^{2}	5.00×10^{2}	5.60×10^{2}	100
Everkool	1.30×10^{1}	2.70×10^{2}	2.80×10^{2}	3.00×10^{2}	3.00×10^{2}	3.00×10^{2}	3.50×10^{2}	100
S&M	9.00	2.80×10^{2}	3.00×10^{2}	3.20×10^{2}	3.40×10^{2}	3.90×10^{2}	4.00×10^{2}	100
Cobb-ji	1.40×10^{1}	2.80×10^{2}	3.00×10^{2}	3.00×10^{2}	3.00×10^{2}	3.80×10^{2}	3.90×10^{2}	100
St. Hubert	9.00	4.00×10^{2}	4.50×10^{2}	4.50×10^{2}	4.70×10^{2}	5.30×10^{2}	5.80×10^{2}	100
Dominion	9.00	3.00×10^{2}	3.20×10^{2}	3.50×10^{2}	3.80×10^{2}	4.50×10^{2}	4.50×10^{2}	100
Gofex	1.00×10^{1}	3.90×10^{2}	4.20×10^{2}	4.20×10^{2}	4.30×10^{2}	4.40×10^{2}	5.50×10^{2}	100
FAECAL COLIFORM								
Mobile	8.00	2.70×10^{2}	3.80×10^{2}	3.80×10^{2}	4.20×10^{2}	4.40×10^{2}	5.00×10^{2}	100
Davis	8.00	1.80×10^{2}	1.80×10^{2}	1.90×10^{2}	2.00×10^{2}	2.00×10^{2}	2.70×10^{2}	100
Rocky	9.00	1.00×10^{2}	1.30×10^{2}	1.50×10^{2}	1.60×10^{2}	1.80×10^{2}	2.40×10^{2}	100
Boadwoo	4.00	1.60×10^{2}	2.00×10^{2}	2.00×10^{2}	2.50×10^{2}	2.60×10^{2}	2.90×10^{2}	100
Everkool	7.00	9.00×10^{1}	1.20×10^{2}	1.40×10^{2}	1.80×10^{2}	2.50×10^{2}	3.20×10^{2}	100
S&M	6.00	1.20×10^{2}	1.40×10^{2}	1.80×10^{2}	1.80×10^{2}	2.00×10^{2}	2.70×10^{2}	100
Cobb-ji	8.00	1.30×10^{2}	1.70×10^{2}	1.80×10^{2}	1.80×10^{2}	1.80×10^{2}	2.00×10^{2}	100
St. Hubert	8.00	7.00×10^{1}	2.20×10^{2}	2.30×10^{2}	2.70×10^{2}	2.80×10^{2}	3.00×10^{2}	100
Dominion	6.00	1.60×10^{2}	1.80×10^{2}	2.60×10^{2}	2.80×10^{2}	2.80×10^{2}	3.00×10^{2}	100
Gofex	5.00	1.00×10^{2}	2.20×10^{2}	2.60×10^{2}	2.90×10^{2}	3.00×10^{2}	3.10×10^{2}	100
E. COLI								
Mobile	3.00	1.50×10^{1}	1.50×10^{1}	1.70×10^{1}	1.90×10^{1}	2.00×10^{1}	2.00×10^{1}	100
Davis	3.00	9.00	1.00×10^{1}	1.20×10^{1}	1.20×10^{1}	1.20×10^{1}	1.30×10^{1}	59
Rocky	3.00	6.00	7.00	7.00	9.00	1.20×10^{1}	1.60×10^{1}	100
Boadwoo	3.00	9.00	9.00	9.00	1.20×10^{1}	1.20×10^{1}	1.20×10^{1}	88
Everkool	3.00	4.00	6.00	6.00	1.20×10^{1}	1.20×10^{1}	1.90×10^{1}	50
S&M	3.00	3.00	6.00	6.00	7.00	7.00	9.00	39
Cobb-ji	3.00	4.00	6.00	6.00	7.00	7.00	7.00	62
St. Hubert	3.00	3.00	1.10×10^{1}	1.20×10^{1}	1.20×10^{1}	1.40×10^{1}	1.90×10^{1}	100
Dominion	3.00	9.00	1.50×10^{1}	1.50×10^{1}	1.50×10^{1}	1.50×10^{1}	1.60×10^{1}	100
Gofex	3.00	6.00	6.00	6.00	1.20×10^{1}	1.20×10^{1}	1.20×10^{1}	93

Table 4.1 b: Geometric mean indicator bacterial numbers in factory bagged sachet water stored at normal atmospheric temperatures for six weeks

ENTEROCOCCI								
Mobile	1.40×10^{1}	2.80×10^{2}	3.50×10^{2}	3.50×10^{2}	3.80×10^{2}	4.20×10^{2}	4.80×10^{2}	100
Davis	1.00×10^{1}	1.50×10^{2}	2.50×10^{2}	2.70×10^{2}	2.80×10^{2}	3.00×10^{2}	4.00×10^{2}	100
Rocky	1.10×10^{1}	2.00×10^{2}	2.00×10^{2}	2.30×10^{2}	2.60×10^{2}	3.00×10^{2}	3.80×10^{2}	100
Boadwoo	2.00	3.10×10^{2}	3.70×10^{2}	3.50×10^{2}	4.00×10^{2}	4.10×10^{2}	4.70×10^{2}	100
Everkool	5.00	1.80×10^{2}	2.80×10^{2}	3.00×10^{2}	3.00×10^{2}	3.10×10^{2}	4.40×10^{2}	100
S&M	3.00	1.20×10^{2}	1.60×10^{2}	1.60×10^{2}	1.90×10^{2}	2.10×10^{2}	3.00×10^{2}	100
Cobb-ji	2.00	5.00×10^{1}	1.40×10^{2}	1.60×10^{2}	1.70×10^{2}	1.90×10^{2}	3.00×10^{2}	93
St. Hubert	8.00	1.20×10^{2}	1.20×10^{2}	1.20×10^{2}	1.80×10^{2}	1.80×10^{2}	2.50×10^{2}	100
Dominion	4.00	1.00×10^{2}	2.00×10^{2}	2.20×10^{2}	2.70×10^{2}	2.70×10^{2}	2.80×10^{2}	100
Gofex	4.00	8.00×10^{1}	2.00×10^{2}	2.10×10^{2}	2.50×10^{2}	3.10×10^{2}	3.70×10^{2}	100
								%↓
SALMONELLA								
Mobile	3.00	3.00	3.00	3.00	3.00	4.00	4.00	100
Davis	3.00	3.00	3.00	3.00	3.00	4.00	4.00	100
Rocky	3.00	3.00	3.00	4.00	6.00	6.00	9.00	100
Boadwoo	3.00	3.00	3.00	6.00	6.00	9.00	9.00	100
Everkool	3.00	4.00	6.00	7.00	7.00	9.00	1.20×10^{1}	100
S&M	3.00	3.00	3.00	3.00	7.00	4.00	6.00	100
Cobb-ji	3.00	3.00	3.00	4.00	4.00	6.00	6.00	100
St. Hubert	3.00	3.00	4.00	4.00	6.00	6.00	9.00	100
Dominion	3.00	3.00	3.00	3.00	3.00	3.00	4.00	100
Gofex	3.00	3.00	3.00	3.00	3.00	3.00	4.00	100
								%↓
TOTAL VIABLE COUNT								
Mobile	4.13×10^{5}	9.30×10^{5}	1.22×10^{6}	1.69×10^{6}	1.67×10^{6}	1.68×10^{6}	2.05×10^{6}	100
Davis	3.13×10^{5}	9.26×10^{5}	1.14×10^{6}	1.47×10^{6}	1.92×10^{6}	1.93×10^{6}	1.93×10^{6}	100
Rocky	4.30×10^{5}	8.56×10^{5}	1.53×10^{6}	1.56×10^{5}	1.56×10^{6}	1.60×10^{6}	2.03×10^{6}	100
Boadwoo	1.30×10^{5}	8.20×10^{5}	1.15×10^{6}	1.53×10^{6}	1.55×10^{6}	1.60×10^{6}	2.43×10^{6}	100
Everkool	1.23×10^{5}	1.26×10^{6}	1.56×10^{6}	1.90×10^{6}	1.93×10^{6}	1.98×10^{6}	2.95×10^{6}	100
S&M	3.13×10^{5}	1.64×10^{6}	1.67×10^{6}	2.81×10^{6}	2.86×10^{6}	3.25×10^{6}	3.76×10^{6}	100
Cobb-ji	4.33×10^{4}	8.52×10^{5}	2.27×10^{6}	3.36×10^{6}	3.36×10^{6}	3.95×10^{6}	3.95×10^{6}	100
St. Hubert	7.66×10^{4}	7.96×10^{5}	1.20×10^{6}	1.41×10^{6}	1.61×10^{6}	1.76×10^{6}	1.95×10^{6}	100
Dominion	8.60×10^{4}	4.83×10^{5}	4.70×10^{5}	8.17×10^{5}	1.28×10^{6}	2.23×10^{6}	1.45×10^{6}	100
Gofex	4.30×10^{4}	3.13×10^{5}	4.96×10^{5}	6.86×10^{5}	9.10×10^{5}	1.56×10^{6}	4.47×10^{6}	100

Table 4.2 a: Geometric mean indicator bacteria numbers in factory bagged sachet water stored in the refrigerator for six weeks

BRANDS	INITIAL	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	%
TOTAL COLIFORM								
Mobile	1.50×10^{1}	2.20×10^{2}	1.80×10^{2}	1.50×10^{2}	9.00×10^{1}	8.00×10^{1}	4.00×10^{1}	74
Davis	1.00×10^{1}	1.50×10^{2}	1.00×10^{2}	8.00×10^{1}	8.00×10^{1}	5.00×10^{1}	4.00×10^{1}	81
Rocky	1.10×10^{1}	2.50×10^{2}	1.90×10^{2}	1.80×10^{2}	1.20×10^{2}	1.00×10^{2}	1.00×10^{2}	100
Boadwoo	9.00	3.50×10^{2}	2.80×10^{2}	1.00×10^{2}	1.00×10^{2}	8.00×10^{1}	8.00×10^{1}	100
Everkool	1.30×10^{1}	2.00×10^{2}	1.90×10^{2}	1.50×10^{2}	1.20×10^{2}	1.00×10^{2}	1.00×10^{2}	92
S&M	9.00	2.80×10^{2}	2.40×10^{2}	1.90×10^{2}	1.70×10^{2}	1.50×10^{2}	1.20×10^{2}	100
Cobb-ji	1.40×10^{1}	1.50×10^{2}	1.30×10^{2}	1.00×10^{2}	1.00×10^{2}	8.00×10^{1}	7.00×10^{1}	74
St. Hubert	9.00	2.40×10^{2}	1.50×10^{2}	1.20×10^{2}	1.00×10^{2}	1.00×10^{2}	1.00×10^{2}	100
Dominion	9.00	2.80×10^{2}	2.60×10^{2}	2.20×10^{2}	2.20×10^{2}	2.00×10^{2}	1.40×10^{2}	100
Gofex	1.000×10^{1}	2.20×10^{2}	2.00×10^{2}	1.80×10^{2}	1.80×10^{2}	1.70×10^{2}	1.60×10^{2}	100
FAECAL COLIFORM								%↓
Mobile	8.00	1.80×10^{2}	1.30×10^{2}	1.00×10^{2}	9.00×10^{1}	8.00×10^{1}	4.00×10^{1}	100
Davis	8.00	1.00×10^{2}	8.00×10^{1}	5.00×10^{1}	4.00×10^{1}	3.00×10^{1}	3.00×10^{1}	79
Rocky	9.00	2.20×10^{2}	1.50×10^{2}	1.30×10^{2}	1.00×10^{2}	9.00×10^{1}	6.00×10^{1}	100
Boadwoo	4.00	2.00×10^{2}	1.80×10^{2}	1.70×10^{2}	8.00×10^{1}	6.00×10^{1}	4.00×10^{1}	100
Everkool	7.00	1.50×10^{2}	1.10×10^{2}	1.00×10^{2}	9.00×10^{1}	7.00×10^{1}	6.00×10^{1}	100
S&M	6.00	2.60×10^{2}	2.20×10^{2}	1.30×10^{2}	1.20×10^{2}	1.00×10^{2}	8.00×10^{1}	100
Cobb-ji	8.00	9.00×10^{1}	5.00×10^{1}	4.00×10^{1}	4.00×10^{1}	4.00×10^{1}	4.00×10^{1}	82
St. Hubert	8.00	1.70×10^{2}	9.00×10^{1}	8.00×10^{1}	7.00×10^{1}	6.00×10^{1}	5.00×10^{1}	100
Dominion	6.00	2.00×10^{2}	1.00×10^{2}	1.00×10^{2}	9.00×10^{2}	9.00×10^{1}	8.00×10^{1}	100
Gofex	5.00	1.50×10^{2}	1.20×10^{2}	1.00×10^{2}	1.00×10^{2}	9.00×10^{1}	8.00×10^{1}	100
E. COLI								
Mobile	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Davis	3.00	4.00	3.00	3.00	3.00	3.00	3.00	_
Rocky	3.00	6.00	6.00	3.00	3.00	3.00	3.00	_
Boadwoo	3.00	9.00	3.00	3.00	3.00	3.00	3.00	_
Everkool	3.00	7.00	3.00	3.00	3.00	3.00	3.00	_
S&M	3.00	1.20×10^{1}	3.00	3.00	3.00	3.00	3.00	_
Cobb-ji	3.00	4.00	3.00	3.00	3.00	3.00	3.00	_
St. Hubert	3.00	9.00	4.00	3.00	3.00	3.00	3.00	_
Dominion	3.00	9.00	4.00	4.00	3.00	3.00	3.00	_
Gofex	3.00	7.00	4.00	4.00	3.00	3.00	3.00	_

(-) Means no percentage (%) change

Table 4.2 b: Geometric mean indicator bacteria numbers in factory bagged sachet water stored in the refrigerator for six weeks

ENTEROCOCCI								%↓
Mobile	1.40×10^{1}	8.00×10^{1}	6.00×10^{1}	5.00×10^{1}	5.00×10^{1}	4.00×10^{1}	3.00×10^{1}	56
Davis	1.00×10^{1}	7.00×10^{1}	5.00×10^{1}	4.00×10^{1}	4.00×10^{1}	3.00×10^{1}	1.00×10^{1}	35
Rocky	1.10×10^{1}	1.00×10^{2}	5.00×10^{1}	4.00×10^{1}	4.00×10^{1}	3.00×10^{1}	2.00×10^{1}	47
Boadwoo	2.00	1.50×10^{2}	1.20×10^{2}	1.20×10^{2}	1.00×10^{2}	1.00×10^{2}	8.00×10^{1}	100
Everkool	5.00	1.30×10^{2}	1.30×10^{2}	1.10×10^{2}	1.10×10^{2}	1.00×10^{2}	1.00×10^{2}	100
S&M	3.00	1.00×10^{2}	1.00×10^{2}	9.00×10^{1}	8.00×10^{1}	7.00×10^{1}	6.00×10^{1}	100
Cobb-ji	2.00	1.50×10^{2}	1.40×10^{2}	1.30×10^{2}	1.00×10^{2}	1.00×10^{2}	1.00×10^{2}	84
St. Hubert	8.00	1.50×10^{2}	1.40×10^{2}	1.20×10^{2}	1.10×10^{2}	1.00×10^{2}	9.00×10^{1}	100
Dominion	4.00	1.50×10^{2}	1.30×10^{2}	1.20×10^{2}	1.10×10^{2}	1.10×10^{2}	9.00×10^{1}	100
Gofex	4.00	1.50×10^{2}	1.60×10^{2}	1.20×10^{2}	1.10×10^{2}	8.00×10^{1}	6.00×10^{2}	96
SALMONELLA								
Mobile	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Davis	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Rocky	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Boadwoo	3.00	4.00	3.00	3.00	3.00	3.00	3.00	_
Everkool	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
S&M	3.00	4.00	3.00	3.00	3.00	3.00	3.00	_
Cobb-ji	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
St. Hubert	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Dominion	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Gofex	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
TOTAL VIABLE COUNT								%↓
Mobile	4.13×10^{5}	1.12×10^{6}	1.12×10^{6}	7.50×10^{5}	7.46×10^{5}	4.30×10^{4}	4.00×10^{4}	_
Davis	3.13×10^{5}	1.20×10^{6}	7.86×10^{5}	5.20×10^{5}	9.30×10^{4}	3.00×10^{4}	2.00×10^{4}	_
Rocky	4.30×10^{5}	1.13×10^{5}	7.46×10^{5}	8.30×10^{4}	5.00×10^{4}	4.60×10^{4}	4.30×10^{4}	_
Boadwoo	1.30×10^{5}	1.10×10^{5}	9.00×10^{5}	8.23×10^{5}	6.60×10^{4}	4.40×10^{5}	4.40×10^{4}	100
Everkool	1.23×10^{5}	1.14×10^{6}	1.13×10^{6}	7.86×10^{5}	7.56×10^{5}	4.26×10^{5}	4.23×10^{5}	100
S&M	3.13×10^{5}	1.90×10^{6}	1.50×10^{6}	1.31×10^{6}	1.23×10^{6}	7.53×10^{5}	4.60×10^{5}	100
Cobb-ji	4.33×10^{4}	7.93×10^{5}	4.16×10^{5}	3.76×10^{5}	1.43×10^{5}	1.16×10^{5}	1.00×10^{4}	100
St. Hubert	7.66×10^{4}	8.13×10^{5}	4.13×10^{5}	4.10×10^{5}	3.83×10^{5}	3.76×10^{5}	5.60×10^{4}	100
Dominion	8.60×10^{4}	7.83×10^{5}	7.76×10^{5}	4.46×10^{5}	1.17×10^{6}	1.00×10^{5}	6.30×10^{4}	100
Gofex	4.30×10^{4}	7.30×10^{6}	4.33×10^{5}	4.23×10^{5}	4.06×10^{5}	4.03×10^{5}	3.73×10^{5}	100

(-) Means no percentage (%) change

Table 4.3 a: Geometric mean indicator bacteria numbers in factory bagged sachet water stored at room temperatures for six weeks

BRANDS	INITIAL	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	WEEK 6	%↑
TOTAL COLIFORM								
Mobile	1.50×10^{1}	2.80×10^{2}	3.00×10^{2}	3.60×10^{2}	2.80×10^{2}	2.90×10^{2}	3.00×10^{2}	100
Davis	1.00×10^{1}	3.20×10^{2}	2.90×10^{2}	3.00×10^{2}	3.00×10^{2}	3.80×10^{2}	3.60×10^{2}	100
Rocky	1.10×10^{1}	1.80×10^{2}	2.60×10^{2}	3.00×10^{2}	2.40×10^{2}	2.80×10^{2}	2.50×10^{2}	100
Boadwoo	9.00	2.00×10^{2}	1.50×10^{2}	2.00×10^{2}	1.50×10^{2}	1.80×10^{2}	1.20×10^{2}	100
Everkool	1.30×10^{1}	2.50×10^{2}	2.00×10^{2}	2.50×10^{2}	3.00×10^{2}	2.00×10^{2}	1.80×10^{2}	100
S&M	9.00	3.50×10^{2}	2.60×10^{2}	2.80×10^{2}	2.20×10^{2}	2.00×10^{2}	1.50×10^{2}	100
Cobb-ji	1.40×10^{1}	3.20×10^{2}	2.80×10^{2}	3.00×10^{2}	3.80×10^{2}	2.20×10^{2}	2.50×10^{2}	100
St. Hubert	9.00	2.60×10^{2}	1.40×10^{2}	2.40×10^{2}	1.20×10^{2}	1.80×10^{2}	1.90×10^{2}	100
Dominion	9.00	3.00×10^{2}	2.80×10^{2}	3.00×10^{2}	2.60×10^{2}	2.50×10^{2}	2.00×10^{2}	100
Gofex	1.000×10^{1}	3.80×10^{2}	3.00×10^{2}	3.20×10^{2}	2.80×10^{2}	3.00×10^{2}	3.20×10^{2}	100
FAECAL COLIFORM								%↑
Mobile	8.00	1.80×10^{2}	2.50×10^{2}	2.80×10^{2}	1.80×10^{2}	1.90×10^{2}	1.20×10^{2}	100
Davis	8.00	2.00×10^{2}	1.50×10^{2}	2.00×10^{2}	2.00×10^{2}	2.20×10^{2}	1.00×10^{2}	100
Rocky	9.00	1.50×10^{2}	1.00×10^{2}	1.80×10^{2}	1.60×10^{2}	1.80×10^{2}	1.80×10^{2}	100
Boadwoo	4.00	1.80×10^{2}	1.20×10^{2}	1.80×10^{2}	1.00×10^{2}	1.50×10^{2}	9.00×10^{1}	100
Everkool	7.00	1.80×10^{2}	1.10×10^{2}	1.50×10^{2}	1.80×10^{2}	1.20×10^{2}	5.00×10^{1}	100
S&M	6.00	1.90×10^{2}	1.90×10^{2}	2.40×10^{2}	9.00×10^{1}	1.00×10^{2}	9.00×10^{1}	100
Cobb-ji	8.00	2.00×10^{2}	9.00×10^{1}	1.90×10^{2}	1.80×10^{2}	1.60×10^{2}	1.00×10^{2}	100
St. Hubert	8.00	2.10×10^{2}	1.40×10^{2}	1.70×10^{2}	1.20×10^{2}	9.00×10^{1}	8.00×10^{1}	100
Dominion	6.00	2.50×10^{2}	2.00×10^{2}	2.20×10^{2}	2.00×10^{2}	1.10×10^{2}	9.00×10^{1}	100
Gofex	5.00	3.00×10^{2}	1.90×10^{2}	2.60×10^{2}	1.90×10^{2}	2.00×10^{2}	1.80×10^{2}	100
E. COLI								%↓
Mobile	3.00	1.60×10^{1}	7.00	1.20×10^{1}	1.50×10^{1}	9.00	7.00	100
Davis	3.00	1.20×10^{1}	7.00	9.00	9.00	7.00	4.00	100
Rocky	3.00	1.30×10^{1}	3.00	6.00	1.10×10^{1}	6.00	6.00	100
Boadwoo	3.00	1.10×10^{1}	9.00	1.60×10^{1}	7.00	4.00	4.00	100
Everkool	3.00	9.00	6.00	9.00	9.00	7.00	3.00	_
S&M	3.00	1.60×10^{1}	1.10×10^{1}	1.50×10^{1}	3.00	4.00	3.00	_
Cobb-ji	3.00	1.20×10^{1}	9.00	1.20×10^{1}	4.00	3.00	3.00	_
St. Hubert	3.00	1.90×10^{1}	6.00	9.00	4.00	3.00	3.00	_
Dominion	3.00	1.60×10^{1}	1.30×10^{1}	1.60×10^{1}	1.10×10^{1}	7.00	3.00	_
Gofex	3.00	1.90×10^{1}	7.00	9.00	3.00	4.00	6.00	100

(-) Means no percentage (%) change

Table 4.3 b: Geometric mean indicator bacteria numbers in factory bagged sachet water stored at room temperatures for six weeks

ENTEROCOCCI								
Mobile	1.40×10^{1}	1.20×10^{2}	1.40×10^{2}	1.80×10^{2}	1.50×10^{2}	1.00×10^{2}	1.50×10^{2}	98
Davis	1.00×10^{1}	1.90×10^{2}	1.00×10^{2}	1.30×10^{2}	1.60×10^{2}	1.40×10^{2}	1.80×10^{2}	90
Rocky	1.10×10^{1}	2.70×10^{2}	3.30×10^{2}	3.80×10^{2}	1.80×10^{2}	1.80×10^{2}	1.80×10^{2}	100
Boadwoo	2.00	2.00×10^{2}	1.80×10^{2}	2.00×10^{2}	1.00×10^{2}	8.00×10^{1}	1.00×10^{2}	100
Everkool	5.00	2.90×10^{2}	1.50×10^{2}	2.80×10^{2}	1.50×10^{2}	1.20×10^{2}	1.50×10^{2}	100
S&M	3.00	2.30×10^{2}	2.00×10^{2}	3.20×10^{2}	2.00×10^{2}	9.00×10^{1}	9.00×10^{1}	100
Cobb-ji	2.00	2.60×10^{2}	1.90×10^{2}	2.50×10^{2}	1.80×10^{2}	1.50×10^{2}	1.70×10^{2}	100
St. Hubert	8.00	1.80×10^{2}	2.10×10^{2}	1.90×10^{2}	1.80×10^{2}	1.20×10^{2}	2.00×10^{2}	100
Dominion	4.00	1.60×10^{2}	2.60×10^{2}	2.80×10^{2}	2.40×10^{2}	1.90×10^{2}	2.60×10^{2}	100
Gofex	4.00	2.50×10^{2}	3.00×10^{2}	2.90×10^{2}	1.50×10^{2}	1.10×10^{2}	1.60×10^{2}	100
SALMONELLA								
Mobile	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Davis	3.00	3.00	3.00	3.00	3.00	4.00	3.00	_
Rocky	3.00	3.00	3.00	3.00	3.00	3.00	3.00	_
Boadwoo	3.00	3.00	3.00	6.00	4.00	3.00	3.00	_
Everkool	3.00	6.00	4.00	9.00	3.00	3.00	3.00	_
S&M	3.00	4.00	3.00	6.00	3.00	3.00	3.00	_
Cobb-ji	3.00	3.00	4.00	4.00	3.00	3.00	3.00	_
St. Hubert	3.00	4.00	3.00	9.00	3.00	3.00	3.00	_
Dominion	3.00	3.00	3.00	6.00	3.00	3.00	3.00	_
Gofex	3.00	3.00	3.00	4.00	3.00	3.00	3.00	_
TOTAL VIABLE COUNT								%↓
Mobile	4.13×10^{5}	8.46×10^{5}	1.53×10^{6}	1.59×10^{6}	1.67×10^{6}	1.39×10^{6}	2.01×10^{6}	100
Davis	3.13×10^{5}	9.00×10^{5}	6.03×10^{5}	1.32×10^{6}	1.72×10^{6}	1.94×10^{6}	1.53×10^{6}	100
Rocky	4.30×10^{5}	4.56×10^{5}	1.13×10^{6}	2.47×10^{6}	1.13×10^{6}	1.57×10^{6}	2.28×10^{6}	100
Boadwoo	1.30×10^{5}	1.16×10^{6}	1.64×10^{6}	9.83×10^{5}	9.93×10^{5}	8.83×10^{5}	1.70×10^{6}	100
Everkool	1.23×10^{5}	2.02×10^{6}	1.30×10^{5}	1.61×10^{6}	7.33×10^{5}	1.59×10^{6}	1.26×10^{6}	100
S&M	3.13×10^{5}	1.35×10^{6}	6.56×10^{5}	1.30×10^{6}	1.63×10^{6}	8.00×10^{6}	8.76×10^{5}	100
Cobb-ji	4.33×10^{4}	8.00×10^{6}	4.80×10^{5}	7.93×10^{5}	1.30×10^{6}	7.60×10^{5}	1.16×10^{6}	100
St. Hubert	7.66×10^{4}	1.01×10^{6}	4.60×10^{5}	1.26×10^{6}	1.15×10^{6}	7.80×10^{5}	4.56×10^{5}	100
Dominion	8.60×10^{4}	1.12×10^{6}	4.00×10^{5}	2.17×10^{6}	2.04×10^{6}	1.99×10^{6}	8.33×10^{5}	100
Gofex	4.30×10^{4}	5.30×10^{5}	4.16×10^{5}	8.33×10^{5}	1.62×10^{6}	1.27×10^{6}	1.20×10^{6}	100

(-) Means no percentage (%) change

Table 4.4: Mean total coliform numbers at different temperatures and statistical comparison between the brands

Geometric mean/100 mL Sample

Sachet water brands	Initial TC Levels	Normal atmospheric Temperature (30°c)	%change in TC levels at N. A.T	Refrigerator Temperature (4°c)	%change in TC levels at REF	Room temperature (26.1°c)	%change in TC levels at R.T
Mobile	1.50×10^{1}	5.56×10^{2c} (±65.42)	134	1.09×10^{2a} (±68.02)	74	3.01×10^{2b} (±29.94)	112
Davis	1.00×10^{1}	4.19×10^{2c} (±54.92)	151	7.60×10^{1a} (±39.33)	81	3.23×10^{2b} (±36.74)	141
Rocky	1.10×10^{1}	3.15×10^{2c} (±65.42)	141	1.47×10^{2a} (±60.22)	108	2.49×10^{2b} (±41.19)	130
Boadwoo	9.00	3.71×10^{2b} (±126.24)	170	1.36×10^{2a} (±118.62)	124	1.64×10^{2a} (±32.04)	132
Everkool	1.30×10^{1}	2.99×10^{2c} (±27.57)	123	1.38×10^{2a} (±44.12)	92	2.26×10^{2b} (±44.72)	112
S&M	9.00	3.35×10^{2b} (±48.34)	165	1.84×10^{2a} (±59.13)	138	2.35×10^{2a} (±69.47)	149
Cobb-Ji	1.40×10^{1}	3.22×10^{2b} (±47.22)	118	1.01×10^{2a} (±30.17)	74	2.87×10^{2b} (±56.01)	113
St. Hubert	9.00	4.76×10^{2b} (±64.50)	182	1.28×10^{2a} (±55.05)	122	1.82×10^{2a} (±54.56)	137
Dominion	9.00	3.70×10^{2b} (±64.11)	170	2.15×10^{2a} (±48.99)	145	2.63×10^{2a} (±37.82)	154
Gofex	1.00×10^{1}	4.39×10^{2c} (±55.65)	164	1.84×10^{2a} (±21.68)	126	3.15×10^{2b} (±34.45)	149

Figures in brackets are standard deviation, TC: Total coliform, N.A.T: Normal Atmospheric Temperature, REF: Refrigerator, R.T: Room Temperature

Table 4.5: Mean faecal coliform numbers at different temperatures and statistical comparison between the brands

Geometric mean/100 mL Sample

Sachet water brands	Initial FC Levels	Normal atmospheric temperature (30°c)	%change in FC level at N.A.T	Refrigerator (4°c)	%change in FC level at REF	Room temperature (26.1°c)	%change in FC level at R.T
Mobile	8.00	3.92×10^{2c} (±77.05)	184	9.36×10^{1a} (±47.61)	116	1.93×10^{2b} (±56.92)	151
Davis	8.00	2.01×10^{2b} (±33.86)	145	4.93×10^{1a} (±28.81)	79	1.73×10^{2b} (±44.91)	138
Rocky	9.00	1.54×10^{2a} (±47.75)	165	1.15×10^{2a} (±56.12)	148	1.55×10^{2a} (±31.25)	163
Boadwoo	4.00	2.22×10^{2b} (±39.37)	193	7.00×10^{1a} (±67.65)	131	1.32×10^{2a} (±39.33)	165
Everkool	7.00	1.67×10^{2b} (±86.87)	128	9.24×10^{1a} (±32.04)	103	1.21×10^{2a} (±49.56)	114
S&M	6.00	1.76×10^{2a} (±52.31)	152	1.39×10^{2a} (±71.67)	140	1.38×10^{2a} (±64.81)	140
Cobb-Ji	8.00	1.72×10^{2b} (±23.38)	143	4.75×10^{1a} (±20.00)	82	1.46×10^{2b} (±47.19)	135
St. Hubert	8.00	2.08×10^{2b} (±83.29)	160	7.97×10^{1a} (±43.20)	113	1.28×10^{2a} (±49.30)	137
Dominion	6.00	2.37×10^{2c} (±58.54)	180	1.04×10^{2a} (±44.72)	137	1.67×10^{2b} (±63.69)	161
Gofex	5.00	2.32×10^{2b} (±78.91)	144	1.04×10^{2a} (±25.03)	108	2.16×10^{2b} (±48.58)	140

Figures in brackets are standard deviation, FC: Faecal coliform, N.A.T: Normal Atmospheric Temperature, REF: Refrigerator, R.T: Room Temperature

Table 4.6: Mean *Escherichia coli* counts at different temperatures and statistical comparison between the brands

		Geometric mean/100 mL Sample					
Sachet water brands	Initial *E.coli* Levels	Normal atmospheric temperature (30°c)	%change in *E. coli* levels at N.A.T	Refrigerator (4°c)	%change in *E. coli* levels at REF	Room temperature (26.1°c)	%change in *E.coli* levels at R.T
Mobile	3.00	1.75×10^{1b} (±2.34)	111	3.00^{a} (±0.00)	-18	1.04×10^{1b} (±3.95)	72
Davis	3.00	1.14×10^{1c} (±1.38)	59	3.15^{a} (±0.41)	-25	7.59^{b} (±2.68)	33
Rocky	3.00	9.31^{b} (±3.66)	102	3.78^{a} (±1.55)	20	6.73^{b} (±3.73)	72
Boadwoo	3.00	1.04×10^{1b} (±1.64)	88	3.60^{a} (±2.45)	5	7.50^{b} (±4.59)	56
Everkool	3.00	8.56^{b} (±5.60)	50	3.46^{a} (±1.63)	-12	6.72^{b} (±2.40)	33
S&M	3.00	6.02^{ab} (±1.97)	39	3.78^{a} (±3.67)	3	6.76^{b} (±6.09)	48
Cobb-Ji	3.00	6.06^{ab} (±1.17)	62	3.15^{a} (±0.41)	2	6.00^{b} (±4.36)	62
St. Hubert	3.00	1.04×10^{1b} (±5.19)	112	3.78^{a} (±2.40)	20	5.77^{a} (±6.15)	58
Dominion	3.00	1.39×10^{1c} (±2.56)	107	3.97^{a} (±2.34)	7	9.57^{b} (±5.18)	78
Gofex	3.00	8.49^{b} (±3.29)	93	3.80^{a} (±1.55)	20	6.65^{b} (±5.80)	70

Figures in brackets are standard deviation, N.A.T: Normal Atmospheric Temperature, REF: Refrigerator, R.T: Room Temperature

Table 4:7 Mean enterococci numbers at different temperatures and statistical comparison between the brands

Geometric mean/100 mL Sample

Sachet water brands	Initial enterococci Levels	Normal atmospheric temperature (30°c)	%change in enterococci levels at N.A.T	Refrigerator (4°c)	%change in enterococci levels at REF	Room temperature (26.1°c)	%change in enterococci levels at R.T
Mobile	1.40×10^{1}	3.71×10^{2c} (±68.31)	138	4.93×10^{1a} (±17.22)	56	1.38×10^{2b} (±27.57)	98
Davis	1.00×10^{1}	2.64×10^{2c} (±80.68)	112	3.45×10^{1a} (±20.00)	35	1.47×10^{2b} (±33.47)	90
Rocky	1.10×10^{1}	2.55×10^{2b} (±69.40)	120	4.11×10^{1a} (±28.05)	47	2.41×10^{2b} (±87.56)	118
Boadwoo	2.00	3.82×10^{2b} (±45.83)	180	1.10×10^{2a} (±24.01)	121	1.34×10^{2a} (±55.74)	131
Everkool	5.00	2.92×10^{2c} (±83.05)	143	1.13×10^{2a} (±13.66)	102	1.79×10^{2b} (±74.57)	122
S&M	3.00	1.82×10^{2b} (±61.97)	140	8.19×10^{1a} (±16.33)	103	1.70×10^{2b} (±87.96)	147
Cobb-Ji	2.00	1.49×10^{2ab} (±80.85)	93	1.18×10^{2a} (±22.80)	84	1.96×10^{2b} (±44.72)	104
St. Hubert	8.00	1.55×10^{2a} (±52.31)	112	1.16×10^{2a} (±23.17)	100	1.77×10^{2a} (±31.62)	118
Dominion	4.00	2.12×10^{2b} (±68.31)	130	1.17×10^{2a} (±20.41)	104	2.27×10^{2b} (±46.65)	133
Gofex	4.00	2.14×10^{2b} (±99.93)	124	1.11×10^{2a} (±45.79)	96	1.96×10^{2b} (±80.25)	120

Figures in brackets are standard deviation, N.A.T: Normal Atmospheric Temperature, REF: Refrigerator, R.T: Room Temperature

Table 4.8: Mean *salmonella* numbers at different temperatures and statistical comparison between the brands

Geometric mean/100 mL Sample

Sachet water brands	Initial *salmonella* Levels	Normal atmospheric temperature (30°c)	%change in *salmonella* levels at N.A.T	Refrigerator (4°c)	%change in *salmonella* levels at REF	Room temperature (26.1°c)	%change in *salmonella* levels at R.T
Mobile	3.00	3.30^{a} (±0.52)	-18	3.00^{a} (±0.00)	-25	3.00^{a} (±0.00)	-25
Davis	3.00	3.30^{a} (±0.52)	-22	3.00^{a} (±0.00)	-28	3.15^{a} (±0.41)	-26
Rocky	3.00	4.45^{b} (±2.32)	12	3.00^{a} (±0.00)	-17	3.00^{a} (±0.00)	-17
Boadwoo	3.00	5.45^{b} (±2.51)	17	3.15^{a} (±0.41)	-22	3.53^{a} (±1.21)	-12
Everkool	3.00	7.09^{c} (±2.74)	47	3.00^{a} (±0.00)	-17	4.24^{b} (±2.42)	8
S&M	3.00	3.53^{a} (±1.21)	-3	3.15^{a} (±0.41)	-14	3.53^{a} (±1.21)	-3
Cobb-Ji	3.00	4.16^{a} (±1.37)	29	3.00^{a} (±0.00)	0	3.30^{a} (±0.52)	8
St. Hubert	3.00	5.00^{b} (±2.16)	45	3.00×10^{0a} (±0.00)	0	3.97×10^{0b} (±2.34)	22
Dominion	3.00	3.15^{a} (±0.41)	2	3.00×10^{0a} (±0.00)	-2	3.37×10^{0a} (±1.22)	8
Gofex	3.00	3.15×10^{0a} (±0.41)	4	3.00×10^{0a} (±0.00)	0	3.15×10^{0a} (±0.41)	2

Figures in brackets are standard deviation, N.A.T: Normal Atmospheric Temperature, REF: Refrigerator, R.T: Room Temperature

Table 4.9: Mean total viable counts at different temperatures and statistical comparison between the brands

		Geometric mean/100 mL Sample					
Sachet water brands	Initial TVC Levels	Normal Atmospheric temperature (30°c)	%change in TVC levels at N.A.T	Refrigerator (4 °c)	%change in TVC levels at REF	Room temperature (26.1°c)	%change in TVC levels at R.T
Mobile	4.13×10^{5}	1.49×10^{6b} (±397872.68)	9	3.26×10^{5a} (±490587.40)	-3	1.46×10^{6ab} (±382692.26)	8
Davis	3.13×10^{5}	1.49×10^{6b} (±445383.73)	10	1.74×10^{5a} (±482397.52)	-6	1.24×10^{6ab} (±505259.01)	8
Rocky	4.30×10^{5}	1.65×10^{6b} (±411412.53)	10	9.40×10^{4a} (±278524.27)	-11	1.31×10^{6b} (±763258.65)	8
Boadwoo	1.30×10^{5}	1.43×10^{6b} (±469674.36)	13	3.19×10^{5a} (±347446.93)	1	1.19×10^{6ab} (±355252.59)	12
Everkool	1.23×10^{5}	1.87×10^{6b} (±571660.53)	13	7.18×10^{5a} (±317025.18)	6	1.36×10^{6ab} (±434244.48)	11
S&M	3.13×10^{5}	2.54×10^{6b} (±855510.76)	14	1.08×10^{6a} (±517087.87)	7	1.52×10^{6b} (±2818339.82)	10
Cobb-Ji	4.33×10^{4}	2.64×10^{6b} (±1201943.1)	18	1.66×10^{5a} (±284394.09)	-3	1.23×10^{6b} (±2914101.61)	12
St. Hubert	7.66×10^{4}	1.40×10^{6b} (±417101.15)	22	3.22×10^{5a} (±240668.03)	9	7.87×10^{5ab} (±344861.71)	17
Dominion	8.60×10^{4}	9.58×10^{5a} (±675683.58)	9	5.21×10^{5a} (±397470.33)	4	1.22×10^{6a} (±739502.45)	11
Gofex	4.30×10^{4}	9.37×10^{5a} (±1560475.1)	38	4.49×10^{5a} (±133207.61)	31	8.76×10^{5a} (±464076.90)	38

Figures in brackets are standard deviation, TVC: Total Viable Counts, N.A.T: Normal Atmospheric Temperature, REF: Refrigerator, R.T: Room Temperature

4.2 Microbial indicator numbers on different brands of sachet water sold on the street of Kejetia

Of the ten brands selected at random and tested for microbial indicator numbers on the factory plastic-bagged sachet drinking water, all contained total coliforms, faecal coliforms, *E. coli*, enterococci, and pathogenic salmonella (Table 4.10-4.12).

4.2.1 Vendors handling practices effect on the microbial numbers on different brands of factory bagged plastic sachet water

Following the varying handling practices of the factory bagged sachet drinking water by vendors, bacterial indicator counts recovered on the different brands of sachet water ranged from 8.00 to 3.80×10^1 for total coliforms, 6.00 and 3.00×10^1 for faecal coliforms, 3.00 and 1.90×10^1 for *E. coli* and 1.00 and 1.60×10^1 for enterococci (Table 4.10).

Average bacterial numbers were generally high on the Rocky brand; 2.77×10^1 for total coliforms, 2.11×10^1 for faecal coliforms and 1.14×10^1 for *E. coli*. However, the S&M brand recorded higher numbers for enterococci (8.57) (Table 4.10).

Statistically significant differences were recorded between brands for total coliform ($p<0.017$) and *E. coli* ($p<0.007$) numbers. However, no statistically significant differences were recorded between brands for faecal coliform ($p>0.05$) and enterococci ($p>0.05$) numbers (Table 4.10).

Table 4.10: Geometric mean microbial indicator numbers on factory plastic - bagged sachet water sold within the Kumasi metropolis

Total coliforms

Sachet water brands	Mean count	Range
Mobile	1.16×10^{1}	$8.00 – 1.50\times 10^{1}$
Davis	1.86×10^{1}	$1.70\times 10^{1} – 2.00\times 10^{1}$
Rocky	2.77×10^{1}	$2.00\times 10^{1} – 3.80\times 10^{1}$
Boadwoo	1.09×10^{1}	$8.00 – 1.60\times 10^{1}$
Everkool	2.38×10^{1}	$1.80\times 10^{1} – 3.00\times 10^{1}$
S&M	2.13×10^{1}	$1.50\times 10^{1} – 2.80\times 10^{1}$
Cobb-Ji	2.04×10^{1}	$1.50\times 10^{1} – 3.00\times 10^{1}$
St. Hubert	2.50×10^{1}	$1.50\times 10^{1} – 4.00\times 10^{1}$
Dominion	1.25×10^{1}	$1.00\times 10^{1} – 1.50\times 10^{1}$
Gofex	2.72×10^{1}	$2.40\times 10^{1} – 3.00\times 10^{1}$

Faecal coliforms

Sachet water brands	Mean count	Range
Mobile	7.83	$6.00 – 1.00\times 10^{1}$
Davis	1.10×10^{1}	$1.00\times 10^{1} – 1.20\times 10^{1}$
Rocky	2.11×10^{1}	$1.80\times 10^{1} – 2.60\times 10^{1}$
Boadwoo	4.31	2.00 – 8.00
Everkool	1.50×10^{1}	$1.40\times 10^{1} – 1.60\times 10^{1}$
S&M	1.34×10^{1}	$9.00 – 1.80\times 10^{1}$
Cobb-Ji	1.59×10^{1}	$1.00\times 10^{1} – 2.70\times 10^{1}$
St. Hubert	1.19×10^{1}	$7.00 – 3.00\times 10^{1}$
Dominion	8.90	$8.00 – 1.10\times 10^{1}$
Gofex	1.25×10^{1}	$1.00\times 10^{1} – 1.50\times 10^{1}$

Escherichia coli

Sachet water brands	Mean count	Range
Mobile	4.16	3.00 – 6.00
Davis	7.86	6.00 – 9.00
Rocky	1.14×10^{1}	$7.00 – 1.90\times 10^{1}$
Boadwoo	3.30	3.00 – 4.00
Everkool	1.06×10^{1}	$9.00 – 1.20\times 10^{1}$
S&M	7.23	6.00 – 9.00
Cobb-Ji	9.74	$7.00 – 1.20\times 10^{1}$
St. Hubert	5.81	4.00 – 7.00
Dominion	6.65	6.00 – 7.00

Gofex	8.14	

Enterococci

Sachet water brands	Mean count	Range
Mobile	6.00	3.00 – 9.00
Davis	2.47	1.00 – 5.00
Rocky	7.83	6.00 – 1.00× 10^1
Boadwoo	3.04	2.00 – 7.00
Everkool	7.56	6.00 – 9.00
S&M	8.57	7.00 – 1.00× 10^1
Cobb-Ji	5.31	5.00 – 6.00
St. Hubert	7.11	4.00 – 1.00× 10^1
Dominion	6.21	5.00 – 8.00
Gofex	8.32	4.00 – 1.60× 10^1

4.2.2 Vendors handling practices effect on *Salmonella* numbers on different brands of factory bagged plastic sachet water

Average *Salmonella* numbers on the different brands of the factory bagged sachet water were generally low and varied between 3.30 and 7.65 with no statistically significant differences ($p>0.05$) between the brands (Table 4.11).

Table 4.11: Geometric mean *Salmonella* counts on factory plastic - bagged sachet water

Sachet water brands	Mean count	Range
Mobile	5.01	3.00 – 7.00
Davis	3.30	3.00 – 4.00
Rocky	7.65	4.00 – 1.60× 10^1
Boadwoo	4.38	3.00 – 7.00
Everkool	7.23	6.00 – 9.00
S&M	4.82	4.00 – 7.00
Cobb-Ji	4.00	4.00 – 4.00
St. Hubert	5.09	3.00 – 1.10× 10^1
Dominion	5.28	3.00 – 7.00
Gofex	3.30	3.00 – 4.00

Salmonella counts were performed on 10 samples each of factory plastic - bagged sachets

4.2.3 Vendors handling practices effect on heterotrophic bacteria total viable numbers on different brands of factory bagged plastic sachet water sold in the Kumasi metropolis

Of the ten randomly selected factory plastic-bagged sachet drinking water samples sold on the streets of the Kumasi metropolis, numbers of heterotrophic bacteria varied between 4.13×10^5 and 1.98×10^6. Averagely, counts were low (7.61×10^5) on Dominion and high (1.20×10^6) on Boadwoo (Table 4.12).

There were no statistically significant differences in total viable counts between the brands ($p > 0.05$) (Table 4.12).

Table 4.12: Geometric mean total viable count on factory plastic - bagged sachet water

Sachet water brands	Mean count	Range
Mobile	1.11×10^6	$7.76 \times 10^5 - 1.51 \times 10^6$
Davis	8.90×10^5	$4.13 \times 10^5 - 1.53 \times 10^6$
Rocky	8.59×10^5	$4.90 \times 10^5 - 1.16 \times 10^6$
Boadwoo	1.20×10^6	$4.63 \times 10^5 - 1.98 \times 10^6$
Everkool	1.17×10^6	$1.13 \times 10^6 - 1.23 \times 10^6$
S&M	9.61×10^5	$6.03 \times 10^5 - 1.30 \times 10^6$
Cobb-Ji	9.07×10^5	$7.66 \times 10^5 - 1.24 \times 10^6$
St. Hubert	1.14×10^6	$7.66 \times 10^5 - 1.67 \times 10^6$
Dominion	7.61×10^5	$4.20 \times 10^5 - 1.26 \times 10^6$
Gofex	8.07×10^5	$5.33 \times 10^5 - 1.19 \times 10^6$

Total viable counts (TVCs) were performed on 10 samples each of factory plastic - bagged sachets

4.3 Observational Checklist on the Hygiene and Sanitation Status of Vendors Selling Factory-Bagged Drinking Water

Results of the survey showed that 70% of vendors presented a poor shabby appearance with only 30% being relatively well dressed and showing some level of cleanliness. It was also observed that 95% of the vendors did not wash their hands at all throughout the day (Table 4.13). Vendors were also observed to be using varying water carrying receptacles in their trade; 70% aluminum pans, 20% plastic bowls and 10% metals baskets. Sanitary conditions in and around the environment where these vendors freeze their water or organized them into the carrying receptacles were 80% generally poor with uncovered bins, insects, goats and fowls in the vicinity (Table 4.13).

Table 4.13: Observations on Hygienic practices by vendors of sachet water

Questions	Yes	No
	Hygiene	
Did the vendor appear clean?	30%	70%
Was the vendor appropriately dressed?	20%	80%
Did the vendor handle money when adding ice-blocks to cool the water?	90%	10%
Did the vendor wash their hands after purchasing the water from distributors before cooling?	5%	95%
What did the vendors use in selling the water?		
Aluminum pans	70%	
Metal baskets	10%	
Plastic bowls	20%	
	Sanitation	
How would you rate the vendor premises?		
Very clean		
Fairly clean		20%
Poor	80%	
Were there animals or Garbage around the area where vendors operate?	30%	70%
How would you rate the overall sanitation of the surroundings where the vendors operated?		
Vey clean		
Fairly clean		10%
Poor	90%	

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

This study has showed that plastic-bagged sachet drinking water sold on the streets and other outlets within the Kumasi Metropolis has varying microbial populations. Ideally, well sealed plastic-bagged sachet drinking water would be expected to be free of microorganisms, however, bacterial numbers were on average (10^2) total coliforms, (10^2) faecal coliforms, (10^1) *Escherichia coli*, (10^2) enterococci, and (10^1) for *Salmonella* in all the ten brands studied (Table 1-3). The reason for the high bacterial counts in the different brands could be several. Firstly, the quality of the source water before treatment. Most sachet water producers are known to draw their water from shallow contaminated wells which become contaminated through surface runoffs, especially during the rainy season. Secondly, most of the plastic bags used in bagging the water are not kept under hygienic conditions. Some are even observed to have grown mouldy before use. Lack of treatment for prevention of bacterial growth in some of the brands sold in Kumasi may also partly explain the observed higher rate of contamination in accordance with the findings of other studies conducted (Hunter and Burge, 1987; Warburton *et al.*, 1986). In Africa, about 340 million people do not have access to a disease and parasite free water source. Due to inadequate environmental sanitary measures, water supply and poor sanitation service 70 percent of diseases in Ghana is brought on by the quality of drinking water (http://www.ghanaontop.com).

5.1.1 Bacterial numbers in factory-bagged plastic sachet drinking water stored at different temperatures

Factory-bagged sachet water stored under varying temperature regimes, normal atmospheric temperatures (32.0°C), room temperatures (26.1°C) and in the refrigerator (4°C) also had varying microbial populations. Samples stored under normal atmospheric conditions increased in microbial numbers by more than 100% after six week of storage with statistically significant differences ($p<0.001$ between the weeks. This could be due to their exposure to unfavorable temperature conditions. Most bacteria are found to positively increase in number under an optimal temperature range of 25 – 45^{o}C. The factory-bagged sachet water were often exposed to natural sunlight, rain and in liquid environment for six weeks which makes it favorable for bacteria growth, especially thermotolerant coliforms. A study by Dodoo *et al.* (2006) reported that sachet water stored at 40ºC (sun exposure) which was similar to the sachet water being sold in open air markets or on streets by vendors recorded total coliform counts as high as 98 million CFU/100ml and found 45% of the samples contaminated. Most small scale producers of sachet drinking water tend to use common methods in decreasing bacterial growth by boiling before packaging for sale. This could reduce the desired quality of the water while also allowing for microbial entry if not carefully handled and or monitored. Although the microbial quantity levels in processed water are often initially low, they can evolve rapidly to high levels during storage (Stickler, 1992).

At the refrigeration temperature of 4^{o}C, there was a general decrease in the levels of bacteria after six weeks of storage. This might be so because lower temperatures are often not suitable for mesophilic organisms and might have contributed to the decrease in bacterial numbers since it slows down the vital activities and may affect the viability of the organisms. It was observed that most of the brands were not kept under cold conditions by the vendors and distributors thereby enhancing increases in the bacterial load. Although, the Ghana Environmental Protection Agency Act (GEPA Act 490, 1994) indicates that drinking water products should not be exposed to sunlight, most of the vendors and distributors do not comply with these directives. Reasons for this could be that these vendors and distributors do not have the capacity to do so, they are not informed of the consequences and also the regulations are not enforced.

The room temperature (26.1^{o}C) used for this study might have affected the optimal growth of the *E. coli*, thus accounting for the decrease in their numbers. Compared to total coliforms, faecal coliforms, enterococci, *Salmonella* and heterotrophic bacteria numbers which showed significant increases, the *E. coli* might be much more sensitive to temperature, hence the decrease. The optimal temperature for mesophilic organisms ranges from 25 to 37^{o}C (http://www.disknet.com/indiana_biolab/b062.htm). A study conducted by Nsaze and Babarinde (1999) in the United Arab Emirates demonstrated that these organisms multiply more easily between 25 and 37°C.

5.1.2 Vendors handling practices effect on the microbial numbers on different brands of factory bagged plastic sachet water

Poor handling practices of the sachet bags did contribute to the presence of the variable microbial populations, as shown in this study. Most vendors were illiterates from poor family backgrounds and did not operate under any rules and regulations. The cooling processes and equipments used by these vendors also influenced the microbial load for sachet drinking water. It was observed that equipments such as ordinary rubber buckets with tops, coolers in poor conditions and utensils used to store retailed sachet drinking water were in poor hygienic conditions; utensils were left uncovered, water for sale kept in unsuitable containers; areas of sale unhygienic and thus encouraged flies, wild birds, and fowls roamed freely in the vicinity. Another uncertain event was the infrequent washing of hands before placing the hand in the sac or container for sachet water, while the water used in washing hands were often dirty. In a study in Ibadan, Nigeria, Ajayi *et al.* (2008) reported higher numbers of coliforms in sachet water compared to bottled water; evidence of poor hygienic practices and handling methods. In a similar study, Ashbolt, (2004) attributed contamination of sachet drinking water to poor personal hygiene of handlers and general environmental hygiene. According to Obiri-Danso *et al.*, (2003), hand filled and hand-tied sachet water was of poor microbial quality and occurrence of indicator organisms in the water constitutes serious threat to the community.

Earlier investigations conducted on the safety of drinking water in Ghana have shown that bottled water was often sold under very high hygienic conditions. This is because most of these shops were managed by persons with at least secondary education thus resulting in higher hygienic practices. In factory – bagged sachet water, the containers used in packaging are produced in very large quantities, stored over long period of time and used when needed (Obiri-Danso *et al*., 2003; Ferreira *et al*., 1994; Leclerc, 1994). Through the handling practices, storage, production and transportation to the consumer, the sachet may also get contaminated.

The Food and Drugs Board (FDB) of Ghana has published guidelines for small-scale producers of sachet-packaged drinking water. However, many of these producers have failed to comply with these guidelines as is evident by the high microbial populations in all the brands studied. Also the World Health Organization guidelines (2008) on the bacteriological quality of drinking water state that *Escherichia coli* of faecal coliforms must not be detectable in any 100ml of the water (Appendix 6). This study shows that *Escherichia coli* counts (10^1) and faecal coliform counts (10^2) in all the water samples did not meet the WHO guideline (2008).

There may be no evidence that high counts of heterotrophic bacteria may have led to any health problems but they can be good indicators of the overall quality of production (Ferreira *et al.*, 1994). Hence, the study shows that there could be some health risk in the intake of sachet drinking water without modifications in processing, handling, sources and quality of bags used. This was affirmed in a survey carried out at the Komfo Anokye Teaching Hospital (KATH) from 2006 – 2009 on patients with water related diseases. In 2008, of the 175 patients who reported of typhoid infections between ages 0 – 30 years, about 27.4% were children. From 2006 – 2009, diarrhea and cholera cases reported for outpatient and inpatients at the Komfo Anokye Teaching Hospital (KATH) were mostly adults. In addition, cases of typhoid, diarrhea, and cholera are of common occurrence and reported every year at the Komfo Anokye Teaching Hospital (KATH). The high prevalence of diarrhea among children and infants can be traced to the use of unsafe drinking water and unhygienic practices (Tortora *et al.*, 2002).

5.2 CONCLUSION

The study shows that due to the poor handling practices by vendors of plastic-bagged sachet drinking water in the Kumasi Metropolis the bag is highly contaminated with bacteria on the outside. The quality of the drinking water in the plastic-bag also deteriorates on exposure varying environmental temperatures.

Most of sachet drinking water do not comply with the WHO bacteriological quality on safe drinking water and the Food and Drugs Board guidelines of Ghana on small-scale sachet drinking water.

5.3 RECOMMENDATIONS

- The need for producers of factory-bagged drinking water in Ghana to improve their production operations, especially in terms of hygiene, and to ensure strict compliance with guidelines as set by Ghanaian's standard regulatory body;

- That Food and Drugs Board of Ghana monitors all producers and publish on a regular basis the list of producers, who have registered their products;

- Ghana Standard Board (GSB) should make sure that vendors and distributors have the products stored properly;

- Food and Drugs Board should conduct tests on these products and alert consumers about those which are unwholesome products;

- There should be effective awareness campaign amongst the producers to avoid contamination resulting from human activities;

- Training should focus on the need for good personal hygiene and compliance with good manufacturing practices;

- Control of hygiene certificates by staff of organizations seeking a license for the production of factory-bagged packaged drinking water should be a useful measure before approval is given.

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APPENDICES

Appendix 1: TWOWAY ANOVA results with parameters at different storage temperatures

Annex i: Log values of total coliform

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum	5	45486	9097	3.27	
Brand	10	1306791	130679	46.94	<.001
Conditions	2	1644046	822023	295.29	<.001
Brand. Conditions	20	561847	28092	10.09	<.001
Residual	160	445400	2784		
Total	197	4003569			

Annex ii: Log values of faecal coliform

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum	5	105266	21053	10.25	
Brand	10	576571	57657	28.07	<.001
Conditions	2	464295	232148	113.01	<.001
Brand. Conditions	20	228653	11433	5.57	<.001
Residual	160	328669	2054		
Total	197	1703455			

Annex iii: Log values of *Escherichia coli*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum	5	384.498	76.900	8.69	
Brand	10	685.090	68.509	7.74	<.001
Conditions	2	1391.038	695.519	78.57	<.001
Brand. Conditions	20	515.495	25.775	2.91	<.001
Residual	160	1416.335	8.852		
Total	197	4392.457			

Annex iv: Log values of enterococci

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum	5	10941	2188	0.71	
Brand	10	586803	58680	18.99	<.001
Conditions	2	784773	392386	127.00	<.001
Brand. Conditions	20	463702	23185	7.50	<.001
Residual	160	494326	3090		
Total	197	2340545			

Annex v: Log values of *Salmonella*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum	5	12.122	2.424	1.94	
Brand	10	77.229	7.723	6.17	<.001
Conditions	2	63.947	31.974	25.56	<.001
Brand. Conditions	20	74.191	3.710	2.96	<.001
Residual	160	200.178	1.251		
Total	197	427.666			

Annex vi: Log values of total viable count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Sample stratum	5	2.200×10^{12}	4.401×10^{11}	0.64	
Brand	10	4.543×10^{13}	4.543×10^{12}	6.65	<.001
Conditions	2	4.211×10^{13}	2.106×10^{13}	30.82	<.001
Brand. Conditions	20	1.340×10^{13}	6.698×10^{11}	0.98	0.489
Residual	160	1.093×10^{14}	6.832×10^{11}		
Total	197	2.125×10^{14}			

Appendix 2: ONEWAY ANOVA results with parameters for the brands

Annex i: Log values of total coliform

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Brand	9	1182.80	131.42	3.08	0.017
Residual	20	852.67	42.63		
Total	29	2035.47			

Annex ii: Log values of faecal coliform

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Brand	9	618.17	68.69	2.23	0.065
Residual	20	616.00	30.80		
Total	29	1234.17			

Annex iii: Log values of *Escherichia coli*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Brand	9	211.633	23.515	3.71	0.007
Residual	20	126.667	6.333		
Total	29	338.300			

Annex iv: Log values of enteroccoci

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Brand	9	123.333	13.704	1.70	0.155
Residual	20	161.333	8.067		
Total	29	284.667			

Annex iv: Log values of *Salmonella*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Brand	9	84.967	9.441	1.21	0.342
Residual	20	156.000	7.800		
Total	29	240.967			

Annex v: Log values of total viable count

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Brand	9	9.647	1.072	0.53	0.834
Residual	20	4.030	2.015		
Total	29	4.994			

Appendix 3: Cases of typhoid perforations from the Komfo Anokye Teaching Hospital (KATH) from 2006 – 2009 between the age of 0 – 30 years

Typhoid Perforations	0 - 5	6 -10 yrs	11-15yrs	16-20yrs	21- 25 yrs	26- 30 yrs	TOTAL
2006	15	18	11	10	15	10	96
2007	14	32	23	28	15	9	138
2008	48	29	22	27	12	14	175
2009	19	30	21	15	13	9	125

Appendix 4: Cases of adult inpatient diarrhea (21 – 65+ years) at Komfo Anokye Teaching Hospital from 2006 – 2008

Year	21 - 30 yrs		31 - 40 yrs		41 – 50 yrs		51 – 64 yrs		65+ Yrs		TOTAL	
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
2006	4	2	3	1	1	3	0	1	1	2	9	9
2007	1	1	2	1	0	0	1	0	4	2	8	4
2008	1	0	0	2	0	0	0	1	0	1	1	4

Appendix 5: Cases of outpatient cholera patients reported at the Komfo Anokye Teaching Hospital from 2006 – 2009 between age group of 1 – 60+ years.

Year	1 - 4 yrs		5 - 14 yrs		15 - 44 yrs		45 - 59 yrs		60+ yrs		TOTAL	
	M	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*	*M*	*F*
2006	5	4	4	2	0	3	25	26	5	2	39	37
2007	1	2	0	0	4	12	14	55	3	5	18	74
2008	0	0	0	0	1	0	0	2	0	0	1	2
2009	0	0	0	0	0	0	0	73	0	0	73	0

Appendix 6: WHO Guideline values for verification of microbial quality

Organisms	Guideline Values
All water directly intended for drinking	
E.coli or thermotolerant coliform bacteria	Must not be detectable in any 100-ml Sample
Treated water entering the distribution System	
E.coli or thermotolerant bacteria	Must not be detectable in any 100-ml Sample
Treated water in the distribution system	
E.coli or thermotolerant bacteria	Must not be detectable in any 100-ml Sample

Appendix 7: WHO Categorization of drinking-water systems based on compliance with performance and safety targets

	Proportion (%) of samples negative for *E.coli*		
	Population size:		
Quality of water system	<5000	5000–100000	>100000
Excellent	90	95	99
Good	80	90	95
Fair	70	85	90
Poor	60	80	85