IS THE SHELF LIFE OF BOTTLED WATER A CAUSE FOR CONCERN?

by

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DECLARATION

I, the undersigned, Yvone Lieketseng Liee, hereby declare that this dissertation is the result of my own independent work/investigation. I have not previously in its entirety or in part submitted it to any university for degree purposes. Other sources are acknowledged by giving explicit references. A bibliography is appended. I hereby give consent for my dissertation, if accepted, to be available for photocopying and for interlibrary loan, and for the title and the summary to be made available to outside organizations.

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DEDICATION

This study is dedicated to my family who always believed in me and prayed for my prosperity in education. They taught me that the "sky is the limit". It is also dedicated to my son, Kamohelo Liee who was so lovingly patient while Mommy studied.

ABSTRACT

Bottled water like any drinking water used for human consumption should be safe and wholesome to ensure adequate public health protection. This is due to potential health effects of concern such as endocrine disruption, toxicity teratogenicity, mutagenicity and carcinogenicity. Despite the number of regulatory bodies, publications on bottled water and speculations on its public health significance, many questions remain to be answered. One of the questions is whether the shelf life of bottled water is a cause for concern. The aim of the study was to determine the shelf-life of various commercial bottled waters by monitoring the variation in microbiological, chemical and aesthetic qualities of bottled water. A total of five commercial bottled water brands (A, B, C, D, E) each containing bottles from the same batch consisting of spring water, mineral water and bottled tap water were purchased directly after being bottled from different distributors around Gauteng in South Africa. All samples were stored at room temperature with artificial lighting and controlled temperature for a year thus mimicking typical conditions in retail outlets, supermarkets and in homes. Analyses were conducted over a period of 12 months, at monthly intervals**.** Within days of being purchased, high Heterotrophic plate counts (HPC) bacteria exceeding drinking water alert level >5 000 cfu/ml was common in four bottled water brands. Growth succession occurred during the period of study as various algal species were growing and accumulating on all bottled water tested. Total coliforms (TC), faecal coliforms (FC) and *E.coli* were not detected in all the bottled water tested. Yeasts and moulds were also not detected in all the bottled water. There were insignificant variations during the period of study for turbidity, pH, TDS, conductivity, and colour. These did not indicate any potential impact on aesthetic quality of bottled water. Two bottled water brands had hardness measures as low as 11mg/ℓ as $CaCO_3$ making the water too soft which has an effect on taste. Radioactive substances, trihalomethanes, heavy metals, pesticides and other chemical contaminants were not found at levels that can be detrimental to human health.

Key words: Bottled water, shelf-life, water quality, health concern, chemical, biological and aesthetic quality

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LIST OF ACRONYMS, SYMBOLS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrometry
AIDS	Acquired Immunodeficiency Syndrome
ATCC	American Type culture collection
cfu	Colony Forming Unit
CO	Cytochrome oxidase
DWAF	Department of Water Affairs
E.coli	Escherichia coli
EC	European Commission
EU	European Union
FC	Faecal coliform
FDA	Federal Drug Administration
HACCP	Hazard Analysis Critical control Points
HIV	Human Immunodeficiency Virus
HPC	Heterotrophic plate count
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
MF	Membrane Filtration
NTU	Nephelometric Turbidity Units
ONPG	*o*-nitrophenyl-ß-D-galactopyranoside
SANAS	South African National Accreditation System
SANS	South African National Standards
TC	Total coliform
TDS	Total dissolved solids
THM	Trihalomethane
USA	United States of America
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

LIST OF ACRONYMS, SYMBOLS AND ABBREVIATIONS

S*pp*	Species
As	Arsenic
Cd	Cadmium
Pb	Lead
Ba	Barium
Sb	Antimony
V	Vanadium
Zn	Zinc
Cr	Chromium
Ni	Nickel
Se	Selenium
Cu	Copper
Ca	Calcium
Co	Cobalt
Mo	Molybdenum
Be	Beryllium
Mn	Manganese
Fe	Iron
Al	Aluminium
U	Uranium
NO_3^-	Nitrates
SO_4^{2-}	Sulphate
B	Boron
F^-	Fluoride
K	Potassium
Na	Sodium
Cl^-	Chloride

LIST OF ACRONYMS, SYMBOLS AND ABBREVIATIONS

Cr	Chromium
Ca	Calcium
Mg	Magnesium
Ni	Nickel
Pb	Lead
mg	Milligram
mg/ℓ	Milligram per Liter
µg/ ℓ	Microgram per Liter
<	Less than
>	Greater than
%	Percentage
°C	Degrees Celsius
mℓ	Milliliter
mS/m	millisiemens per metre

CHAPTER 1 INTRODUCTION

1.1 BACKROUND

Any potable water that is intended for public consumption, bottled, distributed and offered for sale, is regarded as bottled water (Ehlers *et al.*, 2004). Bottled water can be obtained from natural springs, wells, boreholes, municipal systems or other sources which are considered to be safe, of sanitary quality and fit for human consumption. This is regarded as natural bottled water. Mineralised bottled water is potable water with added salts (Cabral *et al*., 2002). According to the South African Department of Health (DoH) Act, Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act no.54 of 1972), bottled water means" water that is packaged in sealed containers of various forms and capacities, and which is offered for sale as a foodstuff for human consumption, but does not contain sugars, sweeteners, flavourings or any other foodstuff" (DoH, 2006).

The consumption of bottled water in countries such as North America, Greece and South Africa has significantly increased during the last decade (Kokkinakis *et al*., 2008). In many parts of the world, non-carbonated bottled water has become more popular than the carbonated brand, being a substitute for tap water in many homes. In Canada the bottled water industry boom began in the early 1980's and has shown a significant growth since then. The industry continued its rapid growth over the past decade. This trend has been observed worldwide, but the rates of increase vary from country to country. In New Zealand, per capita consumption of bottled water has increased at a rate of 20% per year (from 1997 to 2004) (Doria, 2006). In Eastern Europe countries and in the Asia-Pacific region, the consumption increased by the about 13% per year, and in the USA and Western Europe, this rate was about 6% per year (from 1997 to 2004) (Doria, 2006).

1.2 USE OF BOTTLED WATER-THE CONSUMER'S PERSPECTIVE

Various reasons have been reported for the higher trend of bottled water use in many countries. Some reasons include:

- Consumer awareness about increasing water pollution;
- Deficiencies in municipal water supplies in terms of aesthetic, chemical and microbiological water quality;
- Successful marketing strategies of bottled water by the bottling companies;
- The easy availability and reasonable pricing has popularized the utilisation of bottled drinking water by a number of people who can afford it; and
- Bottled water is generally considered safe and is taken for granted by people without question. For example 'spring water' is perceived as a pristine, natural source of water.

Bottled mineral water has long been consumed as a safer alternative in countries with reticulated water of uncertain quality (Cabral *et al*., 2002). However, consumers should be aware that bottled water is not necessarily safer than tap water (Ehlers *et al*., 2004).

1.3 PUBLIC HEALTH ASPECTS AND CONCERNS ABOUT BOTTLED WATER USE

Although the consumption of bottled water is increasing, there are many concerns, these include:

- Poor regulation by different member states;
- Uncertainty of the shelf-life and possible health implications; and
- Uncertainty of water quality status.

1.3.1 Bottled water Regulations

In most countries bottled water is regulated as a food commodity. Criteria have been set for physical, chemical and biological aspects as well as for organoleptic properties of water. In most situations the bottled water quality has to comply with treated drinking water guidelines or standards.

1.3.1.1The South African perspective

The Department of Health in South Africa regulates the entire bottled water industry according to the Foodstuffs, cosmetics and disinfectants. Regulations from the act are revised on an ongoing basis to ensure public health protection (DoH, 2006). This includes bottled water from springs, wells, boreholes, municipal supplies and other sources. However, these sources are known vehicles for enteric pathogens such as bacteria, parasites and viruses (Ehlers *et al*., 2004). It is stipulated in the legislation that 'antimicrobial' treatments should be used in order to conserve the microbial fitness for human consumption. From source to marketing, drinking water should conform to the microbiological requirements set for bottled water (http://www.doh.gov.za/docs/regulations/2006/reg0718.pdf).

The bottled water industry in South Africa is considered to be lucrative. Sales from this industry amounts to R1.7 billion a year with an expected annual growth of 25 percent expected for the next decade (http://www.nbws.org/). While this industry might be attractive, it might trigger ripple effects like small businesses supplying the public with a sub-standard quality of bottled water (http://www.nbws.org/). In 2003 the South African bottled mineral water market increased by an estimated 20 percent including consumption from all sectors, such as retail wholesale and imports. This market growth was attributed to two clearly defined factors such as health and convenience (http://www.nbws.org/).

In order to understand microbiological quality of bottled water, a random survey was conducted by Ehlers *et al.* in 2004. This was a proactive response to an increase in the number of people drinking bottled water. Findings were that, eight out of ten samples of the bottled water were free from pathogenic microbes and that bottled water generally complied with current drinking water legislation (Ehlers *et al*.,2004). According to SANBWA any harmful bacteria must be absent. However, these bacteria are not specified in the regulation (Ehlers *et al.*, 2004).The South African Bureau of Standards (SABS) specifies a Heterotrophic Plate Count (HPC) limit of less than 100cfu/mℓ for bottled water. This limit has been endorsed by the Department of Water Affairs (DWA) and the National Department of Health. Faecal coliform bacteria indicate the presence of faecal pollution in the water. These faecal coliforms should be 0cfu/mℓ in bottled natural water for human consumption purposes (Ehlers *et al.*, 2004).

The South African National Bottled Water Association (SANBWA) is the recognised regulatory body of the South African water industry. The role SANBWA plays is to ensure the quality standards of its members are being adhered to by bottlers and it protects the consumers against misinterpretation from within the industry. The South African Standards were adopted with European quality guidelines, with which to regulate and monitor the quality of local bottlers (www.sanbwa.org.za).

1.3.1.2 An international perspective

Bottled water has been implicated as the source of cholera, typhoid fever as well as traveller's disease in countries such as Portugal and Spain (Ehlers *et al.*, 2004). The Centres for Disease Control and Prevention have reported a 1994 cholera outbreak that occurred in the Marianas Island, a trust territory of the US. In-plant contamination played a major role in this incident. Cabral and Fernandez described the fungal spoilage of bottled mineral water in Argentina (Cabral & Fernandez, 2002).

1.3.1.3 Regulation of bottled water in the United States of America (USA)

In the United States of America bottled water is regulated as food by the Food and Drug Administration under the Federal Food, Drug, and Cosmetic act (FFDCA). Municipal water is not regulated as food by FDA but rather as a commodity by the Environmental Protection Agency (EPA). Bottled Water is one of the most extensively regulated food products under the FDA's jurisdiction amongst food products. Bottled water is deemed to be in unpure if:

- A poisonous substance may render it injurious to human health;
- It contains industrial contaminants, unapproved pesticides or other substances harmful to human health; and
- It becomes contaminated during processing by dirt or other debris. (www.waterbottlers.com).

The U.S Food and Drug Administration (FDA) regulate bottled water. The FDA has established specific regulations for bottled water in Title 21 of the code of Federal regulations (21CFR) that includes the required standard of identity regulations that define different types of bottled water such as spring water, mineral water and standard quality regulations that establish allowable levels for contaminants (chemical, physical, microbial and radiological) in bottled water (Prosnick & Kim, 2002).

Specific FDA regulations in the bottled water area cover current Good Manufacturing Practice (CGMP) regulations for the processing and bottling of bottled drinking water. Labelling regulations (21 CFR part 101) and CGMP regulations for foods in general also apply to bottled water. Recent regulatory activity on bottled water includes adoption of allowable levels of certain disinfectants and disinfection by-products in the quality standard for bottled water and publication of a feasibility study on the appropriate methods for providing consumers with information on the contents of bottled water (Prosnick & Kim, 2002).

1.3.1.4 Regulation of bottled water in Europe

Bottled waters must meet the highest safety standards. Bottled water is carefully protected at source, in the bottling process and in the bottle itself according to Hazard Analysis Critical Control Points (HACCP) principles. HACCP is a food safety system which identifies steps critical to consumer safety imposes controls and highlights corrective action where necessary. Before official approval is given to start bottling water a rigorous set of rules laid down by national authorities have to be met in order to ensure that practices are hygienic. Following approval, every bottler has to continue to prove on a regular basis that the water is safe to drink. In addition, there is regular testing of all water destined for bottling to ensure that it is microbiologically safe. Bottled water sources must also be carefully protected to ensure that they do not get contaminated.

The fact that there is no clarity on the prescribed minimum duration of shelf-life of bottled water at national level emphasizes the need for this study in South Africa. The main aim of this study was to establish the shelf-life of the commercially available bottled water brands in SA. Rosenberg (2003) reported that people have a perception that tap water is of inferior quality while bottled water is pure. Few people will consider that bottled water may have caused the symptoms of their sickness. Although it is rare to get sick from drinking bottled water, isolated incidents of outbreaks from its consumption have been reported (Rosenberg, 2003).

1.3.2 Uncertainty of bottled water quality status

It is important to specify the quality of bottled water at the point and time of bottling and to continue monitoring the quality of the water while it is in use. However, this is not the case in most instances. Of major concern has been the microbial quality of bottled water given the acute nature attributed to waterborne diseases that fall within this class (Ehlers, 2004, Kokkinakis *et al*., 2008). Microbial surveys carried out worldwide indicate various problems with bottled water such as high heterotrophic plate count (HPC) levels, fungal spoilage, presence of resistant antibiotics, Pseudomonas species, the presence of *Staphylococcus aureus, Aeromonas hydrophyla*, typhoid fever and traveller's diarrhoea (kokkinakis *et al*., 2008). Increased illness from contamination of bottled water due to *Campylobacter* infection has been reported (Evans *et al*., 2003)

Bacterial indicators do not always indicate the presence of pathogenic viruses and protozoa. Compared to coliform bacteria, viruses and protozoan cysts are known to be more persistent in the environment and more resistant to chemicals used for water treatment processes. It has been confirmed that microbial contamination of bottled natural water is most likely to occur due to improperly cleaned equipment and bottles, failure of ozonation or Ultra Violet (UV) equipment or due to contamination of the water by workers (Ehlers *et al.*, 2004). In all the studies conducted the chemical quality remains uncertain (Lal and Kaur, 2006, Ehler, 2004).

As a result, the growth of bacteria in stored bottles has been reported due to bottling materials that during the storage can release organic matter and provide additional substrates for the microbial growth. It has also been observed that there is possibility of pathogen ultra microcell presence in the raw water that can pass through the filters, survive and grow slowly in the final water. Under improper and /or prolonged storage conditions of bottled water can grow to levels that may be harmful to human health (Kokkinakis *et al.*, 2008) especially immune-compromised individuals such as the infirm, elderly, young infants, people living with HIV/AIDS, people on immunosuppressive chemotherapy, and transplant patients (Ehlers et a*l*.,2004).

The chemical quality of bottled water although believed to be fairly stable with time can be of concern if not monitored. This is always the case with radioactivity. Natural waters originating from springs or wells may contain natural radionuclides predominantly of the uranium-radium decay series. Concentrations vary over a wide range depending on the bedrock of the aquifer from which the spring flows, the pH of the water at the source of radionuclides transport, the time each well was pumped, the amount of pumped water, the presence of any water treatment prior to bottling of the water and the relative depths of the wells if more than one well is utilized. Water is not likely to be a major source of natural radionuclides intake compared to food, yet the increasing contribution of natural radionuclides in bottled water to the total ingestion dose should be assessed for radiation protection reasons (Kralick *et al*., 2003).

1.3.3 Bottled water shelf-life

The shelf-life of Bottled water and its potential health effects on humans impacts thereof has not been established. Bottled water has been implicated as the source of outbreaks of cholera, typhoid fever as well as traveller's disease in countries such as Portugal and Spain (Ehlers *et al*., 2004).Recently, Nor virus (previously known as Norwalk like viruses) sequences were detected in these European brands of mineral water (Ehlers *et al*., 2004). None of these regulations discussed in preceding sections address the aspect of bottled water shelf-life. The assumption is that once bottled, the water will remain safe for consumption until it is opened.

The aim of this research was to monitor the microbiological, chemical and aesthetic qualities of bottled water at different intervals in order to determine the impact of the shelf-life of bottled water on water quality. The study has been therefore designed to assess the possibility of variation in physical, chemical, and microbiological water quality with time using different brands of bottled water.

1.4 RESEARCH QUESTION

Despite the number of regulatory bodies, publications on bottled water and speculations on its public health significance, many questions remain to be answered. There are established regulations for bottled water nationally and internationally. However none of these regulations give an indication of acceptable minimum and maximum duration of bottled water shelf-life and possible human health concerns thereof. This emphasizes the importance of the current study.

1.5 AIMS AND OBJECTIVES

1.5.1 Aims

- To monitor the microbiological, chemical and aesthetic qualities of bottled water at different intervals.
- To determine the shelf-life of various commercial bottled waters when stored at room temperature, as it is common in grocery stores and often at homes.

1.5.2 Objectives

The study is set out to address five objectives:

- Determine the variability of microbial and chemical quality of commercially available bottled water upon storage;
- Compare the results generated in this study to SANS 1657:2003 (South African Bureau of Standard for bottled water);
- Determine the optimum shelf-life of the bottled water brands tested using the testing parameters; and to
- Make relevant recommendations based on the results of this study.

CHAPTER 2 POTENTIAL HAZARDS OF BOTTLED WATER WITH REGARD TO SHELF-LIFE

2.1 INTRODUCTION

Since the early 1990s there has been a growing interest in providing drinking water that is trusted by consumers. Drinking water of a variety of qualities is packaged as bottled water and sold for public consumption throughout the world. Trends in sales and consumption of bottled water have risen significantly in the last two decades in both developed and developing countries (Peh *et al*., 2010). Bottled water sales worldwide exceed $5.7billion (Rosenberg, 2003). Large quantities of bottled water (between 40 percent – 60 percent globally) consisting of packaged tap water which, in some cases, may have been processed have been sold (Doria, 2006). Actually, a natural drinking water either spring or mineral water moved quickly from commodity to fashion (Peh *et al*., 2010).

There is still no clarity on the reason for the increase of consumption of bottled water and surveys conducted indicate different reasons for choosing bottled water over tap water. The drinking habits of consumers are also diverse and depend on environmental and economic factors (Nsanze *et al*., 1999). For example, bottled mineral drinking water is extensively marketed in the United Arab Emirates (UAE) and the whole Arabian Peninsula (Nsanze *et al*., 1999). Plenty of bottled water is consumed daily by the population because this region is mainly a desert with high temperatures, low humidity and no free-flowing water (Nsanze *et al*., 1999). Demographic variables can somehow influence bottled water usage and these variables include ethnic group, age income, occupation and gender. These patterns vary according to regions and country. Bottled water sales tend to be higher amongst African - Americans, Asians and Hispanic groups, which typically have lower incomes than whites (Doria, 2006). It was hypothesized that ethnic differences mirror the variability of water system quality between urban, suburban and rural areas. This is attributed to memories of the past problems caused by deficient tap water systems in deprived areas (Doria, 2006).

Consumers have expressed some dissatisfaction with the tap water quality, especially the organoleptic aspects (that is water quality characteristics that can be detected using the senses of taste, odour and sight). Taste tends to be more prevalent (Doria, 2006). Surveys conducted in Canadian regions, respondents identified organoleptics to be the main contributor for not consuming tap water and consumers opted to consume bottled water instead. The French survey (IFEN, 2000) resulted in the same findings. Doria further highlighted a study conducted by Falahee and Mc Rae in 1995 where blind comparison was done on different waters by British students (Doria, 2006). The outcome of the cases indicated that consumers preferred water with high mineral content derived from bottled and borehole waters (Doria, 2006).

Consumer perception of risk has been one of the factors. Consumers perceive tap water to have more negative impact on their health due to the use of chemicals during its production than bottled water. Hence, the use of bottled water is seen as a way of healthy living. For example a survey conducted in the US, indicated that consumers regard drinking tap water as a risk and drinking bottled water as a safer alternative. The growth of the bottled water market is also associated with 'healthy foods'. Organic-food buyers are more likely to drink bottled water than tap water. As cited by Hammit (1990) 70% organic food buyers drink bottled water and consumers in general prefer products that are presented as having higher health benefits (Doria, 2006).

This has resulted in consumers being exposed to different hazards since there are many types of bottled water. The biggest challenge facing the bottled water industry is the assigning of a realistic shelf-life given these different types of bottled water. It is known that some of these types of bottled water are not chemically treated before consumption. The different types of bottled water are summarized in Table 1.

2.1.1 Different types and uses of bottled water

According to the Department of Health in South Africa regulations from the Foodstuffs, cosmetics and disinfectants Act (DoH 2006) recognizes different types of bottled waters. In these regulations the various types of bottled water have been assigned to various meanings/definitions as stipulated in Table 1.

Table 1: Types of Bottled Water (DoH, 2006)

Types of Bottled Water	Definitions
Bottled Water	Water that is packed in sealed containers of various forms and capacities, and which is offered for sale as a foodstuff for human consumption, but does not contain sugars, sweeteners, flavourings or any other foodstuffs.
Bulk water	Non bottled water in direct contact with the surface of the transportation vessel and the atmosphere.
Carbonated natural water	Natural water which, after possible treatment and packaging, has been made effervescent by the addition of carbon dioxide from an origin other than that of the natural water
Decarbonised natural water	Natural water which, after possible treatment and packaging, has lower carbon dioxide content than that at emergence and does not visibly and spontaneously give off carbon dioxide under normal conditions of temperature and pressure
Naturally-carbonated natural water	Natural water which, after possible treatment and reincorporation of gas from the same source and packaged, taking into consideration normal technical tolerance, has the same content as carbon dioxide as it would if carbon dioxide were spontaneously and visibly given off under normal conditions of temperature and pressure
Natural mineral water	Bottled natural water which contains mineral salts in various proportions and which is characterized by the presence of trace elements and other substances such as calcium, magnesium, sodium and potassium
Natural Water	Bottled water derived from an underground formation which has not been modified and has not undergone treatment
Natural water with added carbon dioxide	Natural water which, after possible treatment and packaging, has more carbon dioxide content than such natural water had at its source
Non-carbonated natural water	Natural water which, by nature and after possible treatment and packaging, taking into consideration usual technical tolerance, does not contain free carbon dioxide in amounts larger than are necessary to keep the hydrogen carbonate salts present in water dissolved
Prepared Water	Bottled that has undergone any treatment acceptable for bottled waters that may originate from any type of water supply e.g. distilled tap water, tap water with added minerals etc.
Spring Water	Bottled water sourced from an underground formation from which water flows naturally to the surface of the earth, and which is collected from the spring or borehole tapping the underground formation, and which may be classified as a "natural water" or as" water defined by origin"
Water defined by its origin	Bottled Water originating from an underground or surface water system, which flows naturally from its source e.g. artesian water

2.2 Bottled water Shelf-life and potential related risks thereof

There is always anxiety about the quality of bottled water because it is stored for longer periods and not always preserved under the recommended conditions or because containers and bottles are re-used without adequate cleaning and disinfection (Amiridou and Voutsa, 2011). It is, however, indisputable that bottled water has been used as an alternative to tap water. This is still current practice. Some cited reasons for this change of consumer behaviour include:

- Public concern about the quality of tap water;
- The presence of disinfection by-products;
- The shortage of water in tourist areas especially during warm seasons;
- Consistent quality of bottled water; and
- Efficient marketing of bottled water. (Rosenberg *et al*., 2003, Ehlers *et al*., 2004)

In the United States of America, bottled water is date stamped for two years. During this time the bottled water should be placed in a dark, cool, dry area away from any solvents or chemicals. According to the International Bottled Water Association (IBWA), no suggestion has been made as to the limitation of the bottled water shelf-life. The two year expiration date acts as a lot number and is for stock rotation purposes. It does not mean that the product is substandard after that date. Thus according to the association, bottled water purchased in bulk is good indefinitely if stored appropriately, that is unopened in a cool, dry place away from odours and toxic substances (www.ehso.com).

2.2.1 Physical and aesthetic parameters of bottled water

Whether organoleptic problems are just nuisances or actual health threats (such as the almond like odour of cyanide), they are certainly the traits of drinking water that consumers first notice (Dietrich, 2006). From an evolutionary perspective, humans are hard-wired to notice differences and to proceed with caution when they are found. Consumers smell, taste and look at their water. Water that is not palatable, although safe, will be avoided (Dietrich, 2006). Most companies design their bottles in a way that aim to 'highlight' their positioning as a brand with cutting-edge style

(Doria, 2006). Plastic containers used for bottling from production at the manufacturing plant until the time of consumption, are said to be exposed to a wide variety of chemicals that leach from the container itself. The packaging most widely used is polyethylene terephthalate (PET). These bottles contain a variety of additives, catalyst chemicals that are involved in the plastic synthesis process, chemicals that impart physical stability and resistance to packaging, and odour-scavenger substances that eliminate the smell associated with chemicals leaching from plastic. It is further indicated that upon long-term storage, some of these chemicals could potentially leach from the plastic into the bottled water itself and impair the quality of this water.

2.2.2 The chemical quality of bottled water

It has been reported that the chemical quality or composition of different types of bottled water are largely influenced by geological and hydro-geological aspects of aquifers from which they are drawn (Peh *et al*., 2010). This has resulted in trace elements being introduced into water that is used to produce bottled water (Ikem *et al*., 2002, Botezatu *et al*., 2005, Walner *et al.,* 2008, Krachler and Shotyk, 2009). Some chemicals are introduced into treated water during drinking water production. The presence of organics, radionuclides, nitrates and nitrites in drinking water can lead to cancer, other human body malfunctions and chronic illness (Ikem *et al*., 2002).

2.2.2.1 Micro and Macro elements (inorganic) constituents of concern

Several trace elements (Ag, Be, Li, Ge, Sb, Sc, Te, Th, and U) are not monitored regularly in bottled waters. As a result, there is extremely limited data on the abundance and potential health impact of many toxic trace elements (Krachler and Shotyk, 2009). According to South African National Standard SANS 1657:2003, bottled water should not contain elements listed in column I, Table 2 in greater quantities than those indicated on column II, Table 2. The regulation further stipulates that bottled water with elements in column I, Table 2 in quantities greater than the acceptable range in column III of the Table 2 represents contamination to levels that are considered adverse to human health (DoH, 2006).

Table 2: Maximum allowable levels for permissible elements present in bottled water (DoH, 2006)

Substance	Maximum Level(mg/ℓ)	Acceptable range(mg/ℓ)
Antimony	0,005	0,005-0,01
Arsenic	0,01(as total arsenic)	0,01 – 0,05
Barium	0,7	-
Borate	5,0 (as total boron	-
Bromate	0,01	-
Cadmium	0,003	0,003-0,005
Chromium	0,05(as total chromium)	0,05 – 0,1
Copper	0,5	0,5-1,0
Cyanide	0,07	0,07-0,2
Lead	0,01	0,01-0,05
Manganese	0,5	0,05-0,1
Mercury	0,001	0,001-0,002
Nickel	0,02	0,02-0,15
Nitrate	50 (calculated as nitrate)	-
Selenium	0,01	0,01-0,02

Water is known as a universal solvent and even in its natural state it may contain substantial quantities of chemical elements in high concentrations. Therefore it may vary over a wide range depending on the aquifer lithology from which the spring flows and the presence of any water processing treatment methods prior to bottling of the water. At elevated concentrations some elements can be harmful to human health and can cause morphological abnormalities, mutagenic effects, reduced growth, and increased mortality in humans. Anthropogenic contaminants can also be found in the water. They can include (e.g. pesticides, fertilizers and nutrients), industrial chemicals (e.g. hydrocarbon derivatives and heavy metals) and many more (Guler, 2007). Chemical contaminants in drinking water pose a health risk for all of us, although sensitive groups may be more vulnerable to these pollutants than the general population. This population includes infants, the elderly, as well as people with weakened immune systems due to viral infections, immune disorders, cancer, chemotherapy or recent organ transplantation. Mistrust of tap water quality, causes to consumers to turn to bottled water hoping to find a guarantee of safety and quality (Naidenko *et al.,* 2008). Inorganic substances in water have received most of the attention because of their potentially harmful effects. These include heavy metals such as aluminium, arsenic, barium, cadmium, chromium, lead, mercury, and macro inorganic elements such as nitrate /nitrite (Wenhold & Faber, 2009). Some drinking water (tap and bottled) is grossly polluted at its source such as rivers, streams, and underground aquifers. A study conducted in California by Environmental Working Group (EWG) found 38 pollutants in 10 bottled water brands. In this study, bottled waters were purchased and test results revealed the chemical pollutants (that exceeded safety standards) listed in Table 3 including possible health effects (Naidenko *et al*., 2008).

Table 3: Some chemical contaminants detected in bottled waters, Naidenko *et al.* ,2008

Contaminant(s)	Possible Health effects
Fluoride	Fluoride is important for the integrity of bones and teeth; it protects teeth against dental caries. In South Africa fluoridation of drinking water to a concentration of up to 0,7mg/ℓ is mandatory. Regulation related to bottled waters in South Africa, specifies that bottled water containing more than 1mg/ℓ should be labelled "contains fluoride". If it contains more than 2mg/ℓ the label must indicate that the product is not suitable for infants and children younger than 7 years(DoH, 2006)
Nitrate	Nitrate is a fertilizer ingredient that widely pollutes drinking water nationwide. It poses particular risks for infants, who are susceptible to a form of methemoglobinemia, or blue baby syndrome, caused by nitrate replacing the oxygen normally carried by red blood cells. Exposure to elevated levels of nitrate in drinking water for pregnant women has been linked to possible adverse reproductive and developmental effects (www.ewg.org).
Ammonia	Ammonia enters the water from fertilizer runoff, leaching septic tanks, and erosion of natural deposits. It can occur as a pollutant in tap water. Ammonia volatilizes into the air and people are exposed primarily by breathing in it. It can trigger an asthma attack in some peoples (www.ewg.org).
Radioactivity: Radium-228 and Strontium-90	In humans and animal exposure to radioactivity causes a wide range of health effects including bone, liver, lung, and brain tumours, leukaemia, skin damage and blood damage (www.ewg.org).
Arsenic	Arsenic is a metal that enters the water by erosion of natural deposits. Inorganic arsenic has pesticide properties and is toxic to people upon inhalation or ingestion. Health implications associated with excessive consumption include cancer, cardiovascular or blood toxicity, developmental toxicity, endocrine toxicity, gastrointestinal or liver toxicity, kidney toxicity, neurotoxicity, reproductive toxicity and skin sensitivity (www.ewg.org).
Boron	Boron gets into drinking water from naturally occurring and human sources. Contamination of boron can come directly from urban and industrial wastewater and indirectly from soil runoff. In animal studies ingestion of boron has been linked with toxicity to male reproductive tract (testicular lesions) and developmental toxicity (www.ewg.org).

2.2.2.2 Organic contaminants of concern

Bottled water producers use a variety of aquatic sources for their products, such as springs, wells, borehole and municipal water. Organic chemicals have been widely detected in ground water. Most commonly found organic chemicals in drinking water systems are tetrachloroethylene or perchloroethylene, trichloroethylene, 1,1,1–trichloroethane and vinylidene chloride or 1,1-dichloroethylene. There are concerns that some of these volatile organic chemicals cause cancer, but data on this and reproductive effects are mainly from animal studies, data from human studies are limited. The most common synthetic organic chemicals used are by-products produced when chlorine is being used to disinfect the water. When chlorine reacts with natural organic material in the water the trihalomethanes (THMs) are produced. The THMs include chemicals such as chloroform (dichloromethane), bromoform, bromodichloromethane and dichlorodibromomethane.

Chloramines on the other hand are used as an alternative to disinfect the water and produces fewer THMs. Chloramines are less effective than chlorine. Kidney dialysis facilities remove chloramines because they can damage red blood cells (De Sanctis *et al.,* 2004). The THMs in treated water represent a higher cancer risk than most toxic pollutants. Bottled water companies usually use distillation, deionization and reverse osmosis to remove organic chemicals, chlorine, and other organic taste and odour causing chemicals (USEPA, 2003).

Table 4: Some organic contaminants that have been identified in bottled water (EWG, 2008).

Organic Contaminants	Possible Health effects
Total Trihalomethanes (TTHM's): Chloroform Bromoform Bromodichloromethane Chlorodibromomethane	These by-products are linked to cancer and reproductive problems. They form when disinfectants react with the residual pollution in the water.
Haloacetic acid: Dichloroacetic Acid Trichloroacetic acid	Haloacetic acids are genotoxic and carcinogenic, they are known to produce metabolic disturbances. Haloacetic acids are also linked to defects in embryos grown outside the womb (the whole embryo cell culture).
Phthalates(Chemicals used in plastic production)	Exposure is linked to hormone disruption effects.

2.2.2.3 Radioactivity in bottled water

Natural waters originating from springs or wells may contain radionuclides predominantly of the uranium decay series. As a rule, mineral water springs run across highly mineralized rocks and by so doing may contain radionuclides (Botezatu *et al*., 2005). The geological sources of natural mineral water are known as aquifers, which may be of different types and vary greatly in terms of their depth, horizontal extent, composition and permeability (Botezatu *et al*., 2005). Radioactive minerals occur irregularly in the bedrock, similarly to other minerals and they dissolve easily in water (Botezatu *et al*., 2005). Concentrations vary over a wide range depending on the bed rock of the aquifer from which the spring flows, the pH of the water at the source of the radionuclide transport, the time each well was pumped, the amount of pumped water, the presence of any water treatment prior to bottling of the water and relative depths of the wells if more than one well is used (Kralik *et al*., 2003).

Concentrations of ^{226}Ra, ^{222}Rn and ^{210}Pb were analyzed in domestic bottled waters commercially available in Austria and it was found that their levels vary over a large range (Kralik *et al*., 2003). Elevated concentrations of ^{226}Ra and ^{210}Pb were restricted to mineral waters. The highest concentrations of ^{226}Ra were found in water bottled from wells in the South East region of the country. Radiation doses from natural radionuclides in mineral water were low compared to average total doses of 2.4mSv/y from internal and external radiation in Austria (Kralik *et al*., 2003).

A study of mineral water / natural water conducted in Italy showed a similar concern. Monitoring of natural radioactivity in some bottled mineral waters produced in Italy was considered necessary due to the importance of bottled mineral water in human diet with special regard to children in the lactation period. Gross alpha and beta activities including ^{226}Ra, ^{238}U, ^{234}U and ^{210}Po concentrations were measured. The results revealed that the concentrations (mBqℓ^{-1}) of ^{226}Ra, ^{238}U, ^{234}U and ^{210}Po ranged from <10, 00 to 52, 5 from <0,17 to 89, from <0,17 to 7 900 and <0,04 to 21,01 respectively (Desideri *et al.,* 2007). A similar survey was conducted in Romania (Botezatu *et al*., 2005).

It has also been discovered that natural radionuclides from the uranium and thorium decay series are omnipresent in the earth's crust and are leached by surface and groundwater, especially groundwater. ^{228}Ra, ^{228}Ra, ^{238}U, ^{234}U, ^{210}Pb and ^{210}Po were investigated (Walner *et al.,* 2008). From the measured activities concentrations the committed effective doses for adults and babies were calculated and compared to the total indicative dose of 0,1mSv/a given in European Commission (EC) Drinking Water Directive as a maximum dose. The dominant portion of the committed effective dose was due to the radium isotopes, the dose from ^{228}Ra in most samples clearly exceeded the dose from ^{226}Ra (Walner *et al*., 2008).

2.2.3 Microbiological quality of bottled water

Bottled water can be the source of the causative agent in cholera, typhoid fever outbreaks and traveller's disease. Bacteria (the most common pathogen), has been found at various stages in the production of bottled water and from commercial bottled water samples (Ikem *et al*., 2002). Some microorganisms have been isolated from bottled water as causative agents to some diseases (Table 5). There may be considerable risk to humans, especially children exposed to bottled water containing microbiological entities. It is known that children may be exposed due to their behaviours, greater gastrointestinal absorption and a lower threshold for adverse health effects (Ikem *et al*., 2002).

Table 5: Microorganisms isolated from bottled water

Microorganisms isolated	Possible cause	Reference
-Total coliform (TC) -*Escherichia coli* -Heterotrophic Plate Count (HPC) bacteria	Isolated bacteria were from unprocessed water and these microorganisms were not detected in processed water. Possible cause was animal husbandry activities on the surrounding mountain contaminating the well through rainfall permeability.	(Kokkinakis *et al.*, 2008)
-TC -*E.coli* -HPC bacteria -Enterococcus Faecalis	Contamination was due to raw water source, treatment process employed and hygienic practices observed in production.	(Oyedeji *et al.,* 2010)
-HPC bacteria	Possible contamination was likely to occur due to improperly cleaned equipment and bottles, failure of ozonation or UV equipment or contamination of the water by workers.	Ehlers *et al*, 2004
HPC Bacteria *Acinetobacter lwoffi* Bacillus species Micrococcus species Acinetobacter spp *Steno maltophilia* *Psedomonas species* *Flav.meningosepticum* *Erwinia spp* *Edwardsiella lictaluri* *Staph epidermidis*	-Microbial contamination in 20ℓ bottles was higher than in 1,5ℓ bottles. The notable difference was that 20ℓ bottles were reusable, and it was difficult to clean with no possibility of sterilization. -Bacteria protozoa and fungi have often been observed in long-standing residual water in some of these bottles used. -Bottles were stored at varying temperature between 4 and 45°C,contamination was highly aided by temperature	Nsanze ,*et al.* 1999
Total Coliform (TC) Faecal coliform (FC)	Contamination was experienced during handling and purification process and also in washing and filling phase of the bottles.	(Robles,1999)
Penicillium citrinum, *P. glabrum* and other penicillium species *Cladosporium cladosporioides* and *Alternaria alternada*	species were isolated in spoiled samples	(Cabral *et al.*, 2002).
A alternate and *Penicilium citrinum*	species isolated in spoiled samples and these have the potential to be toxic	(Cabral *et al.*, 2002).

Although waterborne diseases associated with consumption of commercial bottled water are not common, such cases have been reported. Outbreaks traced to consumption of bottled water include cited incidents where in 1974 a cholera outbreak in Portugal occurred (Rosenberg, 2003). *Vibrio cholera* was isolated from two springs that supplied water to a commercial bottling plant (Rosenberg, 2003). The source water was safe and the contamination was caused by sewage infiltration through a limestone stratum. It was revealed that individuals ingested the non-carbonated mineral water contracted the disease and no cases of cholera were reported among those who consumed carbonated water (Rosenberg, 2003). Further cases were reported by the centre for Disease Control and production in 1994, where the cholera outbreak occurred in Marianas Island and the cause of the outbreak was in-plant contamination (Rosenberg, 2003). Incidents of outbreaks tend to have a long-lasting impact on public behaviour. An outbreak was reported in Sydney 1998, Cryptosporidium and Giardia affected the Australian bottled water market. This incident continued to remain in the minds of consumers (Doria, 2006). Other cases of illness that were traced to bottled water consumption include typhoid fever and traveller's diarrhoea (Rosenberg, 2003).

Historical disease outbreaks in tap water utilizing ground water supply is still of concern (Reynolds; 2005). Untreated sources are subjected to contamination from animal populations and excessive runoffs during severe storms, resulting in water pollution. Ground water is considered to be safe and free of disease causing microbes. The most dangerous form of groundwater water pollution by these microbes is human enteric viruses, ulcer causing bacteria, and protozoa (cryptosporidium). Of the 751 waterborne disease outbreaks that occurred in the US from 1971 – 2000, 467 (62 percent) of the cases were linked to ground water systems. During the 1999 - 2000 surveillance period, 29 of the 39 (74,4 percent) drinking water outbreaks, included an outbreak associated with bottled water (Reynolds, 2005)

The study from the University of Texas addressed the bottled water safety once it has been opened. The aim was to assess whether it was safe to drink out of the bottle of water and seal it up later. The outcome of the study revealed that, the bacterial count of bottled water went from one colony per millilitre to 38 000 colonies per millilitre when stored at room temperature after 48 hours. The study further revealed that refrigerating the product showed improved results with 50 percent fewer colonies. According to a study conducted by Nsanze *et al.* (1999: 1) organisms multiply more easily between 25°C to 37°C, the common indoor temperature, whereas refrigeration or high temperatures (around 42°C) reduce or maintain the status of the microorganisms present in the water.

2.2.3.1 Conditions that regulate the growth of yeasts and Moulds in bottled water

Mould growth in non-carbonated bottled water can be a problem especially when polyterephtalate (PET) bottles are being used. Filamentous fungi in water do not pose a health hazard to human health and hence they are not pathogenic to humans. However, some species can cause illness. The disease can occur in humans if the fungi produce mycotoxins. These metabolites are toxic and can induce tumours in several animals (Criado *et al*., 2005). The presence of spores or certain fungi is not always followed by toxin production but there are conditions that allow toxin production as compared to those which give way to growth.

The study conducted by Criado and Cabral (2005) focused on storage conditions that can influence the growth of moulds in bottled mineral water and to establish whether the toxic metabolites could be released into the water under storage conditions (Criado *et al.,* 2005). In this study, it was discovered that, storage time has the most important influence on mould growth. The research revealed that after 5 months of storage conditions, compounds (the plasticizer additive such as di-n-butyl phthalate, residues from the polymerization process, degradation compounds) migrate from PET packaging material into mineral water. These compounds serve as nutrients (organic matter) for mould growth. *A.alternata* and *P.citrinum* strains were toxicologically characterized. Both strains produced mycotoxins *in vitro*, and *P.citrinum* produced citrinin in mineral water, posing a potential health risk for consumers (Criado *et al.*, 2005).The study further emphasized the fact that light and temperature did not influence mould growth. Fungus was able to grow in wide temperature and the light ranges being important for their morphogenesis and germination but they were not a somatic requirement for them (Criado *et al*., 2005).

Studies were conducted and several publications describe the microbial flora of bottled waters, speculations made highlighted the significance in public health (Cabral *et al.*, 2002). It is however difficult to know which component of the flora represents an increase in the flora of ground water and which are those that can be attributed to contamination during or after extraction. Cabral *et al.,* 2002 conducted a study to determine fungal spoilage of bottled mineral water (Cabral *et al.*, 2002). The aim was to isolate and identify fungal species from spoiled bottled mineral water, to determine if propagules of those species were present in water without visible microbial growth and also to determine whether or not a relationship exists between the presences of fungal propagules. In this study filamentous fungus which do not pose any health risk were isolated and fungi with the potential to be toxic i.e. *Alternaria alternate* and *P.citrinum* were also isolated (Cabral *et al*., 2002).

2.2.4 Bottled water storage

Bottled water stored for weeks or sometimes for months on the grocery shelf or stored in homes is said to be in closed system, unlike drinking water taken from the tap that flows through the water pipes. Disinfection tends not to be synonymous with sterilization. Any bacteria present will attach itself to the sides and bottom of the container and multiply at the expense of whatever organic matter is present in the water (Rosenberg, 2003).Storage at ambient temperatures will aid the multiplication of contaminants contained there-in (Nsanze *et al.*, 1998).

2.2.4.1 Leaching of chemicals during poor storage conditions

Many questions have been raised about the possible migration of chemicals from the bottles during storage time especially under poor storage conditions such as high temperatures and exposure to the sun. Many studies have reported the presence of organic contaminants in bottled water that may affect the organoleptic characteristics of water and pose health risks to consumers. (Amiridou and Voutsa, 2011). Organic compounds could be the source of carbon to inherent microorganisms which also have implications for odour and taste thresholds.

The presence of xenobiotics in bottled waters represents a complex problem and the origin of several substances is not clearly established. An investigation of bisphenol A, nonylphenol, octylphenol and phthalates in bottled water was conducted by assessing the effect of environmental factors and storage time on the occurrence of chemical substances after exposure to outdoor conditions for 15 and 30 days respectively (Amiridou and Voutsa, 2011). The study exhibited higher concentrations for Bisphenol A in bottled water from polycarbonate containers. Other compounds were detected at lower levels in bottled water purchased from the local market (Amiridou and Voutsa, 2011). Antimony, a regulated contaminant that poses both acute and chronic health effects in drinking water has been found to leach from bottled water material (Westerhoff *et al*., 2008). High temperature storages have been found to promote the leaching of Antimony from PET bottles into the water (Westerhoff *et al*., 2008).

2.2.5 General Discussions

The literature reveals that there is no stipulated bottled water shelf-life assigned to all the different types of bottled water. Therefore, this makes it difficult to make generalisations about the duration of bottled water shelf-life because of many factors that can influence the quality of this type of water. These factors include the type and source water, the effectiveness of the disinfection process used at the bottling plant, contamination that may occur at any time during and after processing, the effects of storage conditions and storage at the point of retail, and transportation conditions. All these factors pose a concern as to the health of the public consuming bottled water. Literature reveals that microbial contaminants are present naturally in water either from the surface of the earth or from the ground. These microbes may persist in bottled water if not stored properly (i.e. if bottled water stored at relatively warm temperatures for extended periods of time) with no residual disinfectant in it. The microbial colony can grow and overpopulate causing serious illness to consumers with compromised immune systems. Bacteria, protozoa, and fungi have often been observed in long-standing residual water in some of the bottled waters.

Fungus with the potential to be toxic i.e. *Alternaria alternate* and *P.citrinum* were detected in bottled water stored for prolonged periods. In order to protect public health, bottled water companies need to monitor and protect the water source used for bottling - for example, springs and wells from surface intrusion and other environmental influences. This is to ensure that the surface water contaminants such as *Cryptosporidium* and *Giardia* are not present. The literature further indicates that pollution at the source (ground water, streams, and springs) if not monitored can contain chemical pollutants (organic and inorganic). The chemical contaminants in bottled water such as lead, arsenic, benzene can build up to high levels and cause a range of health complications. If carcinogens are frequently consumed through bottled water, they can build up in the human body and increase the risk of cancer.

Based on the above observations from the literature the current study has been designed such that it would be possible to assess the variation of bottled water quality with that of shelf-life and determine if the later is of concern which is the main objective of this study. The study has been designed to determine the shelf-life of commercial bottled water brands consisting only of still water (i.e. spring water, mineral water, bottled tap water). The research methodology and design for the study is discussed in detail in **Chapter 3**.

CHAPTER 3 RESEARCH METHODOLOGY AND DESIGN

3.1 INTRODUCTION

The purpose of this Chapter is to present the research methodology and design used in establishing if shelf-life of bottled water is of human health concern. The methodology used for the assessment of water quality constituents of concern is outlined and an explanation is given for the experimental design used and for the selection of the various parameters. In sections 3.2 and 3.3 of this Chapter, the research design and experimental techniques used are discussed.

3.2 RESEARCH DESIGN

A total of five commercial bottled Water brands (brand A= mineral water, brand B = mineral water, brand C = mineral water, brand D = bottled tap water, brand E= spring water) each containing bottles from the same batch were purchased directly after being bottled from different distributors around Gauteng in South Africa. The bottled waters were selected based on their availability and popularity. These bottled waters were in plastic bottles (clear and coloured) with plastic screw caps. All samples were transported in a cooler box with ice bricks at the temperature between 6-8°C.The bottled water samples were stored at room temperature with artificial lighting and controlled temperature. All the bottled water brands collected were exposed to artificial lighting for up to one year, thus mimicking typical conditions in retail outlets, supermarkets and in homes. Analyses were conducted over a period of 12 months, in order to identify the effects of the shelf-life of the bottled water.

Monthly analyses involved the removal of duplicate sealed bottles from the same batch of each brand. The first set of analysis was conducted within three days of purchase. Upon storage, the room temperature was monitored at storage temperature between (17°C and 25°C). Microbiological water quality determinants (heterotrophic plate count, total and faecal coli forms including *E.coli*) were analyzed during the twelve months period. Analyses for yeasts and moulds were performed bi-annually. Analysis to detect algal formation was conducted quarterly a year. Physical, chemical (inorganic and organic determinants such as total trihalomethanes and dissolved organic carbon) were analyzed three times in a year and compared with SANS 1657: 2003 edition 1.3 bottled natural water standards. The standards for drinking water (SANS 241) were also used since some guidelines were lacking in bottled water guidelines.

3.3 EXPERIMENTAL TECHNIQUES

3.3.1 Microbiological analysis

3.3.1.*1 The detection of heterotrophic plate count (HPC)*

All five bottled water brands were analyzed for Heterotrophic plate counts using the pour plate technique. Yeast extract agar was used for the detection of heterotrophic plate count (HPC) bacteria. Using a sterile pipette, 1 and 5ml of water samples were aseptically dispensed into sterile Petri dishes. This was done with a set of controls where no sample was added and sterile yeast extract agar was poured into the plate to serve as a negative control. AES APS300 automated preparator was used to dispense the agar onto the plates. The preparator used was programmed at sterilizing temperature of 121 ± 3°C, sterilizing time of 15 minutes and dispensing temperature of 46 ± 23°C. The plates were left to solidify at incubation temperature of 35 ± 1°C. The plates count was determined after 48 ± 3 hours with a set of controls and the results were expressed as number of colony forming units (cfu) per millilitre of water.

Yeast extract agar was dispensed into a clean plate (without any sample) to test the sterility of the medium. The growth test was done to ensure that the medium would support the growth of the organisms. Agar was poured into an empty Petri-dish and left to solidify. The agar was inoculated by streaking out from the *E. coli* culture onto the agar surface.

3.3.1.2 Enumeration of yeasts and moulds

For the enumeration of yeasts and moulds, all five bottled waters were prepared according to ISO 7954 using Rose Bengal chloramphenicol agar at a temperature of 25°C. The enzymatic digest of soybean meal in the agar provides the vitamins and nitrogen source required for the organisms growth and the high concentration of dextrose served as an energy source. Rose Bengal incorporated in the agar served as a selective agent to inhibit bacterial growth and restricted the growth of rapidly growing moulds.

3.3.1.3 The detection of faecal coliforms (FC)

All bottled water brands were inspected for the presence of FC and TC. Membrane filter technique was used to isolate the presence of FC using m-FC agar. 100ml of the water sample was filtered through sterile 0.45µm bacteriological filter using a sterile membrane filter assembly. After filtration, the filter was aseptically removed using sterile forceps, placed on sterile m-FC agar plates and incubated at 44.5 ± 0.2°C for 18 to 24 hours. Before incubation, the plates were incubated with a set of positive and negative controls using American Type Culture Collection (*Escherichia coli* ATCC 25922 for positive control and *Enterococcus faecium* ATCC 19434). After incubation, the plates were observed for typical FC-like colonies and checked against a set of controls, the results were expressed as numbers of FC per 100ml.

3.3.1.4 The detection of total coliforms (TC)

The membrane filter technique accredited was used to detect the presence of TC bacteria using m-endo agar-Les following the same filtration method. Selective agar (i.e. m-Endo Agar Les) was used and the plates were incubated at 35 ±1 °C for 18 to

24 hours. The plates were incubated with a set of positive and negative controls (*Staphylococcus aureus* ATCC 25923 as a negative control and *Enterobacter aerogenes* ATCC 13048 as a positive control). After incubation, the plates were observed for TC-like colonies and checked against a set of controls and the results expressed as the number of TCs per 100ml.

3.3.1.5 Determination of E.coli

Verification:

Confirmation of red colonies with the characteristic metallic sheen from M-Endo Agar LES counted as total coliforms (TC). All shades of blue colonies produced on M-FC Agar were counted as faecal coliforms (FC) by the cytochrome oxidase (CO) test for indophenol, *o*-nitrophenyl-ß-D-galactopyranoside (ONPG) test for ß-D-galactosidase and indole production from tryptone water.

TC gives an ONPG (+), CO (-) and Indole (-) reaction.

FC gives an ONPG (+), CO (-) and Indole (-) reaction.

E. coli gives an ONPG (+), CO (-) and Indole (+) reaction.

Inoculation:

A sterile inoculation loop was used to pick growth from the centre of a well-isolated colony and inoculated on appropriate media for each of the isolates for verification in the following order:

Nutrient agar plate for CO tests, and incubated at 35 $\pm$ 1 °C.

Tube with tryptone water for indole production, and incubated at 44.5 ± 0.2 °C.

Tube with ONPG-broth and incubated at 35 $\pm$ 1 °C.

Inoculate the positive / negative controls with the relevant reference cultures

Reading of results: ONPG-broth

Positive test (+): Yellow colour within 20 minutes to 24 hours

Negative test (-): Colourless after 24 hours (Media can be turbid due to growth, but without the production of the enzyme)

Indole production from tryptone water

5 drops of KOVACS' reagent were added to each tube after incubation. The assessment was done as follows;

Positive test (+): A red / pink ring at the surface of the medium in the alcohol layer.

Negative test (-): No colour development at the alcohol layer; takes the colour of the KOVACS' reagent (yellow).

Cytochrome oxidase

The test is performed with Oxidase strips. The required quality control was performed on the strips. Sterile platinum or disposable inoculation loop was used to take some growth from the 24 hour culture on the nutrient agar plate and spread growth onto the reaction zone. (The Nichrome needle should be avoided as a false positive reaction might be observed).The results /reactions were recorded as follows:

Positive test (+): Development of a blue or blue-violet colour after approximately 20 to 60 seconds. Comparison to the colour scale on the container was made.

Negative test (-): No colour change should be observed within 60 seconds.

3.3.1.6 The detection of Algal species

Phytoplankton identification and enumeration was used to detect various algal species. All bottled water samples were pressurized to deflate the floating mechanism of some phytoplankton species. Different bottled water samples were dispensed into a marked sedimentation chamber in which all the phytoplankton present was sedimented by centrifugation of water samples. The phytoplankton species were identified and enumerated using an inverted light microscope. For precision and accuracy, the biomass concentrations of replicate phytoplankton analysis of the same bottled water brand should be within the 2 standard deviation (tolerance) and 80 percent similarities between replicate analysis. Concentrations were expressed as cells/ml. Quality controls for sediment chambers were carried out after washing; the whole chamber floor was evaluated for the presence of algae. The percentage uncertainty for the phytoplankton identification and enumeration method was 56 percent, at a coverage factor of 2 and a level of confidence of 95 percent.

3.3.2 Determination of physical determinants

3.3.2.1 Determination of Total Dissolved Solids

Because some bottled waters tend to have high mineral content such as calcium, magnesium, chloride, or sulphate, measures were taken to prevent a water trapping crust from forming. The bottled water samples were filtered through a glass-fibre filter, evaporated and dried in a weighed dish and dried to constant weight at 180 ± 2°C. The increase in dish weight represented the total dissolved solids. This method was done according to the Rand Water Analytical Services laboratory method number 2.1.2.04.1.

3.3.2.2 Determination of Colour

The colour of the bottled water samples was expressed in terms of the amount of light scattered at a specific wavelength of between 400 to 700 nm and expressed as mg/ℓ Pt Co. The verification standard was analysed after every 10^{th} sample. The uncertainty of this method was determined during initial method validation using a control chart (2 x standard deviation/known concentration).The procedure used followed the Rand Water Analytical Services laboratory method number 2.1.2.08.1.

3.3.2.3 Determination of Turbidity

Turbidity was measured using turbidity meter to determine the clarity of a natural body of various bottled waters. The procedure consisted of comparing the intensity of light scattered by the sample under defined conditions with the intensity of light scattered by a standard reference suspension under the same conditions. The higher the intensity of scattered light, the higher the turbidity. Turbidity of water samples was expressed in nephelometric turbidity units (NTU). Verification checks were performed daily using the secondary Gelex standard that was adjusted with the most recent calibration procedure. The lower detection limit is 0, 06 NTU, the linear dynamic range was 0 to 7 500 NTU, the estimated error was at 95 percent and the confidence level was 5 percent.

3.3.2.4 Determination of pH, Conductivity and Alkalinity

Metrohm Tiamo autotitrator was utilized for the automated determination of pH, conductivity and total alkalinity. The measurements were made according to the Rand Water Analytical Services laboratory method number 2.1.3.01.2., where conductivity was measured in units of millisiemens per metre (mS/m), pH in pH units and alkalinity (in mg/ℓ as CaCO3). Potassium Chloride (KCℓ) was used as a verification standard when measuring conductivity. A pH buffer of 4 and 7 (commercially available in a liquid state) was used as calibration and verification standard when measuring the pH, and a sodium carbonate (Na_2CO3) solution was used as a standard when measuring alkalinity. The verification standard was placed at the beginning of the sample run and one after every 10th sample. The results were plotted on the alkalinity, pH and conductivity control chart.

3.3.3 Determination of inorganic chemical determinants

Most inorganic determinants (measured in µg/l), which may have direct health impact if such water is consumed, were determined using various accredited laboratory methods. Microelements such as cadmium, chromium, cobalt, lead, nickel, cyanide and vanadium were measured using inductively coupled plasma-Atomic Emission Spectroscopy according to the Rand Water Analytical Services laboratory method number 2.1.4.01.1 and the unit of measurement is µg/ℓ This method was also used to determine the mineral composition of calcium, magnesium, sodium, potassium and other determinants such as copper, iron, manganese, lead, zinc, aluminium, boron and molybdenum. Ion chromatography (Metrohm 761 Compact IC–system A) was the analysis method used to determine the common anions such as fluoride, chloride, nitrite, bromide, nitrate and sulphate and chloride. The verification standard was run after every 10th sample during the analysis. Atomic absorption spectroscopy was to determine arsenic, selenium, antimony and mercury. Uranium, selenium and barium were analyzed using I-ICP MS.

3.3.4 Determination of Organic chemical determinants

Gas Chromatography with electron capture detection (GC-ECD) and headspace auto sampler was used on all bottled water samples to determine trihalomethanes (Chloroform (CHCL3), Dibromochloromethane (CHBr2CL), bromodichloromethane (CHBrCL2) and bromoform (CHBr3). Bottled water samples were prepared with the internal standard (i.e. 1, 2 dibromoethane), isothermically heated and injected into gas chromatograph. Analyses were performed using an electron capture detector and a computerized data analysis program. The quality control standard was analysed after every tenth sample. The blank chromatogram was checked to ensure that the system was free from contamination.

The organic molecules were broken down to single carbon units in order to be measured quantitatively. The bottled water received was measured using ultraviolet (UV) light-catalysed persulfate oxidation of organics in the sample to form CO_2. The CO_2 produced was tuned to the absorptive wavelength of CO_2 where the microprocessor calculates the area of peaks produced and compares them to the peak area of the calibrated standard stored in the memory. The instruments used have the wavelength with the limit of detection of 0.70 mg/ℓ and uncertainty of measurement of 1,40 mg/ℓ.

3.4 LIMITATIONS OF THE STUDY

The study only involved the testing of the final product. No attempts were made to sample the actual bottling water sites and source areas as this fall outside the scope of this project. Full spectrum of analysis could not be conducted monthly due to budget constraints which limits the size of the data set and makes it difficult to apply statistical assessment in some instances. The bottled water brands were only purchased around Gauteng which limits the understanding of the problem on a national scale and the number of bottled water brands used for this study.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 INTRODUCTION

The Chapter addresses the monitoring of the microbial flora, chemical and aesthetic qualities of bottled water at different intervals to determine the impact of any on the shelf-life of bottled water. In order to assess the possibility of variation in physical, chemical, and microbiological water quality with time using different brands of bottled water, bottled water standard (SANS 1657 endorsed by the South African Departments of Health) was used to compare results attained. In cases where the standard was not specified, drinking water standard SANS 241 was used to compare with attained results and where both standard (i.e SANS 1657 and SANS 241) were not indicated, World Health Organization guidelines were used to compare results.

4.2 Measurement of physical determinants

Physical parameters are selected in this study to assess the impact of shelf-life on the aesthetic quality of the bottled water. These parameters include the taste, odour, appearance, and colour of the water. Turbidity on the other hand is used to measure the clarity of the water. Total dissolved solids (TDS) and electrical conductivity (EC) are the two main determinants used to determine salt content.

4.2.1 Total dissolved solids(TDS)

TDS constitutes all the dissolved solids in the water. It is usually comprised of inorganic minerals(salts), small amounts of organic material and soluble minerals (Fe and Mn). The inorganic minerals(salts) are commonly found in nature and are deposited by the weathering of the sedimentary rocks and erotion of the earth's surface. TDS gives an indication of whether or not all suspended solids were removed when the water passed through a fine filter during water treatment processes since some bottled waters are derived from tap water (such as brand D). According to SANS 1657 if the product contains more than 1000 mg/ℓ of total dissolved solids, a statement should be made to the effect that excessive consumption (which should be defined) might have a deuretic effect. High levels of TDS may also cause an objectionable taste,odor and colour to the water.In the case of mineral water, the label should state whether the product has a high (≥1000 mg/ℓ), medium (500 mg/ℓ to 1000 mg/ℓ), low (≤500 mg/ℓ TDS level. It is evident from Figure 1 that there was no significant TDS variation during the period of study from brands A,B,C,D andE. Hence the TDS concentration varied between brands since water came from different sources. TDS was within drinking water standard of 1000 mg/ℓ with average TDS values of 47 mg/ℓ for brand A, 263 mg/ℓ for brand B, 218 mg/ℓ for brand C, 218 mg/ℓ for brand D and 76 mg/ℓ for brand E (Figure 1). The TDS for all bottled water tested was within the reccommended limits and won't have negative aesthetic effects.

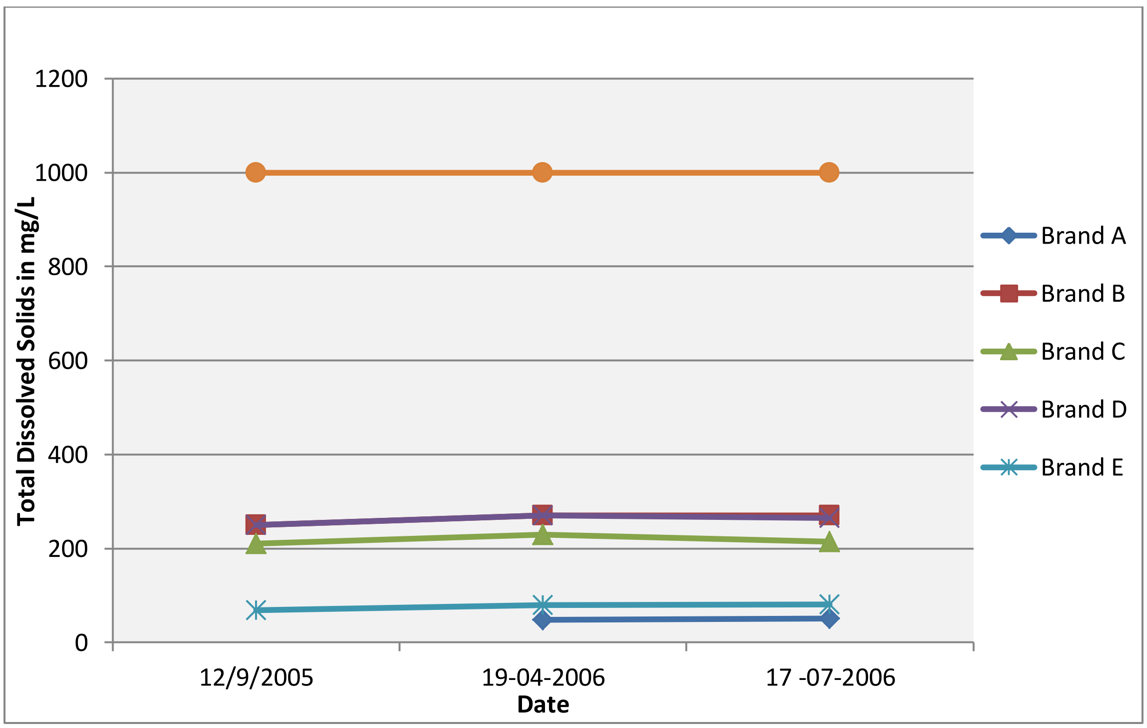

Figure 1: Total dissolved solids results

4.2.2 Colour

Drinking Water should ideally have no visible colour. Colour in drinking water is usually due to the presence of coloured organic matter associated with the humus fraction of soil, aquatic plants, organic matter such as humus, peat or decaying plant matter. It may also result from the contamination of the water source with industrial effulent and may be first indication of a harzadous situation. Most consumers can detect colour above 15m mg/ℓ Pt in a glass of water or non-coloured bottled water containers. According to WHO levels of colour below 15 mg/ℓ Pt are usually acceptable to consumers, but acceptability may vary. High colour could also indicate a high propensity to produce by-products from the disinfection process. The results for colour as indicated in Figure 2 showed that there was a slight variation in colour, in Brand B: 7 mg/ℓ pt and Brand E: 6 mg/ℓ pt during the initial stage of bottled water shelf-life. This could have been due to bottles that were not shaken properly before being analyzed. For the remainder of the storage period colour remained constant at 5 mg/ℓ Pt during the period of study.

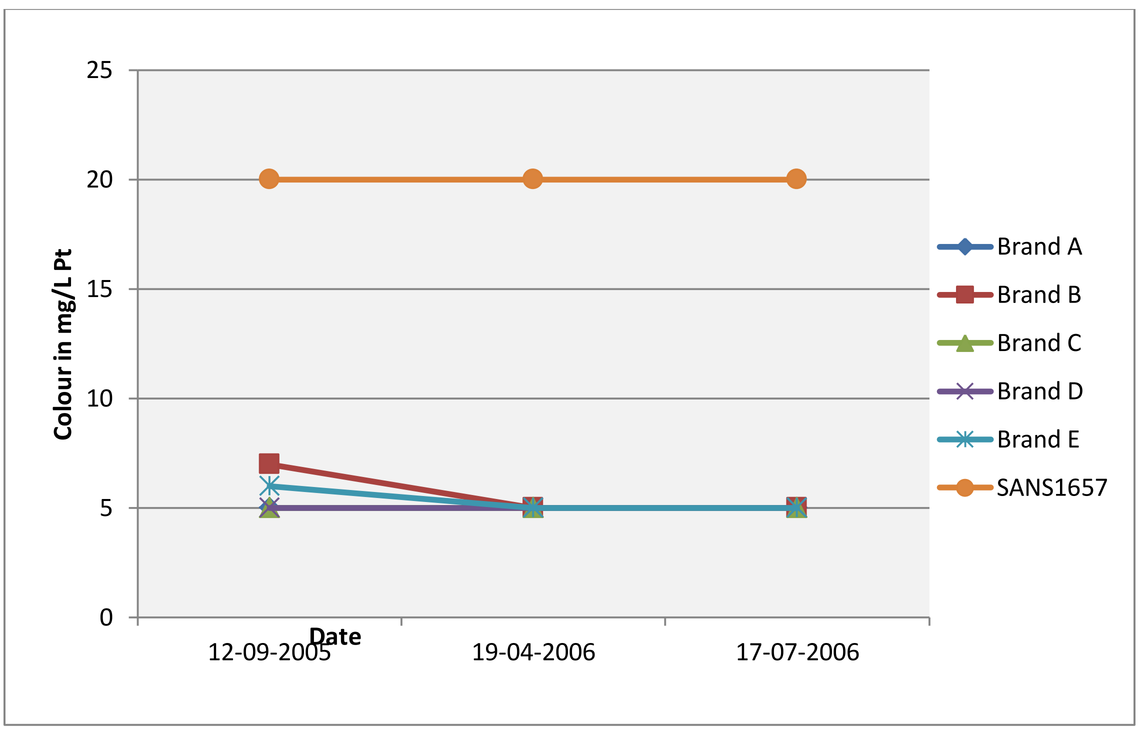

Figure 2: Results for colour

4.2.3 Conductivity

Conductivity is a measurement of the ability of the water to conduct electricity. Conductivity for brand A ranged between 5.1 and 6.5 mS/m with mean value of 5.7 mS/m (Figure 3). In bottled water, brand B, the conductivity ranged between 40 and 43 mS/m with a mean value of 42 mS/m (Figure 3), while in Brand C it ranged between 31 and 39 with a mean value of 38 mS/m (Figure 3). Brand D results ranged between 41 and 43 mS/m with a mean value of 42 mS/m while in brand E results ranged between 6.2 and 8.5 mS/m with a mean value of 7 mS/m (Figure 3). Brand B, C and D were characterised by a higher conductivity (still within SANS 241 of <150) than brand A and E. Aesthetic problems of the water with conductivity as high as 150 mS/m, are that it tastes salty and water with conductivity higher than 300 mS/m, water fails to quench the thirst. Sensitive groups are children under the age of one year, people on salt-restricted diets, such as heart and kidney patients and individuals with chronic diarrhoea.

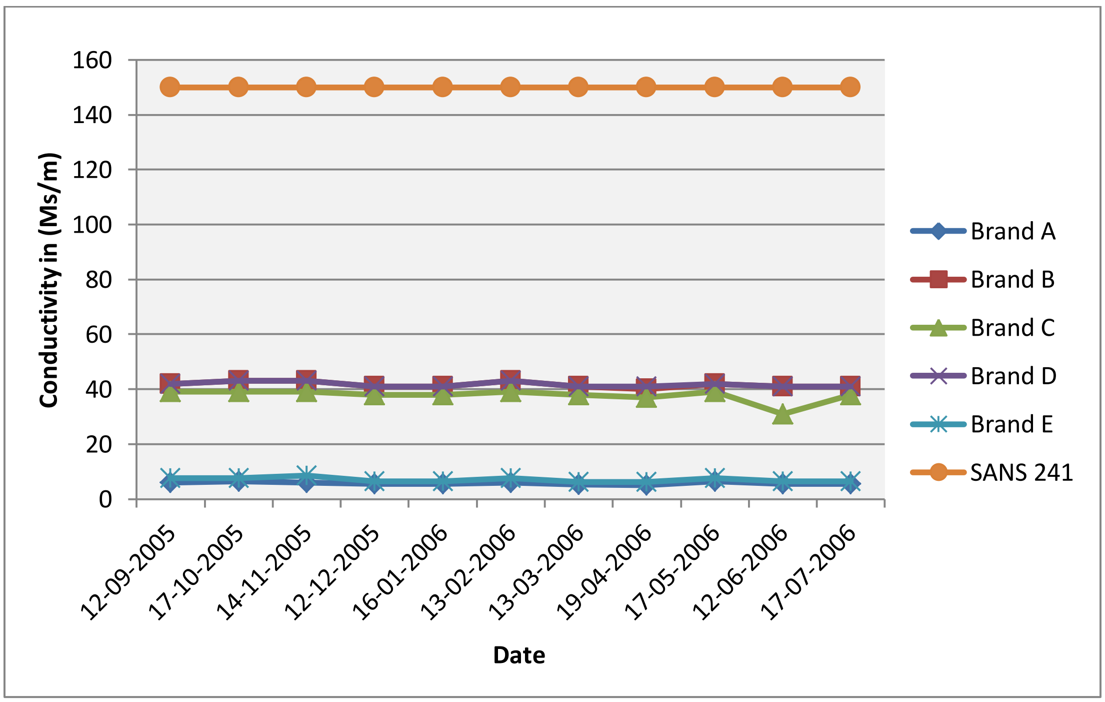

Figure 3: Results for conductivity

4.2.4 Hardness

The hardness in drinking water varies depending on the rocks and soils of the area that the water comes from and the treatment process used. Most bottled water brands chosen come from different regions around South Africa. According to the WHO there was some indication that very soft waters may have an adverse effect on mineral balance. Maximum permissible level for hardness established is 500 mg/ℓ according to WHO guidelines. Water hardness range as Follows: Soft (0-50 mg/ℓ as CaCO3), moderately soft (50-100 mg/ℓ as CaCO3), slightly hard (100-150 mg/ℓ as CaCO3), moderately hard (150-200 mg/ℓ as CaCO3), Hard (200-300 mg/ℓ as CaCO3) and very hard (>300 mg/ℓ as CaCO3). There was slight variation during the period of study for Brand A,B,C, and E , with Brand A and E being regarded as soft (Figure 4). Brand B and D were slightly hard and Brand C was regarded as being hard. Hard water is not a health hazard. In fact, hard drinking water generally contributes a small amount towards total calcium and magnesium in human dietary needs. In some instances, where dissolved calcium and magnesium are very high, water could be a major contributor of calcium and magnesium to the diet.

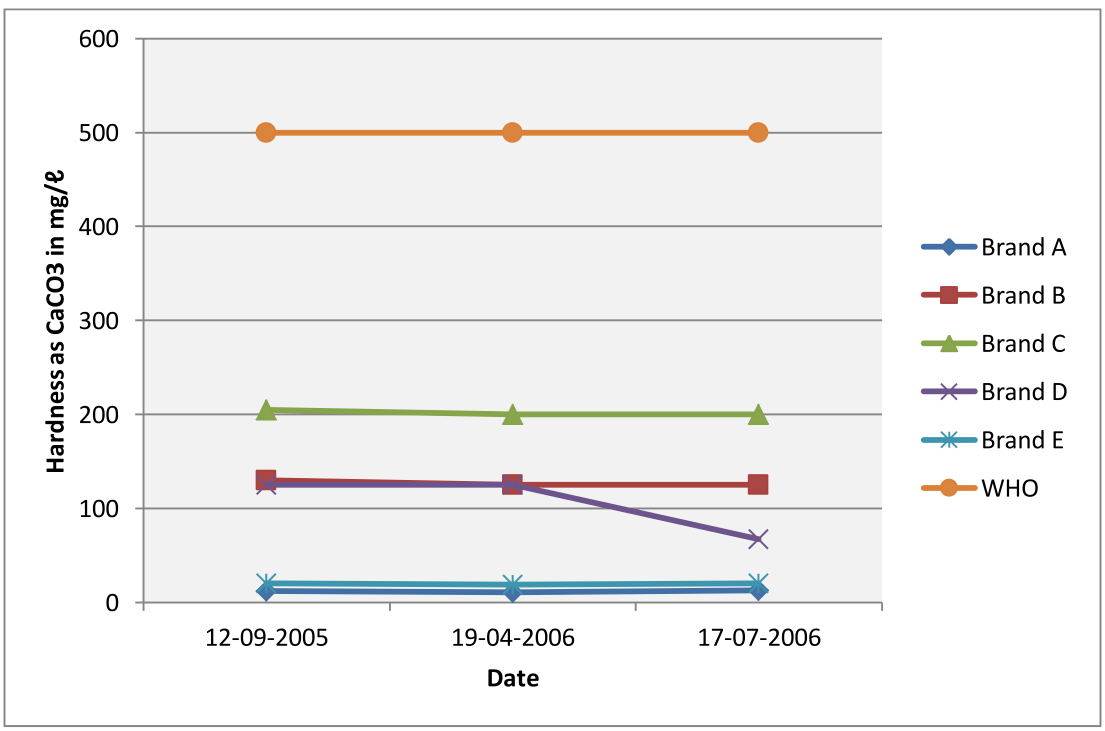

Figure 4: Results for hardness

4.2.5 The pH

pH is one of the most important operational water quality determinants.The closer pH gets to 1, the more acidic the water becomes. According to SANS 241 drinking water standard pH limits for drinking water are supposed to be between ≥ 5.0 to ≤ 9.5 (pH units). The pH values ranged from 6 to 7.7 and no significant variations were noticed during the period of study. International standards for drinking water suggest that pH less than 6.5 or greater than 9.2 would impair the potability of the water. All the brands tested were within the specified standard between 5.0 and 9.5 with average values of 6. *pH* average values were 6 for brand A, 7,2 for brand B, 7,7 for brand C, 7,1 for brand D and 7.0.for brand E.

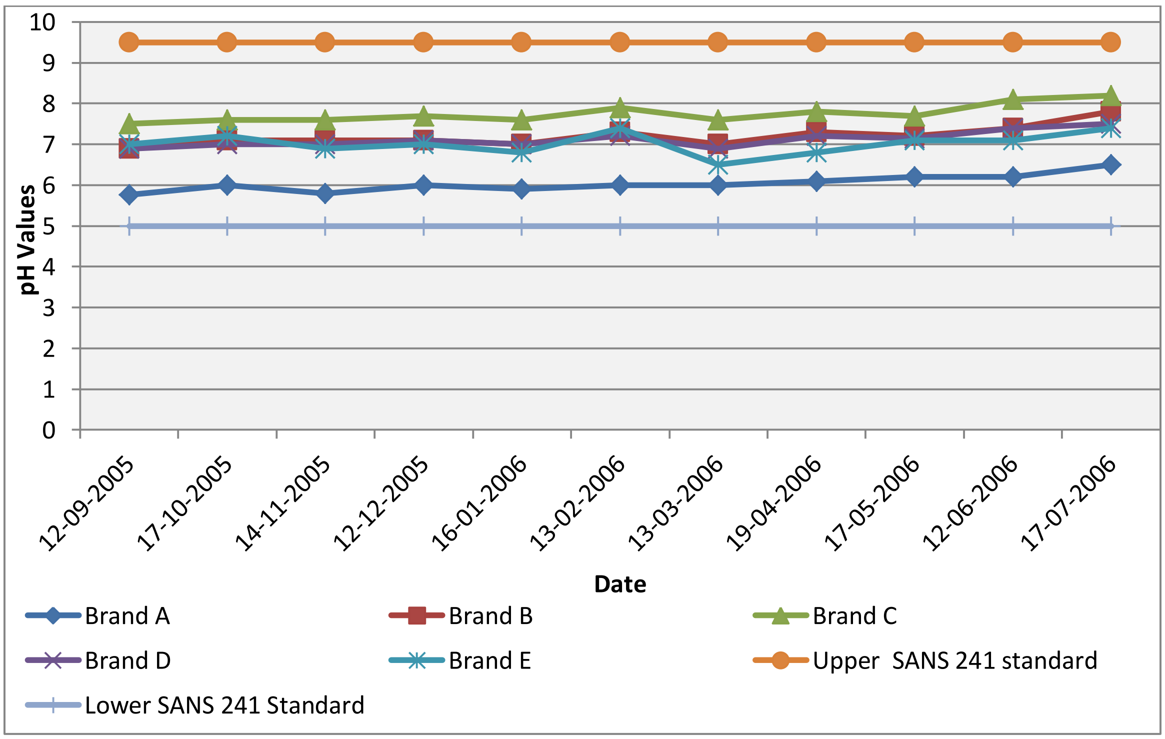

Figure 5: Results for pH

4.2.6 Alkalinity

Alkalinity gives an indication of how much acid water can be received without changing the pH. Alkalinity comes from rocks and soils, salts and certain plants activities. If an area's geology contains large quantities of calcium carbonate ($CaCO3$, limestone), water bodies tend to be more alkaline. The World Health Organization suggested guideline values for alkalinity as Low alkalinity < 50 mg/ℓ as $CaCO_3$, Medium alkalinity 50-250 mg/ℓ as $CaCO_3$, High alkalinity > 250 mg/ℓ as $CaCO_3$. There were no particular trends noticed from all bottled water brands tested as alkalinity from brand A, B, C, D and E fluctuated slightly over time (Figure 6). Alkalinity for Brand A was low ranging from 9, 8 to 14 mg/ℓ as $CaCO_3$ with average value of 12.43 mg/ℓ as $CaCO_3$. Alkalinity for brand B was medium with results ranging from 155 to 210 mg/ℓ as $CaCO_3$ with average results of 144 as mg/ℓ $CaCO_3$. Brand C had medium alkalinity ranging from 140 to 144 mg/ℓ as $CaCO_3$ with average results of 191 mg/ℓ as $CaCO_3$. Alkalinity for Brand D was low and ranged from 140 to 144 mg/ℓ as $CaCO_3$ with average value of 143 mg/ℓ as $CaCO_3$ (Figure 6). Brand E had low alkalinity ranging from 33 to 37 mg/ℓ as $CaCO_3$ with average results of 35 mg/ℓ as $CaCO_3$. Some water treatment plants use lime in their treatment processes to produce tap water and some bottled water such as brand D used tap water as a source. But when water (especially drinking water) becomes too alkaline, treatments, such as reverse osmosis, distillation and deionization, are used to remove excessive amounts of minerals. Most bottled water companies use these methods during their bottling process.

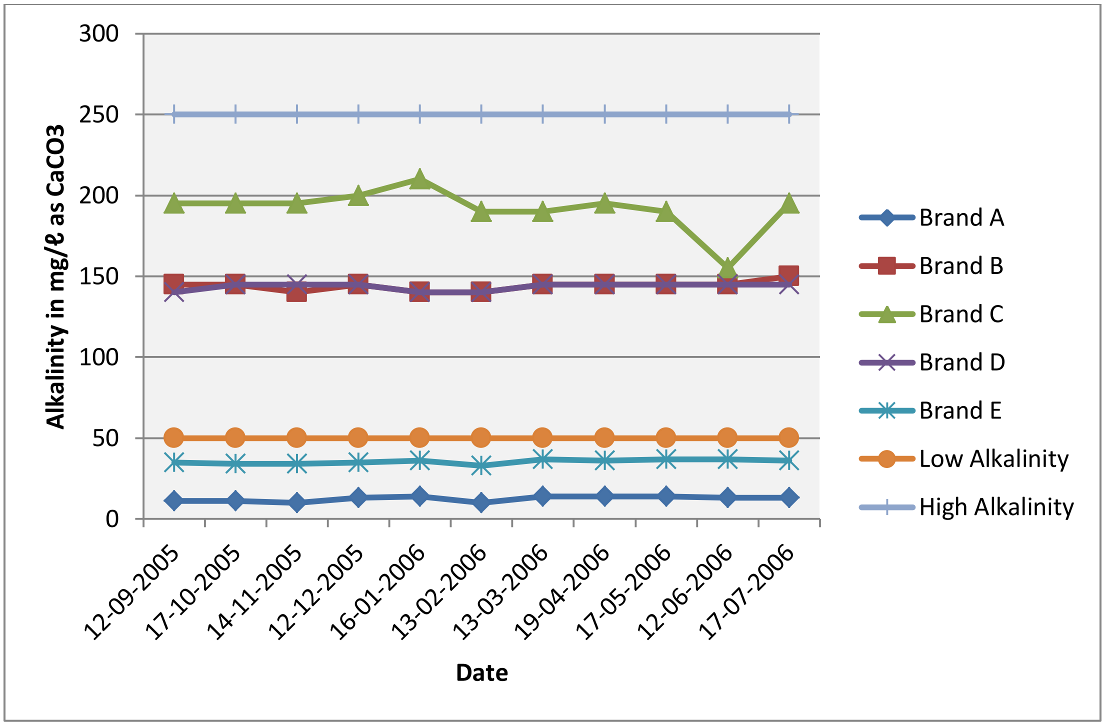

Figure 6: Results for Alkalinity

4.2.7 Turbidity

Turbidity is the amount of cloudiness in the water. Clarity of water is important in producing products destined for human consumption and manufacturing uses. Turbidity in water is caused by suspended matter, such as clay, silt, finely divided organic and inorganic matter, soluble coloured organic compounds, plankton and other microscopic organisms. According to WHO appearance of water with a turbidity of less than 5 NTU is usually acceptable to consumers. Bottled water standards recommend Turbidity of 1NTU for the finished product and all bottled water brands fell within this limit. The results shown below indicate that Brand A, B, D, and E a slight variation in turbidity values between 0, 15 and 0,19NTU (Figure 7) during the period of study, which is still within acceptable WHO guidelines. Turbidity in drinking water (bottled water) may be caused by particulate matter that may be present from the source water as a consequence of inadequate filtration, presence of inorganic particulate matter in some groundwater such as Brand B (underground water was used as a source) and bacteria in the bottle.

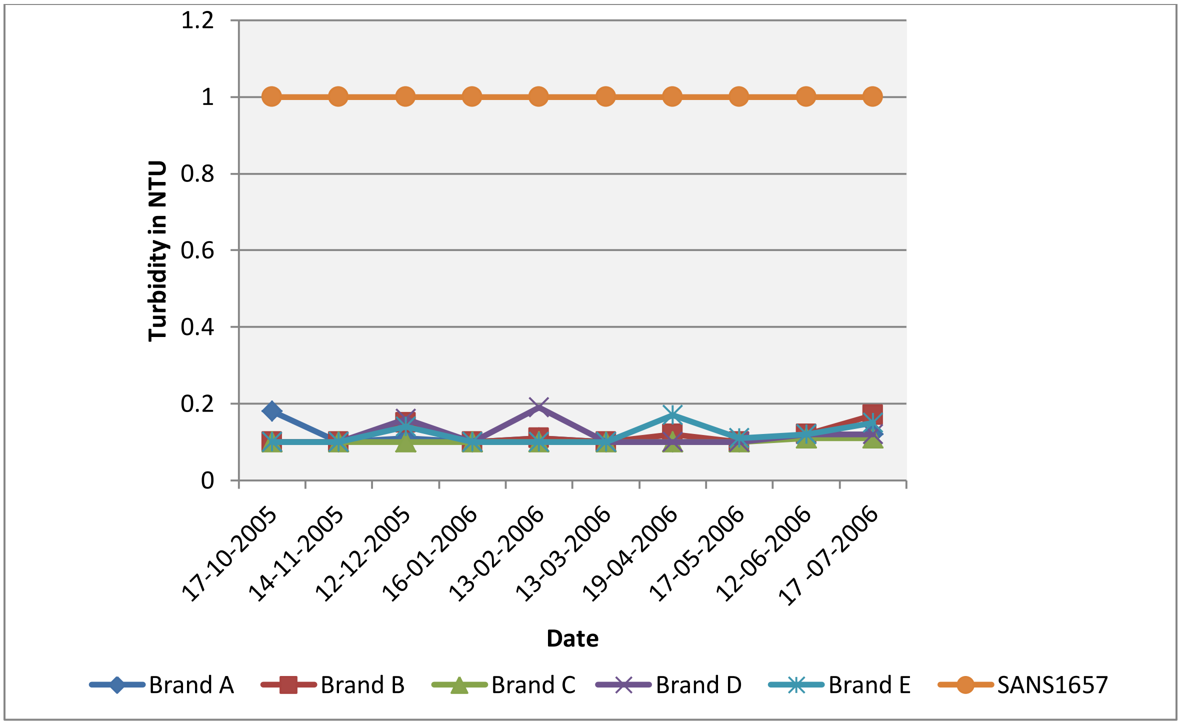

Figure 7: Results for Turbidity

4.3 Microbiological water quality results

In order to assess the effects of bottled water shelf-life, microbiological determinants such as HPC counts, coliform bacteria, yeasts and moulds and algal species were used to assess the impact thereof.

4.3.1 Detection of Heterotrophic plate count (HPC)

Figures 8 to 12 below indicate the HPC results detected from all bottled water brands tested. The counts for the bottled water tested ranged between 1 and 106 900 with a mean value of 28 100 for brand A, a range between 1 and 90 000 with a mean value of 16 700 for brand B, a range between 1 and 57 000 with a mean value of 5400 for brand C, a range between 100 and 36 000 with a mean value of 7 900 for brand D and a range between 1 and 39 000 with a mean value of 7 400 for brand E (Figures 8 to 12). The HPC findings of the investigated bottle water brands (Brand A, Brand B, Brand C, Brand D, Brand E) exceeded the alert levels for the drinking water standard of

>5 000 cfu/mℓ and SANS 1657 limit of less than 100cfu/mℓ in most cases which is a cause for concern.

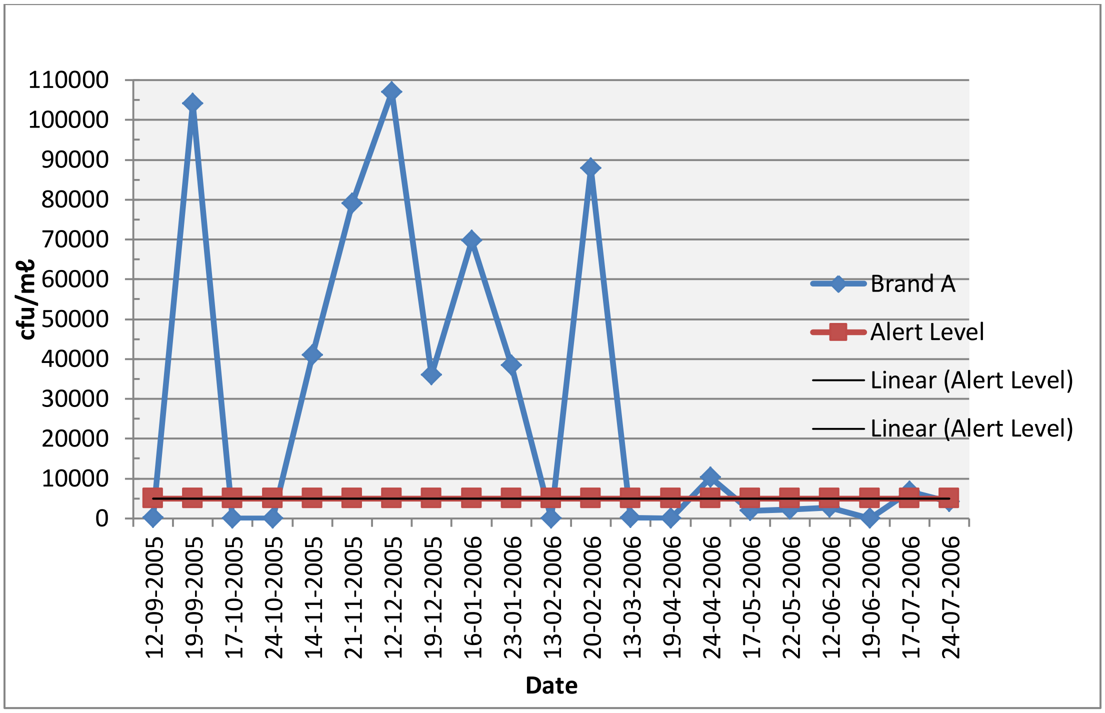

Figure 8: Detection of Heterotrophic Plate count in Brand A

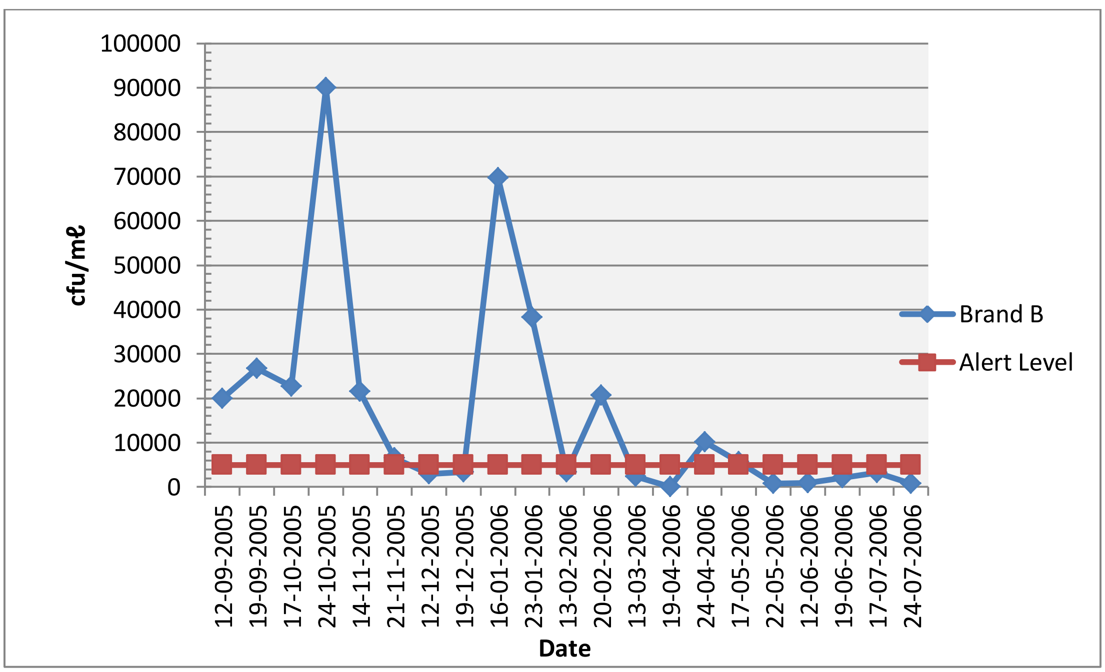

Figure 9: Detection of Heterotrophic Plate count in Brand B

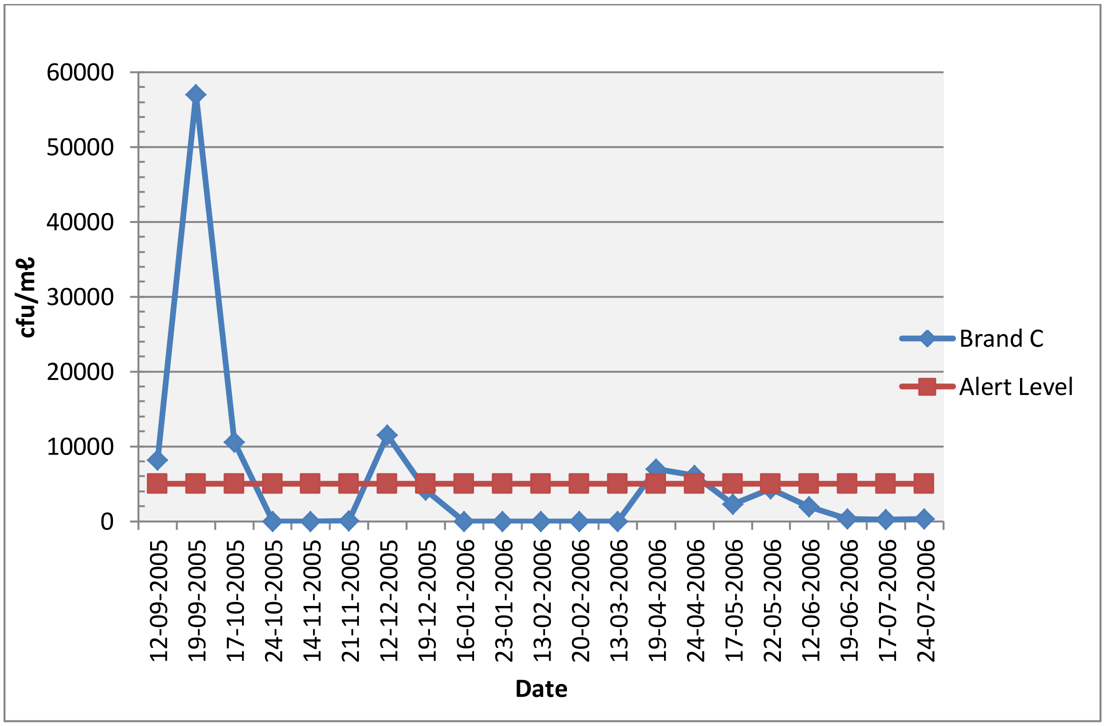

Figure 10: Detection of Heterotrophic Plate count in Brand C

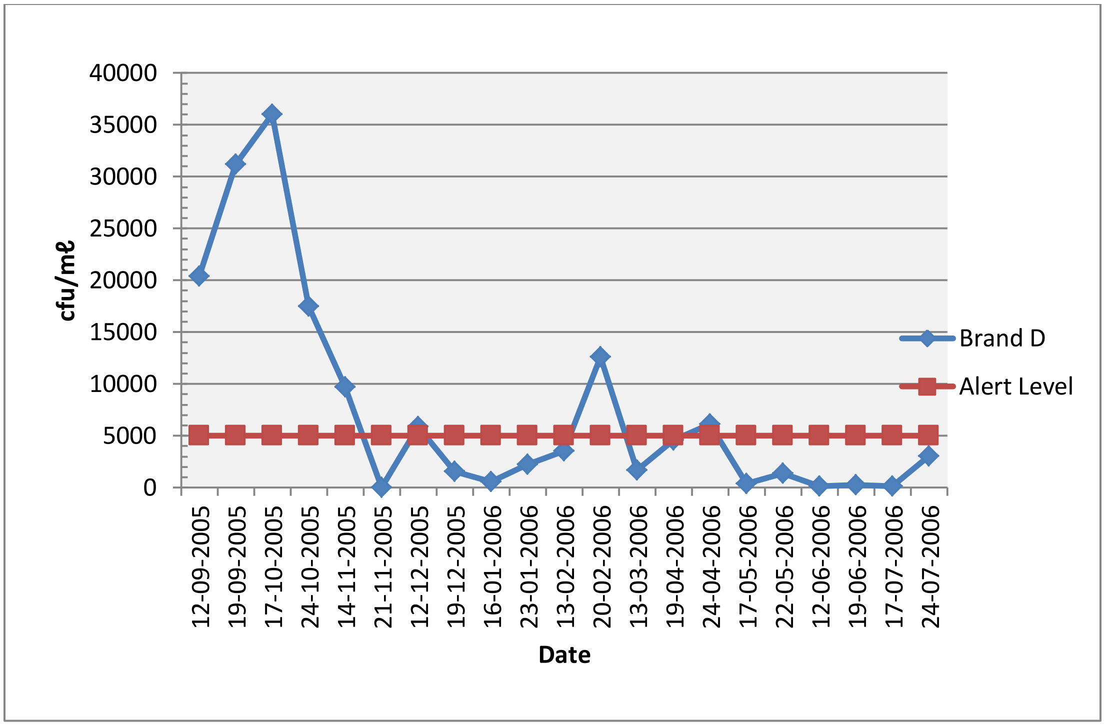

Figure 11: Detection of Heterotrophic Plate count in Brand D

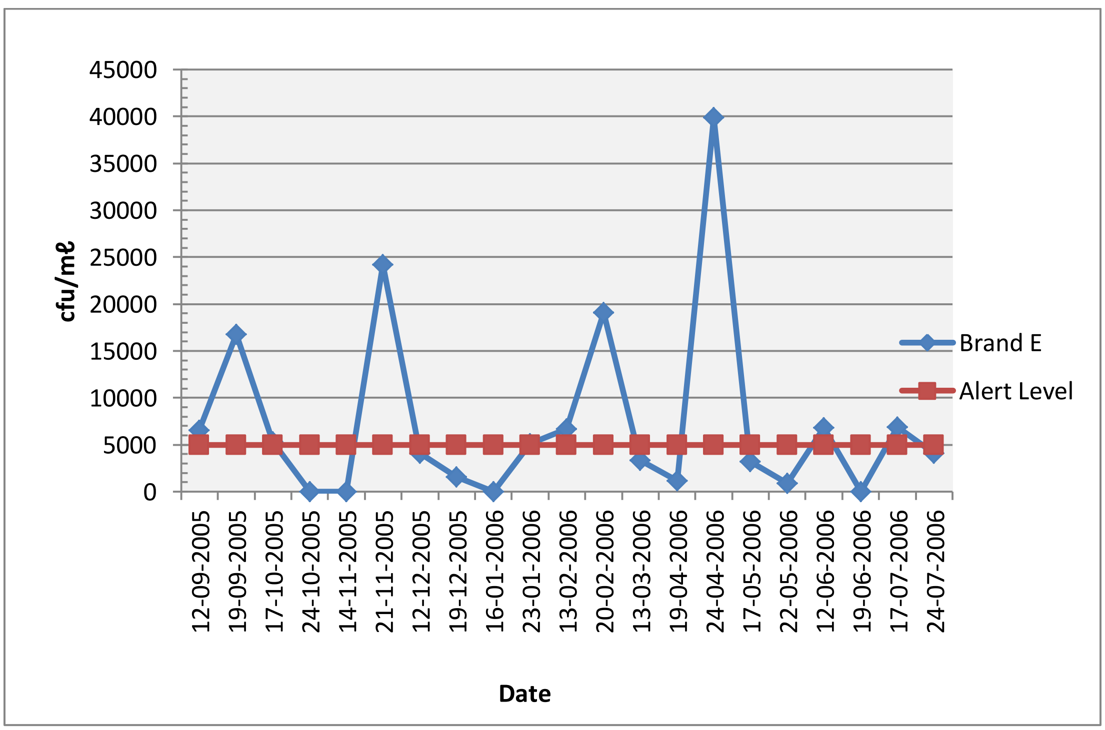

Figure 12: Detection of Heterotrophic Plate count in Brand E

According to SANS 1657, the total viable colony count should not be more than that which results from the normal increase in the bacterial content of the water at the source (bacterial levels at the source were not stipulated on the bottles). Therefore, there is no clarity on what the limit of the HPC should be. During the storage period the investigated bottled water for brand A and E showed a characteristic pattern that varied and fluctuated through the time of the storage period except the last stage of the storage period. During this last stage both brands (A and E) had a similar trend where HPC had depleted to counts below and slightly above the alert level (Figures 8 to 12). This could be due to lack of nutrients in the water that were used up throughout the storage period. It is possible that the microorganisms could not compete and utilise available nutrients and as a result they were slowly dying off. Brand C and D shows a logarithmic growth with HPC slightly increasing and reaching peak values and decreased to counts below and slightly above the alert level during the last stage of the storage period (Figures 10 and 11). This particular trend (i.e. HPC decreasing during the last stage) is seen with brand A and brand E (Figures 8 and 12). Brand B shows a high HPC count of >20 000 cfu/mℓ in the initial stage of analysis reaching peak values and in the last stage follows a similar pattern of HPC decreasing to levels below and slightly above the alert level. Contributing factors to these high HPC counts observed could be due to prolonged storage, storage temperature, typical natural flora of the source water used for different brands and lack of availability of nutrients in the water.

*All maximum results for HPC were expressed correct to the nearest hundred for HPC.

4.3.2 Detection of TC, FC, E.coli and yeast and moulds

Table 6, below shows the number of analyses completed and the results attained. During the period of study, no total and faecal coliform bacteria were detected from any of the bottled water brands tested. Total and faecal coliform bacteria demonstrate faecal pollution in water and food and the counts of these indicator bacteria should be 0cfu/100mℓ (Ehlers *et al.,* 2004). Further verification was not required in order to detect presence of *E.coli* as indicated on Table 6. Moulds were believed not to grow into visible bodies in mineral water products containing low amounts of nutrients (Criado *et al.,* 2005). Polyethylene Terephthalate (PET) and its plasticizer material (e.g. Di-*n*-butyl phthalate) during prolonged storage can release organic matter that could provide additional substrates for the microbial growth. After three years of storage at room temperature no yeasts or moulds were detected.

Table 6: Detection of TCs, FCs, yeasts and moulds

Year	No of complete analyses	Compliance (as a percentage)				
		Yeast	Mould	TC	FC	EC
2005	8	N/A	N/A	100	100	100
2006	13	N/A	N/A	100	100	100
2007	N/A	N/A	N/A	N/A	N/A	N/A
2008	2	100	100	N/A	N/A	N/A

N/A- Not Analyzed

4.3.3 Detection of algal species

Table 7 shows the phytoplankton constituents and species composition that indicated changes in water quality due to prolonged storage. All the bottled water tested was stored at room temperature with artificial lighting to mimic typical conditions of the supermarket and in homes.

Table 7: Results of Algal analysis for different bottled water brands

Bottled Waters	Date	Algal Species detected	Results(cells/ml)
Brand A	Sep-05	Algal species not detected	0
	Jan-06	Bacillariophyceae	34
		pennate diatoms	34
		organic pollution	34
	Apr-06	Euglenophyceae	34
		trachelomonas Species	34
		organic pollution	34
	Jul-06	Bacillariophyceae	103
		centric Diatoms	69
		pennate diatoms	34
		Filter clogging	69
Brand B	Sep-05	Algal species not detected	0
	Jan-06	Chlorophyceae	34
		Monoraphidium	34
	Apr-06	Algal species not detected	0
		Algal species not detected	0
	Jul-06	Algal species not detected	0
		Algal species not detected	0
and C	Sep-05	Centric Diatoms	34
	Jan-06	Chlorophyceae	34
		Monoraphidium	34
	Apr-06	Bacillariophyceae	138
		Aulacoseira species	103
		Centric diatoms	34
	Jul-06	Chlorophyceae	0
		Coccomonas species	0
Brand D	Sep-05	Algal species not detected	0
	Jan-06	Chlorophyceae	586
		Monoraphidium species	586
	Apr-06	Chlorophyceae	0
		Coccomonas species	0
	Jul-06	Chlorophyceae	0
		Coccomonas species	0
Brand E	Sep-05	Algal species not detected	0
	Jan-06	Monoraphidium species	34
	Apr-06	Aulacoseira species	138
		centric Diatoms	34
		Filter clogging	172
	Jul-06	Coccomonas species	0

Brand A

Within a week of purchase, no algal species were detected in brand A (mineral water). Prolonged storage after four months revealed presence of (Bacillariophyceae) diatoms at 34cells/ mℓ. These diatoms are microscopic in nature and are commonly found in freshwater where they live free floating or attached to a substrate. They are usually used to determine the quality of the water. After seven months of storage (Euglenophyceae) *Trachelomonas* species 34 cells/mℓ was detected; trachelomonas species is mostly found in fresh water rich in organic matter. In the Euglenophyceae, a photosynthetic genus and species such as *Trachelomonas* possess *lorica* deposited by iron and magnesium. It is clear that there was growth succession from the algal species detected. Bacillariophyceae 103 cells/ mℓ (centric diatoms and pinnate diatoms) were detected; therefore it was evident that prolonged storage of brand A resulted in algae growing and increasing in numbers over time (Table 7).

Brand B

Within a week after purchase, no algal species were detected in brand B (mineral water). Four Months storage resulted in Chlorophyceae 34 cells/ mℓ commonly referred to as green algae being detected. Thereafter, the remaining storage period no algae were detected. This could be due to growth succession period where algae die off in order to allow other species to reoccur (Table 7).

Brand C

Within a week after purchase, a centric diatom 34 cells/ml was detected in brand C (mineral water from unspecified source). This was followed by dominant algal species *Chlorophyciae (monoraphidium)* in 34 cells/mℓ found in other brands (i.e. brand B, D and E) after 4 four months of storage (Table 7). Growth succession occurred after seven months of storage and different algal species were detected *Bacillariophyceae* 138 cells/ml, *Aulacoseira* species 103 cells/ml and centric diatoms 34 cells/mℓ. After 11 months of storage no algal species were detected. It is evident from these results that, during the period of study algae became persistent when bottled water was stored for a prolonged period and growth succession resulted in a variety of algal species.

Brand D:

Within a week of purchase, no algal species were found in brand D (bottled water sourced from the tap). After four months of storage, *Chlorophyciae (monoraphidium)* 586cells/ml was detected in high numbers and thereafter no algal species were found. This could be due to growth period where algae was dying off and allowing reoccurrence of other algal species in its succession growth phase (Table 7).

Brand E:

Within a week after purchase, no algal species were detected in brand E (spring water).Dominant species (i.e. *monoraphidium*) 34 cells/ml was found. This was followed by *Aulacoseira species* 138 cells/ml, centric diatoms 34 cells/ml and other species. Thereafter, no algal species were detected (Table 7).

4.4 Inorganic determinants results

Not all chemicals will be present in a water supply; if present they may not be found at levels of concern. The determinants investigated include those that pose a health risk to human health when they are present in excessive quantities and those that are beneficial to human health as part of human daily intake requirements. The results below will address various determinants and the effects thereof on the shelf-life of various bottled water brands tested.

4.4.1 Heavy metals

These are chemical elements with a specific gravity that is at least 5 times the specific gravity of drinking water. In small quantities, certain heavy metals are nutritionally essential for a healthy life. Toxic metals can be present in industrial, municipal, and urban runoff, which can be harmful to humans and aquatic life. There are over 50 elements that can be classified as heavy metals, 17 of which are considered to be both very toxic and relatively accessible. Toxicity levels depend on the type of metal, its biological role, and the type of organisms that are exposed to it. Some bottled waters are derived from ground water therefore; heavy metals at elevated concentration can contaminate these sources making drinking water (including bottled water) unsafe to drink if not monitored.

4.4.1.1 Arsenic (As)

Arsenic is widely distributed throughout the Earth's crust.Arsenic is introduced into drinking-water sources primarily through the dissolution of naturally occurring minerals and ores. Except for individuals who are

Occupationally exposed to arsenic, the most important route of exposure is through the oral intake of food and beverages. There are a number of regions where arsenicmay be present in drinking-water sources, particularly groundwater, at elevatedconcentrations. Arsenic in drinking-water is a significant cause of health effects in some areas, and arsenic is considered to be a high-priority substance for screening in drinking-water sources. Concentrations are often highly dependent on the depth to which the well is sunk (WHO, 2004). Figure 13 indicates that arsenic was below the specified bottled water standard of 0,05 mg/ℓ and complied with SANS 1657 of 0,05 mg/ℓ. A similar trend was observed for brands A, B, C, D and E. Arsenic remained constant throughout bottled water shelf-life. Therefore, there was no significant variation during the period of study. Although it may be found in surface water, ground water could be the main source of arsenic contamination. Concentration in ground water in some areas can be elevated as a result of erosion from local rocks.

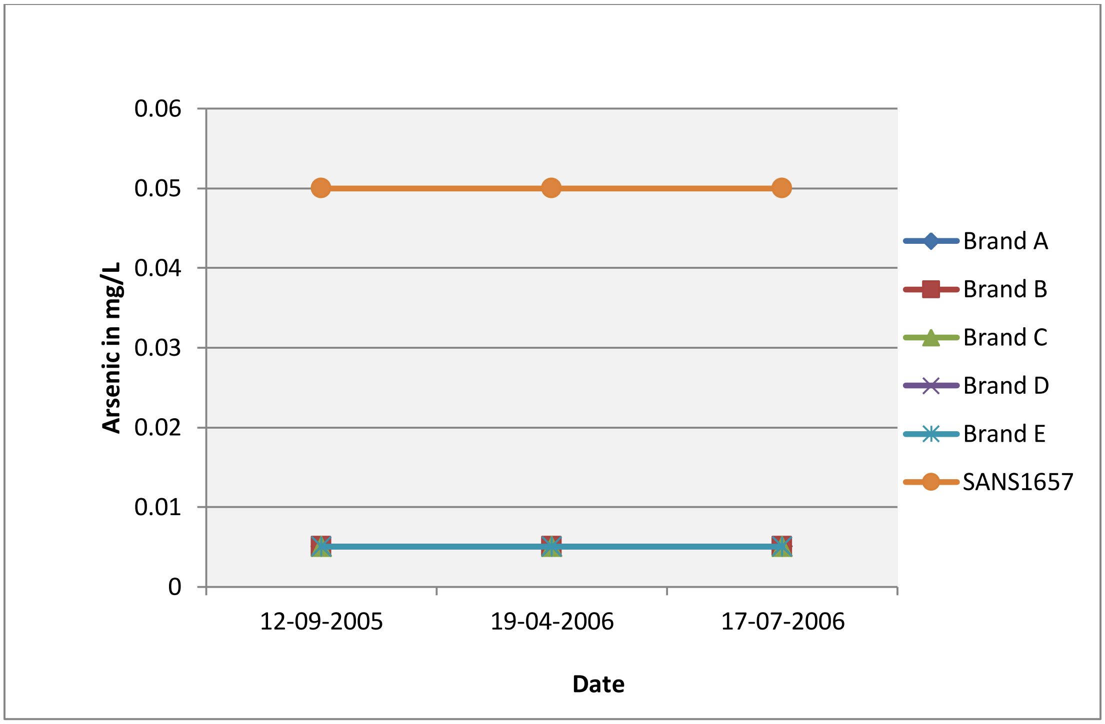

Figure 13: Results for Arsenic

4.4.1.2 Cadmium (Cd)

Cadmium occurs naturally in the earth's crust. It can enter water from disposal of waste water from households or industries. It stays in the liver and kidneys, causing the kidneys to be overloaded and causes health damage. Contamination in drinking-water may be caused by impurities in the zinc of galvanized pipes and solders and some metal fittings. Food is the main source of daily exposure to cadmium (WHO, 2004) (Figure 14 shows that Cadmium was below the detection limit 0,003 mg/ℓ and 0, 0025 mg/ℓ and complied with SANS 1657 of 0, 01 mg/ℓ. A similar trend was observed for brand A, B, C, D and E as cadmium remained constant (below detection limit) throughout the bottled water shelf-life. Therefore, there was no significant variation during the period of study .The slight decrease in final results was due to the laboratory detection limit being changed from 0,003mg/ℓ to 0, 0025 mg/ℓ. Fertilizers often contain cadmium in them which can easily enter the soil and as a result cadmium can enter water in that way. Therefore, it should be monitored in source water during water treatment processes before bottling.

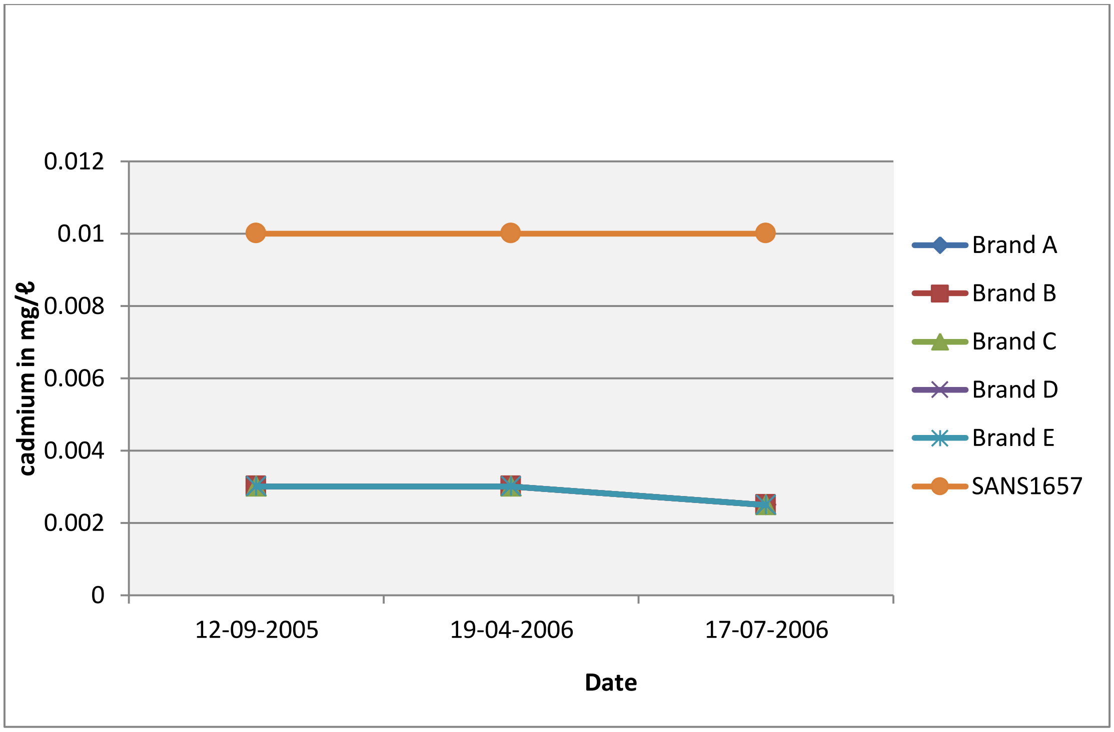

Figure 14: Results for Cadmium

4.4.1.3 Lead (Pb)

Lead is a soft metal widely used for application in meal products, cables, pipelines, paints and pesticides. The amount of lead dissolved from the plumbing system depends on several factors, including pH, temperature, water hardness and standing time of the water, with soft, acidic water being the most plumb solvent. Lead targets organs such as kidney and liver and causes adverse health effects (WHO, 2004).Therefore, water sourced from the tap such as Brand D can contain lead if treatment systems are not managed accordingly. The graph below indicates that lead was below the detection limit and complied with SANS 1657 limit of less than 0, 05 mg/ℓ. A similar trend was observed for brand A, B, C, D and E as lead remained constant throughout the bottled water shelf-life (Figure 15). Therefore, there was no significant variation during the period of study. The slight decrease in final results was due to the laboratory detection limit being changed from 0,01mg/ℓ to 0,008 mg/ℓ.

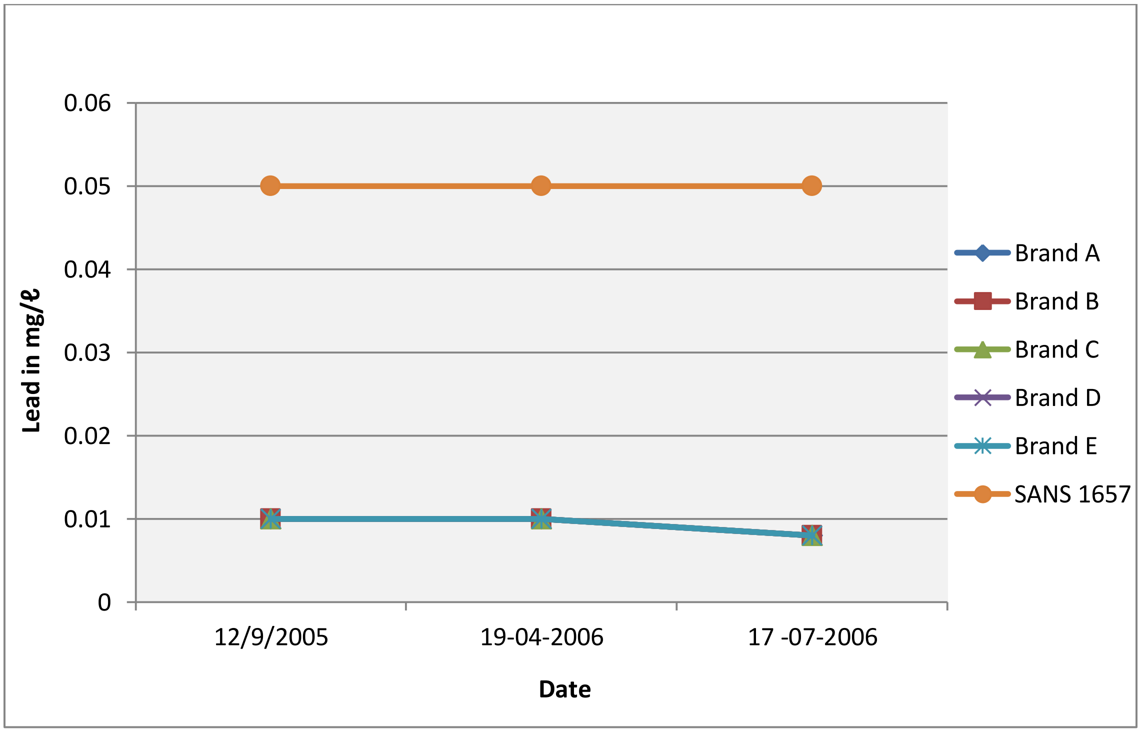

Figure 15: Results for Lead

4.4.1.4 Barium (Ba)

Barium (Ba) is a metal derived from natural sources, it occurs combined with other elements, such as sulfur,carbon and oxygen. Barium (Ba) in water comes primarily from natural sources. Food is the primary source of intake for the non-occupationally exposed population. However, where barium levels in water are high, drinking-water may contribute significantly to total intake (WHO.2004). The results as indicated on the graph below show that prolonged storage results in barium slightly increasing during the period of study for all the brands tested.This could be due to barium reacting with available sulfur,carbon and oxygen present in the bottled water tested. Barium complied with SANS 1657 of 1 mg/ℓ for all bottled water brands tested.Hence, the amount of barium that was detected in water was not high enough to become a health concern.

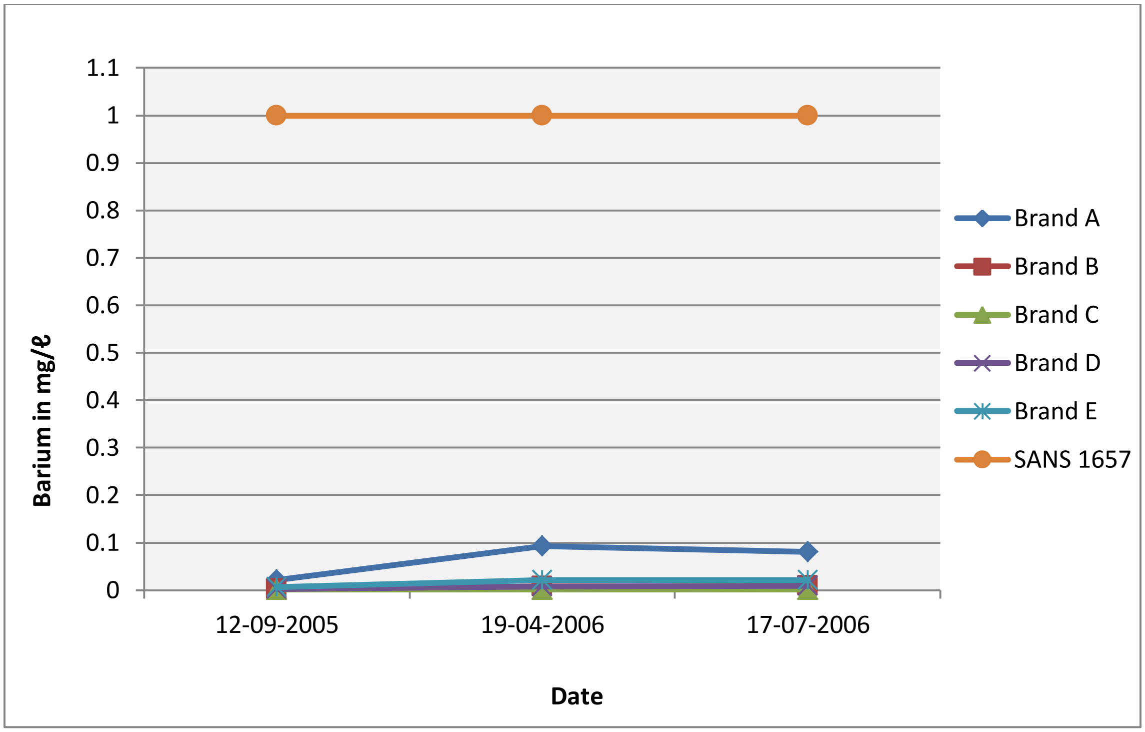

Figure 16: Results for Barium

4.4.1.5 Antimony (Sb)

Antimony (Sb) is naturally present in water, rocks and soil. Antimony was considered as a possible replacement for lead in solders, but there is no evidence of any significant contribution to drinking-water concentrations from this source. Daily oral uptake of antimony appears to be significantly higher than exposure by inhalation, although total exposure from environmental sources, food and drinking-water is very low compared with occupational exposure.Figure 17 below indicates that Antimony was below the detection limit of <5µg/ℓ in all brands. There was no significant variation during the period of study for all the bottled water investigated. All bottled water brands complied with SANS 241 drinking water standard of <10µg/ℓ.

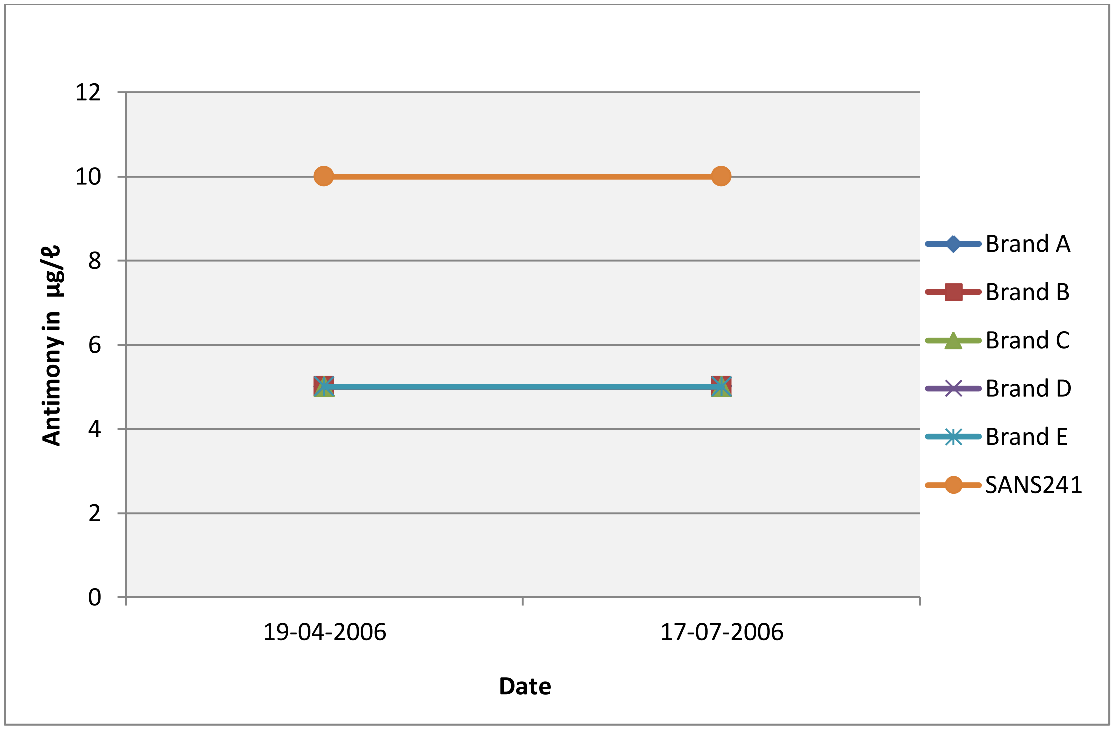

Figure17: Results for antimony

4.4.1.6 Vanadium (V)

Vanadium *(V)* occurs in the earth's crust, water and soil. The route of exposure for humans to vanadium can be via inhalation and oral ingestion. Vanadium is toxic to humans and animals and acute exposure to it may cause eye, nose, lung and throat irritation. The other health effects that vanadium has on humans are cardiac and vascular disease, damage to the nervous system, bleeding of the liver and kidneys, headaches and dizziness. It affects human health when consumed in high concentrations. Figure 18 below indicates that vanadium was below the detection limit of <0,03 mg/ℓ. There was no significant variation during the period of study for all the bottled water investigated. All bottled water brands complied with SANS 241 drinking water standard of <0,2 mg/ℓ. The concentration of vanadium in drinking water depends on the geographical location.

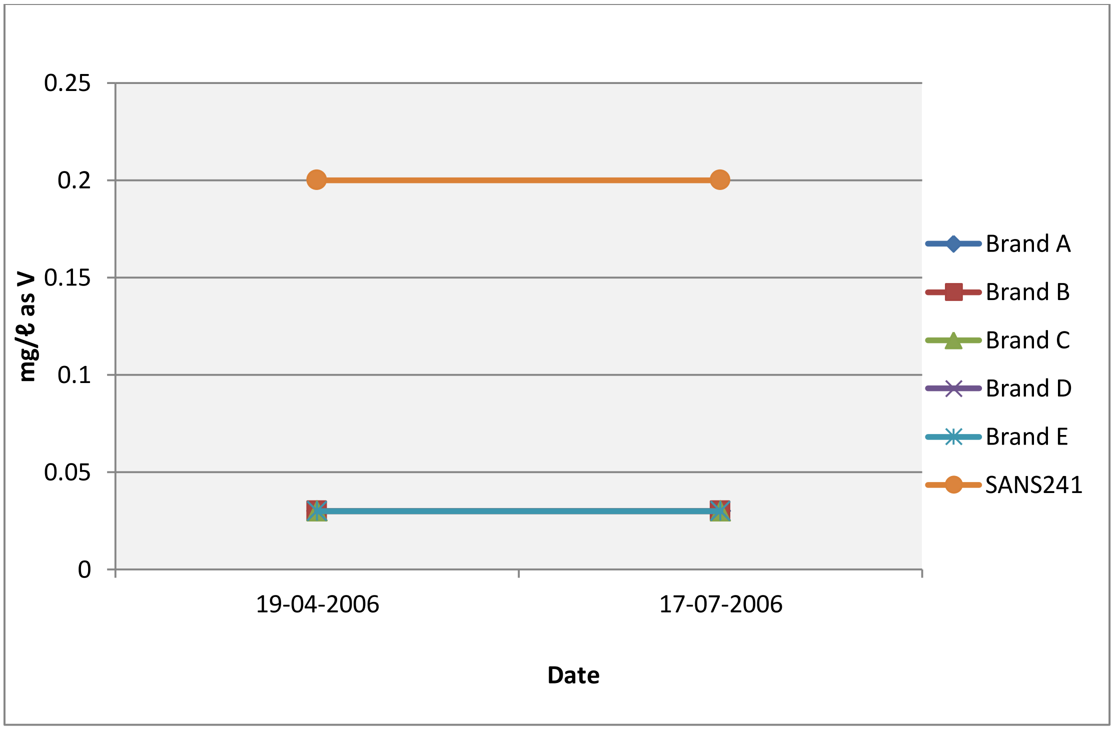

Figure 18: Results for Vanadium

4.4.2 Essential elements

4.4.2.1 Zinc (Zn)

Zinc occurs naturally in small amounts. However, most zinc occurs in groundwater due to human activity. Zinc is an essential trace element found in virtually all food and potable water in the form of salts or organic complexes. The diet is normally the principal source of zinc. Although levels of zinc in surface water and groundwater normally do not exceed 0.01and 0.05 mg/ℓ, respectively, concentrations in tap water can be much higher as a result of dissolution of zinc from pipes. However, drinking-water containing zinc at levels above 3 mg/ℓ may not be acceptable to consumers (WHO, 2004). The concentration of zinc in surface and ground water is low. The results for zinc showed that there was no significant difference in the concentrations found in all the bottled water tested (Figure 19). For Brands A and E, concentrations varied during the period of study with the highest concentration of 0,3 mg/ℓ in both brands. Brand B, C and D showed no particular trend as concentrations varied during the period of study with the highest concentration of 0,09 mg/ℓ for brand B and 0,08 mg/ℓ for brand C and D. However, the concentration in tap water can be high due to corrosion of zinc= coated pipes. Zinc is not toxic, but acute exposure to high concentrations of zinc may cause nausea, vomiting, abdominal cramps and diarrhoea. It is important that zinc is monitored during water treatment processes and ground water especially if the water is used for bottling.

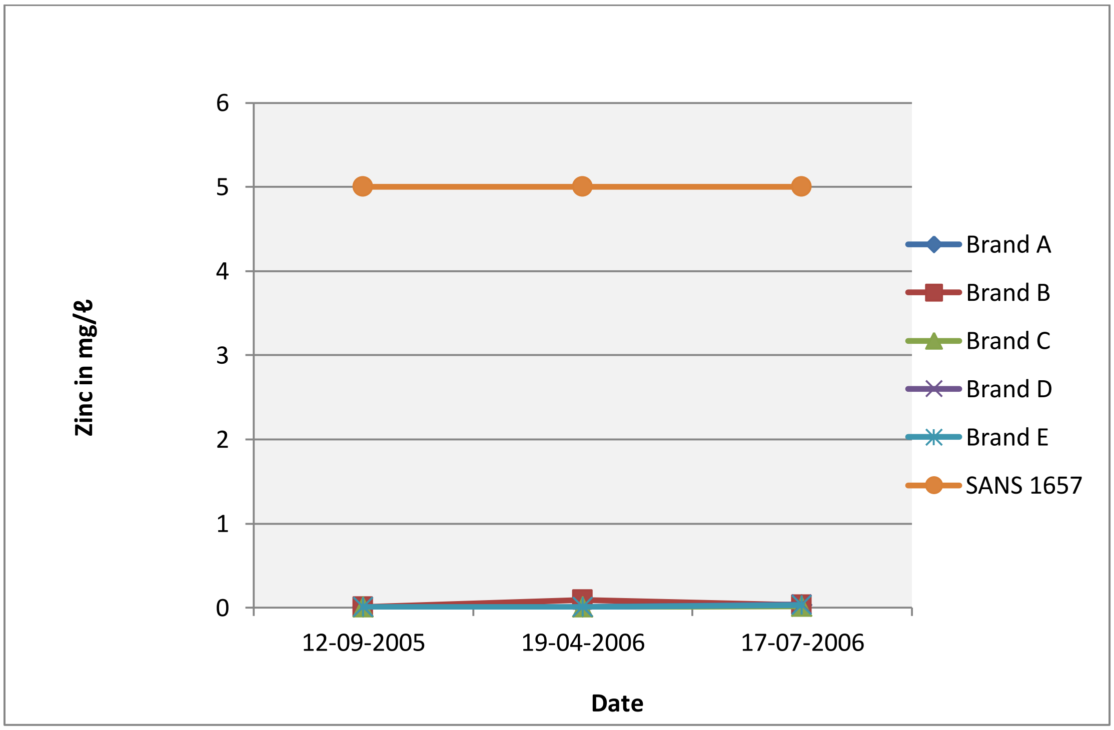

Figure 19: Results for Zinc

4.4.2.2 Chromium (Cr)

Chromium is a metal that occurs in the soil, rocks and ores. Other than occurring naturally in the environment, it can be introduced by industrial effluents. It can be found in waters only in trace amounts. It is also present in the human body. Daily intake strongly depends upon feed levels. Another form of chromium (i.e. trivalent chromium) is an essential trace element for humans. It combines with insulin to remove glucose from blood and metabolises fat. The placenta is the organ with the highest chromium amounts. Figure 20 below indicates that chromium was below the detection limit and complied with SANS 1657 limit of less than 0,05mg/ℓ. The WHO *International guidelines for drinking-water* recommended a maximum allowable concentration of 0.05 mg/ℓ for chromium (WHO, 2004).Chromium for brand A, B, C, D and E remained constant throughout bottled water shelf-life. Therefore, there was no significant variation during the period of study.

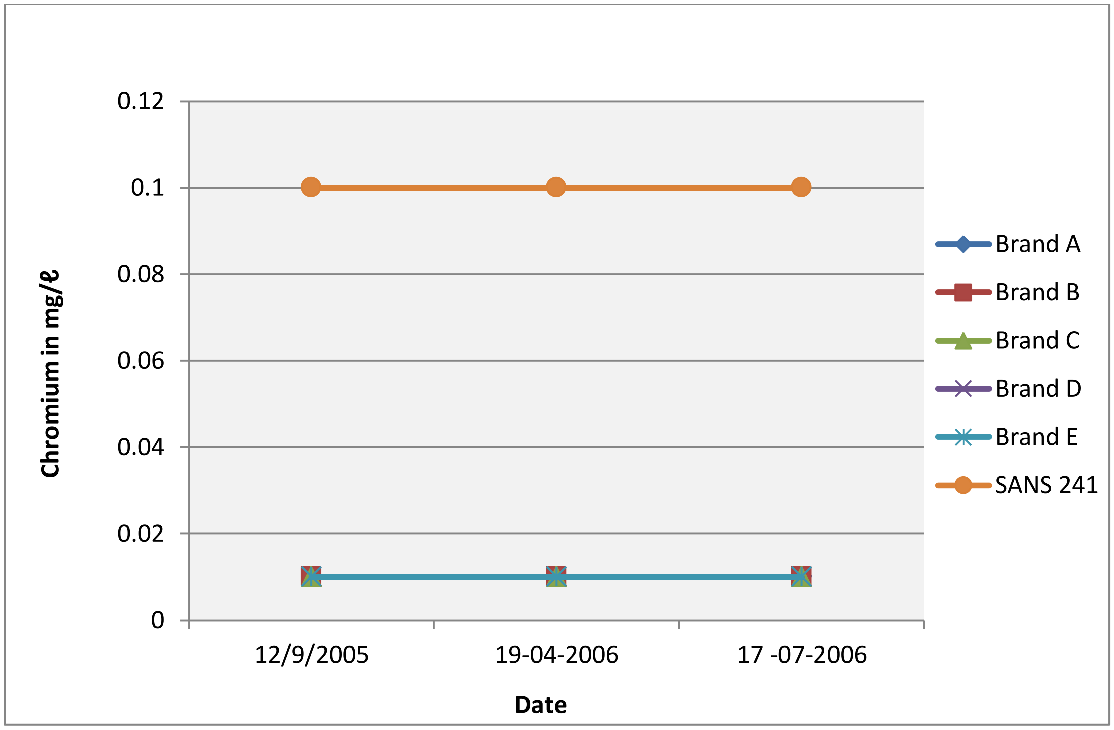

Figure 20: Results for Chromium

4.4.2.3 Nickel (Ni)

The major contributor to the total daily oral intake of nickel in humans is food and water is a minor contributor. If water has stood for an extended period of time in water pipes, nickel contribution from water may be significant. Therefore, measures need to be taken if bottled water is sourced from the tap (nickel-plate taps). High nickel doses may result in liver and kidney toxicity (NHMRC, 2004). The most common adverse health effect of nickel in humans is an allergic reaction (USEPA, 2002).Figure 21 below indicates that nickel was below the detection limit of <0,05 mg/ℓ. A similar trend was observed for brand A, B, C, D and E (Figure 21). There was no significant variation during the period of study for all the bottled water investigated. All bottled water brands complied with SANS 241 drinking water standard of <0,15 mg/ℓ (Figure 21).

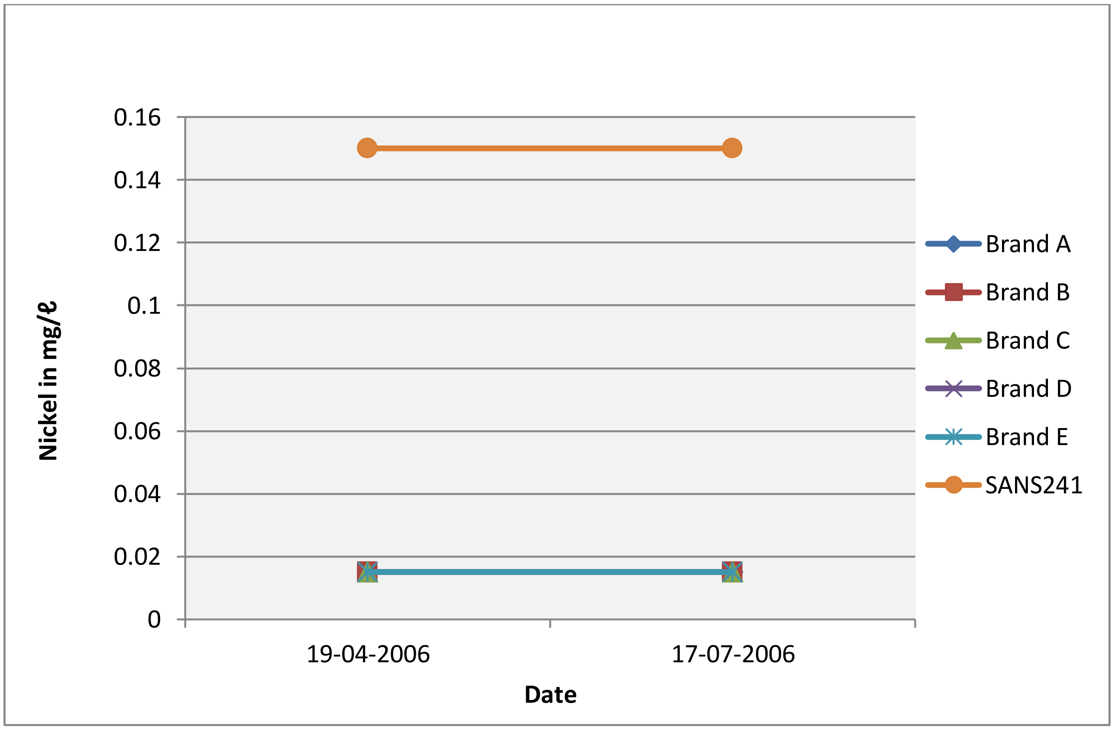

Figure 21: Results for Nickel

4.4.2.4 Selenium (Se)

Sources of selenium in water are copper metal cleanser, lead refinery effluents and municipal wastes. Selenium is toxic and short-term exposure at elevated concentrations can cause nausea, diarrhoea, vomiting and fatigue. Long-term exposure to selenium in drinking water can cause damage to hair, fingernails, nervous system, kidneys and liver tissue. There is no evidence of selenium causing cancer. Figure 22 below indicates that Selenium was below the detection limit of 0,005 mg/ℓ and complied with SANS 1657 limit of less than 0,01mg/ℓ. A similar trend was observed for brands A, B, C, D and E as selenium remained constant throughout bottled water shelf-life. Therefore, there was no significant variation during the period of study.

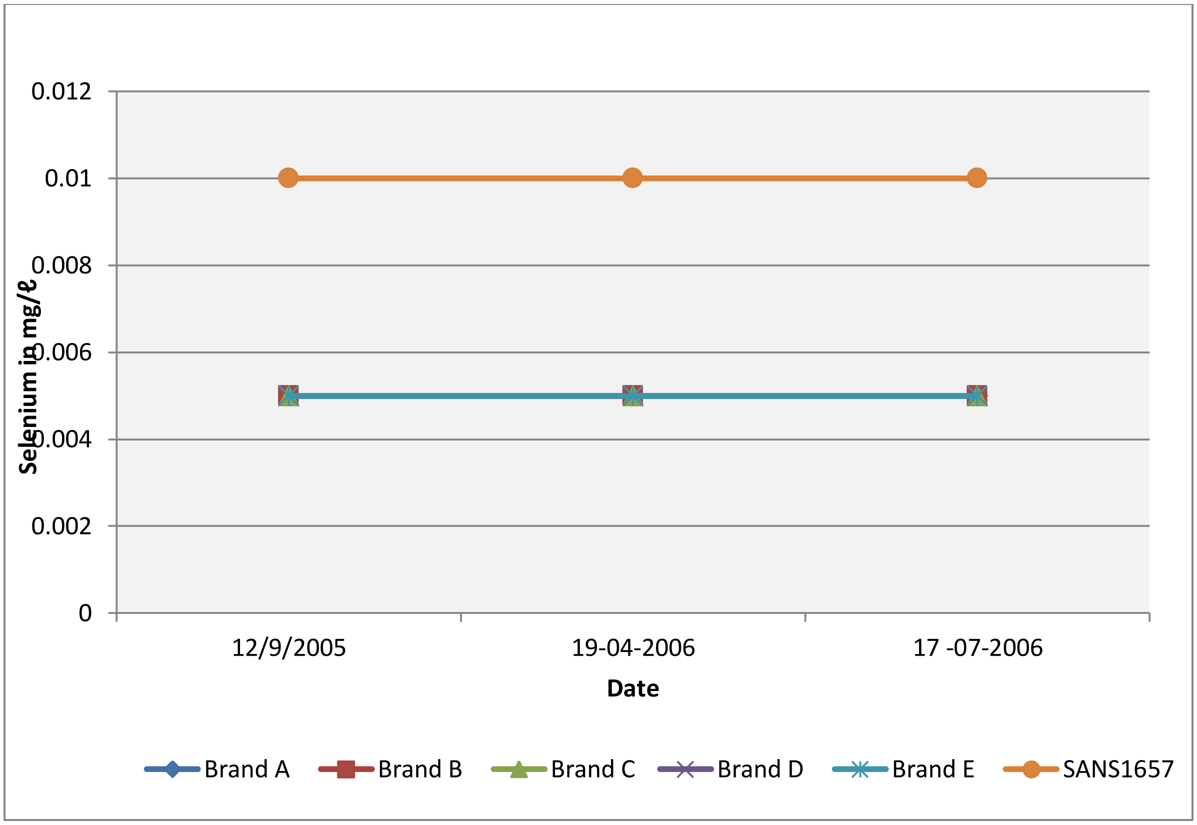

Figure 22: Results for Selenium

4.4.2.5 Copper (Cu)

Copper is the metal that occurs naturally in rocks, soil water, sediment and air. Hence, copper can work its way into the water by dissolving from copper pipes in plumbing material. The longer the water has stagnated in the pipes, the more copper it is likely to have absorbed. It is however essential for human health however, copper at concentrations >5 mg/ℓ can also give rise to taste problems. It serves as a normal constituent in the blood. Concentrations of copper are highest in the liver, brain, heart, and kidney. Muscle contains a low concentration of copper, but because of its large mass, skeletal muscle contains almost 40 percent of all copper in the body. Immediate effects from consuming elevated levels of copper include vomiting, diarrhoea, stomach cramps, and nausea. The human body has a natural mechanism for maintaining appropriate levels of copper. However, children under one year old have not yet developed this mechanism and, as a result, are more vulnerable to the toxic effects of copper. All the bottled water brands that were investigated showed that copper increased in concentration and decreased during the final stage of storage period (Figure 23). They also complied with SANS 1647 bottled water standard of 1.0 mg/ℓ.

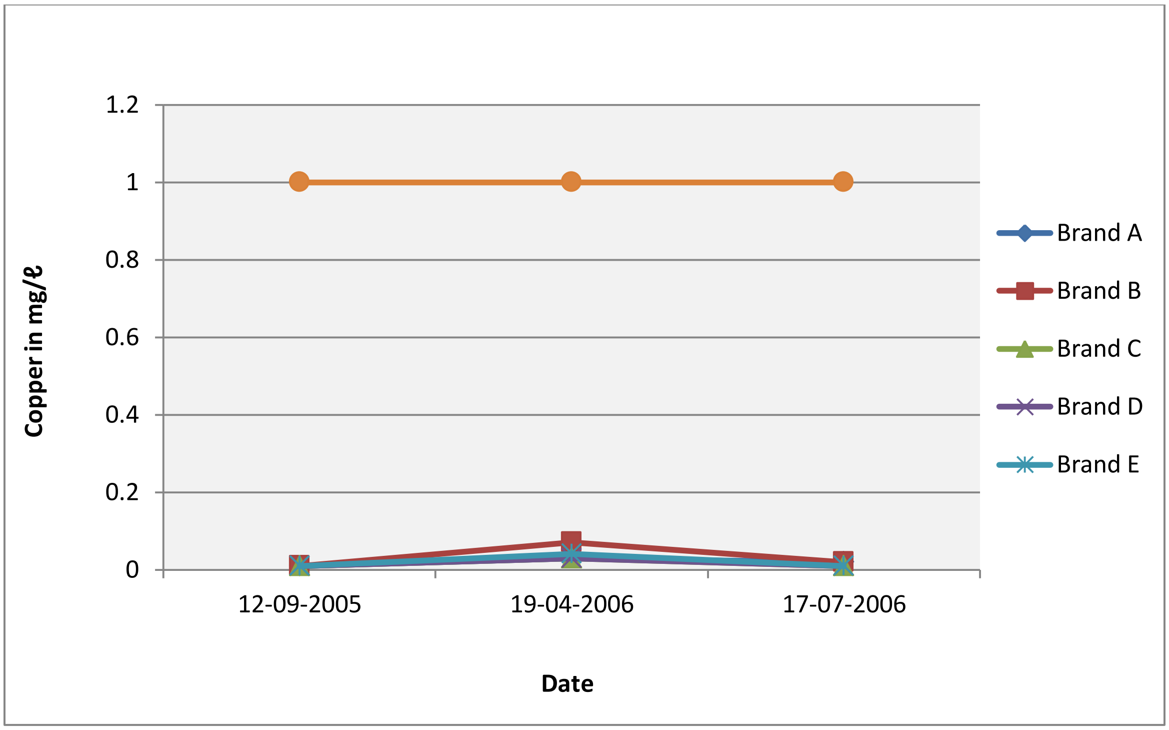

Figure 23: Results for Copper

4.4.2.6 Calcium (Ca)

Calcium is the most abundant mineral in the body; it makes up about 1.5% to 2% of the body weight and 39% of the total body minerals. Approximately 99% of the calcium exists in the bones and teeth. Calcium plays an important role in building healthy teeth and bones. It is vital to every cell of the body for muscle function, nerve transmission, blood clotting and many other uses. A lack of calcium can cause osteoporosis (a disease in which the bones become extremely porous) (http://www.sahealthinfo.org/nutrition/vitaminminerals/minerals/calcium.htm)

No particular trend was noticed in all the bottled water investigated (Figure 24). Brands A and E showed that calcium concentrations varied during the period of study, this is seen with fluctuation in the results observed. In brands B and C calcium decreased in concentration and thereafter remained constant (Figure 24). Brand D remained constant and decreased particularly in the last stage of the storage period. Calcium concentrations observed had average values of 2,3 for brand A,33 for brand B,40 for brand C,27 for brand D and 4,7 for brand E. All the bottled water tested complied with SANS 241 drinking water standard of <150mg/ℓ.

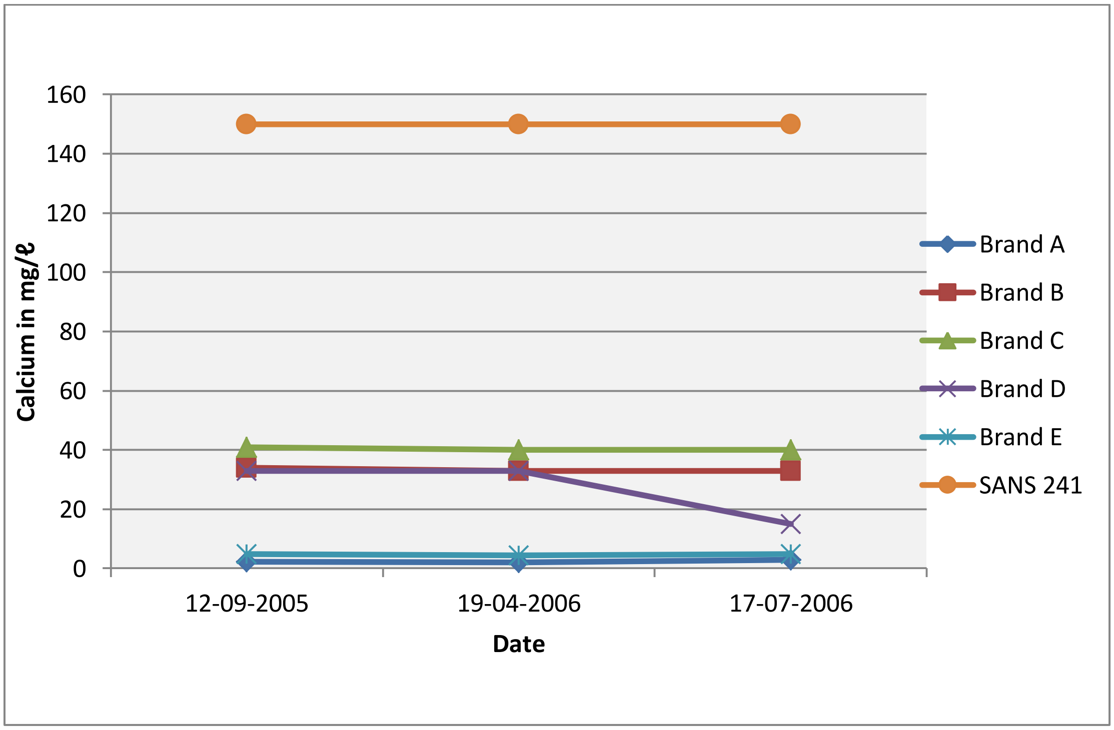

Figure.24: Results for Calcium

4.4.2.7 Cobalt (Co)

Cobalt is an essential element for humans and forms part of Vitamin B12. It is widely dispersed in the environment. It is used to treat anaemia with pregnant woman, because it stimulates the production of red blood cells. Total daily intake of cobalt varies and may be as much as 1mg (http://www.lenntech.com/periodic/elements/co.htm).Figure 25 below indicates that cobalt was below the detection limit of less than 0,05 mg/ℓ and complied with SANS 241. Cobalt remained constant during the period of study. Therefore, there was no significant variation during the period of study for all the bottled water investigated (Figure 25).

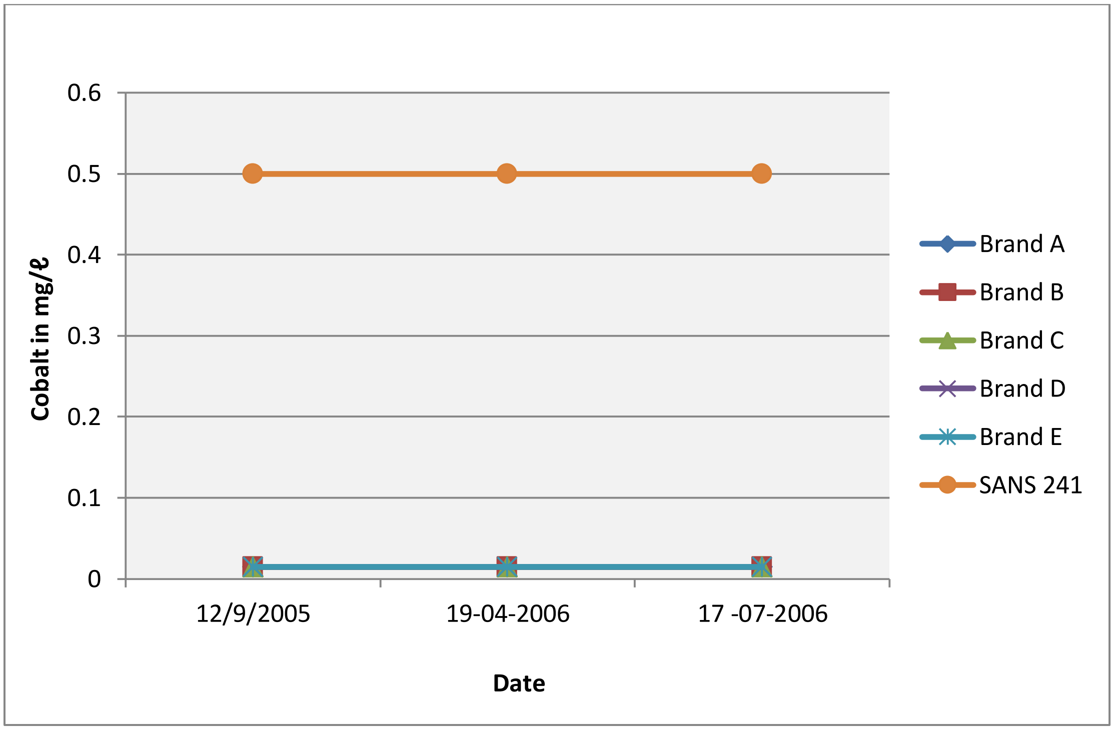

Figure 25: Results for Cobalt

4.4.2.8 Molybdenum (Mo)

Molybdenum is considered to be an essential element, with an estimated daily requirement of 0.1–0.3 mg/ℓ for adults. No data are available on the carcinogenicity of molybdenum by the oral route. Additional toxicological information is needed on the impact of molybdenum on bottle-fed infants (WHO, 2004).Figure 26 below indicates that molybdenum was below the WHO guideline value of less than 0,07 mg/ℓ .

Concentrations in drinking-water are usually less than 0.01 mg/ℓ, although concentrations as high as 200 mg/ℓ have been reported in areas near mining sites. A similar trend was observed for brand A, B, C, D and E .There was no significant variation during the period of study for all the bottled water investigated (Figure 26).

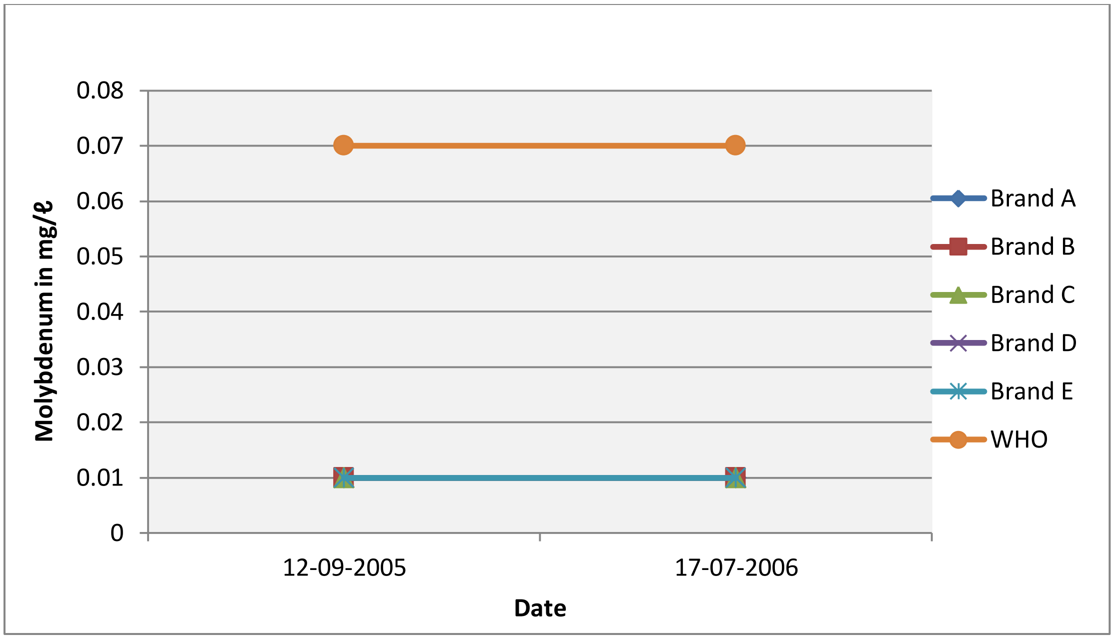

Figure 26: Results for Molybdenum

4.4.2.9 Beryllium (Be)

According to WHO Berylium is unlikely to occur in drinking water and there is no guidline values for it. The values only repesent the detection limit of <0,02 mg/ℓ. All the bottle water brands did not show any variations during the period of study (Figure 27).

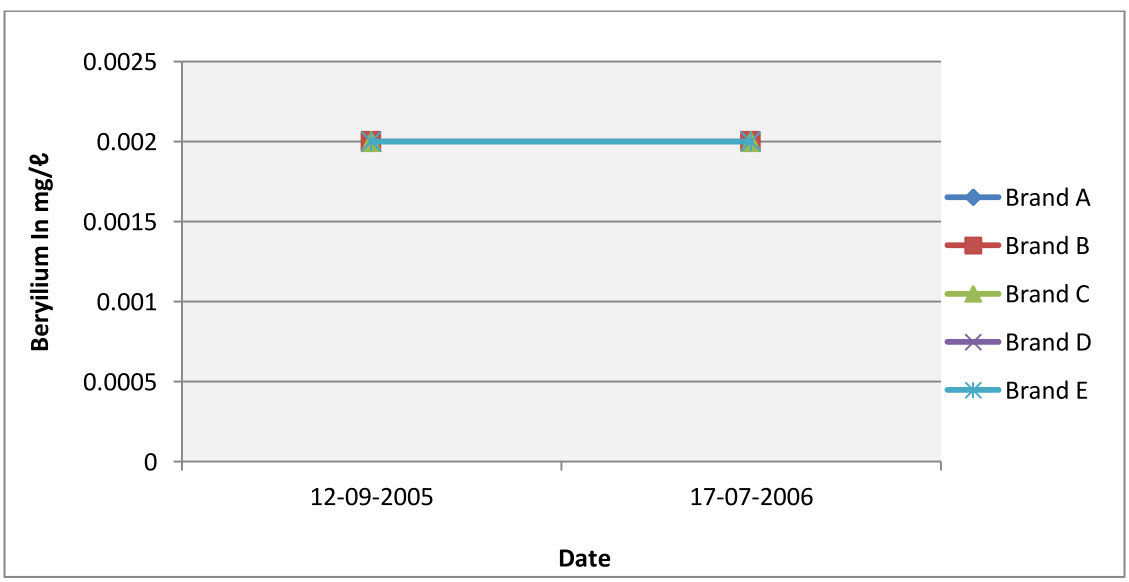

Figure 27: Results for Beryllium

4.4.3 Metals of aesthetic significance

4.4.3.1 Manganese (Mn)

Manganese is one of the most abundant metals in the Earth's crust, usually occurring with iron. Manganese is an essential element for humans and other animals and occurs naturally in many food sources. The most important oxidative states for the environment and biology are Mn2+, Mn4+ and Mn7+. Manganese is naturally occurring in many surface water and groundwater sources, particularly in anaerobic or low oxidation conditions. Levels in fresh water typically range from 1 to 200 mg/ℓ , although levels as high as 10 mg/litre in acidic groundwater have been reported; higher levels in aerobic waters usually associated with industrial pollution (WHO,2004).There was no significant variation in manganese concentration from all the brands tested (Figure 28). However, Brand D (bottled tap water) showed a slight increase in Mn concentrations. Average concentrations of 0,007 mg/ℓ for Brand A, 0,005 mg/ℓ for brand B, 0,005 mg/ℓ for brand C, 0,01 mg/ℓ for brand D and 0.007 mg/ℓ for brand E. Manganese concentrations were within bottled water standard 0,5 mg/ℓ for all bottled water brands investigated.

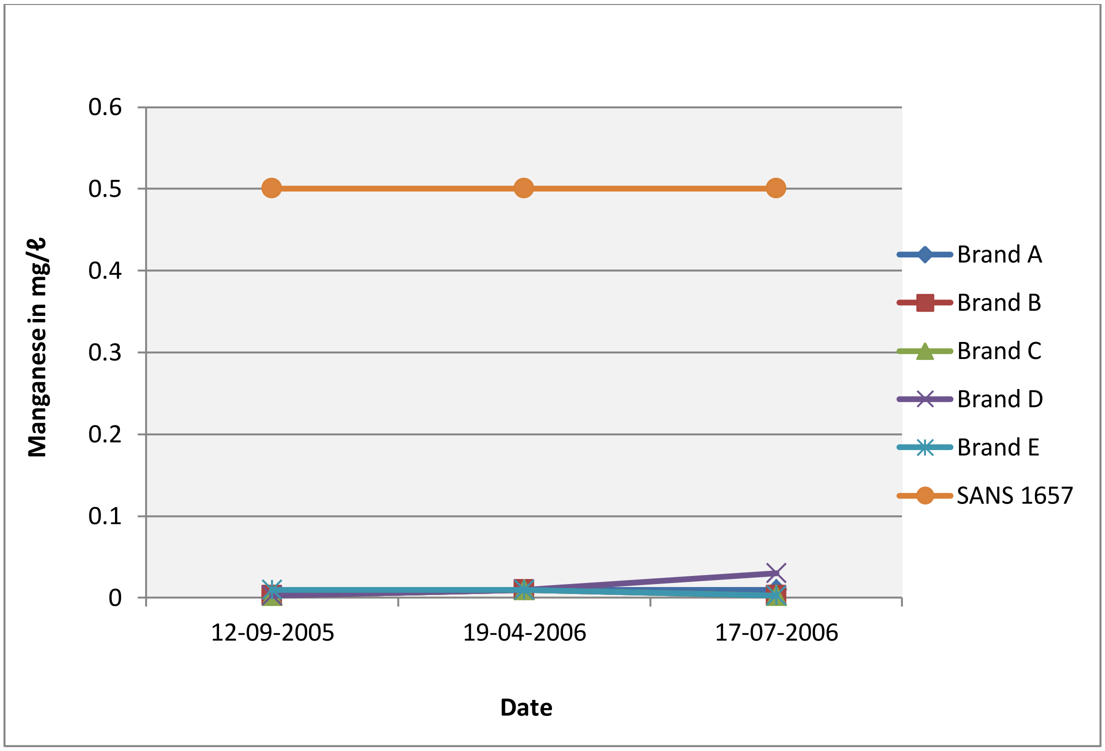

Figure 28: Results for Manganese

4.4.3.2 Iron (Fe)

Iron is a metallic element found in the earth's crust. Water seeping through the soil and rock can dissolve minerals containing iron and hold it in solution. Occasionally, iron pipes also may be a source of iron in water. Iron is not considered hazardous to health. In fact, iron is essential for good health because it transports oxygen in the blood (WHO,2004). SANS 1657 recommended limit for iron in bottled water is 0,2mg/ℓ this limit is based on taste and appearance rather than on any detrimental health effect. Results for iron observed indicated that brands A, B, C, and E showed similar characteristic patterns of iron concentration decreasing during the period of study (Figure 29). Hence, all these brands consisted of mineral water and spring water. Brand D (bottled tap water), remains an exception and iron concentration increased slightly during the period of study (Figure 29). The reason for this type of behaviour is unknown. Although present in your drinking water, iron is seldom found at concentrations greater than 10 mg/ℓ. However, as little as

0,3mg/ℓ can cause water to turn a reddish-brown colour. Iron concentrations were within bottled water standard 0.2mg/ℓ for all bottled water brands investigated

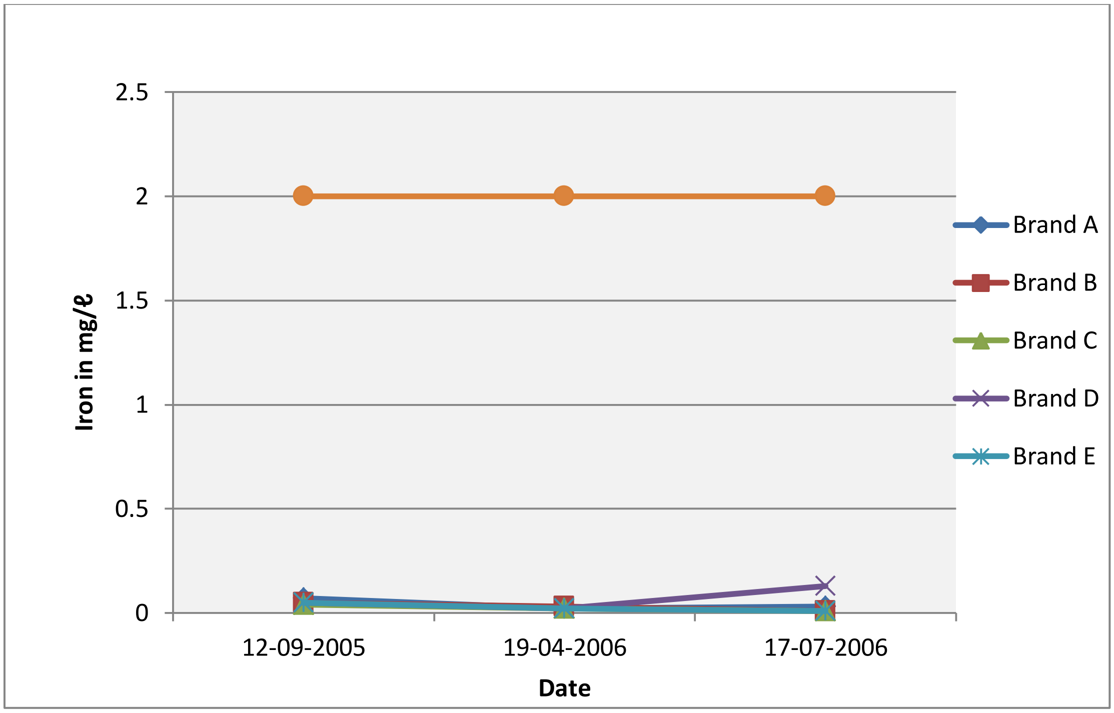

Figure 29: Results for Iron

4.4.3.3 Aluminium (Al)

Aluminium is the most abundant metallic element and constitutes about 8% of the earth's crust. Aluminium salts are widely used in water treatment as coagulants to reduce organic matter, colour, turbidity and microorganism levels. Such use may lead to increased concentrations of aluminium in finished water.Where residual concentrations are high, undesirable colour and turbidity may ensue. Concentrations of aluminium at which such problems may occur are highly dependent on a number of water quality parameters and operational factors at the water treatment plant. In humans, aluminium and its compounds appear to be poorly absorbed, although the rate and extent of absorption have not been adequately studied for all sectors of the population. The degree of aluminium absorption depends on a number of parameters, such as the aluminium salt administered, pH (for aluminium speciation and solubility), bioavailability and dietary factors. Brand A, B, C, D and E showed the same characteristic pattern as shown in Figure 30. Concentrations increased slightly followed by a decrease in the final stage. Average values (0,01 mg/ℓ for brand A, 0,01 mg/ℓ for brand B, 0,01 mg/ℓ for Brand C, 0,01 mg/ℓ for brand D and 0,01 mg/ℓ for brand E) for all the bottled water brands investigated did not indicate any reason for concern. Aluminium concentrations were within bottled water standard SANS 1657 limit of 0,15mg/ℓ.

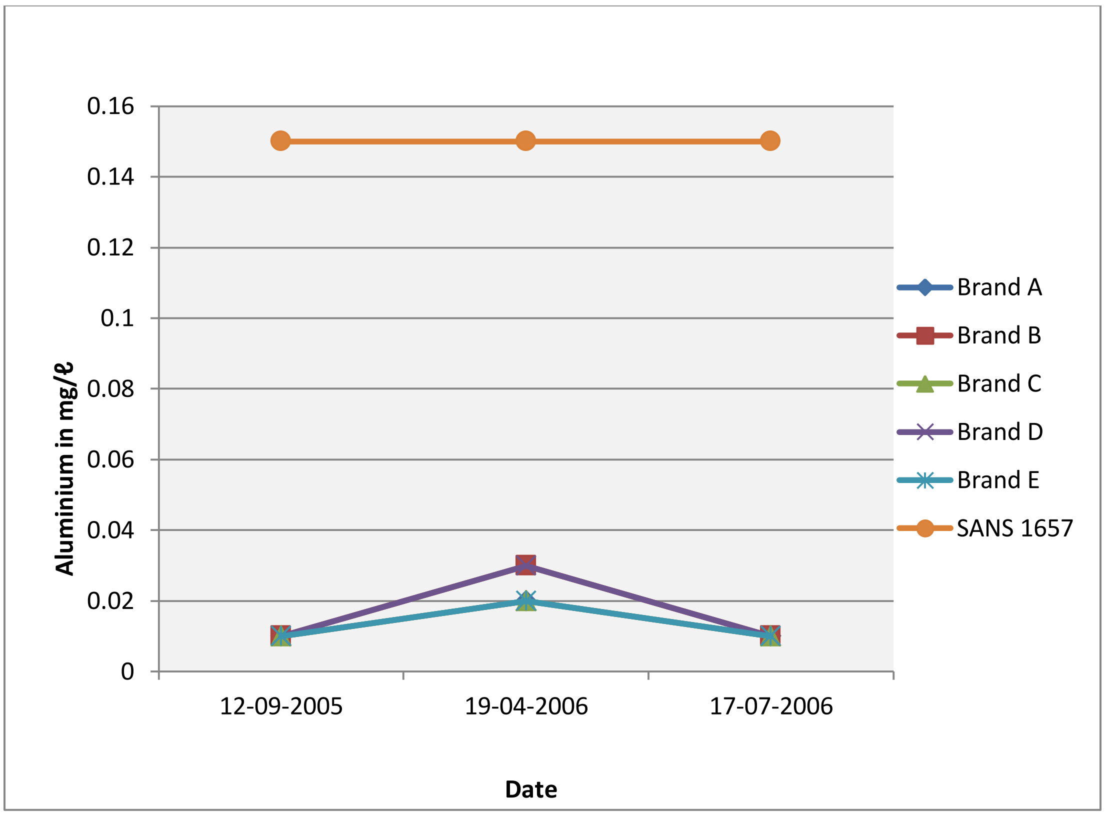

Figure 30: Results for Aluminium

4.4.4 Radioactive Substances

Radioactive constituents of drinking-water can result from:

—naturally occurring radioactive species (e.g., radionuclides of the thorium and uranium decay series in drinking-water sources), in particular radium-226/228 and a few others;

—technological processes involving naturally occurring radioactive materials (e.g. the mining and processing of mineral sands or phosphate fertilizer production);

—manufactured radionuclides (produced and used in unsealed form), which
might enter drinking-water supplies as a result of regular discharges and, in particular, in case of improper medical or industrial use and disposal of radioactive materials, such incidents are different from emergencies, which are outside the scope of these Guidelines, and

—past releases of radionuclides into the environment including water sources. The contribution of drinking-water to total exposure is typically very small and is due largely to naturally occurring radionuclides in the uranium and thorium decay series. Radionuclides from the nuclear fuel cycle and from medical and other uses of radioactive materials may, however, enter drinking-water supplies. The contributions from these sources are normally limited by regulatory control of the source or practice, and it is normally through this regulatory mechanism that remedial action should be taken in the event that such sources cause concern by contaminating drinking-water . Due to the low levels of radionuclides typically found in drinking-water supplies, acute health effects of radiation are not a concern for drinking-water supplies. The effective dose of radiation received by a person is, in simple terms, the sum of the equivalent doses received by all tissues or organs, weighted for “tissue weighting factors.” These reflect different sensitivities to radiation of different organs and tissues in the human body. The SI unit for the equivalent and effective dose is the sievert (Sv), where 1 Sv = 1 J/kg.To reflect the persistence of radionuclides in the body once ingested, the committed effective dose is a measure of the total effective dose received over a lifetime (70 years) following intake of a radionuclide (internal exposure) (WHO,2004).

4.4.4.1 Uranium (U)

WHO guideline value for uranium is 0,015 mg/ℓ. Results showed that uranium concentration for brand B (mineral water from underground sources), D (bottled water sourced from tap water) and brand E (spring water) increased to levels that were insignificant than other brands (Figure 31). However, these concentrations were within WHO guideline of 0,015 mg/ℓ. Brand A and C did not change during the period of study and remained constant throughout. The reason for this behaviour is unknown.

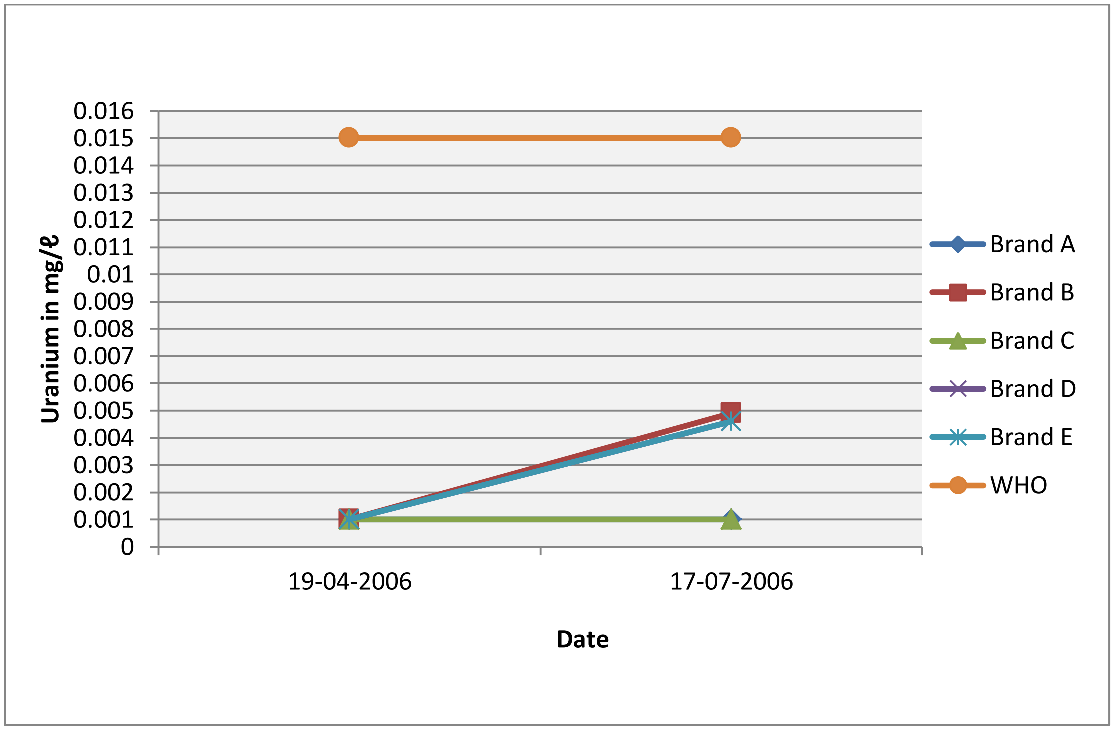

Figure 31: Results for Uranium

4.4.5 Other inorganic elements

4.4.5 .1 Nitrates (NO_3)

Sources of nitrogen and nitrates may include runoff or seepage from fertilized agricultural lands; municipal and industrial waste water, private sewage disposal systems, urban drainage and decaying plant debris. Although low levels of nitrates may occur naturally in water, sometimes higher levels, which are potentially dangerous to infants, are found. Infants are especially susceptible because their stomach juices are less acidic and therefore are conducive to the growth of nitrate-reducing bacteria. Adults can consume large quantities of nitrates in drinking water or food with no known ill effects; their stomachs produce strong acids that do not promote the growth of bacteria that convert nitrate to nitrite. Therefore, water (including bottled water) that is high in nitrates should not be used for preparing infant formula or in any other way that could result in consumption by a baby (http://www.idph.state.il.us/envhealth/factsheets/NitrateFS.htm.) Observations from bottled water brands showed brands A and E to have a similar trend of nitrate concentration fluctuation, while brands B and D, nitrate concentration increased during the period of study and nitrate concentration for brand C remained constant throughout the study (Figure 32). All bottled water tested complied with SANS 1657 limit of 10 mg/ℓ with average concentrations of 1.2mg/ℓ for brand A, 0.89 mg/ℓ for brand B,1.6 mg/ℓ for brand C, 1.2 mg/ℓ for brand D and 0.75 mg/ℓ brand E.

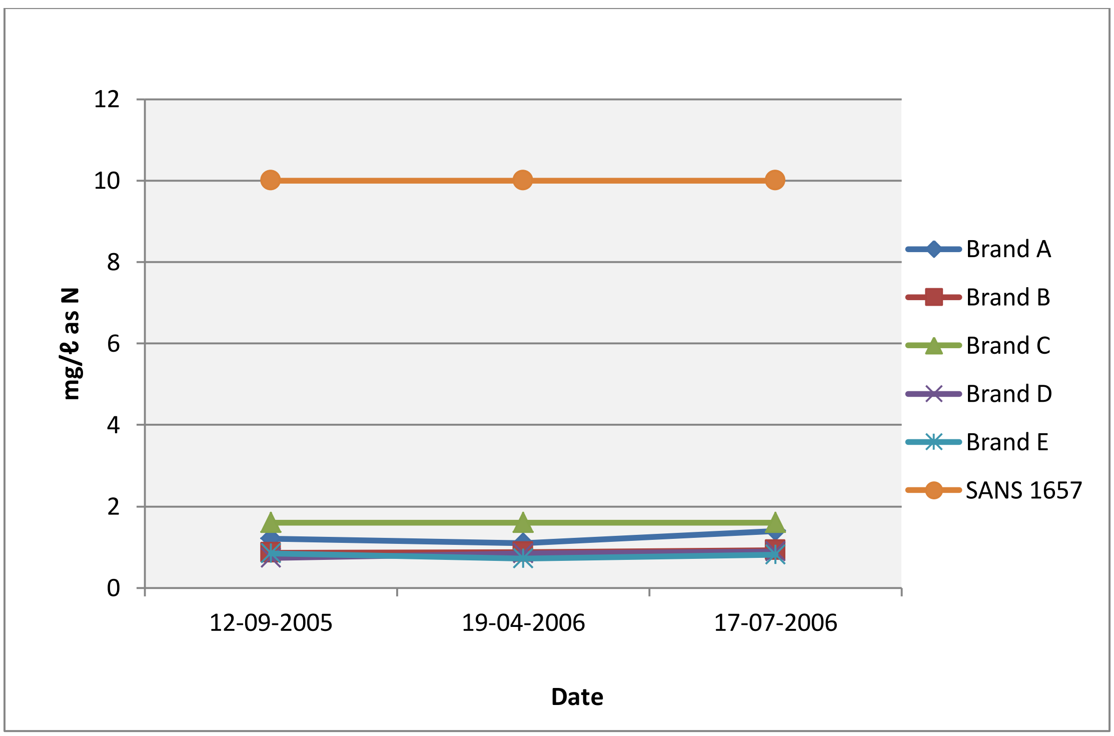

Figure 32: Results for Nitrate

4.4.5.2 Sulphate (SO_4)

Sulphate occurs naturally in numerous minerals and is used commercially, principally in the chemical industry. They are discharged into water in industrial wastes and through atmospheric deposition; however, the highest levels usually occur in groundwater and are from natural sources. In general, the average daily intake of sulfate from drinking-water, air and food is approximately 500mg, food being the major source. No health-based guideline is proposed for sulphate. However, because of the gastrointestinal effects resulting from ingestion of drinking-water containing high sulphate levels(WHO,2004). The results in Figure 33 showed insignificant increase in sulphate concentrations during the period of study for brand B,C,D. While with brand A and E concentrations remained constant throughout the period of study. All results attained were compliant with SANS 241 drinking water standard of 400mg/ℓ with average values of 5mg/ℓ for brand A, 25 mg/ℓ for brand B, 7mg/ℓ for brand C, 2mg/ℓ for brand D and 5 mg/ℓ for brand E.

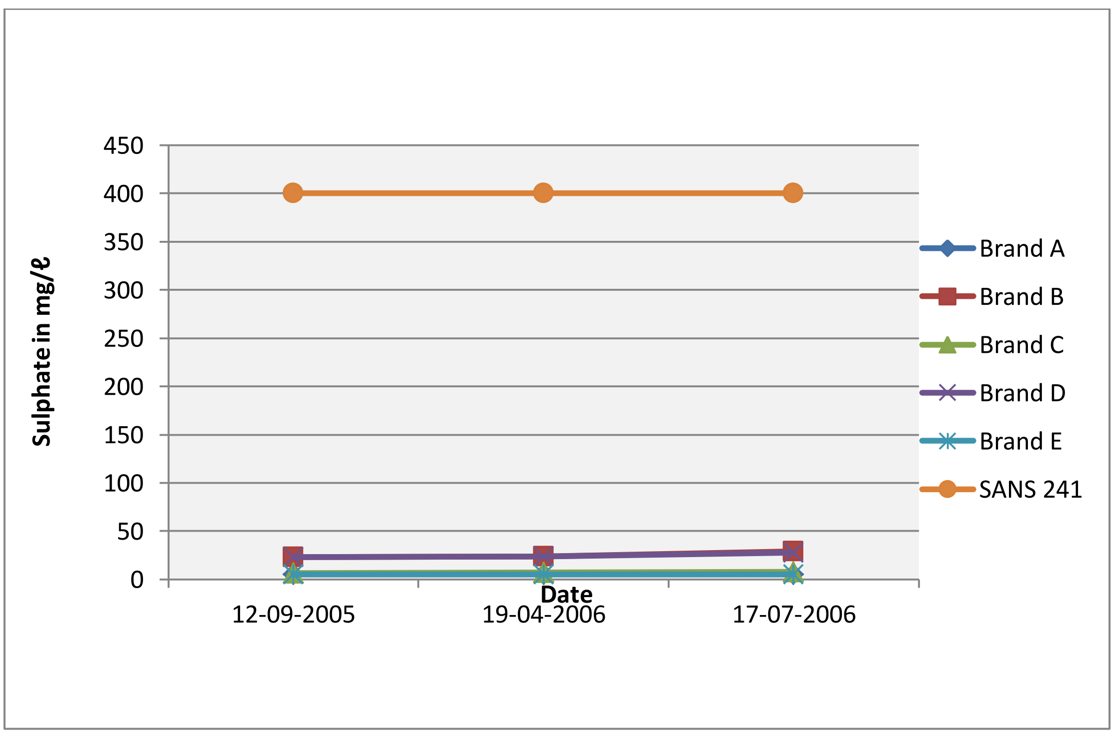

Figure 33: Results for Sulphate

4.4.5.2 Boron (B)

Boron is not present in water in elemental form. It can be found in volcanic spring waters containing boric acid. Exposure to high concentrations of boron may lead to skin eruptions, depression, and reproductive malfunction in men as well as developmental abnormalities. Boron is an endocrine disruptor. Exposure to it can lead to miscarriages, severe birth deformities and reduced fertility. It is an essential element that can affect the metabolism and utilization of other substances such as calcium, copper, magnesium and nitrogen (WHO, 2003).The results in Figure 34 indicated similar growth pattern amongst bottled water brands investigated. Prolonged storage of bottled water can result in bottled water concentration changing depending on the source water used. No significant change was observed from all the bottled water brands investigated. The graph below (Figure 34) indicates that all bottled water brands tested complied with the WHO guideline value of 0, 5 mg/ℓ with average values of 0.01mg/ℓ for all bottled water brands investigated.

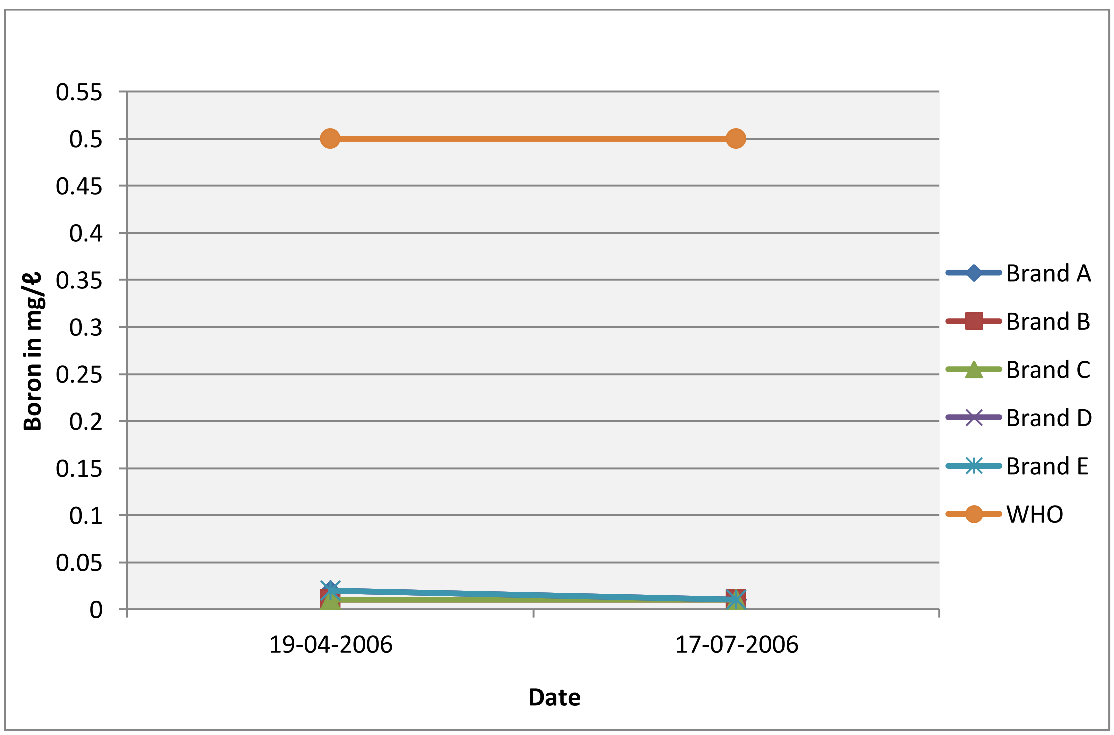

Figure 34: Results for Boron

4.4.6 Beneficial Minerals

4.4.6.1 Fluoride (F)

The most important source of fluoride is drinking water. High fluoride dose exposure for extended periods produces skeletal fluorosis (brittle bones) and discoloured teeth (Ncube and Schutte, 2005). Concentrations lower than recommended levels for drinking water to provide protection may result in dental caries, especially in children (Ncube and Schutte, 2005). However, fluoride may contain varying concentrations of naturally occurring fluoride depending on the water source. No particular trend was noticed over time as fluoride concentration fluctuated for all bottled water brands investigated with the highest fluoride concentrations observed in brands B and D (Figure 35). There were variations during the period of study as fluoride concentrations fluctuated from all the bottled water tested. Results showed brand A to have a fluoride maximum concentration of 0,07 mg/ℓ, 0,44mg/ℓ for brand B, 0,14 mg/ℓ for brand C, 0,45 mg/ℓ for brand D and 0,19 mg/ℓ for brand E (Figure 35). All the bottled water brands tested complied with the SANS 241:2006 drinking water standard of <1 mg/ℓ, with average concentrations of 0,07 mg/ℓ for brand A, 0.33 mg/ℓ for brand B, 0,12 mg/ℓ for brand C, 0,43 mg/ℓ for brand D and 0,15 mg/ℓ for brand E.

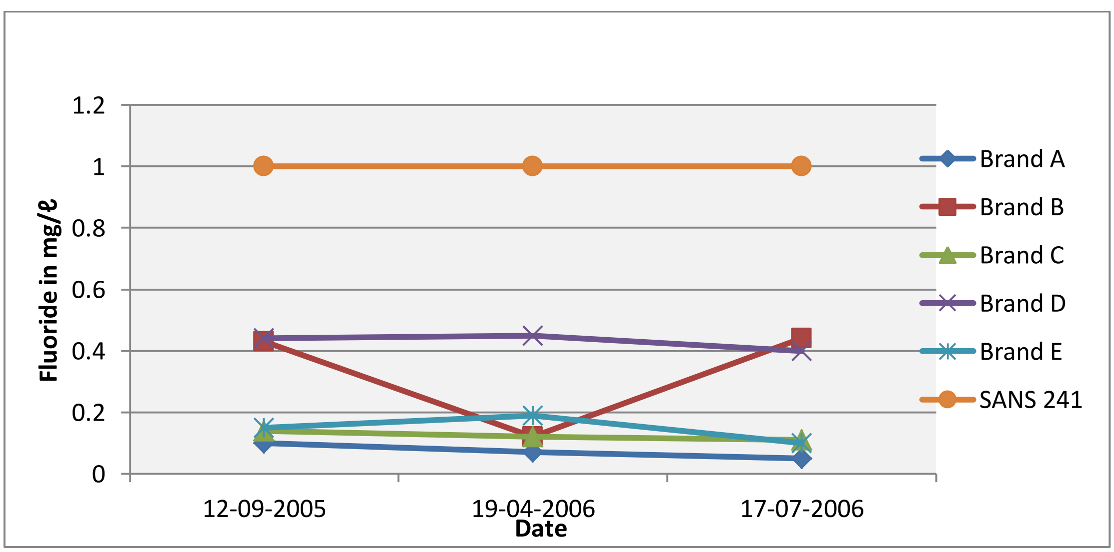

Figure 35: Results for fluoride

4.4.6.2 Magnesium (Mg)

The adult human body contains approximately 20 to 28g of Mg, of which approximately 60 percent is found in bone, 26 percent in muscle, and the remainder in soft tissues and body fluids. Although excess Mg can inhibit bone calcification, magnesium in excess from dietary sources including supplements, are unlikely to result in toxicity. The results of the bottled water investigated, revealed that magnesium remained fairly stable during the period of study and no significant variations were observed. As shown on the graph below (Figure 36),all bottled water investigated complied with SANS 241 drinking water standard of <70 mg/ℓ.

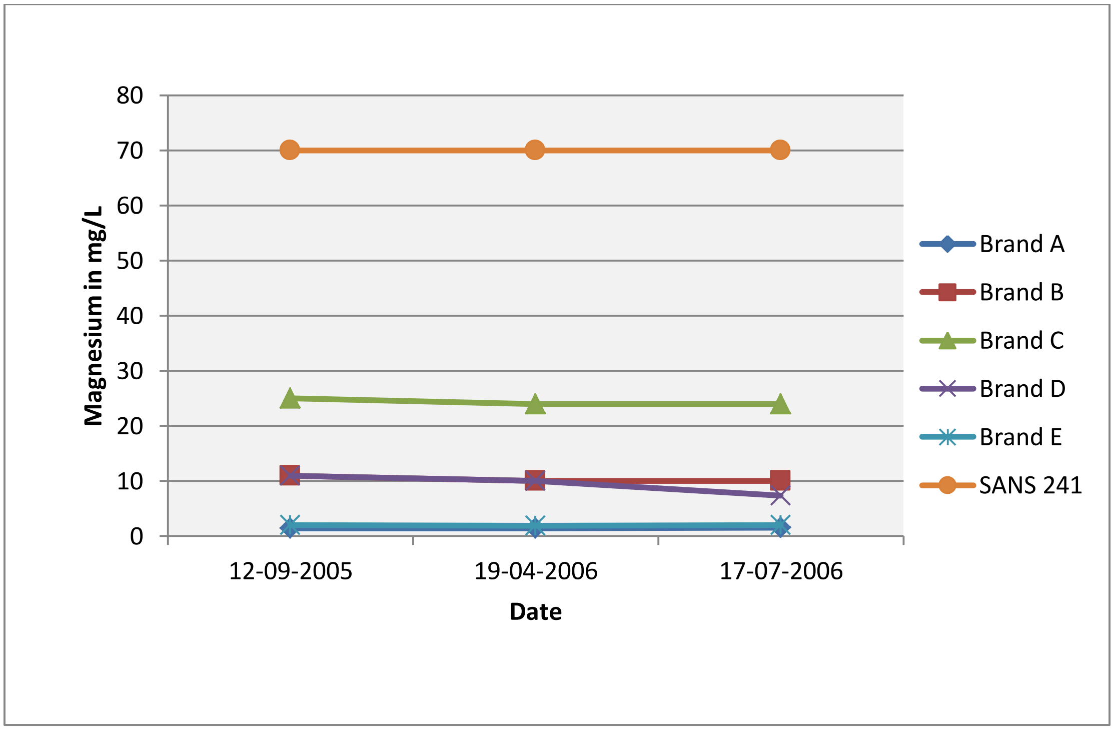

Figure 36: Results for Magnesium

4.4.6.3 Potassium (K)

Levels of potassium are usually low in drinking water and do not pose any health risk to humans. A treatment process such as reverse osmosis effectively removes potassium in drinking water. The results of the bottled water investigated, revealed that pottassium remained fairly stable during the period of study and no significant variations were observed. As shown on the graph below (Figure 37),all bottled water investigated complied with SANS 241:2006 drinking water standard of <50 mg/ℓ .

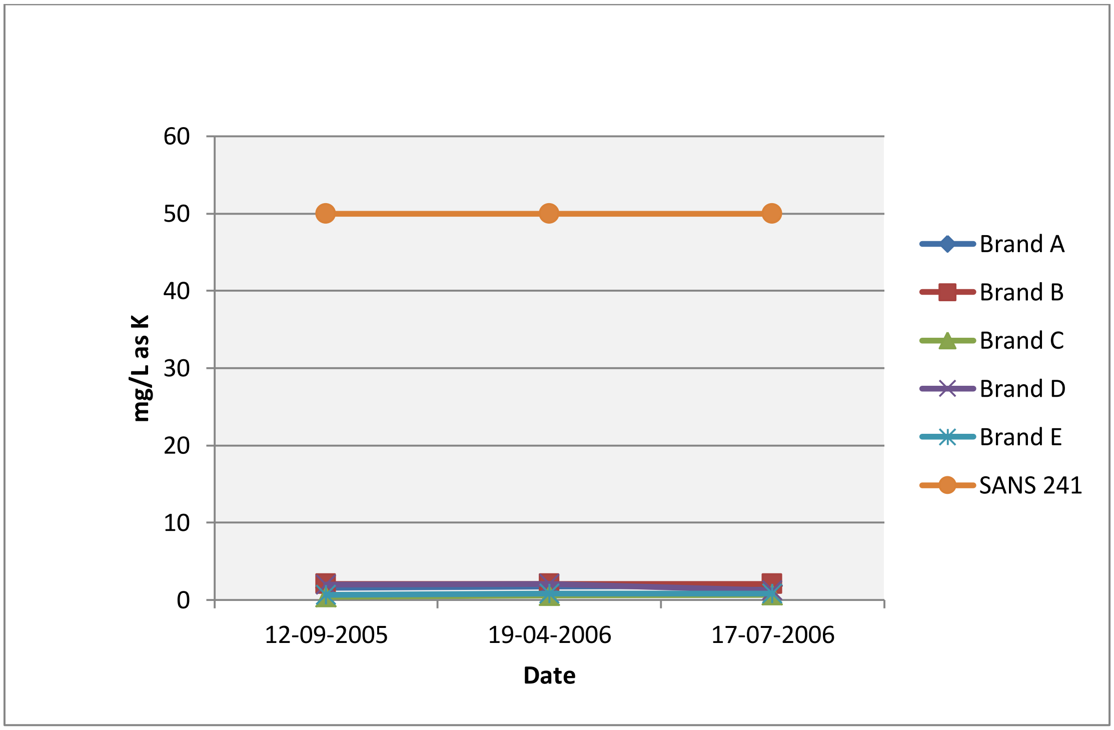

Figure 37: Results for Potassium

4.4.6.4 Sodium (Na)

Sodium salts (e.g., sodium chloride) are found in virtually all food (the main source of daily exposure) and drinking-water. Although concentrations of sodium in potable water are typically less than 20 mg/ℓ, they can greatly exceed this in some countries. The levels of sodium salts in air are normally low in relation to those in food or water. It should be noted that some water softeners can add significantly to the sodium content of drinking-water. No firm conclusions can be drawn concerning the possible association between sodium in drinking-water and the occurrence of hypertension. Therefore, no health based guideline value is proposed. However, concentrations in excess of 200 mg/ℓ may give rise to unacceptable taste (WHO, 2004). All bottled water tested (Brands A, B, C, D and E) showed a logarithmic growth pattern of increasing and decreasing levels of sodium (Figure 38). Brand B (mineral water from underground source) and brand D bottled (tap water) turned out to have more sodium content than other brands (A, C and E). All bottled water tested complied with SANS 241 drinking water standard of <200 mg/ℓ with average sodium concentrations of 5.6 mg/ℓ for brand A, 35 mg/ℓ for brand B, 4,8mg/ℓ for brand C, 27 mg/ℓ for brand D and=7,4 mg/ℓ for brand E. Concentrations in potable water are typically less than 20 mg/ℓ; hence they can exceed this limit in some countries.

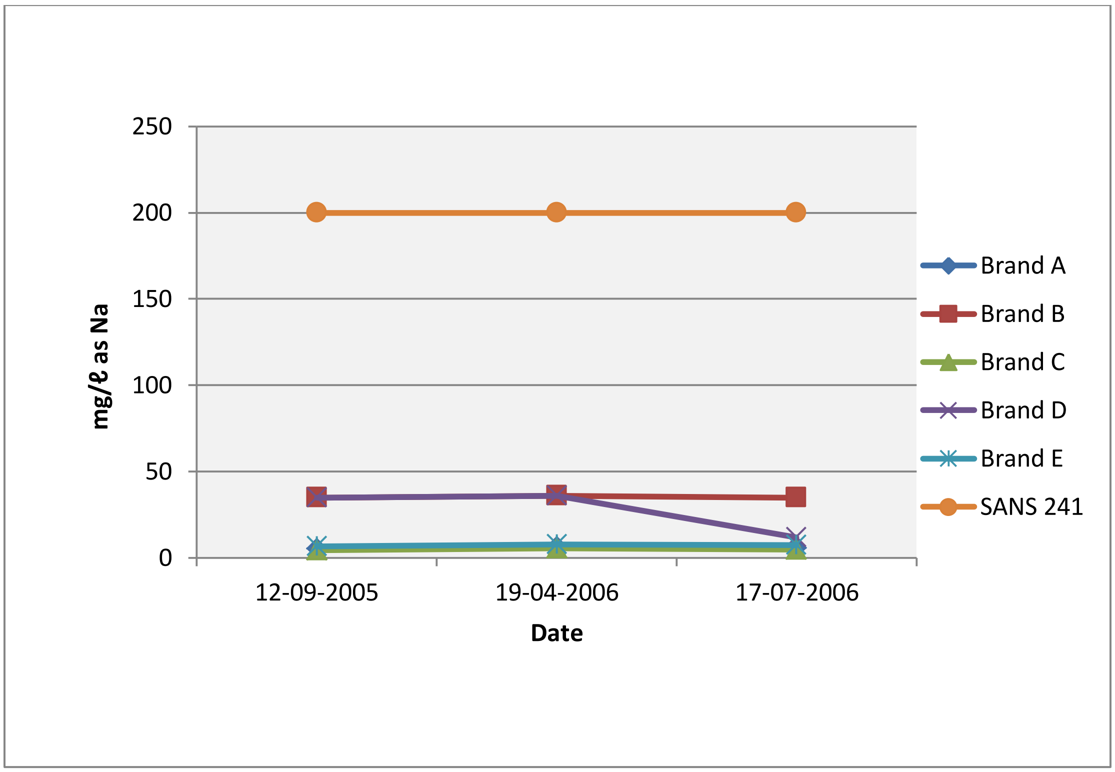

Figure 38: Results for Sodium

4.4.6.5 Chloride (Cl)

The presence of chloride in natural waters is due to the effluents from chemical industries, wastewater treatment plants, road salting and agricultural runoff. It is also present in water, rocks, soil and many foods. Chloride may also contribute towards total dissolved solids in drinking water. High concentrations of chloride in drinking water may result in a salty taste giving bottled water the same salty taste. Chloride is an essential element for humans (WHO, 2003). Observations from Figure 39 showed that brand B (mineral water from underground source) and brand D (bottled tap water) tend to have more Chloride content than other brands (A, C and E). This increase in chloride is seen with sodium in both brands therefore, brand B and D are characterised by a salty taste due to sodium chloride. All bottled water brands tested remained constant during the period of study. Average values (9 mg/ℓ for brand A, 27 mg/ℓ for brand B, 5 mg/ℓ for brand C, 28 mg/ℓ for brand D and 5 mg/ℓ for brand E) for chloride concentrations complied with SANS 241 drinking water standard of < 200 mg/ℓ. There is no evidence that intake of chloride in large quantity in drinking water can harm humans.

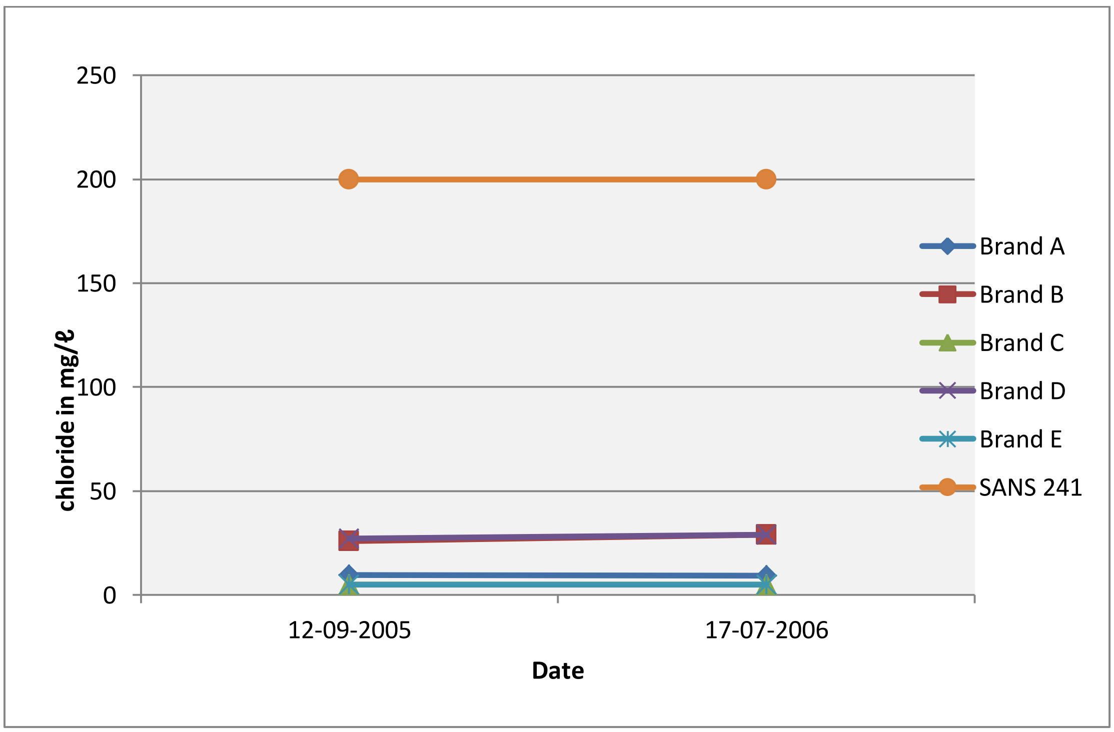

Figure 39: Results for Chloride

4.5 Organic determinants results

4.5.1Trihalomethanes (THMs)

Trihalomethanes consist mainly of Chloroform, Bromodichloromethane, Dibromochloromethane and bromoform and they are a family of chemicals created when chlorine is used to disinfect the water (chlorine reacts with organic matter in the water to form THMs and other by-products). The amount of each THM formed depends on the temperature, pH, chlorine and bromide ion concentrations. Studies conducted revealed that people and animals exposed to THMs in their tap water have the risk of suffering from the colon,kidney or liver cancer and a higher potential to suffer from reproductive disorders uch as spontaneous abortion and birth defects (Nieuwenhuijsen *et al*.,2000,Leavens *et al*.,2007) . There was no variation during the period of study from all bottled water brands tested (Figure 40). This is typical as most bottled water derived from other sources (e.g. underground, springs) have insufficient traces of chlorine or no chlorine at all in order to react with organic material in the water. Chloroform is commonly found in tap and bottled water (sourced from the tap); it is of public health concern because consumption in excessive amounts can be carcinogenic. Figure 41 shows that chloroform was below the detection limit of <0,328 μg/ℓ (Figure 41). There was no variation during the period of study from any bottled water brands tested (i.e. brand A, B, C, D and E). Bottled water brands were within the WHO guideline value of 0,2mg/ℓ . Bromodichloromethane was below the detection limit of <0,558 μg/ℓ (Figure 42). Dibromochloromethane and bromoform indicated no significant variation during the period of study as they were within guideline value of 0,1mg/ℓ with detection limits below <1.168 and 0,543 μg/ ℓ respectively (Figures 43 and 44).

Gas Chromatograph-ECD used to test for various trihalomethanes, have different detection limits for various trihalomethanes tested.

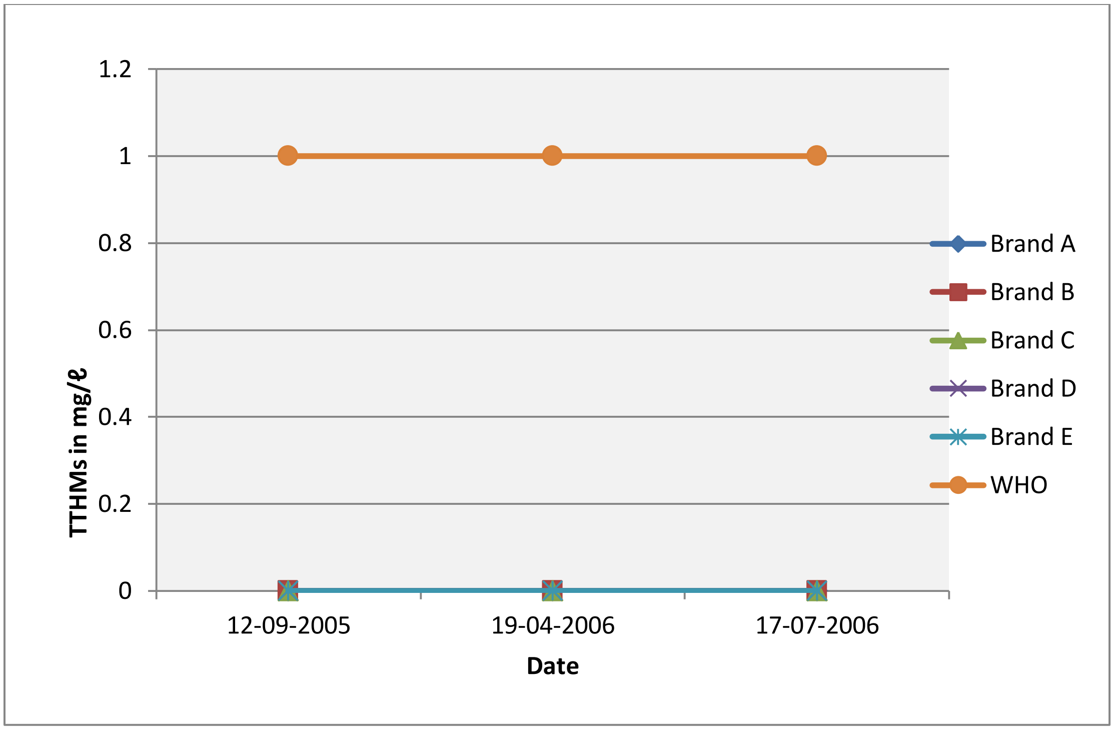

Figure 40: Results for Total trihalomethanes

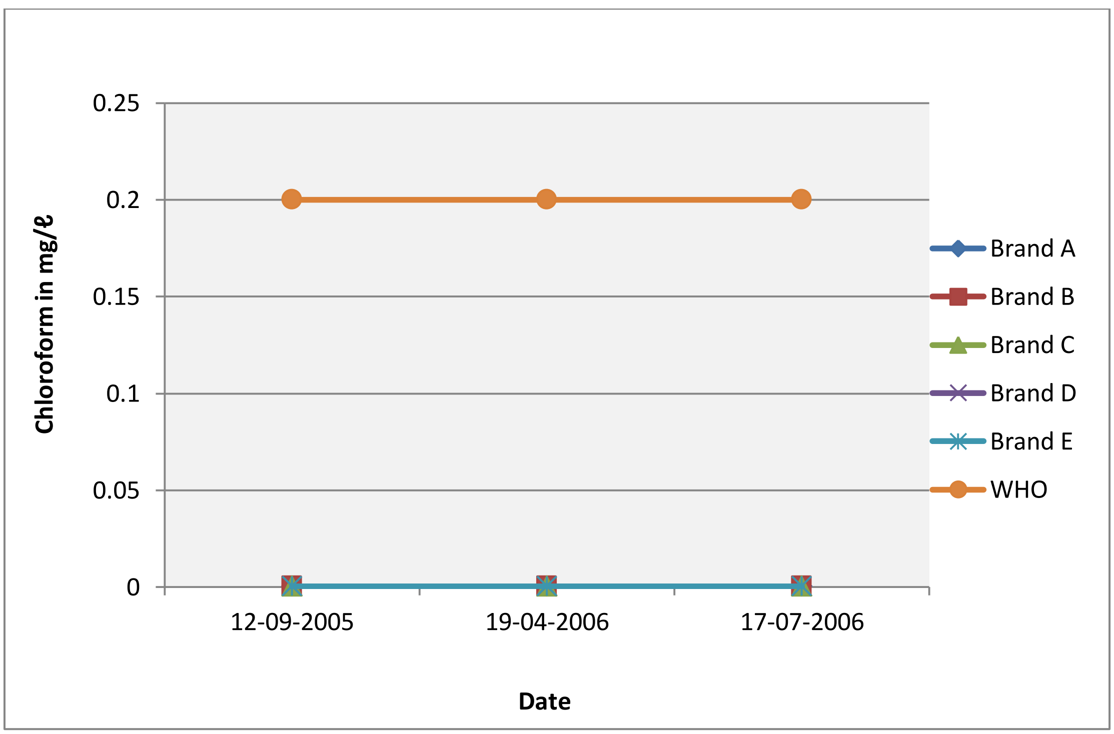

Figure 41: Results for Chloroform

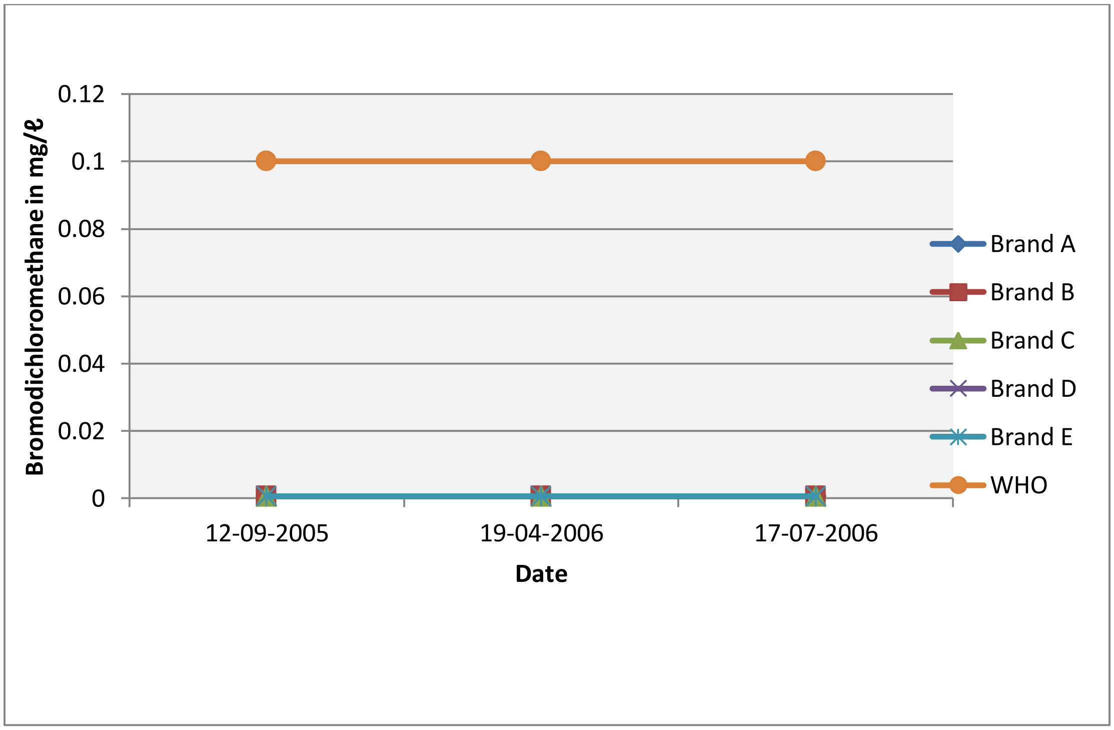

Figure 42: Results for Bromodichloromethane

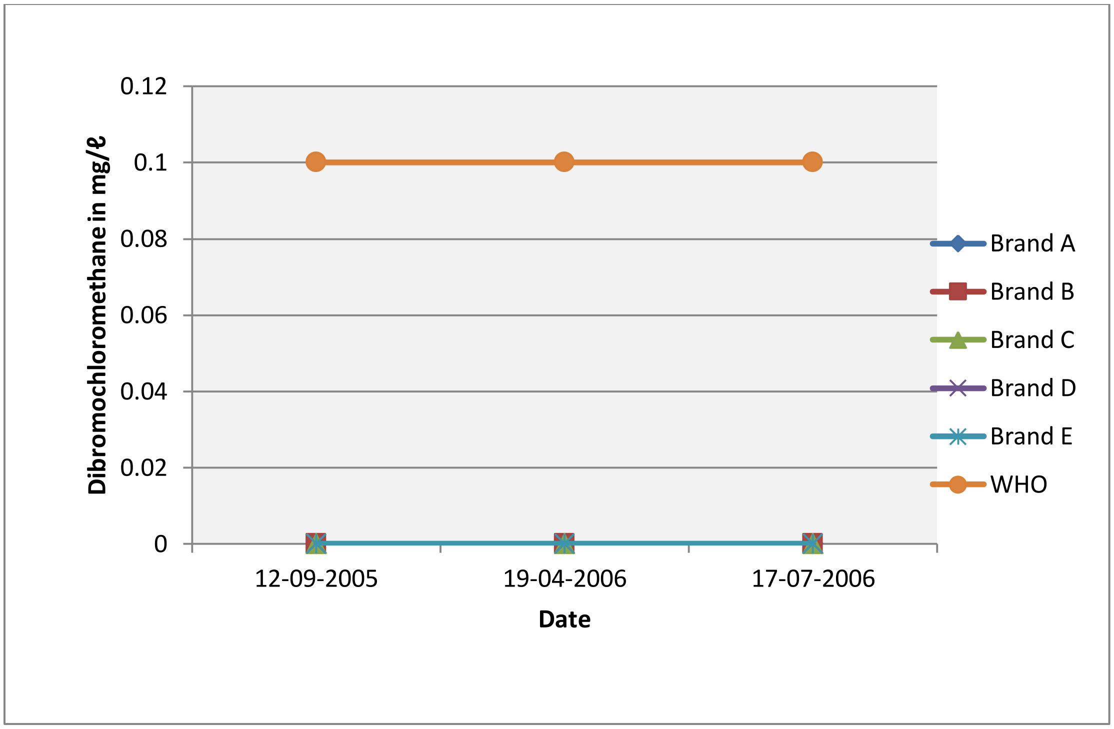

Figure 43: Results for Dibromochloromethane

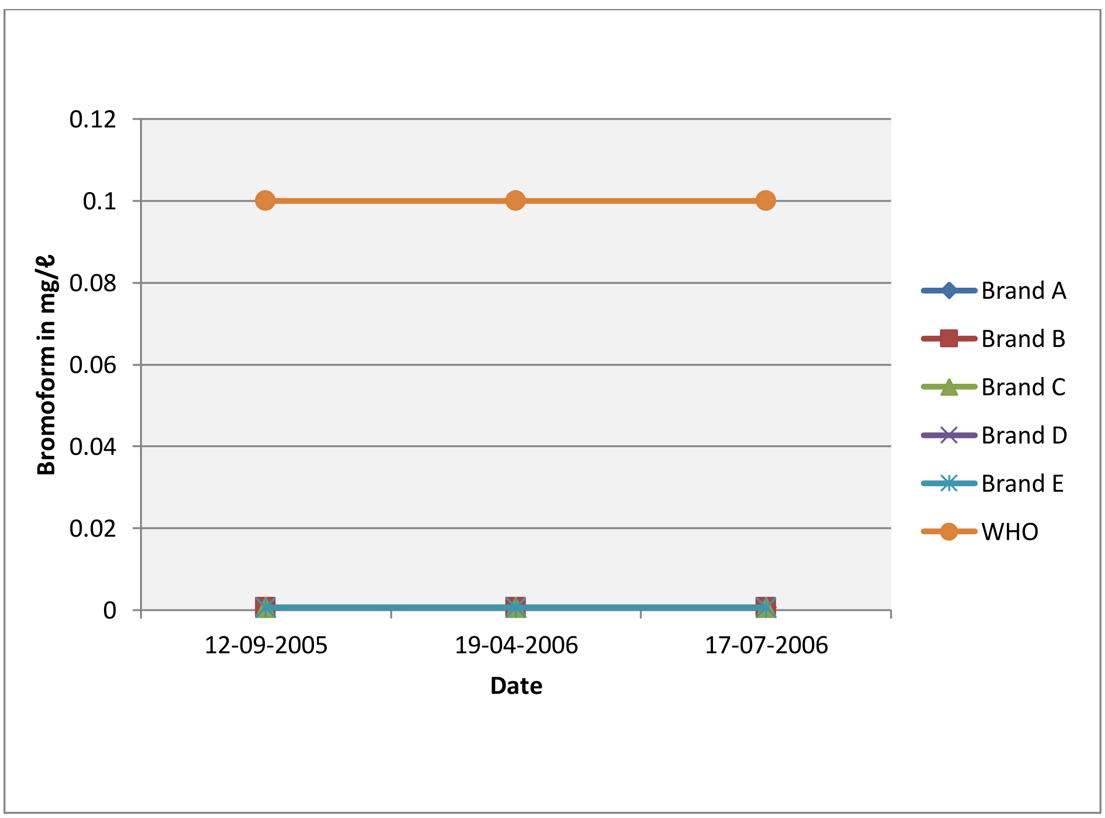

Figure 44: Results for Bromoform

4.5.2 Pesticides

Pesticides can arise from agricultural activities. They are also used to control weeds. Their presence can be affected by many factors resulting from application and movements following rainfall or inappropriate waste disposal methods (WHO, 2004). There are various pesticide substances used and atrazine was used in this study as a representative for other pesticides as it is widely used as a herbicide in South Africa.

4.5.2.1 Atrazine

Atrazine is a selective pre and post-emergence herbicide**.** According to WHO it has been found in surface water, ground water and drinking water at levels below 10µg/ℓ (0,01 mg/ℓ) as a result of its mobility in soil (WHO,2004).The results below showed that atrazine from all the bottled water brands tested were below the detection limit of 0,0001 mg/ℓ . The results were used to indicate its presence in bottled water and not its variability during the period of study. Atrazine was compliant with the WHO guideline value of 0,002 mg/ℓ.

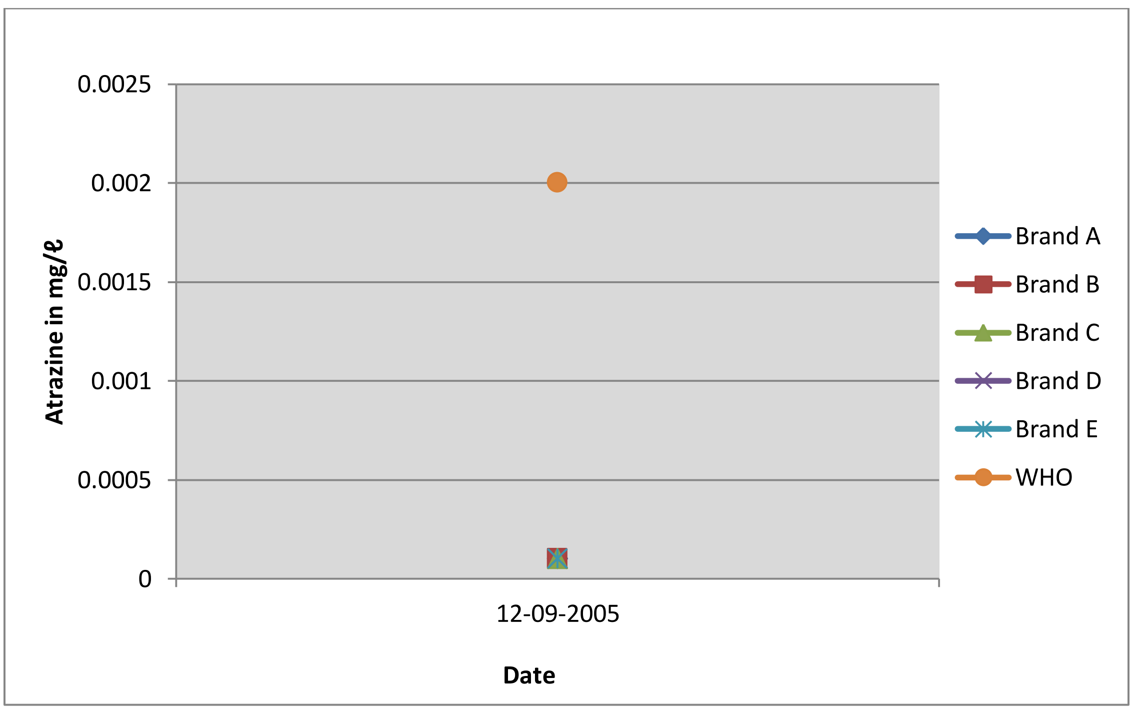

Figure 45: Results for Atrazine

CHAPTER 5 GENERAL DISCUSSIONS

Bottled Water is generally of good quality for drinking, but if not properly protected during bottling, transit and storage could be a subject of contamination (Ehlers, 2004). In general HPC is considered to be harmless however; studies conducted in other countries as well as in South Africa suggest a potential health risk associated with HPC bacteria present in drinking water. In this study a more appropriate way to look at the effect of shelf-life affecting microbiological quality was used to analyse the bottled water brands during the period of study. Within a week high HPC bacteria was common in all bottled water tested. It exceeded the alert levels for drinking water standard of >5000 cfu/mℓ and SANS 1657 limit of less than 100cfu/mℓ in most cases. According to SANS 1657, the total viable colony count should not be more than that which results from the normal increase in the bacterial content of the water at the source (bacterial levels at the source were not stipulated on the bottles). Therefore, there is no clarity on what the limit of the HPC should be. These high counts are likely to depend on the natural flora of the source water and during bottling. Further studies conducted found an association between high numbers of

HPC bacteria and gastroenteritis. It was also found that members of the HPC bacteria produce virulence factors that can put individuals especially immuno-compromised individuals at risk of HPC infections.

This study found that prolonged storage of bottled water at room temperature and artificial lighting, results in a variety of algal species growing and accumulating through time during their growth succession stages. This is of concern as a number of algae and other organisms can also produce substances that may impart a pungent earthy taste and odour resulting in water of unacceptable quality. While these may not be a health risk they can make the water unpalatable. Other algal species found, are those that can contribute to organic pollution in the water. Algae not only produce neuro and hepatotoxins that could be detrimental to the consumer's health, but algal products may also act as trihalomethane precursors and a source of carbon for microbiological and other heterotrophic growth. Indicator bacteria such as coliforms (TC and FC) were not detected in any of the bottled water tested. Likewise, yeasts and moulds were not detected in any of the bottled water tested.

The results found that physical parameters tested such as turbidity, pH, TDS, conductivity, and colour did not indicate any reason for concern. Variations over time for these parameters were within recommended guidelines. They have potential aesthetic effects (causing colour, taste and odour problems) if they are present in unacceptable levels. However, hardness of the bottled water tested was of concern. The hardness of water relates to the amount of calcium, magnesium and sometimes iron in the water. The more minerals present, the harder the water. Soft water may contain sodium and other minerals or chemicals; however, it contains very little calcium, magnesium or iron. It was evident from this study that brand A (mineral water) and brand E (spring water) were regarded as being soft with hardness values as low as 11 and 19 respectively. According to WHO ecological and analytical epidemiological studies, there is a significant inverse relationship between hardness and drinking water. However the degree of hardness in the water may affect its acceptability to the consumer in terms of scale deposition (WHO, 2004).

The results for nitrate and sulphate could be of health concern if found in high concentrations in drinking water. The health concern related to nitrate is that microorganisms present in the water can change nitrate to nitrite. This is a health concern for the body because it causes haemoglobin in the blood to change to methemoglobin. Methemoglobin reduces the amount of oxygen that can be carried in the blood, resulting in cells throughout the body being deprived of sufficient oxygen to function properly. The condition called methemoglobinemia can result. This study has shown variations and increases in nitrate concentration to be of no concern when bottled water was stored for prolonged periods. Sulphate on the other hand can be of health concern if found at higher levels. According to Darbi, Viraraghavan, Jin, Braul, Orkal (2003:160) there were reported cases where infants experienced gastroenteritis with diarrhoea and dehydration upon ingesting water that had high levels of sulphate (650-1150 mg/ℓ) .Prolonged storage for bottled water tested has shown insignificant increase in sulphate concentrations that were within the required drinking water standard.

CHAPTER 6 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The study has shown that the levels of bacteria increase quickly to maximum levels after six weeks of unrefrigerated shelf-life. Hence, bottled water stored at room temperature for prolonged periods can result in a high HPC population that can cause health problems for individuals, especially among the sensitive population.

Prolonged storage of bottled water results in algal species causing negative aesthetic effects and organic pollution and hence the presence of algae can pose both health and aesthetic problems.

Physical parameters such as hardness could be problematic if the water is too soft resulting in water that may not be acceptable to consumers in terms of taste and scale deposition.

Storage temperature for long periods plays a major role as it can impact on the acceptability of other inorganic constituents and enhance the growth of microorganisms resulting in the offensive tastes and odours.

Chemical parameters remained constant and others varied over time. These variations were too insignificant to be of major concern to the people drinking bottled water. Bottled water shelf-life is not of concern when considering the chemical quality as it is fairly stable. The concern is in the original chemical quality at the time of bottling.

6.2 RECOMMENDATIONS

It is important to specify the quality of bottled water at the point and time of bottling and to continue monitoring the quality of the water while it is in use. Hence, it is recommended that bottled water providers should provide the quality of source water in the form of test reports to SANBWA as well as the national Department of Water Affairs Regulation unit.

More stringent standards are required in regulating the bottled water industry in South Africa in monitoring potential radiological, biological and chemical pollutants in source water used (ground water, streams, springs).Bottled water companies need to manage algae from the source water used for bottling in order to prevent water that could aesthetically be unacceptable. Retailers and consumers at home need to ensure that bottled water purchased should be stored at cool temperatures to avoid microorganisms from multiplying when left at room temperature for prolonged periods.

A review of policies and procedures governing the bottling, storage and usage of bottled water produced in South Africa is recommended to ensure adequate protection of local, national and international consumers.

REFERENCES

AMIRIDOU, D. & VOUTSA, D. 2011. Alkyl phenol and phthalates in bottled waters. *Journal of Hazardous Materials,* 185: pp. 281-286.

BOTEZATU, E., IACOB, O., ELISEI, G., & CAPITANU, O. 2005. Exposure of the population through mineral water consumption. *Radioactivity in the Environment,* 7: p.1099.

CABRAL, D., & PINTO, V.E.F.2002. Fungal spoilage of bottled mineral water. *International Journal of Food Microbiology,* (72): pp.73 – 76.

CBWI (the Canadian bottled water industry).2008.Bottled water from Canada. [Online]. Available at <http://www4.agr.gc.ca/AAFC-AAC/display-afficher.do?id=1171644581795 >. Accessed: 12/7/2010.

CRIADO, M.V., FERNANDEZ, V.E., BADESSARI, A., & CABRAL, D. 2005. Conditions that regulate the growth of moulds inoculated into bottled mineral water. *International Journal of Food Microbiology,* 99:pp. 343 – 349.

DARBI, A., VIRARAGHAVAN, T., JIN, Y., BRAUL, L., & ORKAL, D.2003.Sulphate removal from water. *Water Quality journal,* 38:pp.169- 170.

DE SANCTIS, R., DE BELLIS, R ., SCESA, C., MANCINI, U., CUCCHIARINI, L., & DACHÀ, M. 2004 . *In vitro* protective effect of *Rhodiola rosea* extract against hypochlorous acid-induced oxidative damage in human erythrocytes. *BioFactors, 20*(3):pp.147-159.

DoH.2006. Regulations relating to all bottled waters, Foodstuffs, Cosmetics and Disinfectants Act, 1972 (Act no. 54 of 1972) Regulation no. R. 718 Department of Health, Republic of South Africa, pp. 3 -13.

DoH (department of health) [Online]. Available at: <http://www://doh.gov.za>. Accessed: 2/12/2010.

DORIA, M.F. 2006.Bottled Water versus tap water: understanding consumer's preferences. *Journal of Water and Health,* 04.2: pp.271-276.

DESIDERI, D., MELI, M.A., FEDUZI, L., ROSELLI, C., RONGONI, A., & SAETTA, D.2007. ^{226}Ra, ^{238}U, ^{234}U and ^{210}Po concentrations of bottled mineral waters in Italy and their dose contribution .*Journal of Environmental Radioactivity,* 94: pp. 86-97.

EH (Environmental Health).2010.Fact Sheet. [Online]. Available at <http://www.idph.state.il.us/envhealth/factsheets/NitrateFS.htm.)>. Accessed: 22/02/2011

EHSO (Enviromental, Health and Safety Online).1996.Frequently asked Questionsabout drinking water, tap water and bottled water and your health. [Online]. Available <athttp://www.ehso.com/ehshome/DrWater/drinkingwaterfaqs.php#safer>. Accessed: 12/02/2011

De Sanctis. R, De Bellis. R , Scesa. C., Mancini. U, Cucchiarini. L, & Dachà. M 2004 *In vitro* protective effect of *Rhodiola rosea* extract against hypochlorous acid-induced oxidative damage in human erythrocytes. *BioFactors,* 20(3):147-159.

DIETRICH, A.M. 2006. Aesthetic issues for drinking water. *Journal of water and health,* 04:11-14.

EFBW (European federation of bottled waters). Safety and Quality. [Online]. Available at <http://efbw.eu/safety.php?classement=01>. Accessed: 2/12/2010.

EHLERS, M.M., VAN ZYL, W.B., PAVLOV, D.N., & MULLER, E.E. 2004. Random survey of the Microbial quality of bottled. *Water SA,* 30(2): pp. 203 - 210.

EHNIB (environmental health investigations branch).1990. Health Concerns related to Nitrate and Nitrite in private well water. [Online]. Available at <http://www.ehib.org/paper.jsp?paper_key=NITRATE_NITRITE_FACT_SHEET>. Accessed: 14/01/2011.

EHSO (environment, health and safety online). 2006. Frequently asked questions on bottled water. [Online]. Available at <http://www.ehso.com/ehshome/DrWater/drinkingwaterfaqs.php>. Accessed: 14/ 01/ 2011.

EWG (environmental working group).Bottled water quality investigation.10 Major Brands, 38 Pollutants [Online]. Available at <http://www.ewg.org/BottledWater/Bottled-Water-Quality-Investigation/Bottled-Water-Quality-Investigation-Test-Results>. Accessed: 14/ 09/ 2010.

EVANS, M.R., RIBEIRO, C.D., & SALMON, R.L. 2003.Hazards of healthy living: Bottled Water and Salad vegetables as risk factors for Campylobacter infection. *Research* 9(10): pp. 1219 – 1224.

GULER, C. 2007. Evaluation of maximum contaminant levels in Turkish bottled drinking waters utilizing parameters reported on manufacturers labelling and Government-issued production licenses. *Journal of Food Composition and*

Analysis, (20): pp.262-272.

IKEM, A., ODUEYUNGBO, S., EGIEBOR, N.O., NYAVOR. K .2002. Chemical quality of bottled waters from three cities in eastern Alabama. *The Science of the Total Environment,* 285:pp. 165-175.

KOKKINAKIS, E.N., FRAGKIADAKIS, G.A., & KOKKINAKIS, A.N. 2008. Monitoring microbiological quality of bottled water as suggested by HACCP. *Methodology Food Control,* 19: pp.957 – 961.

KRALIK, C., FRIEDRICH, M., & VOJIR, F. 2003. Natural radionuclides in bottled water in Austria. *Journal of Environmental Radioactivity,* 65:pp.233 – 241.

KRACHLER, M. & SHOTYK, W. 2009. Trace and ultratrace metals in bottled waters: survey of sources worldwide and comparison with refillable metal bottles. *Science of the Total Environment,* 407: pp. 1089-1096

LAL, M. & KAUR, H. 2006. A microbiological study of bottled mineral water marketed in Ludhiana Indian. *Journal of Public Health,* (1): pp. 31 – 32.

LEAVENS,T.L.,BLOUNT,B.C.,DeMARINI,D.M.,MADDEN,M.C.,VALENTINE.J.L.,CASE.M.W.,SILVA,L.K.,WARREN,S.H.,HANLEY,N.M., & PEGRAMS,R.A. 2007. Disposition of bromodichloromethane in Humans following Oral and dermal exposure. *Toxicological sciences,* 99(2): pp.432-445.

LENNTECH.2009. Cobalt. [Online]. Available at <http://www.lenntech.com/periodic/elements/co.htm> .Accessed: 23/04/2011.

NAIDENKO, O., LEIBA, N., SHARP, R., & HOULIHAN, J. 2008. Water Quality Investigation: 10 Major Brands, 38 Pollutants. [Online]. Available at <http://www.ewg.org>. Accessed: 5/3/2011.

National Health and Medical Research Council (NHMRC), 2004. National water quality management strategy: Australian drinking water guidelines 6, Australian Government.

NCUBE, E.J & SCHUTTE, F.C. 2005 The occurrence of Fluoride in South African groundwater: A water quality and health problem. *Water SA,* 31(1): 35-40.

NIEUWENHUIJSEN, M.J., TOLEDANO, M.B., EATON,N.E., FAWELL,J., & ELLIOT,P.2000. Chlorination disinfection by-products in water and their association with adverse reproductive outcomes. *Occupational and Environmental medicine*, 57:73-85.

NBWS (National bottled water standards).2009. Bottled water standards in South Africa. [Online.] Available at <http://www.nbws.org>. Accessed: 12/7/2010.

NSANZE, H. & BABARINDE, Z. 1999. Microbiological quality of Bottled drinking water in the UAE and effect of storage at different temperatures. *Environment International,* 25(1): 53-57.

NRDC (natural resources defence council).2005. Bottled water contamination [Online]. Available at <www.nrdc.org/water/drinking/bw/chap3.asp>. Accessed: 12/7/2010.

OYEDEJI, O., OLUTIOLA, P.O., & MONINUOLA, M.A. 2010. Microbiological quality of packaged drinking water brands marketed in Ibadan metropolis and IIe-lfe city in South Western Nigeria. *Journal of Microbiology Research,* Vol 4(1): 096-102.

POSNICK, L.M. & KIM, H. 2002. Bottled water regulation and the FDA*. Food and safety magazine* Aug/Sept p.4.

ROBLES,E.,RAMIREZ,P.,GONZALEZ,M.E.,SAINZ,M.D.,MARTINEZ,B.,DURAN ,A.,MARTINEZ,M.E.1999. NAME OF ARTICLE MISSING. *Water, air and Soil Pollution,* 113: pp.217-226.

ROSENBERG, F.A. 2003. The Microbiology of bottled Water. *Clinical Microbiology Newsletter,* 25(6):41- 43.

REYNOLDS, K.A. 2005. The Microbial quality and Safety of Bottled Water. Water conditioning and purification, pp. 39-40.

PEH, Z., ŠORŠA, A., HALAMIC, J. 2010. Composition and variation of major and trace elements in Croatian bottled waters. *Journal of Geochemical Exploration,* 107: 227-237.

POSNICK, L.M. & KIM, H. 2002. Bottled water regulation and the FDA. Food and safety magazine Aug/Sept pp4.

SANBWA (South African National Bottled Water Association). 2011. About bottled water frequently asked questions. [Online]. Available at <http://www.sanbwa.org.za/water_faq.asp >. Accessed: 12/7/2010.

SA HEALTH INFO (South African Health information). Nutrition.2006. [Online].

Available at
<http://www.sahealthinfo.org/nutrition/vitaminminerals/minerals/calcium.htm>. Accessed: 12/7/2010.

SANS (South African National Standards) 1657,2003. Bottled natural Water, Edition 1.3

SANS(South African National Standards) 241,2006.Drinking Water, Edition 6.1

WESTERHOFF, P., PRAPAIPONG, P., SHOCK, E., HILLAIREAU, A. 2008. Antimony leaching from polyethylene Terephthalate (PET) plastic used for bottled drinking water. *Water Research,* 42:551-556.

WALNER, G., WAGNER, R., & KATZLBERGER, C. 2008. Natural radionuclides in Austrian mineral water and their sequential measurement by fast methods. *Journal of Environmental Radioactivity,* 99: 1090-1094.

World Health Organization (WHO). 2003. Background document for Development of WHO guidelines for drinking water quality, *World Health Organization*, Geneva.

World Health Organization (WHO). 2004. Atrazine in drinking Water, Background document for Development of WHO guidelines for drinking water quality, *World Health Organization*, Geneva.